Olsen and Liang *Molecular Brain* (2017) 10:45
DOI 10.1186/s13041-017-0325-8

Molecular Brain

REVIEW Open Access

Role of $GABA_A$ receptors in alcohol use disorders suggested by chronic intermittent ethanol (CIE) rodent model

Richard W. Olsen[1*] and Jing Liang[1,2]

Abstract

GABAergic inhibitory transmission is involved in the acute and chronic effects of ethanol on the brain and behavior. One-dose ethanol exposure induces transient plastic changes in $GABA_A$ receptor subunit levels, composition, and regional and subcellular localization. Rapid down-regulation of early responder δ subunit-containing $GABA_A$ receptor subtypes mediating ethanol-sensitive tonic inhibitory currents in critical neuronal circuits corresponds to rapid tolerance to ethanol's behavioral responses. Slightly slower, α1 subunit-containing $GABA_A$ receptor subtypes mediating ethanol-insensitive synaptic inhibition are down-regulated, corresponding to tolerance to additional ethanol behaviors plus cross-tolerance to other GABAergic drugs including benzodiazepines, anesthetics, and neurosteroids, especially sedative-hypnotic effects. Compensatory up-regulation of synaptically localized α4 and α2 subunit-containing $GABA_A$ receptor subtypes, mediating ethanol-sensitive synaptic inhibitory currents follow, but exhibit altered physio-pharmacology, seizure susceptibility, hyperexcitability, anxiety, and tolerance to GABAergic positive allosteric modulators, corresponding to heightened alcohol withdrawal syndrome. All these changes (behavioral, physiological, and biochemical) induced by ethanol administration are transient and return to normal in a few days. After chronic intermittent ethanol (CIE) treatment the same changes are observed but they become persistent after 30 or more doses, lasting for at least 120 days in the rat, and probably for life. We conclude that the ethanol-induced changes in $GABA_A$ receptors represent aberrant plasticity contributing critically to ethanol dependence and increased voluntary consumption. We suggest that the craving, drug-seeking, and increased consumption in the rat model are tied to ethanol-induced plastic changes in $GABA_A$ receptors, importantly the development of ethanol-sensitive synaptic $GABA_A$ receptor-mediating inhibitory currents that participate in maintained positive reward actions of ethanol on critical neuronal circuits. These probably disinhibit nerve endings of inhibitory GABAergic neurons on dopamine reward circuit cells, and limbic system circuits mediating anxiolysis in hippocampus and amygdala. We further suggest that the $GABA_A$ receptors contributing to alcohol dependence in the rat and presumably in human alcohol use disorders (AUD) are the ethanol-induced up-regulated subtypes containing α4 and most importantly α2 subunits. These mediate critical aspects of the positive reinforcement of ethanol in the dependent chronic user while alleviating heightened withdrawal symptoms experienced whenever ethanol is absent. The speculative conclusions based on firm observations are readily testable.

Keywords: $GABA_A$ receptors, Rodent model of alcoholism, Chronic intermittent ethanol, Inhibitory synaptic plasticity

* Correspondence: rolsen@mednet.ucla.edu
[1]Department of Molecular and Medical Pharmacology, David Geffen School of Medicine at UCLA, Los Angeles, CA 90095, USA
Full list of author information is available at the end of the article

BioMed Central

© The Author(s). 2017 **Open Access** This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The Creative Commons Public Domain Dedication waiver (http://creativecommons.org/publicdomain/zero/1.0/) applies to the data made available in this article, unless otherwise stated.

Background

Definition of alcohol use disorders

Alcohol use disorders (AUD) are defined as alcohol abuse and alcohol dependence clinically defined as drinking—or being sick from drinking—that interferes with taking care of one's home or family, or causes job troubles, or school problems, creating large problems both for society and for the drinkers themselves [1, 2]. AUD represent a substantial public health problem worldwide. According to the World Health Organization (WHO) 2015 report, the harmful use of alcohol results in approximately 3.3 million deaths per year world-wide [3]. Approximately 7.2% or 17 million adults in the United States ages 18 and older had an AUD in 2012. This includes 11.2 million men and 5.7 million women. Adolescents can be diagnosed with an AUD as well, and in 2012, an estimated 855,000 adolescents ages 12–17 had an AUD [2, 4].

Molecular actions of ethanol on the brain, $GABA_A$ receptors, and other potential ion channel targets, development of drug dependence after chronic ethanol

$GABA_A$ receptors ($GABA_A$Rs) have long been implicated in mediating at least part of the actions of ethanol (EtOH) in mammalian brain. The molecular mechanism(s) of action for intoxicating doses of EtOH have been especially of interest even before the advent of the Research Society on Alcoholism. In recent years, however, the focus of EtOH research has shifted to identifying a protein receptor-based target for EtOH, and several ligand-gated ion channels (LGIC), which include NMDA- [5] and non-NMDA-type glutamate receptors [6, 7], serotonin 5-HT3 receptors [8], inhibitory glycine receptors, purinergic receptors (P2X) [9, 10], and $GABA_A$Rs [11–13], as well as voltage-gated ion channels (VGIC): G-protein coupled inwardly rectifying K^+ channels (GIRK) [14], and Big Potassium (BK) channels, have been implicated in ethanol's actions on the brain. Whether EtOH acts directly or indirectly on these membrane channel proteins is not totally established. EtOH is accepted to have a GABA-mimetic effect. However, some important effects of EtOH on $GABA_A$R-mediated inhibition may be presynaptic [15–18]. Nevertheless, in either case (presynaptic or postsynaptic action on GABAergic transmission), considerable evidence favors the direct action on specific protein targets. We believe there is strong evidence for direct action on some channels, especially $GABA_A$Rs. In this mini-review we give a brief review of evidence suggesting $GABA_A$Rs involvement in AUD, with a detailed summary of the Chronic Intermittent Ethanol (CIE) rodent model, emphasizing studies in our lab.

To date, the mechanisms for how excess EtOH consumption leads to alterations in the human brain that produce alcohol dependence remain murky. The formation of AUD is a chronic and complex process. EtOH affects brain function by interacting with multiple neurotransmitter systems, especially disruption of the delicate balance between GABA, the primary inhibitory neurotransmitter, and glutamate, the major excitatory neurotransmitter in the central nervous system (CNS) [19]. Short-term alcohol exposure tilts this balance toward CNS depression, while under long-term alcohol exposure, the brain attempts to compensate by bringing the balance back toward equilibrium. These neurobiological changes present behaviorally as the development of tolerance to EtOH's sedative effects. When EtOH consumption is abruptly discontinued or reduced, these compensatory changes are no longer opposed by the presence of EtOH, thus leading to the excitation of neurotransmitter systems and the development of the alcohol withdrawal syndrome (AWS) [20].

Evidence suggesting $GABA_A$Rs involvement in AUD

Several lines of evidence suggest a possible role of $GABA_A$Rs in AUD. Here is a list of some of these; space does not allow a thorough review of these subjects nor a thorough evaluation of the pros and cons for the theoretical connection, but present some examples.

1) Human genetic link of AUD and $GABA_A$Rs.
2) Plasticity of neurotransmission triggered by experience (learning and memory), including exposure to neuroactive drugs, with development of dependence. Concept of over-stimulation by agonists or positive allosteric modulators inducing down-regulation of target receptors and compensatory additional receptor changes.
3) Acute EtOH and $GABA_A$Rs. Direct action on $GABA_A$R subtypes.
4) Effects of in vivo chronic administration of EtOH: internalization of $GABA_A$R subtypes and altered subunit gene expression, subtype cell surface levels, and localization involving trafficking.
5) Correspondence of time course of EtOH-induced plastic changes in $GABA_A$R subtypes with behavioral alterations associated with EtOH withdrawal and dependence development. The CIE rodent model of alcohol dependence.

1). Genetics.

AUD is a complicated behavioral disorder with complex genetic involvement. Genes encoding a cluster of $GABA_A$R subunits *GABRA4, GABRA2, GABRB1, and GABRG1* on chromosome 4 are associated with certain aspects of alcoholism in humans. Gene clusters are well known to exhibit co-regulation of expression. There are several $GABA_A$R subunit gene clusters, and some have

been reported to show developmentally controlled co-expression of the gene products [21], suggesting some combination of these proteins acting together functionally in some way, presumably the heteropentameric α4βγ or α2β1γ1 subtypes, could affect alcohol behavior. Single nucleotide polymorphisms (SNPs) in the chromosome 4 $GABA_AR$ subunit genes are highly associated with alcohol abuse and dependence [22–24]. In fact the important α2 subunit [25] shows the highest association with AUD of any gene in the human genome [26]. Why these genes show behavioral association is not clear, but some animal evidence suggests that the α2 subunit-containing $GABA_ARs$ participate functionally in critical neurocircuitry involved in the positive reinforcing effects of EtOH including anxiolysis [27–30] (discussed below), as they are for benzodiazepines (BZs) [31–34], and other drugs of abuse, such as cocaine [35]. We posit that the α2-$GABA_ARs$ are needed for the development of EtOH dependence, with evidence below. Increased expression and function of these $GABA_ARs$ might be associated with dependence, and reduced expression and function somehow associated with less susceptibility to developing dependence. Note that both the α4 [36] and δ [37] $GABA_AR$ subunits in ventral striatum (nucleus accumbens in dopamine reward circuit) are also required for high levels of voluntary EtOH consumption (Commentary [38]).

2). Plasticity of neurotransmission triggered by experience (learning and memory), including exposure to neuroactive drugs, and development of dependence.

Synaptic plasticity is most often described, to be as simplistic as possible, as a strengthening or weakening of synaptic strength in response to activating that synapse. This is probably best typified by the phenomenon of long-term potentiation (LTP) in the hippocampus as a synaptic model of memory [39]. In this model, tetanic (100 Hz for 1 s) stimulation of the perforant pathway input to hippocampal field CA1 results in LTP of excitatory synapses and plastic changes in the synaptic AMPA- and NMDA-type glutamate receptors, changing their expression levels, or subunit composition, and/or localization [40]. The mechanisms proposed for producing synaptic plasticity are many, involving either presynaptic or postsynaptic changes or both [41]. The suggested postsynaptic mechanisms involve protein phosphorylation-controlled membrane insertion, removal, rearrangement of receptors, or mysterious change in receptor conductance [42, 43]. This is usually but not always considered a use-dependent synaptic strengthening. On the other hand, use-dependent down-regulation of neurotransmitter receptors is a well-described phenomenon [44, 45]. The ratio of excitation to inhibition is regarded as so important that a new concept called *scaling* has been put forward (e.g., [46]), in which compensatory changes in excitation or inhibition accompany any perturbation of the other (inhibition or excitation). Nevertheless, examples abound in which the deciding factor for aberrant plasticity is reduced GABAergic inhibitory function, which seems particularly susceptible to derangement. These examples cover several chronic drug models as well as epilepsy. Application of GABAergic positive allosteric modulator (PAM) drugs, or even GABA itself, to the mammalian cerebral cortex produces withdrawal signs upon removal, such that even an hour exposure can produce long-lasting focal seizures upon termination, the so-called "GABA withdrawal syndrome" [47–49] and that modified $GABA_ARs$ are found in many types of human and experimental epilepsy [50–54]. Status epilepticus induces massive synaptic release of GABA and protein phosphorylation-dependent down-regulation of synaptic $GABA_ARs$ [55, 56], leading to plastic changes in other $GABA_AR$ subtypes including extrasynaptic ones [57]. Likewise, administration of, and in some cases withdrawal from, any $GABA_AR$ PAM drug, including neurosteroids [58], BZs [59, 60], and anesthetics [61] can induce $GABA_AR$ down-regulation, compensatory plasticity, producing tolerance and withdrawal and aberrant plasticity involving $GABA_ARs$ and associated negative effects on behaviors. We present evidence that EtOH is also a PAM with this potential for harm via chronic over-stimulation-induced aberrant plasticity, and in fact, involvement in AUD.

3). Acute EtOH and $GABA_ARs$.

Single or acute alcohol consumption is an alcohol intake that occurs over a short period of time.

The effects of single alcohol consumption depend on alcohol concentration and the amount of intake. EtOH concentrations in the brain can vary in a range from a few millimolar after one drink to more than 100 millimolar, which induces sleep in a naïve individual. As a CNS depressant, EtOH in a concentration range of ≥5 ~ 10 mM (about 3 drinks) leads first to a feeling of being 'high' or 'buzzed': mood elevation, talkativeness, increased socialization, disinhibition of shyness, and grandiose thoughts, followed by sedation accompanied by decreased attention, impaired decision making, impaired coordination/ locomotion, alterations in memory, mood changes, and lethargy [15]. These behavioral changes are accompanied by an apparent increase in $GABA_AR$ inhibition and decreased glutamatergic excitation [62, 63]. The legal limit for driving a car in the USA is 0.08% [64], about 17 mM in serum and something similar in brain CSF [65]. A large number of animal experiments have shown EtOH effects on the brain. EtOH is accepted to have a GABA-mimetic effect, and an acute anxiolytic effect, which is at least in part related to the potentiation of GABAergic neurotransmission in the

basolateral amygdala (BLA) [66]. However, as with the case of glutamate receptor synaptic plasticity in LTP [41], and in alcohol actions [5], in addition to postsynaptic $GABA_AR$ interactions of EtOH [11, 67, 68], some important effects of EtOH on $GABA_AR$-mediated inhibition may be presynaptic [16, 17]. In vitro studies on neurons in brain slices, or in culture, or even brain membrane homogenates, demonstrate that application of EtOH at 20 ~ 100 mM stimulates GABA-activated Cl^- channels ($GABA_ARs$: [69, 70]). In studies of effects on neurons using patch clamp recordings in slices prepared after intraperitoneal injection in rats of EtOH (3 g/kg), a rapid down-regulation of $GABA_AR$ phasic and tonic inhibitory currents was observed in hippocampus within 5 ~ 15 min. This change was accompanied by plastic changes in $GABA_AR$ subunit cell surface levels and localization consistent with a net subunit switch [65, 71]. These effects of acute EtOH exposure on $GABA_ARs$ are transient and reversible; understanding the process of $GABA_ARs$ interacting with EtOH from the time of exposure to recovery can provide valuable information for how dependence develops with long term EtOH exposure.

Several lines of evidence support direct action of EtOH on $GABA_ARs$. $GABA_ARs$ have been implicated in mediating the anxiolytic, mood-enhancing, and motor incoordination effects of alcohol at blood alcohol levels of 10 ~ 30 mM [11, 62, 68, 70, 72, 73]. $GABA_AR$ antagonists reduce EtOH effects in vivo, while agonists and PAMS enhance EtOH effects [62]. Systemic EtOH enhances $GABA_AR$-mediated inhibition of target cells but does not show much direct action on such cells [74, 75]. Enhancement of $GABA_AR$ synapses is widely observed (e.g., [76]) but some reports noted that these EtOH actions on $GABA_AR$ synapses could be presynaptic [16, 17]. Others demonstrated direct enhancement of $GABA_AR$ function by EtOH in the assay in brain membane homogenates containing synaptoneurosomes [77, 78] and in primary cultured neurons [79]. In neurons recorded from brain slices, α4/6βδ $GABA_AR$ subtype-mediated tonic inhibitory currents are uniquely sensitive to alcohol (≤30 mM) EtOH concentrations [80–84]. High EtOH sensitivity (≤10 mM) has also been reported in recombinantly expressed α4/6βδ receptors [85], with significant β3 selectivity [86]. Other workers (e.g., Borghese et al., [87]), did not see EtOH effects on $GABA_AR$ currents. Clearly they are region and cell-type specific and of variable amplitude.

4). Effects of in vivo chronic administration of EtOH: internalization of $GABA_AR$ subtypes, altered subunit gene expression, subtype cell surface levels, and trafficking/subcellular localization.

The finding of EtOH-induced $GABA_AR$ plasticity was based on earlier observations on ionotropic glutamate receptors [43] and actions of BZs on $GABA_ARs$ [88]. Chronic administration of BZs leads to tolerance to the traditional 'agonist' effects of diazepam. The effect of chronic agonist BZs on $GABA_AR$ modulation by BZs was at first interpreted as 'uncoupling' of receptors for GABA and BZs [89]. However, Gallager and colleagues [90] used implanted dialysis tubing to administer diazepam continuously for many days and observed reduction in $GABA_AR$-mediated transmission in several brain areas, *not* just reduction of BZ modulation of $GABA_AR$ synapses. Poisbeau et al. [91] pointed out the 'silencing' of $GABA_AR$ synapses in some regions of hippocampus during flurazepam withdrawal. Primus et al. [92] demonstrated uncoupling of BZ modulation of GABA binding to $GABA_ARs$ in membrane homogenates after exposure of recombinant cells expressing $GABA_ARs$ to one hour or more of BZs prior to homogenization. But, this was explained by our observation [93] that the enhancement of BZ binding produced by GABA was retained by the receptor protein after BZ treatment of the cells, because the homogenization resulted in membrane vesicles (endosomes) which exhibited BZ binding inside the vesicles that was insensitive to GABA which could not penetrate the membranes to reach the receptor binding sites, whereas the radioactive BZ could enter. Brief treatment of the membrane vesicles with osmotic shock, centrifugation, and resuspension in fresh assay buffer allowed detection of the same number of $GABA_AR$-BZ binding sites with intact GABA enhancement. This was consistent with the receptor being internalized and no longer functioning at the cell surface, i.e., down-regulation of $GABA_AR$ proteins that are sensitive to a given BZ after over-stimulation by that BZ.

However, tolerance to diazepam and its congeners was accompanied by an increase in efficacy for inverse agonists; further, chronic administration of inverse agonists and lead to 'chemical kindling' of seizures [94, 95]. This led to a teeter-totter model of receptor plasticity, suggested as a change in set point of intrinsic activity for allosteric modulatory drugs, with unknown structural explanation [94]. This idea was supported by the observation of [96] that tolerance to chronic diazepam was reduced persistently after a single exposure to the BZ antagonist flumazenil. However, others [97] showed that kindling by a negative allosteric modulator (NAM) β-carboline could co-exist with diazepam tolerance in mice treated chronically, suggesting the two events are independent. Nevertheless, there was more new information in addition to the evidence by Gallager and colleagues and many others including us for loss of $GABA_ARs$ and not just BZ modulation, apparently due to internalization of BZ-modulated $GABA_ARs$ [71, 98, 99]. Importantly, the cloning of a family of $GABA_AR$ subunit genes and demonstration of a family of heteropentameric receptor subtypes differing in localization as well as

pharmacology and regulatory mechanisms would appear to account for a complicated tolerance pattern for BZs of differing chemical structure [89].

Ticku and colleagues [62] showed EtOH-induced plasticity of $GABA_AR$s including functional reduction of $GABA_AR$-mediated transmission, and increased efficacy for excitatory inverse agonists like Ro-15-4513 (partial inverse agonist) and β-carbolines, accompanied by an up-regulation of diazepam-insensitive (DZ-IS) binding of [^{3}H]Ro15–4513 in forebrain and cerebellum [100], shown later to be due to α4 and α6 subunits, respectively. This is homologous to observations with other GABAergic drugs like BZs. A similar effect of chronic EtOH exposure (CIE [67]) is apparent, producing down-regulation of EtOH- (and diazepam-) sensitivity of $GABA_AR$s, but increased sensitivity to inverse agonist BZ-site NAMs, explained by EtOH-induced $GABA_AR$ plasticity.

Similar increases in $GABA_AR$ α4 subunit and smaller changes in some other subunits were observed by several groups in rodents treated with chronic EtOH, e.g., Ticku and colleagues [101, 102]; and Morrow and colleagues [103, 104]. Measurements by most groups did not include significant withdrawal periods, but Biggio and colleagues examined both chronic EtOH and withdrawal [105, 106]. Our results are described below.

5). The chronic intermittent ethanol (CIE) rodent model of alcohol dependence.

Twenty-five years ago, Kokka and Olsen established a rat model of the kindling hypothesis of alcohol dependence in humans [107, 108] and investigated the possible role of $GABA_AR$s [109]. In kindling, a sub-threshold stimulus such as an electrical stimulus or chemical convulsant drug is administered, and repeated with a defined duration, until the same stimulus produces a frank seizure on its own, and this supersensitivity is long-lasting. The kindling hypothesis of dependence development for CNS depressant drugs, including EtOH, sedative-hypnotics, and BZ addiction [110–112], was proposed based on the kindling of seizures.

The CIE model and its relationship to human alcoholism

CIE: 1991–2006

The rodent CIE regimen, with 5–6 g/kg EtOH administered to rats by gavage per day for at least 40 days (40–60 d) [111], was found to reduce the seizure threshold to the GABAergic convulsant drug pentylenetetrazol (PTZ, a $GABA_AR$-chloride channel blocker), and this change lasted at least 40 d after EtOH was stopped (Fig. 1); importantly, the persistence of the changes (*kindling*) was dependent on the intermittent regimen, with repeated cyclical CNS depression and rebound hyperexcitable mini-withdrawal (Fig. 1a): providing an equivalent amount of EtOH continuously without repeated intermittent withdrawal produced a single serious withdrawal with seizures, but within a couple days there was no remaining effect on the animal, unlike with the CIE regimen (Fig. 1b). Other workers have demonstrated that the intermittent administration of EtOH, including

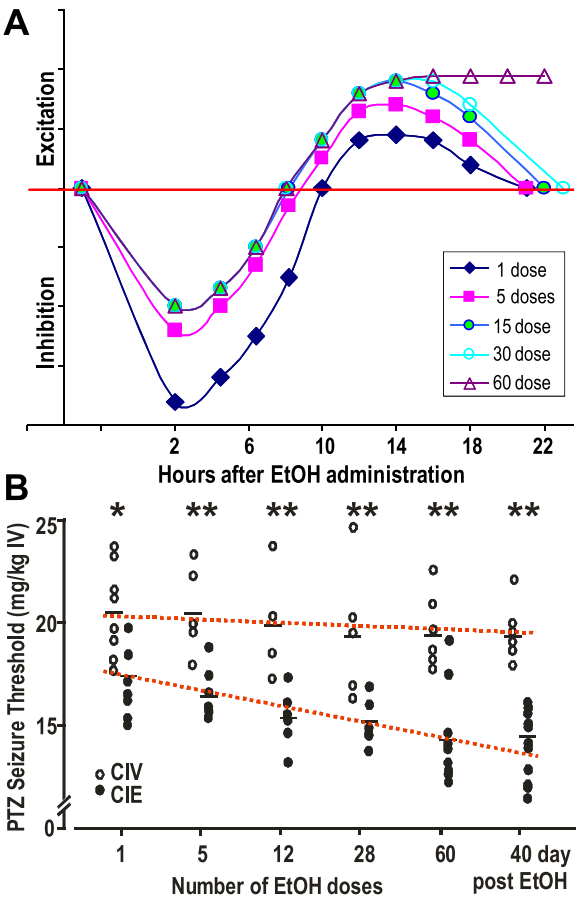

Fig. 1 Time course of behavioral state and PTZ seizure threshold in rats given EtOH by gavage. **a**. Cartoon representation of behavioral state over time after administration of EtOH by oral intubation (gavage) in rat. EtOH exhibits maximum absorption into the brain by ~2 h, accompanied by behavioral depression. As the EtOH leaves the brain, activity (arbitrary units, amplitude depends on dose) returns to normal. Before the EtOH is even eliminated, the behavioral activity returns to normal and overshoots to produce a rebound hyperexcitability (withdrawal), then returns to normal by 24 h (blue diamonds). CIE after 5 doses (pink squares), reduces initial depression (tolerance) and slows return to normal with heightened severity of rebound hyperexcitability. After 60 doses (open triangles) in rats (30 in mice) the heightened withdrawal does not return to normal and stays elevated for at least 40–120 days, possibly for life [109]. This is the CIE 'kindled' state. **b**. Effect of CIE on PTZ seizure threshold: persistent decrease after cessation of EtOH treatment. EtOH, 5.0 g/kg/48 h, was given by oral intubation; PTZ seizure threshold was measured 18 h after EtOH. CIV rats tested at the same times as the CIE rats showed no significant changes in PTZ seizures. Horizontal bars indicate mean PTZ seizure threshold. ** $p < 0.01$. Reproduced from Kokka et al. (1993) [109] with permission. * $p < 0.05$

periods of deprivation, can increase voluntary consumption [113, 114].

The chronic repetition of the mini-withdrawals leads to a persistent state of AWS in which the withdrawals become more severe and long-lasting, eventually becoming permanent. In other words, repetition turns a relatively normal brain activity involving plasticity into a pathological condition of uncontrolled hyperactivity. This is reminiscent of the kindling phenomenon in epilepsy research, in which seizures can be triggered by subconvulsant stimuli after they have been repeated over and over [115, 116]; eventually, seizures can become spontaneous, and once they do, they can occur for the rest of the person's life. One facet of human alcohol dependence is increased seizure susceptibility, and delirium tremens and frank seizures are triggered by withdrawal from EtOH in very heavy abusers [117]. Greater susceptibility and/or severity of seizures is produced by greater periods of EtOH abuse and by previous withdrawals and/or withdrawal seizures. When the number of previous exposures and withdrawal episodes reaches a certain threshold, the severe withdrawal (AWS) [118] becomes persistent, possibly permanent. This led to the conclusion of a kindling-like phenomenon in human EtOH dependence [107, 112, 119, 120]. However, a significant reduction in seizure threshold can be measured during the mini-withdrawals experienced in rats after EtOH administration [109, 121]. This suggests that seizure susceptibility is, first, an integral component of withdrawal. Second, the increased severity and persistence of seizure susceptibility are signs of and critical ingredients of alcohol dependence. Numerous animal models employ this kindling-like regimen of intermittent episodes of EtOH intoxication and withdrawal, termed *chronic intermittent ethanol* (CIE) [113, 122–125].

We showed that in CIE, $GABA_AR$ binding was not much affected throughout the brain but that $GABA_AR$ function, assessed with a neurochemical assay of GABA-stimulated $^{36}Cl^-$ flux in brain slices, was impaired specifically in hippocampal formation, but not in inferior colliculus, several lobes of cortex, thalamus, striatum, or cerebellum. Using extracellular electrode recording in hippocampal slices in collaboration with Dr. Igor Spigelman, we demonstrated a parallel reduction in paired-pulse inhibition [126] that was consistent with the increase in behavioral seizure susceptibility. Veatch and Gonzalez [127] presented similar evidence that intermittent EtOH with multiple withdrawals led to elevated excitability specifically in hippocampus, as detected by electroencephalography (EEG). We have further shown small changes in BZ modulation of $GABA_AR$ radioligand binding accompanied by a significant elevation in the $GABA_AR$ α4 subunit mRNA assessed by in situ hybridization histochemistry; the increase was relatively larger in hippocampus than in thalamus, despite higher levels of the subunit in thalamus [128]. This is consistent with elevated BZ-insensitive $GABA_AR$ and behavioral and cellular tolerance to BZ. Indeed, with intracellular sharp electrode recordings in hippocampal slices, we showed a reduction in allosteric modulation of $GABA_AR$-mediated postsynaptic potentials by BZ and steroids but not by EtOH. EtOH enhancement of evoked synaptic potentials was, if anything, increased [126, 129]. In situ hybridization and reverse transcriptase-polymerase chain reaction (RT-PCR) revealed several changes in $GABA_AR$ subunits in CIE rat brain, including elevated γ2S in hippocampus and increased binding of the imidazo-benzodiazepine radioligand [^{3}H]Ro15–4513 to diazepam-insensitive sites in cerebellum and forebrain, considered to involve the α6 and α4 subunits, respectively; we also showed $GABA_AR$ subunit mRNA level changes consistent with altered expression [130].

EtOH exposure causes changes in rodent brain $GABA_AR$ subunit composition and function, playing a crucial role in EtOH withdrawal symptoms and dependence. We showed [81, 131, 132] that CIE treatment and withdrawal results in decreased EtOH-enhanced δ subunit-containing $GABA_AR$-mediated extrasynaptic current (Fig. 2a) correlated to down-regulated δ subunit (Fig. 2b). This is accompanied by increased EtOH sensitivity of $GABA_AR$ miniature postsynaptic currents (mIPSCs, Fig. 2a) correlated with hippocampal α4βγ2 subtypes including up-regulated α4 (Fig. 2b), and synaptic location demonstrated by post-embedding immunogold labeling electron microscopy (Fig. 2c-d).

Using subunit-specific antibodies, we measured $GABA_AR$ subunits by Western blotting in CIE rat hippocampus and demonstrated significant, persistent elevation in the α4 and γ2 subunits with a decrease in α1 and δ—in other words, a net "subunit switch" of α1 to α4 and δ to γ2. Using reverse transcription polymerase chain reaction (RT-PCR) assays, we found that CIE led to elevated mRNA levels for γ2S but not γ2L, as well as γ1 subunit but not α2; CIE-treated animals were shown to exhibit increased anxiety in the elevated plus maze assay, and behavioral tolerance to the sedative action of EtOH, BZ, and neurosteroids [131]. Steroids and BZ showed reduced enhancement of $GABA_AR$ synaptic and tonic inhibitory currents in hippocampal neurons recorded by patch-clamp electrodes in slices from CIE rats [132].

The changes found after CIE treatment did not appear to involve any gross pathology in either brain or liver [126]. Microscopic examination of tissue sections revealed no evident changes in the morphology and location of GABA-synthesizing neurons in hippocampus, thalamus, or neocortex [128]. Unbiased stereological cell

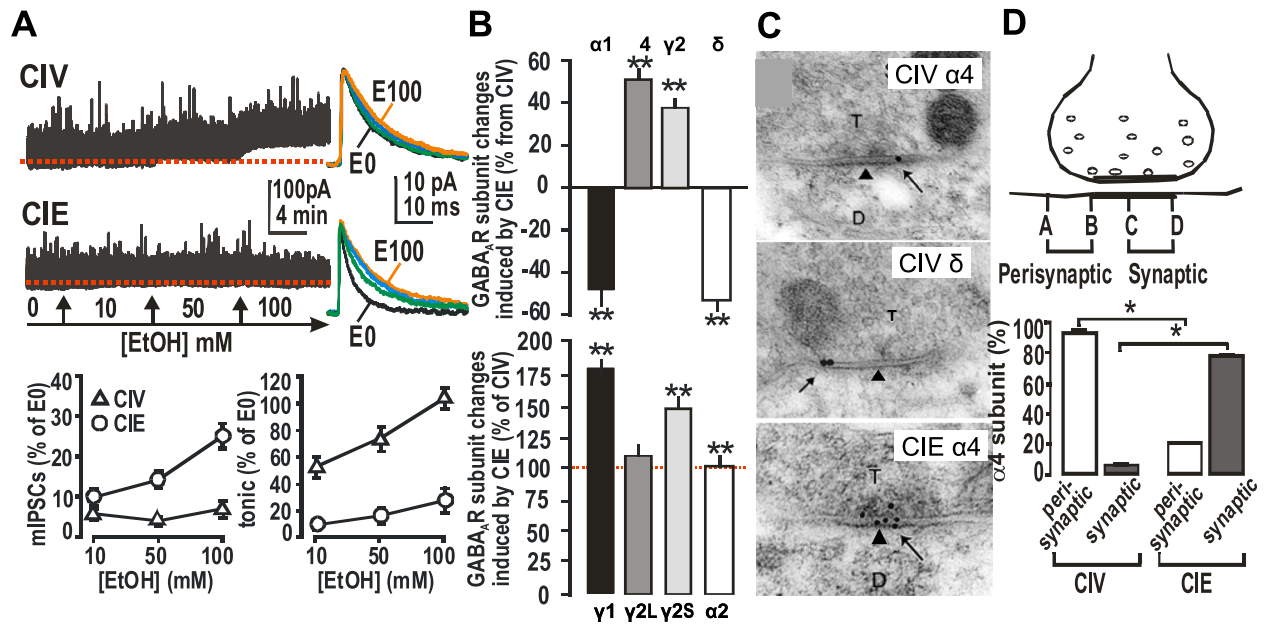

Fig. 2 Plastic changes in GABA$_A$R subunits and currents in rat hippocampal formation induced by CIE. A. EtOH-enhanced mIPSCs observed in hippocampal slices from CIE vs. CIV. Top left of A, recordings from CIV and CIE, including exposure to various concentrations of EtOH in the recording chamber. Top right of **a**, averaged mIPSC from each period response to EtOH applications during the recordings (left of **a**). Bottom of **a**, Summary of mIPSC area and tonic current for EtOH vs. pre-EtOH application. Redrawn from Liang et al., [81]. **b**. Upper: Summary of Western blot analyses of hippocampal GABA$_A$R subunit peptides after CIE compared with CIV. Data are presented as percent changes from control peptide levels mean ± SEM. (n = 10 ~ 12 rats). ** $p < 0.01$, t-test. **b** Lower: GABA$_A$R subunit mRNA levels assayed by PCR, normalized to the unchanged reference gene GADPH. Data are expressed as percentage of CIV group (control) mean ± SEM, ** $p < 0.01$, t-test. **c**. Post-embedding immunogold labeling reveals a change in α4 but not in δ subunit location from perisynaptic to synaptic sites in the molecular layer of the DG after CIE. In CIV (top and middle of **c**), colloidal gold labeling of the α4 subunit (arrows) was present on or near the plasma membrane of dendrites that contacted axon terminals (T). Gold particles were found predominantly at the outer edges of symmetric synapses (arrows) but not at the center of these synapses (arrowheads). After CIE (bottom of **c**), labeling for α4 was found mainly in the center of symmetric synapses (arrows). **d**. Quantitative analysis showed that perisynaptic labeling was found at 93% of α4-labeled synapses (open bar) in CIV (n = 3). In CIE (n = 3), perisynaptic labeling was observed at 22% (open bar) of labeled synapses, but synaptic labeling was evident at 78% of labeled synapses (black bar). *$p < 0.001$ vs. CIV. In contrast to the α4 labeling, δ subunit labeling (arrow) in CIE was present at perisynaptic locations but not within the synaptic contact (arrowhead). Figs. **a**, **c**, and **d** are reproduced from Liang et al. [81] with permission. Figs. **b** are redrawn from Cagetti et al. [131]

counts in the nucleus accumbens of NeuN-stained sections showed no differences between CIE, single-dose EtOH, and vehicle-treated animals (I Spigelman, N Ahmad, J Liang, and RW Olsen, unpublished). This result is not consistent with evidence that exposure to a single very high dose of EtOH with blood levels of over 300 mg/dL, as experienced in human binge drinking, or to a very high level of cumulative alcohol exposure, as in human chronic alcohol abuse, produced significant neuronal cell death [133, 134]. We found no evidence for a significant increase in newborn neurons or for stem cell death in dentate gyrus (DG) of CIE rats versus normal controls (I Spigelman, J Liang, RW Olsen, and F Crews, unpublished). Thus, in our hands, high blood levels of EtOH administered by gavage, exceeding 250 mg/dL for several hours but not exceeding 275 mg/dL [65] were insufficient or too brief to produce the damage reported by other extreme exposures to EtOH. Nevertheless, CIE treatment is definitely a severe, abnormal stress to the brain.

CIE rats exhibit impaired hippocampal-specific spatial learning deficits [135], possibly due to decreased levels of neurosteroids. Neurosteroids (endogenous neuroactive steroids acting as GABA$_A$R-PAMs: Smith [58]) may be increased by acute EtOH and decreased by chronic EtOH [136, 137], and thus could participate in GABA$_A$R plastic changes induced by EtOH [59, 138]. CIE rodents have not been observed to exhibit spontaneous seizures but this has not been studied with sufficient care to conclude that there are none.

CIE: 2007–2017

With the observations of remarkable GABA$_A$R plasticity induced by CIE, we attempted to learn molecular mechanisms and functional relevance through studies to determine the minimum dose, duration, and frequency of

EtOH administration required to produce the changes. We found that a single high, intoxicating, dose of EtOH administered by gavage was able to induce many of the same changes in behavior, $GABA_AR$ subunit composition, and hippocampal neuron pharmacology seen in CIE, but the changes were transient [65]. Thus, we showed that within 1 h the α4 and δ subunits, but not the α1 or γ2 subunits, were reduced at the cell surface, accompanied by loss of EtOH enhancement of tonic inhibitory currents but no change in synaptic pharmacology. Thus, the first target of EtOH action, the extrasynaptic δ subunit-containing $GABA_ARs$ [68] are the first to respond with plastic changes. After 24 h but not at 1 h, one could detect increased cell surface and increased total levels of γ2 and α4 subunits, decreased levels of α1 subunit, and a tolerance to BZ enhancement of both extrasynaptic and synaptic currents (Fig. 3a, b). These changes are probably the result of altered gene expression; they may be triggered somehow by the reduced tonic inhibition or even the reduced synaptic inhibition seen at several hours post-EtOH. Altered protein synthesis may also be initiated by the EtOH exposure itself, but requires a longer time to reach experimental detectability. At 12 ~ 24 h, the animals exhibited tolerance to BZ- and high dose EtOH-induced loss of righting reflex (LORR), and the synaptic currents became more sensitive to EtOH (as in CIE), but they returned to normal within a few days. This included the δ subunit remaining low for 1 ~ 2 days and then returned to normal [65]. All the changes require the CIE regimen to become more persistent, fortunately for human alcohol users, who have the option to refrain from chronic use. Failure to do so is called AUD.

$GABA_AR$ plasticity induced by CIE demonstrated a correlation between the degree of tolerance induced for

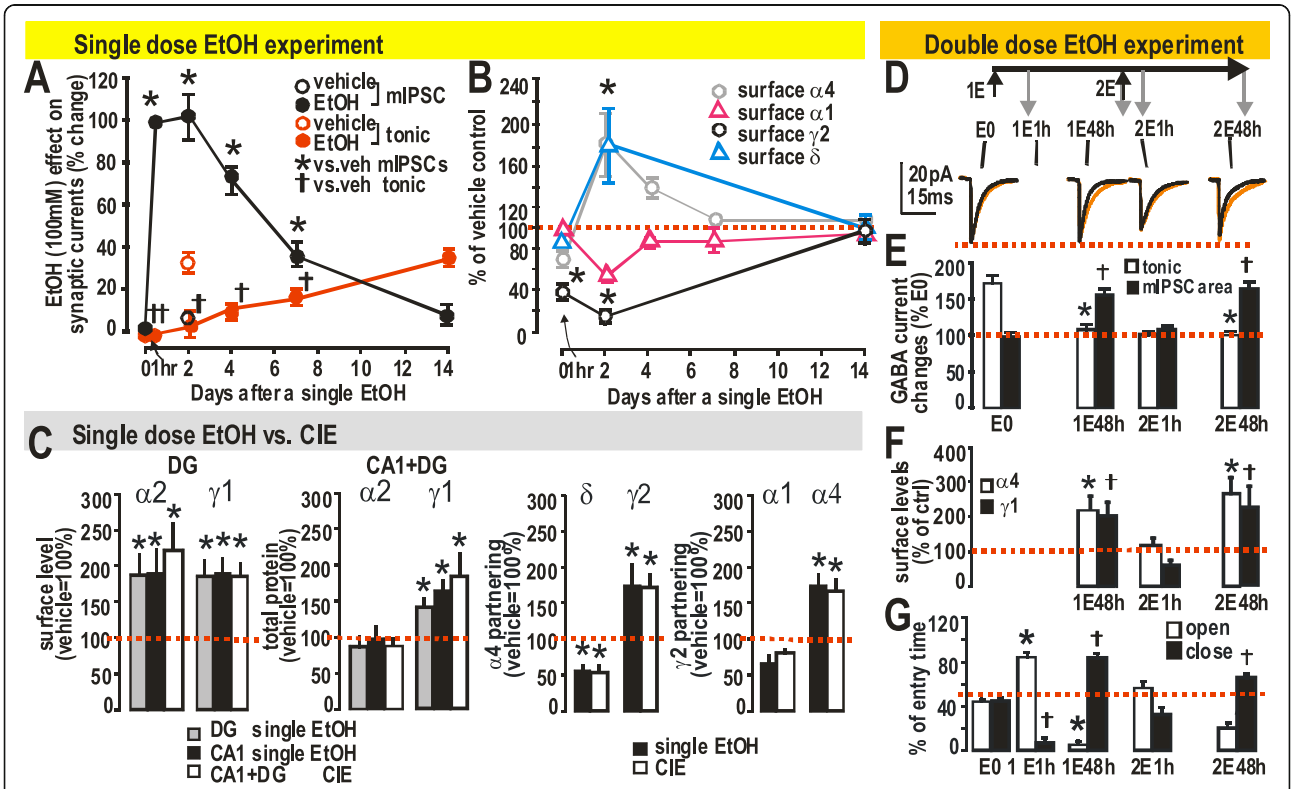

Fig. 3 EtOH-induced plasticity of $GABA_AR$ subunits and currents in rat after single-dose EtOH, CIE, and two-pulse EtOH. **a**: Summary of changes in mIPSCs, and **b**: inhibitory tonic currents after single-dose EtOH vs. pre-EtOH application (redrawn from Liang et al. [65]). A single dose EtOH induces loss of EtOH-sensitive tonic current and gain of EtOH-sensitive mIPSCs. Mean ± SEM are shown as % of vehicle-treated controls (red dashed line, $n = 4–6$. * $p < 0.05$). **c**: Biochemical analysis of $GABA_AR$ subunit plasticity in rat DG within 24 h after single-dose EtOH compared with the changes induced by CIE, 40-d withdrawal. Surface protein levels of $GABA_AR$ subunits measured using protein cross-linking and Western blotting. Mean ± SEM as % of vehicle-treated controls (red dashed line, n = 4–6. * $p < 0.05$). The α2 and γ1 subunits cell surface expression are up-regulated by both one-dose EtOH and CIE, γ1 total peptide is up-regulated, but not α2; and the heteropentameric subunit partnerships up-regulated are α4βγ2 and α2β1γ1. **d**, Upper panel: The protocol of double-dose EtOH experiment. **d**, Lower panel: Averaged mIPSC from each time point response to EtOH applications during the recordings. **e**: Summary of acute EtOH-induced changes in tonic current and mIPSCs ($n = 5$). **f**: Quantification of surface levels of $GABA_AR$ (n = 4–6) by Western blots for $GABA_AR$ α4 and γ1 after cross-linking in slices. **g**: Anxiety assayed by EPM ($n = 6$). The duration time rats stayed in arms (% of total 5 min). **e**,**f**,**g**: all bars are compared to the control (E0 value for that parameter): * p , 0.05; † $p < 0.05$. In **e**, the control level (dashed red line, at 100%) applies only to mIPSCs; in **f**, the red line refers to control (100%) for both subunits; in **g**, the dashed red line corresponds to the E0 point for either open or closed arms. **c**,**d**,**e**,**f**,**g**: from Lindemeyer et al., [30] with permission

a series of GABAergic sedative-hypnotic drugs to produce LORR and the degree of tolerance induced for the same drugs to enhance $GABA_AR$-mediated tonic inhibitory currents in hippocampal neurons [139]. On the other hand, the anticonvulsant and anxiolytic actions of $GABA_AR$ PAMs (EtOH, neurosteroids, propofol, barbiturates, as well as the GABA analogue gaboxadol show little tolerance [81, 131, 132, 135, 139]).

However, the CIE rodents and the one-dose EtOH-treated animals show elevated sensitivity of $GABA_AR$-mediated mIPSCs to modulation by low mM EtOH in the recording chamber [81]. In CIE we observed an increase in α4βγ2 $GABA_ARs$, including movement of the α4 into the postsynaptic membrane. The δ subunit was not elevated and did not accumulate in the synaptic membrane, and the increased EtOH modulation of mIPSCs was also observed in the alcohol- naïve knockout (KO) mouse for both $GABA_AR$ α4 subunit [140] and δ subunit (J Liang, RW Olsen, and I Spigelman (2002), unpublished) and might account for the lack of reduction in many EtOH behaviors in these mice [141–143]. Furthermore, we posited that these EtOH-sensitive $GABA_ARs$ are apparently up-regulated by EtOH treatment, and might be positioned in brain locations where they might mediate the continued EtOH-sensitivity in regions needed for the *positive reinforcement reward as well as anxiolytic efficacy of EtOH in the dependent individual, animal or human*. Therefore, we asked: what might be the subunit composition of $GABA_ARs$ accounting for this increased sensitivity to EtOH of mIPSCs? Below we describe our discovery of a $GABA_AR$ subtype that meets these requirements.

We have also extended the CIE model to the mouse [111]; the mouse required a slightly modified regimen of EtOH administration due to higher metabolism, but we managed to achieve similar EtOH-induced $GABA_AR$ plasticity as in rats. The goal is to establish a short term intermittent EtOH (SIE) mouse model to replicate the information found in the established chronic intermittent EtOH (CIE) model. In comparison to the CIE model, the SIE mouse model can be more easily combined with genetic technology for in depth studies of the underlying mechanisms of alcoholism. C57Bl/6 mice were separated into short intermittent vehicle (SIV) and SIE groups. SIV and SIE mice were gavaged drinking water or ethanol respectively, every other day for five doses and from day 11th, once a day for 30-d. SIV mice served as the control group. We evaluated behavioral changes after two day and 40-d withdrawal from SIE and compared with CIE. The results are consistent with previous reports and indicate that SIE mice, like CIE rats, have greater anxiety, hyperexcitability, and tolerance to acute EtOH-induced LORR than SIV [111]. Then we analyzed genetically engineered animals with $GABA_AR$ subunits knocked out, in, or down [30, 140, 141, 144, 145]. The α4KO mouse showed reduced $GABA_AR$-mediated tonic inhibition throughout the brain and reduced electrophysiological and behavioral effects of gaboxadol [144], including also reduced modulation of tonic currents by low mM EtOH [140], but, disappointingly, normal behavioral responses to EtOH [141, 142]. We did establish in preliminary evaluation that the α4KO mice showed a *blunted* effect of CIE treatment, especially elimination of tolerance development to the intoxicating effects of EtOH, including sedative-hypnotic, motor-incoordinating effects [146]. We extended the model to primary cultured hippocampal neurons [147], where certain variables could be more closely controlled than in the animal. Exposure of the cultured neurons (cultured at embryonic age 18-d, and studied at ≥15-d in vitro (DIV), but not earlier, at which time, they exhibited both δ subunit expression and EtOH-enhanced tonic inhibitory currents, showed a rapid down-regulation of EtOH-enhanced tonic inhibitory currents as well as down-regulation of δ subunit, mimicking the EtOH effect in vivo [147].

Both covalent biotinylation of cell surface proteins (technique most suitable for cultured monodisperse cells) and crosslinking of cell surface proteins, to exclude them from the SDS gel during electrophoresis (technique most suitable for brain slices) that the rapid, within hours, and likely minutes, down-regulation of α4βδ $GABA_ARs$ by EtOH exposure involves protein internalization (endocytosis). In the case of δ subunit, this is clathrin-dependent [71]. This is consistent, as described above, with the extrasynaptic δ subunit-containing $GABA_AR$ as early responders to low millimolar EtOH, and likely requires a conformational change in the intracellular domain of δ to allow it to bind the clathrin accessory subunit when the $GABA_AR$ protein binds GABA 'too long,' as when the GABA concentration is prolonged at a high concentration, or by the presence of a PAM like EtOH to enhance GABA binding. Terunuma et al. [56] showed that during status epilepticus, presumed massive synaptic GABA release and binding to synaptic (α1, 2,and 3) subunit-containing $GABA_AR$ molecules exhibit internalization triggered by the prolonged activated protein conformation with the β3 subunit becoming a substrate for a phosphatase that removes phosphate and allowing endocytosis. This mechanism was ruled out for the δ-containing $GABA_AR$ [71]. The down-regulation of δ-$GABA_ARs$ reverts to normal after some hours to days of EtOH removal but fails to normalize after multi-dose CIE regimen [65, 81]. We have argued that this is probably not due to cell death or damage. One possibility under consideration is the possible loss of a δ membrane surface location-stabilizing protein factor, either the fragile X protein FMRX or

another protein exhibiting increased translation regulated by FMRX. Mice lacking FMRX were found to lose cell surface $GABA_AR$ δ subunit without change in total δ protein [148].

Although most measurements were made, justified by region-specific changes in $GABA_AR$ pharmacology and expression related to EtOH action, in the hippocampal formation, changes in EtOH-sensitive $GABA_AR$ s throughout the CNS are likely (basolateral amygdala: [149–151]; ventral tegmental area: [152], nucleus accumbens: [153]), thus affecting many behaviors. These would be expected to show regional and cell type specificity if they depend on the presence of the $GABA_AR$ subtypes that we found are down-regulated (δ, α1) or up-regulated (α4, α2) by EtOH exposure. We suggest that the plastic changes in hippocampus are a model for changes in other regions and these could well involve areas/circuits critical for both the dopamine reward system (ventral striatum/nucleus accumbens and ventral tegmental area), and for maintained anxiolysis (amygdala, hippocampus) in the EtOH-dependent individual, rodent or human.

The latest news on the CIE rodent model of AUD

Acute and chronic EtOH intoxication in rats increased surface levels of $GABA_AR$ α2 and γ1 subunit protein in hippocampus, using cross-linking and Western blots. CIE and single-dose EtOH administration up-regulate $GABA_ARs$ composed of α2β1γ1 subunits that bind to gephyrin, demonstrated by co-immunoprecipitation (co-IP) experiments [30]. In order to determine which subunits partner with γ1, both γ1 and γ2 co-IP (positive control) Western blots were probed for α1, α2, α4, and α5. In contrast to γ2, which was found to associate with different α subunits, γ1 primarily co-assembled with the α2 subunit (Fig. 3c). The γ1 antibody did not co-IP γ2 and vice versa. The preferred β subunit partner for the α2γ1-containing $GABA_ARs$ was identified by co-IPs with β1-, β2-, or β3-specific antibodies, probing for γ1 and γ2. The γ1 preferentially formed a receptor complex with the β1 subunit, with a small extent with β3 and no β2. By contrast, the γ2 equally partnered with β1 and β3 and somewhat less with β2. These data identify $GABA_ARs$ composed of α2, β1, and γ1 subunits in hippocampal CA1 and DG regions that are found to be up-regulated after CIE and single-dose EtOH exposure (Fig. 4). The selective partnering of γ1 with α2 allows use of γ1 as a marker for the up-regulated pool of cell surface α2 subunits (a minor subtype of α2, which preferentially partners with γ2). Western blotting with a gephyrin antibody suggests at least some postsynaptic localization of γ1-containing receptors at inhibitory synapses. We also showed by co-IP studies on solubilized membrane proteins from hippocampus of CIE-treated rats that the previously reported [81] up-regulated α4 and γ2 subunits and down-regulated α1 and δ subunits are accompanied by a net switch in partnering of α4 from δ to γ2 and partnering of γ2 from α1 to α4; the new α2 is selectively partnered with γ1, β1, and gephyrin [30]. This demonstrates that the up-regulated $GABA_AR$ subtypes are α4βγ2 and α1β1γ1. These up-regulated subtypes are probably satisfactory

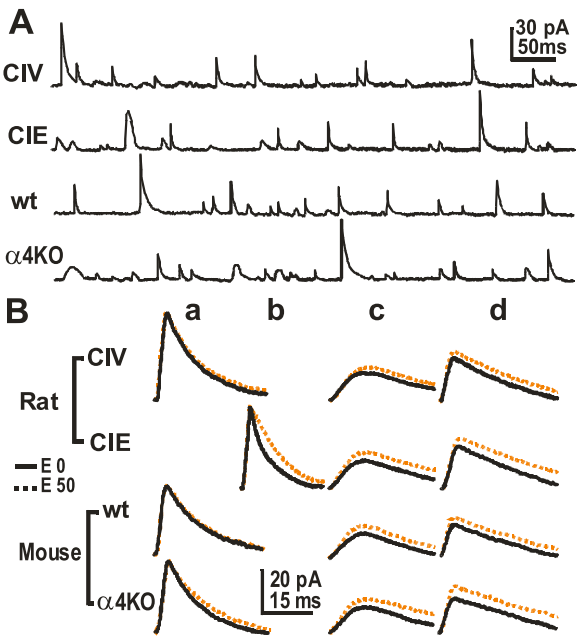

Fig. 4 Hippocampal cells mIPSC kinetics patterns for $GABA_AR$ subtypes in CIE rats and α4KO mice. **A**: mIPSC sample traces of CIE- vs. CIV-treated rats and α4KO and WT mice in hippocampal DG cells. **B**: Averaged mIPSC shape patterns detected by DataView revealed 3–4 relatively abundant distinct templates. In CIV, mIPSC patterns 'a', 'c', and 'd' were detected. Pattern 'a' is a standard shape, typical rise and decay kinetics; patterns 'c' and 'c' are slow rise-slow decay patterns correlated in abundance (not shown here) with α2 subunit subtypes. Three patterns of mIPSCs were also detected in CIE, but the 'a' pattern was not seen in CIE, and replaced by the slower decay pattern 'b'. See text for interpretation that 'a' is mainly α1 and 'b' is mainly α4 subunit subtypes (as in Liang et al., 2006). Patterns of mIPSCs in WT and α4KO mice are similar to CIV rats, with peaks 'a', 'c', and 'd'. However, the abundance of pattern 'd' was increased in CIE relative to CIV and in the α4ko mouse relative to WT. Since the CIE but not CIV, and a4KO mouse but not WT exhibited EtOH-enhanced mIPSCs, we examined recordings of these four animal groups with 50 mM EtOH (E50, dashed line) compared to without EtOH (E0, solid line) in the recording chamber. Peak pattern 'a' was not significantly enhanced by EtOH, but 'b', 'c', and 'd' were enhanced. Peak 'b' in CIE correlates with up-regulated α4, and is not seen in the α4KO mouse. Peak 'd' is up-regulated in both CIE rat and α4KO mouse, as is the α2 subunit surface expression, and peak 'd' has slow kinetics consistent with the α2 subunit subtypes. Its increase in abundance correlates with the increased average stimulation by EtOH in the recording chamber for both CIE and α4KO. Reproduced from Lindemeyer et al. [30] with permission

for replacing the lost synaptic and extrasynaptic inhibitory currents normally mediated by the EtOH-induced $GABA_AR$ subtypes [67].

Time-dependent changes of α4- and α2γ1-containing $GABA_AR$ subtypes are tightly correlated with up- and down-regulation of EtOH-sensitive mIPSCs and withdrawal anxiety following one or two doses of EtOH. The α2β1γ1 and α4βγ2 receptor subtypes have a similar, not easily distinguishable pharmacological profile so we could not unambiguously distinguish them based on pharmacology. In order to understand better the process of CIE-induced synaptic restructuring, we studied effects on rats given a single dose and double dose of EtOH (Fig. 3d, e, f, g). Animals gavaged with a single-dose EtOH (5 g/kg) repeated at 48 h, show within 1 ~ 2 h a parallel loss of α4 and γ1 (marker for α2), loss of EtOH-enhanced mIPSCs in hippocampal slice patch-clamp recordings, and loss of withdrawal signs seen at 48 h after the 1st dose of EtOH (tolerance to EtOH- and BZ-LORR; increased anxiety using the elevated plus maze (EPM) technique, and sensitivity to PTZ seizures). Testing again at 48 h after the 2nd EtOH dose showed return in parallel of all the above: anxiety, EtOH-sensitive mIPSCs, and the up-regulated α2 and α4 (Fig. 3d, e, f, g). Thus either the α2 or α4 might mediate the EtOH-sensitive mIPSCs. Forty-eight hour after the 1st EtOH dose ("one-dose"), the changes already described are seen (behavioral withdrawal, including tolerance to EtOH and BZ sedation and LORR; increased hyperactivity including increased sensitivity to PTZ seizures; and increased anxiety in EPM; loss of EtOH-enhanced tonic inhibitory $GABA_AR$ currents, but appearance of EtOH-enhanced mIPSCs; and down-regulation of δ and α1 and beginning of up-regulation of α4βγ2 $GABA_AR$. Now a 2nd EtOH is administered. Within 1 ~ 2 h, the EtOH-sensitive mIPSCs are gone; the elevated surface α4 and γ1 (marker for the subset of α2-$GABA_AR$, α2β1γ1 subtype) are gone; and the withdrawal anxiety is reduced. At 48 h after the 2nd EtOH, all these parameters return to the levels seen 48 h after the 1st EtOH dose: EtOH-sensitive mIPSCs are present; α4 and γ1(α2) subunits are present; withdrawal anxiety is present (Fig. 5). Thus both the α4- and α2-containing $GABA_AR$ subtypes are changing rapidly up and down after EtOH, one or two doses, and this approach cannot distinguish which might be more important for EtOH-sensitive mIPSCs; possibly both are important [30].

CIE induces up-regulation of one or more $GABA_AR$ subtypes with slow mIPSC decay kinetics. To better understand how changes in subunit combinations alter $GABA_AR$ function and responsiveness to acute EtOH, we measured mIPSCs in DGCs from hippocampal slices of CIV (control) and CIE-treated rats (Fig. 4A), and analysed them for shape patterns using the optimally scaled template method [154] implemented in DataView software to identify kinetic patterns of mIPSCs (e.g., fast rise and fast decay, slow rise and slow decay). Then, we used these identified patterns as templates to detect

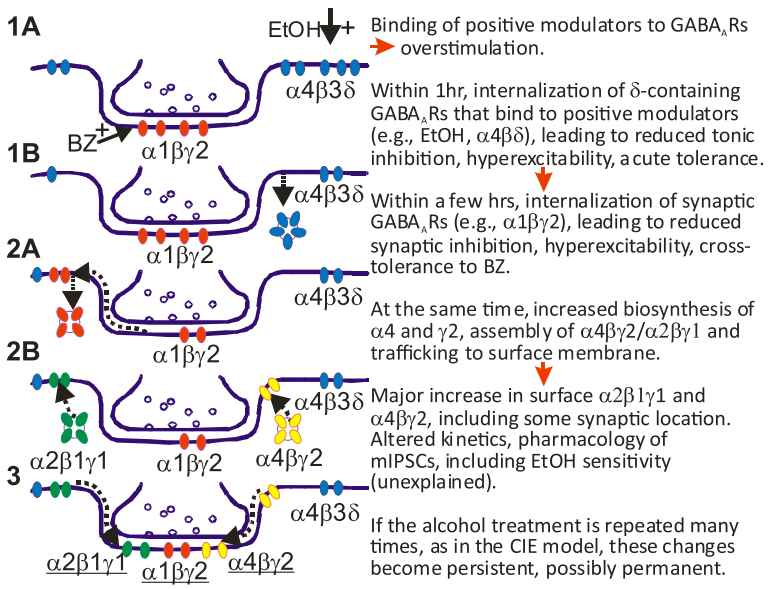

Fig. 5 A Reasonable Hypothesis of $GABA_AR$ Subunit Plasticity Induced Within Two Days by One Dose of EtOH. Administration of EtOH to rats leads to changes of physio-pharmacological properties in GABAergic ionotropic receptor-mediated inhibitory synaptic transmission in hippocampus. The text at right of figure explains the time course of EtOH-induced plasticity, and how these same changes become persistent after CIE treatment. Reproduced from Lindemeyer et al. [30] with permission

differently shaped mIPSCs in the recording traces. An acceptable error level was set which is the degree of similarity an event must have to the templates to be included in the search results. We observed that mIPSCs exhibited a few relatively consistent waveform patterns in the recordings. The detected mIPSC peak patterns were averaged, mIPSC patterns were classified (Fig. 4B, a, b, c, and d), and their kinetic decay constants τ were determined, and % abundance of each template was counted (not shown in figure presented) in a sufficiently large epoch of recordings as in Fig. 4A.

We reasoned that different $GABA_AR$ subtypes have been claimed to be recognizable as different subunit-containing subtypes based on mIPSC kinetics. Different α subunit-containing native or recombinant $GABA_ARs$ with αβγ2 [155–157] could be distinguished from each other, α1 faster than α2, and can be detected in neurons by the peak-shapes of their mIPSCs, which provide a 'fingerprint' for individual α subunits including α2. Recombinant α4β2γ2 have accelerated deactivation compared to their α1 or α5 counterparts, correlating with up-regulated α4 subunit in a hyperexcitable model examining hippocampal slices in a neurosteroid-withdrawn rat [158]. Also, the γ1 subunit-containing receptors (especially with α2) exhibit slower activation and deactivation rates than the respective γ2-containing $GABA_ARs$ expressed in engineered synapses [159]. mIPSC rise time is sensitive to multiple physical variables of synaptic transmission other than receptor subunit composition [160] However, the decay time is less sensitive to these variables as they are rather random, but more sensitive to the nature of the postsynaptic receptor-channels, such as rates of channel closing and agonist dissociation [161] mIPSC shape is highly sensitive to synaptically released peak GABA concentrations and durations [162], but [quote], "differential expression of $GABA_AR$ α subtypes with either a variable or constant ratio from synapse-to-synapse and cell-to-cell, allows them to fulfil individual cellular requirements in network dynamics" [163].

CIV animals exhibited three distinct mIPSC waveform patterns (Fig. 4B): one standard pattern ('a', abundance ~48%), and the other two both display a slower decay pattern ('c', ~37%; and 'd', ~16%). CIE animals likewise showed three distinct mIPSC shape patterns, but one was changed: a 'fast' decay pattern (peak pattern 'b', ~42%); and two apparently similar to CIV patterns with a slow decay (peak pattern 'c', ~22%); and a very slow decay pattern (peak pattern 'd', ~36%). The standard peak pattern 'a' seen in CIV had disappeared in CIE, whereas the ratio of 'c' to 'd' had reversed, from ~2:1 (CIV) to ~2:3 (CIE). Also, importantly, pattern 'd' had clearly increased in abundance, while 'c' may have decreased [30].

To better understand the different pattern of peaks possibly carried by particular $GABA_AR$ subtypes, we extended this analysis to genetically engineered α4KO mice (Fig. 4B). The patterns of mIPSCs in WT mice, untreated. 'a', abundance 46%; 'c', abundance 36%; 'd', 18% are similar to CIV rats, while α4KO mice show mIPSC waveform patterns 'a' (abundance ~36%), 'c' (~35%), with increased abundance of 'd' (~29%) (Fig. 4B [abundance not shown in figure]).

EtOH (50 mM) perfused into the recording chamber potentiated mIPSCs by prolonging decay time and/or increasing charge transfer (area under the curve), as previously observed Liang et al., [81]. For CIE rats, we therefore examined whether EtOH (50 mM) application enhanced the current of the various types of mIPSCs detected (Fig. 4B). We found that acute EtOH potentiated some specific $GABA_AR$ mIPSCs. The area of the mIPSCs increased greatly in CIE pattern 'd' with EtOH in the recording chamber (Fig. 4B), as did its abundance as a fraction of total mIPSCs in the recording trace. In vitro sensitivity to EtOH modulation correlated in time with the up- and down-regulation of the α4- and especially the α2-containing $GABA_AR$ subtype species (Fig. 4B). The mIPSC peak pattern 'a' was previously [81] correlated with the down-regulated α1 subtypes, and the peak pattern 'b' was correlated to the CIE-up-regulated synaptic α4-subtype. But what $GABA_AR$ subtypes account for peaks 'c' and 'd'? These cells also contain α2- and α5-$GABA_AR$ subtypes. The α2 are considered synaptic and the α5 primarily extrasynaptic [164, 165]. Peak 'd' is almost certainly an up-regulated α2 subtype. To summarize, two novel $GABA_AR$ subtypes are up-regulated after acute EtOH treatment and CIE. Cell surface levels of both subtypes are tightly synchronized over one- or two-dose EtOH administration with changes in anxiety behavior and the abundance of EtOH-enhanced mIPSCs. We directly related changes in surface expression of $GABA_AR$ subunits (down-regulation of α1 and δ, up-regulation of α4, α2, γ1, and γ2) with a decrease in heteropentameric extrasynaptic α4βδ- and synaptic α1βγ2-containing $GABA_ARs$ and an increase in postsynaptic α4βγ2- and α2β1γ1-containing $GABA_ARs$ in hippocampal neurons (Fig. 5).

Up-regulated α2 subtypes correlated with the appearance of synaptic currents enhanced by EtOH (>10 mM). EtOH-enhanced mIPSCs have also been observed in untreated α4KO mice [140, 145], in which the EtOH-sensitive subtype cannot contain α4. The α2 subunit is co-localized with gephyrin and presynaptic glutamic acid decarboxylase (GAD) at both DGC cell bodies and axon initial segments [163] and is up-regulated in the hippocampus of α4KO mice [140, 145]. The decrease in α1βγ2- and gain of α4βγ2- and α2β1γ1-containing $GABA_ARs$ change the kinetics and pharmacological

properties of mIPSCs. We previously found decreased diazepam or zolpidem enhancement of mIPSC decay constants and a markedly increased area by the imidazobenzodiazepine partial inverse agonist Ro15–4513 in hippocampal slices after CIE [65, 81, 131, 132, 139], and single-dose treatment in vivo [65]. These pharmacological and subunit changes were reproduced in primary cultured embryonic hippocampal neurons after 15-d in vitro, 24 h after exposure for 30 min to EtOH (50 mM) [147].

Fig. 5 shows a reasonable hypothesis of $GABA_AR$ plasticity induced by EtOH in rat hippocampus (updated from Liang et al., [65]). This shows how synaptic and extrasynaptic $GABA_AR$ subtypes change rapidly in surface expression after in vivo exposure to EtOH and that the plastic changes become persistent after CIE treatment. Note that in this simplified cartoon we have grouped all the game players in a single synapse, which is not likely to be the actual situation.

AUD as an aberrant plasticity phenomenon of $GABA_ARs$ in brain [67]

EtOH induces down-regulation of the first responder receptors, which produces acute tolerance to EtOH, and also triggers the loss of additional $GABA_AR$ subtypes resulting in hyper-excitability. Adaptations to correct this change do restore inhibition, but it is abnormal, and the animals remain hyperexcitable. Although the EtOH-sedating $GABA_AR$ are gone, the replacement $GABA_ARs$ exhibit EtOH-enhanced synaptic $GABA_AR$ inhibitory currents [81]. The CIE-treated rats that show 'kindling' to the $GABA_AR$ channel blocking convulsant drug PTZ-induced seizures [109], and increased anxiety [131], and tolerance to sedative-hypnotic effects produced by EtOH, BZ, and all GABAergic sleep aids (and likely drug-resistant insomnia in man) [139], do not exhibit tolerance to the anxiolytic action of EtOH in the dependent CIE rats [81] and presumably in dependent humans. We posit that the retained sensitivity to the anxiolytic effects of EtOH is important to development of withdrawal-promoted drinking. The hallmark of alcohol addiction is increased drinking and this has been demonstrated by many to result from CIE treatment in rodents [113, 124]. All these behavioral features of alcohol addiction are persistent for 4 ~ 12 months, and probably for life [67, 109]. We have learned that the new EtOH-enhanced synaptic $GABA_AR$ in CIE are the up-regulated α4βγ2 and, especially, α2β1γ1.

The behavioral changes of AWS can be explained by persistently reduced $GABA_AR$-mediated inhibition due to EtOH-induced plasticity of $GABA_ARs$. When this becomes persistent due to the CIE treatment, this can be termed 'aberrant plasticity' [109]. The receptors for the very important rapid neurotransmitters glutamate, and especially GABA, are liable to aberrant plasticity and in a position to do the most harm [166]. In the case of CIE, the treated individual has all the signs of AWS which is an extreme hyperexcitable condition, contributory to increased EtOH consumption. Anxiety (feeling stressed), insomnia, and increased seizure susceptibility (kindling?), also aspects of AWS, would seem to be critical aspects of dependence development [67, 107, 120, 167]. However, we do not know what additional factors, including susceptibility genes, if any, are required to generate actual addiction (alcoholism).

Conclusions, discussion, speculation

Remaining questions about the rodent CIE model

The CIE animal model exhibits EtOH-induced plastic changes in $GABA_AR$ subunit composition and localization. Acute EtOH induces transient changes in a prescribed temporal sequence, starting with decreased extrasynaptic α4βδ, followed by decreased synaptic α1βγ2 detectable within hours but possibly triggered earlier; about the same time a detectable increase in α4βγ2, including surface expression and synaptic localization, is observed in hippocampus [65] and nucleus accumbens [153], as well as increased synaptic α2βγ, primarily α2β1γ1-gephyrin in hippocampal formation [30] and basolateral amygdala (BLA) [150]. The major question remaining is, '*How do these changes become persistent* after EtOH administration that produces a certain number (30~60) of cycles of behavioral depression and hyperexcitable rebound mini-withdrawals?'

Attempts to answer this question have included more detailed analysis of the nature and time course of changes in the subunit composition, both total and surface expression, as well as subtype subunit partnering measured by co-immunoprecipitation and Western blotting, including receptor-associated proteins, in hippocampal formation or microdissected DG or CA1. This has been correlated with alcohol intoxication and withdrawal behaviors and patch clamp recordings of $GABA_AR$ currents in hippocampal slices to determine channel amplitudes, kinetics, and pharmacology, including sensitivity to modulation by EtOH applied in the recording chamber. We have also extended the CIE model to the mouse, and analyzed genetically engineered animals with $GABA_AR$ subunits knocked out, in, or down [30, 140, 141, 144, 145]. Also, we extended the model to primary cultured hippocampal neurons [147], where certain variables could be more closely controlled than in the animal. To determine the nature of the changes more precisely, we attempted to pinpoint the regulated step(s) to aspects of protein cell biology: transcription, translation, assembly, and trafficking, including membrane surface expression? We examined the possible role of associated proteins, protein phosphorylation and/or

neurosteroids. In some cases, we attempted to determine whether all the changes seen were occurring in the same cells.

The rapid removal of α4βδ and somewhat slower build-up of α4βγ2 appears to involve de novo synthesis of α4 as well as assembly selectively of α4βγ2 and membrane insertion, plus synaptic localization [81], not normal for α4-$GABA_A$R [168]. The regulation of α4 transcription has been demonstrated to involve up-regulation of immediate early gene transcription factors, like heat shock proteins elevated by EtOH exposure [169] and/or BDNF, elevated by seizures [170, 171], and/or by microRNAs, possibly suggesting epigenetic mechanisms [172]. We have speculated (below, also Lindemeyer et al., [31]) that the DGC $GABA_A$R synapses after CIE treatment may be abnormal in some way to explain unusual physiology and pharmacology, such as mIPSC kinetics and sensitivity to low millimolar EtOH modulation. This could involve an associated protein, possibly gephyrin/ collybistin [169–171], or even PSD-95 (Lindemeyer AK, Liang J, Olsen RW (2013), unpublished), normally part of glutamate receptor synapses [43]. Once formed, these synapses might be for some reason resistant to turnover and/or reversion to the normal structures, perhaps due to aberrant matrix structure [173–175].

The α1-$GABA_A$R expression and surface localization have been demonstrated to be regulated in vivo by a complex region- and cell-specific protein kinase A and protein kinase C system [53, 176]. In vitro studies in cultured neurons helped clarify the timing and interactions of the various phosphorylation events relevant to both gene expression and trafficking triggered by EtOH exposure [177, 178]. As mentioned above [145], we found in mice lacking the GABAAR α4 subunit that α1- and α2-$GABA_A$Rs were prevalent contributors to the mIPSCs in DGC which were enhanced by EtOH in the recording chamber and were rapidly down-regulated by EtOH exposure, unlike in naïve wild type mice or rats, consistent with early internalization of early responder-$GABA_A$Rs to EtOH in vivo. We also found that the α2-$GABA_A$R subtype of synaptic current that is most abundantly up-regulated in cell surface expression after CIE and that is most sensitive to EtOH modulation in the recording chamber [30]. Since most of the up-regulated pool of α2 in this region and possibly elsewhere such as amygdala [150], is complexed in a heteromeric $GABA_A$R with α2β1γ1 [30], a rare subtype in most regions, and this might produce synapses differing from 'normal, including low turnover and persistent phenotype.

Suggested importance of $GABA_A$R plasticity in AUD and role of α2 subunit

Investigating the relevant $GABA_A$R subtypes for a causative role in CIE/AUD, we tested the U. Rudolph α2KO mouse [179] in the two-bottle choice paradigm to estimate voluntary EtOH consumption and found these animals to exhibit lower acceleration of drinking than wild type [180]. However, the α4KO mouse of Homanics [140] showed higher than wild type level drinking (Fig. 6). This α2KO result conflicts with results from both D. Stephens' lab [181] and A. Harris' lab [182]. Behavioral scientists will understand that variable results are routine in studying different strains of animals in different labs with slightly different methodology, so more study is needed to clarify this situation, but clearly the

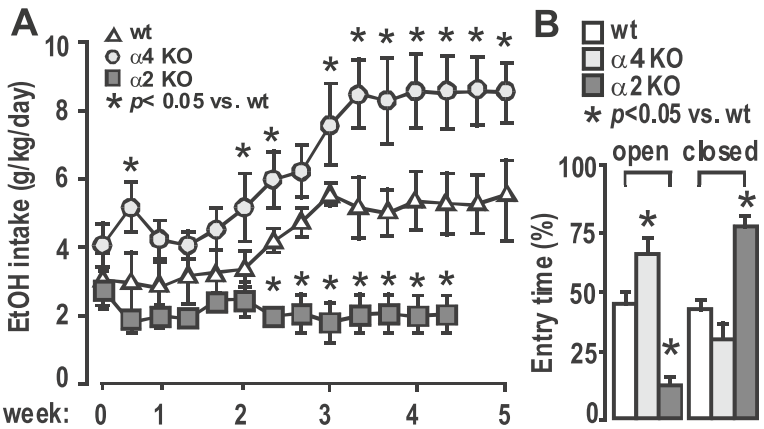

Fig. 6 Two-Bottle Choice Assessment of EtOH Drinking by $GABA_A$R Wild Type and α2KO and α4KO Mice. **a**. EtOH preference assayed by voluntary access to EtOH (15%) in the 2 BC. (Δ, WT [C57/BL/6]; O, α4KO (G Homanics); and □, α2KO (U Rudolph), n = 6–8). After the 3rd week, the EtOH intake in the α4KO group became significantly higher than that in the WT group. In contrast, the α2KO group did not show EtOH preference. **b**. Anxiety assay after 3 weeks of 2 BC measured by EPM (n = 6 ~ 8). The α4KO EPM data show reduced anxiety relative to wild type, while the α2KO exhibit more anxiety

α2-$GABA_A$R subtypes are candidates of interest in AUD. We mentioned above that evidence suggests that the α2 subunit-containing $GABA_A$Rs participate functionally in critical neurocircuitry involved in the positive reinforcing effects of EtOH [27–30], as they are for BZ [32, 33], and other drugs of abuse [35]. We posit that the α2-$GABA_A$Rs are needed for the development of EtOH dependence. Increased expression and function might be associated with dependence, and reduced expression and function somehow associated with less susceptibility to developing dependence. This is consistent with genetic association of GABRA2 with alcoholism [26].

Is the α2-$GABA_A$R in critical brain regions and subcellular membrane locations critical for the anxiolytic response to EtOH and for the elevated drinking in the dependent CIE mouse? If so, it will establish insights into EtOH dependence and drinking and possible therapies that will set the stage for the next generation of questions to answer. For example, how does the EtOH-induced plasticity, namely induction of α2β1γ1 occur, and can it be prevented, or remedied, e.g., with subtype-selective drugs? How do the EtOH-induced plastic changes become persistent, and can that be prevented, or remedied? Is the switch at the level of gene expression or protein trafficking? Where do genetic differences, which we know affect human alcoholism, manifest in such an addiction model? Are the α2β1γ1 and α4 gene cluster SNPs really important? One factor that might be critical for addiction and individual differences is stress [183]: how important is it? Is it possible the $GABA_A$R-enhancing (calming) neurosteroids (metabolites of progesterone and corticosterone) participate at this level? We are giving a lot of credit to $GABA_A$R plasticity in certain circuits: what about $GABA_A$R changes elsewhere and the behaviors affected (e.g., [28])? How about the glutamate receptor plasticity that has also been observed? How do those interact with $GABA_A$R changes? It is likely that numerous neuropsychiatric disorders, not just drug abuse, involve aberrant receptor plasticity and this may be complicated by chronic therapy with negative or positive allosteric modulatory drugs (NAM or PAM) for the receptors involved. Successful therapy for AUD based on $GABA_A$R plasticity would be impetus for more research in the receptor plasticity field.

Administration of any $GABA_A$R-PAM drug, including EtOH, neurosteroids [58], benzodiazepines [60, 89], and anesthetics [61], can induce $GABA_A$R down-regulation, compensatory plasticity, producing tolerance and withdrawal, as well as aberrant plasticity involving $GABA_A$Rs and associated negative effects on behaviors. Neurosteroid $GABA_A$R-PAMs have been demonstrated to produce a hyperexcitable model upon withdrawal, accompanied by anxiety, reduced $GABA_A$R-mediated inhibition, and tolerance to BZs [58, 184], with many changes mirroring with minor differences those reviewed here for acute and chronic EtOH administration. It has also been suggested that neurosteroids (endogenous neuroactive steroids acting as $GABA_A$R-PAMs [58] may a) actually mediate some (but clearly not all) pharmacological actions of EtOH [185]; b) may be increased by acute EtOH and decreased by chronic EtOH [136, 137] and thus participate in $GABA_A$R plastic changes induced by EtOH [59, 138]; c) be particularly sensitive to sex (progesterone) and stress (deoxycorticosterone) endocrine status, since one- or two-step metabolites of the hormones are endogenous $GABA_A$R PAM neurosteroids [58], and appear to change important brain functions during the menstrual cycle and play a neuropsychiatric role in premenstrual syndrome [186], puberty [187], pregnancy [188], post-partum depression [189], involving $GABA_A$R plasticity, both by inducing changes in $GABA_A$R expression and localization [98], and by modulating $GABA_A$R sensitivity to EtOH [58, 190]. It is currently difficult to assess the importance and detailed role of neurosteroids in AUD involving $GABA_A$Rs, but this remains an area of interest requiring more research.

Abbreviations

AUD: Alcohol use disorder; AWS: Alcohol withdrawal syndrome; BZ: Benzodiazepine; CIE: Chronic intermittent ethanol; CIV: Chronic intermittent vehicle; DGC: Dentate gyrus cells; EPM: Elevated plus maze; EtOH: Ethanol; $GABA_A$R: $GABA_A$ receptor; LGIC: Ligand-gated ion channel; NAM: Negative allosteric modulator; NMDA: N-methyl-D-aspartate; PAM: Positive allosteric modulator; RT-PCR: Reverse transcription polymerase chain reaction; SIE: Short intermittent ethanol; SIV: Short intermittent vehicle

Acknowledgements

We especially thank for their assistance with the research and many helpful discussions, Drs. A. Kerstin Lindemeyer, Igor Spigelman, X. Max Shao, Martin Wallner, and Daryl L. Davies. We also thank Michael Scott, Bill Kwon, and other members of the UCLA undergraduate student team for animal treatments and behavioral assays.

Funding

Supported by NIAAA (grant # AA021213), NINDS, and NIGMS of the Office of Extramural Research, National Institutes of Health.

Availability of data and materials

Not applicable.

Authors' contributions

RWO and JL wrote the manuscript, including preparation of figures, and revised the manuscript. We collaborated for 15 years on the research project reviewed in major sections of the article. Both authors read and approved the final manuscript.

Ethics approval

Human research: not applicable.

Animal research: all data presented are published in peer-reviewed journals as cited, except those data presented in Fig. 6 (unpublished). The animals used in those experiments were cared for in the AAALAC-approved vivarium facilities at UCLA, and the research protocols approved (#1993–099-61, #2002–047-08, and #2015–083-01) by the UCLA campus IACUC, monitored by the UCLA Office of Animal Research Oversight.

Consent for publication

Not applicable.

Competing interests
The authors declare that they have no competing interests.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Author details
[1]Department of Molecular and Medical Pharmacology, David Geffen School of Medicine at UCLA, Los Angeles, CA 90095, USA. [2]Titus Family Department of Clinical Pharmacy, School of Pharmacy, University of Southern California, Los Angeles, CA 90089, USA.

Received: 29 May 2017 Accepted: 5 September 2017
Published online: 20 September 2017

References

1. NIH/NIAAA: National Institute on Alcohol Abuse and Alcoholism FIVE YEAR STRATEGIC PLAN 2006. FY07–11 https://www.pubsniaaanihgov/publications/StrategicPlan/ NIAAASTRATEGICPLANhtm.
2. NIH/NIAAA: Alcohol use disorder: A comparison between DSM–IV and DSM–5. NIH Publication 2016 No 13–7999.
3. WHO: Alcohol. Fact sheet Updated January 2015. http://www.whoint/mediacentre/factsheets /fs349/en/.
4. MayoClinic: Alcohol use disorder. 2015 http://www.mayoclinicorg/diseases-conditions/alcohol-use-disorder/basics/definition/con-20020866.
5. Lovinger D, White G, Weight F. Ethanol inhibits NMDA-activated ion current in hippocampal neurons. Science. 1989;243(4899):1721–4.
6. Costa E, Olivera D, Meyer D, Ferreira V, Soto E, Frausto S, Savage D, Browning M, Valenzuela C. Fetal alcohol exposure alters neurosteroid modulation of hippocampal N-methyl-D-aspartate receptors. J Biol Chem. 2000;275(49):38268–74.
7. Frye G, Fincher A. Sustained ethanol inhibition of native AMPA receptors on medial septum/diagonal band (MS/DB) neurons. Br J Pharmacol. 2000;129(1):87–94.
8. Lovinger D, White G. Ethanol potentiation of 5-hydroxytryptamine3 receptor-mediated ion current in neuroblastoma cells and isolated adult mammalian neurons. Mol Pharmacol. 1991;40(2):263–70.
9. Davies D, Asatryan L, Kuo S, Woodward J, King B, Alkana R, Xiao C, Ye J, Sun H, Zhang L, et al. Effects of ethanol on adenosine 5′-triphosphate-gated purinergic and 5-hydroxytryptamine receptors. Alcohol Clin Exp Res. 2006;30(2):349–58.
10. Davies D, Machu T, Guo Y, Alkana R. Ethanol sensitivity in ATP-gated P2X receptors is subunit dependent. Alcohol Clin Exp Res. 2002;26(6):773–8.
11. Aguayo L, Peoples R, Yeh H, Yevenes G. $GABA_A$ receptors as molecular sites of ethanol action. Direct or indirect actions? Curr Top Med Chem. 2002;2(8):869–85.
12. Roberto M, Madamba S, Moore S, Tallent M, Siggins G. Ethanol increases GABAergic transmission at both pre- and postsynaptic sites in rat central amygdala neurons. PNAS. 2003;100(4):2053–8.
13. Davies A, Pierce-Shimomura J, Kim H, VanHoven M, Thiele T, Bonci A, Bargmann C, McIntire S. A central role of the BK potassium channel in behavioral responses to ethanol in C. Elegans. Cell. 2003;115(6):655–66.
14. 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(12):1091–7.
15. Davies M. The role of $GABA_A$ receptors in mediating the effects of alcohol in the central nervous system. J Psychiatry Neurosci. 2003;28(4):263–74.
16. Siggins G, Roberto M, Nie Z. The tipsy terminal: presynaptic effects of ethanol. Pharmacol Ther. 2005;107(1):80–98.
17. Weiner J, Valenzuela C. Ethanol modulation of GABAergic transmission: the view from the slice. Pharmacol Ther. 2006;111(3):533–54.
18. Breese G, Criswell H, Carta M, Dodson P, Hanchar H, Khisti R, Mameli M, Ming Z, Morrow A, Wallner M, Olsen R, et al. Basis of the gabamimetic profile of ethanol. Alcohol Clin Exp Res. 2006;30(4):731–44.
19. Valenzuela C. Alcohol and neurotransmitter interactions. Alcohol Health Res World. 1997;21(2):144–8.
20. Bayard M, McIntyre J, Hill K, Woodside J Jr. Alcohol withdrawal syndrome. Am Fam Physician. 2004;69(6):1443–50.
21. Fillman S, Duncan C, Webster M, Elashoff M, Weickert C. Developmental co-regulation of the β and γ-$GABA_A$ receptor subunits with distinct α subunits in the human dorsolateral prefrontal cortex. Int J Dev Neurosci. 2010;28(6):513–9.
22. Porjesz B, Almasy L, Edenberg H, Wang K, Chorlian D, Foroud T, Goate A, Rice J, O'Connor S, Rohrbaugh J, et al. Linkage disequilibrium between the β frequency of the human EEG and a $GABA_A$ receptor gene locus. PNAS. 2002;99(6):3729–33.
23. Edenberg H, Dick D, Xuei X, Tian H, Almasy L, Bauer L, Crowe R, Goate A, Hesselbrock V, Jones K, et al. Variations in GABRA2, encoding the α2 subunit of the $GABA_A$ receptor, are associated with alcohol dependence and with brain oscillations. Am J Hum Genet. 2004;74(4):705–14.
24. Enoch M. The role of $GABA_A$ receptors in the development of alcoholism. Pharmacol Biochem Behav. 2008;90(1):95–104.
25. Borghese C, Harris R. Alcohol dependence and genes encoding α2 and γ1 $GABA_A$ receptor subunits: insights from humans and mice. Alcohol Res. 2012;34(3):345–53.
26. Olfson E, Bierut L. Convergence of genome-wide association and candidate gene studies for alcoholism. Alcohol Clin Exp Res. 2012;36(12):2086–94.
27. Liu J, Yang A, Kelly T, Puche A, Esoga C, June HL Jr, Elnabawi A, Merchenthaler I, Sieghart W, June HL Sr, Aurelian L. Binge alcohol drinking is associated with GABAA α2-regulated toll-like receptor 4 (TLR4) expression in the central amygdala. Proc Natl Acad Sci U S A. 2011;108(11):4465–70.
28. Koob G. A role for GABA mechanisms in the motivational effects of alcohol. Biochem Pharmacol. 2004;68(8):1515–25.
29. Roh S, Matsushita S, Hara S, Maesato H, Matsui T, Suzuki G, Miyakawa T, Ramchandani VA, Li TK, Higuchi S. Role of GABRA2 in moderating subjective responses to alcohol. Alcohol Clin Exp Res. 2011;35(3):400–7.
30. Lindemeyer A, Shen Y, FYazdani F, Shao X, Spigelman I, Davies D, Olsen R, Liang J. α2 subunit-containing $GABA_A$ receptor subtypes are up-regulated and contribute to alcohol-induced functional plasticity in rat hippocampus. Mol Pharmacol. 2017;92(2):101–12.
31. Löw K, Crestani F, Keist R, Benke D, Brünig I, Benson J, Fritschy J, Rülicke T, Bluethmann H, Möhler H, et al. Molecular and neuronal substrate for the selective attenuation of anxiety. Science. 2000;290(5489):131–4.
32. Tan K, Rudolph U, Luscher C. Hooked on benzodiazepines: $GABA_A$ receptor subtypes and addiction. Trends Neurosci. 2011;34(4):188–97.
33. Engin E, Bakhurin K, Smith K, Hines R, Reynolds L, Tang W, Sprengel R, Moss S, Rudolph U. Neural basis of benzodiazepine reward: requirement for α2 containing $GABA_A$ receptors in the nucleus accumbens. Neuropsychopharmacology. 2014;39(8):1805–15.
34. Engin E, Smith K, Gao Y, Nagy D, Foster R, Tsvetkov E, Keist R, Crestani F, Fritschy J, Bolshakov V, et al. Modulation of anxiety and fear via distinct intrahippocampal circuits. Elife. 2016;5:e14120. doi:10.7554/eLife.14120.
35. Dixon C, Morris H, Breen G, Desrivieres S, Jugurnauth S, Steiner R, Vallada H, Guindalini C, Laranjeira R, Messas G, et al. Cocaine effects on mouse incentive-learning and human addiction are linked to α2 subunit-containing $GABA_A$ receptors. PNAS. 2010;107(5):2289–94.
36. Rewal M, Jurd R, Gill T, He D, Ron D, Janak P. α4-Containing $GABA_A$ receptors in the nucleus accumbens mediate moderate intake of alcohol. J Neurosci. 2009;29(2):543–9.
37. Nie H, Rewal M, Gill T, Ron D, Janak P. Extrasynaptic δ-containing $GABA_A$ receptors in the nucleus accumbens dorsomedial shell contribute to alcohol intake. PNAS. 2011;108(11):4459–64.
38. Olsen R. Extrasynaptic $GABA_A$ receptors in the nucleus accumbens are necessary for alcohol drinking. PNAS. 2011;108(12):4699–700.
39. Bliss T, Lomo T. Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. J Physiol. 1973;232(2):331–56.
40. Lüscher C, Nicoll R, Malenka R, Muller D. Synaptic plasticity and dynamic modulation of the postsynaptic membrane. Nat Neurosci. 2000;3(6):545–50.
41. Bliss T, Collingridge G. Expression of NMDA receptor-dependent LTP in the hippocampus: bridging the divide. Mol Brain. 2013;6:5. doi:10.1186/1756–6606–6-5.
42. Bliss T, Collingridge G, Kaang B, Zhuo M. Synaptic plasticity in the anterior cingulate cortex in acute and chronic pain. Nat Rev Neurosci. 2016;17(8):485–96.
43. Nicoll R. A brief history of long-term potentiation. Neuron. 2017;93(2):281–90.
44. Greengard P. A conversation with Paul Greengard. Interview by Eric J Nestler. Annu Rev Pharmacol Toxicol. 2013;53:1–16.
45. Snyder S. A life of neurotransmitters. Annu Rev Pharmacol Toxicol. 2017;57:1–11.

46. Kilman V, van Rossum M, Turrigiano G. Activity deprivation reduces miniature IPSC amplitude by decreasing the number of postsynaptic $GABA_A$ receptors clustered at neocortical synapses. J Neurosci. 2002;22(4):1328–37.
47. Brailowsky S, Kunimoto M, Menini C, Silva-Barrat S, Riche D, Naquet T. The GABA-withdrawal syndrome: a new model of focal epileptogenesis. Brain Res. 1988;442(1):175–9.
48. Silva-Barrat C, Champagnat J, Brailowsky S, Menini C, Naquet R. Relationship between tolerance to $GABA_A$ agonist and bursting properties in neocortical neurons during GABA-withdrawal syndrome. Brain Res. 1989;498(2):289–98.
49. Frey G. γ-Aminobutyric acid changes in alcohol withdrawal. In: Porter RJ, Mattson RH, Cramer JA, Diamond I, editors. Alcohol and Seizures: Basic Mechanisms and Clinical Concepts. Philadelphia: FA Davis Company; 1991. p. 87–101.
50. Coulter D. Epilepsy-associated plasticity in γ-aminobutyric acid receptor expression, function, and inhibitory synaptic properties. Int Rev Neurobiol. 2001;45:237–52.
51. Olsen R, DeLorey T. GABA and glycine. In: Basic Neurochemistry: Molecular, Cellular, and Medical Aspects. Edited by Siegel GJ AB, Fisher SK, Albers RW, Uhler MD. (Sixth ed.): Philadelphia: Lippincott-Raven. 1999, pp. 335–346.
52. Houser C, Zhang N, Peng Z. Alterations in the distribution of $GABA_A$ receptors in epilepsy. Source: Jasper's Basic Mechanisms of the Epilepsies [Internet] 4th edition. Noebels AM, Avoli, M, Rogawski MA, Olsen RW, Delgado-Escueta AV, editors. Bethesda (MD): National Center for Biotechnology Information (US). 2012.
53. Brooks-Kayal A, Russek S. Regulation of $GABA_A$ receptor gene expression and epilepsy. In: SourceJasper's Basic Mechanisms of the Epilepsies [Internet] 4th edition: Noebels JL, Avoli M, Rogawski MA, Olsen RW, Delgado-Escueta AV, editors. Bethesda (MD): National Center for Biotechnology Information (US). 2012.
54. Macdonald R, Kang J, Gallagher M. $GABA_A$ receptor subunit mutations and genetic epilepsies. In: Jasper's Basic Mechanisms of the Epilepsies [Internet] 4th edition Edited by Noebels JL Avoli M, Rogawski MA, Olsen RW, Delgado-Escueta AV, editors.: Bethesda (MD): National Center for Biotechnology Information (US). 2012.
55. Naylor D, Liu H, Wasterlain C. Trafficking of $GABA_A$ receptors, loss of inhibition, and a mechanism for pharmacoresistance in status epilepticus. J Neurosci. 2005;25(34):7724–33.
56. Terunuma M, Xu J, Vithlani M, Sieghart W, Kittler J, Pangalos M, Haydon P, Coulter D, Moss S. Deficits in phosphorylation of $GABA_A$ receptors by intimately associated protein kinase C activity underlie compromised synaptic inhibition during status epilepticus. J Neurosci. 2008;28(2):376–84.
57. Joshi S, Kapur J. $GABA_A$ receptor plasticity during Ssatus epilepticus. In: Noebels JL, Avoli M, Rogawski MA, Olsen RW, Delgado-Escueta AV, editors. Jasper's Basic Mechanisms of the Epilepsies [Internet] 4th edition Bethesda (MD): National Center for Biotechnology Information (US) 2012.
58. Smith S. Neurosteroid effects in the central nervous system: the role of the $GABA_A$ receptor. Boca Raton: CRC Press; 2003.
59. Biggio G, Purdy R, editors. Neurosteroids and brain function. Int Rev Neurobiol. 2001:46.
60. Follesa P, Cagetti E, Mancuso L, Biggio F, Manca A, Maciocco E, Massa F, Desole M, Carta M, Busonero F, et al. Increase in expression of the $GABA_A$ receptor α4 subunit gene induced by withdrawal of, but not by long-term treatment with, benzodiazepine full or partial agonists. Brain Res Mol Brain Res. 2001;92(1–2):138–48.
61. Curran M, Newman LM, Becker G. Barbiturate anesthesia and alcohol tolerance in a rat model. Anesth Analg. 1988;67(9):868–71.
62. Ticku M. Drug modulation of $GABA_A$-mediated transmission. Sem Neuroci. 1991;3:211–8.
63. Weight F, Aguayo L, White G, Lovinger D, Peoples R. GABA- and glutamate-gated ion channels as molecular sites of alcohol and anesthetic action. Adv Biochem Psychopharmacol. 1992;47:335–47.
64. Evett J, Finley C, Nunez A, Britt L, Huff J. Judicial outcome for the intoxicated driver admitted to a regional trauma center. Acad Emerg Med. 1994;1(3):254–7.
65. Liang J, Suryanarayanan A, Abriam A, Snyder B, Olsen RW, Spigelman I. Mechanisms of reversible $GABA_A$ receptor plasticity after ethanol intoxication. J Neurosci. 2007;27(45):12367–77.
66. Silberman Y, Ariwodola O, Weiner J. β1-Adrenoceptor activation is required for ethanol enhancement of lateral paracapsular GABAergic synapses in the rat basolateral amygdala. J Pharmacol Exp Ther. 2012;343(2):451–9.
67. Olsen R, Spigelman I. $GABA_A$ receptor plasticity in alcohol withdrawal. In: Jasper's Basic Mechanisms of the Epilepsies, 4th edition. Noebels: Avoli M, Rogawski MA, Olsen RW, Delgado-Escueta AV. editors. New York: Oxford University Press; 2012. pp. 562–573.
68. Olsen R, Hanchar H, Meera P, Wallner M. $GABA_A$ receptor subtypes: the "one glass of wine" receptors. Alcohol. 2007;41(3):201–9.
69. Harris R, Allan A. Alcohol intoxication: ion channels and genetics. FASEB J. 1989;3(6):1689–95.
70. Suzdak PD, Schwartz RD, Skolnick P, Paul SM. Ethanol stimulates γ-aminobutyric acid receptor-mediated chloride transport in rat brain synaptoneurosomes. PNAS. 1986;83(11):4071–5.
71. Gonzalez C, Moss S, Olsen R. Ethanol promotes clathrin adaptor-mediated endocytosis via the intracellular domain of δ-containing $GABA_A$ receptors. J Neurosci. 2012;32(49):17874–81.
72. Wallner M, Hanchar HJ, Olsen RW. Low-dose alcohol actions on α4β3δ $GABA_A$ receptors are reversed by the behavioral alcohol antagonist Ro15-4513. PNAS. 2006;103(22):8540–5.
73. Wallner M, Olsen R. Physiology and pharmacology of alcohol: the imidazobenzodiazepine alcohol antagonist site on subtypes of $GABA_A$ receptors as an opportunity for drug development? Br J Pharmacol. 2008;154(2):288–98.
74. Lee R, Smith S, Chapin J, Waterhouse B, Shimizu N, Maddux B, Woodward D. Effects of systemic and local ethanol on responses of rat cerebellar Purkinje neurons to iontophoretically applied γ-aminobutyric acid. Brain Res. 1995;687(1–2):1–11.
75. Palmer M, Hoffer B. GABAergic mechanisms in the electrophysiological actions of ethanol on cerebellar neurons. Neurochem Res. 1990;15(2):145–51.
76. Carta M, Mameli M, Valenzuela C. Alcohol enhances GABAergic transmission to cerebellar granule cells via an increase in Golgi cell excitability. J Neurosci. 2004;24(15):3746–51.
77. Leidenheimer N, Harris R. Acute effects of ethanol on $GABA_A$ receptor function: molecular and physiological determinants. Adv Biochem Psychopharmacol. 1992;47:269–79.
78. Suzdak P, Glowa J, Crawley J, Schwartz R, Skolnick P, Paul S. A selective imidazobenzodiazepine antagonist of ethanol in the rat. Science. 1986;234(4781):1243–7.
79. Mehta A, Ticku M. Ethanol potentiation of GABAergic transmission in cultured spinal cord neurons involves γ-aminobutyric acidA-gated chloride channels. J Pharmacol Exp Ther. 1988;246(2):558–64.
80. Hanchar H, Dodson P, Olsen R, Otis T, Wallner M. Alcohol-induced motor impairment caused by increased extrasynaptic $GABA_A$ receptor activity. Nat Neurosci. 2005;8(3):339–45.
81. Liang J, Zhang N, Cagetti E, Houser CR, Olsen RW, Spigelman I. Chronic intermittent ethanol-induced switch of ethanol actions from extrasynaptic to synaptic hippocampal $GABA_A$ receptors. J Neurosci. 2006;26(6):1749–58.
82. Wei W, Faria LC, Mody I. Low ethanol concentrations selectively augment the tonic inhibition mediated by δ subunit-containing $GABA_A$ receptors in hippocampal neurons. J Neurosci. 2004;24(38):8379–82.
83. Jia F, Chandra D, Homanics G, Harrison N. Ethanol modulates synaptic and extrasynaptic $GABA_A$ receptors in the thalamus. J Pharmacol Exp Ther. 2008;326(2):475–82.
84. Herman M, Roberto M. Cell-type-specific tonic GABA signaling in the rat central amygdala is selectively altered by acute and chronic ethanol. Addict Biol. 2016;21(1):72–86.
85. Sundstrom-Poromaa I, Smith DH, Gong QH, Sabado TN, Li X, Light A, Wiedmann M, Williams K, Smith SS. Hormonally regulated α4β2δ $GABA_A$ receptors are a target for alcohol. Nat Neurosci. 2002;5(8):721–2.
86. Wallner M, Hanchar HJ, Olsen RW. Ethanol enhances α4β3δ and α6β3δ γ-aminobutyric acid type a receptors at low concentrations known to affect humans. PNAS. 2003;100(25):15218–23.
87. Borghese C, Stórustovu S, Ebert B, Herd M, Belelli D, Lambert J, Marshall G, Wafford K, Harris R. The δ subunit of γ-aminobutyric acid type a receptors does not confer sensitivity to low concentrations of ethanol. J Pharmacol Exp Ther. 2006;316(3):1360–8.
88. Tallman J, Paul S, Skolnick P, Gallager D. Receptors for the age of anxiety: pharmacology of the benzodiazepines. Science. 1980;207(4428):274–81.
89. Biggio G. Chloride Channels and Their Modulation by Neurotransmitters and Drugs. New York: Raven Press; 1988.
90. Gallager D, Lakoski J, Gonsalves S, Rauch S. Chronic benzodiazepine treatment decreases postsynaptic GABA sensitivity. Nature. 1984;308(5954):74–7.

91. Poisbeau P, Williams S, Mody I. Silent $GABA_A$ synapses during flurazepam withdrawal are region-specific in the hippocampal formation. J Neurosci. 1997;17(10):3467–75.
92. Primus R, Yu J, Xu J, Hartnett C, Meyyappan M, Kostas C, Ramabhadran T, Gallager D. Allosteric uncoupling after chronic benzodiazepine exposure of recombinant γ-aminobutyric acid (a) receptors expressed in Sf9 cells: ligand efficacy and subtype selectivity. J Pharmacol Exp Ther. 1996;276(3):882–90.
93. Ali N, Olsen R. Chronic benzodiazepine treatment of cells expressing recombinant $GABA_A$ receptors uncouples allosteric binding: studies on possible mechanisms. J Neurochem. 2001;79(5):1100–8.
94. Little H, Nutt D, Taylor S. Kindling and withdrawal changes at the benzodiazepine receptor. J Psychopharmacol. 1987;1(1):35–46.
95. Corda M, Giorgi O, Orlandi M, Longoni B, Biggio G. Chronic administration of negative modulators produces chemical kindling and $GABA_A$ receptor down-regulation. Adv Biochem Psychopharmacol. 1990;46:153–66.
96. Gonsalves S, Gallager D. Persistent reversal of tolerance to anticonvulsant effects and GABAergic subsensitivity by a single exposure to benzodiazepine antagonist during chronic benzodiazepine administration. J Pharmacol Exp Ther. 1988;244(1):79–83.
97. Schneider H, Stephens D. Co-existence of kindling induced by the β-carboline, FG 7142, and tolerance to diazepam following chronic treatment in mice. Eur J Pharmacol. 1988;154(1):35–45.
98. Jacob T, Moss S, Jurd R. $GABA_A$ receptor trafficking and its role in the dynamic modulation of neuronal inhibition. Nat Rev Neurosci. 2008;9(5):331–43.
99. Kuver A, Smith S. Flumazenil decreases surface expression of α4β2δ $GABA_A$ receptors by increasing the rate of receptor internalization. Brain Res Bull. 2015;120:131–43.
100. Mhatre M, Mehta AK, Ticku MK. Chronic ethanol administration increases the binding of the benzodiazepine inverse agonist and alcohol antagonist [^{3}H]RO15-4513 in rat brain. Eur J Pharmacol. 1988;153(1):141–5.
101. Mhatre MC, Ticku MK. Chronic ethanol administration alters γ-aminobutyric acidA receptor gene expression. Mol Pharmacol. 1992;42(3):415–22.
102. Mehta A, Ticku M. Prevalence of the $GABA_A$ receptor assemblies containing α1-subunit in the rat cerebellum and cerebral cortex as determined by immunoprecipitation: lack of modulation by chronic ethanol administration. Brain Res Mol Brain Res. 1999;67(1):194–9.
103. Keir W, Morrow A. Differential expression of $GABA_A$ receptor subunit mRNAs in ethanol-naive withdrawal seizure resistant (WSR) vs. withdrawal seizure prone (WSP) mouse brain. Brain Res Mol Brain Res. 1994;25(3–4):200–8.
104. Kumar S, Kralic JE, O'Buckley TK, Grobin AC, Morrow AL. Chronic ethanol consumption enhances internalization of alpha1 subunit-containing $GABA_A$ receptors in cerebral cortex. J Neurochem. 2003;86(3):700–8.
105. Follesa P, Biggio F, Caria S, Gorini G, Biggio G. Modulation of $GABA_A$ receptor gene expression by allopregnanolone and ethanol. Eur J Pharmacol. 2004;500(1–3):413–25.
106. Sanna E, Talani G, Obili N, Mascia M, Mostallino M, Secci PP, Pisu MG, Biggio F, Utzeri C, Olla P, et al. Voluntary ethanol consumption induced by social isolation reverses the increase of α4/δ $GABA_A$ receptor gene expression and function in the hippocampus of C57BL/6J mice. Front Neurosci. 2011;5:15. doi:10.3389/fnins.2011.00015.
107. Becker H. Kindling in alcohol withdrawal. Alcohol Health Res World. 1998; 22(1):25–33.
108. Brown G, Jackson A, DN S: Effects of repeated withdrawal from chronic ethanol on oral self-administration of ethanol on a progressive ratio schedule. Behav Pharmacol 1998, 9(2):149–161.
109. Kokka N, Sapp DW, Taylor AM, Olsen RW. The kindling model of alcohol dependence: similar persistent reduction in seizure threshold to pentylenetetrazol in animals receiving chronic ethanol or chronic pentylenetetrazol. Alcohol Clin Exp Res. 1993;17(3):525–31.
110. Becker HC, Veatch LM, Diaz-Granados JL. Repeated ethanol withdrawal experience selectively alters sensitivity to different chemoconvulsant drugs in mice. Psychopharmacol (Berl). 1998;139(1–2):145–53.
111. Wong S, Nguyen A, Tan K, Kamrava B, Olsen R, Liang J. Novel alcoholism mouse model exhibits $GABA_A$ receptor-mediated changes. J UCLA Students. 2014;27:39–45.
112. Brown M, Anton R, Malcolm R, Ballenger J. Alcohol detoxification and withdrawal seizures: clinical support for a kindling hypothesis. Biol Psychiatry. 1988;23(5):507–14.
113. Rimondini R, Arlinde C, Sommer W, Heilig M. Long-lasting increase in voluntary ethanol consumption and transcriptional regulation in the rat brain after intermittent exposure to alcohol. FASEB J. 2002;16(1):27–35.
114. Simms JASP, Medina B, Abernathy KE, Chandler LJ, Wise R, Bartlett SE. Intermittent access to 20% ethanol induces high ethanol consumption in long-Evans and Wistar rats. Alcohol Clin Exp Res. 2008;32(10):1816–23.
115. Goddard G, McIntyre D, Leech C. A permanent change in brain function resulting from daily electrical stimulation. Exp Neurol. 1969;25(3):295–330.
116. McNamara JO. Kindling: an animal model of complex partial epilepsy. Ann Neurol. 1984;16 Suppl:S72–6.
117. Trevisan LA, Boutros N, Petrakis IL, Krystal JH. Complications of alcohol withdrawal: pathophysiological insights. Alcohol Health Res World. 1998;22(1):61–6.
118. Goldstein D. Physical dependence on ethanol: its relation to tolerance. Drug Alcohol Depend. 1979;4(1–2):33–42.
119. Booth BM, Blow FC. The kindling hypothesis: further evidence from a U.S. national study of alcoholic men. Alcohol Alcohol. 1993;28(5):593–8.
120. Breese G, Overstreet D, Knapp D. Conceptual framework for the etiology of alcoholism: a "kindling"/stress hypothesis. Psychopharmacol (Berl). 2005;178(4):367–80.
121. McQuarrie D, Fingl E. Effects of single doses and chronic administration of ethanol on experimental seizures in mice. J Pharmacol Exp Ther. 1958;124(3):264–71.
122. Ehlers C, Slawecki C. Effects of chronic ethanol exposure on sleep in rats. Alcohol. 2000;20(2):173–9.
123. McCown TJ, Breese GR. Multiple withdrawals from chronic ethanol "kindles" inferior collicular seizure activity: evidence for kindling of seizures associated with alcoholism. Alcohol Clin Exp Res. 1990;14(3):394–9.
124. O'Dell L, Roberts A, Smith R, Koob G. Enhanced alcohol self-administration after intermittent versus continuous alcohol vapor exposure. Alcohol Clin Exp Res. 2004;28(11):1676–82.
125. Veatch L, Becker H. Electrographic and behavioral indices of ethanol withdrawal sensitization. Brain Res. 2002;946(2):272–82.
126. Kang M, Spigelman I, Sapp D, Olsen R. Persistent reduction of $GABA_A$ receptor-mediated inhibition in rat hippocampus after chronic intermittent ethanol treatment. Brain Res. 1996;709(2):221–8.
127. Veatch L, Gonzalez L. Repeated ethanol withdrawal produces site-dependent increases in EEG spiking. Alcohol Clin Exp Res. 1996;20(2):262–7.
128. Mahmoudi M, Kang M, Tillakaratne N, Tobin A, Olsen R. Chronic intermittent ethanol treatment in rats increases $GABA_A$ receptor α4-subunit expression: possible relevance to alcohol dependence. J Neurochem. 1997;68(6):2485–92.
129. Kang M, Spigelman I, Olsen R. Alteration in the sensitivity of $GABA_A$ receptors to allosteric modulatory drugs in rat hippocampus after chronic intermittent ethanol treatment. Alcohol Clin Exp Res. 1998;22(9):2165–73.
130. Petrie J, Sapp D, Tyndale R, Park M, Fanselow M, Olsen R. Altered $GABA_A$ receptor subunit and splice variant expression in rats treated with chronic intermittent ethanol. Alcohol Clin Exp Res. 2001;25(6):819–28.
131. Cagetti E, Liang J, Spigelman I, Olsen RW. Withdrawal from chronic intermittent ethanol treatment changes subunit composition, reduces synaptic function, and decreases behavioral responses to positive allosteric modulators of $GABA_A$ receptors. Mol Pharmacol. 2003;63(1):53–64.
132. Liang J, Cagetti E, Olsen RW, Spigelman I. Altered pharmacology of synaptic and extrasynaptic $GABA_A$ receptors on CA1 hippocampal neurons is consistent with subunit changes in a model of alcohol withdrawal and dependence. J Pharmacol Exp Ther. 2004;310(3):1234–45.
133. Collins M, Zou J, Neafsey E. Brain damage due to episodic alcohol exposure in vivo and in vitro: furosemide neuroprotection implicates edema-based mechanism. FASEB J. 1998;12(2):221–30.
134. Nixon K, Crews F. Binge ethanol exposure decreases neurogenesis in adult rat hippocampus. J Neurochem. 2002;83(5):1087–93.
135. Cagetti E, Pinna G, Guidotti A, Baicy K, Olsen RW. Chronic intermittent ethanol (CIE) administration in rats decreases levels of neurosteroids in hippocampus, accompanied by altered behavioral responses to neurosteroids and memory function. Neuropharmacology. 2004;46(4):570–9.
136. Cagetti E, Baicy KJ, Olsen RW. Topiramate attenuates withdrawal signs after chronic intermittent ethanol in rats. Neuroreport. 2004;15(1):207–10.
137. Snelling C, Tanchuck-Nipper M, Ford M, Jensen J, Cozzoli D, Ramaker M, Helms M, Crabbe J, Rossi D, Finn D. Quantification of ten neuroactive steroids in plasma in withdrawal seizure-prone and -resistant mice during chronic ethanol withdrawal. Psychopharmacol (Berl). 2014;231(17):3401–14.
138. Ramaker M, Ford M, Fretwell A, Finn D. Alteration of ethanol drinking in mice via modulation of the $GABA_A$ receptor with ganaxolone, finasteride, and gaboxadol. Alcohol Clin Exp Res. 2011;35(11):1994–2007.

139. Liang J, Spigelman I, Olsen RW. Tolerance to sedative/hypnotic actions of GABAergic drugs correlates with tolerance to potentiation of extrasynaptic tonic currents of alcohol-dependent rats. J Neurophysiol. 2009;102(1):224–33.
140. Liang J, Suryanarayanan A, Chandra D, Homanics GE, Olsen RW, Spigelman I. Functional consequences of $GABA_A$ receptor α4 subunit deletion on synaptic and extrasynaptic currents in mouse dentate granule cells. Alcohol Clin Exp Res. 2008;32(1):19–26.
141. Chandra D, Werner DF, Liang J, Suryanarayanan A, Harrison NL, Spigelman I, Olsen RW, Homanics GE. Normal acute behavioral responses to moderate/high dose ethanol in $GABA_A$ receptor α4 subunit knockout mice. Alcohol Clin Exp Res. 2008;32(1):10–8.
142. Cushman J, Moore M, Jacobs N, Olsen R, Fanselow M. Behavioral pharmacogenetic analysis on the role of the α4 $GABA_A$ receptor subunit in the ethanol-mediated impairment of hippocampus-dependent contextual learning. Alcohol Clin Exp Res. 2011;35(11):1948–59.
143. Mihalek RM, Bowers BJ, Wehner JM, Kralic JE, VanDoren MJ, Morrow AL, Homanics GE. $GABA_A$-receptor δ subunit knockout mice have multiple defects in behavioral responses to ethanol. Alcohol Clin Exp Res. 2001;25(12):1708–18.
144. Chandra D, Jia F, Liang J, Peng Z, Suryanarayanan A, Werner DF, Spigelman I, Houser CR, Olsen RW, Harrison NL, et al. $GABA_A$ receptor α4 subunits mediate extrasynaptic inhibition in thalamus and dentate gyrus and the action of gaboxadol. PNAS. 2006;103(41):15230–5.
145. Suryanarayanan A, Liang J, Meyer E, Lindemeyer A, Chandra D, Homanics G, Sieghart W, Olsen R, Spigelman I. Subunit compensation and plasticity of synaptic $GABA_A$ receptors induced by ethanol in α4 subunit knockout mice. Front Neurosci. 2011;5:110.
146. Kelson B, Lim V, Bucley S, Jeong K, Tu C, Basa A, Lam T, Spigelman I, Olsen R, Liang J. Chronic alcohol-induced plasticity of $GABA_A$ receptor is blunted in α4 subunit KO mice. Abstr Soc Neurosci. 2010 #3652.
147. Shen Y, Lindemeyer AK, Spigelman I, Sieghart W, Olsen RW, Liang J. Plasticity of $GABA_A$ receptors after ethanol pre-exposure in cultured hippocampal neurons. Mol Pharmacol. 2011;79(3):432–42.
148. Zhang N, Peng Z, Tong X, Lindemeyer A, Cetina Y, Huang C, Olsen R, Otis T, Houser C. Decreased surface expression of the δ subunit of the $GABA_A$ receptor contributes to reduced tonic inhibition in dentate granule cells in a mouse model of fragile X syndrome. Exper Neurol. 2017; in press
149. Díaz M, Christian D, Anderson N, McCool B. Chronic ethanol and withdrawal differentially modulate lateral/basolateral amygdala paracapsular and local GABAergic synapses. J Pharmacol Exp Ther. 2011;337(1):162–70.
150. Lindemeyer A, Liang J, Marty V, Meyer E, Suryanarayanan A, Olsen R, Spigelman I. Ethanol-induced plasticity of $GABA_A$ receptors in the basolateral amygdala. Neurochem Res. 2014;39(6):1162–70.
151. Herman M, Contet C, Justice N, Vale W, Roberto M. Novel subunit-specific tonic GABA currents and differential effects of ethanol in the central amygdala of CRF receptor-1 reporter mice. J Neurosci. 33(8):3284–98.
152. Arora D, Nimitvilai S, Teppen T, McElvain M, Sakharkar A, You C, Pandey S, Brodie M. Hyposensitivity to γ-aminobutyric acid in the ventral tegmental area during alcohol withdrawal: reversal by histone deacetylase inhibitors. Neuropsychopharmacology. 2013;38(9):1674–84.
153. Liang J, Lindemeyer AK, Suryanarayanan A, Meyer EE, Marty VN, Ahmad SO, Shao XM, Olsen RW, Spigelman I. Plasticity of $GABA_A$ receptor-mediated neurotransmission in the nucleus accumbens of alcohol-dependent rats. J Neurophysiol. 2014;112(1):39–50.
154. Clements J, Bekkers J. Detection of spontaneous synaptic events with an optimally scaled template. Biophys J. 1997;73(1):220–9.
155. Lavoie A, Tingey J, Harrison N, Pritchett D, Twyman R. Activation and deactivation rates of recombinant $GABA_A$ receptor channels are dependent on α-subunit isoform. Biophys J. 1997;73(5):2518–26.
156. McClellan A, Twyman R. Receptor system response kinetics reveal functional subtypes of native murine and recombinant human $GABA_A$ receptors. J Physiol. 1999;515(Pt 3):711–27.
157. Vicini S. New perspectives in the functional role of $GABA_A$ channel heterogeneity. Mol Neurobiol. 1999;19(2):97–110.
158. Smith S, Gong Q. Neurosteroid administration and withdrawal alter $GABA_A$ receptor kinetics in CA1 hippocampus of female rats. J Physiol. 2005;564(Pt 2):421–36.
159. Dixon C, Halbout B, King S, Stephens D. Deletion of the $GABA_A$ α2-subunit does not alter self administration of cocaine or reinstatement of cocaine seeking. Psychopharmacol (Berl). 2014;231(13):2695–703.
160. Capogna M, Pearce R. $GABA_A$,slow: causes and consequences. Trends Neurosci. 2011;34(2):101–12.
161. Otis TS, Mody I. Modulation of decay kinetics and frequency of $GABA_A$ receptor-mediated spontaneous inhibitory postsynaptic currents in hippocampal neurons. Neuroscience. 1992;49(1):13–32.
162. Lagrange AH, Botzolakis EJ, Macdonald RL. Enhanced macroscopic desensitization shapes the response of α4 subtype-containing $GABA_A$ receptors to synaptic and extrasynaptic GABA. J Physiol. 2007;578(Pt 3):655–76.
163. Kerti-Szigeti K, Nusser Z. Similar $GABA_A$ receptor subunit composition in somatic and axon initial segment synapses of hippocampal pyramidal cells. Elife. 2016;5 https://doi.org/10.7554/eLife.18426.
164. Brünig I, Scotti E, Sidler C, Fritschy J. Intact sorting, targeting, and clustering of γ-aminobutyric acid a receptor subtypes in hippocampal neurons in vitro. J Comp Neurol. 2002;443(1):43–55.
165. Olsen R, Sieghart W. International Union of Pharmacology. LXX. Subtypes of γ-aminobutyric acid(a) receptors: classification on the basis of subunit composition, pharmacology, and function. Update. Pharmacol Rev. 2008;60(3):243–60.
166. Chandler L, Harris R, Crews F. Ethanol tolerance and synaptic plasticity. Trends Pharmacol Sci. 1998;19(12):491–5.
167. Heilig M, Egli M, Crabbe J, Becker H. Acute withdrawal, protracted abstinence and negative affect in alcoholism: are they linked? Addict Biol. 2010;15(2):169–84.
168. Zhang N, Wei W, Mody I, Houser C. Altered localization of $GABA_A$ receptor subunits on dentate granule cell dendrites influences tonic and phasic inhibition in a mouse model of epilepsy. J Neurosci. 2007;27(28):7520–31.
169. Pignataro L, Varodayan F, Tannenholz L, Harrison N. The regulation of neuronal gene expression by alcohol. Pharmacol Ther. 2009;124(3):324–35.
170. Brooks-Kayal A, Raol Y, Russek S. Alteration of epileptogenesis genes. Neurotherapeutics. 2009;6(2):312–8.
171. Grabenstatter H, Cogswell M, Cruz Del Angel Y, Carlsen J, Gonzalez M, Raol Y, Russek S, Brooks-Kayal A. Effect of spontaneous seizures on $GABA_A$ receptor α4 subunit expression in an animal model of temporal lobe epilepsy. Epilepsia. 2014;55(11):1826–33.
172. Bekdash R, Harrison N. Downregulation of Gabra4 expression during alcohol withdrawal is mediated by specific microRNAs in cultured mouse cortical neurons. Brain Behav. 2015;5(8):e00355. doi:10.1002/brb3.355.
173. Fritschy J, Brünig I. Formation and plasticity of GABAergic synapses: physiological mechanisms and pathophysiological implications. Pharmacol Ther. 2003;98(3):299–323.
174. Petrini E, Ravasenga T, Hausrat T, Iurilli G, Olcese U, Racine V, Sibarita J, Jacob T, Moss S, Benfenati F, et al. Synaptic recruitment of gephyrin regulates surface $GABA_A$ receptor dynamics for the expression of inhibitory LTP. Nat Commun. 2014;5:3921. doi:10.1038/ncomms4921.
175. Li J, Casteels T, Ingvorsen C, Honoré C, Courtney M, Huber KVM, Schmitner N, Kimmel RA, Romanov RA, Sturtzel C, Lardeau C-H, Klughammer J, Farlik M, Sdelci S, Vieira A, Avolio F, Briand F, Baburin I, Májek P, Pauler FM, Penz T, Stukalov A, Gridling M, Parapatics K, Barbieux C, Berishvili E, Spittler A, Colinge J, Bennett KL, Hering S, Sulpice T, Bock C, Distel M, Harkany T, Meyer D, Superti-Furga G, Collombat P, Hecksher-Sørensen J, Kubicek S. Artemisinins Target $GABA_A$ Receptor Signaling and Impair Cell Identity. Cell. 2017;168:1–15.
176. Kumar G, Khanum F. Neuroprotective potential of phytochemicals. Pharmacogn Rev. 2012;6(12):81–90.
177. Carlson S, O'Buckley T, Thomas R, Thiele T, Morrow A. Altered $GABA_A$ receptor expression and seizure threshold following acute ethanol challenge in mice lacking the RIIβ subunit of PKA. Neurochem Res. 2014;39(6):1079–87.
178. Werner D, Kumar S, Criswell H, Suryanarayanan A, Fetzer J, Comerford C, Morrow A. PKCγ Is required for ethanol-induced increases in $GABA_A$ receptor α4 subunit expression in cultured cerebral cortical neurons. J Neurochem. 2011;116(4):554–63.
179. Crestani F, Löw K, Keist R, Mandelli M, Möhler H, Rudolph U. Molecular targets for the myorelaxant action of diazepam. Mol Pharmacol. 2001;59(3):442–5.
180. Olsen R, Lindemeyer A, Shao X, Shen Y, Rudolph U, Spigelman I, Liang J. Chronic EtOH-Induced up-regulation of EtOH-sensitive GABAR mIPSCs: Responsible GABAR subtypes and relationship to increased EtOH consumption. Alcoholism: Clinical and Experimental Research. 2014;38(Suppl-1):12A.
181. Dixon C, Walker S, King S, Stephens D. Deletion of the gabra2 gene results in hypersensitivity to the acute effects of ethanol but does not alter ethanol

self administration. PLoS One. 2012;7(10):e47135. doi:10.1371/journal.pone.0047135.
182. Blednov Y, Benavidez J, Black M, Chandra D, Homanics G, Rudolph U, Harris R. Linking $GABA_A$ receptor subunits to alcohol-induced conditioned taste aversion and recovery from acute alcohol intoxication. Neuropharmacology. 2012;67:46–56.
183. Norman K, Seiden J, Klickstein J, Han X, Hwa L, DeBold J, Miczek K. Social stress and escalated drug self-administration in mice I. Alcohol and corticosterone. Psychopharmacol (Berl). 2015;232(6):991–1001.
184. Gulinello M, Gong Q, Li X, Smith S. Short-term exposure to a neuroactive steroid increases α4 $GABA_A$ receptor subunit levels in association with increased anxiety in the female rat. Brain Res. 2001;910(1–2):55–66.
185. Morrow A, Khisti R, Tokunaga S, McDaniel J, Matthews D. GABAergic neurosteroids modulate selective ethanol actions: mechanisms and significance. In: Smith S, editor. Neurosteroid effects in the central nervous system: the role of the $GABA_A$ receptor. Boca Raton, FL: CRC Press; 2004. p. 219–45.
186. Maguire JL, Stell BM, Rafizadeh M, Mody I. Ovarian cycle-linked changes in $GABA_A$ receptors mediating tonic inhibition alter seizure susceptibility and anxiety. Nat Neurosci. 2005;8(6):797–804.
187. Afroz S, Parato J, Shen H, Smith S. Synaptic pruning in the female hippocampus is triggered at puberty by extrasynaptic $GABA_A$ receptors on dendritic spines. Elife. 2016;5 doi:10.7554/eLife.15106.
188. Licheri V, Talani G, Gorule A, Mostallino M, Biggio G, Sanna E. Plasticity of $GABA_A$ receptors during pregnancy and postpartum period: from gene to function. Neural Plast. 2015:170435. doi:10.1155/2015/170435.
189. Maguire J, Mody I. $GABA_AR$ plasticity during pregnancy: relevance to postpartum depression. Neuron. 2008;59(2):207–2136019.
190. Abramian A, Comenencia-Ortiz E, Modgil A, Vien T, Nakamura Y, Moore Y, Maguire J, Terunuma M, Davies P, Moss S. Neurosteroids promote phosphorylation and membrane insertion of extrasynaptic $GABA_A$ receptors. PNAS. 2014;111(19):7132–7.

Submit your next manuscript to BioMed Central and we will help you at every step:

- We accept pre-submission inquiries
- Our selector tool helps you to find the most relevant journal
- We provide round the clock customer support
- Convenient online submission
- Thorough peer review
- Inclusion in PubMed and all major indexing services
- Maximum visibility for your research

Submit your manuscript at
www.biomedcentral.com/submit

BioMed Central