INTRODUCTION

The tension-reduction hypothesis postulates that one factor which motivates ethanol consumption is ethanol-mediated relief from ‘stress’ (Cappell and Herman, 1972; Labouvie, 1986; Pohorecky, 1990, 1991). Under these circumstances, ethanol consumption is envisioned as a type of self-medication. In support of this hypothesis are clinical studies demonstrating that ethanol can decrease physiological stress responses (Levenson et al., 1987) and individuals report that stress and anxiety can increase ethanol consumption and craving (Ludwig and Stark, 1974; Labouvie, 1986; Billingham et al., 1993; Smith et al., 1993). Studies in animal models have also shown that ethanol functions as an anxiolytic (Thatcher-Britton and Koob, 1986; Pohorecky, 1990), and that increases in anxiety and stress are associated with increased ethanol consumption (Bowers et al., 1997; Moller et al., 1997a,b). Taken together, these data suggest that an interaction between ethanol and stress may influence ethanol consumption, craving and relapse.

Two neuropeptides which have been implicated in modulating stress responses, in an opposing fashion, are corticotropin-releasing factor (CRF) and neuropeptide Y (NPY). CRF is a potent anxiogenic agent (Sutton et al., 1982; Thatcher-Britton et al., 1986; Dunn and File, 1987; Moreau et al., 1997) and NPY is an anxiolytic agent (Heilig and Murison, 1987; Pich et al., 1993; Wahlestedt et al., 1993; Yamada et al., 1996). The interplay and balance between these two peptide systems has been suggested to be important in the regulation of stress, anxiety, and depression (Heilig et al., 1994; Yamada et al., 1996; Ehlers et al., 1997b). These two peptidergic systems are of particular interest because acute and chronic ethanol exposure alters activity in both CRF and NPY systems, as well as the overall functioning of
the hypothalamic–pituitary–adrenal (HPA) axis. For example, the hypercortisolaemia which has been reported in some actively drinking alcoholics (Proto et al., 1985; Veldman and Meinders, 1996) has been suggested to be the result of increased CRF secretion (Vargas et al., 1992). In rodents, acute ethanol challenge increases circulating adrenocorticotropic hormone (ACTH) levels (Rivier et al., 1984, 1990; Lee and Rivier, 1997; Ogilvie et al., 1997). This effect is blocked by the administration of anti-CRF antibodies (Rivier et al., 1984). Lastly, CRF mRNA levels in the paraventricular nucleus of the hypothalamus are increased (Rivier et al., 1990), whereas CRF peptide levels in the hypothalamus are decreased following chronic ethanol exposure (Rivier et al., 1984). In contrast, hypothalamic NPY levels are decreased following chronic ethanol exposure and subsequently increase following long-term withdrawal (Ehlers et al., 1998). These alterations in endogenous peptide levels as a result of ethanol exposure suggest that these peptides may influence responses to stress, and subsequently stress-related ethanol intake, following ethanol withdrawal and abstinence.

Chronic ethanol exposure has also been reported to produce changes in neurophysiological functioning (Ehlers and Chaplin, 1991; Rogers and Hunter, 1992; Tremwel and Hunter, 1994). Following 20–30 weeks of forced exposure to ethanol in a liquid diet, alterations in the electrical properties of the hippocampus have been reported after 6–8 weeks of ethanol abstinence (Rogers and Hunter, 1992; Tremwel and Hunter, 1994). Ehlers and Chaplin (1991) have reported that after 4 weeks of chronic ethanol vapour inhalation, reductions in the amplitude of late positive components of event-related potentials (ERPs) can be observed after 2 weeks of withdrawal from ethanol. These data indicate that prolonged ethanol exposure and subsequent ethanol withdrawal can result in long-lasting changes in the neurophysiological function of the central nervous system. However, there have been few studies where neurophysiological investigations of stress-related peptides have extended past 2–3 weeks of ethanol withdrawal. This is a clinically important time period, because relapse in alcoholics often results after several months of sobriety.

The present study had two purposes. First, the effects of 6 weeks of chronic ethanol treatment on EEG activity and auditory ERPs following a prolonged period of ethanol abstinence (10–15 weeks) were examined to determine if a neurophysiologically based protracted abstinence syndrome is evident following prolonged ethanol withdrawal. Second, changes in electroencephalogram (EEG) activity and ERPs were examined following intracerebroventricular infusion of CRF or NPY to determine if neurophysiological responses to these peptides are altered during protracted abstinence.

METHODS

Subjects

Thirty-four male Wistar rats were used in this study. At the start of the experiment, the weight of the rats ranged from 234–293 (mean ± SD = 265 ± 14) g. Rats were housed two per cage in standard cages for the duration of the experiment. The light/dark cycle was maintained at 12 h light/12 h dark (lights on at 06:00). Rats were maintained on an ad libitum diet. Animal care was in accordance with NIH and institutional guidelines.

Chronic ethanol exposure procedure

One week after being received, rats were divided into two groups. An ethanol-exposure group (EtOH group; n = 19) was exposed to ethanol for 6 consecutive weeks (7 days/week) in inhalation chambers which have previously been described in detail (Rogers et al., 1979; Roberts et al., 1996). Control subjects (n = 15) were housed in similar chambers but were exposed to air vapour. Ethanol vapour was infused into the chambers, from 05:00 to 07:00 daily resulting in ethanol vapour levels ranging from 22 to 28 mg ethanol/l O₂. Rats were weighed and blood ethanol levels were determined once a week, except for week 2. Blood samples were collected from the tip of the tail. Blood ethanol levels were analysed using an enzymatic assay (Sigma).

Surgical procedure

Two to three weeks after chronic ethanol exposure was terminated, both groups of rats were implanted with screw electrodes in the skull, a bipolar electrode aimed at the amygdala, and a stainless-steel cannula aimed at the lateral ventricle (24 gauge, 7.5 mm). Rats were anaesthetized with an intraperitoneal injection of 50 mg/kg of sodium
pentobarbital. Atropine (0.06 mg, subcutaneous injection) was administered to minimize respiratory suppression during and after surgery. Screw electrodes were placed in the skull overlying the frontal cortex (AP +3.0 mm, ML ± 3.0 mm) and parietal cortex (AP +3.0 mm, ML ± 4.0 mm). A third screw electrode that was grounded was placed posterior to lambda overlying the cerebellum. Stereotaxic coordinates for the amygdala electrode (AP −1.0 mm, ML ± 5.3 mm, DV −8.5 mm) and the lateral ventricular cannula (AP −0.6 mm, ML ± 2.0 mm, DV −3.2 mm) were determined from the Pellegrino atlas (Pellegrino et al., 1979) with the tooth bar set at 5.0 mm above the interaural line. Electrode connections were made to a 5-pin Amphenol connector. The entire assembly was anchored to the skull with dental acrylic and anchor screws.

Electrophysiological recording procedures

EEGs were recorded from rats after being placed in a BRS/LVE recording chamber and attached to a microdot recording cable. Ten minutes of EEG were recorded on a Sensorium polygraph and digitized at a rate of 128 Hz with a low pass filter of 70 Hz and a time constant of 0.3 s (0.53 Hz). Consecutive 4 s epochs of EEG were decomposed with a Fourier transformation over a spectrum of 1–64 Hz. Individual spectra for each 4 s epoch were averaged after being edited for artefact using a computer automated detection program. Artefact trials were verified by visual analysis of spectral distributions. Artefact over a frequency range of 1–20 Hz was identified if average power was >2000 µV²/octave (frontal cortex and parietal cortex) or >5000 µV²/octave (amygdala). These data were then compressed into eight frequency bands: 1–2, 2–4, 4–6, 6–8, 8–16, 16–32, 32–50, 1–50 Hz. Mean power and the coefficient of variation (standard deviation/mean; CV) for each band was then calculated as previously described (Ehlers and Havstad, 1982). EEG was recorded from frontal cortex-ground, parietal cortex-ground, and amygdala-ground.

Event-related potentials (ERPs) were recorded immediately following EEG recordings. ERP recording sessions lasted ~10 min. ERPs were elicited using a three-tone auditory ‘oddball’ paradigm which is modelled after passive ERP paradigms employed in our human investigations (Kaneko et al., 1996). The rationale for using this three-tone paradigm arises from the selective generation of the P300 (P3) ERP component (i.e. a positive potential which occurs 200–400 ms after stimulus presentation) in response to infrequently presented tones. When using this paradigm, two of the three tones are presented with low frequency (rare: 10%, noise: 6%; see below for details) in comparison to the third tone (84%) and, as a result, elicit P3 ERP components. In addition to eliciting a P3 component, the noise tone also elicits a startle response which allows for assessment of neurosensory reactivity in the subjects.

Auditory stimuli were presented from a speaker attached to the top of the recording chamber which was located 45 cm above the subjects. Three tones were presented during ERP sessions. All tones were presented for 20 ms with rise and fall times of <1 ms. Type 1 tones (standard tone: 1000 Hz square wave, 75 dB) were presented 84% of the time. Type 2 tones (rare tone: 2000 Hz square wave, 85 dB) were presented 10% of the time. Type 3 tones (noise tone; white noise, 100 dB) were presented 6% of the time. Individual ERP trials were 1000 ms in duration (200 ms pre-stimulus + 800 ms post-stimulus). Each trial was separated by variable time intervals ranging from 500 to 1000 ms. Standard tone, rare tone and noise tone presentation was randomized with at least one presentation of a standard tone between each rare tone, no more than six standard tones between each rare tone, and no more than 12 trials between the presentation of noise tones. Each session consisted of 312 individual tone presentations.

ERP data were digitized at a rate of 256 Hz. Each wave component was quantified on the basis of peak amplitude, latency to peak amplitude from tone presentation, and polarity. Pre-stimulus baseline activity was determined from average EEG activity 100 ms prior to tone presentation. Each ERP component was identified with an automated peak detection program and confirmed by visual inspection. Movement artefact (i.e. voltages exceeding ± 400 µV) was assessed with an automated computer detection program and eliminated following confirmation by visual analysis. Individual ERP trials for each tone type were then averaged for each subject. ERPs were collected for the following leads: (1) frontal cortex-ground; (2) parietal cortex-ground; (3) amygdala-ground. ERP components were identified based on the largest amplitude peak within a specified latency range.
Latency windows used to identify ERP components for frontal cortex-ground were: N10 = 0–30 ms, P1 = 30–60 ms, N1 = 50–100 ms, P2 = 100–175 ms, N2 = 175–250 ms. Latency windows used to identify the ERP components for parietal cortex-ground were: N10 = 0–30 ms, P1 = 30–60 ms, N1 = 50–100 ms, P2 = 100–175 ms, N2 = 175–250 ms, P3A = 225–300 ms, P3B = 300–375 ms. Latency windows used to identify the ERP components for amygdala-ground were: N10 = 0–30 ms, P1 = 30–60 ms, N1 = 50–100 ms, P2 = 75–150 ms, N2 = 125–200 ms, P3A = 225–300 ms, P3B = 300–400 ms. These data analyses have been previously described (Ehlers et al., 1991).

**Drugs**

NPY and CRF were obtained from the Salk Institute, courtesy of Dr J. E. Rivier. All rats were administered saline, 0.50 μg CRF, and 5.0 μg NPY (i.e. total of three injections) using a Randomized Block design. Each peptide was dissolved in a physiological saline (0.9% w/v NaCl) vehicle immediately prior to infusion. Intracerebroventricular (ICV) infusions (5 μl) were performed over a 1-min period with stainless-steel (30 gauge, 7.5–8.0 mm) injectors attached to 10 μl Hamilton syringes via polyethylene tubing. Injectors were kept in place for 1 min following the completion of each infusion. Infusions were administered 20 min before EEG recording (i.e. 30 min before ERP recording).

**General procedures**

The treatment groups were first exposed to 6 weeks of chronic ethanol exposure (EtOH group; \( n = 19 \)) or 6 weeks of air vapour exposure (Con group; \( n = 15 \)). Following recovery from surgery and a protracted ethanol abstinence (10–15 weeks), EEG and ERPs were recorded following infusion of saline, CRF, or NPY as described above. At the end of the experiment, rats were anaesthetized with an intraperitoneal injection of sodium pentobarbital (100 mg/kg). Toluidine blue (2 μl) was infused into the ventricle for verification of cannula placement. After decapitation, the brains were extracted and frozen on dry ice. Each brain was then sectioned (60 μM) and depth electrode placement was histologically verified.

**Statistical analysis**

Statistical analysis was completed using Systat for the Macintosh (Systat, Inc.). Body weight differences between the groups: (1) at the beginning of the experiment; (2) 2 weeks after surgery; (3) at the end of the experiment were examined using independent \( t \)-tests (\( P < 0.05 \)). Differences in body weight between the two groups during the period of chronic ethanol exposure were examined using a two-way repeated measures (RM) analysis of variance [weight \( \times \) week]. For analysis of baseline differences in EEG and ERPs, a between-group one-way analysis of variance (ANOVA) was used to examine EEG and ERPs following the saline infusion. For analysis of the effects of CRF and NPY (compared to saline) on EEG and ERPs, within-subject one-way RM ANOVAs were used. For within-group one-way RM ANOVA, only subjects which had usable infusions for all drugs (saline, NPY, and CRF) were employed. All \( P \)-values obtained from RM ANOVA were corrected using the Greenhouse–Geisser adjustment. Significance values for EEG and ERP were \( P < 0.05 \) at least.

**RESULTS**

Prior to chronic ethanol exposure, there were no statistically significant differences (\( t = –1.917, \text{df} = 32, \ P = 0.064 \)) in body weight between the control group (270 ± 12 g; mean ± SD) and EtOH group (261 ± 14). During the period of chronic ethanol exposure, the control group weighed significantly [\( F(1,32) = 35.81, \ P < 0.0001 \)] more than the EtOH group (Table 1). The control group gained ~20 g/week, but the EtOH group only increased body weight by ~10 g/week. However, following withdrawal, the weight differences disappeared, such that immediately prior to testing there were no statistically significant differences in body weight between the EtOH and control groups (Table 1: week 10–15). During chronic ethanol exposure, the EtOH group sustained average blood ethanol levels of 181.7 ± 4.2 mg/dl (Table 1).

Two subjects in the EtOH group and one subject from the control group were not included in EEG and ERP data analysis, due to poor recordings during the habituation phase of the experiment. In addition, one rat from the EtOH group was excluded from analysis of the amygdala due to poor recording quality. The final number of subjects for between-group baseline analysis in the EtOH group was...
17 \((n = 16\) for amygdala\) and in the control group was 14. For statistical analyses of the effects of CRF and NPY, three additional rats in the EtOH group were excluded due to bad injections or excessive artefact (EtOH group, \(n = 14\), \(n = 13\) for amygdala; control group, \(n = 14\)). Proper placement of the electrodes in the amygdala and of the cannula in the ventricle was confirmed histologically in all the subjects used in the final data analysis.

**EEG results**

Examination of the resting EEG following ICV saline infusion revealed no statistically significant differences in EEG power between the EtOH and control groups in the frontal cortex (Fig. 1), or amygdala (Fig. 2). In the parietal cortex, power in the 6–8 Hz frequency band was significantly greater \([F(1, 29) = 6.73, P = 0.015]\) in the EtOH group in comparison to the control group (Fig. 1, bottom). Significant differences in the coefficient of variation (CV), an index of EEG stability, were observed between the two groups. In the frontal cortex, a statistically significant decrease in the CV in the 6–8 Hz frequency bands \([F(1, 29) = 10.28, P = 0.003]\) was observed.

Infusion of CRF and NPY into the lateral ventricle resulted in significant changes in EEG activity in each group. Infusion of CRF in the EtOH group increased power in the frontal and parietal cortices. In the frontal cortex, CRF resulted in significant increases in 1–2 Hz \([F(1, 13) = 10.38, P = 0.007]\) and 32–50 Hz \([F(1, 13) = 10.60, P = 0.001]\) frequency bands of the EtOH group with similar non-significant increases observed in the 2–4, 4–6 and 6–8 Hz frequency bands (Fig. 1, top). In the control group, statistically significant increases in EEG power in the frontal cortex were observed in the 32–50 Hz \([F(1, 13) = 15.22, P = 0.002]\) frequency band. In the parietal cortex, CRF increased EEG power in the 4–6 Hz \([F(1, 13) = 9.89, P = 0.008]\), 6–8 Hz \([F(1, 13) = 17.01, P = 0.001]\), 32–50 Hz \([F(1, 13) = 27.18, P = 0.003]\), and 1–50 Hz \([F(1, 13) = 4.99, P = 0.004]\) frequency bands in the EtOH group (Fig. 1, bottom). In the control group, CRF decreased EEG power in the 1–2 Hz frequency band \([F(1, 13) = 12.80, P = 0.003]\) in the parietal cortex. CRF infusion also increased EEG stability in the ETOH group, as measured by statistically significant decreases in the CV in the 16–32 Hz \([F(1, 13) = 10.34, P < 0.007]\) and 32–50 Hz \([F(1, 13) = 35.15, P < 0.0001]\) frequency bands (Table 2) in the frontal cortex.

NPY infusion into the lateral ventricle significantly decreased power in the parietal cortex in both the EtOH and control groups, but only decreased power in the EtOH group in the amygdala. In the parietal cortex, NPY decreased power in the 8–16 Hz \([\text{EtOH: } F(1, 13) = 15.71, P = 0.002]\; \text{control: } F(1, 13) = 9.18, P = 0.008]\), 16–32 Hz \([\text{EtOH: } F(1, 13) = 13.56, P = 0.003]\; \text{control: } F(1, 13) = 18.05, P = 0.0009]\), and 32–50 Hz \([\text{control: } F(1, 3) = 21.08, P < 0.0001]\) frequency bands (Fig. 2, top). In the amygdala NPY decreased power in the EtOH group in the 8–16 Hz \([F(1, 12) = 10.32, P = 0.007]\) frequency band, but NPY did not alter power in the control group (Fig. 2, bottom). There were no statistically significant effects of NPY on EEG stability as measured by the coefficient of variation in either group in any lead.
**ERP results**

In the parietal cortex, a one-way ANOVA between groups reported statistically significant \( F(1, 29) = 6.98, P = 0.013 \) differences in the latency of the P3B wave elicited by the rare tone. The latency of the P3B wave in response to the rare tone was greater in the EtOH group compared to the control group following saline infusion (Fig. 3, bottom). In the frontal cortex, there were statistically significant reductions in the amplitude of the P2 ERP component to the standard \( F(1, 29) = 12.89, P = 0.001 \), rare \( F(1, 29) = 9.14, P = 0.005 \), and noise tone \( F(1, 29) = 9.88, P = 0.004 \) in the EtOH group compared to the control group (Fig. 3, top). There were no significant differences in ERPs in the amygdala under baseline conditions. There were no significant effects of CRF on ERPs evoked from any of the leads. NPY infusion significantly decreased the amplitude of the N1 ERP component in the EtOH group in response to the rare \( F(1, 13) = 32.21, P < 0.0001 \) and noise \( F(1, 13) = 16.98, P = 0.001 \) tones in the frontal cortex, but there were no effects of NPY on the N1 in the control group (Fig. 4).

**DISCUSSION**

The increases in cortical EEG power seen during protracted ethanol withdrawal in this study are consistent with data previously reported from this laboratory (Ehlers and Chaplin, 1991). While examining the time course of changes in EEG and ERPs following 4 weeks of chronic ethanol vapour inhalation, Ehlers and Chaplin (1991) reported
Fig. 2. Mean band power in the parietal cortex and amygdala in the ethanol (EtOH) and control groups following intracerebroventricular saline and neuropeptide Y (NPY) infusions. Solid bars represent the EtOH group (parietal cortex, Pctx, n = 14; amygdala, Amyg, n = 13) and open bars represent the control group (n = 14). Error bars represent the standard error of the mean. Sal = saline. *Significant difference compared to the saline condition within each group for the same band (P < 0.05).

Table 2. Effects of chronic ethanol (EtOH) treatment on electroencephalogram stability in the frontal cortex as measured by the coefficient of variation

<table>
<thead>
<tr>
<th>Power frequency band (Hz)</th>
<th>Saline infusion</th>
<th>CRF infusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EtOH group (n = 14)</td>
<td>Control group (n = 14)</td>
</tr>
<tr>
<td>1–2</td>
<td>0.58 ± 0.01</td>
<td>0.68 ± 0.04</td>
</tr>
<tr>
<td>2–4</td>
<td>0.47 ± 0.02</td>
<td>0.53 ± 0.05</td>
</tr>
<tr>
<td>4–6</td>
<td>0.51 ± 0.02</td>
<td>0.59 ± 0.08</td>
</tr>
<tr>
<td>6–8</td>
<td>0.51 ± 0.02*</td>
<td>0.66 ± 0.05</td>
</tr>
<tr>
<td>8–16</td>
<td>0.61 ± 0.04</td>
<td>0.70 ± 0.05</td>
</tr>
<tr>
<td>16–32</td>
<td>0.37 ± 0.03</td>
<td>0.49 ± 0.08</td>
</tr>
<tr>
<td>32–50</td>
<td>0.28 ± 0.02</td>
<td>0.23 ± 0.03</td>
</tr>
<tr>
<td>1–50</td>
<td>0.29 ± 0.01</td>
<td>0.36 ± 0.05</td>
</tr>
</tbody>
</table>

Values are means ± SD. *Significant difference (P < 0.05) from the control group in the same brain region. †Significant difference from saline infusion in the same frequency band (P < 0.05).
that 24 h after the end of chronic ethanol exposure cortical EEG was characterized by increased power in the same frequency bands which were affected in the present study (4–16 Hz). However, the increases in EEG power were found to dissipate 2 weeks following cessation of ethanol exposure (Ehlers and Chaplin, 1991). In the present study, a persistent increase in theta band power (6–8 Hz) in the parietal cortex was observed 10–15 weeks following cessation of ethanol exposure. Significant and persistent increases in EEG stability were also observed in the EtOH group.

The increase in EEG stability and power observed in the cortical leads might indicate enhanced arousal or anxiety, as the potent anxiogenic CRF (Thatcher-Britton et al., 1986; Dunn and File, 1987;
Moreau et al., 1997) has previously been reported to induce increases in EEG power and stability (Ehlers et al., 1986, 1997b; Ehlers, 1998), whereas anxiolytics, such as benzodiazepines and ethanol, decrease EEG stability (Ehlers and Chaplin, 1991; Robledo et al., 1994). The prolonged neurophysiological indications of arousal/anxiety observed in the present study are consistent with human studies where increased arousal and neuronal hyperexcitability have been reported (Porjesz and Begleiter, 1981) following chronic ethanol exposure. These data also indicate that a subtle state of CNS arousal/anxiety may persist following ethanol exposure. They are also consistent with studies which report increased locomotor responses to amphetamine, cocaine, and CRF after acute ethanol withdrawal (>2–3 weeks), even when behavioural baselines prior to drug administration are equivalent (Ehlers and Chaplin, 1987; Manley and Little, 1997).

The effects of chronic ethanol exposure on ERPs have previously been reported in rats (Ehlers and Chaplin, 1991). The present study replicates the finding of decreased P2 amplitudes observed 2 weeks after a 4-week ethanol exposure (Ehlers and Chaplin, 1991). Further, the blunted response of ethanol-exposed rats, compared to controls, is consistent with data on the N1–P2 ERP component in alcoholics (Porjesz et al., 1980). In comparison to age-matched controls, alcohols do not display differential responses to rare, versus frequently presented stimuli, and display an overall blunted response to all stimuli. This same observation was made in the ethanol-treated rats in the present study. The N1–P2 complex is sensitive to differences in stimulus modality and attentional processes. The deficit in the N1–P2 complex in alcoholics has been suggested to result from a ‘sensory filtering’ impairment (Porjesz et al., 1980). The overall blunted response of the ethanol-treated subjects to the auditory stimuli presented in this study suggests that a similar ‘sensory filtering’ deficit might be present.

In the present study, chronic ethanol exposure resulted in an increased latency of the P3 ERP component in the parietal cortex. An effect of chronic ethanol exposure on late positive ERPs in the rat is consistent with reports of the P3 ERP wave component of alcoholics having an increased latency (Porjesz et al., 1987; Pfefferbaum et al., 1991) and/or decreased amplitude (Porjesz et al., 1980, 1987; Pfefferbaum et al., 1991; Bauer et al., 1994). Decreases in amplitude and increases in latency of P3 ERP components are suggestive of impaired and/or slowed cognitive processing. Individuals at high risk for alcoholism or substance abuse also have decreased amplitude/increased latency of P3 components prior to any significant ethanol exposure (Begleiter et al., 1984; Berman et al., 1993). These data have been suggested to indicate a pre-existing neurophysiological deficit which might serve as a marker of susceptibility to alcohol abuse, alcoholism, or substance abuse (Begleiter et al., 1984; Pfefferbaum et al., 1991; Berman et al., 1993). The present data suggest that the altered P3 wave components observed in alcoholics could be the result not only of a pre-existing difference in neurophysiological function, but also an effect of long-term ethanol exposure. One could speculate that individuals with pre-existing slowed P3 latencies or decreased P3 amplitudes may even be more susceptible to the effects of chronic ethanol exposure on late positive ERPs.

The observation of increased neurophysiological responses of the EtOH group to CRF is consistent with a previous report of an enhancement of the locomotor activating effects of CRF in chronically ethanol-treated rats (Ehlers and Chaplin, 1987). The enhanced response to CRF might be partially modulated by an up-regulation of CRF receptors in response to decreased CRF levels. Such decreases in CRF levels in the hypothalamus following chronic ethanol exposure have been reported previously (Rivier et al., 1984). It has also recently been reported that chronic ethanol exposure decreases NPY levels in the hypothalamus but following withdrawal there is a rebound increase in NPY levels (Ehlers et al., 1998). Therefore, it is reasonable to postulate that compensatory changes in NPY levels and receptors could account for altered responses to NPY in the ethanol-treated group. In addition, given the postulated interaction between these two peptides (Heilig et al., 1994; Ehlers et al., 1997a), it is possible that the increased responsiveness to CRF and NPY is a result of alterations in the balance of CRF-NPY tone in the central nervous system. Further studies employing site-specific micro-injections of these compounds would be necessary to elucidate more fully the mechanism of these altered responses.

In summary, the present study examined the effects of chronic ethanol exposure on the EEG and ERPs in rats during a protracted abstinence period.
These data suggest that chronic ethanol exposure and subsequent withdrawal results in persistent changes in EEG and ERPs indicative of increased arousal/anxiety and impaired stimulus evaluation. These data are consistent with previous findings from this laboratory (Ehlers and Chaplin, 1991), and extend the effects of ethanol exposure to a protracted abstinence period of 10–15 weeks. These effects, particularly the effects on late positive ERP components, are also consistent with those reported for alcoholics (Begleiter et al., 1984; Pfefferbaum et al., 1991; Berman et al., 1993) and suggest that altered P3 morphology in alcoholics may represent both a pre-existing state and a chronic effect of ethanol exposure. These long-term neurophysiological changes may serve as a partial substrate which results in greater susceptibility of ethanol-treated rats to the effects of centrally administered CRF and NPY. Overall, these data support the hypothesis that after prolonged abstinence from alcohol, altered responsivity to neuropeptides implicated in stress and anxiety responses (i.e. CRF and NPY) may influence relapse and ethanol consumption in alcoholics.

Acknowledgements — The authors would like to thank David Cloutier for his assistance in the statistical analysis of these data, Maury Cole for his assistance in the maintenance of the ethanol vapour chambers and determination of blood ethanol levels, James Havstad, PhD for developing the EEG and ERP software, and Susan Lopez for her assistance in the data analysis. This work has been supported by AA06059 and AA00223 to C.L.E.

REFERENCES


Levenson, R. W., Oyama, O. N. and Meek, P. S. (1987) Greater reinforcement from alcohol for those at risk:


