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# Effect of chronic ethanol treatment *in vivo* on excitability in mouse cortical neurones *in vitro*

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**1** The effects of cessation of chronic ethanol ingestion on seizure activity *in vivo* and on the characteristics of the evoked synaptic potentials in cortical neurones *in vitro* have been investigated in mice. Withdrawal from chronic ethanol treatment increased handling seizure ratings in mice between 4 and 16 h post-withdrawal. This ethanol-induced increase in seizure rating was unaffected by carbamazepine (30 mg $kg^{-1}$) but significantly reduced at a higher concentration (130 mg $kg^{-1}$).

**2** Intracellular recordings were made from cortical layer II neurones *in vitro* from control mice and from mice following chronic ethanol ingestion. Evoked synaptic potentials were generated in these neurones through intralaminar stimulation.

**3** Neurones from control mice displayed an evoked potential consisting of a fast excitatory postsynaptic potential (e.p.s.p.) mediated by AMPA-type glutamate receptors and an inhibitory postsynaptic potential (i.p.s.p.) mediated via $GABA_A$ receptors. Application of pentylenetetrazole (PTZ) or bicuculline onto these neurones inhibited the i.p.s.p., caused a large increase in both the amplitude and duration of the e.p.s.p. and initiated spontaneous excitatory activity. The resulting large evoked e.p.s.p. was mediated via both NMDA- and AMPA-type glutamate receptors.

**4** Most neurones (77%) from ethanol treated mice displayed an evoked potential which comprised a large e.p.s.p. and no i.p.s.p. The e.p.s.p. consisted of several distinct components and in addition these neurones displayed spontaneous paroxysmal depolarizing shifts. This multi-component e.p.s.p. was mediated through both NMDA- and AMPA-type glutamate receptors. A population (23%) of neurones from ethanol treated mice exhibited evoked potentials which possessed both inhibitory and excitatory components and these neurones were effectively identical to those obtained from control mice.

**5** Carbamazepine reduced the duration of the e.p.s.p. in neurones from ethanol treated mice and in PTZ-treated control neurones.

**6** Prolonged ethanol ingestion is known to create a neurochemical imbalance in cortical neurones resulting in abnormal neurotransmission. The present study highlights the functional consequences that arise as a result of these neurochemical changes leading to over-excitation of neurones and pronounced epileptiform activity.

**Keywords:** $GABA_A$ receptors; NMDA receptors; synaptic transmission; epileptiform activity

## Introduction

The cessation of chronic ethanol intake or 'ethanol withdrawal' is an experimental procedure recognized to produce seizures in mice (Goldstein & Pal, 1971). This convulsant activity is associated with an increase in excitatory neurotransmission in a number of brain areas, specifically the hippocampus. Long-term treatment with ethanol causes an increase in hippocampal N-methyl-D-aspartate (NMDA) receptor mRNA (Snell *et al*., 1996) and an increase in MK801 binding (Gulya *et al*., 1991). These neurochemical changes constitute an adaptive response to the acute effect of ethanol which is to inhibit NMDA-mediated neurotransmission (Lovinger *et al*., 1990; Wright *et al*., 1996). The concomittant increase in NMDA receptor level correlates well with the functional hyperexcitability found in the hippocampus following ethanol withdrawal (Morrisett, 1994; Shindou *et al*., 1994).

Acute ethanol treatment also inhibits NMDA-mediated events in the neocortex (Gonzales & Woodward, 1990; Takadera *et al*., 1990; Wright *et al*., 1996) and chronic treatment leads to an increase in NMDA receptor mRNA and MK801 binding (Follesa & Ticku, 1995; Hu & Ticku, 1995). However, in the cortex the effects of ethanol are not restricted to the NMDA receptor as they may be in the hippocampus (Criswell *et al*., 1993). Thus acute application of ethanol onto cortical neurones enhances synaptically evoked $\gamma$-aminobutyric $acid_A$ ($GABA_A$)-mediated potentials (Aguayo, 1990; Proctor *et al*., 1992) whilst chronic treatment leads to a reduction in $GABA_A$ mRNA (Mhatre & Ticku, 1992).

Despite the well characterized neurochemical changes that occur in cortical neurones following chronic ethanol treatment, the effects on neuronal function in this brain area have not been studied electrophysiologically. In the present study the changes in neuronal excitability in mouse cortex following chronic ethanol treatment were investigated by use of intracellular recording techniques. The observations in ethanol-treated animals were compared with the effects of the chemoconvulsant, pentylenetetrazole and the actions of the anti convulsant, carbamazepine were also examined.

## Methods

### *Ethanol administration*

Male TO mice (Bantin & Kingman, Hull, U.K., 18–22 g) were housed in pairs under 12 h light/dark cycle (lights on at 07 h 00 min). Mice received a 20% w/v liquid diet (Leiber & Decarli Dyet #710027, Pennsylvania, U.S.A.) for 3 days and those animals allocated to the ethanol treatment groups were then given an 8% v/v absolute ethanol addition to the liquid diet on the start of the fourth day. The mice were maintained on this diet for a further 7 day period. Control mice were also prepared which were maintained on liquid diet only without the addition of ethanol for a period of 10 days. On each experimental day (day 11) one animal was killed and brain slices

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prepared whilst the second animal was deprived of ethanol and subsequently rated at 2 hourly intervals for handling seizures according to the methods of Ritzmann and Tabakoff (1976) and Goldstein and Pal (1971). Briefly, mice were picked up by the tail and rated as follows; 0 – correspond to no reaction, 1 – correspond to a slight tremor, 2 – correspond to a clonic seizure after mouse being turned 360°C, 3 – correspond to a clonic seizure after mouse being turned 180°C and 4 – correspond to an instantaneous clonic seizure upon being handled. To examine the susceptibility of the ethanol withdrawal-induced seizures to carbamazepine, mice received a subcutaneous injection of either vehicle (1% carboxymethylcellulose/0.1% Tween 80) or carbamazepine (30 – 130 mg $kg^{-1}$). Mice were then evaluated for seizure activity at six hours following ethanol withdrawal. Data from handling seizures were subjected to an individual Mann Whitney U-test comparing each separate time point or dose with vehicle treated animals.

### *Preparation of brain slices and electrophysiology*

Mice were permitted access to alcohol until the point of death. Mice were killed by cervical dislocation and the brains dissected to obtain a block of tissue containing midbrain. Coronal brain slices (300 $\mu$m) were cut in artificial cerebrospinal fluid (aCSF; see Drugs and solutions) pre-gassed with 95% $O_2$/5% $CO_2$ at 2 – 4°C with a rotorslicer (DSK). Slices were then incubated for 2 h in aCSF at room temperature before the commencement of recording to allow withdrawal from the ethanol *in vitro*. Recordings were then made between 2 and 8 h following slice preparation. The timing of these recordings was selected to coincide with the period during which the mice demonstrated handling seizure activity *in vivo*. For recording, single slices were pinned to the Sylgard coated base of a Perspex recording chamber and perfused with aCSF (5 ml $min^{-1}$) at 37°C via a gravity-fed perfusion system. The recording temperature was maintained by passing the aCSF through a water-jacketed reservoir connected to a thermocirculator (Harvard).

Intracellular recordings were made from cortical layer II neurones by an Axoclamp 2A amplifier and data were filtered at 1 kHz. Intracellular electrodes were fabricated from fibre-filled borosilicate glass (Clark Electromedical; 1.2 mM o.d.; 0.69 mM i.d.) with a Flaming Brown P-87 electrode puller and had resistances of between 80 MΩ and 100 MΩ when filled with K-acetate (1.5 M). Acquisition of data was performed online via an IBM clone PC equipped with a Digidata 1200B interface (Axon Instruments). For recording and analysing data, the pClamp programme was used (Axon Instruments: version 6). Data were also recorded on video tape, evoked potentials were monitored continuously on a Tektronix oscilloscope and pen recorder printouts of the data were made on a chart recorder (Gould). Neurone input resistance was monitored by the injection of hyperpolarizing current (200 pA, 300 ms) through the recording electrode. Synaptic potentials were generated by electrical stimulation (5 – 15 V, 0.01 Hz, bipolar) of the intralaminar layer II pathway lateral to the site of recording. Data are expressed as the mean ± s.e.mean.

### *Statistics*

Statistical comparisons of the data from the different populations of animals (control and ethanol-treated) were performed by use of two-way analysis of variance. Differences between pre and post drug application were then tested for by use of a paired Student's $t$ test. $P$ values less than 0.05 were taken to indicate significance.

### *Drugs and solutions*

Artificial cerebrospinal fluid (aCSF) consisted of (in mM): NaCl 126, KCl 5, $MgCl_2$ 1.2, $NaH_2PO_4$ 1.2, $CaCl_2$ 2.4, $NaHCO_3$ 26 and glucose 10 and was bubbled with 95% $O_2$/5% $CO_2$.

The following drugs were used; pentylenetetrazole (PTZ, Sigma), 6-nitro-7-sulphamoylbenzo(f)-quinoxaline-2,3-dione (NBQX; Tocris Cookson), D-(−)-2-amino-5-phosphonovaleric acid (D-APV; Tocris Cookson), carbamazepine (Parke-Davis Pharmaceuticals, Ann Arbor, MI.), tetrodotoxin (TTX, Sigma), bicuculline (Sigma), phaclofen (Tocris Cookson) and ethanol (Fisons). All drugs were reconstituted as stock solutions in water, with the exception of NBQX which was dissolved in DMSO (10 mM) and diluted to the required concentration in aCSF before application to the tissue through the perfusion system.

## Results

### *Ethanol withdrawal-induced seizure*

Significant increases in seizure ratings were observed in ethanol-treated animals between 4 and 16 hours following with-

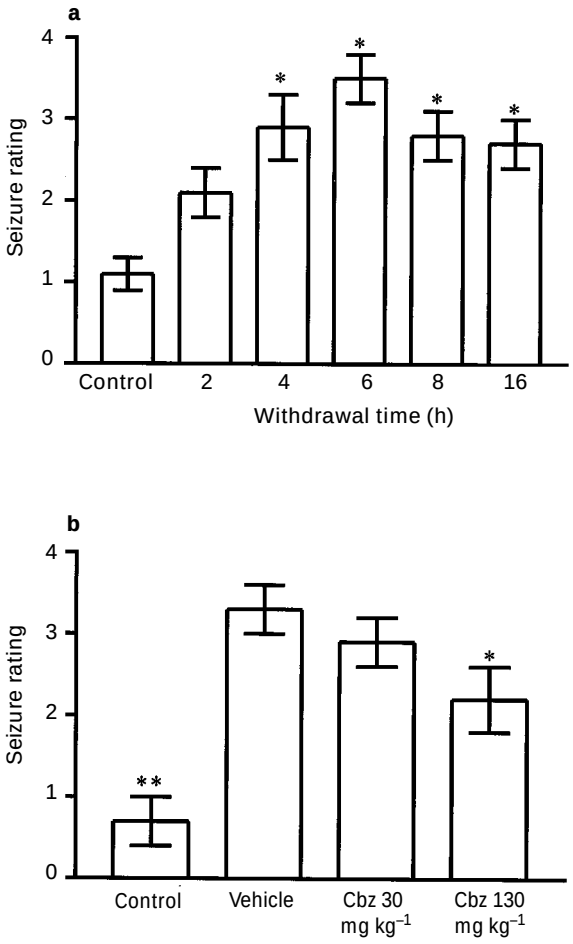


**Figure 1** The effect of cessation of chronic ethanol intake on handling seizures in mice. (a) Following 7 days ethanol ingestion, animals were withdrawn and evaluated for handling seizures at 2 h intervals. Mice in control groups received no ethanol treatment. Significant increases in seizure rating were found 4 h following ethanol withdrawal and were maximal 6 h post withdrawal ($P<0.05$). (b) The effect of carbamazepine (Cbz) on seizure rating was also examined. Ethanol-treated mice received carbamazepine (30 and 130 mg $kg^{-1}$, s.c.) or vehicle at the time of ethanol withdrawal and were compared with control mice at 6 h post withdrawal. Ethanol treated mice administered with vehicle exhibited a significantly increased seizure rating when compared to control mice ($P<0.01$). Carbamazepine (130 mg $kg^{-1}$) significantly reduced the seizure rating compared to vehicle treated mice ($P<0.05$). However, at a lower dose (30 mg $kg^{-1}$) carbamazepine was without effect. Each column represents median seizure rating and error bars represent the 1st and 3rd quartiles ($n=8-10$).

drawal of ethanol. This effect was maximal at 6 h post-withdrawal and then declined over the following 10 h (Figure 1). The sensitivity of the seizure activity to carbamazepine was also evaluated. Carbamazepine (30 and 130 mg $kg^{-1}$, s.c.) was administered upon cessation of ethanol intake and seizure activity was examined 6 h later. Carbamazepine (30 mg $kg^{-1}$) had no effect on the handling seizure scores when compared to vehicle. However, at higher doses (130 mg $kg^{-1}$) carbamazepine significantly reduced the seizure rating (Figure 1).

### *Electrophysiology*

Data are presented from 56 recordings (from 47 mice), 26 from neurones from chronically ethanol treated mice (from 23 mice) and 30 from control neurones (from 24 mice). No significant differences were observed in the resting membrane potential (control $66.1 \pm 1.4$ mV, ethanol treated $67.6 \pm 1.4$ mV, not significant) or in the input resistance (control $77.7 \pm 5.2$ MΩ, ethanol treated $78.9 \pm 3.8$ MΩ, not significant) of the neurones recorded from the two groups of animals. In both ethanol-treated and control mice, stimulation of the intralaminar cortical layer II pathway produced an evoked potential which was completely abolished by bath application of tetrodotoxin (1 μM, $n=4$; not shown).

### *Evoked potentials in cortical neurones from control mice*

Neurones from control mice did not display any spontaneous electrical activity at resting membrane potential (Figure 2A). The evoked potentials obtained from these neurones could be divided into two distinct groups: 70% (21/30, Type A) of these control neurones displayed an evoked potential which comprised of an initial fast excitatory post-synaptic potential (e.p.s.p.) followed by an inhibitory post-synaptic potential (i.p.s.p. Figure 2A). The e.p.s.p. was relatively small (amplitude $21.4 \pm 2.9$ mV, duration $138.6 \pm 14.2$ ms) and was rarely of sufficient amplitude to elicit a full action potential whilst the i.p.s.p. was longer in duration but was similarly small in amplitude (amplitude $-2.4 \pm 0.3$ mV, duration $263.8 \pm 19.1$ ms). In the remaining neurones, (9/30, 30%, Type B) a larger e.p.s.p. was evoked (amplitude $36.8 \pm 4.6$ mV, duration $828.3 \pm 159.3$ ms) which was not accompanied by an inhibitory component. The e.p.s.p. in these neurones comprised of a single component and was normally of sufficient amplitude to produce several full action potentials.

Application of pentylenetetrazole (PTZ, 10 mM, $n=21$) had not effect upon the resting membrane potential of type A neurones (control $-66.4 \pm 1.6$ mV, +PTZ $-67.2 \pm 2.1$ mV, not significant) but increased both the amplitude (control $21.7 \pm 2.9$ mV, +PTZ $50.4 \pm 1.8$ mV; $P<0.05$) and duration (control $135.8 \pm 9.1$ ms, +PTZ $1281.5 \pm 122.5$ ms; $P<0.05$) of the excitatory component of the evoked potential (Figures 3 and 4) and inhibited the i.p.s.p. (control amplitude $-2.3 \pm 0.3$ mV, control duration $272.2 \pm 22.8$ ms; Figure 3). Washout of the PTZ from the recording chamber resulted in a gradual reduction in the excitatory component to near control values (amplitude $17.1 \pm 1.5$ mV, duration $324.3 \pm 86.6$ ms) combined with a return of the inhibitory component (ampli-

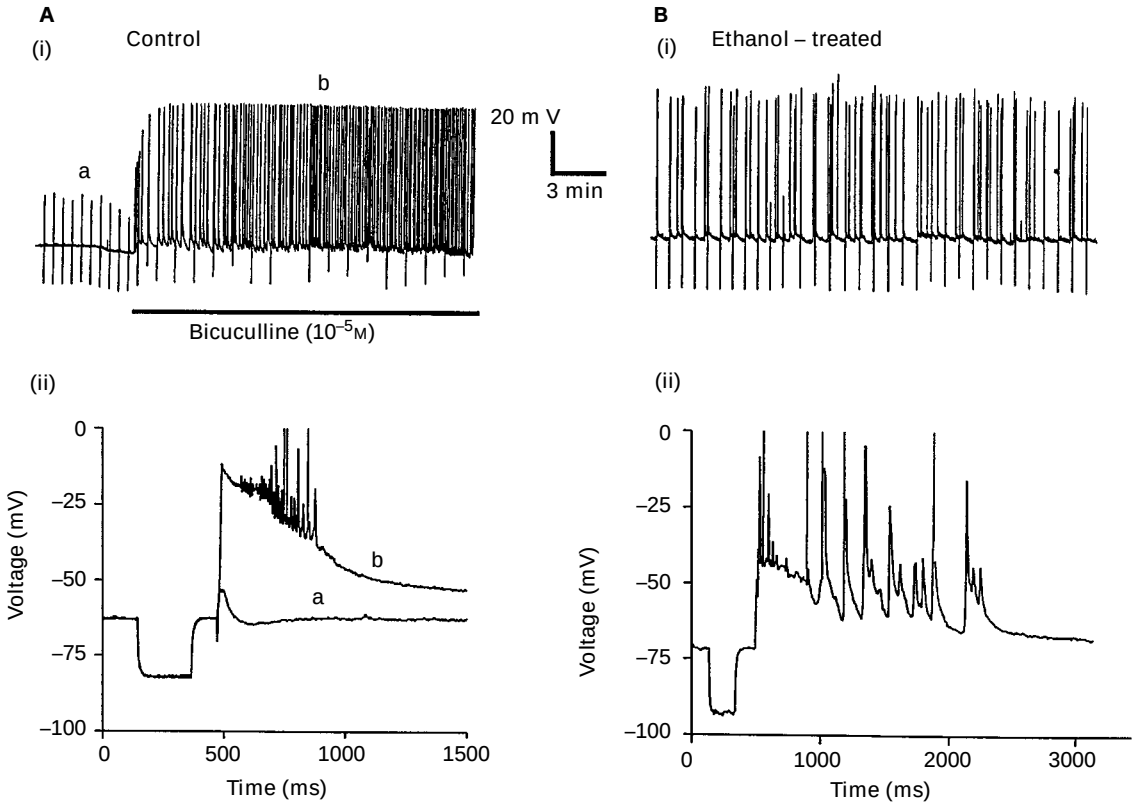


**Figure 2** Intracellular recordings from layer II cortical neurones from ethanol-treated and control mice. Evoked potentials were generated through stimulation of the intralaminar pathway. The upper panels (i) in each case represent pen recorder traces from typical recordings and lower panels (ii) depict single representative evoked potentials taken from the recordings in (i). In each case, downward deflections denote the injection of a hyperpolarizing current and upward deflections represent depolarizing synaptic activity. (A) Neurones from control mice did not display any spontaneous synaptic activity (A(i)a) and evoked potentials comprised of a fast e.p.s.p. followed by a long i.p.s.p. (A(ii)a; type A). Application of bicuculline (20 μM) to these control neurones increased the amplitude of e.p.s.p. and initiated spontaneous paroxysmal depolarising shifts (PDS, A(i)b). Bicuculline abolished the inhibitory component of the evoked response and increased both the amplitude and duration of the e.p.s.p. (A(ii)b). (B) Intracellular recordings were also made from neurones from mice following chronic ethanol ingestion. These neurones were not quiescent but displayed spontaneous PDS-like activity in addition to the evoked potentials (B(i); type C). The evoked responses in these neurones comprised of a long e.p.s.p. consisting of several regenerative components. Neurones from ethanol-treated mice did not display any inhibitory component to the evoked potential (B(ii)).

tude $-3.7 \pm 0.9$ mV, duration $253.7 \pm 32.3$ ms). Application of PTZ (10 mM) to the type B neurones increased both the amplitude (control $36.8 \pm 4.6$ mV, +PTZ $54.6 \pm 2.5$ mV; $n=9$, $P<0.05$) and the duration (control $828.3 \pm 159.3$ ms, +PTZ $1307.6 \pm 163.9$ ms, $P<0.05$) of the evoked e.p.s.p. and this was also reversible upon washing.

PTZ exerts its proconvulsant action through an inhibitory effect at the benzodiazepine-$GABA_A$ receptor ionophore (De Deyn & McDonald, 1989). To confirm the nature of the GABA receptors underlying the i.p.s.p. in type A neurones, the effects of the selective antagonists bicuculline ($GABA_A$) and phaclofen ($GABA_B$) were examined. Application of phaclofen (200 $\mu$M, 15 min; $n=3$) had no effect on the amplitude or duration of the evoked events (not shown). However, bicuculline (20 $\mu$M; Bic) increased both the amplitude (control $28.0 \pm 11.1$ mV, Bic $57.2 \pm 3.2$ mV; $n=3$, $P<0.05$) and duration (control $264.3 \pm 129$ ms, Bic $832.3 \pm 155.6$ ms; $P<0.05$) of the excitatory component and abolished the inhibitory component of the evoked potential (Figure 2A). Application of either bicuculline or PTZ also produced spontaneous excitatory potentials. These paroxysmal depolarizing shifts (PDS) were similar in amplitude and duration to the evoked events associated with cortical pathway activation (Figures 2A and 5).

In type A neurones, which displayed both excitatory and inhibitory components to the evoked potential, D-APV (20 $\mu$M, 15 min) had no effect either on the amplitude (control $20.33 \pm 1.9$ mV, +D-APV $22.2 \pm 2.5$ mV; $n=4$, not significant) or duration (control $144 \pm 22.7$ ms, +D-APV $108 \pm 18.5$ ms, not significant) of the e.p.s.p. However, when both D-APV (20 $\mu$M) and NBQX (10 $\mu$M, 5 min; $n=4$) were co-applied to the neurone the evoked response was completely inhibited (Figures 3 and 4). The effects of NBQX were not readily reversible. These data demonstrate that the fast e.p.s.p. observed in neurones from control mice is mediated entirely by $\alpha$-amino-3-hydroxy-5-methyl-4-isoxazolapropionate (AMPA)-type glutamate receptors.

Type A neurones from control mice were also treated with PTZ (10 mM) and the sensitivity of the evoked potentials to glutamate antagonists was examined. In the presence of PTZ, neither D-APV (20 $\mu$M) nor NBQX (10 $\mu$M), significantly affected the amplitude (control $50.2 \pm 4.9$ mV, +D-APV $41.2 \pm 13.8$ mV, +NBQX $40.3 \pm 14.0$ mV; $n=4$, not significant) but both agents reduced the duration (control $1314.5 \pm 123.1$ ms, +D-APV $475.8 \pm 176$ ms, +NBQX, $452.8 \pm 165.8$ ms; $n=4$, $P<0.05$ in each case) of the e.p.s.p. (Figures 3 and 4). When NBQX (10 $\mu$M) and D-APV (20 $\mu$M) were co-applied to the neurones it was not possible to generate an evoked potential (Figures 3 and 4). These results show that the NMDA receptors are present in the neurones from control mice but are not activated during the evoked potential under control conditions.

### *Evoked potentials in cortical neurones from mice following chronic ethanol ingestion*

The evoked potentials in neurones from ethanol-treated mice were also found to consist of two distinct types. The majority of neurones recorded from these mice (20/26, 77%, Type C) displayed an evoked potential which consisted of a large e.p.s.p. comprising of up to 8–10 distinct regenerative excitatory components (amplitude $31.8 \pm 2.5$ mV, total duration $1013.6 \pm 133.4$ ms) and was not followed by an i.p.s.p. (Figure 2B). In addition, 50% of type C neurones (10/20) also displayed spontaneous excitatory activity at the resting membrane potential (Figure 2). This spontaneous activity ranged from an occasional action potential to pronounced spontaneous paroxysmal depolarizing shifts which were up to 50–60 mV in amplitude and several hundred milliseconds in duration. The remaining neurones in this group (6/26; 23%,

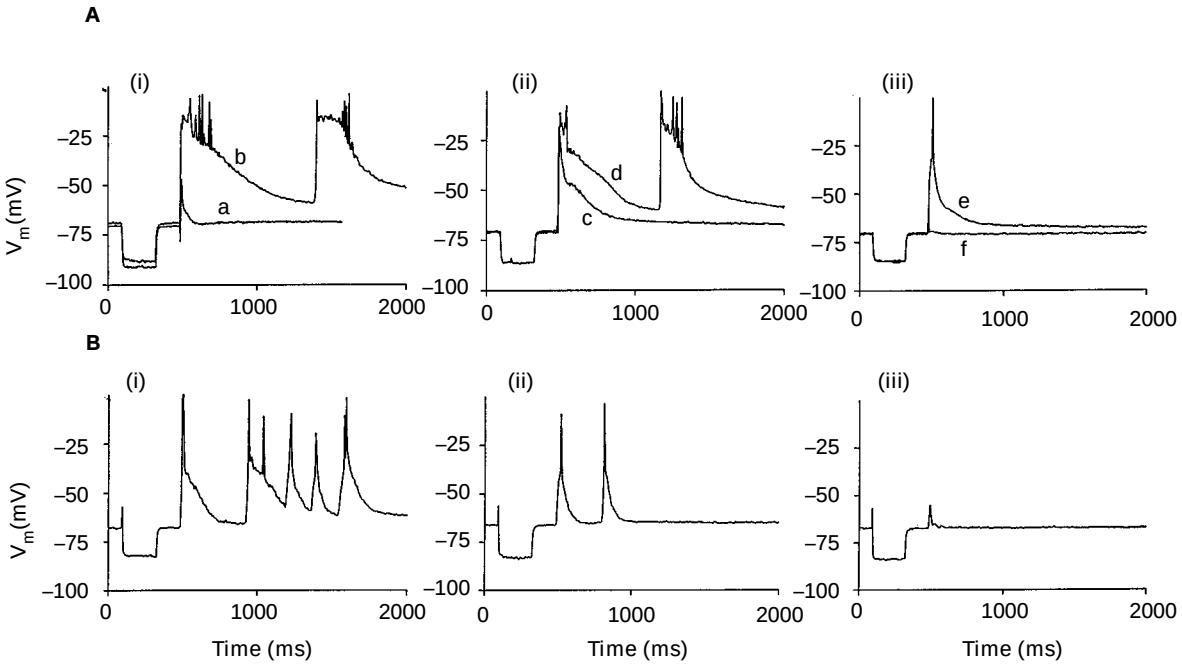


**Figure 3** The effect of glutamate and GABA receptor antagonists on evoked synaptic potentials in cortical neurones from control mice (A) and ethanol-treated mice (B). (A(i)) Control evoked potentials consisted of a fast e.p.s.p. followed by a long i.p.s.p. (a). Pentylenetetrazole (PTZ, 10 mM) abolished the inhibitory component of the evoked potential and increased both the amplitude and duration of the excitatory potential (b). (A(ii)) Application of D-APV (20 $\mu$M) in the continued presence of PTZ, reduced the duration of the e.p.s.p. (c) and this effect was reversible upon removal of D-APV from the perfusate (d). (A(iii)) With PTZ still present, application of NBQX (10 $\mu$M) also markedly reduced the duration of the e.p.s.p. (e) and when D-APV and NBQX were both applied to the neurone in the presence of PTZ, the evoked potential was completely inhibited (f). (B(i)) Evoked potentials from mice following chronic ethanol ingestion comprised a large excitatory component consisting of several regenerative components. These neurones did not display any inhibitory component to the evoked potential. (B(ii)) Application of D-APV (20 $\mu$M) reduced the duration of the evoked potential and the number of components contributing to this potential. (B(iii)). In the continued presence of D-APV, application of NBQX (10 $\mu$M) completely inhibited the evoked response.

Type D) displayed evoked potentials similar to those observed in control animals. These neurones possessed both an excitatory (amplitude 20.3±1.3 mV, duration 134.33±9.1 ms) and inhibitory (amplitude −1.4±0.2 mV, duration 214.3± 33.1 ms) component and did not display any spontaneous excitatory activity. These Type D neurones appeared to be unaffected by the chronic ethanol treatment and thus were not used in further experiments. Their characteristics were similar in all respects to the evoked potentials generated from control neurones (Type A) depicted in Figures 1 and 2.

Chronic treatment with ethanol is known to cause alterations in the levels of glutamate receptors within specific brain areas (Hu & Ticku, 1995). Experiments were therefore performed to determine the relative roles of the NMDA- and the AMPA-type glutamate receptors in these evoked potentials. Application of D-APV (20 $\mu$M; 15 min, $n=5$) to type C neurones had no effect on the amplitude (control 27.0±4.7 mV, +D-APV 29.3±2.6 mV) but significantly reduced the duration (control 1237.2±283.7 ms, +D-APV 256.6±101.8 ms, $P<0.05$) of the evoked potentials (Figures 3 and 4). When NBQX (10 $\mu$M) was added to the perfusate, in the continued presence of D-APV (20 $\mu$M) it was not possible to generate either an excitatory or an inhibitory evoked potential in these neurones (Figures 3 and 4). These data suggest that both NMDA- and AMPA-type glutamate receptors contribute to the hyperexcitability observed in neurones from chronically ethanol treated mice.

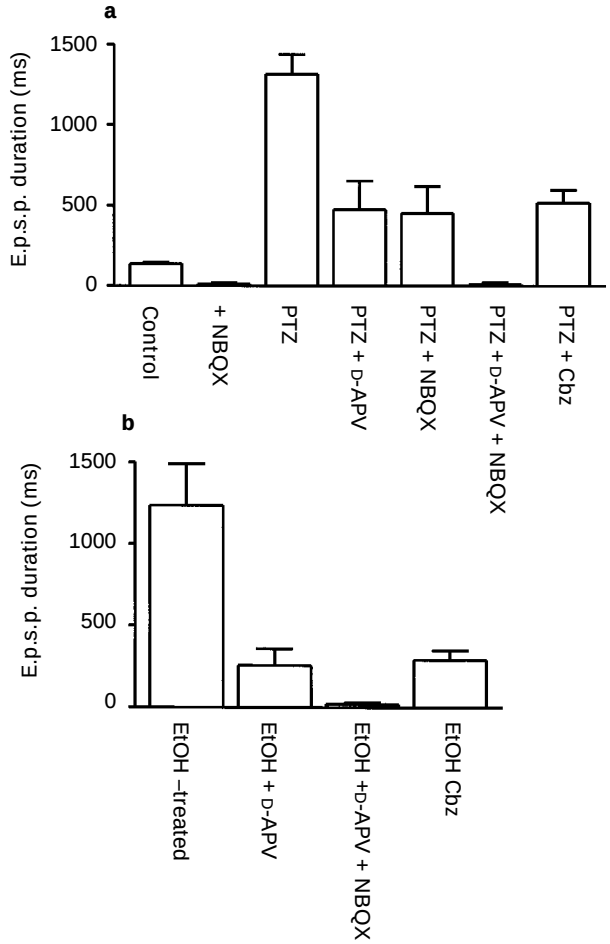


**Figure 4** The effect of glutamate antagonists on the e.p.s.p. duration in cortical neurones from control (a) and ethanol-treated (b) mice. In control mice (a), the e.p.s.p. was inhibited by NBQX (10 $\mu$M) and enhanced by PTZ (10 mM). The PTZ-enhanced e.p.s.p. was inhibited by both NBQX (10 $\mu$M) and D-APV (20 $\mu$M) and by carbamazepine (Cbz, 100 $\mu$M). In mice following chronic ethanol (EtOH) ingestion (b), the large e.p.s.p. was inhibited by both NBQX (10 $\mu$M) and D-APV (20 $\mu$M) and by carbamazepine (100 $\mu$M). All values are mean±s.e.mean and $n$ values were between 3 and 21.

### *The effect of carbamazepine on chronic ethanol and PTZ-induced hyperexcitability*

The hyperexcitability observed in cortical neurones following chronic ethanol intake and upon treatment with PTZ may provide an insight into the mechanism by which these procedures cause seizures *in vivo*. Seizures caused by the chronic ingestion of ethanol were found to be sensitive to carbamazepine and it was therefore of interest to determine whether the cellular hyperexcitability caused by chronic ethanol treatment was also sensitive to this anticonvulsant. In type C neurones from mice subject to chronic ethanol ingestion, application of carbamazepine (100 $\mu$M, 15 min) had no effect on the amplitude (control 42.3±3.1 mV, carbamazepine 39.33±3.4 mV; $n=4$, not significant) but significantly reduced the duration of e.p.s.p. (control 961.8±265 ms, +carbamazepine 290.3± 58.6 ms, $P<0.05$; Figures 4 and 5). Removal of the carbamazepine from the perfusate resulted in the duration of the e.p.s.p. returning to control values (834.6±169.9 mV).

Similar experiments were performed in control mice where the sensitivity of the PTZ-induced hyperexcitability to carbamazepine was examined. Application of carbamazepine (100 $\mu$M, 30 min) to neurones (Type A) treated with PTZ

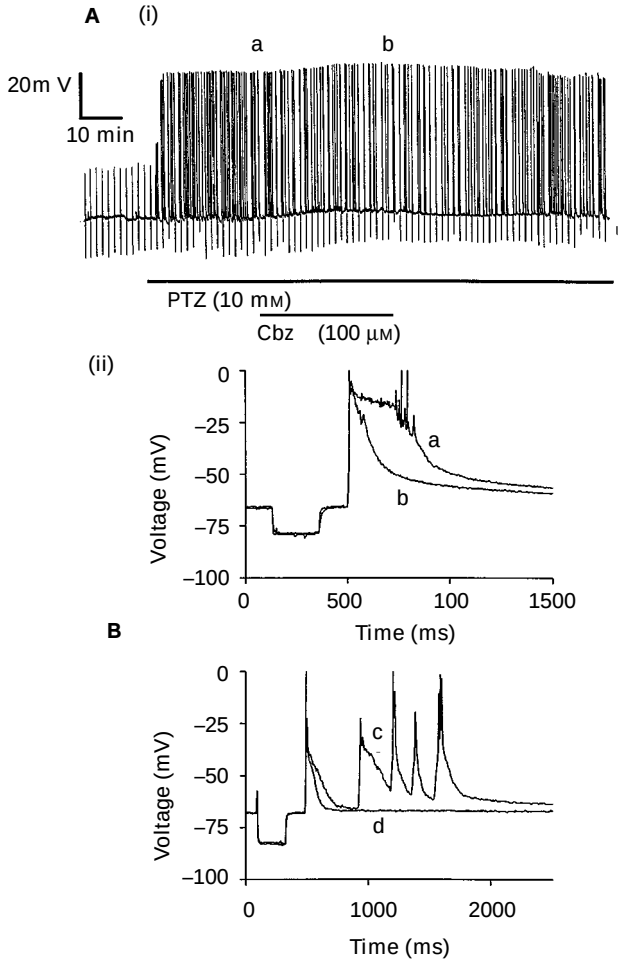


**Figure 5** The effect of carbamazepine on pentylenetetrazole (PTZ)- and ethanol-induced hyperexcitability in mouse cortical neurones. (A) In control neurones (type A), PTZ (10 mM) initiated spontaneous paroxysmal depolarizing synaptic activity (A(i)a) and increased both the amplitude and duration of the excitatory evoked potential (A(ii)a). Application of carbamazepine (Cbz, 100 $\mu$M) in the continued presence of PTZ reduced the spontaneous activity (A(i)b) of the neurone and also reduced both the amplitude and duration of the PTZ-enhanced e.p.s.p. (A(ii)b). (B) Following chronic ethanol ingestion, cortical neurones displayed large excitatory evoked potentials consisting of several discrete components (Bc; type C). Application of carbamazepine (100 $\mu$M) resulted in a decrease in the number of components and in the total duration of the evoked potential (Bd).

(10 mM) had no effect on the amplitude (PTZ 54.6±2.4 mV, PTZ+carbamazepine, 55.8±2.9 mV; $n=5$, not significant) of the e.p.s.p. but significantly reduced the duration (PTZ 1155.2±122.6 ms, PTZ+carbamazepine 516.4±78.3 ms, $P<0.05$) of this response (Figures 4 and 5).

## Discussion

Chronic intake of ethanol in the mouse is known to cause neurochemical changes in both excitatory and inhibitory pathways within the cerebral cortex (Mhatre & Ticku, 1992; Snell *et al*., 1996). The present study reveals the functional consequences of the neurochemical imbalance caused by the chronic ethanol intake. Neurones from ethanol-treated animals (type C) were characterized by large e.p.s.ps which consisted of several distinct components and also by a complete lack of any inhibitory component to their evoked potential. Both of these features are consistent with the observed increase in NMDA mRNA and the decrease in $GABA_A$ mRNA (Mhatre & Ticku, 1992; Hu & Ticku, 1995) following chronic ethanol treatment. The present study also demonstrated that ethanol withdrawal resulted in a profound increase in handling seizure rating. Carbamazepine reduced the cellular activity observed in neurones from ethanol-treated mice and was also effective at reducing the seizure activity observed in these animals. These observations support the notion that the excitability observed in cortical neurones *in vitro* reflects the seizure activity observed *in vivo*.

### *Evoked responses in neurones from control mice*

The relative contribution of the glutamate and GABA receptors plays a critical role in determining the amplitude and duration of the responses of cortical neurones to synaptic input. The majority of control neurones (70%, Type A) displayed a fast e.p.s.p. which was unaffected by the NMDA receptor antagonist, D-APV but which was inhibited by NBQX. This indicates that in neurones from control mice, the fast e.p.s.p. is mediated exclusively via AMPA-type glutamate receptors. The marked increase in the amplitude and duration of the e.p.s.p. upon application of bicuculline, coupled with the lack of effect of phaclofen indicates that the i.p.s.p. is mediated through $GABA_A$ receptors. In the remaining 30% of these control neurones (Type B), the evoked potentials did not display an i.p.s.p. However, application of PTZ still resulted in an increase in both the duration and amplitude of the e.p.s.p. This suggests that despite the lack of a pronounced i.p.s.p., the evoked potential in these neurones is still influenced by inhibitory GABAergic transmission.

Pentylenetrazole (PTZ) is an epileptogenic agent (Speckmann & Walden, 1993) which reversibly inhibits GABA responses in mouse spinal neurones with an $IC_{50}$ of 1.1. mM (De Deyn & Mcdonald, 1989). A specific interaction between PTZ and the picrotoxin site on the benzodiazepine-GABA receptor-ionophore complex has been found by Ramanjaneyulu & Ticku, (1984). In the present study, PTZ increased the amplitude and duration of the excitatory component of the evoked potential and completely inhibited the i.p.s.p. in neurones from control mice. The resulting e.p.s.p. was reduced by both D-APV and by NBQX indicating that it is mediated through both NMDA- and AMPA-type glutamate receptors. The observation that NMDA receptors are present on these control neurones but do not play any role in the evoked potential under normal conditions suggests that the $GABA_A$-mediated i.p.s.p. serves to attenuate the e.p.s.p. functionally.

### *Hyperexcitability in neurones from ethanol treated mice*

Application of PTZ to neurones from control mice reveals an e.p.s.p. which is partly mediated via NMDA-type glutamate receptors. It is therefore clear that NMDA receptors are functionally present in control mice, although they do not seem to be involved in mediating the evoked e.p.s.p. in this pathway. There is no apparent difference in the duration of the e.p.s.p. in neurones from ethanol-treated mice and in control neurones following PTZ treatment (PTZ-treated control neurones, 1281±122 ms; neurones from ethanol treated mice, 1013±133 ms). In addition, the proportion of the e.p.s.p. that is mediated via the NMDA receptor does not differ between the two groups of animals (74% in PTZ-treated control neurones; 79% in neurones from ethanol treated mice). Thus, these data suggest that the NMDA receptor population in cortical neurones has not been significantly altered following chronic ethanol treatment.

In neurones from control mice (Type A), the $GABA_A$-mediated i.p.s.p. functionally restricts the duration of the e.p.s.p. and inhibits the recruitment of the NMDA receptors to the e.p.s.p. In neurones from ethanol-treated mice (Type C), the contribution made by NMDA receptors to the evoked potential is not altered when compared to control neurones in which GABA transmission has been inhibited. This may therefore suggest that the NMDA receptor is largely unaffected by chronic ethanol treatment. The marked hyperexcitability and lack of i.p.s.p. in neurones from ethanol-treated mice may therefore be indicative of a reduction in GABA transmission following chronic ethanol intake.

The presence of an inhibitory component to the evoked potential in a small number of neurones from ethanol treated mice (Type D) indicates that GABA transmission is reduced but not completely abolished. The reasons for this are unclear. However, the profound effects of ethanol treatment, observed in the majority of neurones (Type C) illustrate that chronic ethanol does increase cellular excitability. Clearly, this excitability is mediated through both NMDA and non-NMDA glutamate receptors. However, excitatory potentials of a similar magnitude were also observed in control neurones upon blockade of GABAergic transmission. Thus it is conceivable that the increase in excitability observed in ethanol treated mice does not result from an increase in glutamatergic transmission but rather from a decrease in inhibitory input. The data from the present study do not preclude an increase in excitatory transmission as a contributing factor to the observed hyperexcitability. However, a reduction in GABAergic transmission clearly plays a major role in the abnormal cellular excitability displayed by cortical neurones following chronic ethanol treatment.

Previous work (Proctor *et al*., 1992; Criswell *et al*., 1993) has illustrated the differential effects demonstrated by ethanol between cortical and hippocampal neurones. The hyperexcitability observed in hippocampal neurones following chronic ethanol has been suggested to arise primarily through an increase in excitatory transmission (Whittington *et al*., 1995) and not through a decrease in inhibitory input (Whittington *et al*., 1992). Molleman & Little (1995) showed that the increase in hippocampal excitability observed upon withdrawal of ethanol results, at least in part, from an increase in non-NMDA glutamate receptors and not through a decrease in GABA transmission. In addition, Snell *et al*. (1996) found an increase in both NR1 and NR2A NMDA subunits in hippocampal neurones following ethanol treatment. Clearly, therefore, the actions of ethanol within the hippocampus involve modulation of both expression and function of glutamate receptors. However, the actions of ethanol within cortical neurones may differ from those observed within the hippocampus. Thus Criswell and coworkers (1993) and Proctor *et al*. (1992) observed that ethanol potently augments GABA responses in cortical neurones whilst having no effect on GABA responses in hippocampal neurones. Wright *et al*. (1996) demonstrated that ethanol inhibits NMDA-activated currents in cortical neurones and Hu & Ticku (1995) found an increase in MK-801 binding following chronic ethanol intake. In the cortex, therefore, the effects of chronic ethanol may not be restricted to the glutamate receptors as they appear to be in the hippocampus but may also extend to the modulation of $GABA_A$ receptors. The results from the present study suggest that, whilst NMDA and non-

NMDA receptors are important, a decrease in GABAergic transmission may also contribute to the observed hyperexcitability in cortical neurones and further illustrate the specific effects of ethanol within discrete brain areas.

### *The effects of carbamazepine on PTZ- and ethanol-induced hyperexcitability*

Carbamazepine is a well established anticonvulsant therapy (Fraser, 1996) and has been shown to be effective in the clinical management of alcoholism (Erstad & Cotugno, 1995). Furthermore, carbamazepine has been demonstrated to reduce significantly the ethanol withdrawal reaction in the rat (Ulrichsen *et al*., 1986). It exerts its anticonvulsant action by enhancing sodium channel inactivation and thereby reducing the firing of action potentials (Macdonald & Kelly, 1995). In the present study, carbamazepine had no effect on the amplitude of the e.p.s.p. in neurones from ethanol-treated mice or from PTZ-treated neurones, but did reduce the duration of the e.p.s.p. in both groups. Carbamazepine (130 mg $kg^{-1}$) was also effective at reducing ethanol withdrawal-induced handling seizures but was without effect at a lower dose (30 mg $kg^{-1}$). From the present study it is unclear how closely the *in vitro* concentrations used correlate with the effective *in vivo* dose. Upper therapeutic anticonvulsant levels in man are approximately 50 $\mu$M (Ulrichsen *et al*., 1986) with toxicity at higher doses. Thus the concentration of carbamazepine used *in vitro* in the present study (100 $\mu$M) may not be indicative of the efficacy of antiepileptic agents in this model. However, the effects of carbamazepine were both rapid in onset and large in magnitude. Further experiments examining the concentration-dependence of these effects *in vitro* may yet reveal e.p.s.p. depressant effects at therapeutically relevant concentrations.

The results of the present study highlight the functional consequences that arise as a result of the neurochemical disturbances within cortical neurones exposed to chronic ethanol treatment. The profound electrophysiological changes in these cortical neurones that accompany the cessation of chronic ethanol intake may well play a role in the development of seizure activity observed *in vivo*. The data described here suggest that a reduction in GABAergic neurotransmission contributes to the hyperexcitability of cortical neurones following ethanol treatment, although a secondary more subtle increase in the NMDA receptor population may also play a role in the epileptiform activity displayed by these neurones.

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