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Ethanol reduces the phase locking of neural activity in human and rodent brain

Cindy L. Ehlers^{1,*}, Derek N. Wills¹, and James Havstad

¹Department of Molecular and Integrative Neuroscience, The Scripps Research Institute, La Jolla, CA USA

Abstract

How the neuromolecular actions of ethanol translate to its observed intoxicating effects remains poorly understood. Synchrony of phase (phase locking) of event-related oscillations (EROs) within and between different brain areas has been suggested to reflect communication exchange between neural networks and as such may be a sensitive and translational measure of ethanol's effects. Using a similar auditory event-related potential paradigm in both rats and humans we investigated the phase variability of EROs collected from 38 young men who had participated in an ethanol/placebo challenge protocol, and 46 adult male rats given intraperitoneal injections of ethanol/saline. Phase locking was significantly higher in the delta frequencies in humans than in rats. Phase locking was also higher for the rare (target) tone than the frequent (non-target) tone in both species. Significant reductions in phase locking to the rare (target) tone in the delta, theta, alpha, beta and gamma frequencies, within and between brain sites, was found at one hour following ethanol as compared to placebo/saline administration in both rats and humans. Reductions in phase locking in the alpha frequencies in the parietal cortex were found to be correlated with blood ethanol concentrations. These findings are consistent with the hypothesis that ethanol's intoxicating actions in the brain include reducing synchrony within and between neuronal networks, perhaps by increasing the level of noise in key neuromolecular interactions.

Keywords

EEG; ERO; ERP; phase locking index; ethanol; time series analysis

1. Introduction

The neurobehavioral effects of ethanol range from mild euphoria, anxiolysis and disinhibition to impaired coordination, ataxia, decreased mentation, slurred speech, nausea and vomiting, and finally to respiratory failure, coma and death, depending on the dose imbibed (Schuckit, 1995). The presumed neuromolecular basis for these behavioral effects includes actions on lipids, at high doses, and more direct molecular targets such as enzymes, neurotransmitter receptors, and ion channels at doses that typically produce intoxication in humans (Harris et al., 2008; Vengeliene et al., 2008). While much progress has been made on identifying molecular targets for ethanol, less insight has been gained into how ethanol's

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Correspondence: Dr. C.L. Ehlers, The Scripps Research Institute, Department of Molecular and Integrative Neurosciences, 10550 North Torrey Pines Road, SP30-1501, La Jolla, CA 92037, USA. cindy@scripps.edu.

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actions at the molecular level may translate to its behavioral and intoxicating effects. The global behavioral effects of ethanol most likely require the integration of a number of functional neuronal areas distributed over the brain that are in constant interaction with each other. It has been suggested that such large scale integration and communication within the brain could be mediated by groups of neurons that oscillate within a specific frequency range and enter into precise phase-locking, or synchrony, over a limited period of time (Hipp et al., 2011; Lachaux et al., 1999; Sauseng and Klimesch, 2008;). Measures of neuronal phase synchrony of event-related neurophysiological events may therefore be a sensitive way to measure the effects of ethanol on local and global neural networks.

Event-related potentials (ERPs) are a series of negative and positive voltage deflections that are time locked typically to either sensory or cognitive events. They consist of several components that are averaged from the ongoing EEG that generally occur between 50 and 1000 msec. It has been suggested that the stimuli that evoke ERP components, like the P300, influence oscillatory changes within the dynamics of ongoing EEG rhythms (Basar-Eroglu and Basar, 1991; Demiralp et al., 2001a, 2001b; Karakas et al., 2000a, 2000b; Schurmann et al., 1995, 2001; Yordanova and Kolev, 1996;). This synchronization or enhancement of ongoing EEG oscillations by a time locked cognitive and/or sensory process is termed an event-related oscillation (ERO) (Basar et al., 2000; Begleiter and Porjesz, 2006; Roach and Mathalon, 2008). EROs are thought to arise by a “phase re-ordering” of the background EEG in several frequency bands (Basar, 1980; Makeig et al., 2002). EROs are typically estimated by a decomposition of the EEG signal into phase and magnitude information for a range of frequencies and then changes in those frequencies are characterized with respect to their energy and phase relationships over a millisecond time scale with respect to task events.

Event-related oscillations over the spectral range of the EEG (1-50 Hz) have been suggested to underlie a number of different cognitive processes. For instance, event-related alpha oscillations have been attributed to attentional resources, semantic memory, and stimulus processing (Basar et al., 1997; Klimesch et al., 1994, 1997a, 1997b), whereas, beta and gamma oscillations have been associated with sensory integrative processes (Basar et al., 2001a, 2001b; Schurmann et al., 1997). Oscillations in the delta and theta frequency ranges have been associated with signal detection, decision-making, conscious awareness, recognition memory and episodic retrieval (Basar et al., 1999c, 2001c, 2001d; Doppelmayr et al., 1998; Gevins et al., 1998; Klimesch et al., 1994, 2001; Schurmann et al., 2001). It has been suggested that high frequency oscillations (above 30 Hz) reflect synchronization of neuronal ensembles that are interacting over short distances in response to primarily sensory processes (Bressler and Freeman, 1980; Ohl et al., 2003), whereas, lower frequency oscillations (1-4 Hz) are generated by synchronization of ensembles interacting at longer distances during higher cognitive processing (Kopell et al., 2000; Lubar, 1997).

Cognitive and behavioral processes most likely involve the coordination of a large number of neurons into assemblies that are in communication within individual brain areas as well as across different subsystems (Damasio, 1990; Neuenschwander et al., 1996; Sejnowski, 1986; Singer 1990, 1993). Recent studies suggest that this may be indexed by stimulus-dependent neuronal synchronization (phase locking) of EROs. Phase locking of EROs can be measured in both humans (Sauseng and Klimesch, 2008; Roach and Mathalon, 2008) and more recently in rodent models (Criado and Ehlers, 2009, 2010a, 2010b; Ehlers and Criado, 2009), allowing for translational studies to be conducted.

We have argued that ethanol may produce its effects on micro as well as macro electrophysiology by introducing an increased level of noise or randomness in neuronal processing. Several studies provide data that are descriptively supportive of this idea. For

instance, Aston-Jones et al. (1982) demonstrated that low doses of ethanol, although having no effect on the mean spontaneous discharge of rat locus coeruleus neurons, significantly increased the variability in the latency at which those neurons fired in response to sensory stimuli. At the level of the EEG, we have previously demonstrated that consumed ethanol produces increased randomness as indexed by a decrease in the nonlinear structure of EEG oscillations (Ehlers, 1992; Ehlers et al., 1998b). The present study was conducted to further explore that hypothesis by measuring the effects of ethanol on ERO phase synchrony (phase locking) in both humans and animals. We predicted that an ethanol-induced increase in the randomness of neuromolecular interactions would result in a reduction of synchrony or phase locking both within a neuronal population and between neuronal populations. To assess this hypothesis, we explored three main questions: (1) Do phase locking measures of EROs differentiate infrequent from frequent tones in rats and humans? (2) Do phase locking measures of EROs differentiate placebo from ethanol in both humans and rats? and (3) Do these measures correlate with a person's subjective report of intoxication and/or blood ethanol concentration?

2. Results

2.1 Phase locking in response to frequent and infrequent tones in rats and humans

Thirty-eight (38) human participants completed the ethanol/placebo challenge study and had valid electrophysiological data available for the current analyses. These individuals had a mean $\pm$ SE age of 20.53 ± 0.34 yrs, and 11.89 ± 1.09 yrs of education. They drank an average of 4.5 ± 0.56 days per month, and consumed an average of 5.18 ± 0.58 drinks per occasion. Forty-six rats completed the protocol, had data available for analyses, and had verified electrode locations.

To address the first major research question: Multivariate Analysis of Variance (MANOVA) with repeated measures was used to determine if the values for phase locking index (PLI) for the three electrode locations in the human (human: Frontal Cortex (FZ), Central Cortex (CZ), Parietal Cortex (PZ), and in rat (rat: Frontal cortex (FCTX), dorsal hippocampus (DHPC), amygdala (AMYG) were higher following the rare (target) stimuli as compared to the frequent (non-target tone) within the ROI frequencies and time intervals under the placebo condition. MANOVA revealed that highly significant increases were seen in the PLI obtained following the infrequent (target) tone as compared to the frequent (non-target) tone in all brain sites in all of the ROI time frequency intervals in humans (Wilk's Lambda=0.393; $F=67.32$; $df=5,218$; $p<0.0001$) (Grand mean: frequent tone = 0.401 ± 0.006 , range=0.133-0.876, infrequent tone = 0.586 ± 0.006 , range=0.182-0.933) and in rats (Wilk's Lambda=0.300; $F=121.0$; $df=5,260$; $p<0.0001$) (Grand mean: frequent tone = 0.366 ± 0.007 , range=0.064-0.8461, infrequent tone = 0.648 ± 0.008 , range=0.150-0.982). These results are presented in figure 1. Similar highly significant findings were found in phase difference lock index (PDLI) between FZ and PZ in humans (Wilk's Lambda=0.415; $F=61.43$; $df=5,218$; $p<0.0001$) (Grand mean: frequent tone = 0.761 ± 0.005 , range=0.368-0.993, infrequent tone = 0.873 ± 0.004 , range=0.516-0.996) and between FCTX and DHPC, DHPC and AMYG and between FCTX and AMYG in the rats (Wilk's Lambda=0.366; $F=88.06$; $df=5,254$; $p<0.0001$) (Grand mean: frequent tone = 0.434 ± 0.005 , range=0.08-0.999, infrequent tone = 0.642 ± 0.006 , range= 0.2-1.0). PDLI was found to be significantly higher in humans and in rats following the infrequent (target) tone as compared to the frequent (non-target) tone in all of the ROI time frequency intervals. Phase locking was higher in humans for the frequent tone than in rats. In all these analyses repeated measures were Bonferonni corrected and all analyses met criteria for significance ($p<0.0001$). Additionally, it was found that the amount of phase locking in humans in cortical sites is higher in the delta frequencies, whereas, in rats the greatest increases in phase locking to the stimulus occurs in beta and gamma frequencies at FCTX, DHPC, and AMYG.

2.2 Phase locking in response to ethanol in rats and humans

The second major research question concerned whether ethanol as compared to placebo produced reductions in phase locking of EROs within and between electrode locations in humans and rats. In these sets of studies only effects on the infrequent and/or target tones were analyzed in order to reduce potential multiple comparisons. In the human participants, repeated measures ANOVA revealed that ethanol produced a significant reduction in the phase lock index (PLI) to the infrequent (target) tone in the delta ($F=8.58$, $df=1,37$, $p<0.006$), beta ($F=4.9$, $df=1,37$, $p<0.03$) and gamma frequency range in frontal cortex (FZ) ($F=6.2$, $df=1,37$, $p<0.017$) and in central cortex (CZ) in the delta ($F=8.96$, $df=1,37$, $p<0.00543$), theta ($F=11.59$, $df=1,37$, $p<0.006$), alpha ($F=68.5$, $df=1,37$, $p<0.006$), beta (300-800 ms) ($F=6.9$, $df=1,37$, $p<0.013$) and gamma ($F=6.97$, $df=1,37$, $p<0.012$) frequencies. Significant reductions in PLI were also seen at parietal sites (PZ) in the delta ($F=8.26$, $df=1,37$, $p<0.007$), theta ($F=10.84$, $df=1,37$, $p<0.002$), and alpha ($F=11.89$, $df=1,37$, $p<0.001$) frequency ranges. Grand averages of the PLI values for the entire group of subjects ($n = 38$) for the ethanol and placebo condition are presented in Figure 2. Figure 3a gives the mean ($\pm$ SE) values for the PLI for each frequency range for the three electrode locations for the ethanol and placebo condition, for the infrequent/target tone in the human participants. The phase difference lock index (PDLI) for the electrode pair FZ and PZ, for ethanol and placebo condition is shown in Figure 4a. Reductions in PDLI at 60 minutes following ethanol as compared to placebo were found in the delta ($F=5.99$, $df=1,37$, $p<0.019$) and theta ($F=4.6$, $df=1,37$, $p<0.038$) frequency ranges. No significant differences were seen for the electrode pairs FZ and CZ, or CZ and PZ, most likely because the leads were closer together.

Ethanol produced similar results on PLI values collected following the infrequent tone in the rats. Reductions in PLI were found in frontal cortical sites (FCTX) in the delta ($F=4.58$, $df=1,45$, $p<0.04$), theta ($F=5.32$, $df=1,45$, $p<0.025$), alpha ($F=5.3$, $df=1,45$, $p<0.025$), beta ($F=27.3$, $df=1,45$, $p<0.0001$) and gamma ($F=6.5$, $df=1,45$, $p<0.013$) frequency ranges in response to alcohol as compared to saline. In contrast, in the dorsal hippocampus (DHPC) and amygdala (AMYG), alcohol produced significant decreases in PLI only in the beta frequencies in the AMYG (AMYG: $F=4.28$, $df=1,45$, $p<0.036$). Grand averages of the PLI values for the entire group of rats ($n = 46$) for the ethanol and placebo condition for the infrequent tone are presented in Figure 5. Figure 3b gives the mean ($\pm$ SE) values for the significant PLI findings for the three electrode locations for the ethanol and saline condition. Significant differences from ethanol and placebo conditions, for the infrequent tone, were found for phase difference lock index (PDLI) from electrode pairs, FCTX and AMYG, and FCTX and DHPC. Reductions in phase locking at 60 minutes following ethanol were found in the theta ($F=3.99$, $df=1,42$, $p<0.05$) and beta ($F=4.88$, $df=1,42$, $p<0.032$) frequency ranges between FCTX and AMYG; the delta ($F=24.3$, $df=1,45$, $p<0.0001$), theta ($F=11.8$, $df=1,45$, $p<0.001$), beta ($F=10.29$, $df=1,45$, $p<0.002$) and gamma ($F=4.13$, $df=1,45$, $p<0.047$) frequency ranges between FCTX and AMYG as seen in Figure 4b.

2.3 Phase locking values, level of intoxication, and blood ethanol concentrations in humans

The third major research question explored was whether the subjectively experienced effects of alcohol and/or blood ethanol concentrations (BEC's) were associated with alcohol-induced changes in PLI or PDLI to the infrequent/target tones in the human participants, using Spearman and Pearson correlations. The total score on the Subjective High Assessment Scale (SHAS) at 60 minutes following ethanol ingestion was found to be significantly associated with PLI changes in the beta frequency range in FZ using both the Pearson and ($r=.337$, $p<0.04$) Spearman ($r=.412$, $p<0.01$) correlations (see figure 6). Administration of 0.56 g/kg ethanol produced a mean $\pm$ SD (g/dL) BEC of 0.077 ± 0.02 at

60 min after beverage ingestion. BEC's were found to be significantly associated with changes in PLI in the alpha frequency range in PZ using both the Pearson ($r = -.327$, $p < 0.045$) and Spearman ($r = -.325$, $p < 0.046$) correlation (see figure 6). No significant associations were found between PDLI values and SHAS and BEC measures. Blood ethanol concentrations were not available for the rats; however, our previously published pharmacokinetic data suggest that BECs for a 1.5 G/kg dose at 60 minutes following the injection yields a BEC of .1-.15 G/dL.

3. Discussion

Macroelectrophysiological recordings reflect the activity of large-scale neuronal assemblies; exactly how these neural assemblies organize to generate behavior is largely unknown. However, a body of knowledge is beginning to emerge that suggests that the phase locking of frequency specific, neuro-oscillatory activity within and between neural assemblies may underlie the processes whereby the brain organizes and communicates information (Basar et al., 1999a, 1999b; Roach and Mathalon, 2008; Sauseng and Klimesch, 2008 for reviews). Phase locking of event-related oscillations (EROs) represents a methodology whereby neuronal synchrony can be quantified and compared among experimental conditions in both man and animals providing thereby a translatable measure with which to explore the neural basis of behavior.

3.1 Auditory oddball stimuli produce phase locking in humans and rats

Significant phase locking of EROs was found to occur within and between brain areas in both humans and rats, as has been previously described (Fell et al., 2008; Freunberger et al., 2009; Grabner et al., 2007; Gysels and Celka, 2004; Hanslmayr et al., 2005; Jensen, 2005; Moore et al., 2006; Munk et al., 1996; Roelfsema et al., 1997; Schack et al., 2005; Siapas et al., 2005; van Wingerden et al., 2010). In the present study, a similar auditory event-related potential paradigm was used in male humans and male rats in order to explore effects of ethanol on phase locking of EROs in specific frequency bands. Our study is unique in that the same stimuli, data recording, and analysis techniques were used in human and rats, although the rats were not required to respond to the stimuli, which limits some of the applicability. We found that the degree of phase locking at cortical sites in humans was highest in the delta frequencies; however, in rats it was highest in the beta and gamma frequencies. Several authors have suggested that oscillations in specific frequency ranges may underlie specific mental functions. For instance, event-related alpha oscillations have been attributed to attentional resources, semantic memory, and stimulus processing (Basar et al., 1997; Klimesch et al., 1994, 1997a, 1997b), whereas, beta and gamma oscillations have been associated with sensory integrative processes (Basar et al., 2001a, 2001b; Schurmann et al., 1997). Oscillations in the delta and theta frequency ranges have been associated with signal detection, decision-making, conscious awareness, recognition memory and episodic retrieval (Basar et al., 1999c, 2001c, 2001d; Doppelmayr et al., 1998; Gevins et al., 1998; Klimesch et al., 1994, 2001; Schurmann et al., 2001). Further it has been suggested that high frequency oscillations (above 30 Hz) reflect synchronization of neuronal ensembles that are interacting over short distances in response to primarily sensory processes (Bressler and Freeman, 1980; Ohl et al., 2003), whereas, lower frequency oscillations (1-4 Hz) are generated by synchronization of ensembles interacting at longer distances during higher cognitive processing (Kopell et al., 2000; Lubar, 1997). Our data demonstrating that humans, who are responding to the stimuli show more phase locking in the delta frequencies whereas rats, who are not required to respond to the stimuli have more phase locking in the high frequency oscillations. These data further suggest the possibility that humans may possess a greater ability to synchronize neuronal ensembles over longer distances than rodents who are more specialized to respond to primary sensory processes over shorter distances.

3.2 Infrequent tones produce higher phase locking than frequently presented tones

In our study, in both humans and rats, the presentation of an auditory infrequently presented (rare/target) stimulus produced a robust and highly significant increase in phase locking of EROs, as compared to the frequently presented stimulus, both within and between brain areas in all frequency bands. We suggest that in this simple sensory paradigm the most likely explanation of this finding is that it represents a change in neural state associated with attending to a more novel, possibly environmentally relevant noise. However, since the humans are required to respond to the stimuli and the rats are not it is entirely possible that the increase in synchrony has different meanings in the two species. These findings are, however, consistent with a previous study that evaluated phase locking of EROs using a complex motor-learning task (Sauseng et al., 2007). In that task, long-range theta phase coherence was stronger in the novel condition compared to learned sequences, independent of task-difficulty. The authors interpreted those findings as a reflection of an increase in the amount of sensory information necessary to integrate novel sequences as compared to learned sequences. Our studies demonstrate that changes in the stimulus characteristics in simple auditory tasks can also produce widespread changes in phase locking over a number of brain areas in the full range of the EEG frequencies (1-50 Hz). It has been suggested that the processing of sensory information, such as those used in our simple auditory task, is primarily guided by automatic “bottom up” processes that do not require as much mental processing (Klimesch et al., 2007). Such effects may be mediated by mesencephalic reticular activation of cortical activity (Munk et al., 1996).

3.3 Ethanol reduces ERO phase locking in humans and rats

The main goal of the present study was to investigate the effects of ethanol on phase locking of EROs in order to give further insight into the actions of ethanol on the brain. Although a number of studies have presented data suggesting that phase locking of EROs is correlated with various cognitive functions, few studies have accomplished psychopharmacological studies using this methodology. In the present study phase locking to the rare (target) tone in the delta, theta, alpha, beta and gamma frequencies, within and between brain sites, was found at one hour following ethanol was significantly reduced as compared to placebo administration in both rats and humans. Two other studies have reported changes in brain synchrony following pharmacological challenge. In one study, administration of cholinergic antagonists was found to reduce interregional phase synchronization in humans (Wink et al., 2006). In another study, muscarinic blockade was shown to reduce the interaction between theta phase and gamma amplitude in the mouse (Hentschke et al., 2007). The results of these studies have been interpreted as indicating that phase synchronization may play a causal role in memory processes (Sauseng and Klimesch, 2008). Acute alcohol ingestion, especially at higher doses, can cause transient but significant decreases in memory function, that could be associated with reductions in phase locking, but it also produces a number of other behavioral effects including the sensation of intoxication which is presumably the reason for its consumption (Fernandez-Serrano et al., 2011).

3.4 Phase locking in the alpha frequencies is associated with blood ethanol concentrations

In the present study, although reductions in phase locking were found in all frequency ranges investigated, only changes at alpha frequencies were associated with blood ethanol concentrations. EEG power in the alpha frequency ranges is a sensitive measure of the effects of ethanol on the brain (Ehlers and Schuckit, 1988). Alpha activity has been associated with level of alcohol intoxication in previous studies (Ehlers and Schuckit, 1991, Ehlers et al., 1999). In fact, Lukas et al. (1986, 1989) have reported that episodes of EEG alpha activity closely correlated with the subjective experience of ethanol-induced euphoria. It has been suggested that synchronized alpha may reflect the degree of inhibitory activity of

central executive processes involved in modifying information processing (Klimesch et al., 2007). Ethanol is also known to produce an increase in motoric and cognitive disinhibition, interpreted as a loss of executive functioning (Rose and Duka, 2007). Additionally, behavioral under-control has been associated with risk for alcohol dependence (Slutske et al., 2002; Zucker et al., 2008). Further studies will be necessary to link changes in alpha phase locking, ethanol consumption, disinhibitory behaviors and executive functioning.

3.5 Phase locking in the beta frequencies is associated with subjective measures of intoxication

An increase in phase locking at beta frequencies, in frontal cortex, was found to correlate selectively and significantly with the human participant's overall ratings of their level of ethanol intoxication. Beta activity in the EEG has also been long associated with risk for the development of alcoholism (Campanella et al., 2009; Ehlers and Schuckit, 1990; Porjesz et al., 2005; Rangaswamy et al., 2004). Findings from the Collaborative Study on the Genetics of Alcoholism (COGA) have demonstrated that genetic linkage and linkage disequilibrium between the beta frequencies of the EEG and a GABA_A receptor gene on chromosome 4 (Porjesz et al., 2002). The GABA_A receptor gene has also been found to be associated with the diagnosis of alcohol dependence in that dataset (Edenberg et al., 2004). Interestingly, many of the behavioral effects of ethanol overlap with the effects of GABA_A receptor agonists, whereas decreases in ethanol responses can be produced by inverse agonists and antagonists (see Grobin et al., 1998; Kumar et al., 2009, for reviews). Although some of the effects of low doses of ethanol (30-100 nM) appear to be mediated by interactions with GABA_A receptors, ethanol has effects on a number of other molecular targets, such as other ligand gated ion channels, enzymes, as well as other proteins (Harris et al., 2008).

3.6 Ethanol reduced nonlinear structure in the EEG

We have previously suggested that low doses of ethanol may produce effects on multiple brain systems by introducing an increased level of noise or randomness in neuronal processing as indexed by a decrease in nonlinear structure in the cortical EEG (Ehlers, 1992; Ehlers et al., 1998b). In our previous studies, using a different data set, we demonstrated that several of the measures that formally index nonlinear structure provided evidence to suggest that EEG collected during mild alcohol intoxication in young men has less nonlinear structure than EEG data collected after placebo consumption (Ehlers et al., 1998b). Measures of EEG determinism, space-filling properties of the attractor, and redundancy were also found to be predictive of the level of intoxication as reported by the subject (Ehlers et al., 1998b). Linear measures of the EEG (EEG spectra), on the other hand, failed to be predictive of the behavioral measures of intoxication. These findings, together with the present data demonstrating that ethanol reduces phase locking of EROs, are consistent with the hypothesis that low doses of ethanol increase the noise level in neuronal processing at the level of the EEG leading to its behavioral effects.

3.7 Conclusion

Our study used the same auditory stimuli in both rats and humans in order to investigate the phase variability of EROs following alcohol administration. Phase locking was significantly higher in the delta frequencies in humans than in rats. Phase locking was also higher for the rare (target) tone than the frequent (non-target) tone in both species. Significant reductions in phase locking to the rare (target) tone in the delta, theta, alpha, beta and gamma frequencies, within and between brain sites, was found at one hour following ethanol as compared to placebo/saline administration in both rats and humans. Increases in phase locking in the beta frequencies in frontal cortex were found to correlate significantly with the human participants overall ratings of their level of intoxication; whereas, reductions in phase locking in the alpha frequencies in the parietal cortex was found to be correlated with

blood ethanol concentrations. These findings are consistent with the hypothesis that ethanol's actions in the brain include reducing synchrony within and between neuronal networks, perhaps by increasing the level of noise in key neuromolecular interactions.

4. Experimental procedures

4.1 Human participants

Males between the ages of 18 and 25 years were recruited using a combination of venue-based method and a respondent-driven procedure that has been described elsewhere (Ehlers et al., 1998a). Following telephone screening with research staff to complete a questionnaire (Schuckit, 1984; Schuckit and Gold, 1988) that was used to select individuals who met eligibility for the study; all subjects signed informed consent, and the study was approved by The Scripps Research Institute Internal Review Board and Indian Health Council. Participants were excluded from further evaluation if they met diagnostic criteria for alcohol or other substance dependence, or other major Axis I psychiatric disorders according to criteria outlined in the Third Diagnostic and Statistical Manual Disorders (DSM-III) (American Psychiatric Association, 1980). Individuals were also excluded from this study if they were taking prescribed medication, had any major medical condition, had never drank alcohol, or had abstained from alcohol over the previous 6 months.

4.2 Ethanol challenge protocol in human participants

Men ($n = 47$) who met inclusion criteria were invited to participate individually in two test sessions, approximately 1 week apart, which consisted of baseline evaluations and subsequent challenges with placebo and ethanol. Of the original 47 participants 38 records were still available on readable media in order to be used in the present analyses. Participants were instructed not to use alcohol or any other drugs for 3 days prior to testing. On both test days, each man arrived at the laboratory at approximately 8:00 A.M. after fasting overnight and was provided a standardized low-fat breakfast. Baseline measurements were taken and at about 9:00 A.M., a placebo or ethanol beverage was administered in random order, using a placebo ethanol administration device (Mendelson et al., 1984). The ethanol beverage was 0.75 ml/kg of 95% ethanol (0.56 g/kg) as a 20%-by-volume solution in a caffeine-free and sugar-free soda. The placebo beverage was made using the same mixer with 3 ml of 95% ethanol floated on top. Subjects were instructed to drink at a steady pace and to consume the beverage over 7 min. ERPs were collected at 60 min after beverage intake. Subjective ratings of alcohol's effects were determined using the Subjective High Assessment Scale (SHAS, see Schuckit, 1984) and blood samples for subsequent determination of blood ethanol concentrations (BECs) were also collected at 60 minutes following the beverage as described previously (Ehlers et al., 1998a).

4.3 ERP collection and analyses in human subjects

Three channels of ERP data (FZ, CZ, PZ, and referenced to linked ear lobes with a forehead ground, international 10–20 system) were obtained by using gold-plated electrodes with impedance held below 5K ohms. An electrode placed left lateral infraorbitally and referenced to the left earlobe was used to monitor both horizontal and vertical eye movement. ERP signals were recorded on a Nihon-Kohden polygraph amplified (sensitivity 7 microvolts/mm, time constant 0.1 s, 35 Hz low pass). Signals were transferred to an Apple computer and digitized at a rate of 256 Hz. The EEG amplifier input range corresponding to the full range of the 12-bit analog-to-digital converter was about ± 250 microvolts. Periodic calibration results were used to scale the digitized EEG to microvolts. Auditory stimuli and ERPs were elicited using an oddball plus “noise” paradigm described previously (Ehlers et al., 1996, 1998a). Each subject was instructed to depress a counter each time he

detected a rare tone. Individual trials containing excessive eye movement artifact as well as trials where the EEG exceeded ± 250 microvolts ($< 5\%$ of the trials) were eliminated.

4.4 Animal subjects and ethanol administration

The experimental subjects were 54 experimentally naive male Wistar rats weighing 250–356 g. At least 2 weeks prior to the experimental procedures rats were surgically prepared with recording electrodes in hippocampus (AP -3.0 , ML ± 3.0 , DV -3.0), and amygdala (AP -1.0 , ML ± 5.3 , DV -8.5). Stainless steel screw electrodes were also placed over frontal cortex and in the bony calvarium 3 mm posterior to lambda. Rats were administered ethanol (1.5 G/Kg and saline intraperitoneally (I.P.)) on separate days and the order of the administration was randomized. Ethanol was given in a 20% solution in saline. Electrophysiological recording were collected sixty minutes following drug/saline administration (Ehlers et al., 1998c). All experimental protocols were approved by the Institutional Animal Care and Use Committee at the Scripps Research Institute and were consistent with the guidelines of the NIH Guide for the Care and Use of Laboratory Animals (NIH Publication No. 80-23, revised 1996).

4.5 ERP Collection and Analyses in animal subjects

ERPs were elicited by auditory stimuli that were presented through a small speaker centered approximately 20 cm above the rat's head. EEG signals were recorded on a Nihon Kohden polygraph with sensitivity set at 75 microvolts/mm, using a bandpass of 0.3–35 Hz. ERPs were elicited by an acoustic “oddball” plus noise paradigm identical to that described above for the human subjects. Signals were transferred to an Apple computer and digitized at a rate of 256 Hz. The EEG amplifier input range corresponding to the full range of the 12-bit analog-to-digital converter was about ± 250 microvolts. Periodic calibration results were used to scale the digitized EEG to microvolts. Trials containing excessive movement artifact were eliminated prior to averaging ($< 5\%$ of the trials). Following the study, the rats that survived all procedures were decapitated and their brains immediately frozen on dry ice and stored at -80°C for verification of electrode locations. Of the 54 rats 46 had available data, free of artifacts, and with electrode locations verified in the correct positions.

4.6 ERO, PLI, and PDLI analyses

ERO, PLI (phase lock index) and PDLI (phase difference lock index) analyses were accomplished from the same datasets that were used to generate ERP data reported in two previous publications in humans and animals (Ehlers et al., 1998a, 1998b). Data from each ERP trial generated by the auditory stimuli were prepared for time-frequency analysis by subtracting from each trial its mean prestimulus value, windowing the first and last 100 msec with a cosine function, and padding with zeroes for a total of 512 data points. The time-frequency analysis of EROs utilizes the S transform (Stockwell et al., 1996). The computer code for this is based closely on a C language S transform subroutine downloaded from the NIMH MEG Core Facility web site (<http://kurage.nimh.nih.gov/meglab/>).

An S transform calculates a complex spectrum at each time point in the time series. The time-frequency points saved from each S transformation are from 100 ms before to 900 ms after the onset of the stimulus, and from 1 Hz through 50 Hz at intervals of 0.5 Hz.

An S transformation at time t and frequency f has real and imaginary parts

$$S(t, f) = \text{Re}S(t, f) + i\text{Im}S(t, f)$$

where i is the square root of minus 1. The cosine and sine of the phase angle at this time-frequency point are

$$\begin{aligned}\cos\phi(t, f) &= \operatorname{Re} S(t, f) / |S(t, f)| \\ \sin\phi(t, f) &= \operatorname{Im} S(t, f) / |S(t, f)|\end{aligned}$$

where the vertical bar pair indicates magnitude, here and below. The cosine and sine of phase angle are calculated from the S transformation without having to calculate the phase angle.

PLI is a measure of synchrony of phase angle over trials, as a function of frequency and of time relative to the start of the stimulus for each trial. The range of PLI is from zero to 1.0, with high values at a time and frequency indicating little variation, among trials, of phase angle at that time and frequency. PLI is defined as

$$PLI(t, f) = |\langle \cos\phi(t, f) + i \sin\phi(t, f) \rangle|$$

where the angle bracket pair indicates mean value over eligible trials, here and below. Eligibility depends on the stimulus type and absence of significant artifact. This definition is based on cosine and sine of phase angles that are calculated from S transformations without calculating phase angles. This definition is mathematically equivalent to the definition in Schack and Klimesch (2002).

PDLI is a measure of constancy over trials of the difference in phase angle between two channels, as a function of frequency and of time relative to the start of the stimulus for each trial. The range of PDLI is from zero to 1.0, with high values at a time and frequency indicating little variation, among trials, of phase angle difference between channels of the pair, at that time and frequency. PDLI is defined for frequency f at time t as:

$$PDLI(t, f) = |\langle \cos(\phi_A(t, f) - \phi_B(t, f)) \rangle + i \langle \sin(\phi_A(t, f) - \phi_B(t, f)) \rangle|$$

where ϕ_A and ϕ_B are phase angles of channels A and B, respectively. This definition of PDLI is equivalent to a definition of PLV, phase lock value, in Brunner et al. (2005). By means of some standard trigonometric identities the equation above is equivalent to the following, which, as for PLI, does not require that the phase angles be calculated:

$$PDLI(t, f) = \left| \frac{\langle \cos\phi_A(t, f)\cos\phi_B(t, f) + \sin\phi_A(t, f)\sin\phi_B(t, f) \rangle + i \langle \sin\phi_A(t, f)\cos\phi_B(t, f) - \cos\phi_A(t, f)\sin\phi_B(t, f) \rangle}{2} \right|.$$

The EEG amplifier low-pass filters for humans and for animals were set to 35 Hz, but the time-frequency analysis extended to 50 Hz, resulting in signal attenuation and phase angle changes that are dependent on frequency but are constant over time. PLI and PDLI analyses are measures of stability of phase angle over time, so the filters have no significant effects on the analyses reported in this paper.

Rectangular regions of interest (ROIs) were defined within the time-frequency analysis plane by specifying, for each ROI, a band of frequencies and a time interval relative to the stimulus onset time. Time 0 in these definitions is the onset of the stimulus. The ROI frequencies in both human and animal studies were: delta (1-4 Hz), theta (4-7 Hz), alpha (7-13 Hz), beta (13-30 Hz) and gamma (30-50 Hz). The ROI time intervals were delta (200-500 ms), theta (10-400 ms), alpha (0-300 ms) beta (0-300 ms) gamma (0-300ms) ROI time intervals were selected based on ERO energy in specific ERP component locations

(N1, P3) in previous ERP studies (Ehlers et al., 1998a). Using mean values over trials, for each of S transform energy (square of magnitude), PLI amplitude, and PDLI amplitude, the maximum value and its location in frequency and latency (time with respect to onset of stimulus) were calculated for each ROI, for each electrode location or, for PDLI, for each pair of electrode locations.

4.7 Statistical analyses

The first set of analyses were aimed at determining, during the placebo/saline condition, whether the degree of phase locking within and between electrodes locations was higher following the rare (target) tone as compared to the frequent (non-target) tone. Multivariate Analysis of Variance (MANOVA) with Bonferroni corrections for multiple comparisons was used to determine if the values for PLI and PDLI for the three electrode locations in the human and rat (human: FZ, CZ, PZ), (rat: frontal cortex, dorsal hippocampus, amygdala) were higher following the rare (target) stimuli as compared to the frequent (non-target tone) within the ROI frequencies and time intervals. The second question focused on whether significant effects of ethanol compared to placebo/saline could be detected on PDLI and PLI values within the time frequency ROI's following the rare tone in humans and rats. To address this question, ANOVA was conducted with PDLI and PLI values as dependent variables comparing drug condition (ethanol, placebo) for the defined time frequency ROI's. The third hypothesized question focused on whether differences in response to ethanol as indexed by PLI and PDLI values in the ROIs of interest were significantly associated with either blood ethanol concentrations or level of intoxication in the human participants. In these exploratory analyses linear regression was used to determine if a significant part of the variance in the PDLI/PLI measures following ethanol could be accounted for by: (1) the total score on the Subjective High Assessment Scale (SHAS) or (2) the blood ethanol concentrations (BEC's).

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ABBREVIATIONS

AMYG Amygdala

ANOVA	Analysis of Variance
BECs	Blood ethanol concentrations
CZ	Central Cortex
DHPC	Dorsal Hippocampus
EEG	Electroencephalogram
EROs	Event-related oscillations
ERPs	Event-related potentials
FCTX	Frontal Cortex (in rat)
FZ	Frontal Cortex
GABA_A	Gamma amino butyric acid A
MANOVA	Multivariate analysis of Variance
P300	Event related potential component occurring around 300 msec following a stimulus
PDLI	phase difference lock index
PLI	phase locking index
PLV	phase locking value
PZ	Parietal Cortex
ROI	region of interest
SHAS	Subjective high assessment scale

HIGHLIGHTS

Phase variability of auditory event-related oscillations (EROs) was measured in humans and rats.

Phase locking of EROs was higher for the rare (target) tone than the frequent (nontarget) tone in both species.

Ethanol produced significant reductions in phase locking in both rats and humans.

Increases in phase locking in the beta frequencies correlated significantly with overall ratings of intoxication.

Reductions in phase locking in the alpha frequencies correlated with blood ethanol concentrations.

Figure 1.

Grand mean values for the phase locking index (PLI) of event-related oscillations (EROs) 60 minutes following placebo/saline administration. MANOVA revealed that the infrequent (target) tone (gray bars), as compared to the frequent (non-target) tone (black bars), produced significant increases in phase locking in all time frequency ranges (delta (1-4 Hz), theta (4-7 Hz), alpha (7-13 Hz), beta (13-30 Hz), and gamma (30-50 Hz)), and in all electrode locations, in humans (n=38, left bars), (FZ= midline frontal cortex, CZ=midline central cortex, PZ=midline parietal cortex) and in rats (n=46, right bars) (FCTX=frontal cortex, DHPC=dorsal hippocampus, AMYG=amygdala).

Figure 2.

Grand averages of phase locking index values (PLI) of event-related oscillations (EROs) for 38 young men participating in an ethanol/placebo challenge study. Each graph depicts a time-frequency representation of PLI values in the delta, theta, alpha, beta, and gamma bands following the rare tone in three electrode locations (FZ=midline frontal cortex, CZ=midline central cortex, PZ=midline parietal cortex), at 60 minutes following ethanol or placebo administration. In each graph frequency (Hz) is presented on the Y-axis, time regions of interest on the X-axis (msec) and PLI is presented as color equivalents as indicated on the bar at the bottom of each graph. Ethanol, as compared to placebo produced significant reductions in phase locking at all three electrode sites.

Figure 3.

Mean values for the phase locking index (PLI) of event-related oscillations (EROs) 60 minutes following ethanol (black bars) and placebo/saline (white bars) administration for the infrequent (target) tone in the delta (1-4 Hz), theta (4-7 Hz), alpha (7-13 Hz), beta (13-30 Hz), and gamma (30-50 Hz), frequency ranges for the three electrode locations in humans (n=38), in the upper graph (a.), (FZ=midline frontal cortex, CZ=midline central cortex, PZ=midline parietal cortex) and in rats (n=46) in the lower graph (b.) (FCTX= frontal cortex, DHPC=dorsal hippocampus, AMYG=amygdala). Ethanol, as compared to placebo produced significant reductions in phase locking at all three electrode sites in humans and in rats.

Figure 4.

Mean values for the phase difference lock index (PDLI) of event-related oscillations (EROs) 60 minutes following ethanol (black bars) and placebo/saline (white bars) administration for the infrequent (target) tone in the delta (1-4 Hz), theta (4-7 Hz), alpha (7-13 Hz), beta (13-30 Hz), and gamma (30-50 Hz), frequency ranges. In humans ($n=38$), in the upper graph (a.), phase differences were calculated between midline frontal cortex (FZ) and midline parietal cortex (PZ); and in rats ($n=46$), in the lower graph (b.) phase differences were calculated between frontal cortex (FCTX) and dorsal hippocampus (DHPC), frontal cortex (FCTX) and amygdala (AMYG), and dorsal hippocampus (DHPC) and amygdala (AMYG). Ethanol, as compared to placebo produced significant reductions in phase difference lock index in humans and in rats.

Figure 5.

Grand averages of phase locking index values (PLI) of event-related oscillations (EROs) for 46 rats that were administered ethanol and saline (control). Each graph depicts a time-frequency representation of PLI values in the delta, theta, alpha, beta, and gamma bands following the rare tone in three electrode locations (FCTX=frontal cortex, DHPC=dorsal hippocampus, AMYG=amygdala), at 60 minutes following ethanol or saline administration. In each graph frequency (Hz) is presented on the Y-axis, time regions of interest on the X-axis (msec) and PLI is presented as color equivalents as indicated on the bar at the bottom of each graph. Ethanol, as compared to saline, produced significant reductions in phase locking at all three electrode sites.

Figure 6.

Individual data points for phase locking index (PLI) of event related oscillations (EROs) at 60 minutes following ethanol administration in human participants. In the upper graph, PLI for the alpha frequency range in parietal cortex (PZ) is plotted on the Y-axis and blood ethanol concentration (BEC g/dL) is plotted on the X-axis. In the lower graph PLI for the beta frequency range in frontal cortex (FZ) is plotted on the Y-axis and total score on the subjective high assessment scale (SHAS) is plotted on the X-axis. Significant correlations were found for PZ Alpha PLI and BEC as well as FZ Beta PLI and total SHAS.