

# Effect of Acamprosate on Magnetic Resonance Spectroscopy Measures of Central Glutamate in Detoxified Alcohol-Dependent Individuals

## *A Randomized Controlled Experimental Medicine Study*

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**Context:** Acamprosate is approved for the treatment of alcoholism, but its mechanism of action remains unclear. Results of animal studies suggest that a persistent hyperglutamatergic state contributes to the pathogenesis of alcoholism and that acamprosate may exert its actions by intervening in this process. Human translation of these findings is lacking.

**Objective:** To examine whether acamprosate modulates indices of central glutamate levels in recently abstinent alcohol-dependent patients as measured using proton nuclear magnetic resonance spectroscopy (<sup>1</sup>H-MRS).

**Design:** A 4-week, double-blind, placebo-controlled, randomized controlled experimental medicine study, with <sup>1</sup>H-MRS measures obtained on days 4 and 25.

**Setting:** An inpatient research unit at the NIH Clinical Center.

**Patients:** Thirty-three patients who met the DSM-IV criteria for alcohol dependence and who were admitted for medically supervised withdrawal from ongoing alcohol use.

**Intervention:** Four weeks of acamprosate (initial oral loading followed by 1998 mg daily) or matched placebo, initiated at the time of admission.

**Main Outcome Measures:** The glutamate to creatine ratio as determined using single-voxel <sup>1</sup>H-MRS in the anterior cingulate. Exploratory neuroendocrine, biochemical, and behavioral outcomes were also collected, as were safety- and tolerability-related measures.

**Results:** There was a highly significant suppression of the glutamate to creatine ratio across time by acamprosate (time × treatment interaction:  $F_{1,29} = 13.5$ ,  $P < .001$ ). Cerebrospinal fluid levels of glutamate obtained in a subset of patients 4 weeks into abstinence were uncorrelated with the MRS measures and unaffected by treatment but were strongly correlated ( $R^2 = 0.48$ ,  $P < .001$ ) with alcohol dependence severity. Other exploratory outcomes, including repeated dexamethasone–corticotropin-releasing hormone tests, and psychiatric ratings were unaffected. Among tolerability measures, gastrointestinal symptoms were significantly greater in acamprosate-treated individuals, in agreement with the established profile of acamprosate.

**Conclusion:** The MRS measures of central glutamate are reduced across time when acamprosate therapy is initiated at the onset of alcohol abstinence.

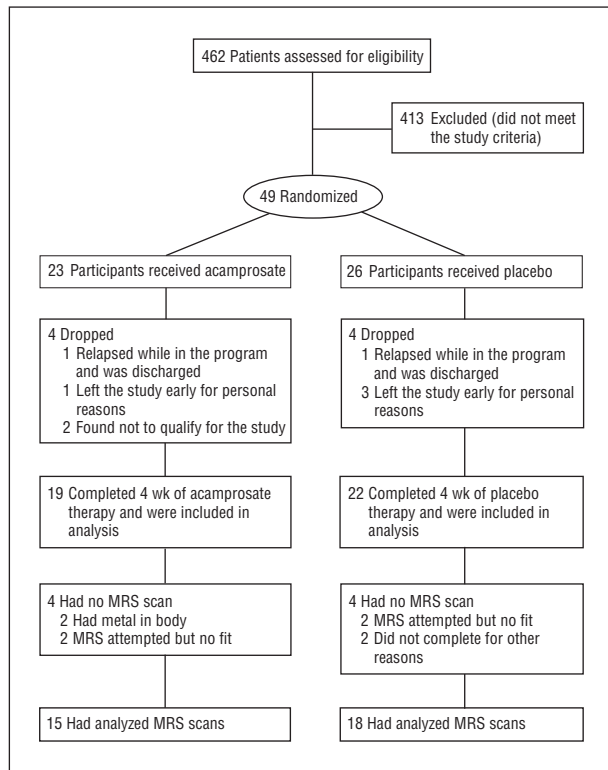
**Trial Registration:** clinicaltrials.gov Identifier: NCT00106106

*Arch Gen Psychiatry.* 2010;67(10):1069-1077

**D**YSREGULATION OF CENTRAL glutamatergic function has been proposed as a key factor underlying the neural and behavioral abnormalities of alcoholism and as a mechanism that could be targeted by pharmacotherapies.<sup>1-4</sup> Brain microdialysis in experimental animals has directly shown a progressive increase in extracellular glutamate with consecutive cycles of intoxication and withdrawal.<sup>5</sup> A persistent hyperglutamatergic state may be

linked to progression of dependence because it is expected to result in chronic Ca<sup>2+</sup> (calcium ion)–mediated hyperphosphorylation of the transcription factor CREB,<sup>6</sup> a molecular switch into a state of impaired reward circuitry function.<sup>7</sup> The persistent switch from low to high alcohol preference that occurs in experimental animals after prolonged intermittent brain alcohol exposure is accompanied by upregulation of glial glutamate transporter (GLAST) gene expression.<sup>8</sup> Upregulation of GLAST expression has also

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**Figure 1.** CONSORT (Consolidated Standards of Reporting Trials) graph showing the flow of the study participants. MRS indicates magnetic resonance spectroscopy.

been reported in postmortem brain tissue from alcohol-dependent individuals.<sup>9</sup> GLAST is critical for clearance of extracellular glutamate,<sup>10</sup> and its upregulation after prolonged brain alcohol exposure presumably reflects an adaptation to elevated extracellular glutamate levels. However, despite the extensive animal literature pointing to a role of glutamatergic dysregulation in the pathogenesis of alcohol dependence, limited human data are available to translate these findings.

A translational tool to address the role of glutamatergic dysregulation in alcoholism may be offered by acamprosate, a medication approved for the treatment of alcohol dependence that reduces craving and relapse.<sup>11</sup> Despite some negative studies,<sup>12</sup> meta-analysis of available studies supports the efficacy of acamprosate to increase abstinence.<sup>13</sup> Severity of dependence may be a critical factor in determining the efficacy of acamprosate because the medication robustly suppresses escalated alcohol intake in rats with a prolonged history of dependence but is ineffective in nondependent rats consuming modest amounts of alcohol.<sup>8</sup> Acamprosate modulates glutamatergic transmission through presently unknown, possibly multiple actions.<sup>4</sup> A foundation for translational research on the role of glutamatergic function in alcohol dependence was provided by experiments with null mutants for the clock gene *Per2*. In these animals, elevated brain levels of extracellular glutamate were found by microdialysis due to impaired glutamate clearance by GLAST. As predicted, these rats showed markedly elevated levels of alcohol intake. Acampro-

sate normalized the elevated glutamate levels in the *Per2* mutants, and this was accompanied by a marked reduction in voluntary alcohol intake.<sup>14</sup> These findings prompt the hypothesis that the clinical efficacy of acamprosate in humans may be related to an ability to suppress central glutamatergic transmission.

Indices of central glutamate levels can be obtained in humans using proton nuclear magnetic resonance spectroscopy (<sup>1</sup>H-MRS). This approach faces considerable technical challenges and is complicated by the fact that synaptic glutamate comprises only a minute fraction of the total glutamate in the brain. Despite these challenges, the neurobiologic relevance of MRS-generated brain glutamate indices is suggested, for example, by consistent findings of decreased glutamatergic measures in the anterior cingulate of individuals with major depression<sup>15-17</sup> and by the observation that effective electroconvulsive therapy normalized these MRS findings in depressed patients.<sup>16</sup> A previous MRS study<sup>18</sup> has suggested acute modulation of central glutamate levels by intravenous acamprosate infusion in a small group of healthy volunteers. A challenge for MRS studies of brain glutamate has been to resolve measures of glutamate from those of glutamine. Published studies,<sup>18,19</sup> including that examining the effects of acamprosate in healthy volunteers,<sup>18</sup> have, therefore, typically reported a composite measure, commonly referred to as *Glx*, that originates from the C2 protons common to glutamate and glutamine and is detected at a chemical shift of 3.75 ppm.

Recently, advances in spectrum acquisition and analysis techniques have allowed the isolation of an unobstructed glutamate signal that originates from the C4 proton of glutamate, detected at 2.35 ppm.<sup>20</sup> Herein, we used this technique to evaluate whether acamprosate modulates central glutamate levels in alcohol-dependent individuals after the initiation of abstinence. Two secondary objectives were also addressed. First, we examined whether MRS indices of central glutamate are related to levels of glutamate obtained in cerebrospinal fluid (CSF) or other patient characteristics. Second, we explored whether central glutamate level is related to measures of corticotropin-releasing hormone (CRH) (also referred to as corticotropin-releasing factor) system function. Neuroadaptations that encompass the CRH system are key in alcohol dependence,<sup>6</sup> and activity of glutamatergic synapses can be directly modulated by CRH.<sup>21</sup> We, therefore, used the dexamethasone-CRH test, a neuroendocrine probe of CRH function,<sup>22</sup> and examined whether dexamethasone-CRH responses would be affected by acamprosate in parallel with MRS measures of central glutamate.

## METHODS

### PARTICIPANTS AND CLINICAL ASSESSMENTS

The flow of participants through the study is shown in **Figure 1**, and descriptive participant characteristics are given in **Table 1**. Alcohol-dependent individuals in early withdrawal were admitted to a 28-day inpatient protocol at the National Institute on Alcohol Abuse and Alcoholism Inpatient Unit in the NIH Clinical Center. All participants underwent telephone pre-

screening. Individuals were excluded if they had received any psychiatric medications in the 2 weeks preceding the study or had a severe psychiatric illness, such as dementia or a psychotic disorder. Pregnant women and those with severe complicating medical conditions or human immunodeficiency virus infection were also excluded. To be eligible, individuals had to be in significant withdrawal, with a Clinical Institute Withdrawal Assessment of Alcohol Scale (CIWA-Ar)<sup>23</sup> score greater than 8, or have a blood alcohol level greater than 0.10 g/dL on admission and be expected to experience significant alcohol withdrawal. (Complete eligibility criteria are available at <http://www.clinicaltrials.gov>.) Informed consent was obtained in accordance with the Declaration of Helsinki and the NIH institutional review board.

Participants were assessed using the *Structured Clinical Interview for DSM-IV Axis I Disorders*<sup>24</sup> and the Addiction Severity Index.<sup>25</sup> Severity of alcohol dependence was assessed using the Alcohol Dependence Scale (ADS),<sup>26</sup> and alcohol consumption during the preceding 3 months was quantified using timeline follow-back.<sup>27</sup> Withdrawal intensity was evaluated using the CIWA-Ar Scale every 4 hours while awake for the first 5 days after admission. Diazepam was given as necessary at CIWA-Ar scores greater than 15. Total benzodiazepine dose and the dose received in the 24 hours before the first scan were recorded and used to control for possible medication effects in subsequent analyses. Psychiatric symptoms were assessed twice weekly using the self-report version of the Comprehensive Psychopathological Rating Scale.<sup>28</sup> A clinical blood chemistry panel was obtained weekly from each patient. Sleep quality, a potentially important manifestation of central nervous system excitability, was assessed using the Pittsburgh Sleep Quality Index (PSQI).<sup>29</sup> Visual analog scales (VASs) were used to assess common aspects of general well-being. Throughout the study, all individuals participated in a standard behavioral inpatient alcohol rehabilitation program but did not receive any prescription medications other than diazepam as described previously herein. Vitamin B<sub>1</sub> (thiamine hydrochloride) supplementation was provided according to clinical guidelines.

## PHARMACOLOGIC INTERVENTION

After achieving a blood alcohol level of 0 g/dL, participants were randomized to receive acamprosate or matching placebo. Randomization was conducted by the NIH Clinical Center pharmacy and was isolated from investigators and clinical staff. Double-blinding was achieved by encapsulating commercially obtained acamprosate and manufacturing matching placebo capsules. For individuals randomized to receive active treatment, the first 3 acamprosate doses were 1332 mg every 8 hours in an attempt to more rapidly achieve active plasma concentrations, followed by 666 mg of acamprosate every 8 hours for the remainder of the study. Plasma concentrations of acamprosate were determined on days 2, 4, and 26 by the SWEDAC (Swedish Board for Accreditation and Conformity Assessment)-accredited Clinical Pharmacology Laboratory of the Karolinska Institute, Stockholm, Sweden.

## MAGNETIC RESONANCE SPECTROSCOPY

The MRS was performed on days 4 and 25 after initiation of randomized treatment. Scans were performed using a 3-T scanner and the echo time-averaged PRESS sequence previously published to detect the resonance line of glutamate at 2.35 ppm and average out the interferences from glutamine, N-acetylaspartate (NAA), and the macromolecules<sup>20</sup> (**Figure 2**). The acquisition parameters were repetition time, 3 seconds; echo

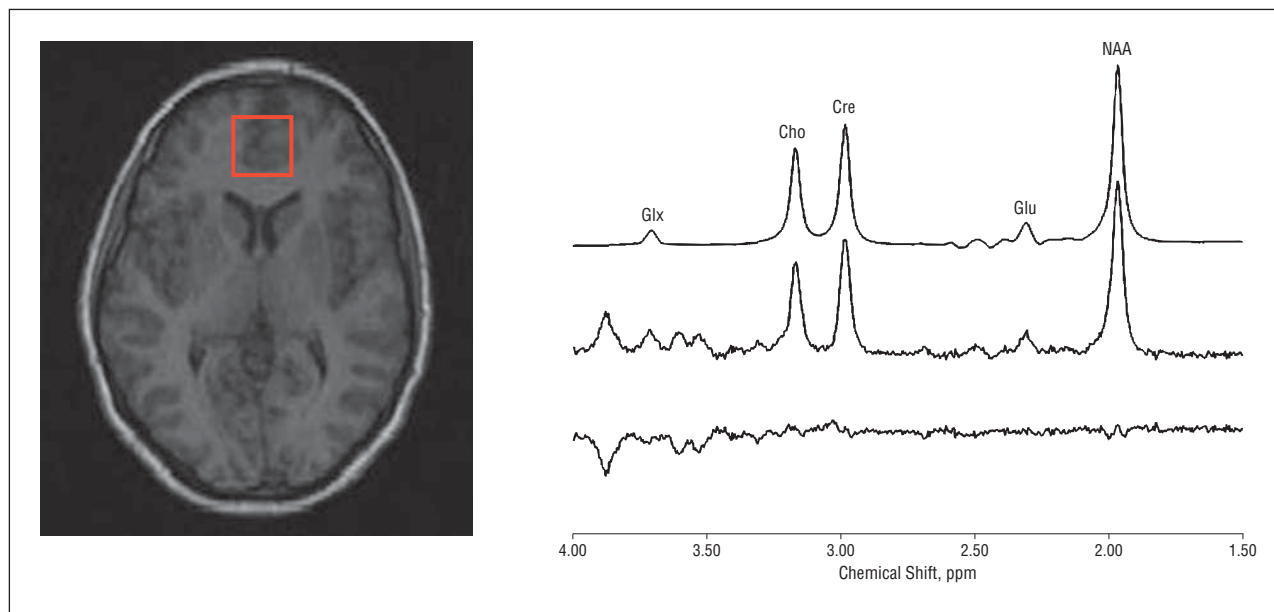
**Table 1. Baseline Descriptive Variables for the Participants Who Completed Both MRS Scans and Generated Spectra of Acceptable Quality for Analysis**

Variable	Acamprosate Group (n=15)	Placebo Group (n=18)
Sex, M/F, No.	9/6	10/8
Race, No.		
White	9	13
Black	6	5
Age, mean (SEM), y	32.7 (1.3)	34.0 (1.2)
Weight, mean (SEM), kg	74.6 (4.3)	72.0 (3.9)
Body mass index, mean (SEM) <sup>a</sup>	24.4 (1.0)	23.8 (1.0)
Addiction Severity Index score, mean (SEM)		
Alcohol	0.67 (0.04)	0.73 (0.05)
Drug	0.14 (0.04)	0.10 (0.04)
Employment	0.75 (0.07)	0.61 (0.07)
Family	0.38 (0.06)	0.32 (0.05)
Legal	0.24 (0.08)	0.13 (0.05)
Medical	0.42 (0.10)	0.21 (0.07)
Psychological	0.40 (0.07)	0.29 (0.04)
Alcohol Dependence Scale score, mean (SEM)	28.00 (1.77)	25.72 (1.31)
Timeline follow-back, 90 d, mean (SEM)		
Total drinks, No.	1015.01 (142.05)	1198.71 (136.05)
Drinking days, No.	69.29 (5.70)	79.00 (4.61)
Heavy drinking days, No.	62.71 (7.75)	78.65 (4.62)
Drinks/d, No.	14.48 (1.78)	14.57 (1.41)
Initial blood alcohol, mean (SEM), g/dL	0.11 (0.03)	0.10 (0.03)
Peak score on the Clinical Institute Withdrawal Assessment of Alcohol Scale, mean (SEM)	14.60 (1.06)	12.83 (1.39)
Diazepam required for withdrawal, No.	13	7
Total diazepam dose in entire group, mean (SEM), mg	22.0 (8.4)	22.8 (7.7)
Smokers, No.	13	15
Fagerström score for nicotine dependence, mean (SEM)	3.8 (0.8)	3.8 (0.7)
Comorbid diagnoses, No.		
Mood disorder	7	11
Anxiety disorder	5	8
Other substance use disorders	9	9
Laboratory values, mean (SEM)		
Serum GGT	79.87 (23.13)	120.94 (34.51)
Serum albumin	4.18 (0.08)	4.31 (0.09)
Serum ALT/GPT	72.33 (14.78)	65.89 (14.56)
Serum ALP	71.27 (4.00)	70.39 (4.96)
Serum AST/GOT	80.00 (14.36)	95.17 (23.24)
CDT%	0.14 (0.03)	0.10 (0.02)

Abbreviations: ALP, alkaline phosphatase; ALT/GPT, alanine transaminase, also known as glutamic pyruvate transaminase; AST/GOT, aspartate transaminase, also known as glutamic oxaloacetic transaminase; CDT, carbohydrate-deficient transferrin; GGT, gamma-glutamyltranspeptidase; MRS, magnetic resonance spectroscopy.

<sup>a</sup>Calculated as weight in kilograms divided by height in meters squared.

interval, 6 milliseconds; echo number, 32; and excitation number, 4. Measurement was made from a 2.5 × 2.5 × 2.5-cm<sup>3</sup> voxel in the area of the anterior cingulate (Figure 2). We chose the anterior cingulate region for MRS because the frontal lobe has been implicated in alcoholism.<sup>30</sup> Locating the MRS voxel in the anterior cingulate ensured that the data were collected from a



**Figure 2.** Proton nuclear magnetic resonance spectroscopy was performed using a 3-T scanner and the echo time-averaged PRESS sequence, which generates a chemical shift signal for glutamate without contamination from glutamine. Measurement was made from a single  $2.5 \times 2.5 \times 2.5\text{-cm}^3$  voxel in the area of the anterior cingulate (red box, inset). A sample spectrum is shown. From top to bottom: the automatically fitted model, the raw spectrum, and the residual of the automatic fit and raw spectrum. Cho indicates choline (3.35 ppm); Cre, creatine (3.02 ppm); Glu, glutamate (2.35 ppm); Glx, composite glutamate and glutamine (3.75 ppm); and NAA, *N*-acetylaspartate (2.02 ppm). See the "Magnetic Resonance Spectroscopy" subsection of the "Methods" section for details.

homogeneous tissue region that contained predominantly gray matter. Technical details about the MRS analysis are provided in the eAppendix (<http://www.archgenpsychiatry.com>).

#### CSF SAMPLING AND GLUTAMATE ANALYSIS

Samples of CSF were obtained, as previously described,<sup>31</sup> on days 5 and 26 after initiation of treatment (in each case, 1 day after MRS). Briefly, on the morning of the study, participants remained in bed except for a brief use of the restroom at approximately 7 AM. At approximately 9:30 AM, blood samples were collected by venipuncture immediately before lumbar puncture. Lumbar puncture was performed in the left lateral decubitus position. After obtaining 5 mL for clinical analysis, 12 mL of CSF was collected in a single aliquot, thoroughly mixed, and immediately placed on ice and quickly stored at  $-70^\circ\text{C}$ . Analysis of CSF glutamate was performed as described elsewhere.<sup>32</sup> Briefly, a 10- $\mu\text{L}$  aliquot of sample was derivatized with 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate and was analyzed using ultraperformance liquid chromatography (Aquity UPLC; Waters Corp, Milford, Massachusetts) with fluorescent detection using an amino acid kit (MassTrak; Waters Corp).

#### DEXAMETHASONE-CRH TEST

The dexamethasone-CRH test<sup>22</sup> was performed, as described elsewhere,<sup>33</sup> on day 6 or 7 and then again on day 27 or 28 after the initiation of treatment (in each case, 1 day after CSF sampling). Briefly, participants received 1.5 mg of dexamethasone (Dexacortol; Organon, Oss, the Netherlands) at 11 PM. The next day, a standard lunch was served, and an intravenous catheter was inserted before 2 PM. Participants remained in bed. Baseline blood samples were collected at 3 PM, followed by the administration of 100  $\mu\text{g}$  of human CRH and serial blood collections for the analysis of corticotropin and cortisol during the next 3 hours.

#### STATISTICAL PROCEDURES

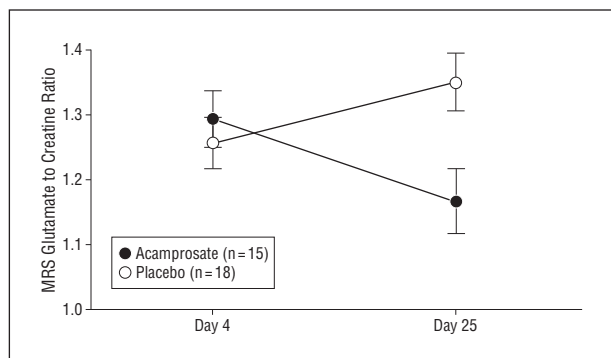
Individuals who received medication through the 4 weeks of the experimental study ( $n=41$ ) were considered for the analyses. The final MRS analysis was restricted to 33 of the 41 completers for whom measurable spectra could be obtained on both scans (Figure 1). For the behavioral measures, some questionnaires were not returned or were incorrectly filled out, leading to exclusion of that individual, as reflected in the degrees of freedom indicated for the respective analysis.

Data were examined for homogeneity of variances and distribution and were analyzed using general linear models (Statistica 6.0; StatSoft, Tulsa, Oklahoma). One-way (baseline characteristics) or repeated-measures (primary and secondary outcomes) analysis of variance was used, the latter with treatment (acamprosate vs placebo) as a between-subjects factor and time as a within-subject factor. According to a predefined data analysis plan, potential contributing variables (baseline characteristics, smoking status, diagnoses of comorbid psychiatric disorders, initial and peak CIWA-Ar Scale scores, and benzodiazepine dose) were evaluated by initial inclusion in the model and were retained if they contributed significantly or reduced the residual variance; they were otherwise dropped from the model. Baseline data and secondary outcomes were corrected for multiplicity of testing using the Holm-Bonferroni method.<sup>34</sup> The VAS ratings of tolerability were not corrected to avoid a bias against detecting adverse effects.

### RESULTS

#### BASELINE CHARACTERISTICS AND PLASMA CONCENTRATIONS OF ACAMPROSATE

The randomized groups did not differ on baseline variables, including alcoholism severity and withdrawal, neither in the subgroup of participants who successfully com-



**Figure 3.** Using magnetic resonance spectroscopy (MRS), suppression of glutamate levels across time was observed in the cingulate cortex of acamprosate-treated alcohol-dependent patients after the initiation of abstinence ( $P < .001$ ). Data are given as means. Error bars represent SEM. For detailed statistics, see the “Main Outcome: MRS Measures of Glutamate” subsection of the “Results” section.

pleted both MRS scans and generated spectra that could be analyzed (Table 1) nor in the full set of participants who received medication through the 4 weeks of the study (eTable). Among participants with 2 viable MRS scans, a larger proportion of patients taking acamprosate vs placebo required 1 or more benzodiazepine doses for initial withdrawal treatment (Fisher exact test, 2-tailed:  $P = .03$ ). However, the mean total benzodiazepine dose did not differ between the groups (Table 1 and eFigure), and the 9 patients who received any dose within 24 hours of the first MRS scan were evenly distributed between the groups (5 in the acamprosate group and 4 in the placebo group). By the time of the second MRS scan, all participants had been free of benzodiazepines for a minimum of 3 weeks. Steady-state acamprosate levels were achieved as predicted by pharmacokinetic modeling of the loading procedure. Mean (SEM) plasma concentrations of acamprosate were 248.2 (29.0) ng/mL on day 2, 250.6 (43.2) ng/mL on day 4, and 246.4 (26.7) ng/mL on day 26 of the study. Acamprosate was undetectable at any time point in the plasma of 5 randomly selected placebo-treated individuals included in the analysis as negative controls.

### MAIN OUTCOME: MRS MEASURES OF GLUTAMATE

The MRS glutamate measure showed good reliability, with a coefficient of variation of 13%. Acamprosate robustly suppressed central glutamate levels across time as measured by MRS (treatment  $\times$  time interaction:  $F_{1,29} = 13.5$ ,  $P < .001$ ) (Figure 3). Post hoc tests (Newman-Keuls) showed that on the second scan, the control glutamate levels in the acamprosate group were significantly lower than those in the placebo group ( $P = .04$ ). The effect size for this reduction, as measured using Cohen  $d$ , was approximately 0.95, that is, “large.” The acamprosate group also showed a significant decrease in central glutamate levels from the first to the second scan ( $P = .04$ ). In contrast, the placebo group showed a trend toward an increase in central glutamate levels across time ( $P = .09$ ). In this model, sex was retained because it somewhat reduced residual variance, although the original model that

**Table 2. Specificity of Glutamate Findings Assessed by Additionally Analyzing the Ratios of *N*-acetylaspartate (NAA) and Choline (Cho) to Creatine (Cr)<sup>a</sup>**

Measure	$F^b$	$df$	$P$ Value	Scan	Mean (SEM)	
					Acamprosate Group	Placebo Group
NAA to Cr	0.53	1,31	.47	1	1.42 (0.04)	1.41 (0.03)
				2	1.50 (0.03)	1.45 (0.03)
Cho to Cr <sup>c</sup>	0.11	1,30	.78	1	0.89 (0.04)	0.88 (0.04)
				2	0.87 (0.03)	0.88 (0.03)

<sup>a</sup>In contrast to the glutamate to creatine ratio, the corresponding metric for the other metabolites was stable across time and unaffected by treatment.

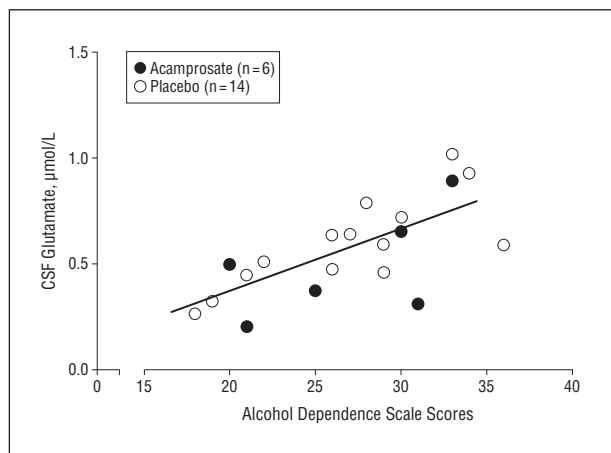
<sup>b</sup>Results are presented for the time (scan 1 vs scan 2)  $\times$  treatment interaction effect. There were no significant main effects of time or treatment for either measure.

<sup>c</sup>Analysis of the Cho ratio included age as a covariate in the model, hence the different degrees of freedom.

did not include sex was also significant ( $F_{1,31} = 10.1$ ,  $P = .003$ ). None of the other potentially contributing variables was significant, showed a trend for significance, or reduced residual variance. Variables that did not contribute significantly to the model ( $P > .50$ ) were measures of withdrawal severity (initial and peak CIWA-Ar Scale scores), benzodiazepine treatment (yes/no), the total benzodiazepine dose received, and the benzodiazepine dose received in the 24 hours before the scan, indicating that neither withdrawal intensity nor medication effects were confounds in the MRS analysis. Furthermore, results were not affected by comorbid diagnoses of mood, anxiety, and other substance use disorders or by smoking status (yes/no) or level of nicotine dependence (measured using the Fagerström score). The acamprosate effect was also robust in that it remained when the analysis was restricted to individuals in whom automated fit of spectra was successful (n=27; placebo group: 15; acamprosate group: 12; treatment  $\times$  time:  $F_{1,23} = 5.9$ ,  $P = .02$ ). Because the MRS measure of glutamate is based on the ratio of glutamate to creatine, we also explored whether the results might be confounded by changes in creatine levels. In contrast to its effect on the glutamate to creatine ratio, acamprosate had no significant or trend-level effect on the NAA to creatine ratio or the choline to creatine ratio, and these measures were also stable across time (Table 2). This suggests that the effect of acamprosate to suppress the glutamate to creatine ratio is specific and unlikely to be caused by changes in creatine levels.

### EXPLORATORY SECONDARY OUTCOMES

Levels of CSF glutamate in the subset of participants in whom both spinal taps were successful (n=20, placebo group: 14, acamprosate group: 6) were unaffected by treatment ( $F_{1,17} = 0.23$ ,  $P = .63$ ). Furthermore, in participants in whom both measures were obtained, CSF glutamate showed no correlation or trend for correlation with MRS measures in the early ( $R^2 = 0.03$ ,  $P = .46$ ) or the late ( $R^2 < 0.01$ ,  $P = .97$ ) phase. However, a highly significant correlation between alcohol dependence severity, measured as ADS scores, and CSF glutamate level



**Figure 4.** Cerebrospinal fluid (CSF) levels of glutamate were successfully measured 4 weeks after the initiation of abstinence in a subset of patients (n=20). Levels of CSF glutamate were unaffected by acamprosate treatment and did not correlate with brain magnetic resonance spectroscopy measures. However, severity of alcohol dependence, as measured using the Alcohol Dependence Scale, and CSF glutamate were strongly correlated, irrespective of treatment ( $R^2=0.48$ ,  $P<.001$ ). Additional analyses suggested that this did not reflect other patient characteristics.

was found on the second spinal tap ( $R^2=0.48$ ,  $P<.001$ ) (**Figure 4**). Because this correlation included patients receiving acamprosate and placebo, it was further explored using stepwise regression, with treatment and baseline characteristics as predictors. Forward and backward procedures converged on a model that retained only the ADS score as a predictor. In contrast to the correlation between ADS and central glutamate level found on the second spinal tap, no such correlation was found on the first spinal tap ( $R^2=0.22$ ,  $P=.30$ ).

Diurnal cortisol level was unaffected by treatment in early withdrawal (main effect:  $F_{1,21}=0.87$ ,  $P=.36$ ; treatment  $\times$  time:  $F_{7,147}=0.98$ ,  $P=.45$ ) and late withdrawal (main effect:  $F_{1,25}=1.09$ ,  $P=.31$ ; treatment  $\times$  time:  $F_{7,175}=1.91$ ,  $P=.07$ ). Similarly, corticotropin and cortisol responses in the dexamethasone-CRH test were unaffected by treatment in the early study phase (corticotropin: main effect,  $F_{1,26}=0.01$ ,  $P=.92$ ; treatment  $\times$  time,  $F_{9,234}=1.79$ ,  $P=.07$ ; cortisol: main effect,  $F_{1,27}=0.94$ ,  $P=.34$ ; treatment  $\times$  time,  $F_{9,243}=1.58$ ,  $P=.12$ ) and the late study phase (corticotropin: main effect,  $F_{1,27}=0.82$ ,  $P=.37$ ; treatment  $\times$  time,  $F_{9,243}=0.90$ ,  $P=.53$ ; cortisol: main effect,  $F_{1,27}=0.02$ ,  $P=.89$ ; treatment  $\times$  time,  $F_{9,243}=0.40$ ,  $P=.94$ ). There was also no treatment effect when corticotropin and cortisol responses were analyzed as area under the curve (data not shown).

#### SAFETY, TOLERABILITY, AND BEHAVIORAL MEASURES

No serious adverse effects were associated with acamprosate treatment. None of the participants dropped out because they could not tolerate acamprosate, although 1 individual taking placebo dropped out after 2 days because she thought that the study drug was causing auditory hallucinations. Acamprosate was well tolerated. We found no treatment effects on withdrawal ratings, psychiatric symptoms or sleep (CIWA-Ar Scale, Compre-

hensive Psychiatric Rating Scale, or PSQI), or blood chemistry measures. Among VAS measures, there was a main treatment effect ( $F_{1,34}=5.37$ ,  $P=.03$ ) and a treatment  $\times$  time interaction ( $F_{4,136}=3.05$ ,  $P=.02$ ) on "sleepiness" and a main treatment effect ( $F_{1,34}=4.7$ ,  $P<.04$ ) and a treatment  $\times$  time interaction ( $F_{4,136}=2.40$ ,  $P=.05$ ) on "stomachaches" but not on other measures. Patients taking acamprosate showed higher sleepiness on day 8 (Newman-Keuls post hoc test,  $P=.01$ ) but not on subsequent days. Stomachaches were absent during the first week but arose with time and were significantly higher in patients taking acamprosate on day 18 (Newman-Keuls post hoc test,  $P=.02$ ). There was no treatment effect of acamprosate on the amount of benzodiazepines required to treat withdrawal ( $F_{1,39}=0.03$ ,  $P=.87$ ).

#### COMMENT

The key finding of the present study is that acamprosate, given to alcohol-dependent individuals on initiation of abstinence, markedly suppressed MRS measures of central glutamate during 4 weeks of treatment. Advances in spectrum acquisition and analysis allowed us to obtain a glutamate signal mostly uncontaminated by glutamine. Because steady-state plasma concentrations of acamprosate were reached already on day 2, the delayed onset of acamprosate action is unlikely to be explained by pharmacokinetics. Interpretation of the MRS measures on day 4 of acamprosate treatment may be complicated because severity of alcoholism, acute withdrawal, benzodiazepines, and acamprosate may all have an effect on the anterior cingulate cortex glutamate concentration at this time point. For 2 reasons, we do not believe that these are major limitations. First, measures of dependence severity, withdrawal intensity, and benzodiazepine use did not contribute to the results when included in the analysis. Second, and more important, no effect of acamprosate was observed at the early time point, when the impact of these confounds might be expected. Instead, the acamprosate effect was observed at a time when participants had been free of withdrawal symptoms and benzodiazepine for approximately 3 weeks. Furthermore, analyses of other brain metabolites, such as NAA and choline, suggest that the acamprosate effect is specific and is not driven by changes in, for example, creatine levels.

Limited data are available on MRS measures of glutamate in alcohol-dependent individuals during withdrawal and early protracted abstinence. One previous study,<sup>19</sup> using a combined glutamate plus glutamine measure, did not find changes across time in alcohol-dependent individuals or a difference between patients and controls after controlling for tissue composition. This study did find an effect of smoking status on the combined MRS measure. We, therefore, controlled for smoking status as a potential confound in the current study but did not find any effect of this factor. Because all but 5 of the participants were smokers, we did not have adequate power to assess an independent effect of smoking, which was not an objective of this study. Another article<sup>35</sup> reported serial MRS scans in alcohol-

dependent individuals during early abstinence but did not provide measures of glutamate or glutamine, which were stated to be too hard to resolve.

Although important methodological differences exist, a picture of acamprosate action emerges from the present findings that is in general agreement with available animal data.<sup>3,4,36</sup> When alcohol abstinence is initiated in alcohol-dependent individuals, brain levels of glutamate show a tendency to increase in placebo-treated individuals but are suppressed by acamprosate treatment. These data were obtained from the cingulate cortex, and it remains to be established whether other brain areas are similarly affected. Nevertheless, to the extent that a persistent rise in the glutamate level contributes to craving and relapse in alcoholism, as has been commonly hypothesized, these data support the notion that acamprosate may exert its therapeutic effect by counteracting this pathophysiologic process. These data do not directly address whether elevated levels of brain glutamate are present in alcohol-dependent patients compared with healthy participants. The spectroscopic method used to determine central glutamate levels relies on a ratio vs creatine. The present control data with NAA and choline ratios make it unlikely that acamprosate treatment per se would confound the glutamate measure by affecting creatine levels. It remains unknown, however, whether alcohol dependence might affect the spectroscopy results, for example, through structural changes, in ways that would differentially affect the glutamate and the choline signal, making a comparison between alcohol-dependent and healthy individuals difficult to interpret.

A hyperglutamatergic state has also been implicated in the hyperexcitability of alcohol withdrawal.<sup>3,4,36</sup> The ability of acamprosate to suppress central glutamate levels might, therefore, also be expected to suppress acute alcohol withdrawal symptoms. However, in agreement with a previous study,<sup>37</sup> we did not find an acamprosate effect on acute withdrawal. These findings are consistent with the delayed nature of the acamprosate effect on central glutamate levels observed in this study. Acute withdrawal symptoms subside within 3 to 5 days, whereas no effect of acamprosate on MRS measures of central glutamate was found after 4 days. Acamprosate was inactive at this time point despite steady-state plasma levels that were ultimately effective. Pharmacokinetics could, nevertheless, be relevant for the lack of acamprosate effect on acute withdrawal because it is not known whether brain concentrations are in immediate equilibrium with the plasma compartment. Alternatively, the delayed onset of acamprosate action may reflect a slow, possibly indirect pharmacodynamic mechanism. Finally, the present evaluation of sleep quality using the PSQI also did not show a significant effect of acamprosate. This is in contrast to a previous study<sup>38</sup> in which acamprosate had a beneficial effect on polysomnographic measures of sleep architecture. The delayed nature of acamprosate actions may be equally critical in this case because the previous study<sup>38</sup> initiated acamprosate treatment 8 days before the onset of withdrawal.

We found that CSF levels of glutamate were not correlated with central glutamate levels as measured using

MRS and were unaffected by acamprosate treatment. In contrast, CSF glutamate levels in protracted withdrawal were strongly correlated with severity of alcohol dependence. Two mutually nonexclusive mechanisms may account for these observations. First, increased CSF levels of glutamate are observed in stroke and trauma, where they are caused by an efflux of cytosolic glutamate from neurons and astrocytes.<sup>39</sup> Significant loss of gray matter occurs across time in alcoholism,<sup>30</sup> and animal models have directly demonstrated cell death after a period of intoxication.<sup>40,41</sup> Thus, an efflux of cytosolic glutamate from damaged cells, similar to that observed with trauma and stroke, might occur after a period of intoxication. Second, a gradient of glutamate is normally maintained between blood and the CSF compartment by an energy-dependent transport mechanism across the choroid plexus endothelium, protecting the nervous system from high (approximately 0.2mM) plasma glutamate concentrations. Thiamine deficiency resulting from heavy alcohol use results in an impaired ability of choroid plexus endothelium cells to maintain the blood-CSF glutamate gradient.<sup>42</sup> If the degree of this impairment increases with the severity of alcohol dependence, a correlation of the type that was observed would be expected. Although we provided standard clinical thiamine supplementation, it has been suggested that impairment of choroid plexus endothelium-dependent transport in chronic alcohol dependence may become lasting.<sup>42</sup>

Both of the mechanisms discussed herein point to sources of CSF glutamate other than the transmitter pool. The observation that CSF glutamate levels were unaffected by the pharmacologic intervention is also consistent with this notion. In contrast, the pool of glutamate reflected by MRS was uncorrelated with CSF levels and was sensitive to acamprosate. This indicates that it originates from a pool distinct from that contributing to glutamate in CSF and one that is likely to be more closely related to neurotransmission. Finally, the present data leave unresolved why CSF levels of glutamate in protracted but not early abstinence were strongly correlated with alcohol dependence severity. It may be speculated that CSF levels in early withdrawal are mostly affected by state-related factors with high individual variability, such as nutritional status. After a month in the highly standardized environment of the inpatient care unit, state-related factors may play a lesser role, whereas the remaining variance is, to a higher extent, accounted for by traitlike factors, such as alcoholism severity.

Finally, upregulation of hypothalamic-pituitary-adrenal axis reactivity, measured by dexamethasone-CRH responses, has been reported in the first weeks after initiation of alcohol abstinence<sup>43</sup> and may offer a window on central CRH activity, which is suggested by animal studies to be upregulated after a prolonged history of brain alcohol exposure.<sup>44</sup> We, therefore, explored whether a modulation of dexamethasone-CRH test responses by acamprosate would indicate that glutamatergic and CRH-related neuroadaptations in alcoholism are related to each other. However, in agreement with another recent study,<sup>45</sup> we found no effect of acamprosate on the dexamethasone-CRH response in neither early

nor protracted abstinence. Probes more directly tapping into central CRH function, such as positron emission tomography ligands for central CRH receptors, may be needed to address a possible relationship between neuroadaptations that encompass glutamate and CRH systems in alcoholism.

In conclusion, we find that <sup>1</sup>H-MRS is a valuable non-invasive translational tool to study measures of glutamate function in alcoholism. Although it cannot be excluded that this finding reflects a lowering of glutamate levels by acamprosate therapy in a compartment not directly relevant to neurotransmission, this interpretation is made less likely by the concordance between the present finding and available animal data.<sup>5,14</sup> Magnetic resonance spectroscopy offers an attractive surrogate marker for early human evaluation of candidate therapeutics that target the glutamatergic system.

**Submitted for Publication:** August 26, 2009; final revision received March 1, 2010; accepted April 6, 2010.

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**Author Contributions:** Drs Umhau, Momenan, and Schwandt contributed equally to the article.

**Financial Disclosure:** None reported.

**Funding/Support:** This study was supported by intramural research funding from the National Institute on Alcohol Abuse and Alcoholism.

**Online-Only Material:** The eAppendix, eTable, and eFigure are available at <http://www.archgenpsychiatry.com>.

**Additional Contributions:** Katy Rice, BA, assisted with data collection and entry; Monte J. Phillips, BS, and Karen Smith, MLS, helped with manuscript preparation; and NIH Clinical Center Pharmaceutical Development Services formulated the drug and the placebo.

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### Correction

**Errors in Text.** In the Original Article titled “Modification of Cognitive Performance in Schizophrenia by Complexin 2 Gene Polymorphisms” by Begemann et al, published in the September issue of the *Archives* (2010; 67[9]:879-888), 3 errors occurred in the text. On page 879, in the “Objective” section of the “Abstract,” the last sentence should have read, “Because synaptic dysfunction plays a key role in schizophrenia, the complexin 2 gene (*CPLX2*) was examined in the first phenotype-based genetic association study (PGAS) of GRAS.” On page 880, the first sentence of the first full paragraph should have read, “In the present study, we opted for an alternative approach to study genetic causes of the schizophrenic phenotype: a phenotype-based genetic association study (PGAS).” On page 883, the subheading in the right column should have read, “Phenotype-Based Genetic Association Study.”