

AMPA Receptor Potentiation can Prevent Ethanol-Induced Intoxication

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We present a substantial series of behavioral and imaging experiments, which demonstrate, for the first time, that increasing AMPA receptor-mediated neurotransmission via administration of potent and selective biarylsulfonamide AMPA potentiators LY404187 and LY451395 reverses the central effects of an acutely intoxicating dose of ethanol in the rat. Using pharmacological magnetic resonance imaging (phMRI), we observed that LY404187 attenuated ethanol-induced reductions in blood oxygenation level dependent (BOLD) in the anesthetized rat brain. A similar attenuation was apparent when measuring local cerebral glucose utilization (LCGU) via C¹⁴-2-deoxyglucose autoradiography in freely moving conscious rats. Both LY404187 and LY451395 significantly and dose-dependently reversed ethanol-induced deficits in both motor coordination and disruptions in an operant task where animals were trained to press a lever for food reward. Both prophylactic and acute intervention treatment with LY404187 reversed ethanol-induced deficits in motor coordination. Given that LY451395 and related AMPA receptor potentiators/ampakines are tolerated in both healthy volunteers and elderly patients, these data suggest that such compounds may form a potential management strategy for acute alcohol intoxication. *Neuropsychopharmacology* (2008) **33**, 1713–1723; doi:10.1038/sj.npp.1301562; published online 12 September 2007

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INTRODUCTION

Alcohol misuse has severe implications for individuals' health and places a heavy economic burden on primary health-care services. It is estimated that in the United States alone the annual health-care expenditure on alcohol misuse is over \$26 billion (Harwood, 2000) with accident and emergency departments placed under particular strain by intoxicated patients (Charalambous, 2002). Despite this, the cellular and molecular actions of alcohol are still not fully understood. There is, however, substantial evidence that the intoxicating effects of alcohol may be mediated, at least in part, through the glutamatergic system (Tsai *et al*, 1995). Historically, it was thought the most likely mechanism for this modulation was the inhibitory action of ethanol at the NMDA subtype of the glutamate receptor. Preclinical studies have shown acute exposure to ethanol dose-dependently attenuates NMDA-evoked currents in hippocampal (Lovinger *et al*, 1990) and locus coeruleus neurons (Frölich *et al*, 1994), blocks extracellular striatal increases in

glutamate caused by focal application of NMDA (Carboni *et al*, 1993), and protects against NMDA-induced convulsions (Kulkarni *et al*, 1990) in rats. Furthermore, the NMDA antagonists ketamine, MK-801, and phencyclidine substitute for ethanol in animal drug discrimination studies (Grant *et al*, 1991; Colombo and Grant, 1992; Schecter *et al*, 1993), and ketamine has ethanol-like effects in healthy subjects (Krystal *et al*, 1994) and recently detoxified alcoholics (Krystal *et al*, 1998).

More recently, however, evidence from *in vitro* studies has suggested ethanol may also modulate AMPA, as well as NMDA, receptor-mediated glutamatergic transmission. Ethanol attenuates AMPA-induced currents in rat CA1 hippocampal (Martin *et al*, 1995), cortical (Wirkner *et al*, 2000), septum/diagonal band (Frye and Fincher, 2000), and spinal cord motor neurons (Wang *et al*, 1999) and mouse cortical and hippocampal neurons (Möykkynen *et al*, 2003). Moreover, the degree of inhibition of these currents by ethanol in rat cortical neurons is equivalent to that observed on NMDA-induced currents (Wirkner *et al*, 2000). AMPA-induced depolarizations of rat locus coeruleus neurons are also depressed by ethanol (Wang *et al*, 1999), as are the amplitude of Ca²⁺ signals in response to AMPA in rat cerebellar Purkinje neurons (Gruol *et al*, 1997).

Although an attenuation of the hypothermic response to ethanol in mice lacking the AMPA GLUR1 receptor (Cowen *et al*, 2003) and a reduction in GLUR1 receptor levels

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following neonatal ethanol exposure in rat (Bellinger *et al*, 2002) have been reported, there is a surprising lack of *in vivo* studies investigating interactions between ethanol and AMPA receptor activity. We aimed to address this issue and report a series of imaging and behavioral studies demonstrating that increasing AMPA receptor-mediated neurotransmission via systemic administration of the biarylsulfonamide AMPA potentiators LY404187 and LY451395 attenuates the response to acute ethanol administration in the rat *in vivo*. LY404187 and LY451395 are potent, selective, and centrally active positive allosteric modulators of AMPA receptor-mediated neurotransmission, which increase ion channel flux in the presence of agonist by suppressing desensitization and/or deactivation of the receptors (O'Neill *et al*, 2004). *In vitro*, biarylsulfonamides increase AMPA currents in isolated hippocampal and Purkinje neurons (Gates *et al*, 2001) and AMPA receptor-mediated synaptic inputs in frontal cortex (fCTX) pyramidal neurons (Quirk and Nisenbaum, 2002). *In vivo* they are active in rodent models of memory and cognition (Quirk and Nisenbaum, 2002) and display an antidepressant profile in behavioral models (Li *et al*, 2001). They have also been shown to increase brain-derived neurotrophic factor (BDNF) both *in vitro* and *in vivo* (O'Neill *et al*, 2004).

We previously conducted a pharmacological magnetic resonance imaging (phMRI) study examining the effects of AMPA receptor-mediating compounds on blood oxygenation level-dependent contrast (BOLD), a proposed functional correlate of neuronal activity (Ogawa *et al*, 1992), in the rat brain (Jones *et al*, 2005). Further in-depth analyses of these data revealed LY404187 attenuated decreases in BOLD MR contrast that were apparent in animals receiving a vehicle solution containing 30% ethanol. To examine this interaction further, we undertook a series of experiments to determine whether AMPA receptor potentiation could reverse the effects of higher, intoxicating doses of ethanol in the freely moving, non-anesthetized rat. Using used C^{14} -2-deoxyglucose (C^{14} -2-DG) autoradiography we observed that ethanol produced widespread decreases in local cerebral glucose utilization (LCGU), which were selectively reversed by LY404187. In demonstration of the interaction between AMPA receptor potentiation and alcohol at a behavioral level, both LY404187 and LY451395 significantly and dose-dependently reversed ethanol-induced deficits in motor coordination and performance in an operant task where animals were trained to press a lever for food reward. LY404187 reversed ethanol-induced deficits in motor coordination when given both before and after ethanol. When administered alone, both compounds had negligible effects on behavior or LCGU. These data provide the first demonstration that increasing AMPA receptor-mediated neurotransmission can reverse the effects of an intoxicating dose of ethanol in the rat.

METHODS

Subjects

All experiments were conducted in accordance with the Animals (Experimental Procedures) Act, 1986, and local ethical requirements. Subjects for phMRI, C^{14} -2-deoxyglucose, rotorod, and tilt-plane studies were male Sprague–Dawley

rats weighing between 210 and 280 g at the start of testing. Food and water were available *ad libitum*. Animals used for VI-30 operant response studies were Lister Hooded rats weighing 250–320 g upon testing. They were kept on a food-restricted diet and maintained at no less than 85% of their free feeding weight. In the VI-30 experiments animals participated in several experiments, and thus had exposure to a variety of drugs at a rate of one dose per week. In all other experiments animals were experimentally naive and only participated in one experiment. Subjects for all experiments were group housed in a temperature-controlled environment (20–22°C).

Drugs

LY404187 and LY451395 were supplied by Eli Lilly and Co. Ltd, Indianapolis, IN. For the phMRI experiment LY404187 was dissolved in 30% ethanol and administered subcutaneously at a volume of 2.0 ml/kg. The 30% ethanol solution was equivalent to 0.6 g/kg ethanol. For the rotorod test LY404187 was dissolved in 5% DMSO and 24% hydroxy-propyl- β -cyclodextrin (Sigma, UK) and administered via oral gavage. For all other experiments LY404187 and LY451395 were dissolved in 5% DMSO and 24% hydroxy-propyl- β -cyclodextrin and administered subcutaneously at a volume of 2.0 ml/kg. Ethanol was diluted with distilled H_2O to a 20% v/v solution and injected intraperitoneally. For the C^{14} -2-DG, tilt plane, and rotorod tests the dose of ethanol was 2.0 g/kg, and for the variable interval 30 s (VI30) task the dose of ethanol was 1.0 g/kg. Corresponding vehicles served as control injections. All solutions were freshly prepared on test days.

Pharmacological MRI

Acquisition. All MRI measurements were performed using a 4.7 T Oxford 200/300 MkII (Oxford Instruments) superconducting magnet; hpag 18 (Oxford Instruments) combined gradients and shims (max 100 mT/m, 12 cm bore size); q63 (Varian) quadrature birdcage coil; and VNMR 6.1B (Varian) software. Animals were placed in an induction chamber and anesthetized with 4% isoflurane in 0.9 l/min medical air and 0.1 l/min medical oxygen before being secured in a stereotaxic head frame and placed into the scanner. To allow remote infusion of drugs during the scan subcutaneous cannulae attached to tubing, which ran outside the scanner, were inserted into each subject. Anesthesia was maintained at 1.2% isoflurane ventilated with the same complement of gases for the remainder of the experiment. Subjects were scanned using a continuous, three echo, gradient-echo (GE) sequence (TE = 5, 10, 15 ms; TR = 940 ms; acquisition matrix = $64 \times 64 \times 24$; FOV = 4 cm^2 ; yielding an isotropic voxel resolution of $0.5 \times 0.5 \times 0.5 \text{ mm}$). Brain volumes of 40 slices were acquired every minute for 180 min. Subjects received either LY404187 dissolved in 30% ethanol or 30% ethanol (equivalent to 0.6 g/kg) alone 30 min into the scan. Following acquisition of the GE time series, a higher in-plane spatial resolution 40 slice brain volume was acquired using a spin-echo anatomical sequence (TE = 40 ms, TR = 2 s, acquisition matrix = $128, 128, 24$; FOV = 4 cm^2 ; at a voxel resolution of $0.25 \times 0.25 \times 0.5 \text{ mm}$). Throughout the scanning procedure

subjects' body temperature was maintained at 37°C using a thermostatically controlled heating blanket with rectal temperature probe. Seven subjects per group were imaged.

Preprocessing. Data were preprocessed as previously described (Jones *et al*, 2005). Gradient-echo images underwent extensive preprocessing prior to statistical analysis. For each GE scan, mean-echo images with an effective TE of 10 ms were created by summation of the 5, 10, and 15 ms images. These images were individually masked with vascular masks derived by applying a coefficient of variance threshold of 15% to suppress signal changes associated with macroscopic vessels, minimizing the contamination of surrounding parenchyma associated with spatial smoothing. Using SPM99 (Institute of Neurology, UK), a motion-correction algorithm was applied to the image time series from each animal corrected for translational and rotational movements during data acquisition (<1 voxel in all animals). An automated Brain Extraction Tool outlined intracerebral structures, creating an extracerebral mask subsequently applied to each subjects' realigned time course. Finally, in SPM99, images were normalized to a rat brain template created by averaging the realigned time series of GE images from a randomly chosen single subject and Gaussian smoothed using a full-width half-maximum kernel of 1 mm ($\times 2$ in-plane resolution) to impose a normal distribution on the data.

Statistical Analysis

Post-processed MRI images were analyzed in SPM99 using fixed effects General Linear Model multisubject covariates designs. This approach conducts a voxel by voxel analysis of brain volumes from each subject at each time point and identifies voxels displaying temporal changes in signal intensity, which correlate with a specified input function. Individual subjects' data are then combined to create Statistical Parametric Maps (SPMs) for each group displaying statistically significant changes in BOLD contrast which correlate with the input function. The input function used here was interpolated from the pharmacokinetic profile of LY404187. The square of translations in the *x*, *y*, and *z* planes during acquisition were entered as covariates of no interest to account for possible confounding effects of motion. Statistical significance for the SPMs was set at $p < 0.05$, corrected for multiple comparisons.

To demonstrate signal change over time in the superior colliculus, an area showing significant changes in BOLD contrast in the SPM analyses, a region of approximately $1.5 \times 1.5 \times 1.5$ mm was manually created on a coronal section from the post-processed template image. The signal intensity of this region was then extracted from each volume for each subject over the 180 min time course using the MarsBar toolbox for SPM99 (Brett *et al*, 2002). The extracted values for each subject were scaled to the mean signal intensity of the baseline data (0–30 min) and then scaled to an arbitrary value. The time course for each group was then plotted over consecutive 15 min intervals.

***C*¹⁴-2-deoxyglucose autoradiography.** The method employed to study cerebral glucose utilization using *C*¹⁴-2-DG in freely moving rats was adapted from that previously

described (Duncan, 1992). Subjects ($n = 6$ –8 per group) were anesthetized with isoflurane/nitrous oxide/oxygen (induction: 3 l/min O₂, 5% isoflurane; maintenance: 3 l/min N₂O, 1.5 l/min O₂, 1.5–5% isoflurane, adjusted to each rat's respiration rate and reflex response). Blunt-dissection was performed to expose the left jugular vein. A cannula (Portex tubing, 0.58 mm \times 0.96 mm) containing heparinized saline was inserted into the jugular vein and secured with surgical thread. The cannula was exteriorized at the base of the neck between the ears. Rats were left for 2–4 h to recover at 37°C. Each animal was housed individually for 24–48 h prior to start of *C*¹⁴-2-DG experiments. At least 2 h prior to the start of the experiment, subjects were placed in observation bowls. They then received an injection of either LY404187 (1.5 mg/kg) or vehicle. After 30 min they were then administered either ethanol (2.0 g/kg) or water. After 25 min a total dose of *C*¹⁴-2-DG (100 μ Ci/kg; Amersham), in 0.5 ml heparinized saline, was injected into the jugular cannula over a 30 s period and the rats were returned to the observation bowls. After 5 min the animals were killed by 3 min exposure to 100% CO₂ and decapitation. The brains were immediately removed, frozen in isopentane, and stored at -70°C .

Coronal frozen sections (20 μ M) were prepared by cryostat, captured on slides (snow-coat extra microslides, Surgipath), and immediately dried at 60°C. The sections were subsequently exposed to Kodak Biomax MR autoradiographic film (Amersham) for ~ 10 days.

Statistical Analysis

*C*¹⁴-2-DG levels were measured in 20 distinct brain regions identified from coronal sections using the rat brain atlas of Paxinos and Watson (1986). Densitometric image analysis was performed using MCID elite software (Imaging research Inc.) using a reference curve of counts per minute vs optical density, calculated from β -emitting *C*¹⁴-microscale standards (Amersham) and used to semiquantify the intensity of signal expressed as nCi/g of tissue in each brain region of interest. To minimize the variation in *C*¹⁴-2-DG intensity between individuals in each group the data were expressed as a ratio of that measured in the corpus callosum for each rat. These data were then used to determine an overall mean intensity for each brain region of interest for the animals in each treatment group. The grouped data were then compared between treatments using one-way analysis of variance (ANOVA) (Neuman-Keuls *post hoc* analysis; $p < 0.05$).

Tilt-Plane Test

The tilt-plane test was based on the apparatus previously described by White *et al* (2002) and consisted of a clear glass box (60 \times 25 \times 20 cm) with a hinge at one end. The box was tilted via a wooden handle attached to the nonhinged end. The angle at which subjects began to slide was measured via a chart next to the handle that was marked in 0.5° units. Each test session consisted of three trials. Subjects were placed inside the glass box at the nonhinged end. The experimenter then slowly tilted the box until the subject lost their footing and began to slide down the apparatus toward the hinged end. The angle at which this

slip occurred was recorded to the nearest 0.5° units, the box was lowered and the procedure repeated a further two times. The mean slip angle from the three trials formed subjects' scores for each test session. If at any point a subject moved to the hinged end before a trial was started they were ushered back to the nonhinged end before the box was tilted. Prior to drug treatment all subjects were exposed to one test session to measure baseline performance.

Two tilt-plane experiments were conducted. The first examined the ability of LY404187 and LY451395 to reverse the behavioral effects of an acute ethanol challenge in the test. To investigate whether AMPA receptor potentiation had any prophylactic effect against the intoxicating effects of ethanol, we also conducted a second experiment where subjects were pre-dosed with LY404187 before being given the ethanol challenge and exposed to the apparatus. For the first experiment subjects received either LY404187 (0.5, 1.5, or 3.0 mg/kg), LY451395 (1.5 or 3.0 mg/kg), or vehicle followed, 30 min later, by a second injection of either ethanol (2.0 g/kg) or vehicle. After 15 min they were reexposed to the apparatus and tested every 15 min for 120 min. For the second tilt-plane experiment subjects received either LY404187 (1.5 or 3.0 mg/kg), ethanol (2.0 g/kg), or vehicle followed, 15 min later by a second injection of either LY404187 (1.5 or 3.0 mg/kg), ethanol, or vehicle. After a further 15 min they were reexposed to the apparatus and tested every 15 min for 120 min. The apparatus was cleaned between test sessions. All groups had a sample size of $n = 10$.

Statistical Analysis

Subjects' scores from each session were converted into percentages of their baseline scores. These data were then analyzed using a two-factor repeated measures ANOVA examining the effects of time and dose. Any significant main effects of dose were followed up with Tukey's HSD tests comparing experimental groups at each time interval with the α level set to 0.0001 to control for multiple comparisons.

Rotorod

Rotorod testing used an accelerating rotorod (Omnitech Electronics, Columbus, OH) connected to an IBM PC computer. For training and testing purposes, the rotorod was set up to accelerate to 17 r.p.m. in 5 s and maintain that speed for 40 s. Rats were given three training trials to learn to maintain posture on the rotorod prior to the actual day of drug testing. The following day subjects received either LY404187 (1.5 or 3.0 mg/kg) or vehicle followed by a second injection of vehicle or ethanol (2.0 g/kg) 60 min later. They were tested on the rotorod 10, 30, or 60 min following the second injection. Animals that maintained posture and did not fall off the rotorod were given a maximum score of 40 s.

Statistical Analysis

The time subjects maintained posture on the rotorod was converted into group means. These were analyzed by a one-way ANOVA examining the effects of dose followed by Dunnett's *t*-tests comparing individual groups.

VI30 Task

Testing was conducted in standard operant chambers housed in sound and light attenuation chambers (Med Associates, UK). Two retractable levers were located either side of a recessed magazine where food pellets (Noyes, 45 mg, Formula P) were delivered from an automatic pellet dispenser. Experimental sessions were controlled and data recorded using programs written in-house using MedPC IV software (Med Associates, UK). The number of correct lever presses made during a 30 min test session was measured.

Rats were kept for a period of at least 7 days before any operant training commenced. During this time, animals were acclimated to the food restriction regime and were handled during weighing and general husbandry procedures. Following this pretreatment period, rats underwent 2 days of magazine approach training. Each daily test session was 30 min long, and comprised the delivery of a pellet for a nose-poke response under a variable interval 60 s schedule of reinforcement (range = 15–105 s). Following magazine approach training, rats were pseudorandomly assigned a lever (left or right) to be reinforced on during lever-press training. Lever assignment was counterbalanced across each squad of animals tested (that is half of the squad received reinforcement for a left lever press, half for a right lever press). Both levers were presented during each training session. Each daily test session was 30 min long and comprised of the delivery of a food pellet for pressing the correct lever under a variable interval 30 s schedule of reinforcement (range = 7–53 s). Pressing the incorrect lever had no consequence. Animals received at least 11 days of lever-press training before their first drug experience. Thereafter, animals received 4 days of lever-press training between drug doses.

All groups had a sample size of $n = 9$ –12. For all experiments, data from the last lever-press training day were used to rank animals on the basis of correct lever-press response rate. Animals were pseudorandomly assigned to drug treatments with respect to these ranks. These data were analyzed to ensure that each treatment group was not significantly different from each other on this day.

For the study of the simple effects of the potentiators, two separate experiments were conducted. Animals received a dose of LY451395 (0.5, 1.5, or 3.0 mg/kg), LY404187 (1.5, 3.0, or 6.0 mg/kg), or vehicle in the holding room. They were returned to their cages for a 45 min pretreatment period. Following this pretreatment period, animals were transported to the test room and placed in operant chambers for testing to commence. Test sessions were 30 min long. Ethanol attenuation studies were conducted in a similar manner, except that when animals received an additional dose of ethanol (1.0 g/kg) or vehicle 15 min before being placed in the test boxes.

Statistical Analysis

A general linear model, *a priori* approach was used for all ANOVAs conducted for measured parameters. A one-way ANOVA, with 'dose' as a between-subjects factor was used. A significant main effect of dose was followed by planned comparisons against the vehicle group in simple studies or the vehicle/ethanol group in ethanol attenuation studies.

RESULTS

AMPA Receptor Potentiation Reverses Ethanol-Induced Decreases in BOLD Signal

During our previous experiment into the effects of AMPA receptor-mediating compounds on BOLD contrast (Jones *et al*, 2005), one group of rats received a vehicle solution comprised of 30% ethanol and 70% distilled H₂O and another group received the AMPA potentiator LY404187 (0.5 mg/kg) dissolved in this same vehicle to achieve solubility. Given that subjects had, therefore, received the equivalent of 0.6 g/kg ethanol, a dose higher than that shown to affect behavior in rats (Prediger and Takahashi, 2005), we considered it pertinent to subject these data to further analysis. In the previous study we employed a fixed-effects general linear model to create SPMs displaying changes in BOLD contrast in animals receiving LY404187 compared to those receiving vehicle. For the current analysis we examined changes in BOLD contrast in the vehicle and LY404187 groups independently. The SPM displaying significant changes in BOLD MR contrast in subjects receiving the ethanol vehicle alone revealed a widespread and significant (corrected $p < 0.05$) decrease in BOLD signal (Figure 1a). No increases in BOLD signal were apparent in this group. In contrast, subjects administered LY404187 (0.5 mg/kg) dissolved in the vehicle solution showed a marked attenuation of this decrease in BOLD MR signal (Figure 1b). Figure 1c shows the BOLD signal throughout the 180 min scan in the superior colliculus, a region displaying significant changes in BOLD contrast in the SPM analyses. Following injection a decrease in BOLD signal was observed in the ethanol vehicle group which was not apparent in animals receiving LY404187 dissolved in the ethanol vehicle.

AMPA Receptor Potentiation Attenuates Ethanol-Induced Decreases in Cerebral Glucose Utilization

In order to further examine neuronal interactions between AMPA receptor-mediated neurotransmission and ethanol in the freely moving, nonanesthetized rat we used semiquantitative C¹⁴-2-DG autoradiography to measure LCGU following LY404187, both alone and in combination with ethanol (Figure 2). As group data were expressed as a ratio of LCGU measured in the corpus callosum for each rat we analyzed absolute values of LCGU in this region. No significant group differences were observed. Thirty minutes after an acute injection of ethanol (2.0 g/kg), there was a widespread decrease in the levels of C¹⁴-2-DG across several brain regions when compared to rats given vehicle only. This reduction in regional glucose utilization was significant in the fCTX, cingulate cortex (cgCTX), dorsal striatum (dSTR), globus pallidus (GP), parietal cortex (pCTX), anteroventricular thalamic nucleus (AV), ventrolateral thalamic nucleus (VL), CA1 and CA2 regions of the hippocampus, hippocampal fissure (HF), substantia nigra *pars compacta* (SNc), and third cerebellar lobule (3CBL; see Figure 2 for a full list of abbreviations). The ethanol-mediated decreases in LCGU in the dSTR, GP, pCTX, AV, VL, HF, SNc, and 3CBL were reversed in rats receiving

LY404187 (1.5 mg/kg) 30 min prior to ethanol ($p < 0.05$). The ethanol-induced decreases in glucose utilization in the fCTX, cgCTX, NAc, CA1, reticular pontine nucleus (PN), and the medial geniculate nucleus (MGN) were not reversed by pretreatment with LY404187. There were small increases in LCGU in the GP, pCTX, AV, VL, MGN, and second cerebellar lobule (2CBL) in rats receiving a single injection of LY404187 in the absence of ethanol (compared with β -cyclodextrin + water control group), but these failed to reach significance.

AMPA Receptor Potentiation Reverses Ethanol-Induced Deficits in Motor Control

The ability of ethanol to disrupt tasks requiring balance and motor coordination is well documented across species (Arvola *et al*, 1958; Liguori *et al*, 1999). We therefore used two established tests of motor coordination, the tilt-plane and rotorod tests for our first investigations of the interaction between ethanol and AMPA receptor potentiation on a behavioral level.

Ethanol (2.0 g/kg) produced severe deficits in motor control in all the tilt-plane experiments. For the first experiment examining the ability of AMPA receptor potentiation to modulate ethanol's effects, pre-dosing with 1.5 and 3.0 mg/kg of both LY404187 (main effect of dose $F_{7,72} = 27.84$, $p > 0.001$) and LY451395 (main effect of dose $F_{5,54} = 44.82$, $p > 0.001$) 30 min before ethanol completely attenuated the ethanol-induced deficits (Figure 3). When we repeated the experiment to determine any prophylactic effects of AMPA receptor potentiation on the effects of ethanol, a similar reversal of these deficits was observed when LY404187 was administered 15 min after the ethanol challenge (main effect of dose $F_{6,63} = 43.55$, $p < 0.001$, Figure 4).

Similarly, ethanol (2.0 g/kg) disrupted performance in the rotorod test. LY404187 (1.5 and 3.0 mg/kg), when administered orally 60 min prior to ethanol, dose-dependently and significantly reversed the ethanol-induced impairment seen at 10, 30, and 60 min after the ethanol injection (all one-way ANOVA, $p < 0.05$, 30 min time point shown in Figure 5).

AMPA Receptor Potentiation Blocks Ethanol's Effects on Schedule Controlled Behavior

We then assessed the interaction between ethanol and AMPA receptor potentiation on schedule-controlled response to food reward. Subjects were trained for a minimum of 11 days to press one of two levers for a food pellet before being tested under a variable interval 30 s (VI30) schedule of reinforcement. As shown in Figure 6 both the 1.5 and 3.0 mg/kg doses of LY404187 and LY451395 (main effect of dose $F_{4,43} = 10.20$, $p < 0.001$) and the 6.0 mg/kg dose of LY404187 (main effect of dose $F_{4,42} = 11.68$, $p < 0.001$) significantly attenuated ethanol-induced deficits in correct lever responding. Neither LY404187 or LY451395 alone had a significant effect on baseline lever-press response rates (both $F < 1$) in the VI-30 assay.

DISCUSSION

Using two technically independent neuroimaging techniques and a range of behavioral measures we have

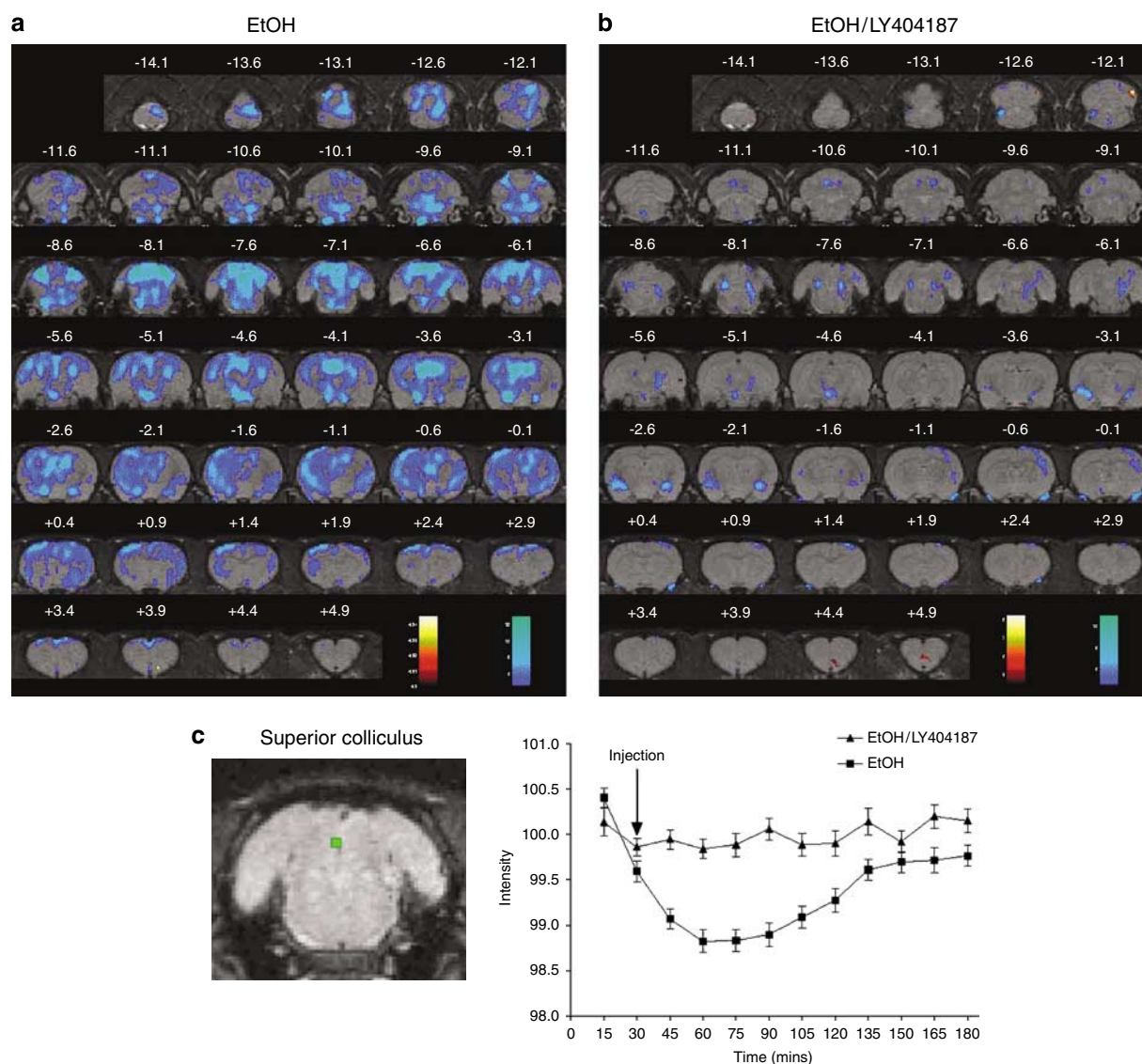


Figure 1 The effects of ethanol and LY404187 dissolved in ethanol (EtOH) on BOLD MR contrast in the rat brain. (a and b) are SPM {t} distribution maps of BOLD MR signal change overlaid onto coregistered spin-echo anatomical templates. (a) Animals receiving 30% EtOH and (b) Animals receiving LY404187 (0.5 mg/kg) in 30% EtOH (EtOH/LY404187). Colored pixels represent significant correlation (thresholded at $p < 0.05$ corrected for multiple comparisons, $T > 4.46$) of signal time course with input function (pharmacokinetic profile of LY 404187). Red represents positive correlation, blue represents negative correlation. Numerical values signify approximate distance from Bregma for each coronal section, according to brain atlas by Paxinos and Watson (1986). EtOH caused significant and widespread decreases in BOLD contrast, which were attenuated by co-administration of LY404187. (c) Changes in BOLD signal over time during the scan in a region showing significant change in the SPM analyses. The region was created on the coronal section -7.6 from bregma and corresponds to the coordinates for the superior colliculus (Paxinos and Watson, 1986). Data presented as group means (\pm SEM).

demonstrated, for the first time, that increasing AMPA receptor-mediated neurotransmission can reverse some of the acute effects of ethanol in the rat. The AMPA potentiator LY404187 reversed the decrease in BOLD MR signal observed in anesthetized rats receiving a vehicle solution equivalent to a low dose of ethanol (0.6 g/kg). Decreases in LCGU observed in freely moving conscious rats receiving 2.0 g/kg ethanol were also reversed by LY404187. Impaired performance in the tilt-plane test of motor control caused by ethanol (2.0 g/kg) was blocked by pretreatment with LY404187 and LY451395 and post-treatment with LY404187. Similarly, LY404187 reversed the effects of ethanol (2.0 g/kg)

on the rotarod test. LY404187 and LY451395 also attenuated the disruptions in previously learned operant responses observed following ethanol (1.0 g/kg). When administered alone, both compounds had negligible effects on behavior and LCGU.

The impairment of motor coordination and disruption of a previously learnt operant response following administration of 2.0 g/kg ethanol are consistent with previous reports that moderate to high doses of ethanol cause behavioral suppression and sedation (Khanna *et al*, 1998; White *et al*, 2002). Our observation that 2.0 g/kg ethanol reduced, to some extent, LCGU in all 19 of the regions analyzed also

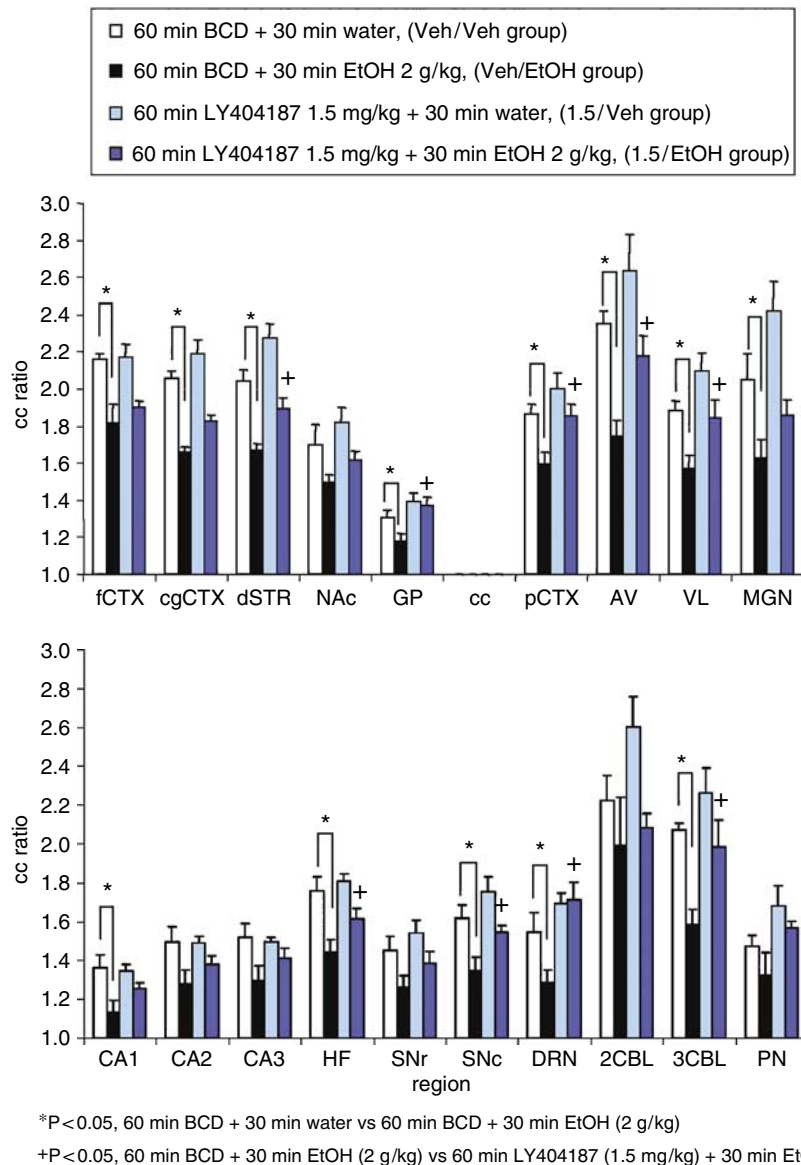


Figure 2 Effect of LY404187 on ethanol challenge in the C^{14} -2-deoxyglucose-treated freely moving rat. Brain regions are listed on the x axis: cingulate cortex (cgCTX), frontal cortex (fCTX), dorsal striatum (dSTR), nucleus accumbens (NAc), globus pallidus (GP), corpus callosum (CC), anteroventral (AV), and ventrolateral (VL) thalamic nuclei, parietal cortex (pCTX), hippocampal regions CA1, CA2, and CA3, the hippocampal fissure (HF; *stratum lacunosum moleculare*; SLM) of the hippocampus, medial geniculate nucleus (MGN), substantia nigra *pars reticulata* (SNr), and *pars compacta* (SNc), the dorsal raphe nucleus (DRN), second cerebellar lobule (2CBL), third cerebellar lobule (3CBL), and the reticular pontine nuclei (PN). The y axis is the amount of LCGU expressed as a ratio of the corpus callosum for each subject. Data are presented as group means (\pm SEM). In comparison to animals receiving the two vehicle solutions (Veh/Veh), LCGU was reduced in all brain regions in subjects receiving ethanol alone (Veh/EtOH). Some of these decreases were blocked by prior administration of LY404187 (1.5/EtOH). LY404187 alone (1.5/Veh) produced no significant changes in LCGU. Significant differences indicated on figure: * $p < 0.05$, Veh/Veh vs Veh/EtOH, + $p < 0.05$ Veh/EtOH vs 1.5/EtOH.

concurs with previous reports of widespread decreases in glucose metabolism following moderate to high doses of ethanol. The effects of ethanol on LCGU in rats appear to be biphasic with lower doses (< 1.0 g/kg) increasing LCGU in the mesocorticolimbic, limbic and extrapyramidal systems, and in neocortical and hypothalamic areas and doses more akin to that used in the current study (≥ 1.0 g/kg) causing decreases throughout limbic, hippocampal, extrapyramidal neocortical, thalamic, and auditory areas (Williams-Hemby and Porrino, 1994; Eckardt *et al*, 1998). Such effects are also

seen clinically with a 25% reduction in whole brain glucose metabolism along with multiple localized reductions including the occipital and parietal cortices, cerebellum, temporal cortex and limbic system reported in intoxicated healthy volunteers (Wang *et al*, 2000).

Given these relatively non-specific behavioral and neuro-anatomical consequences of intoxication it is unsurprising that ethanol's neurochemical effects are diverse and include modulations of GABAergic, opioid, dopaminergic and, serotonergic function (Anton, 1996). However, by demon-

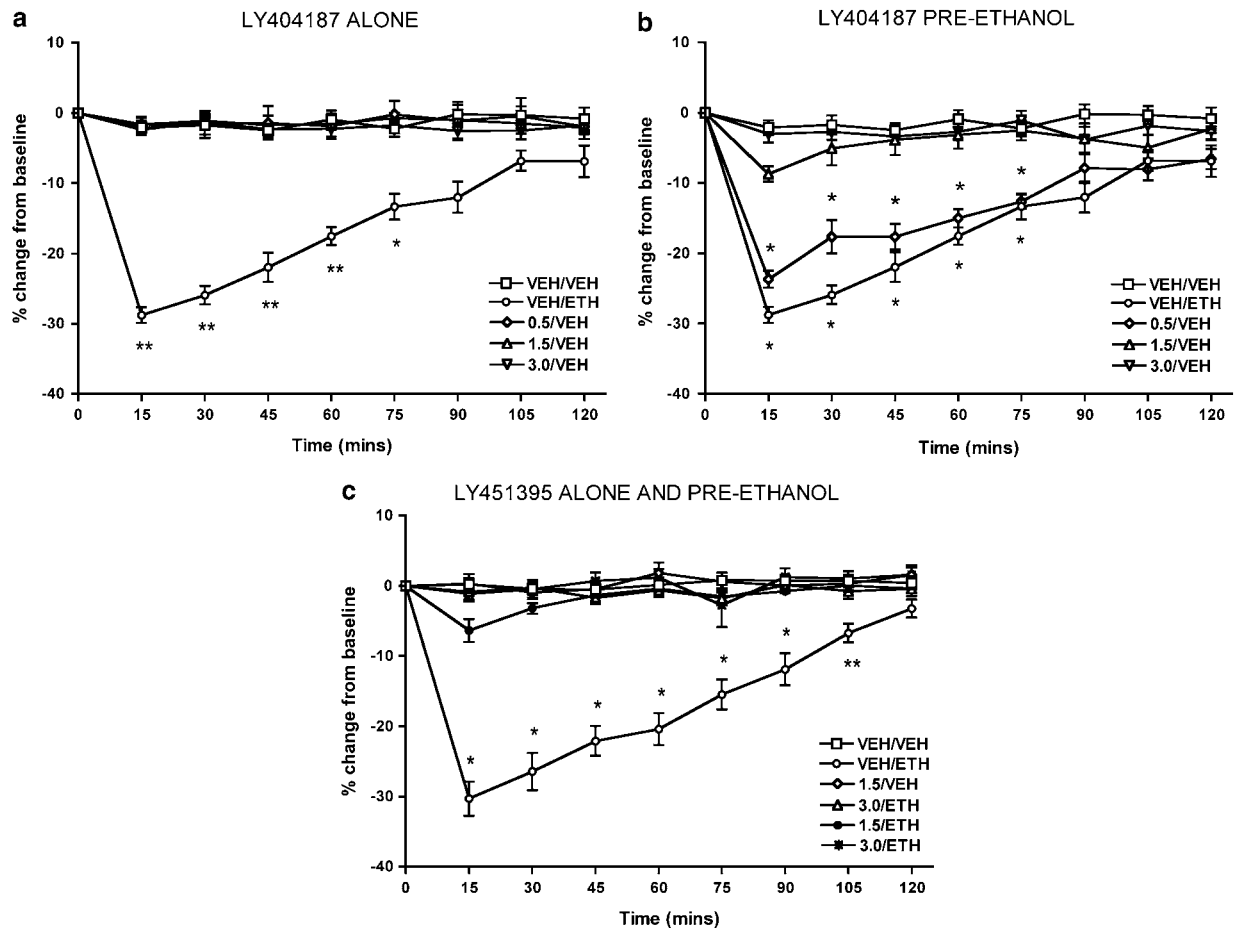


Figure 3 Effect of the AMPA potentiators LY404187 (a and b) and LY451395 (c) alone and prior to ethanol challenge on behavior in the tilt-plane test of motor coordination. Data are presented as group means (\pm SEM) of percentage change in performance compared to baseline (0 min). Ethanol alone (Veh/EtOH) induced deficits in motor coordination on the tilt-plane test that were attenuated by prior administration of 1.5 mg/kg (1.5/EtOH) and 3.0 mg/kg (3.0/EtOH) LY404187 and LY451395. Prior administration of 0.5 mg/kg LY404187 (0.5/EtOH) did not attenuate the effects of ethanol. When administered alone, no dose of LY404187 or LY451395 (0.5/Veh, 1.5/Veh, 3.0/Veh) had an effect on motor coordination. Significant differences (significance level set at $p < 0.0001$ to correct for multiple comparisons) indicated on (a) **Veh/EtOH different from all other groups, (b) *Veh/EtOH different from all groups except 0.5/EtOH, *0.5/EtOH different from all groups except Veh/EtOH, and (c) *Veh/EtOH different from all other groups, **Veh/EtOH different from Veh/Veh and 3.0/EtOH groups.

strating AMPA potentiators reverse ethanol's effects on LCGU, motor coordination and schedule controlled behavior we provide further corroboration that ethanol inhibits AMPA receptor-mediated glutamatergic neurotransmission (Martin *et al*, 1995; Wang *et al*, 1999; Frye and Fincher, 2000; Wirkner *et al*, 2000). Although more studies are needed to elucidate the exact nature of the interaction between ethanol and LY404187 and LY451395 at AMPA receptors, one possibility is that they have opposing effects on receptor desensitization. Following exposure to AMPA or glutamate, AMPA receptors undergo extremely rapid desensitization (Trussell and Fischbach, 1989). Biarylsulfonamide AMPA potentiators have no intrinsic action of their own at AMPA receptors, rather they increase receptor activity by suppressing this desensitization process. Ethanol's inhibition of isolated cortical neurons is reduced via co-administration of cyclothiazide which is also known to significantly reduce receptor desensitization. Furthermore, ethanol's inhibitory effects are dramatically reduced on mutant L497Y GluRA1 receptors which lack desensitization

(Möykkynen *et al*, 2003), suggesting a stabilization of the desensitization process directly opposed to the action of LY404187 and LY451395.

Although our pHMRI results suggest LY404187 reverses the effects of a 30% ethanol solution on BOLD MR contrast, interpretation of these data should be slightly more tentative. Studies examining ethanol's effects using changes in cerebral hemodynamics as markers of brain activity have provided somewhat conflicting results. Human fMRI studies have shown reduced activity in visual regions in response to photic stimulation (Levin *et al*, 1998) and visual perception tests (Calhoun *et al*, 2004) following alcohol, whereas both global and localized increases in regional cerebral blood flow have been observed in humans (Newlin *et al*, 1982; Tiihonen *et al*, 1994) and rats (Lyons *et al*, 1998) after ethanol challenge. One intrinsic confound for such studies is that ethanol itself is vasoactive rendering it potentially difficult to separate its direct effects on the vasculature from neurogenic effects. An additional confound for the current pHMRI study was the use of isoflurane

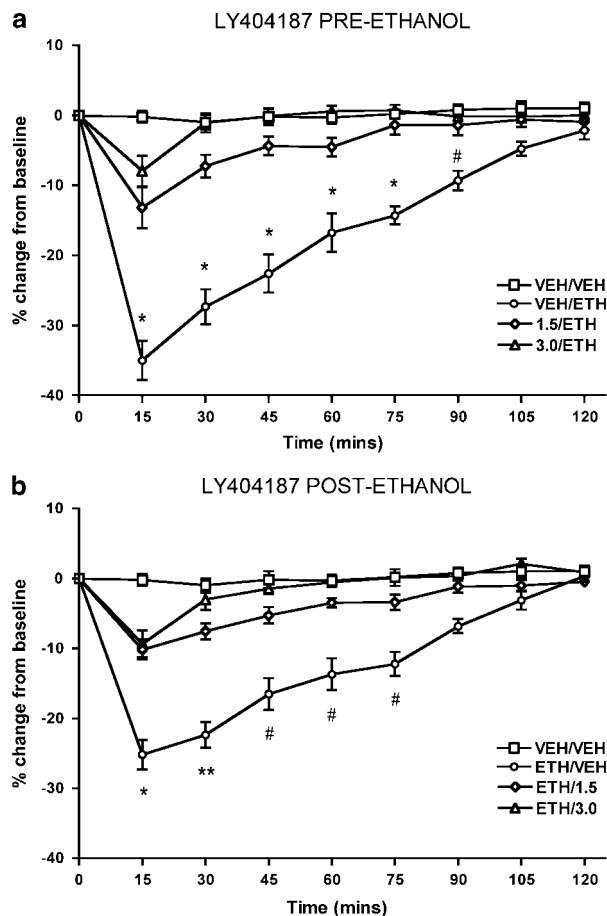


Figure 4 Effect of LY404187 administered before (a) and after (b) acute ethanol challenge on behavior in the tilt-plane test of motor coordination. Data are presented as group means (\pm SEM) of percentage change in performance compared to baseline (0 min). Ethanol alone (Veh/EtOH and EtOH/Veh) induced deficits in motor coordination on the tilt-plane test that were attenuated by 1.5 and 3.0 mg/kg LY404187 given both before (1.5/EtOH, 3.0/EtOH) and after (EtOH/1.5, EtOH/3.0) ethanol. Significant differences (significance level set at $p < 0.0001$ to correct for multiple comparisons) indicated on (a) *Veh/EtOH different from all other groups, #Veh/EtOH different from Veh/Veh and 3.0/EtOH groups, (b) *EtOH/Veh different from Veh/Veh, **EtOH/Veh different from all other groups, #EtOH/Veh different from Veh/Veh and EtOH/3.0 groups.

anesthesia to minimize subjects' head movement during the scan. The exact mode of action of isoflurane remains unclear, yet it is known to share discriminative properties with ethanol, suggesting it may also share its CNS depressant profile (Bowen and Balster, 1997). Hence, additive effects of ethanol and isoflurane cannot be discounted from the current results. However, given we also observed the reversal of ethanol's actions by AMPA receptor potentiation in conscious rats using C^{14} -2-DG, a marker of neuronal activity that is less affected by peripheral changes in cerebral hemodynamics, the existing literature on ethanol's inhibitory actions on AMPA receptors and the current behavioral results, we believe the pHMRI data lends further support to the suggestion that AMPA receptor potentiation reverses the CNS effects of ethanol.

The potential role of AMPA receptor-mediated neurotransmission in alcohol consumption and abuse is complex.

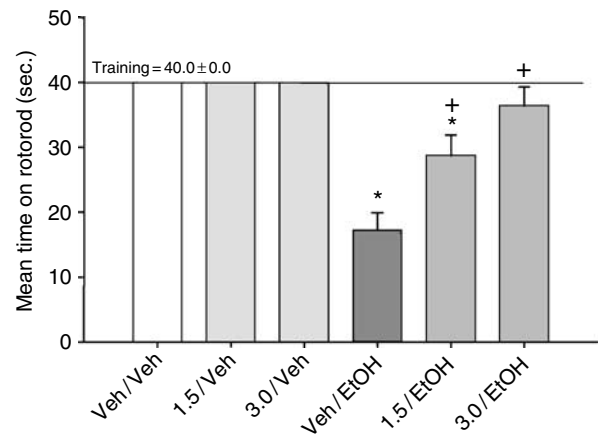


Figure 5 Effects of LY404187 on ethanol-induced deficits in the rotarod test of motor coordination. Data are presented as group means (\pm SEM). Ethanol alone (Veh/EtOH) induced deficits in motor coordination on the rotarod test in comparison to animals receiving the two vehicle solutions (Veh/Veh). These deficits were attenuated by prior administration of 1.5 mg/kg (1.5/EtOH) and 3.0 mg/kg (3.0/EtOH) LY404187. LY404187 administered alone had no effect at either 1.5 mg/kg (1.5/Veh) or 3.0 mg/kg (3.0/Veh). Significant differences indicated on figure * $p < 0.05$ compared to Veh/Veh group, + $p < 0.05$ compared to corresponding time point in the Veh/EtOH group.

Preclinical evidence suggests AMPA receptor antagonists, which reduce cue-induced reinstatement of alcohol-seeking behavior in rats, may form novel therapeutic targets for preventing relapse in chronic alcohol abusers (Sanchis-Segura *et al*, 2006). Conversely, our findings that increasing AMPA receptor activity reverses the effects of a single dose of ethanol raise the intriguing possibility that AMPA potentiators may form a clinical intervention for acute alcohol intoxication. Although clinical trials have been conducted using agents that attenuate some of the behavioral symptoms of acute intoxication by increasing ethanol and acetaldehyde plasma clearance, such as metadoxine (Shpilenny *et al*, 2002), there remains, to date, no treatment capable of reversing the central effects of the drug in man. The benzodiazepine partial inverse agonist Ro15-4513 has similar effects to those observed with LY404187 and LY451395 in our studies, in that it reverses the behavioral effects of acute ethanol in rats. The compound dose-dependently attenuates both observer-rated levels of intoxication when given peripherally before and after 2.0 g/kg ethanol (Suzdak *et al*, 1988) and reductions in open-field locomotion when administered before 0.75 g/kg ethanol (June and Lewis, 1994). Impairments in rotarod performance following 1.5 g/kg ethanol are also reversed via prior infusion of Ro15-4513 into the striatum (Meng and Dar, 1994) and motor cortex (Barwick and Dar, 1998). However, Ro15-4513 has not been tested clinically and the related benzodiazepine partial inverse agonist, FG7142, causes intense anxiety states in healthy volunteers (Horowski and Dorow, 2002). In contrast, LY451395 is well tolerated in healthy volunteers and elderly patients (Jhee *et al*, 2006) rendering AMPA potentiators a more attractive and practical candidate for further development as a possible pharmacological intervention strategy.

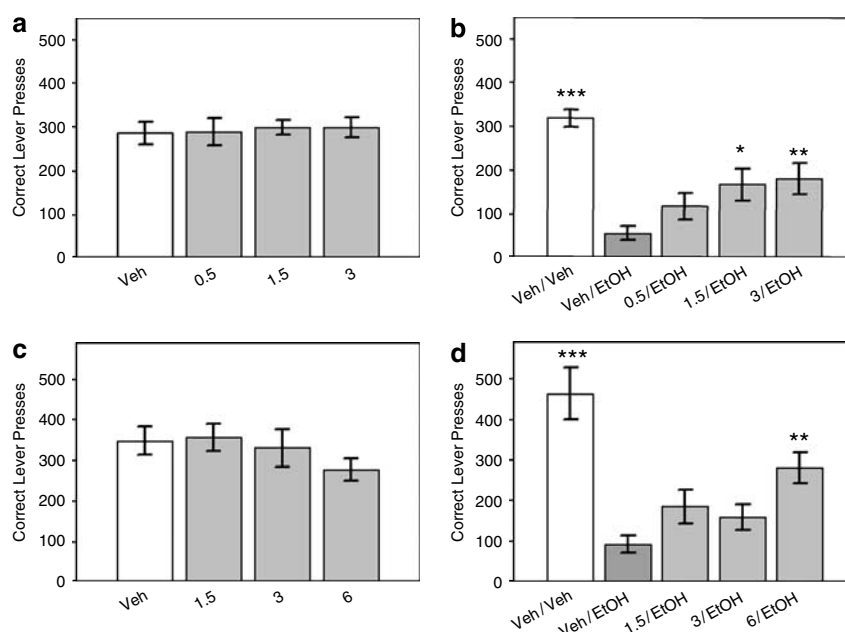


Figure 6 The effect of AMPA potentiators on baseline lever-press rates and ethanol-induced decreases in lever-press rates under a VI-30 schedule of reinforcement. Data are presented as group means (\pm SEM). The y axis of all graphs is the total number of correct presses and the x axis is the drug treatment condition. (a) LY451395 and (c) LY404187 effects on baseline lever pressing. Neither compound significantly affected lever-press rates in isolation. Effects of (b) LY451395 and (d) LY404187 on ethanol-induced decrements in lever pressing. Both compounds were found to significantly attenuate the performance deficit induced by ethanol. Statistical significance indicated on figures is relative to the Veh/EtOH group * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

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DISCLOSURE/CONFLICT OF INTEREST

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