Ethosuximide Reduces Mortality and Seizure Severity in Response to Pentylenetetrazole Treatment During Ethanol Withdrawal

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Abstract

Aims: We recently demonstrated that T-type calcium channels are affected by alcohol abuse and withdrawal. Treatment with ethosuximide, an antiepileptic drug that blocks T-type calcium channels, reduces seizure activity induced by intermittent ethanol exposures and withdrawals. Here, we expand on these findings to test whether ethosuximide can reduce the sensitivity to pentylenetetrazole-induced seizures during ethanol withdrawal.

Methods: We used an intermittent ethanol exposure model to produce withdrawal-induced hyperexcitability in DBA/2J mice.

Results: Ethosuximide (250 mg/kg) reduced seizure severity in mice undergoing ethanol withdrawal with concurrent PTZ treatment (20 mg/kg). Importantly, ethosuximide did not produce rebound excitability and protected against ethanol withdrawal-induced mortality produced by concurrent PTZ treatment (40 mg/kg).

Conclusion: These results, in addition to previous preclinical findings, suggest that ethosuximide should be further evaluated as a safe, effective alternative to benzodiazepines for the treatment of alcohol withdrawal.

INTRODUCTION

Alcohol abuse and dependence can cause compensatory mechanisms that generate a state of hyperexcitability when an individual undergoes detoxification (Rogawski, 2003; Ait-Daoud et al., 2006). These compensatory mechanisms create an imbalance in excitation and inhibition that can persist through withdrawal and may lead to withdrawal seizures. Alcoholics undergoing withdrawal often present with tonic-clonic seizures, and the risk for seizures progressively increases with each successive detoxification (Ballenger and Post, 1978; Brown et al., 1988; Lechtenberg and Worner, 1996; Becker, 1998; Rogawski, 2005). As many as 33% of individuals undergoing alcohol withdrawal develop seizures (Earnest and Yarnell, 1976; Hillbom, 1980; Bratthen et al., 1999; Hillbom et al., 2003; Rogawski, 2005).

Withdrawal seizures are a serious, life-threatening problem that can lead to status epileptics and temporal lobe epilepsy (Rogawski, 2005; Hughes, 2009). Alcohol withdrawal seizures (AWS) are the trigger for status epileptics in up to 25% of the cases reported (Hillbom et al., 2003; Hughes, 2009), and therefore require immediate medical attention. In addition to long-term consequences, one-third of those experiencing AWS develop delirium tremens, and of these, 5–15% die (Erwin et al., 1998).

Both rodent and clinical studies have demonstrated that benzodiazepines, the first line treatment for AWS, are effective in the acute phase of seizures but can exacerbate seizure activity, cause rebound effects when the treatment is removed, and possess high abuse potential (Morris and Victor, 1987; Woods et al., 1987; File and Wilks, 1990;
Numerous compensatory mechanisms are engaged during ethanol withdrawal, including increased calcium channel activity (Walter and Messing, 1999; NGouemo and Morad, 2003). T-type calcium channels (T channels) have been identified as one potential target that contributes to hyperexcitability observed during withdrawal. One T channel isoform, CaV3.2, is inhibited by acute administration of ethanol, through a PKC-dependent mechanism (Shan et al., 2013). During chronic ethanol consumption and withdrawal, T channel expression and function were enhanced (Nordskog et al., 2006; Graet et al., 2011; Welsh et al., 2011). Ethosuximide (ETX), a T channel antagonist, was found to restore certain patterns of sleep activity that had been disrupted in mice undergoing ethanol withdrawal (Wiggins et al., 2013). We also found that ETX reduced the increased spike and wave discharge activity that was observed in mice undergoing ethanol withdrawal, decreased the severity of handling-induced convulsions and the number of tonic-clonic seizures (Riegle et al., 2014). These studies suggest that T channels are a novel target for intervention and that ETX may be a potentially effective treatment for ethanol withdrawal symptoms. However, it is unclear whether the benefit of this treatment extends to reducing generalized, tonic-clonic seizures or reductions in associated withdrawal-induced mortality.

A common strategy to evaluate AWS and test the effectiveness of treatments has been to utilize chemosensitizers to elicit generalized, convulsive (tonic-clonic) seizures in rodents undergoing withdrawal (Szabo et al., 1984; Grant et al., 1990; Kokka et al., 1993; Becker et al., 1998; Stephens et al., 2001; Ripley et al., 2002; Cagetti et al., 2004; Joshi et al., 2005). Here, we used pentylenetetrazole (PTZ), a GABA\(B\) antagonist (Squires et al., 1984; Psarropoulou et al., 1994), to elicit convulsive seizures in mice undergoing an intermittent ethanol exposure and withdrawal paradigm. We evaluated the effects of ETX on the severity of PTZ-induced ethanol withdrawal seizures, rebound excitability and mortality rate.

**METHODS**

For these experiments, we followed guidelines established by the National Institutes of Health and United States Department of Agriculture. All experiments were approved by the Institutional Animal Care and Use Committee of Wake Forest School of Medicine, and included measures to minimize pain and suffering, as well as to reduce the numbers of animals while still achieving the main experimental objectives. All mice used in these studies were group-housed and maintained on a 12 h light/dark cycle (lights off: 6:00 pm, lights on: 6:00 am).

**Experiment 1: Seizure severity**

Male, 6–8 weeks old DBA/2J mice (Jackson Laboratory, Bar Harbor, ME) underwent an intermittent ethanol exposure paradigm as previously described in Riegle et al. (2014). The model was adapted from a previous investigation of ethanol withdrawal seizure progression (Becker and Hale, 1993). Mice were placed in a Plexiglas vapor chamber for each exposure, located in the same room in which the mice were housed. The chamber was filled with volatilized ethanol (95%) utilizing an air pump. Exposure lasted 16 h each day (5:00 pm to 9:00 am). After each withdrawal, mice underwent an 8 h withdrawal period (9:00 am to 5:00 pm). Mice received ethanol exposure and withdrawal for a total of 4 straight days. Control mice received air exposure instead of ethanol and were handled identically as the experimental mice.

All mice were injected subcutaneously with pyrazole (100 mg/kg, Sigma Aldrich, St. Louis, MO, USA), an alcohol dehydrogenase inhibitor used to maintain blood ethanol concentration (BEC) levels, prior to each exposure. BEC measurements were made at the beginning of each withdrawal period as previously described in Riegle et al. (2014). Briefly, a NAD-ADH enzyme assay (Carolina Liquid Chemistries Corp, Winston-Salem, NC, USA) was utilized to measure the ethanol levels, and no differences in BEC levels were found between the treatment groups. Thus, they were combined and averaged. For experiment 1, the BECs (mg/dl) were as follows: first withdrawal, 243.2 ± 12.9; second withdrawal, 205.0 ± 12.0; third withdrawal, 200.5 ± 6.7; fourth withdrawal, 128.2 ± 6.2. BEC values in the fourth withdrawal were significantly lower compared with the first, second and third withdrawal periods (Kruskal–Wallis test with Dunn’s Multiple Comparison post hoc test, \(P < 0.0001\)). A total of 11 mice were excluded from this experiment before analysis due to problems with the vapor chamber resulting in BEC levels that were not between 80 and 200 mg/dl in the fourth withdrawal period.

All ethanol- and air-exposed mice were tested for seizure sensitivity 6–8 h into the fourth withdrawal period. Mice were treated with intraperitoneal (IP) injections of saline or ETX (100 or 250 mg/kg; Sigma Aldrich) 45 min prior to testing for seizure activity. To test for seizure activity, mice were injected with a subthreshold dose of PTZ (20 mg/kg, IP; Sigma Aldrich). Immediately following PTZ injection, mice were video-recorded and monitored for seizure activity for 30 min. A blind reviewer analyzed the seizure activity and scored the seizures based on the following criteria: 0, no seizure activity; 1 tail twitching and/or head nodding; 2, myoclonic activity or tonic; 3, tonic-clonic convolution; 4, severe tonic-clonic convolution resulting in death. Mice that did not have a tonic/clonic seizure were assigned 1800 s and 0 s for latency to and duration of tonic-clonic seizure.

**Experiment 2: Rebound excitability**

Male DBA/2J mice (6–8 weeks old) underwent the intermittent ethanol exposure paradigm as described in experiment 1. There were no differences between treatment groups in BEC values. For experiment 2, the BECs (mg/dl) were as follows: first withdrawal, 298.6 ± 17.9; second withdrawal, 300.7 ± 20.3; third withdrawal, 263.8 ± 22.9; fourth withdrawal, 191.6 ± 15.6. BEC values in the fourth withdrawal were significantly lower compared with the first, second and third withdrawal periods (Kruskal–Wallis test with Dunn’s Multiple Comparison post hoc test, \(P < 0.0001\)). Two mice were excluded from this experiment, one each from the 100 and 250 mg/kg ETX treatment groups because the BEC values for these mice were below 80 mg/dl in the fourth withdrawal.

Mice were administered saline or ETX (100 or 250 mg/kg, IP) at 10:00 am and 1:30 pm during the first three withdrawal periods. Mice were not treated with saline or ETX during the fourth withdrawal period as this was the final, untreated withdrawal period. Seizure activity was assessed 8–10 h into the fourth withdrawal period. To do this, mice were injected with PTZ (20 mg/kg) and scored for seizure activity as described in experiment 1.

**Experiment 3: Mortality**

To assess mortality due to ethanol withdrawal, male DBA/2J mice (6–8 weeks old) underwent the intermittent exposure paradigm as described in experiment 1. For experiment 3, the BECs (mg/dl) were as
Follows: first withdrawal, 259.9 ± 43.4; second withdrawal, 219.0 ± 17.2; third withdrawal, 227.2 ± 16.7; fourth withdrawal, 171.4 ± 15.3. There were no significant differences in BEC values between the treatment groups or between withdrawal periods. No mice were excluded from this experiment.

Mice were administered saline or ETX (250 mg/kg, IP) 45 min prior to testing. At 6–8 h into the fourth withdrawal, mice were tested by injecting 40 mg/kg PTZ (IP). A higher dose of PTZ was used in order to assess the effects of ethanol withdrawal on mortality and whether ETX can be protective. In control mice this dose of PTZ was a non-lethal dose that induced tonic–clonic seizures. Mice were monitored for 1 h following PTZ administration. For this study, a blind reviewer was not used to assess mortality. Tonic–clonic seizure activity was evaluated on whether or not it was present, the mortality rate was assessed, and latencies to death and first tonic–clonic seizure were measured. Descriptive observations were assessed based on the following scale: 0, did not have tonic–clonic activity; 1, tonic–clonic seizure present; 2, tonic–clonic seizure with running and bouncing around cage; 3, tonic–clonic seizure ending with death. The number of tonic–clonic and more severe seizures were recorded for the one hour period of observation.

Statistical analyses
We used nonparametric ANOVA tests to determine differences in seizure score, latency, and duration parameters for experiments 1 and 2. Nonparametric tests were used because sample sizes between groups differed. For experiments 1 and 2, the overall seizure score data did not meet the criteria for the chi-square test. We used a chi-square test to determine if the number of mice with tonic–clonic seizures differed between groups in experiments 1 and 2. We used an ANOVA to determine latency to first seizure and mortality in experiment 3. We also used chi-square analyses to determine if the number of mice with tonic–clonic seizures and mortality rate differed between groups in experiment 3. Appropriate post hoc analyses were performed as needed.

RESULTS
Experiment 1: Seizure severity
In the first experiment, we assessed acute ETX treatment on ethanol withdrawal-induced seizure activity using the chemoconvulsant, PTZ, to elicit convulsive seizure activity. The effects of ETX (100 and 250 mg/kg) are illustrated in Figure. 1(A–C and Table 1. In mice undergoing ethanol withdrawal, a Kruskal–Wallis test confirmed that ETX (250 mg/kg) treatment significantly reduced the seizure score compared with mice treated with saline (Fig. 1A; Dunn’s Multiple Comparison test, $P = 0.0033$; $n = 5–10$ per group; mean ± SEM: AW-Sal, 2.25 ± 0.35; AW-100, 0.88 ± 0.38; AW-250, 0.21 ± 0.15; Air-Sal, 0.30 ± 0.30; Air-250, 0.40 ± 0.40). The lower dose of ETX (100 mg/kg) tested did not produce an effect that was significantly different from saline-treated mice. Air-exposed animals treated with saline or ETX (250 mg/kg) did not exhibit tonic–clonic seizures when administered PTZ (Fig. 1A).

There were significantly more mice with tonic–clonic seizures when treated with saline compared to those treated with ETX (Table 1; Chi-square, $P = 0.024$; $n = 5–10$). Mice treated with the higher dose of ETX (250 mg/kg) were seizure free, as were the air-exposed mice (Table 1).

Overall, there were no significant differences in latency to tonic–clonic seizure (Fig. 1B; Kruskal–Wallis test, $P = 0.0756$; $n = 5–10$; mean ± SEM: AW-Sal, 1336 ± 207.5; AW-100, 1749 ± 51.38; AW-250, 1800 ± 0.0; Air-Sal, 1800 ± 0.0; Air-250, 1800 ± 0.0). Mice that did not have a tonic–clonic seizure were assigned a latency of 1800 s. Only one mouse treated with the lowest dose of ETX had a tonic–clonic seizure.

There was an overall effect on duration of tonic–clonic seizure (Fig. 1C; Kruskal–Wallis test, $P = 0.0240$, $n = 5–10$ per group; mean ± SEM: AW-Sal, 17.40 ± 7.9; AW-100, 2.25 ± 2.25; AW-250, 0.0 ± 0.0; Air-Sal, 0.0 ± 0.0; Air-250, 0.0 ± 0.0); however, the Dunn’s Multiple Comparison post hoc test revealed no differences between groups.
Mice without tonic–clonic seizure activity were assigned a duration of 0 s. Overall, acute treatment with ETX dose-dependently reduced ethanol withdrawal seizure severity.

### Experiment 2: Rebound excitability

We treated mice during each withdrawal period with saline and ETX (100 or 250 mg/kg) and examined seizure activity in a fourth, untreated withdrawal period. We did not observe differences in the seizure score between the groups (Fig. 2A; Kruskal–Wallis test, \( P = 0.9497; n = 6–12 \) per group; mean ± SEM: AW-Sal, 2.79 ± 0.20; AW-100, 2.75 ± 0.36; AW-250, 2.55 ± 0.29). Seventy-five percent of the mice that had been treated with saline, 66.7% that had been treated with 100 mg/kg ETX, and 55.6% that had been treated with 250 mg/kg ETX had tonic–clonic seizures in the untreated, fourth withdrawal period; these values were not significantly different (Table 2; Chi-square test, \( P = 0.8750; n = 6–12 \) per group). Latency to and duration of tonic–clonic seizures were not different between groups (Fig. 2B, C; Kruskal–Wallis test, \( P = 0.9347 \) and 0.7598 respectively; \( n = 6–12 \) per group; mean latency ± SEM: AW-Sal, 799.3 ± 203.5; AW-100, 865.0 ± 318.8; AW-250, 874 ± 293.1; mean duration ± SEM: AW-Sal, 25.83 ± 10.26; AW-100, 29.50 ± 12.87; AW-250, 17.78 ± 7.47). Overall there were no differences between groups, thus suggesting the suppressive effects of ETX on withdrawal seizures do not produce rebound excitability in a fourth untreated withdrawal period.

### Experiment 3: Mortality

Lastly, we sought to determine if acute treatment with ETX (250 mg/kg) could prevent mortality due to convulsive seizures during ethanol withdrawal. All mice treated with ETX during the fourth withdrawal period survived whereas only one mouse treated with saline survived out of eight treated mice (Table 3). ETX treatment significantly reduced mortality (Table 3, Chi-square test, \( P < 0.0001; n = 8 \) per group). We observed that each death followed a severe tonic–clonic seizure. This dose of PTZ (40 mg/kg) induced tonic–clonic seizure activity in air-exposed control mice (Fig. 3A; Table 3); however, none of these mice died (Fig. 3B; Table 3). ETX (250 mg/kg) reduced the number of tonic–clonic seizures compared with ethanol withdrawal and air-exposed mice treated with saline (Table 3, Chi-square test, \( P < 0.0001; n = 8 \) per group). In mice undergoing ethanol withdrawal, the latency to death and first tonic–clonic seizure were significantly greater in mice treated with ETX compared with mice treated with saline (Fig. 3A and B, ANOVA test with Tukey’s Multiple Comparison test, \( P < 0.0001; n = 8 \); mean latency to first T/C: AW-Sal, 155.0 ± 38.7; AW-250, 323.4 ± 36.6; Air-Sal, 247.5 ± 73.16; mean latency to mortality ± SEM: AW-Sal, 1921.0 ± 416.5; AW-250, 3600.0 ± 0.0; Air-Sal, 3600.0 ± 0.0). Mice without tonic–clonic seizure and mice that survived were assigned a latency of 3600 s.

We observed that saline-treated mice undergoing ethanol withdrawal had multiple tonic–clonic seizures that were much more severe compared with the ETX-treated, ethanol withdrawal mice and air-exposed, control mice. These mice exhibited behavior such as running and bouncing around the cage as well as loss of postural control, similar to higher intensity tonic–clonic seizures (Racine, T/C, tonic/clonic.

**Significantly different (Chi-square test, \( P = 0.024 \)).

| Table 1. Summary of T/C seizures in acutely treated mice |
|-----------------------------|-----------------------------|
|                            | Ethanol withdrawal          | Air exposure                  |
|                            | Saline | ETX (100 mg/kg) | ETX (250 mg/kg) | Saline | ETX (250 mg/kg) |
| Mice with T/C seizures**   | 5/10 (50%) | 1/8 (12.5%) | 0/7 (0%) | 0/5 (0%) | 0/5 (0%) |

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Fig. 2. ETX treatment did not produce rebound excitability in a fourth, untreated withdrawal period. All mice presented in the panels underwent ethanol withdrawal. (A) There were no differences in seizure score between saline or ETX (100 and 250 mg/kg) treated mice in a fourth, untreated withdrawal period suggesting that ETX did not produce rebound excitability when treatment was stopped. (B and C) Latency to and duration of tonic–clonic seizure were not different between treatment groups. Mice without a tonic–clonic seizure were assigned a latency to and duration of 1800 sec and 0 sec respectively. Mice were monitored for 30 min (1800 sec). T/C, tonic–clonic seizure; sec, second.

Mice without tonic–clonic seizure activity were assigned a duration of 0 s. Overall, acute treatment with ETX dose-dependently reduced ethanol withdrawal seizure severity.
Mice treated with ETX did not exhibit tonic–clonic seizure activity with the exception of one mouse. In this mouse, the seizure was mild and similar to the tonic–clonic seizures of the air-exposed mice. Air-exposed control mice did not have multiple tonic–clonic seizures as the ethanol withdrawal saline-treated mice did, and the tonic–clonic seizure observed in these mice was much milder with

| Table 2. Summary of mice with T/C seizures following fourth untreated withdrawal |
|------------------|------------------|------------------|
| Ethanol withdrawal | ETX (100 mg/kg) | ETX (250 mg/kg) |
| Mice with T/C seizures | 9/12 (75%) | 4/6 (66.7%) | 5/9 (55.6%) |

The data shown were not significantly different (Chi-square test, \(P = 0.8750\)).

T/C, tonic–clonic.

| Table 3. Summary of acute ETX effects on mortality by ethanol withdrawal seizure |
|------------------|------------------|------------------|
| Ethanol withdrawal | Air exposure |
| Saline | ETX (250 mg/kg) | Saline |
| Seizure mortality*** | 7/8 (87.5%) | 0/8 (0%) | 0/8 (0%) |
| Mice with T/C seizures*** | 8/8 (100%) | 1/8 (12.5%) | 8/8 (100%) |

T/C, tonic/clonic.

***Significantly different (Chi-Square test, \(P < 0.0001\)).

Fig. 3. Acute treatment with ETX (250 mg/kg) reduced ethanol withdrawal-induced mortality. (A) Latency to first tonic–clonic seizure. Mice without tonic–clonic seizures were assigned 3600 sec. ***significant compared with ethanol withdrawal saline and air-exposed mice (\(P < 0.0001\)). (B) Latency to death in each treatment group. Mice that survived were assigned 3600 sec (the amount of time monitored). ***significant compared with ETX-treated and air-exposed mice (\(P < 0.0001\)). (C) Max seizure score assessed for mice undergoing ethanol withdrawal or exposed to air. Acute treatment with ETX (250 mg/kg) during ethanol withdrawal decreased seizure severity compared to what was observed in saline-treated mice undergoing withdrawal or exposed to air. ***P < 0.0001 (D) Number of T/C seizures observed during 1 h of observation. Mice treated with ETX undergoing withdrawal had significantly fewer seizures compared with mice treated with saline undergoing withdrawal. ***P < 0.0001; **P < 0.001; T/C = tonic–clonic; sec, second.

1972).
only loss of postural control. Air-exposed control mice were able to recover after the tonic–clonic seizure. Many of the mice treated with ETX explored and behaved normally without seizure. We analyzed these observations by evaluating maximum seizure score observed and number of tonic–clonic or more severe seizures. ETX-treated mice and air-exposed mice had significantly lower seizure scores and less seizures compared with mice treated with saline undergoing ethanol withdrawal (Fig. 3C and D; ANOVA with Tukey’s Multiple Comparison Test, $P < 0.0001; n = 8$; mean max seizure score: AW-Sal, 2.875 ± 0.125; AW-250, 0.125 ± 0.125; Air-Sal, 1.125 ± 0.125; mean number of T/C seizures: AW-Sal, 3.125 ± 0.639; AW-250, 0.125 ± 0.125; Air-Sal, 1.0 ± 0.0).

**DISCUSSION**

In this study we found that ETX produced a dose-dependent reduction in seizure severity, and did not produce rebound excitability in an untreated withdrawal period. Importantly, ETX also improved survival secondary to PTZ-induced seizures during ethanol withdrawal.

In these experiments, we utilized an intermittent exposure paradigm that included four ethanol exposures and four withdrawal periods. This is a well-established model that consistently generates increased excitability during withdrawal (Becker and Hale, 1993; Becker, 1994; Becker et al., 1997a,b; Veatch and Becker, 2002; Riegle et al., 2014). Previous studies using other mouse strains have established that mice undergoing withdrawal have increased sensitivity to chemoconvulsants and a reduction in seizure threshold (Kokka et al., 1993; Becker et al., 1998; Stephens et al., 2001; Cagetti et al., 2004). We also observed, using DBA/2J mice, that saline-treated mice undergoing withdrawal had increased seizure severity compared with air-exposed, saline-treated mice. In the first experiment, mice undergoing withdrawal exhibited severe tonic–clonic seizures whereas none of the air-exposed, control mice had tonic–clonic seizures after PTZ injection. In the third experiment, increasing the dose of PTZ resulted in tonic–clonic seizures in air-exposed mice; however, these mice only experienced one mild tonic–clonic seizure and all of these mice were able to recover back to normal behavior. Mice undergoing ethanol withdrawal, injected with the higher dose of PTZ, experienced multiple, very severe tonic–clonic seizures that resulted in death in 87.5% (7 out of 8) of the mice. Thus, the mortality observed in these mice can be attributed to the hyperexcitable state induced by ethanol withdrawal.

Acute treatment with ETX dose-dependently reduced PTZ-induced seizures during ethanol withdrawal. In the fourth withdrawal period, mice treated with the higher dose of ETX had a significantly lower seizure score compared with saline-treated mice. ETX treatment also reduced the number of tonic–clonic seizures observed in the fourth withdrawal. Specifically, only one mouse treated with ETX (100 mg/kg) had a tonic–clonic seizure and none of the mice treated with the higher dose of ETX (250 mg/kg) had tonic–clonic seizures, in contrast to 30% of mice that had tonic–clonic seizures in the salinetreated, withdrawal group. There were no differences in latency to or duration of tonic–clonic seizures. These results are most likely due to the increased variability observed among the mice treated with saline undergoing withdrawal. The effects of ETX treatment on seizure severity and number of tonic–clonic seizures are unlikely to be due to differences in ethanol exposure as there were no differences in BEC levels between the different groups of mice undergoing withdrawal. These findings are consistent with our previous investigation demonstrating that ETX reduced spike and wave discharge activity and handling-induced convulsions, both induced by ethanol withdrawal (Riegle et al., 2014).

We further demonstrated that acute ETX treatment reduced mortality caused by severe convulsions during ethanol withdrawal. All mice treated with ETX during the fourth withdrawal period survived despite use of a dose of PTZ that was sufficient to produce death in non-ETX-treated animals. ETX-treated mice had significantly fewer tonic–clonic seizures. Mice undergoing withdrawal that were treated with saline had multiple, severe tonic–clonic seizures. As mentioned earlier, a severe tonic–clonic seizure preceded death in seven out of eight of the mice undergoing withdrawal that had been treated with saline. The effects of ETX were not due to differences in ethanol exposure as there were no differences in BEC levels between the ETX and saline-treated mice undergoing withdrawal.

In previous studies utilizing a similar intermittent exposure paradigm, mice treated with lorzepam or MK-801 during withdrawal suffered from rebound excitability when treatment ceased (Becker and Veatch, 2002; Veatch and Becker, 2005). Under these conditions, withdrawal-induced seizures were exacerbated. We utilized a similar design and treated mice with ETX or saline during the first three withdrawal periods. We then stopped treatment and tested the mice in the fourth withdrawal period, which was considered an untreated withdrawal period. ETX was administered twice during each withdrawal period, once in the morning and once in the afternoon of the first three withdrawal periods, because ETX is metabolized very quickly in rodents with an approximate half-life of 1 h (el Sayed et al., 1978). Seizure activity observed in the fourth withdrawal period was not different between the groups. Thus, prior ETX treatment did not cause rebound excitability in the fourth, untreated withdrawal period. This is significant because benzodiazepines, a primary treatment for AWS, have been shown to cause rebound effects after treatment termination in both rodent and clinical studies (Woods et al., 1987; File and Wilks, 1990; Greenblatt et al., 1990; Rundfeldt et al., 1995; Losher et al., 1996; Ward and Stephens, 1998; Becker and Veatch, 2002; Chouinard, 2004; Veatch and Becker, 2005).

One of the major concerns of AWS is the risk of developing more severe, life-threatening consequences such as status epilepticus and temporal lobe epilepsy. AWS precipitate up to 25% of the reported cases of status epilepticus (Hillbom et al., 2003; Hughes, 2009). Also, there is an increased risk for developing delirium tremens, which can be fatal (Erwin et al., 1998). It is well-established that multiple detoxifications significantly increase the risk for seizures. These factors underscore the importance of early intervention and the need to reduce AWS as it may decrease the overall rate of these more severe consequences. While we have observed promising results with acute ETX treatment, further investigation is necessary. It remains to be determined whether chronic treatment with ETX can alter kindling mechanisms and prevent more severe, long-term consequences. Interestingly, chronic treatment over several months with ETX has been shown to inhibit epileptogenesis in a genetic model of non-convulsive epilepsy (Dezsi et al., 2013).

Our previous investigation demonstrated that ETX reduced both electrophysiological correlates of withdrawal seizure and handling-induced convulsions in mice undergoing withdrawal (Riegle et al., 2014). Our present results extend these observations by demonstrating that ETX can reduce PTZ-evoked tonic–clonic seizures during ethanol withdrawal. ETX did not cause rebound excitability in a fourth, untreated withdrawal period. Most importantly, it protected the mice against withdrawal-induced mortality. These results
emphasize the potential use of ETX as an effective therapeutic option against AWS, and taken together with our prior studies, also implicate T channels as a potential mechanism. Future studies will further assess the specificity and mechanism of these effects.

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**CONFLICT OF INTEREST STATEMENT**

None declared.

**REFERENCES**


