Published in final edited form as:

Neuropharmacology. 2017 August 01; 122: 36–45. doi:10.1016/j.neuropharm.2017.04.007.

EFFECTS OF ACUTE ALCOHOL ON EXCITABILITY IN THE CNS

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Abstract

Alcohol has many effects on brain function and hence on human behavior, ranging from anxiolytic and mild disinhibitory effects, sedation and motor incoordination, amnesia, emesis, hypnosis and eventually unconsciousness. In recent years a variety of studies have shown that acute and chronic exposure to alcohol can modulate ion channels that regulate excitability. Modulation of intrinsic excitability provides another way in which alcohol can influence neuronal network activity, in addition to its actions on synaptic inputs. In this review, we review “low dose” effects [between 2–20mM EtOH], and medium dose effects [between 20–50mM], by considering in turn each of the many networks and brain regions affected by alcohol, and thereby attempt to integrate *in vitro* physiological studies in specific brain regions (e.g. amygdala, ventral tegmental area, prefrontal cortex, thalamus, cerebellum etc..) within the context of alcohol’s behavioral actions *in vivo* (e.g. anxiolysis, euphoria, sedation, motor incoordination).

1. Introduction

Alcohol has many effects on central nervous system (CNS) excitability that may underlie its acute and chronic effects on brain function and behavior. In recent years alcohol effects at the synapse have deservedly received a great deal of attention [1]. The focus on actions of alcohol at the synapse is justified, as modification of CNS inhibitory and excitatory synaptic transmission occurs in response to acute and chronic alcohol (reviewed in this edition by Roberto and Varodayan [67]). It is important to remember, however, that the output of individual neurons (and hence neuronal networks) is dependent on the *intrinsic excitability* of the neuron itself, as well as the synaptic inputs it receives, as such inputs are processed and transduced by the electrical properties of the dendritic tree and soma of the postsynaptic neuron. One of the more interesting recent developments in the field has been a variety of studies showing that acute and chronic exposure to alcohol can modulate voltage-dependent and independent ion channels that regulate excitability. These include so-called “leak” channels that are active around the resting membrane potential (RMP) and contribute to RMP, input resistance and inter-spike intervals. In this way, *plasticity of intrinsic excitability* (PIE) provides another mechanism by which alcohol can influence neuronal network

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activity, by altering the physiological “input-output function” of neurons, expressed in terms of the F-I curve, i.e. neuronal firing rate in response to (synaptic) current input.

The NIH recently issued a Funding Opportunity Announcement to solicit grant proposals, “to define molecular and cellular targets in the brain for the effects of alcohol” at low doses. Reactions to the announcement have been mixed within the alcohol research community, but we strongly support the need for this FOA, in part because of our perception that journals now feature a profusion of studies that report effects of alcohol at very high levels. This raises the likelihood that many such studies are reporting on effects of alcohol that are *epiphenomena*; this is to say, the observed effects of alcohol are real (certainly within a laboratory setting), but may not be biologically relevant to the pharmacological actions of the drug in animals and humans at levels that are *relevant to social intoxication*. While revising this review, we were reminded that a similar climate prevailed in the 1990s, so clearly this is not a new problem for the field [see the mini-review by Deitrich and Harris, 68]. All of the points made so clearly in the earlier commentary continue to ring true today in 2017. For example, “*If the purpose of the experiment is to learn about basic mechanisms that control behavioral reactions to ethanol in an animal model, then the amount of ethanol used in an in vitro experiment should be in the range of concentrations that bring about the behavioral reaction in the animal.*”

In pursuing the discussion of alcohol effects in the CNS, it is helpful to define our terms and to examine precisely what is meant by “low dose alcohol”. In this review, we will define “low dose” effects as those seen when blood alcohol concentrations (BACs) are between 220mM EtOH, while recognizing that this spans a wide range of behavioral effects in humans and the relationship between behavioral intoxication and measurement of BACs is complicated[2]. Very low alcohol levels (1–2mM) produce zero or undetectable effects, in all but alcohol-naïve individuals. Slightly higher levels, associated with minimal social drinking (5–10mM) produce detectable effects on visual acuity, are anxiolytic and elicit signs of modest behavioral disinhibition, while higher levels of blood alcohol (15–20mM) typically produce a degree of sedation and motor incoordination that is considered incompatible with operation of a motor vehicle [and which is illegal in many countries]. We will nevertheless define all of these effects as being within the low dose range (L).

The next range of BACs, between 20–50mM, is still relevant to human intoxication, as a variety of CNS depressant effects dominate, including profound sedation, amnesia, emesis, hypnosis and eventually unconsciousness. All of these effects may occur within this range of BACs - in social drinkers, and especially in “binge drinkers”. We will define all of these effects as being within the medium dose range (M). Finally, there is an additional range of BACs between 50–100mM that is not experienced by normal individuals and so is not normally considered relevant to human intoxication. We should note that these levels are tolerated and frequently achieved by chronic alcoholics seeking to experience sedation, anxiolysis, and hypnosis, and so these levels are relevant to the biology of an unusual (but large and clinically important) class of human drinkers. We define 50–100mM as the high dose range (H) and levels above this (100–200mM), which would be lethal to all but alcoholics, as the very high range (VH). In this context, it should be noted that the

relationship between BACs and behavioral intoxication in humans is complex, and there are interactions with the social environment as well as family history [2].

It is also helpful to know what levels of alcohol are reached in rodent models as the majority of *in vivo* alcohol research is done in mice and rats. In voluntary alcohol consumption rodent models, BACs will typically be around the L range from 5–30 mM in continuous 2-bottle choice [4] and operant procedures [3]. Using intermittent access [4–6] and “drinking in the dark” procedures [7], BACs of individual mice may vary widely, but can reach anywhere within L and M ranges, between 10–50 mM. In forced alcohol exposure methods, BACs can be more readily controlled via ethanol gavage, intraperitoneal injection, and vapor exposure - often in conjunction with the alcohol dehydrogenase inhibitor pyrazole [indeed, in rodents such methods are almost always required to achieve BACs in the H range]. Typically, in these procedures target BACs are within the M range of 30–50 mM. Chronic or intermittent exposure to these levels are known to lead to behavioral symptoms of alcohol dependence and withdrawal [8]. For reasons outlined above, we will focus almost all of this review on work that corresponds to the L and M dose ranges.

There are many ways of organizing a review of this type. One could organize the material chronologically, by the molecular or synaptic targets of alcohol, or simply highlight the new and novel. We have chosen instead to organize the material in two ways: firstly, by considering in turn each of the many networks and brain regions affected by alcohol (see e.g. Figure 1), and thereby attempt to integrate *in vitro* physiological studies in specific brain regions (e.g. amygdala, ventral tegmental area, prefrontal cortex, thalamus, cerebellum etc.) within the context of alcohol’s behavioral actions *in vivo* (e.g. anxiolysis, sedation, motor incoordination). Secondly, as discussed above we have chosen to highlight effects that occur at lower alcohol concentrations, and try to point out the most important and likely existing molecular and cellular targets for alcohol, bearing in mind that the targets for anxiolysis, diuresis, mild cognitive impairment and mild euphoria (all features of social intoxication and within the L range) will probably be more sensitive than the targets that mediate gross motor incoordination, amnesia, emesis and unconsciousness (usually within the M range).

Finally, we have studiously avoided trying to pick winners and losers here, in terms of targets or by critiquing published studies. In this respect, time often plays the role of editor in science more successfully than review authors or journal editors, and it will be apparent before long, which of these studies and targets show the most reliable and reproducible responses to alcohol. In this way we hope to assemble the available evidence and help to summarize and clarify existing knowledge, and perhaps to illuminate areas where there are clearly gaps in our understanding.

2. Cerebellum

The cerebellum is intimately involved in the integration of visual information with motor output, and in fine motor control, but it is also recognized to play an important role in other behavioral activities and has extensive connectivity with non-motor areas such as the prefrontal cortex. In any case, it seems inarguable to consider alcohol actions in the cerebellum as important for its motor effects.

2.1 Low Ethanol (<20mM)

Carta and colleagues [9] assessed the effect of acute ethanol application on GABAergic transmission at Golgi interneuron synapses onto cerebellar granule cells. These authors found that 10 and 20mM ethanol significantly increased sIPSC frequency in granule cells. Additionally, 20mM ethanol increased the tonic current noise variance in these cells. There was no effect of ethanol on evoked IPSCs or glutamatergic transmission at mossy fiber/granule cell synapses. The extrasynaptic GABA_A receptors expressed in cerebellar granule cells have also been implicated in the sensitivity of these cells to low dose ethanol [10]. Hanchar and colleagues found that low ethanol concentrations (3 and 10mM) enhance the tonic GABA current. These authors also reported that a naturally occurring single-nucleotide polymorphism in the gene encoding these receptors further enhances ethanol sensitivity of these extrasynaptic GABA_A receptors, but this effect has not been replicated by other investigators [11]. Cerebellar slices have also been used to assess the effect of ethanol on the excitability of Purkinje cells which are innervated by cerebellar climbing fibers to evoke complex spikes [12]. At a low dose, ethanol (10mM) has been demonstrated to decrease the area of the complex spike. Diaz and Valenzuela [13] found that 20mM ethanol increased sIPSC frequency in cerebellar granule neurons from very young animals (pre-weaning), but this concentration had no effect on tonic inhibition in these cells.

2.2 Medium Ethanol (20–50mM)

As mentioned previously, Carta [9] investigated the effect of ethanol on inhibition of cerebellar granule cells. These authors found that 35 and 50mM ethanol increases sIPSC frequency without effecting IPSC amplitude or decay. This ethanol concentration also increased the tonic current recorded from these cells, inducing an inward current shift. These ethanol-induced increases in GABAergic transmission are occluded by concurrent application of TTX. At 50 mM, ethanol also increases the spontaneous firing frequency of Golgi cells (measured using cell-attached recordings). Hanchar and colleagues [10] found that a medium ethanol concentration (30mM) significantly enhanced tonic GABA currents in cerebellar granule cells. This enhancement was potentiated in granule cells expressing mutant extrasynaptic GABA_A receptors, as was ethanol-induced enhancement of sIPSC frequency; no ethanol-induced changes in sIPSC amplitude or decay time were observed. However, a recent attempt to replicate the results of this study was not successful [11]. Botta et al., [11] found that although a moderately high concentration of ethanol (25mM) increased the amplitude of tonic currents reported in cerebellar granule cells, this effect did not significantly differ in recordings from neurons from wild type and mutant cells. Similarly, although 25mM ethanol increased sIPSC frequency, the subunit polymorphism did not alter the sensitivity of this effect. These discrepancies have yet to be resolved.

A moderately high concentration of ethanol (50mM) has also been shown to increase the frequency of sIPSCs and to enhance paired-pulse facilitation of evoked IPSCs in dissociated rat cerebellar Purkinje neurons [14]. Medium ethanol concentrations (40mM) also increase the frequency and regularity of spontaneous Golgi cell (interneuron) firing and depolarize the membrane potential of these cells [15]. These effects appear to be the result of an ethanol-induced inhibition of the Na⁺/K⁺ ATPase, which controls Golgi cell firing. [12]. This group also demonstrated that moderately high concentrations of ethanol (25 and

50mM) significantly decrease complex spike area in Purkinje cells. At 50mM, ethanol also modulates the late phase of the complex spike, decreasing the amplitude of the after-hyperpolarization, with no effect on calcium transients elicited by climbing fiber stimulation. Application of an mGluR1 receptor antagonist mimicked and occluded the effect on 50mM ethanol on complex spike area, suggesting that interaction with mGluR1 receptors may mediate the effect of ethanol observed in these cells. In support of this, 50mM ethanol inhibited mGluR1-dependent EPSCs recorded from Purkinje neurons in response to climbing fiber stimulation. Ethanol had no effect on AMPA receptor-mediated paired-pulse facilitation, however. Finally, these authors reported that 50mM ethanol inhibits synaptic plasticity at climbing fiber synapses.

Diaz and Valenzuela [13] found that 40mM ethanol increased the frequency of sIPSCs in cerebellar granule neurons obtained from pre-weaning rats. This concentration also increased tonic inhibitory currents in these cells. Belmeguenai and colleagues [16] assessed the effect of a moderately high dose of ethanol (50 mM) on long-term depression at cerebellar parallel fiber synapses onto Purkinje cells in rats, and found that this concentration blocks LTD, but not LTP, at these synapses. 50mM ethanol also reduced the amplitude of voltage-gated calcium currents; this effect was not observed at a lower concentration (20mM). Ethanol also reduced mGluR1-mediated EPSC amplitude at 50mM, but not 20mM, and this effect persisted after washout, suggesting that moderately high concentrations of ethanol induce long-term alterations via an mGluR1 mechanism.

These studies indicate that low and medium concentrations of ethanol produce enhanced synaptic and tonic inhibition of cerebellar granule cells. These actions might be mediated through direct actions of ethanol on tonic and synaptic GABARs, but may also arise indirectly by increasing the excitability of GABAergic Golgi cells. Based on the known circuitry of the cerebellum, increased inhibition of granule cells should lead to a decrease in the synaptic activation of Purkinje cells. Purkinje cells send GABAergic projections to form synapses on the deep cerebellar nuclei, which project from the cerebellum to relay neurons of the thalamus, which in turn project to the cortex (reviewed in [17]). Thus, low and medium doses of ethanol may disrupt fine motor coordination through multiple actions in the cerebellum, which then likely impair thalamic processing of motor information.

3. Ventral Tegmental Area (VTA)

As the site of the cell bodies of the dopaminergic neurons that innervate the nucleus accumbens (nAcc), the VTA is a critical component of the brain's reward system, and as such is one of the critical presumptive sites of action for the euphoric actions of low to medium levels of alcohol. The VTA is also accepted as being involved in the neuroadaptation that takes place during the stage of **positive reinforcement** in the process of alcohol addiction.

3.1 Low Ethanol (<20mM)

In vitro electrophysiological experiments in acute brain slices and in dissociated neurons reveal that ethanol begins to exert effects on the ventral tegmental area (VTA) in the low dose range. At a very low concentration of 1 mM, ethanol has been found to potentiate the

peak current produced when glycine is co-applied onto dissociated rat VTA neurons by 20% [18]. Glycine receptors have long been known to be sensitive targets of alcohol, from work in brain and also in recombinant receptors [69].

Experiments using 10 mM ethanol suggest that the synaptic input onto VTA dopamine (DA) neurons may be altered at this level. At a concentration of 10 mM, ethanol has been found to increase the frequency of spontaneous EPSCs in putative VTA DA neurons by 15–29% [19, 20]. At this concentration, the frequency of spontaneous IPSCs on putative VTA DA neurons has also been observed to decrease by 19% [21].

At 20 mM, it has been shown that the firing rates of VTA neurons are altered. Using whole-cell patch clamp, 20 mM ethanol has been reported to increase the firing rate of dissociated VTA DA neurons by 6% [22]. More recently the cell-attached recording technique has been used to show that DA neurons in the medial VTA in mice are more sensitive to ethanol, and 20 mM ethanol was found to produce an increase in firing rate of 21% [23]. A history of alcohol exposure has been shown to be important in the medial VTA. In brain slices obtained from mice given intermittent access to alcohol during adolescence (PD30–60), 10 mM ethanol has been shown to increase the firing rate of DA neurons in the medial VTA 7.7%, whereas no effect was seen in brain slices from alcohol-naïve mice [24]. While 20 mM ethanol has been found to increase the firing rate of VTA DA neurons in the rat, the firing rates of VTA GABA neurons have been found to decrease by 41% at this concentration [25].

3.2 Medium ethanol (20–50mM)

In vitro electrophysiology has also been used to study the effects of medium doses of ethanol on VTA DA neurons. 40 mM ethanol has been found to decrease the amplitude of the M-type K^+ channel current by 7% in dissociated rat neurons [22]. In addition, this concentration of ethanol has been shown to decrease the peak amplitude of GABA_A receptor-mediated evoked IPSCs in VTA DA neurons by 23% while increasing the paired-pulse ratio of evoked IPSCs by 24% [21]. The amplitude of the depolarizing current I_h , which is carried by HCN channels, has been found to increase by 12% after the administration of 50 mM ethanol.

Initially, the majority of studies investigating alcohol's actions on VTA activity used high concentrations of ethanol. More recently, there have been efforts to identify effects at low and medium concentrations. These studies have found that very low concentrations of acute ethanol (1–10 mM) increase excitatory synaptic currents and decreased inhibitory activity. Direct effects on DA neuron excitability have been observed at slightly higher concentrations (20 mM) and this sensitivity shifts to lower concentrations based on alcohol experience. GABAergic neurons in the VTA also appear to be inhibited by acute alcohol. A summary of the data reported would suggest that acute alcohol in this more relevant dose range likely affects VTA activity by directly modulating voltage-gated ion channels on DA neurons and by shifting activity from ligand-gated ion channels towards excitatory activity. The increase in activity of DA neurons following alcohol would therefore be expected to alter the activity of several downstream regions including the prefrontal cortex, amygdala, thalamic structures, and ventral striatum (nucleus accumbens), among other projection targets of the VTA [26]. The network including the VTA is thought play a central role in the

rewarding properties of alcohol at low concentrations that promote euphoria and may enhance consumption of alcohol to high levels.

4. Hippocampus

The critical role of the hippocampus (and related cortical structures such as the entorhinal cortex) in learning and memory makes these brain areas the presumptive site of action for the amnesic actions of medium levels of alcohol.

4.1 Low Ethanol (<20mM)

One of the earlier patch clamp studies performed in cultured mouse hippocampal neurons determined that concentrations of 40mM ethanol potentiated GABA-gated current, and that increasing concentrations up to 80mM were ineffective in producing further potentiation [27]. Other early work utilizing whole-cell patch clamp recordings in rat hippocampal CA1 neurons showed that 10–50mM concentrations of ethanol enhanced GABA-A receptor mediated fast IPSCs, and that the concentration-dependence of IPSC enhancement constituted a bell-shaped curve, with significant potentiation at 10mM, peaking at 20mM [28]. However, it must be stated that there have been many negative findings over the years in studies that have attempted to repeat these observations. The results of these investigations underscore the importance of using physiologically relevant ethanol concentrations when studying hippocampal excitability and plasticity in response to acute ethanol.

4.2 Medium Ethanol (20–50mM)

More recent work has been performed within a more narrow range of ethanol concentrations for studying physiologically relevant effects. Whole-cell recordings in the dentate gyrus of the mouse hippocampus indicated that the δ subunit-containing GABA-A receptors were potential targets of low ethanol concentrations (30mM), as revealed by the reduced excitability of dentate granule cells associated with enhanced tonic inhibition [29]. The majority of studies contend that 30mM is the most effective ethanol concentration in achieving optimal modulation of GABA-A receptors in hippocampal slice preparations. Some recent work suggests that ethanol modulation of GABA-gated current is dependent on the time course of exposure or by previous exposure to low ethanol concentrations. Using whole-cell patch clamp techniques to measure GABA-gated Cl^- currents in pyramidal CA1 neurons from female rats, Smith et al. determined that 30mM ethanol optimally enhanced GABA-gated current; however, pretreatment with 1mM ethanol diminished this current following application of 30mM ethanol [30]. The efficacy of low ethanol concentrations in modulation of GABA-gated current in the CA1 hippocampal region may therefore be influenced by previous ethanol exposure. Other studies suggest that there may be pivotal developmental differences that account for differential effects of low ethanol concentrations in adolescent (P30–40) versus adult (P70–80) hippocampal neurons. Previous work demonstrates that acute ethanol (30mM) enhances tonic inhibition mediated by GABA-A receptors in dentate granule cells more effectively in adolescent as opposed to adult rats [31]. Other studies have shown that 30mM ethanol promotes firing of hippocampal interneurons, and that this enhancement in frequency of spontaneous action potentials was lower in adolescent rats compared to adults [32]. Furthermore, the amplitude of

hyperpolarization-activated cation current, I_h , was also diminished to a greater extent in adolescent than adult hippocampal interneurons [32]. Interestingly, the enhancement in spontaneous firing rates is restricted to interneurons within stratum oriens [32].

In addition to regional specificity, others have also suggested that adolescent ethanol exposure via behavioral drinking paradigms in rodents may potentiate the effects of physiologically relevant ethanol concentrations in slice preparations. Previous work showed that chronic intermittent ethanol exposure during adolescence enhanced GABA-A mediated tonic inhibition and decreased phasic GABA-gated current in adult rat dentate granule cells upon application of 30mM ethanol [31]. All of these studies discussed above utilize rodent models to study the effects of physiologically relevant ethanol concentrations in humans. However, Ariwodola et al. used 40mM ethanol application in brain slices to perform a direct comparison between rodent and primate dentate granule cell response to low ethanol concentrations (Ariwodola et al., 2003). They found that bath application of ethanol significantly enhanced the area of GABA-A receptor-mediated IPSCs in both the rat and primate (*Macaca fascicularis*) hippocampal slices [33]. Importantly, while there was variability among individual dentate granule cells in each species, there was no difference *between* species in the efficacy or potency of 40mM ethanol to enhance GABA-A receptor-mediated IPSCs [33]. This finding reinforces the translational potential of results from studies of the effects of these ethanol concentrations in rodent models.

The same study also concluded that ethanol significantly diminished NMDA receptor-mediated excitatory postsynaptic currents in both species [33]. Others have also found a link between ethanol and disruption in hippocampal NMDA receptor-mediated signaling using bath application of 50mM ethanol to hippocampal slice preparations. One group found that 50mM ethanol application decreased the frequency of NMDA-R miniature excitatory postsynaptic currents (mEPSCs) and inhibited depolarization-induced potentiation in CA1 hippocampal neurons [34, 35]. More recent work found that application of 40mM ethanol decreased AMPA receptor- and NMDA receptor-mediated field excitatory postsynaptic potentials (fEPSPs) [36]. Kainate receptors (another subtype of glutamate-gated ionotropic receptors) have also been implicated and are critical in the excitation of CA1 hippocampal interneurons. Recordings of spontaneous IPSCs from rat hippocampal slices upon application of 20–40mM ethanol demonstrated inhibited interneuron action potential firing, via a significant inhibition of kainate receptors [37]. Lower ethanol concentrations appear to modulate glutamatergic signaling within the hippocampus as well and may have various molecular targets depending on cell type or region involved. Importantly, 30–40mM concentrations of ethanol are likely ideal for studying physiologically relevant hippocampal mechanisms of ethanol intoxication in both rodent and primate models. However, further work is necessary to determine if certain contextual or prior ethanol experience can promote hippocampal plasticity favoring alternate responses to acute ethanol exposure.

CA1 pyramidal neurons, which provide the major output from the hippocampus, receive direct and indirect input from the entorhinal cortex (reviewed in Basu and Siegelbaum, 2015). Subsets of CA1 neurons have reciprocal connections with the PFC and amygdala, which are respectively important for the proper function of working memory and to mediate

anxiety-related behavior. The effects of alcohol on the hippocampus therefore play a large role in this drug's amnesic and anxiolytic effects.

5. Cerebral Cortex

A variety of cortical areas may be important for the actions of alcohol, most notably the frontal cortex in terms of the cognitive effects of lower doses of alcohol. For the time being, the reliable literature on alcohol effects in many of the clearly delineated cortical areas is somewhat limited, so we have included all such studies together.

5.1 Low Ethanol (<20mM)

In cultured chick neurons, 5–10 mM ethanol elicited maximal GABA response potentiation [38]. Potentiation of I_{GABA} by ethanol (20 mM) has also been seen in cultured mouse cortical neurons [27]. There have been few successful studies in attempts to replicate these findings in more intact systems, however. The effect of acute alcohol exposure on GABA responses has also been examined in the piriform cortex of adult rats subjected to chronic ethanol treatment. Acute ethanol (10 mM) modulated GABA in both chronic drinking and control groups, but a higher percentage of ethanol-induced potentiation was seen in the chronic ethanol group [39]. This was despite the observation that responsiveness to GABA was decreased in the chronic drinking group, indicating that chronic ethanol attenuates sensitivity to GABA, while increasing sensitivity to ethanol-induced GABA potentiation. In the lateral orbital frontal cortex in mice, 11 mM acute ethanol decreased spike frequency and input resistance supporting the idea of an inhibitory action of acute alcohol on excitability via a chloride channel [40]. Other studies have also investigated ethanol modulation of glutamatergic responses. It has been shown that concentrations of 5–10 mM ethanol inhibits NMDA-receptor mediated EPSCs in slices from the posterior cingulate cortex of rats [41]. This inhibition was more potent in cells from juveniles (PD28–32) than from adults (PD95–135). In slices from the primary somatosensory cortex of rats, concentrations of ethanol from 10–20 mM were found to have both potentiating and attenuating modulatory effects on AMPA-induced currents [42]. The outcome of ethanol modulation appeared to be age-dependent: a higher incidence of ethanol-induced potentiation was observed in neurons from rat pups of the PD6 to PD9 age group, while ethanol-induced attenuation was more prevalent in neurons from rat pups of the PD13 to PD15 age group. Other work in rat somatosensory cortical neurons has focused on the effects of ethanol on synaptically evoked responses and membrane properties. The primary effect of ethanol (10 mM) in slices from this region was to reduce neuronal excitability, measured as an increase in rheobase and a decrease in input resistance, repetitive firing, PSP amplitude, and the probability of evoking action potentials [43].

5.2 Medium Ethanol (20–50mM)

In cultured cortical neurons from rats, maximal GABA response potentiation was achieved at 50 mM ethanol [44]. In contrast, GABA current inhibition was seen at 40–50 mM ethanol in cultured chick neurons. In the rat medial frontal cortex, it has been demonstrated that the rate of spontaneous GABA-mediated inhibitory postsynaptic currents (sIPSCs) is unchanged by 50 mM ethanol, indicating that ethanol does not increase GABA release from presynaptic

terminals onto these neurons [14]. Another area of focus has been the effect of ethanol on “persistent activity” in the prefrontal cortex. In slice co-cultures of PFC neurons, it has been shown that concentrations of 25 mM inhibit persistent activity, measured as a reduction in upstate amplitude, duration, and number of spikes per up-state [45]. Other studies have examined the effects of ethanol on persistent activity in pyramidal neurons and fast-spiking GABAergic interneurons. Using a slice co-culture system, it was shown that 44 mM ethanol significantly reduced the average duration of up-states in both types of neuron and had a variable effect on spiking, with a trend towards an increase in pyramidal neurons and a reduction in fast-spiking neurons [46]. Whole-cell recordings in rat cortical slices have been used to determine how ethanol modulates the GABAergic and glutamatergic components underlying persistent activity. Significant inhibition of NMDA receptor-mediated evoked excitatory postsynaptic currents (eEPSCs) was seen after administration of 22 mM ethanol, while concentrations of ethanol up to 44 mM had no effect on AMPA receptor-mediated evoked EPSCs or GABA receptor-mediated IPSCs [47]. This indicates that ethanol primarily affects the postsynaptic NMDA receptor-mediated component of the glutamatergic response. Finally, a contribution of glycine receptors to the inhibitory actions of acute alcohol has been observed. In layer V pyramidal neurons in the mouse lateral orbital frontal cortex, acute alcohol (33–66 mM) reduces neuronal excitability, surprisingly not through an NMDA or GABAergic mechanism, but by acting on a population of strychnine-sensitive glycine receptors that carry a tonic inhibitory current [40, 48] that is known to be present in the frontal cortex [49].

The cerebral cortex shares extensive connections with a wide array of brain areas, so ethanol effects on the cerebral cortex can obviously have wide-ranging behavioral consequences. Some promising candidates for alcohol’s effects include the projections to and from the thalamus that process somatosensory inputs and working memory (as well as participating in the sleep-wake cycle), the projections to the amygdala that help to regulate social and emotional behavior, projections to the hippocampus from the entorhinal cortex as part of the spatial memory circuitry, and the projections to the NAcc and VTA that modulate reward-seeking and risk taking, in addition to reinforcement and drug-seeking [50].

6. Amygdala

The amygdala and associated limbic structures such as the bed nucleus of the stria terminalis (BNST) that make up the extended amygdala, is a critical component of the brain’s fear and stress system, and as such is a presumptive site of action for the anxiolytic actions of low levels of alcohol, as well as being involved in the neuroadaptation that takes place during the stage of **negative reinforcement** in the process of alcohol addiction.

6.1 Low Ethanol (<20mM)

There is some evidence that low doses of ethanol (EtOH) decrease excitability in the central amygdala (CeA) in rats. Roberto and colleagues [51, 52] found that 5 and 11mM EtOH increase evoked IPSC amplitude in CeA neurons. This group later found that this potentiating effect of EtOH may be mediated by CRF1 receptors, as the increased IPSC amplitude observed in CeA neurons in response to 11mM EtOH is absent in CRF1 knockout

mice and blocked by a CRF1 antagonist [53]. Conversely, this group has not demonstrated an effect of low EtOH concentrations on the amplitude of spontaneous IPSCs, nor found evidence that low EtOH concentrations induce paired-pulse facilitation of CeA IPSCs [51–54]. However, they have presented evidence that acute EtOH decreases glutamatergic signaling in the CeA. Specifically, 14mM EtOH was shown to decrease compound EPSPs and EPSCs in this region [55].

Low EtOH concentrations have also been shown to decrease excitability in the basolateral amygdala (BLA). For example, Chung and colleagues [56] examined the effects of EtOH on compound postsynaptic potentials (cPSPs), which are thought to represent spontaneous circuit activity and are dependent on both GABAergic and glutamatergic transmission in the BLA. These authors found that application of 20mM EtOH significantly increased the frequency ratio of cPSPs in slices obtained from young rats (PN16–23). Excitatory neurotransmission in the BLA is also mediated by kainate receptors (KARs), and Lack and colleagues [57] have shown that EtOH decreases KAR-mediated EPSCs in the rat BLA in a concentration-dependent manner. Specifically, these authors found that KAR-mediated synaptic currents were significantly decreased by 20mM EtOH (~26%).

6.2 Medium Ethanol (20–50mM)

Roberto and colleagues [51] have also found that medium doses of EtOH (22 and 44mM) increase GABA_A-mediated IPSCs and IPSPs in most rat CeA neurons. Furthermore, 44mM EtOH increases the amplitude of evoked IPSPs and IPSCs. This effect was not altered by concurrent superfusion of a GABA_B receptor antagonist (in contrast to findings in the hippocampus, nucleus accumbens, and basolateral amygdala). Ethanol decreased paired-pulse ratios (inter-event interval: 50ms) at 44mM, but not at lower concentrations (11 and 22mM). This medium EtOH dose also increased the frequency of sIPSC, and both the frequency and amplitude of mIPSCs, suggesting that ethanol increases inhibitory signaling in this brain region via pre- and post-synaptic mechanisms, with presynaptic effects more obvious at higher doses.

The same group [53] later replicated the finding that 22 and 44 mM EtOH increase IPSC amplitude. However, EtOH had no effect on IPSC amplitude in CRF1 knockout mice. Similarly, superfusion of a CRF1 antagonist ablated the potentiating effect of 44mM EtOH on IPSC amplitude in slices obtained from wild type mice. Herman and colleagues [54] recently investigated these earlier findings more closely by making use of CRF1:GFP reporter mice. These authors found that CRF1-expressing neurons exhibit a GABA_A receptor $\alpha 1$ subunit-mediated tonic conductance, and that EtOH (44mM) increases the firing rate of these cells. Conversely, CeA neurons that did not express CRF1 display a δ -subunit mediated tonic conductance that is enhanced by EtOH, and EtOH superfusion decreases the firing rate of these cells. Interestingly, EtOH application does not alter sIPSC frequency in the majority of CRF1+ neurons, but does increase sIPSC frequency in all CRF1- neurons. Consistent with previous reports by members of this group [51] no EtOH-induced alterations in sIPSC amplitude were observed in either cell population. Finally, retrograde tracing studies revealed that these CRF1-containing neurons project to the BNST. Importantly, superfusion of 44mM EtOH significantly increased the firing rate of all BNST-projecting

CeA neurons, regardless of whether these cells were CRF1+ or CRF1-; the authors conclude that EtOH increases the firing rate of all BNST projections originating in the CeA, of which CRF1+ cells are one subtype.

Interestingly, although chronic exposure to ethanol vapor augments GABA release in the CeA, there does not appear to be any tolerance to the effect of acute ethanol following chronic ethanol inhalation. Roberto and colleagues [52] confirmed their previous findings that medium doses of acute EtOH (22 and 44mM) increase the amplitude of evoked IPSCs and IPSPs in slices from both ethanol-treated and naïve rats. Basal GABAergic transmission appears to be enhanced following chronic ethanol, as IPSCs are larger on average in slices from ethanol treated animals. However, 44mM EtOH enhances evoked GABAergic responses to a similar extent in ethanol-treated and naïve rats (150% of control), and paired pulse ratios are similarly reduced by acute ethanol application in slices from both groups.

Ethanol has also been shown to effect glutamatergic signaling in the CeA. Roberto and colleagues [55] demonstrated that EtOH application decreases compound EPSPs and EPSCs in rat CeA neurons. Further investigation revealed that 44mM EtOH decreases both non-NMDA and NMDA R-mediated EPSPs and EPSCs by ~20%. Interestingly, EtOH inhibition of NMDA currents was significantly increased in slices obtained from animals exposed to chronic ethanol vapor; the authors suggest that these findings indicate sensitization of CeA NMDARs following chronic EtOH treatment. However, the authors do not report any signs of postsynaptic neuronal hyperexcitability in CeA slices obtained from EtOH-exposed animals. Similarly, there is no difference in paired-pulse ratios in slices from EtOH-exposed vs. naïve animals. EtOH application did significantly decrease PPF of NMDAR-mediated EPSPs and EPSCs in slices from EtOH-exposed rats (no change was observed in naïve slices).

An early study in rats [58] found that EtOH reduces the duration of epileptiform activity and NMDA-mediated synaptic responses in BLA neurons. At 50mM, EtOH produces a 40% reduction in the duration of epileptiform activity, while 20mM EtOH decreases this response by 20%. Calton and colleagues [59] later reported that EtOH inhibits NMDA-mediated currents in the rat BLA at medium but not low concentrations. Specifically, these authors found that 44 mM EtOH reduces the amplitude of NMDA receptor-mediated EPSCs by ~30%; no effect of EtOH on NMDA currents was detected at 11 or 22 mM EtOH. These authors later confirmed that EtOH reduces NMDA receptor-mediated synaptic responses in the BLA, and further found that NMDA receptor-mediated depolarizations activate a voltage-dependent regenerative potential that is also reduced by EtOH [60]. In this set of experiments, 44mM EtOH reduced the amplitude of NMDA receptor-mediated EPSPs by up to 30%. The same EtOH concentration significantly reduced spike area and increased the stimulus threshold for spike activation. Finally, these authors report that 44mM EtOH reduces inward currents in BLA pyramidal neurons without shifting the current/voltage relationship of these currents; specifically, EtOH inhibited the steady-state current by ~ 25%.

As mentioned above, Chung and colleagues [56] found that application of 20mM EtOH significantly increases the frequency ratio of cPSPs in BLA slices obtained from young animals (PD16–23); this increase is more pronounced in response to 40mM EtOH. The

authors conclude that EtOH has dose-dependent effects on cPSP frequency that include both enhancement and inhibition, providing evidence for biphasic effects of EtOH in this brain region. As mentioned above, Lack and colleagues [57] have shown that EtOH acutely decreases kainite receptor-mediated EPSCs in the BLA in a concentration-dependent manner. Specifically, these authors found that kainite receptor-mediated synaptic currents were significantly decreased by 20mM EtOH (~26%) and further decreased by 40mM EtOH (~47%). However, low and medium doses of EtOH had no significant effect on NMDA receptor-mediated EPSCs in these experiments.

Neurons within the BLA form reciprocal connections with the PFC and the hippocampus (reviewed in Janak and Tye, 2015). Connections with the PFC are sufficient to alter feeding and freezing behavior, as well as reinstatement of fear conditioning. Connections between the BLA and the hippocampus, on the other hand, have been found to affect anxiety-related and social behavior. Neurons in the BNST region of the extended amygdala connect via both glutamatergic and GABAergic projections to DA neurons of the VTA, as well as by GABAergic projections to the lateral hypothalamus [61]. Both of these circuits have been implicated in reward-seeking behavior. Overall, the actions of alcohol in modulating the excitability of neurons in the amygdala and extended amygdala have the potential to affect social, anxious, and reward behavior as well as associative learning.

7. Thalamus

The thalamus plays important roles in segregating and transmitting sensory information to the appropriate cortical areas, but is also intimately involved in the control of the sleep-wake cycle and in regulating arousal states, by virtue of its central position in connecting a variety of brain stem structures (locus coeruleus, raphe nucleus, pontine cholinergic nuclei etc.) with the neocortex. Because of the importance of the thalamus for control of arousal, this is one of several potential sites at which alcohol might exert its sedative and hypnotic effects.

7.1 Low Ethanol (<20mM)

There are no convincing data on acute alcohol effects on the thalamus at low doses to date.

7.2 Medium Ethanol (20–50mM)

The thalamus is largely made up of relay neurons, and these show both synaptic and non-synaptic (“tonic”) inhibition. Synaptic inhibition in the thalamus seems to be insensitive to medium levels of ethanol, in contrast to findings in the cerebellum and the amygdala, but there is clear evidence for enhanced tonic inhibition in thalamic relay neurons in the mouse, from 30mM-50mM EtOH (Jia et al., 2008). These levels of alcohol are certainly consistent with marked CNS depressant effects of the drug *in vivo*, including profound sedation.

8. Hypothalamic-Pituitary Axis

The hypothalamus is an important part of the brain’s stress system (via its connectivity with the amygdala) and is also critical for integrating the CNS with the autonomic nervous system. In addition, the hypothalamus regulates the endocrine system via its connection with the pituitary. The hypothalamus and pituitary are therefore a potential target of some

importance for alcohol, and this is a topic of considerable extent that has been reviewed in fine detail elsewhere (Wand, 1999). Here we will quickly review the effect of acute alcohol to regulate vasopressin levels and thereby promote diuresis, another well known biological effect of alcohol that occurs at low to medium levels of the drug.

8.1 Low Ethanol (<20mM)

Vasopressin (ADH; antidiuretic hormone) is normally released by the posterior pituitary gland in response to a fall in blood volume or a rise in plasma osmolality, and plays a major role in the regulation of water reabsorption in the kidney. ADH increases the permeability to water of the distal convoluted tubules and collecting ducts through insertion of aquaporin water channels into the apical membrane of renal tubular epithelial cells [62]. Alcohol also acts directly to inhibit the release of vasopressin. Murray [63] showed that administration of a posterior pituitary extract inhibited alcohol-induced diuresis, and it is widely accepted that alcohol exerts its diuretic action by inhibiting vasopressin release. Elegant work by the groups of Lemos and Treistman [64] showed that this effect occurs *in vitro* in preparations of isolated nerve terminals at alcohol levels as low as 10mM and Dopico and others have provided a cellular and molecular level explanation for this action of alcohol, in terms of its ability to enhance the opening of Ca^{2+} -activated K^{+} (BK) channels and thereby reduce the release of vasopressin [65] reviewed in [66]. Although the diuresis elicited by alcohol may be considered to be a peripheral effect of the drug, the mechanism of BK channel modulation by which this occurs is a common one, and one by which alcohol may influence neurons within the CNS, in addition to those in the HPA.

Discussion

The studies reviewed here represent a large fraction of all of the many meaningful physiological studies that have appeared on CNS effects of alcohol at lower (<20mM) to medium concentrations of the drug (20–50mM). It is apparent that alcohol researchers have long ago abandoned a “unitary mechanism” (single target) approach and have also diversified from the “hippocampocentric” view of the brain that prevailed 20 years ago, and are now looking for the origin of the varied and complex behavioral effects of alcohol *within the many distinct CNS structures that subserve those actions*. The necessary sub-division of labor among investigators has allowed what once seemed an impossibly difficult problem to be broken down into more manageable components, and this process of simplification has been extremely helpful in allowing alcohol researchers to ask and answer soluble questions within reasonable time-frames. There is no doubt the drive during the 2000s and since, to focus on the many structures and local circuits involved, has contributed substantially to an increase in the quality and relevance of the scientific work done in the field.

The recent focus on lower alcohol concentrations, promoted by the RFA from NIAAA, will also help to further focus these efforts as the various sites and cellular mechanisms of action are evaluated in the years to come, yielding not only novel scientific insights but also practical results with translational consequences. Importantly, many of the published effects of ethanol on synaptic transmission in the CNS still use very high concentrations of ethanol that are not physiologically relevant. Research on more subtle synaptic (and non-synaptic)

effects of lower concentrations of this drug could provide much needed insight into the effects of ethanol on the brain. The identification of novel substrates of alcohol actions in the CNS will provide new avenues to target, interrupt or modify those maladaptive processes that are set in motion by alcohol abuse and result in long-term alcohol use disorders.

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Highlights

- Ethanol alters neuronal excitability and network activity in critical brain areas to produce its varied behavioral effects.
- Ethanol can alter synaptic transmission and also influence intrinsic excitability in cerebellum, hippocampus, amygdala, VTA, prefrontal cortex and other areas.
- The effects of low (<20mM) to moderate (20–50mM) concentrations of ethanol are most relevant to social intoxication in humans.
- Ethanol has important effects that are pre- and postsynaptic, as well as non-synaptic in nature.

Figure 1.