Long-Term Potentiation in the Rat Hippocampus Is Reversibly Depressed by Chronic Intermittent Ethanol Exposure

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Roberto, M., T. E. Nelson, C. L. Ur, and D. L. Gruol. Long-term potentiation in the rat hippocampus is reversibly depressed by chronic intermittent ethanol exposure. J Neurophysiol 87: 2385–2397, 2002; 10.1152/jn.00145.2001. Alcohol exposure induces multiple neuroadaptive changes in the CNS that can have serious long-term consequences on CNS function including cognitive effects and attenuation of learning and memory. The cellular mechanisms underlying the CNS effects of alcohol have yet to be fully elucidated and are likely to depend on the pattern and dose of alcohol exposure. Using electrophysiological recordings from hippocampal slices obtained from control and chronic alcohol-treated rats, we have investigated the effects of a binge pattern of alcohol abuse on synaptic plasticity in the CNS. The alcohol-treated animals were exposed to ethanol vapor for 12–14 days using an intermittent exposure paradigm (14 h ethanol exposure/10 h ethanol withdrawal daily; blood alcohol levels ~180 mg/dl), a paradigm that models human binge alcohol use. Induction of long-term potentiation (LTP) in the CA1 region of the hippocampus by tetanic stimulation of Schaffer collaterals was completely blocked in slices from the chronic alcohol-treated animals. LTP remained blocked 1 day after withdrawal of animals from alcohol, indicating that the neuroadaptive changes produced by alcohol were not readily reversible. Partial recovery was observed after withdrawal from alcohol for 5 days. Other measures of synaptic plasticity including post-tetanic potentiation and paired-pulse facilitation were also altered by the intermittent alcohol treatment paradigm. The results suggest that alterations in synaptic plasticity induced by chronic intermittent alcohol consumption play an important role in the effects of binge alcohol use on learning and memory function.

INTRODUCTION

One of the principal cognitive effects of alcohol abuse in humans is the attenuation of learning and memory processing (Fadda and Rossetti 1998; Jacobson et al. 1990). The hippocampal region of the brain plays a pivotal role in memory processing (Shors and Matzelt 1997) and is likely to be an important site of alcohol effects that lead to altered cognitive function (Ryabinin 1998). A synaptic mechanism thought to underlie certain types of memory storage in the hippocampus is long-term potentiation (LTP) at the Schaffer collateral-CA1 pyramidal neuron synapse (Abel and Kandel 1998; Bliss and Collingridge 1993; Chen and Tonegawa 1997; Reymann 1993; Sweatt 1999). LTP can be elicited experimentally by a brief high-frequency stimulation of presynaptic afferents and is defined as a long-term increase in the size of the postsynaptic response to synaptic transmission (Bliss and Collingridge 1993). Both activation of N-methyl-d-aspartate (NMDA) receptors as well as inactivation of γ-aminobutyric acid (GABA) receptors are important in the induction and maintenance of LTP in the CA1 region, although the exact interplay between these two processes during LTP induction is still under intense investigation (Gustafsson and Wigström 1990; Gustafsson et al. 1987; Malenka and Nicoll 1999; Mott and Lewis 1991).

Recent studies have shown that both acute (e.g., exposure for tens of minutes) (Blitzer et al. 1990; Givens and McMahon 1995; Morrisett and Swartzwelder 1993; Sinclair and Lo 1986; Steffensen et al. 1993; Sugiuara et al. 1995) and chronic (e.g., exposure for several months) (Durand and Carlen 1984; Peris et al. 1997a) alcohol exposure blocks the induction of LTP in the hippocampus. The acute alcohol-exposure paradigm models CNS effects during alcohol intoxication, whereas the chronic alcohol-exposure paradigm provides information relevant to CNS changes occurring with long-term alcohol abuse such as occurs in alcoholics. Both patterns of alcohol abuse produce memory deficits (White et al. 2000). Thus interactions between alcohol and LTP may be a critical step in the altered memory processes resulting from alcohol abuse. The acute effects of alcohol on hippocampal LTP are thought to result primarily from a direct depressant effect of alcohol on NMDA receptor-mediated currents (Lovingier et al. 1990; Morrisett and Swartzwelder 1993; Schummers et al. 1997). The mechanisms underlying the effects of chronic alcohol exposure on LTP are not well understood and are likely to result from changes in the interactions of several neurotransmitter systems including GABAAergic, glutamatergic, and cholinergic systems (Peris et al. 1997a,b). In addition, a variety of long-lasting or permanent morphological changes of the hippocampal neural circuit occur with chronic alcohol treatment including a 10–40% loss of principal cells (Durand and Carlen 1984) and interneurons (Lescaudron et al. 1986; Scheetz et al. 1987; Walker et al. 1981), effects that are likely to play a major role in the altered synaptic function and plasticity produced by prolonged alcohol exposure.

Another pattern of alcohol abuse that has important physiological and social consequences is binge alcohol consumption. In this case, excessive alcohol consumption occurs on a regular basis for days or weeks followed by a period of abstinence. The binge pattern of alcohol abuse is known to...
produce temporary memory loss (Becker 1994; Maier and Pohorecky 1989), but the underlying mechanisms have yet to be elucidated. In the current study, we have examined hippocampal LTP as a possible substrate for altered memory mechanisms occurring with this pattern of alcohol exposure. To experimentally model a binge pattern of alcohol use as seen in humans, animals (rats) were exposed to a chronic intermittent treatment schedule consisting of daily alternating episodes of alcohol (ethanol) exposure and alcohol withdrawal for a relatively short treatment period, lasting for 2 wk. Blood levels of alcohol were maintained at a level associated with moderate intoxication in humans, ~180 mg/dl (0.18% or 40 mM).

Chronic alcohol abuse even for relatively short periods can result in alcohol dependency, an adaptive condition defined primarily by the appearance of withdrawal signs after cessation of alcohol exposure. The withdrawal syndrome is characterized by both behavioral and electrophysiological parameters (Macey et al. 1996). An early withdrawal period starts immediately after the cessation of alcohol exposure and corresponds to a detoxification associated with a mild hyperexcitability. A more severe withdrawal phase develops within 24 h of the cessation of alcohol exposure and can last for several days (Fadda and Rossetti 1998). Repeated alcohol-withdrawal episodes are known to increase the severity of the subsequent syndrome (Becker 1994) and can lead to neuropathological changes (Becker 1994; Collins et al. 1991; Fadda and Rossetti 1998; Maier and Pohorecky 1989; Zou et al. 1996). In the chronic intermittent exposure paradigm used to model binge alcohol use, the daily, intermittent episodes of alcohol exposure could produce alcohol dependency and daily symptoms of alcohol withdrawal when alcohol is unavailable. In addition, the repeated withdrawal episodes could increase the severity of subsequent withdrawal symptoms as a result of kindling (Macey et al. 1996; Schulteis et al. 1995). Thus the neuroadaptive changes produced by chronic intermittent alcohol exposure could involve effects induced by both exposure to and withdrawal from alcohol. Consequently, in the current study we have also investigated the effect of withdrawal from chronic intermittent alcohol exposure on hippocampal LTP.

Our results show that chronic intermittent alcohol exposure significantly decreases the amount of LTP that can be induced by high-frequency stimulation in the CA1 region of the hippocampus and that this decrease persists for ~24 h after withdrawal from alcohol but shows a partial recovery after a longer period of alcohol withdrawal (5 days). Posttetanic potentiation and paired-pulse facilitation were also altered by the chronic intermittent alcohol treatment, indicating that alcohol-induced alterations in presynaptic mechanisms contribute to the neuroadaptive effects of a binge pattern of alcohol exposure on the CNS. Interestingly, there were no behavioral or physiological signs of alcohol withdrawal with this paradigm of alcohol exposure.

**Methods**

**Chronic ethanol treatment**

Forty-six naive male Wistar rats (40–45 days old; 140–160 g; Charles River) were housed 2–3 per cage with a 6 AM to 6 PM light cycle and with free access to food and water. The animals were divided into two groups, a chronic intermittent ethanol (CIE) treatment group and a control group. The CIE treatment group was exposed intermittently to ethanol on a 14 h on/10 h off cycle for a period of 12–14 days using the vapor inhalation chamber method (Rogers et al. 1979). Control animals were maintained in identical chambers for the same duration as the chronic ethanol-treated animals but were not exposed to ethanol vapor. Animals withdrawn from ethanol for 1 or 5 days were maintained in identical chambers as the CIE-treated and control animals but were not exposed to ethanol vapor during the withdrawal period.

**Blood alcohol level, body weight, and brain weight**

Blood alcohol levels (BALs) of the CIE-treated animals were determined from tail blood samples taken two times per week. Control animals were also routinely bled. When necessary, adjustments in the ethanol vapor concentration were made after the first BAL measurement to achieve a target BAL of 150–200 mg/dl. The mean BAL of all CIE-treated animals was 184 ± 8 (SE) mg/dl (n = 39) during the first week and 179 ± 8 mg/dl (n = 29) during the second week of treatment. The mean body weight of CIE-treated animals and 1-day withdrawn animals were 225 ± 4 g (n = 19) and 236 ± 7 g (n = 6), respectively, compared with a mean body weight of 254 ± 6 g (n = 11) for age-matched control animals. This difference between control and CIE-treated animals was significant (P < 0.05) and presumably reflects a reduced dietary intake in the CIE-treated animals. Whole-brain weight for both CIE-treated animals (1.80 ± 0.03 g, n = 19) and 1-day withdrawn animals (1.79 ± 0.05 g, n = 6) were also smaller than age-matched control animals (1.91 ± 0.05 g, n = 9), but the difference was not significant. Whole-brain weights were estimated by doubling the weight of the unused half of the brain. The mean body weight of 5-day withdrawn animals was 290 ± 5 g (n = 11) compared with a mean body weight of 295 ± 6 g (n = 11) for age-matched control animals. The mean whole-brain weight of 5-day withdrawn animals (1.79 ± 0.1 g, n = 8) was smaller than age-matched control animals (2.00 ± 0.05 g, n = 7), but the difference was not significant.

**Preparation of hippocampal slices**

The animals were weighed, anesthetized with halothane, and decapitated. Brains were rapidly removed and immersed in ice-cold artificial cerebrospinal fluid (ACSF). Hippocampal slices (400 μm) were prepared using a McIlwain tissue chopper (Mickle Laboratory Engineering, Surrey, UK). Slices were maintained (~60 min) until use in a gas-fluid interface perfusion chamber maintained at ~33°C and a flow rate of 0.55 ml/min. Slices from control animals were maintained in normal ACSF, whereas slices from CIE-treated animals were prepared and stored in ACSF containing 150 mg/dl (33 mM) ethanol to prevent physiological changes that may result from ethanol withdrawal. The composition of the control ACSF was (in mM): 130.0 NaCl, 3.5 KCl, 1.25 NaH2PO4, 2.40 NaHCO3, 2.0 CaCl2, 5.0 MgSO4, and 10.0 glucose. During the slicing procedure, the following substitutions were made in the ACSF to maintain slice viability: 0.20 CaCl2 and 12.5 MgSO4. All solutions were gassed continuously with a mixture of 95% O2/5% CO2 (pH 7.2–7.4). Experiments using slices from control and CIE-treated/withdrawn animals were performed on alternate days. On experiment days, animals in the CIE treatment group were maintained in the ethanol vapor chamber until preparation of the hippocampal slices. Slices were prepared in the morning soon after the ethanol exposure period ended.

**Field potential recordings**

Hippocampal slices were transferred to a second gas-fluid interface perfusion chamber for recording (~2 ml/min flow rate, 33°C) and allowed to stabilize for 20–30 min prior to recording. Slices from both control and CIE-treated animals were recorded in normal ACSF. Extracellular field potentials in area CA1 were recorded simultaneously from the stratum pyramidale (somatic region) and s. radiatum
groups. A standard test stimulus was used for most experiments. This study. Only rarely were these criteria not met, and the rate of voltage required to elicit a dendritic voltage (typically between 8 and 30 V) starting at the threshold of the Schaffer collateral-commissural afferent pathway using a concentric bipolar stimulating electrode (Rhodes Medical Instruments, Woodland Hills, CA). To determine the response parameters for each slice, an input/output (I/O) protocol was performed. The slices were stimulated at a range of voltages (typically between 8 and 30 V) starting at the threshold voltage required to elicit the maximum somatic population spike amplitude. Only slices that had a maximum somatic population spike amplitude >5 mV and a maximum dendritic fEPSP amplitude >2 mV were used in this study. Only rarely were these criteria not met, and the rate of failure to meet this criteria did not differ between the treatment groups. A standard test stimulus was used for most experiments. This stimulus was adjusted for each slice such that the dendritic fEPSP was equal to ~50% of the maximal amplitude determined in the I/O relationship. LTP was induced by a single train of high-frequency stimulation (HFS; 100 Hz, 1-s duration) at the same intensity of the test stimulus. The slice was considered to exhibit LTP if the slope of the dendritic fEPSP response remained at an elevated level of >125% of baseline for >60 min following the HFS. Paired-pulse facilitation (PPF) was examined in each slice before and after the HFS using a 40-ms interpulse interval. In all experiments to examine PPF, measurements were made of the dendritic fEPSP slope in both the first and second responses to a pair of stimuli, and the stimulus strength was adjusted such that the amplitude of the first fEPSP of the pair was 50% of the maximal amplitude of the fEPSP determined in the I/O relationship. Measurements were made of the somatic population spike amplitude as well as the slope of the dendritic fEPSP in all protocols. Population spike amplitude was measured from a line extrapolated between the peaks of the two rising components to the peak of the intervening downward deflection (i.e., the spike). The stimulation voltages of the I/O data were normalized such that the voltage required to produce threshold responses of the dendritic fEPSP and population spike were assigned a value of 0 V for each slice, and all stimuli were normalized to this value. For paired-pulse data, the relative amount of facilitation for each slice was expressed as the ratio of the second response with respect to the first response. Compiled data were expressed as the means ± SE. Statistical analyses were done using ANOVA (factorial) and the Fisher’s protected least-significant difference (PLSD) post hoc test. Statistical significance was set at the P < 0.05 level.

RESULTS

CIE treatment blocks LTP expression

In hippocampal slices prepared from control animals, HFS of the Schaffer collaterals reliably produced posttetanic potentiation (PTP) of the CA1 pyramidal neuron synaptic response that lasted for 1–5 min and was followed by LTP that lasted for a minimum of 60 min. The enhancement of the synaptic response during PTP and LTP was evident in both the dendritic fEPSP and somatic population spike (Fig. 1, A and B). The dendritic fEPSP slope measured during PTP and LTP (1 and 60 min after HFS) was 192 ± 9 and 150 ± 8% (n = 19), respectively, of the pre-HFS baseline value (Fig. 1A2). Slices from CIE-treated animals were prepared and maintained in saline containing 150 mg/dl ethanol to prevent ethanol withdrawal and were recorded under ethanol-free conditions shortly after removal of ethanol. In these slices, HFS elicited a significantly smaller PTP of the dendritic fEPSP slope (141 ± 6%, n = 19; P < 0.001). In addition, LTP was not induced in CIE-treated slices (Fig. 1A2); the fEPSP slope returned to near baseline levels shortly after the PTP phase and remained at this level for the remainder of the recording period (106 ± 4% at 60 min). Similar results of CIE treatment were obtained for measures of LTP and PTP from the recordings of the somatic population spike (Fig. 1B, I and 2). Comparison of I/O curves obtained immediately before and 60 min after the HFS showed that in control slices the potentiation of the dendritic fEPSP induced by HFS occurred over a wide range of stimulus strengths, whereas slices from CIE-treated animals did not exhibit a potentiation of the fEPSP at any stimulus intensity (Fig. 1C, I–3). In contrast to PTP and LTP, the basal synaptic responses (dendritic fEPSP and somatic population spike) and input/output (I/O) curves measured before the HFS were comparable in control and CIE treatment groups (Fig. 1C, I and 2).

To assess the possibility that the block in LTP results from a decrease in ability to activate the cells sufficiently during the tetanic paradigm, other stronger tetanic paradigms were tested [e.g., successive trains of stimulation: 3 trains (100 Hz, 1-s duration) at 5 min intervals, at the same intensity of the test stimulus; or a single train (100 Hz, 1-s duration) at the intensity of the maximal response amplitude]. These stronger induction paradigms also did not induce LTP in slices from the CIE-treated rats (data not shown).

To determine if the inhibitory effect of CIE treatment on PTP and LTP expression involves changes in presynaptic mechanisms responsible for neurotransmitter release, PPF (40-ms interpulse interval) was measured in each slice during baseline recordings and after HFS. PPF is characterized by a transient increase in synaptic efficacy during the response to the second pulse of a two-pulse stimulation protocol and is thought to result primarily from residual Ca2+ accumulation within the presynaptic terminals following the first stimulus pulse (Creager et al. 1980; Hess et al. 1987; Konnerth and Heinemann 1983; Zucker 1989). Changes in PPF due to HFS were determined by normalizing paired-pulse ratios measured after HFS to the pretetanus basal ratio. Changes in PPF are inversely related to transmitter release such that enhanced probability of transmitter release is associated with a reduction of PPF, whereas decreased probability of transmitter release is associated with an increase in PPF (Andreasen and Hablitz 1994).

PPF of the dendritic fEPSP was similar in amplitude in slices from control and CIE-treated animals during baseline recordings (before HFS), in accordance with our previous study (Nelson et al. 1999) (Fig. 2B). Immediately after the HFS (i.e., during PTP), PPF was significantly decreased relative to the pre-HFS baseline level in slices from both control and CIE-treated animals, consistent with increased neurotransmitter release during PTP (Zucker 1989). However, the reduction of PPF (relative to pre-HFS baseline PPF) was significantly smaller in slices from CIE-treated animals compared with slices from control animals (Fig. 2B). These results suggest that
Transmitter release was reduced during PTP in slices from CIE-treated animals compared with slices from control animals. PPF returned to near baseline levels following PTP, and was similar in amplitude in slices from CIE-treated and control animals for the remainder of the recording period (60 min post-HFS).

**Ethanol effects on LTP expression are retained after ethanol withdrawal**

To determine the persistence of the effects of CIE treatment, two types of experiments were carried out. In the first set of experiments, slices from the CIE-treated animals were pre-

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**A1**

Control

CIE

Pre-HFS (a) 60 min (c) 1 min (b)

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**B1**

Control

CIE

Pre-HFS (a) 60 min (c) 1 min (b)

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**A2**

% Baseline fEPSP Slope

- Control (n=19)
- CIE (n=19)

Time (minutes)

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**B2**

Pop. Spike Amplit. (mV)

- Control (n=19)
- CIE (n=19)

Time (minutes)

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**C1**

Control

CIE

threshold 1/2 max max

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**C2**

Baseline fEPSP slope (mV/ms)

- Control (n=49)
- CIE (n=30)

Normalized Stimulus Voltage (V)

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**C3**

% Baseline fEPSP Slope

- Control (n=12)
- CIE (n=16)

Normalized Stimulus Voltage (V)
pared and maintained under ethanol-free conditions and allowed to withdraw from ethanol for 2, 4, 6, and 8 h before recordings were made. Slices from control animals were also maintained under ethanol-free conditions for similar periods of time. In a second set of experiments, the CIE-treated animals were withdrawn from the CIE treatment for 1 day (>24 h) prior to preparing the slices. The slices from control and 1-day withdrawn animals were prepared, maintained, and recorded under ethanol-free conditions. For both sets of experiments, measurements of synaptic responses were made under baseline conditions and after HFS, during the period of PTP and LTP. I/O relationships were also determined for each slice.

Results from the first series of experiments showed that ethanol effects on baseline synaptic transmission, PTP, and LTP persisted for up to 8 h after removal of ethanol. Thus the amplitude of PTP was significantly smaller in the withdrawn slices compared with time-matched control slices at all time points tested (2, 4, 6, and 8 h of withdrawal), and the withdrawn slices did not exhibit LTP (Fig. 3), results similar to that observed for slices from the CIE-treated animals (Fig. 1). Comparable results were obtained for measures of LTP and PTP based on the recordings of the somatic population spike (not shown). Baseline I/O curves (measured before HFS) for the dendritic fEPSP slope were also comparable in control and withdrawn slices at all time points measured (2-, 4-, 6-, and 8-h; not shown), results similar to that observed for slices from CIE-treated animals (Fig. 1C2).

Results from the second series of experiments showed that the effects of CIE treatment on PTP and LTP persisted in slices from animals withdrawn from ethanol for 1 day, and that baseline synaptic transmission was also altered by ethanol withdrawal. Thus the amplitude of PTP was significantly smaller in slices from the 1-day withdrawn animals (131 ± 5%, n = 19) compared with slices from control animals (192 ± 9%, n = 19) and the slices from withdrawn animals did not exhibit LTP (Fig. 4A, I and 2), results similar to that observed in slices from animals subjected only to CIE treatment (Fig. 1). Baseline I/O curves for the fEPSP slope measured before HFS were similar to I/O curves measured after HFS in the slices from 1-day withdrawn animals, consistent with the impairment of LTP expression (Fig. 4A, I and 3). Comparable results were obtained for measures of LTP and PTP based on the recordings of the somatic population spike (Fig. 4B, I and 2). In contrast, basal synaptic responses were significantly larger in slices from 1-day withdrawn animals compared with slices from control animals (Fig. 4C2), whereas no significant difference was observed between slices from CIE-treated and control animals (Fig. 1C2).

PPF was also measured in slices from the 1-day withdrawn animals. The amplitude of PPF during baseline recording was significantly smaller in the slices from 1-day withdrawn animals compared with slices from control animals (Fig. 5B), suggesting that the larger basal synaptic response was a result of increased transmitter release. A reduction in PPF relative to baseline PPF was observed during PTP in slices from both control and 1-day withdrawn animals (Fig. 5C), although the reduction was significantly smaller for slices from 1-day withdrawn animals compared with slices from control animals (Fig. 4), results similar to that observed in slices from the CIE-treated animals (Fig. 2). The amplitude of PPF in slices from 1-day withdrawn animals returned to baseline levels following PTP, whereas PPF in control slices remained at a somewhat reduced level (Fig. 5B). Consequently, there was a significant difference between the normalized PPF values (i.e., PPF relative to baseline PPF) between slices from 1-day withdrawn animals and slices from control animals during the period following PTP (Fig. 5C), an effect that was not observed in slices from animals subjected only to CIE treatment (Fig. 2). This difference may relate to the enhanced baseline synaptic responses in slices from the 1-day withdrawn animals.

Taken together, these results show that relatively short-term CIE treatment produces alterations in synaptic plasticity associated with PTP and LTP expression in the hippocampus and that these effects represent a neuroadaptive change that are not readily reversed after removal of ethanol. Moreover, these studies also show that CIE treatment produces neuroadaptive changes in basal synaptic transmission that are not evident immediately after removal of ethanol but are evident 1 day after cessation of CIE treatment.

CIE-treated animals do not show behavioral signs of ethanol withdrawal

Daily intermittent periods of alcohol exposure and withdrawal could result in alcohol dependency as well as a number of symptoms characteristic of the alcohol-withdrawal syndrome that arise when alcohol is unavailable (Schulteis et al. 1995). Thus both exposure to and withdrawal from alcohol...
could be important factors in the induction of neuroadaptive CNS changes produced by CIE treatment. To determine whether the CIE-treated animals in our study exhibited an alcohol-withdrawal syndrome, we assessed their performance on several behavioral tests for ethanol withdrawal severity at the end of the CIE treatment period. Withdrawal signs were evaluated at 0, 2, 4, 6, and 8 h and 1 day of ethanol withdrawal.

**FIG. 2.** CIE exposure alters paired-pulse facilitation (PPF) only during the posttetanic potentiation (PTP) phase. A: sample traces of dendritic field responses evoked by paired-pulse stimulation (40-ms interpulse interval) of Schaffer collateral afferents (↓) recorded in the CA1 of slices taken from CIE-treated and age-matched control animals. Traces represent dendritic fEPSP responses evoked using a stimulus intensity that produced a response equal to 1/2 the maximal baseline dendritic fEPSP amplitude and were taken from pre-HFS baseline recordings (a), immediately following HFS (b), and 60 min following HFS (c). To clearly compare PPF at each time point (a–c), the traces are scaled such that the amplitudes of the responses to the initial pulse at each time point are equivalent. B: mean ± SE paired-pulse ratio in slices taken from CIE-treated and age-matched control animals. C: mean ± SE paired-pulse ratio, expressed as a percentage of the mean baseline value (---), in slices taken from CIE-treated and age-matched control animals. Letters (a–c) in B and C correspond to timepoints depicted in A; ↑, HFS (time = 0). *, significant from age-matched control (P < 0.05, ANOVA/Fisher’s PLSD).

**FIG. 3.** LTP induction remains blocked during acute withdrawal from CIE exposure. A: sample traces of CA1 dendritic field responses evoked by single-pulse stimulation of the Schaffer collateral input (↓) recorded in slices taken from CIE-treated animals and withdrawn from ethanol for 2, 4, 6, and 8 h. Traces represent responses evoked using a stimulus intensity that produced a response equal to 1/2 the maximal baseline dendritic fEPSP amplitude and were taken from pre-HFS baseline recordings (a), immediately following the HFS (b), and 60 min following the HFS (c) in slices withdrawn from CIE exposure for 2, 4, 6, and 8 h and matching control slices.
DISCUSSION

There is general agreement that ethanol impairs cognitive processes such as memory and learning both acutely, during intoxication, and chronically, after long-term ethanol ingestion (Mello 1972; Ryabinin 1998; Walker and Hunter 1978; White et al. 2000). Certain attributes of LTP make it an attractive model for memory processes at the synaptic level (Abel and Kandel 1998; McEachern and Shaw 1996; Shors and Matzel 1997), and investigation of the effects of ethanol on LTP may provide important insights into mechanisms underlying the neuroadaptive effects of ethanol in the CNS. In the current study, we show that HFS induces a large PTP and LTP of the dendritic fEPSP and somatic population spike in hippocampal slices from control animals, whereas PTP is reduced and LTP is not observed in hippocampal slices from animals subjected to a binge pattern of ethanol exposure for a relatively short period (~2 wk). We also show that the effect of ethanol persists 1 day after removal of ethanol but that recovery can occur with longer withdrawal periods, suggesting that the CNS has the capability to recover during periods of abstinence from ethanol if the periods of chronic ethanol intake are relatively short. However, the degree of recovery differs for LTP expression in the somatic versus the dendritic region, a difference that is likely to result from differences in the cellular mechanisms that contribute to the expression of somatic versus dendritic LTP.

The mechanisms underlying the effects of CIE treatment on these synaptic functions remain to be determined. However, a comparison of PTP and PPF in slices from control and CIE-treated animals suggests that presynaptic actions of ethanol are a contributing factor. PTP elicited by HFS was reduced in slices from the CIE-treated animals compared with slices from control animals, whereas synaptic responses evoked by single stimuli measured prior to HFS were similar in slices from CIE-treated and control animals. PTP has been shown to result from increased transmitter release caused by a transient elevation of intracellular Ca\(^{2+}\) in repetitively activated synaptic terminals (Kamiya and Zucker 1994). Therefore the reduced PTP in the CIE-treated animals suggests that transmitter release elicited by HFS at the Schaffer-collateral/CA1 synapse is reduced in slices from the CIE animals compared with slices from control animals. In addition, these results suggest that neuroadaptive changes produced by CIE treatment are most evident when there is high demand on synaptic function such as occurs during HFS.

Alterations in PPF of the dendritic fEPSP observed during PTP are also consistent with altered presynaptic function in the effects of CIE. During PTP, the change in PPF was significantly smaller in slices from CIE-treated animals compared with slices from control animals, whereas during baseline recording prior to HFS, the magnitude of PPF was similar in slices from CIE-treated and control animals. Changes in PPF are inversely related to transmitter release probability. Reduced PPF occurs as a consequence of increased transmitter release during the response to the first stimulus of a paired-pulse stimulation paradigm. Therefore the reduced change in PPF observed during PTP in slices from the CIE-treated animals is indicative of reduced transmitter release relative to control slices during this phase. A reduced level of transmitter release during repetitive stimulation could also contribute to the lack of LTP in slices from the CIE-treated animals. For example, the amount of transmitter released during HFS in slices from the CIE-treated animals may not have been adequate to induce the transient postsynaptic events that trigger the induction of...
LTP such as the activation of NMDA receptors and the elevation of intracellular Ca^{2+} ions. Future studies will address this issue.

The effects of CIE treatment on PTP, PPF, and LTP were still evident in slices from 1-day withdrawn animals, indicating that the effects of ethanol on the mechanisms mediating these events were not readily reversible. However, other aspects of synaptic transmission were altered by withdrawal from CIE treatment. Under baseline conditions, the amplitude of the dendritic fEPSP and PPF in the slices from CIE-treated animals did not differ significantly from the baseline dendritic fEPSP and PPF in slices from control animals. In contrast, the baseline...
FIG. 4. LTP induction remains blocked following 1 day of withdrawal from CIE exposure. A1 and B1: sample traces of dendritic (A1) and somatic (B1) field responses evoked by single-pulse stimulation of the Schaffer collateral input ( ) recorded in the CA1 of slices taken from 1 day withdrawn (WD) and age-matched control animals. A2 and B2: mean ± SE of dendritic fEPSP slope (A2), expressed as a percentage of the mean baseline value ( - - - ), and somatic population spike amplitude (B2), expressed in mV, in slices taken from 1 day WD and age-matched control animals. Letters (a–c) correspond to timepoints depicted in A1 and B1; ↑, HFS (time = 0). C1: sample traces of dendritic responses evoked by single-pulse stimulation of Schaffer collateral afferents ( ) recorded in the CA1 of slices taken from 1 day withdrawn (WD) and age-matched control animals. Traces represent dendritic fEPSP responses taken from I/O protocols shortly before ( - - - ) and 60 min after HFS ( - - - ). C2: mean ± SE baseline dendritic fEPSP slope measured across a range of stimulus intensities in slices from 1 day WD and age-matched control animals. Stimulus intensities in C2 and C3 were normalized to the stimulus intensity that produced the threshold dendritic fEPSP response in baseline I/O protocols (see METHODS). *, significant from age-matched control (P < 0.05, ANOVA/Fisher’s PLSD).

FIG. 5. PPF is altered under baseline conditions and after HFS following 1 day of withdrawal from CIE exposure. A: sample traces of dendritic responses evoked by paired-pulse stimulation (40-ms interpulse interval) of Schaffer collateral afferents ( ), recorded in the CA1 of slices taken from 1 day WD and age-matched control animals. Traces represent dendritic fEPSP responses evoked using a stimulus intensity that produced a response equal to 1/2 the maximal baseline dendritic fEPSP amplitude and were taken from pre-HFS baseline recordings (a), immediately following HFS (b), and 60 min following HFS (c). The traces are scaled such that the amplitudes of the initial pulse responses at each time point (a–c) are equivalent. B: mean ± SE paired-pulse ratio in slices taken from 1-day WD and age-matched control animals. C: mean ± SE paired-pulse ratio, expressed as a percentage of the mean baseline value ( - - - ), in slices taken from 1-day WD and age-matched control animals. Letters (a–c) in B and C correspond to time points depicted in A; ↑, HFS (time = 0). *, significant from age-matched control (P < 0.05, ANOVA/Fisher’s PLSD). dendritic fEPSP was significantly larger in slices from 1-day withdrawn animals compared with slices from control animals and baseline PPF was significantly smaller. The reduced PPF and larger baseline fEPSP suggests that withdrawal from chronic ethanol results in increased transmitter release and are consistent with biochemical studies showing that ethanol withdrawal is associated with increased glutamate release in the hippocampus (Dahchour and De Witte 1999). Changes in postsynaptic mechanisms could also be involved in the enhancement of the baseline dendritic fEPSP. For example, the enhanced synaptic response may represent a potentiated response similar to that occurring during LTP that results from intense synaptic activity during the withdrawal phase in vivo. The withdrawal-induced enhancement of synaptic responses may reflect a relatively mild component of the withdrawal syndrome that could develop into hyperexcitability with more prolonged ethanol exposure or higher ethanol doses.

The effects of CIE treatment on dendritic LTP, PTP, and PPF were reversible when the withdrawal period was extended from 1 to 5 days. Thus PTP, PPF, and dendritic LTP in slices from 5-day withdrawn animals were comparable with that observed in slices from age-matched control animals. In contrast, LTP of the somatic population spike remained significantly smaller in slices from 5-day withdrawn animals compared with slices from age-matched control animals, suggesting only a partial recovery of function was achieved in this cellular region. This difference is likely to result from differences in the cellular mechanisms that contribute to the expression of LTP in the dendritic versus somatic regions. Several intrinsic and extrinsic factors affect the amplitude of the somatic population spike and could be altered by the CIE treatment, including the number and synchrony of neurons firing within the recorded population, the amplitude of the dendritic synaptic potential, the electrical properties of the neurons, and the efficacy of GABAAergic synaptic transmission at the somata (Karssson and Olpe 1989; Rock and Taylor 1986). Of these, GABAAergic synaptic transmission is known to play a critical role in the induction of LTP (Gustafsson and Wigström 1990; Gustafsson et al. 1987; Mott and Lewis 1991; Wigström and Gustafsson 1985), and several studies have shown that chronic ethanol alters GABAAergic synaptic transmission (Frye et al. 1991; Hu et al. 1999; Kang et al. 1996; Peris et al. 1997a,b). Long-term ethanol exposure has been reported to produce persistent changes in GABAAergic transmission in the hippocampus (Kang et al. 1996); this could explain the lack of full recovery of LTP of the population spike observed in our study. We showed previously that paired-pulse inhibition of the population spike, a measure of GABAAergic synaptic transmission at the somatic site of innervation, is not altered by...
short-term CIE treatment (Nelson et al. 1999). However, expression of the neuroadaptive effects of CIE treatment on GABAergic synaptic transmission may require more demanding conditions such as occurs during HFS.

Although LTP recovered in the dendritic region, it is unclear if the recovery reflects a reversal of the effects of CIE treatment or further neuroadaptive changes. HFS-induced LTP in the CA1 region of the hippocampus is known to be NMDA receptor-dependent (Bliss and Lomo 1973). The HFS activates a large number of axonal inputs producing sufficient postsynaptic depolarization to activate NMDA conductances and initiate intracellular mechanisms responsible for LTP induction (Bliss
and Collingridge 1993). Our previous studies showed that NMDA receptor-mediated fEPSPs of CA1 pyramidal neurons were not altered shortly after removal from CIE treatment, but were significantly enhanced following 5–7 days of ethanol withdrawal (Nelson et al. 1999). The expression of NMDA receptor subunits (NMDAR 2A and NMDAR 2B) was also enhanced following 5–7 days of withdrawal from CIE treatment (unpublished data), suggesting that upregulation of postsynaptic NMDA receptors could contribute to the recovery of LTP induction following 5 days of alcohol withdrawal observed in the current study.

To date, studies investigating the effect of chronic ethanol on LTP have used animals subjected to long-term (several months) ethanol exposure and a relatively continuous exposure paradigm, a treatment that induces alcohol dependence and a withdrawal syndrome when ethanol exposure is terminated (Durand and Carlen 1984; Tremwel and Hunter 1994; Walker et al. 1980). This paradigm of ethanol exposure prevents LTP induction in the CA1 region of rat hippocampal slices, an effect that is persistent and can last as long as several months after ethanol withdrawal (Durand and Carlen 1984; Tremwel and Hunter 1994; Walker et al. 1980). The mechanisms underlying the effects of prolonged, chronic ethanol exposure on hippocampal function are still under investigation, but alterations in postsynaptic aspects of synaptic transmission including Ca$^{2+}$ channels, NMDA receptors, and GABA receptors are known to play a prominent role (Fadda and Rossetti 1998; Frye et al. 1991; Grant et al. 1990; Hu et al. 1999; Kang et al. 1996; Lovinger 1997; Peris et al. 1997a,b; Whittington et al. 1995). These mechanisms may also contribute to the effects of CIE treatment on synaptic transmission and plasticity observed in this study. Moreover, LTP is a complex phenomenon involving multiple intracellular mediators including intracellular Ca$^{2+}$, various protein kinases, and immediate-early genes (Soderling and Derkach 2000). Biochemical experiments are underway to determine if CIE treatment-induced effects are mediated by differential intracellular mechanisms.

The chronic ethanol vapor treatment paradigm has been shown to produce a behavioral withdrawal syndrome in rats (Macey et al. 1996) or in mice (Ripley et al. 1996); this correlates with hyperexcitability in slices prepared from chronic ethanol-treated animals (Schulteis et al. 1995). In addition, studies using long-term ethanol drinking paradigms have shown hyperexcitability in hippocampal slices prepared from mice (Whittington and Little 1991; Whittington et al. 1995). However, in our studies, typical behavioral signs of ethanol withdrawal were not evident in the CIE-treated or withdrawn animals and signs of neuronal hyperexcitability were not observed in recordings from hippocampal slices during the acute withdrawal phase. This difference may relate to

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**FIG. 6.** LTP expression is inhibited only in the somatic region after 5 days of withdrawal from CIE exposure. *A* and *B*: sample traces of dendritic responses evoked by single-pulse stimulation of the Schaffer collateral input (↓) recorded in the CA1 of slices taken from 5-day WD and age-matched control animals. *A2* and *B2*: mean ± SE of dendritic fEPSP slope (*A2*), expressed as a percentage of the mean baseline value (─), and somatic population spike amplitude (*B2*), expressed in mV, in slices taken from 5-day WD and age-matched control animals. Letters (*a–c*) correspond to time points depicted in *A* and *B*: ↑, HFS (time = 0). *C*: sample traces of dendritic responses evoked by single-pulse stimulation of Schaffer collateral afferents (↓) recorded in the CA1 of slices taken from 5-day WD and age-matched control animals. *A* and *B*: mean ± SE of dendritic fEPSP slope (*A2*), expressed as a percentage of the mean baseline value (─), and somatic population spike amplitude (*B2*), expressed in mV, in slices taken from 5-day WD and age-matched control animals. *C*: mean ± SE paired-pulse ratio in slices taken from 5-day WD and age-matched control animals. Letters (*a–c*) correspond to time points depicted in *A* and *B*: ↑, HFS (time = 0).

**FIG. 7.** Five days of withdrawal from CIE exposure restores PPF to control levels. *A*: sample traces of dendritic responses evoked by paired-pulse stimulation of Schaffer collateral afferents (↓) recorded in the CA1 of slices taken from 5-day WD and age-matched control animals. Traces represent dendritic fEPSP responses evoked using a stimulus intensity that produced a response equal to 1/2 the maximal baseline dendritic fEPSP amplitude and were taken from pre-HFS baseline recordings (*A*), immediately following HFS (*B*), and 60 min following HFS (*C*). The traces are scaled such that the amplitudes of the initial pulse responses at each time point (*a–c*) are equivalent. *B*: mean ± SE paired-pulse ratio in slices taken from 5-day WD and age-matched control animals. *C*: mean ± SE paired-pulse ratio, expressed as a percentage of the mean baseline value (─), in slices taken from 5-day WD and age-matched control animals. Letters (*a–c*) in *B* and *C* correspond to time points depicted in *A*: ↑, HFS (time = 0).
the BAL levels achieved, the age of the animals at the beginning of the treatment period, the length of the treatment period, or the continuous versus intermittent ethanol exposure paradigm. In studies where chronic ethanol-vapor treatment produced behavioral signs of withdrawal, BALs were ~200 mg/dl (Macey et al. 1996; Schulteis et al. 1995), compared with BALs of ~180 mg/dl in our studies. In addition, in the previous behavioral studies the animals were considerably older at the beginning of the 2-wk chronic ethanol treatment period (by weight, 280–425 g compared with 150 g in the current study), which may increase the vulnerability to ethanol withdrawal effects. Perhaps the most critical difference may relate to the treatment paradigm used. In the previous studies, the animals were continuously exposed to ethanol vapor, whereas in the present study, an intermittent treatment schedule was used to more closely reproduce a pattern of ethanol intake typical of human consumption. Our results show that this pattern of ethanol intake can have pronounced effects on hippocampal synaptic function and hippocampal LTP, a putative cellular substrate of learning and memory, and are consistent with the possibility that short-term chronic alcohol abuse is sufficient to disrupt hippocampal learning and memory formation. However, in contrast to previous studies using long-term, continuous treatment with low doses of alcohol, our results suggest that the effects of short-term, binge-like alcohol intake may be, to some extent, reversible when the period of exposure is relatively brief.

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