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The efficacy of a low dose combination of topiramate and naltrexone on ethanol reinforcement and consumption in rat models

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Abstract

Rationale—Combined medication approaches, by targeting multiple neurotransmitter systems involved in alcohol use disorders (AUDs), may be more efficacious than single-medication approaches.

Objectives—We examined, in animals models of consumption and reinforcement, the combined effects of naltrexone (an opioid antagonist) and topiramate (a GABA/glutamate modulator), two medications that have shown promise for treating AUDs, hypothesizing that their combination would be more efficacious than either alone.

Methods—The effects of naltrexone and topiramate on ethanol consumption were examined in alcohol preferring (P) rats (N=10) and in rats from their background strain (Wistar, N=9) using conditions that induce high levels of consumption (24-hr, 3-bottle, free-choice procedure). Low doses of each medication (1 mg/kg, naltrexone; 10 mg/kg, topiramate) were selected in an attempt to maximize their combined efficacy while minimizing potential side-effects. Their effects on ethanol reinforcement were assessed under a progressive-ratio schedule in additional groups of (N=22) P rats. A moderate dose of topiramate (20 mg/kg) was also included to verify topiramate's efficacy on its own.

Results—In P rats, but not Wistar rats, the combination effectively and persistently reduced consumption; whereas, neither dose alone was effective. The combination and naltrexone alone were equally effective at reducing ethanol reinforcement; however, with the combination, but not naltrexone alone, this effect was selective for ethanol. All treatments produced a similar decrease in home-cage food consumption. The 20 mg/kg dose of topiramate also effectively reduced ethanol consumption and reinforcement.

Conclusions—With greater efficacy and fewer side-effects, the combination shows promise as a treatment for AUDs.

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Keywords

Alcohol Preferring (P) Rats; Ethanol; Naltrexone; Topiramate; Combination Treatment; Reinforcement; Self-Administration

Introduction

Alcohol use disorders (AUDs), which include alcohol abuse and dependence, are responsible for vast health, social, and economic problems. AUDs affect 30% of the U.S. population within their lifetime, but only about a fourth of these individuals receive any kind of treatment (Hasin et al. 2007). Chronic exposure to alcohol results in enduring neuroadaptations in multiple signaling pathways, including opioid, dopamine, glutamate, and γ -aminobutyric acid (GABA) pathways (for reviews see Chastain 2006; Clapp et al. 2008; Koob and Volkow 2009; and Heinz et al. 2009a), that give rise to lasting cognitive and behavioral changes (SAMHSA 2009; for review see Hoffman et al. 2000). These neuroadaptations contribute to the multiple components of AUDs: tolerance, withdrawal, craving (both alcohol craving and withdrawal relief craving), drug reinforcing properties, etc. (for review see Gilpen and Koob 2008; Heinz et al. 2009b; SAMHSA 2009; for review see Hoffman et al. 2000). Currently approved medications are thought to target the neurotransmitter pathways underlying one or more of these components, such as acamprosate, which is believed to reduce withdrawal symptoms and craving through modulation of N-methyl-D-aspartic acid (NMDA) receptors (for review see Rösner et al. 2010). This and other single-pathway targeted pharmacotherapies for AUDs have shown modest therapeutic value over placebo, but greater efficacy is needed. Combination medications, by targeting multiple neurotransmitter pathways implicated in different components of AUDs, are likely to have enhanced efficacy over the traditional single-medication approach.

Naltrexone, an opioid receptor antagonist, is one of three currently approved treatments of AUDs in humans that is particularly effective at decreasing heavy drinking (Chick et al. 2000), likely by reducing the reinforcing effects of alcohol (Volpicelli et al. 1995; King et al. 1997; for review see Heilig and Egli 2006). Treatment with naltrexone also reduces self-administration of ethanol in animal models (Stromberg et al. 1998; Gonzales and Weiss 1998; Middaugh and Bandy 2000). Naltrexone's effects are proposed to be mediated through blockade of mu receptors on medium spiny neurons in the nucleus accumbens that may prevent ethanol-induced dopamine release (for review see Unterwald 2008). There is also evidence to suggest that these effects are mediated via a dopamine-independent mechanism (Self and Nestler 1996). In alcohol-dependent humans, in addition to decreasing alcohol consumption, naltrexone has been shown to block cue-induced activation of the reward pathway (Myrick et al. 2008), reduce craving (O'Malley et al. 2002) and decrease the subjective effects of alcohol (Volpicelli et al. 1995; King et al. 1997). Despite these findings supporting the efficacy of naltrexone in reducing several of the individual components involved in AUDs, its beneficial effects have not consistently translated to enhanced abstinence or reduced consumption in alcohol-dependent individuals. Clinical studies of naltrexone have variable efficacy on reducing alcohol consumption, with meta-analyses showing a small effect size (for review see Garbutt 2010).

Topiramate (a GABA/glutamate modulator) is a currently approved treatment for epilepsy that has also shown promise as a potential treatment for AUDs. *Topiramate facilitates* GABA_A-mediated inhibitory transmission while contemporaneously antagonizing AMPA and *kainate glutamate receptors, among other actions* (Johnson 2004). Studies in humans have shown that treatment with topiramate reduced several measures of alcohol use, such as

number of drinks/day and drinks/drinking day as well increased abstinence (Johnson et al. 2003; Johnson et al. 2007). Topiramate's effects on craving are less clear, with one study reporting lower levels of craving (Johnson et al. 2003), but another showing that while topiramate reduced drinking, it did not decrease reactivity to ethanol-cues or self-reported craving (Miranda et al. 2008). Topiramate has also been reported to reduce ethanol consumption in rats (Knapp et al. 2007; Breslin et al. 2010; Lynch et al. 2011) and in C57BL/J mice (Gabriel and Cunningham 2005). While the mechanism of topiramate's effects on alcohol use has yet to be identified, it was originally postulated that by both inhibiting glutamate and facilitating GABA function, topiramate might suppress corticomesolimbic dopamine, thus decreasing the reinforcing effects of alcohol (Johnson 2004). In preclinical studies, topiramate reduces progressive-ratio (PR) responding for ethanol in rats (Hargreaves and McGregor 2007), supporting the theory that topiramate reduces the reinforcing effects of ethanol; however, these effects appear to be mediated via glutamatergic, rather than dopaminergic signaling (Lynch et al. 2013).

The goal for this study was to determine the effects of naltrexone and topiramate, alone and in combination, in rats that had prolonged access to ethanol under conditions that induce high levels of drinking. Low doses of each medication, which either do not affect or only modestly affect alcohol-related behaviors on their own, were selected in an attempt to maximize their combined efficacy while minimizing potential side-effects. A similar low-dose naltrexone-topiramate combination approach was recently found to effectively reduce alcohol consumption and reinforcement in mice consuming ethanol under sub-chronic and limited access conditions (Navarrete et al. 2013). In this study, effects of treatments on ethanol consumption were assessed in groups of rats given access to ethanol under a 24-hr, three-bottle, free-choice paradigm. These effects were examined in both alcohol-preferring (P) rats and rats of its background strain (Wistar) given our recent work indicating selective effects of topiramate treatment based on a genetic and/or behavioral phenotype (Lynch et al. 2011; Lynch et al. 2013). The effects of treatments were also assessed on ethanol reinforcement in another group of P rats tested under a PR schedule after prolonged exposure under the same free-choice conditions. We hypothesized that this combination treatment, by modulating multiple signaling pathways that are known to be involved in AUDs (i.e. opioids, glutamate, GABA, dopamine), would be more efficacious than either alone at decreasing ethanol consumption and reinforcement.

Methods

Animals and Housing

Male P rats (N=32) from the 73–74th generations were obtained from the Indiana Alcohol Research Center's Animal Production Core (Indianapolis, IN). Male Wistar rats (N=9), the background strain of P rats, were obtained from Charles River Laboratory (Wilmington, MA). The P line of rats has been selectively bred and characterized by numerous studies as a valid animal model of excessive ethanol drinking behavior (Bell et al. 2006). Rats were single-housed in clear, polycarbonate cages in a room maintained on a 12:12 light/dark cycle (lights on at 7:00AM). All animals were allowed a 1 week habituation period before the start of ethanol consumption. Animals were 11–12 weeks old at the start of the experiments. Food and water were provided *ad libitum*, and animals were weighed twice weekly. At the start of the treatment phase, body weights were between 450–550g. By the end of the treatment phase, animals had increased their body weights by approximately 5%. Animal health was monitored daily by trained laboratory staff and all animal protocols were approved by the Animal Care and Use Committee at the University of Virginia.

Drugs

Ethanol solutions (8% v/v, 10% v/v, 16% v/v) were prepared from 190 proof absolute ethyl alcohol (Pharmco-Aaper, Brookfield, CT, USA) and diluted using tap water. Naltrexone HCl and Topiramate HCl were purchased from Sigma-Aldrich (St. Louis, MO). Both compounds were dissolved in 0.9% sodium chloride and sterile water and administered intraperitoneally at a volume of 1 ml/kg.

Experiment 1: Procedure for ethanol consumption

P rats (N=10) and Wistar rats (N=9) were given 24-hr access to two different concentrations of ethanol solution (8 % v/v and 16% v/v) along with a bottle containing water under a free-choice procedure. Intake of ethanol and water solutions were determined daily with fresh solutions presented several times a week. Sipper tubes contained a ball bearing at the tip to prevent leakage. Food intake was measured daily at the same time as liquid consumption. To most accurately model the chronic exposure that is characteristic of human AUDs, we began treatments after a minimum of 6 months of ethanol experience. Prolonged access, defined in previous studies with ethanol experience ranging from 2–16 months, is associated with neurological changes that affect GABA, glutamate, and opioid receptor function (Eravci et al. 2000; Darstein et al. 1998; Höltér et al. 2000).

The effect of topiramate and naltrexone on ethanol consumption was examined on a stable baseline (defined as no increasing or decreasing trend in ethanol consumption, with a variation of less than 1 g/kg/day over three consecutive days) using a within-subjects, Latin-square design with compounds administered in random order. Baseline was re-established prior to each treatment and a minimum of 7 days separated each test session. On test days, a single treatment of topiramate (10 mg/kg), naltrexone (1 mg/kg), their combination (10 mg/kg topiramate/1 mg/kg naltrexone), or an equal volume of saline was administered intraperitoneally during the daily weigh sessions that were conducted between 12:00PM and 1:00PM, with water and ethanol consumption measured 24-hr after each injection. This dose of naltrexone was selected based on previous research showing it to modestly and selectively reduce ethanol self-administration in Wistar rats (Stromberg et al. 1998; Kuzmin et al. 2008). Although higher doses have also been reported to reduce ethanol consumption (2.5–10 mg/kg), these doses also produce non-specific effects (i.e. decrease water consumption, produce motor effects; Bienkowski et al. 1999; Goodwin et al. 2001; Escher et al. 2006). The dose of topiramate was selected based on our previous findings showing that on its own this dose modestly, but selectively decreases both ethanol consumption and reinforcement in P rats tested following a shorter period of ethanol exposure (Breslin et al. 2010; Lynch et al. 2011; Lynch et al. 2013). However, based on pilot data from the current P rats that were given prolonged and excessive ethanol exposure showing that this dose produced variable efficacy, we also included a moderate dose of topiramate (20 mg/kg) to verify its efficacy on its own. Higher doses of topiramate have also been reported to decrease ethanol consumption (25–80 mg/kg), although these doses have also been reported to affect food and water consumption (Gabriel and Cunningham 2005; Hargreaves and McGregor 2007).

Experiment 2: Procedure for ethanol reinforcement

The effects of topiramate and naltrexone on ethanol reinforcement, assessed using a PR schedule, were examined in P rats. Wistar rats were not included in this experiment because levels of responding for ethanol under the PR schedule in this strain were low and unreliable. P rats (N=22) were given access to ethanol under the three-bottle choice procedure described above. After 3 months of daily consumption, ethanol bottles were removed from the home cage and ethanol was only available during daily operant sessions. Food and water remained available *ad libitum* in the home cage.

Rats were trained to self-administer 10% v/v ethanol deliveries (0.1 ml). Testing sessions occurred in operant conditioning chambers within sound attenuating boxes (ENV-018M; Med Associates, St Albans, VT). During the initial training sessions, responding was reinforced under a fixed-ratio 1 (FR1) schedule, in which a single response on the left or right lever led to a delivery of either water (left-lever) or ethanol (right-lever). Training sessions lasted 60 minutes and occurred 6 days/week. Once responding stabilized under these FR1 conditions, typically at around 1–2 months, animals were placed on a PR schedule to measure ethanol reinforcement. With this schedule, animals must respond for ethanol (and water) at higher levels for each subsequent delivery of ethanol in the following steps: 1, 2, 3, 4, 6, 8, 10, 12, 16, 20, 24, 28, 32 etc. After 30 minutes without a response on either lever, the session ended. Testing sessions lasted approximately 45–75 minutes. Ethanol reinforcement was determined by the breakpoint, or the last ratio completed (defined by the number of deliveries obtained within each session). Treatments began after 1–2 months of experience with the PR schedule, so as to be matched for age and ethanol-experience to the animals in Experiment 1. Rats weighed 500g on average during the treatment phase.

The effect of topiramate and naltrexone on ethanol reinforcement was examined on a stable baseline using a within-subject design with compounds administered in random order. On test days, a single treatment of topiramate (10 mg/kg), naltrexone (1 mg/kg), their combination (10mg/kg topiramate/1 mg/kg naltrexone), or an equal volume of saline was administered intraperitoneally 30 minutes prior to the start of the daily testing session. Pilot data of the 10 mg/kg topiramate on ethanol reinforcement were variable as well, so the 20 mg/kg topiramate dose was also administered in this experiment. These pretreatment times were selected based on previous research (Lynch et al. 2013).

Statistical Analysis

The effects of topiramate and naltrexone alone and combined on ethanol consumption and reinforcement were examined by comparing the baseline g/kg consumption or deliveries (averaged across 3 days prior to treatment) to the day of treatment as well as 3 days following treatment using a repeated measures analysis of variance (ANOVA) with separate analyses conducted for P rats and Wistar rats (for Experiment 1). Similar analyses were conducted to examine the effects of treatment on preference for ethanol (total and 8 versus 16) over water, as well as percent change in consumption of ethanol as a function of concentration. Following a significant overall effect, subsequent comparisons of percent change from baseline were examined within each of the four days using univariate ANOVAs. Post hoc comparisons with vehicle and the combination were made using Dunnett's t-test. Similar analyses were used to examine the effects of these treatments on food and water intake in the home cage (Experiments 1 and 2), and water deliveries obtained during PR sessions (Experiment 2). However, when analyzing side effects, we included an analysis of the day of acute treatment only, even if there was no significant overall effect for the 4 testing days (including treatment day and 3 days post-treatment). The effects of the combination treatment versus vehicle were also examined as a function of strain using repeated measures ANOVA. Statistical analyses were performed using SPSS (version 20) with 0.05 as the alpha level for statistical significance for all tests.

Results

Experiment 1: Effect of topiramate and naltrexone alone and combined on ethanol consumption

In P rats, the combination of naltrexone and topiramate (10 mg/kg), but neither of these treatments alone, reduced ethanol consumption on the day of treatment and for several days

following treatment (Fig. 1). Topiramate alone at the 20 mg/kg dose produced a similar effect as the combination treatment. An analysis of ethanol consumption at baseline and after treatment revealed significant effects of day ($F_{4,156}=6.267$, $p<0.001$) and an interaction of day by treatment ($F_{16,156}=2.047$, $p<0.05$; Fig. 1a). To examine the persistence of these treatment effects, we analyzed percent change from baseline levels of ethanol consumption over the four day testing period (treatment day and the 3 post-treatment sessions that followed), which revealed significant overall effects of day ($F_{3,117}=6.992$, $p<0.001$) and treatment ($F_{4,39}=4.444$, $p<0.05$). Post-hoc comparison with vehicle revealed a significant decrease for the combination treatment, and for topiramate alone at the 20 mg/kg dose (p 's <0.05), indicating a persistent decrease in consumption with these treatments. Subsequent comparison of the combination to vehicle within each of the days revealed a significant decrease on the treatment day and for the two sessions that followed ($p=0.05$) with a trend for a decrease on post day 3 ($p=0.06$). The 20 mg/kg dose of topiramate also produced a persistent decrease in consumption, although somewhat less consistently, with significant differences from vehicle on the treatment day and on post-treatment day 2 (p 's <0.05), and a trend for a difference on post-treatment day 3 ($p=0.07$). Further comparison of the combination treatment to each of the dose treatments alone on each of the four testing days revealed a significant difference from the 10 mg/kg topiramate alone on each of the days and a significant difference from naltrexone on post days 2 and 3 (p 's <0.05). The effects of topiramate at the 10 mg/kg dose and naltrexone alone were not different from vehicle on any of the testing days. The combination did not differ from the 20 mg/kg dose of topiramate on any of the testing days.

Further analysis of the effects of combination versus vehicle treatment as a function of concentration (8 versus 16%) revealed that the combination produced a similar reduction in the consumption of both concentrations of ethanol ($p>0.05$) although when analyzed in this way, the difference was statistically significant from vehicle for the 16% concentration of ethanol only ($p<0.05$; see Table 1). Although similar effects of the combination treatment were also observed on preference for ethanol over water, these differences were modest and variable and did not reach statistical significance (see Table 1). In Wistar rats, no effect was observed for any of the treatments on ethanol consumption (p 's >0.05 ; Fig 2). However, when the effects of the combination versus vehicle were analyzed as a function of strain, there was no significant interaction of strain on response to treatment for total g/kg consumption or within 8% or 16% concentrations ($p>0.05$; Table 1), indicating that the combination produced a similar, but a less robust effect, in Wistar versus P rats. Thus, in P rats the combination was more efficacious than either naltrexone or topiramate (10 mg/kg) alone and produced a persistent decrease in consumption. While the efficacious dose of topiramate (20 mg/kg) and the combination produced a similar reduction in consumption, the combination produced a more consistent reduction over the three days post-treatment.

Experiment 2: Effect of topiramate and naltrexone alone and combined on ethanol reinforcement

Prior to operant self-administration, baseline ethanol consumption during 24-hour free access period was 5.15 g/kg (± 0.41) and preference for ethanol was 73.6% (± 4.72 ; baseline is defined as the average of the last 10 days of access). Baseline ethanol consumption under the FR1 schedule was 1.18 g/kg (± 0.06), and was positively correlated with ethanol consumption during 24-hour access ($r=0.562$; $p<0.01$). Average ethanol consumption under the PR schedule (defined as the average of 3 days prior to any experimental manipulation) was 0.19 g/kg (± 0.01) and average breakpoint was 26.4 (± 1.8). Average number of water deliveries obtained was 5.0 (± 0.2) and average breakpoint for water deliveries was 6.2 (± 0.4). The level of ethanol consumption during 24-hr free choice access was not correlated with ethanol consumption ($r=0.228$) or breakpoint ($r=0.224$) under the PR schedule. The

level of ethanol consumption during FR1 was also not correlated with ethanol consumption ($r = 0.072$) or ethanol breakpoint ($r = 0.199$) under the PR schedule.

In P rats, both naltrexone alone and the combination of naltrexone and topiramate (10 mg/kg), but not the 10 mg/kg dose of topiramate alone, reduced PR responding for ethanol (Fig. 3). Topiramate at the 20 mg/kg dose also reduced PR responding for ethanol. An analysis of ethanol deliveries at baseline and after treatment revealed a significant effect of day ($F_{4,348} = 50.442$, $p < 0.001$) and day by treatment interaction ($F_{16,348} = 5.698$, $p < 0.001$; Fig 3a). To examine the persistence of these treatment effects, we analyzed percent change from baseline levels of ethanol deliveries over the four day testing period, which revealed significant overall effects of day ($F_{3,261} = 56.103$, $p < 0.001$) and treatment ($F_{12,261} = 6.472$, $p < 0.001$). Post-hoc comparison with vehicle revealed a significant decrease with naltrexone alone, the combination treatment, as well as with the 20 mg/kg topiramate dose alone (p 's < 0.05). Subsequent comparison to vehicle within each of the testing days revealed significant effects of the naltrexone alone and the combination treatment on the day of treatment, but no significant differences on any of the subsequent days. We also found a decrease in ethanol deliveries with the 20 mg/kg dose of topiramate alone on the day of treatment ($p < 0.05$) and a trend for a reduction on post day 1 ($p = 0.07$) and post day 2 ($p = 0.09$). Comparison of the combination treatment to each of the treatments alone on each of the four testing days revealed a significant difference from the 10 mg/kg topiramate alone on the day of treatment ($p < 0.05$), but no difference from naltrexone alone. No significant effects were observed with 10 mg/kg topiramate. A comparison of naltrexone and the combination to the 20 mg/kg dose of topiramate revealed significant differences for both treatments on the day of administration ($p < 0.05$). Thus, in P rats, naltrexone alone, the combination, and the 20 mg/kg dose of topiramate alone reduced the reinforcing effects of ethanol, with better efficacy observed with the combination as compared to topiramate alone (10 mg/kg), but not naltrexone alone, on the day of treatment. Even in comparison to the efficacious dose of topiramate (20 mg/kg), the combination produced a more robust reduction in ethanol reinforcement.

Side effects of topiramate and naltrexone: PR responding for water deliveries and food and water consumption in the home cage

In Experiment 1, neither food nor water consumption in the home cage was affected by any of the treatments in P rats or Wistar rats. In P rats, separate comparisons of food and water intake at baseline versus on the day of treatment revealed no significant effects of day or treatment ($p > 0.05$; Table 1). In Wistar rats, although there was a trend for an effect of day ($p = 0.069$), there was no effect of treatment on food intake (data not shown). There were no significant effects of any treatment on water intake in Wistar rats (data not shown; p 's > 0.05).

In Experiment 2, naltrexone alone (1 mg/kg), topiramate alone (10 mg/kg), their combination, and the 20 mg/kg dose of topiramate alone reduced food intake, but not water intake, in the home-cage on the day of treatment (Table 2). A comparison of food intake at baseline and on the day of treatment revealed significant effects of day ($F_{1,87} = 39.657$, $p < 0.001$), treatment ($F_{4,87} = 3.441$, $p < 0.05$), and day by treatment ($F_{4,87} = 6.385$, $p < 0.05$). An analysis of percent change from baseline on the day of administration revealed a significant effect of treatment ($F_{4,87} = 5.096$, $p < 0.05$), with post-hoc comparison to vehicle revealing significant differences for each of the treatments (p 's < 0.05 ; Table 1). The effects of the combination were not different from any of the other treatments. No significant effects were observed for any of these treatments on water intake on the day of treatment or any subsequent days (p 's > 0.05) or on food intake after the day of administration (i.e. post-treatment days 1–3; p 's > 0.05).

Water deliveries, as assessed in Experiment 2, were relatively low, and although naltrexone alone reduced deliveries, this effect was modest. A comparison of water deliveries at baseline to the day of administration revealed a significant effect of treatment ($F_{4,87}=7.948$, $p<0.001$), and trends for the effects of day ($F_{1,87}=2.847$, $p=0.095$) and day by treatment ($F_{1,87}=2.107$, $p=0.087$). These effects appear to be accounted for by decreases after naltrexone administration, and post-hoc comparison with vehicle revealed a significant difference ($p<0.05$); however, an analysis of percent change from baseline showed no significant effect of treatment, indicating that this effect of naltrexone was modest (Table 1).

Discussion

In this study, we examined the combined effects of topiramate and naltrexone, medications shown to be effective in treating AUDs in humans, on ethanol reinforcement and consumption in an animal model of prolonged and heavy ethanol experience. Our results demonstrate greater efficacy of the combination of 10 mg/kg topiramate and 1 mg/kg naltrexone compared to either treatment alone on ethanol consumption. The combination was also more efficacious than topiramate alone (at either dose), but not naltrexone alone, on ethanol reinforcement; however, with the combination, but not naltrexone alone, this effect was selective for ethanol. Furthermore, when compared to the higher dose of topiramate alone (20 mg/kg), the combination produced a more consistent reduction in consumption (i.e. reduced for three days post-treatment), and produced a more robust effect on ethanol reinforcement. By decreasing two different ethanol behaviors with greater efficacy and/or fewer side effects than either alone, these results implicate the combination of naltrexone and topiramate as an efficacious and promising treatment for AUDs.

Other combination medications for the treatment of AUDs have been investigated for their potential therapeutic value and have shown promise in both clinical and preclinical studies. For example, the combination of naltrexone and acamprosate was found to reduce drinking and increase abstinence in humans, although it showed little efficacy over naltrexone alone (Anton et al. 2006; Kiefer et al. 2003). Preclinically, the combination of naltrexone and acamprosate reduced ethanol consumption and relapse behavior (Stromberg et al. 2001; Heyser et al. 2003), but consistent with the human studies, it showed little efficacy over naltrexone alone. Additional studies investigating naltrexone with ondansetron (a serotonin 5H-T receptor antagonist) show that this combination reduced alcohol drinking in early onset- alcoholics compared to placebo (Ait-Daoud et al. 2001), as well as ethanol drinking in mice and rats with limited access to alcohol (Le and Sellers 1994). The combination of topiramate and ondansetron has also been reported to effectively reduce ethanol consumption in heavy drinking rats, with better efficacy of the combination than either alone for reducing relapse behavior (Lynch et al. 2011). Additionally, the combination of topiramate and naltrexone was found to reduce ethanol self-administration in mice under limited-access conditions (Navarrete et al. 2013). Here, we found the combination of naltrexone and topiramate reduced both ethanol consumption and reinforcement in models of prolonged ethanol access, with better efficacy of the combination than either alone for reducing ethanol consumption, and better efficacy than topiramate alone for reducing ethanol reinforcement. *While these medications pharmacokinetic interactions with each other are unknown, topiramate has a low interaction potential with other drugs, so its use in combination with naltrexone is unlikely to account for our current findings* (Johannson 1997; Johnson 2000). Taken together, these results support the use of combination medications, and provide specific support for the combination of topiramate and naltrexone, as a potential treatment for AUDs.

Naltrexone is thought to exert its effects on the rewarding properties of ethanol through antagonism of mu-opioid receptors, which influence ethanol response through interaction

with the mesolimbic dopamine system, as well as independently through endorphins (for reviews see Unterwald 2008; Gilpin and Koob 2008). *Consistent with the previous findings for the effects of naltrexone alone* (Gonzales and Weiss 1998; Middaugh et al. 2000), *we found naltrexone to effectively reduce ethanol reinforcement, both alone and in combination with topiramate. Interestingly, this effect of naltrexone alone on ethanol reinforcement did not translate to a reduction in ethanol consumption under these 24-hr free choice conditions. These differential effects of naltrexone on reinforcement versus reinstatement may be due to its relatively short duration of action (its half-life is approximately 8 hours; Misra et al. 1974) such that at this low dose of naltrexone (1 mg/kg) there may have been almost complete clearance before the 24-hour measurement of ethanol intake which may have obscured any decreases in consumption. In support of this idea, previous results with naltrexone and other opioid antagonists alone have shown a reduction in ethanol consumption under limited access conditions, but not under 24-hr access conditions* (Samson and Doyle 1985; Froehlich et al. 1990; Stromberg et al. 1998; Goodwin et al. 2001). *It is also possible, however, that naltrexone's effect on reinforcement versus consumption are different. There is evidence to suggest that ethanol consumption is motivated by more than ethanol's reinforcing effects, particularly following long-term consumption* (Koob and Le Moal, 1997). *Further research examining the time-course for the effects of naltrexone on reinforcement versus reinstatement is needed to address this question.*

Topiramate contemporaneously modulates GABA and glutamate, two neurotransmitter systems involved in drug-taking and reinforcement (for reviews see Koob et al. 1998; Chester and Cunningham 2002). In the current study, in contrast to the effects of naltrexone alone, when topiramate was combined with naltrexone we found not only a reduction in ethanol reinforcement, but also a reduction in consumption that persisted past the day of treatment. These results suggest that topiramate may prolong naltrexone's beneficial effects (or vice versa). While the mechanism underlying this persistent effect on consumption are still unknown, given the relatively fast clearance rates for each medication (Misra et al. 1974; Shank et al. 2000), it is likely due to plastic changes in GABA/glutamate/dopamine transmission.

Surprisingly, although the 20 mg/kg dose of topiramate effectively reduced both ethanol consumption and reinforcement, it was not effective alone at the 10 mg/kg dose. This is surprising because we have previously shown that the 10 mg/kg dose effectively reduces both ethanol consumption (Breslin et al. 2010; Lynch et al. 2011) and ethanol reinforcement (Lynch et al. 2013). However, in these previous studies, the conditions used for ethanol availability and duration of access resulted in lower levels of ethanol intake compared to the current study (i.e. a 2-bottle choice sucrose fade procedure; 4.6 g/kg/day (± 0.1 ; Breslin et al. 2010) and 4.5 mg/kg/day (± 0.1 ; Lynch et al. 2011) compared to 6.5 g/kg/day (± 0.1) in the current study). Similarly, Lynch et al. (2013) used a sucrose-fade procedure in limited access operant sessions, rather than chronic prolonged access prior to operant training, which resulted in lower levels of ethanol deliveries as compared to our study (55.4 deliveries (± 5.9) vs. 79.4 deliveries (± 4.8)). This evidence suggests that the dose-dependent response to topiramate may vary as a function of level of drinking or stage of dependence. As both glutamatergic and GABAergic signaling are known to undergo severe changes in response to chronic and binge-like drinking (for review see Dodd et al. 2000; Grobin et al. 1998), it is possible that the prolonged and excessive intake resulted in widespread changes to these systems, resulting in rightward shift in the dose-effect curve. Other studies have also shown that topiramate's ability to reduce ethanol consumption and reinforcement varies by dose (Gabriel and Cunningham 2005; Breslin et al. 2010; Lynch et al. 2011; Lynch et al. 2013), and currently no optimal dose has been distinguished for clinical models (Wages et al. 2012).

Our data do not show an effect of strain on response treatment; however, the combination of topiramate and naltrexone effectively decreased ethanol consumption in P rats, but did not affect the drinking behavior of rats from the background Wistar strain. This may suggest genetic vulnerability as a factor in the efficacy of this treatment's ability to reduce ethanol consumption. *A lack of an effect in Wistar rats is unlikely due to the use of ineffective doses for this strain, as previous studies using Wistar rats showed decreased ethanol intake using lower (0.25 mg/kg naltrexone; Gonzales et al. 1994) or equal doses (10 mg/kg topiramate; Middaugh and Bandy 2000); however these results were seen under limited access conditions.* In this experiment, we induced high-levels of drinking through the three-bottle choice paradigm. While this resulted in higher levels of ethanol consumption in the P rats (6.5 g/kg/day ($\pm$ 0.1)), the Wistar rats only consumed 1.8 ($\pm$ 0.1) g/kg/day at baseline. It is possible that there was a floor effect in the Wistar rats, in which their low levels of intake did not allow for a significant decrease in consumption. Alternatively, response to treatment could be a function of stage of dependence, although future studies are needed to examine this possibility. It is important to note, however, that while Wistar rats were an important control for background strain in this study, because they consumed such low levels of ethanol, they are unlikely to model a human population that would be treated for AUDs.

One goal of a combination treatment using low doses of both naltrexone and topiramate was to minimize side effects while maximizing its effect on ethanol. In Experiment 1, none of these treatments had an effect on food intake; however, in Experiment 2, each treatment (apart from vehicle) reduced food intake on the day of administration. Conditions in Experiment 1 were likely insensitive to the effects on food in that animals used ethanol as a source of caloric supplement. We confirmed this by removing ethanol from the home cages and measuring food intake differences. With ethanol in the home cage, baseline food intake was 13.9g ($\pm$ 0.48); whereas, after ethanol was removed, food intake increased to 19.0g ($\pm$ 0.60). In Experiment 2, the conditions were more sensitive for detecting effects on food, as animals had access to ethanol only during the daily session, and thus likely did not consume enough ethanol each day for it to have a significant effect on their calorie consumption. However, as food intake was not reduced in Experiment 1, the reduced food consumption is likely based on caloric intake, rather than a direct effect of treatment on feeding behavior. This is not surprising, as topiramate and naltrexone have both been studied as weight loss drugs (Bray et al. 2003; Greenway et al. 2010), and even at the low dose of topiramate, which did not affect alcohol consumption or reinforcement on its own, we saw an effect on food. Notably, however, in contrast to the effects of the combination on alcohol consumption, its effects on food were not persistent. Thus, while the combination did reduce food intake in Experiment 2, it did not produce a significant further reduction of food intake over either naltrexone or topiramate alone and this effect was not persistent.

None of these treatments significantly affected water intake in the home cage (Experiment 1 and 2). However, in Experiment 2, naltrexone non-selectively reduced water deliveries obtained in addition to ethanol. Notably, by adding topiramate, this effect was abolished. This could be due to a locomotor effect, as treatment with 1.0 mg/kg naltrexone has been shown to reduce locomotor activity (Middaugh and Bandy 2000) while 10 mg/kg topiramate increases locomotor activity (Bertges et al 2011). However, we did not see an increase in water deliveries obtained with topiramate alone. Importantly, the combination treatment resulted in no additional side effects over either treatment alone, and even eliminated the non-selective effect on water observed with naltrexone alone.

The mechanism mediating the efficacy for combination of naltrexone and topiramate is still unknown. Navarette et al. (2013) demonstrated that this combination reduced tyrosine hydroxylase in the ventral-tegmental area further than either alone, while simultaneously reducing ethanol reinforcement suggesting that its efficacy may be due to its effects on

ethanol-mediated dopamine release within the mesolimbic pathway. Tyrosine hydroxylase has also been shown to be upregulated after chronic ethanol exposure (Ortiz et al. 1995), *along with the dysfunction of other pathways, such as GABA and opioid peptides that occur in this allosteric state* (Koob, 2003). *More research is needed on ethanol's effects after long-term exposure compared to shorter lengths of chronic ethanol, which are most often used in studies with animal models.* Thus, the combination may be more effective at counteracting changes within *these pathways* induced by prolonged and heavy ethanol experience. How these medications interact to produce or modulate potential side effects is also of interest, and further research is needed.

A limitation of the present study is that only one dose of each treatment was examined. By using low doses, we hoped to more clearly parse out potential synergistic effects of the combination treatment. Topiramate (10 mg/kg) and naltrexone (1 mg/kg) at these doses were ineffective at reducing ethanol consumption on their own, and only through co-administering them did we see a significant effect. Naltrexone alone had a robust effect on ethanol reinforcement, and its effect was not enhanced through the addition of topiramate. This suggests that different mechanisms underlie ethanol consumption versus reinforcement. *For example, the combination of topiramate and naltrexone could be working synergistically to reduce ethanol consumption by reducing its rewarding properties and modulating other components that drive ethanol consumption, such as through withdrawal-relief, which may block compensatory drinking behavior. If different neurotransmitter systems contribute to distinct components of ethanol consumption, this combined medication targeting multiple neurotransmitter pathways is likely a better pharmacotherapeutic approach. Alternatively, this paradigm may not have been sensitive to shorter term effects of naltrexone alone on ethanol consumption given that intake was measured over a 24-hr period.* It would be interesting to determine whether higher doses of these drugs used in combination would have produced a further reduction of ethanol reinforcement over naltrexone alone. However, as we saw non-selective effects on food and water (naltrexone only) from these low doses, higher doses would likely produce even greater side effects.

In summary, these findings support the hypothesis that the combination treatment would be more efficacious than either naltrexone (1 mg/kg) or topiramate (10 mg/kg) alone. The combination effectively and persistently reduced consumption; whereas, neither dose alone was effective. Both the combination and naltrexone alone were equally effective at reducing ethanol reinforcement; however, this effect was selective for ethanol following treatment with the combination, but not naltrexone alone. Additionally, although the higher dose of topiramate (20 mg/kg) also effectively reduced ethanol consumption and reinforcement, the combination produced a more consistent reduction in ethanol consumption as well as a more robust effect on ethanol reinforcement. Interestingly, although both the combination and naltrexone reduced ethanol reinforcement on the day of administration, only the combination's effects on reinforcement translated to an effect on consumption, and this effect persisted for 3 days following treatment. These findings showing increased efficacy with similar, if not fewer, side-effects support the combination of naltrexone and topiramate as a promising new treatment for multiple components of AUDs.

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Highlights

Combination medications show potential in treatment of alcohol use disorders (AUDs).

Naltrexone and topiramate have each shown promise for treating AUDs on their own.

We tested a lose-dose combination of these medications on ethanol self-administration.

We found this combination to be more effective and/or selective than either alone.

The topiramate-naltrexone combination shows promise as a potential treatment for AUDs.

Fig. 1.

The effect of treatment with topiramate (10 or 20 mg/kg), naltrexone (1 mg/kg), and a combination of topiramate and naltrexone (10 mg/kg/1 mg/kg, respectively) on ethanol consumption in P rats, *measured at 24-hour intervals*. (a) Data are plotted as mean ($\pm$ SEM) of the daily g/kg ethanol consumption at baseline (Base), on the day of treatment (0), and for 3 days thereafter (1, 2, 3). (b) Decreases in ethanol consumption are shown as a percent change from baseline for the treatment day and 3 sessions post treatment. An asterisk (*) denotes a significant decrease from baseline when compared to vehicle $p < .05$. A number sign (#) denotes a trend for a decrease from baseline. Each data point represents an N of between 8 and 10. Veh, Vehicle; 10-Top, Topiramate-10 mg/kg; Nal, Naltrexone- 1 mg/kg; 10-Top+Nal, Topiramate 10 mg/kg and Naltrexone 1 mg/kg; 20-Top, Topiramate- 20 mg/kg.

Fig. 2.

The effect of treatment with topiramate (10 mg/kg), naltrexone (1 mg/kg), and a combination of topiramate and naltrexone (10 mg/kg/ 1 mg/kg, respectively) on ethanol consumption in Wistar rats *measured at 24-hour intervals* (a) Data are plotted as mean ($\pm$ SEM) of the daily g/kg ethanol consumption at baseline (Base), on the day of treatment (0), and for 3 days thereafter (1, 2, 3). (b) Decreases in ethanol consumption shown as a percent change from baseline for the treatment day and 3 sessions post treatment. Each data point represents an *N* of between 8 and 10. 10-Top, Topiramate-10 mg/kg; Nal, Naltrexone-1 mg/kg; 10-Top+Nal, Topiramate 10 mg/kg and Naltrexone 1 mg/kg.

Fig. 3.

The effect of treatment with topiramate (10 or 20 mg/kg), naltrexone (1 mg/kg), and a combination of topiramate and naltrexone (10 mg/kg/ 1 mg/kg, respectively) on PR responding for ethanol. (a) Data are plotted as mean ($\pm$ SEM) of the ethanol deliveries obtained at baseline (Base) on the day of treatment (0) and for 3 days thereafter (1, 2, 3). (b) Decreases in ethanol deliveries are shown as a percent change from baseline for the treatment day and 3 sessions post treatment. An asterisk (*) denotes a significant decrease from baseline when compared to vehicle and an ampersand (&) denotes a significant decrease from 20 mg/kg (p 's < 0.05). A number sign (#) denotes a trend for a decrease from baseline. Each data point represents an N of between 15 and 22. Veh, Vehicle; 10-Top, Topiramate-10 mg/kg; Nal, Naltrexone- 1 mg/kg; 10-Top+Nal, Topiramate 10 mg/kg and Naltrexone 1 mg/kg; 20-Top, Topiramate- 20 mg/kg.

Total ethanol consumption (g/kg) and consumption of 8% v/v and 16 v/v concentrations of ethanol for P rats and Wistar rats, before and 24-hr after vehicle treatment and treatment with the combination of topiramate (10 mg/kg) and naltrexone (1 mg/kg).

Table 1

		P Rats			Wistar Rats		
		Baseline	Acute Tx	Change (%)	Baseline	Acute Tx	Change (%)
Vehicle	Total g/kg	6.5 (0.5)	6.5 (0.5)	1.0 (4.4)	2.0 (0.2)	2.2 (0.3)	4.8 (13.4)
	8% g/kg	3.4 (0.5)	2.7(0.5)	-13.1 (13.6)	0.9 (0.2)	0.9 (0.2)	-0.5 (20.5)
	16% g/kg	3.1 (0.5)	3.8 (0.5)	26.3 (26.7)	1.1 (0.1)	1.3 (0.2)	5.7 (15.8)
	EtOH Preference (%)	94.6 (0.8)	92.9 (1.4)	-2.1 (1.3)	37.4 (3.9)	35.1 (4.7)	-7.8 (9.7)
	8% Preference (%)	59.8 (6.1)	54.9 (8.1)	-0.76 (22.8)	23.6 (4.9)	21.0 (4.2)	-0.3 (16.3)
	16% Preference (%)	34.8 (6.0)	37.1 (8.8)	7.7 (19.4)	13.8 (2.0)	14.0 (1.9)	3.5 (17.5)
10-Top + Nal	Total g/kg	6.8 (0.5)	5.6 (0.9)	-20.0 (8.5)*	1.7 (0.1)	1.6 (0.2)	-8.7 (6.5)
	8% g/kg	3.3 (0.7)	2.9 (0.6)	-23.3 (13.8)	0.6 (0.1)	0.7 (0.1)	-0.8 (14.6)
	16% g/kg	3.4 (0.4)	2.8 (0.7)	-29.4 (12.8)*	1.1 (0.1)	0.9 (0.1)	-11.8 (14.3)
	EtOH Preference (%)	94.6 (1.0)	87.7 (2.5)	-7.1 (1.7)	29.2 (2.5)	26.9 (3.1)	-8.2 (5.4)
	8% Preference (%)	56.7 (6.9)	56.8 (8.3)	0.29 (17.1)	14.1 (1.9)	15.9 (2.9)	6.9 (15.4)
	16% Preference (%)	37.9 (6.4)	30.9 (6.5)	-6.5 (22.6)	15.1 (2.5)	11.0 (1.7)	-4.5 (17.9)

An asterisk (*) denotes a significant decrease from baseline (%) when compared to vehicle. Tx, Treatment; 10-Top+Nal, Topiramate-10 mg/kg and Naltrexone-1 mg/kg.

Table 2

Food and water consumption in the home cage for P rats from Experiments 1 and 2, as well as water deliveries obtained during PR sessions in Experiment 2. Data are presented as mean (SEM) at baseline and on the day of treatment.

Treatment	Exp	Home Cage Consumption				PR Responding	
		Food Consumption (g)		H ₂ O Consumption (ml)		Water Deliveries	
		Baseline	Acute Tx	Baseline	Acute Tx	Baseline	Acute Tx
Vehicle	1	18.1 (0.4)	18.4 (0.7)	19.2 (0.5)	19.5 (0.9)	--	--
	2	16.5 (0.7)	16.8 (1.0)	2.4 (0.3)	3.2 (0.6)	4.3 (0.2)	4.2 (0.3)
10-Top	1	18.4 (0.5)	15.8 (0.9)*	19.9 (0.6)	21.1 (0.8)	--	--
	2	15.9 (0.8)	16.2 (0.9)	2.6 (0.3)	3.7 (0.7)	4.5 (0.3)	4.6 (0.3)
Nal	1	18.2 (0.5)	15.9 (0.8)*	20.3 (0.7)	17.8 (1.2)	--	--
	2	16.1 (0.8)	16.2 (1.1)	2.5 (0.3)	3.0 (0.4)	4.0 (0.4)	2.5 (0.5)*
10-Top+Nal	1	18.7 (0.5)	14.3 (0.9)*	19.9 (1.4)	18.7 (1.2)	--	--
	2	15.8 (1.0)	15.8 (1.3)	2.5 (0.3)	3.8 (0.5)	4.7 (0.3)	4.3 (0.4)
20-Top	1	17.0 (0.4)	13.8 (0.7)*	22.0 (0.5)	21.5 (0.8)	--	--
	2	14.2 (0.5)	12.3 (0.8)	2.9 (0.8)	3.5 (1.0)	2.8 (0.2)	2.5 (0.3)

An asterisk (*) denotes a significant decrease from baseline when compared to vehicle. Exp, Experiment; Veh- Vehicle; 10-Top, Topiramate-10 mg/kg; Nal, Naltrexone- 1 mg/kg; 10-Top+Nal, Topiramate 10 mg/kg and Naltrexone 1 mg/kg; 20-Top, Topiramate- 20 mg/kg.