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Nonselective suppression of operant ethanol and sucrose self-administration by the mGluR7 positive allosteric modulator AMN082

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Abstract

Emerging evidence indicates that specific metabotropic glutamate receptors (mGluRs) modulate ethanol self-administration. In general, inhibition of glutamate transmission through blockade of postsynaptic mGluRs, or activation of presynaptic mGluRs, inhibits ethanol self-administration. The goal of this preclinical study was to further characterize mGluR regulation of ethanol self-administration by examining effects of AMN082, an allosteric positive modulator of presynaptic mGluR7 activity. Separate groups of C57BL/6J male mice were trained to self-administer ethanol or sucrose on a fixed-ratio 4 schedule of reinforcement during 1 hour sessions. On test days, mice were pretreated with AMN082 (0, 1.0, 3.0, 5.6, or 10 mg/kg) 30 minutes prior to self-administration sessions. Functional specificity and activity was examined by testing the effects of AMN082 (0 – 10 mg/kg) on open-field locomotor activity and HPA axis function as measured by plasma corticosterone levels. AMN082 (10 mg/kg) produced a significant reduction in ethanol and sucrose reinforced responding, and inhibited locomotor activity. Plasma corticosterone levels were significantly increased following AMN082 (5.6 and 10 mg/kg) suggesting a dose-dependent dissociation between the behavioral and hormonal effects of the compound. These data suggest that activation of mGluR7 by AMNO82 produces non-specific reductions in motivated behavior that are associated with negative effects on motor activity.

Keywords

C57BL/6J mice; alcohol; self-administration; corticosterone; glutamate; mGluR7; AMN082; reinforcement

INTRODUCTION

Glutamate is the primary excitatory neurotransmitter in the mammalian brain. The fast excitatory actions of glutamate are mediated by ionotropic (iGluR) N-methyl-D-aspartate (NMDA), α-amino-3-hydroxi-5-methyl-ioxyzole-4-propionic acid (AMPA) and kainite (KA) receptors. Metabotropic glutamate receptors (mGluRs) mediate slower glutamate responses

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through G-protein coupling to various intracellular signaling cascades that can modulate, or fine-tune, iGluR function (Benquet et al., 2002)

The mGluRs are categorized into three subfamilies based on their sequence homology, pharmacological profiles, and signal transduction mechanisms. Activation of Group I (mGluR1 and 5) receptors stimulates phospholipase C (PLC) which leads to uncaging of Ca^{2+} stores and increased glutamate transmission whereas Group II (mGluR2 and 3) and Group III (mGluR 4, 6, 7, and 8) mGluRs inhibit adenylyl cyclase and decrease cAMP conversion leading to a decrease in glutamate transmission [for reviews see (Gereau and Conn, 1995; Pin and Duvoisin, 1995; Conn and Pin, 1997)]. Group I mGluRs are primarily localized in the postsynaptic membrane where they contribute to regulation of a variety of CNS functions including synaptic plasticity (Baude et al., 1993; Lu et al., 1997), anxiety (Brodkin et al., 2002), and pain (Walker et al., 2001). Alternatively, Group II and Group III mGluRs are found primarily in presynaptic locations where they modulate glutamate release, synaptic plasticity, and other functions (Conn and Pin, 1997; Ferraguti and Shigemoto, 2006). MGluRs are recognized as potential therapeutic drug targets for a number of CNS disorders due to their diverse localization and functions, and their ability to regulate pre- and postsynaptic changes in glutamate neurotransmission (Recasens et al., 2007; Spooren et al., 2001).

Pharmacological evidence supports the targeting of mGluRs as potential treatments for alcoholism. Compounds that block postsynaptic Group I mGluR activity have been shown to alter ethanol self-administration and relapse in animal models. For example, the selective mGluR5 antagonist MPEP decreases ethanol self-administration in mice (Hodge et al., 2006) and rats (Schroeder et al., 2005) and blocks its discriminative stimulus effects (Besheer et al., 2006). In addition, there is evidence that Group II mGluRs modulate ethanol-related behaviors. Activation of presynaptic mGluR2/3 with the selective agonist LY379268 decreases self administration and suppresses reinstatement of cue and stress induced ethanol-seeking behavior following extinction (Backstrom and Hyytia, 2005; Zhao et al., 2006); however, the mGluR2/3 antagonist LY34149 does not affect ethanol self-administration in rats or mice (Hodge et al., 2006; Schroeder et al., 2005). Similarly, the mGluR8 agonist (S)-3,4-DCPG attenuates ethanol self-administration and reinstatement (Backstrom and Hyytia, 2005). Overall, these studies suggest that selective mGluR ligands that decrease glutamate transmission, either by presynaptic or postsynaptic mechanisms, decrease alcohol self-administration.

The Group III receptor mGluR7 is an intriguing target for studies on alcohol related behaviors. MGluR7 is the most highly conserved of all mGluRs and are likely to have a significant physiological role (Flor et al., 1997). They are primarily localized presynaptically and are thought to function as autoreceptors (Shigemoto et al., 1996; Kinzie et al., 1997). Activation of mGluR7 causes accumulation of cAMP which leads to a blockade of P/Q type Ca^{2+} channels and a subsequent decrease in glutamate release (Perroy et al., 2000). mGlu7 receptors are widely distributed throughout the central nervous system, with highest concentrations in the hippocampus, amygdala, prefrontal cortex and locus coeruleus; structures that have been implicated in the reinforcing effects of ethanol (Besheer et al., 2003; Hodge et al., 1996; Hodge and Cox, 1998; June et al., 2001; Roberts et al., 1996; Rodriguez Echandia and Foscolo, 1988; Schroeder et al., 2003). Genetic knockouts of mGluR7 have deficits in fear-conditioning and other amygdala-mediated stress-related responses (Masugi et al., 1999; Mitsukawa et al., 2006) and working memory (Callaerts-Vegh et al., 2006). Furthermore, genetic analysis of a congenic mouse strain that consumes high levels of ethanol compared to controls has identified the mGluR7 gene as being involved in ethanol preference (Vadasz et al., 2007).

Recently, AMN082, a highly specific and efficacious mGluR7 positive allosteric modulator, has become available. AMN082 is orally active and crosses the blood brain barrier making it

ideal for *in vivo* analysis (Mitsukawa et al., 2005). Acute AMN082 administration has antidepressant-like effects in the tail-suspension and forced swim tests (Palucha et al., 2007). However, there are no published studies of the effects of AMN082 addictive behaviors. The aim of the present study was to examine whether AMN082 could specifically modulate ethanol self-administration in C57BL/6J mice.

METHOD

Subjects

8-week old, male C57BL/6J mice (The Jackson Laboratory, Bar Harbor, ME, N = 28) were housed in groups of four in standard Plexiglas cages (17.8W × 29.2L × 12.7H cm) lined with corn cob bedding with a wire stainless steel top. During the course of the experiment, two mice were removed from the ethanol self-administration group due to health problems unassociated with the experimental procedures. Food and water were available *ad libitum* with the exception of the initial four days of training as described below. The mouse vivarium was maintained on a reverse 12 hr light-dark cycle (lights off at 10:00 a.m.). All animals were treated in accordance with the Institutional Animal Care and Use Committee at the University of North Carolina at Chapel Hill and NIH guidelines for the Care and use of Laboratory Animals (National Research Council, 2003).

Self-Administration Procedure

Apparatus—Self-administration sessions were conducted in 16 Plexiglas operant conditioning chambers (Med Associates, Georgia, VT) measuring 15.9 × 14 × 12.7 cm with stainless steel grid floors. Each chamber was housed in a sound-attenuating cubicle equipped with a house fan that provided ventilation and helped mask external noise. The left and right wall of each chamber was equipped with ultra-sensitive stainless steel retractable response levers, one “active” and one “inactive.” Solutions were maintained in 60 ml and 10 ml syringes (for 16 and 1 hr sessions, respectively) mounted on a programmable pump (PHM-100, Med Associates), which delivered 0.014 mL per activation into a stainless steel trough located next to the active response lever. Depending on the schedule of reinforcement (see below), a fixed number of responses on the active lever resulted in the delivery of a solution into the trough. Inactive lever responses did not result in a programmed consequence. Additionally, each chamber has a stimulus light located above each lever that would activate during reward delivery plus a house light that simulated the light schedule of the vivarium. The operant conditioning chambers were interfaced (Med Associates) to an IBM compatible PC, which was programmed to record all lever presses, headpokes, and liquid deliveries.

Ethanol Self-Administration Training—After one week of acclimation to housing conditions, mice (n=16) were given access to 5% sucrose for 48 hours in their home cage. Mice were then deprived of fluids for ~24 hours prior to initial training in the operant conditioning chamber. To facilitate acquisition of the lever pressing behavior, the first 3 training sessions lasted 16 hr in duration beginning with 5% sucrose as the reinforcing solution. During the 1st session, every lever press on the active lever was reinforced by a delivery of 5% sucrose. In the 2nd session the response requirement increased from FR1 to FR2 to FR4 after achieving 15 reinforcements at each schedule of reinforcement. The response requirement for all subsequent sessions remained at FR4. On the 4th day of training, the session was shortened to 1 hr in duration and took place between 1400 – 1600h, 6 days a week (Su – Fr) for the remainder of the study.

Mice (n=16) were then trained to self-administer sweetened ethanol using a sucrose fading procedure during which the ethanol concentration was gradually increased from 0% to 9% (v/v) and sucrose was decreased from 5% to 2% to a final concentration of ethanol (9% v/v) plus

sucrose (2% w/v). A minimum of 2 training sessions were conducted at each concentration. We found in a previous study that self-administration of unsweetened ethanol (10% v/v) during 1 hr sessions led to low response rates in C57BL/6J mice and inconsistent ethanol consumption (ie: the mice lever pressed but did not drink) (Faccidomo et al., 2007); so in this experiment, we used a solution of 2% sucrose/9% ethanol (v/v) throughout the experiment and fluid cups were examined for residual fluid to verify consumption

Effect of AMNO82 on Ethanol Self-administration—During 2 weeks prior to testing, ethanol self-administering mice were given biweekly injections of vehicle prior to testing sessions to acclimate them to the injections. On test days, AMN082 was freshly dissolved in vehicle and injected (i.p.) 30 minutes prior to the beginning of the self-administration sessions. Mice (n=16) received AMN082 (0, 1.0, 3.0, 5.6, or 10.0 mg/kg) in a counter balanced Latin-square design to control for order effects. Test days occurred twice per week (Tuesday and Friday) with two self-administration sessions occurring prior to each test day. AMN082 (17 mg/kg) was given to four mice in the ethanol group; however administration of this dose was discontinued due to animal safety concerns. We observed that this dose produced a high level of inactivity and tremors following administration. These mice behaved normally and responded at baseline levels the following day and remained in the study.

Blood Alcohol Concentration—Four days following the last AMN082 test session in the ethanol self-administration group (n = 15), approximately 20 μl of blood was collected from the tail vein, centrifuged, and plasma was analyzed for blood alcohol concentration using an Analox GL-5 analyser (Analox Instruments, Lunenburg, MA). For each measure, 5 μl of plasma was used to determine blood alcohol concentration (mg/dl).

Effect of AMNO82 on Sucrose Self-administration—To address the potential role of mGluR7 in general reinforcement processes, mice (n=12) were trained to administer a sucrose-only solution. Mice were trained under the same schedule except sucrose was faded from 5% to 2% in the absence of ethanol. After the sessions, mice were returned to their home cages and fluid cups were examined for residual fluid to verify consumption. During the testing phase, the same doses of AMN082 (0, 1, 3, 5.6, or 10 mg/kg) and testing procedure was followed as in the ethanol self-administering mice.

Effect of AMN082 on Locomotor Activity in Ethanol Self-Administering Mice

Apparatus—Potential locomotor effects of AMNO82 were tested in 8 open field Plexiglas chambers (Med Associates, Georgia, VT). Two sets of 16 pulse modulated infrared photobeams were placed on opposite walls at 1-inch centers to record x-y movements. Activity chambers were computer interfaced (Med Associates) for data sampling at 100 millisecond resolution. Following each session the activity chamber was wiped clean with 2.5% glacial acetic acid to limit any confounding odors.

Locomotor testing—Mice (n=14) from the ethanol self-administration group underwent locomotor activity tests to determine if effects of the mGluR7 positive modulator were associated with nonspecific effects on motor ability. Ethanol self-administration sessions continued during this phase of the experiment but were suspended on locomotor test days. Three days following BAC measurement, mice were acclimated to the locomotor chambers during a single 2-hour session to control for novelty-induced hyperactivity on the first day of testing that might not be seen on subsequent test days. One week after the acclimation session, AMN082 (0, 3, or 10 mg/kg) was administered 30 minutes prior to 1 hr locomotor test sessions that occurred on Friday at 2 week intervals. Drug doses were administered according to a randomized Latin-square design to control for order effects.

Effect of AMN082 on Corticosterone Levels in Ethanol Self-Administering Mice

The effects of AMNO82 on plasma corticosterone levels were measured on Wednesday during the same weeks as locomotor testing in the ethanol self-administration mice (n = 14) by injecting the mice with AMNO82 (0, 1, 3, 5.6, or 10 mg/kg) 30 minutes prior to having blood drawn. Plasma collection occurred every seven days at the same time that a self-administration session normally would have occurred (i.e., 23 hours following the previous day's self-administration session which occurred on days when corticosterone and locomotor activity were not assessed). Specifically, mice were placed in a restraint tube (Braintree Scientific, Braintree, MA) and approximately 20 ul of blood was quickly (less than 2 minutes) drawn in heparinized microcentrifuge tubes. Drug dose was randomly assigned in a Latin-square manner to control dose order effects. Immediately after collection, samples were centrifuged for 10 min and the plasma was removed and frozen at −20º C. Plasma levels of corticosterone levels were quantified using a Radioimmuno Assay Kit (MP Biomedicals, Solon, OH) and expressed as ng/mL.

Drugs

The mGluR7 positive allosteric modulator AMN082, N,N′-dibenzhydral-ethane-1,-2-diamine-dihydrochloride (Tocris Bioscience, Ellisville, MO), was dissolved in vehicle (3% DMSO [Sigma Aldrich, St. Louis, MD] in 0.9% saline) and injected i.p. Ethanol (v/v) solutions were prepared by diluting 95% ethanol in tap water. Sucrose solutions (w/v) were prepared by dissolving granulated sucrose in tap water.

Data Analyses

Measures from ethanol and sucrose self-administration studies (total responses, reinforcements delivered, active responses, inactive responses, headpokes, and dose consumed), locomotor assessments (distance traveled), and corticosterone levels were analyzed by one-way repeated measures (RM) ANOVA. Time-course of locomotor activity was analyzed by two-way RM ANOVA. *Post hoc* analyses of significant differences were performed using Dunnet's tests. Blood alcohol concentration was analyzed using a Pearson's product moment correlation. Significance was set at $p < 0.05$ for all statistical tests.

RESULTS

Ethanol and Sucrose Self-administration

All of the mice (n=16) in the ethanol self-administration experiment acquired the behavioral task rapidly and exhibited a stable baseline rate of ethanol intake (g/kg) (Figure 1a, left). Ethanol self-administration occurred for approximately 4 weeks and the average calculated dose consumed during the last 10 days prior to testing was mean ± SEM = 1.33 ± 0.13 g/kg/hr. The mean ± SEM number of reinforcers prior to drug testing was 29 ± 4 and the proportion of active lever responses was 78%, which is much higher than expected by chance (50%) and suggests that the sucrose/ethanol solution was reinforcing. This rate of ethanol self-administration was maintained between (mean ± SEM g/kg/hr = 1.38 ± 0.11 g/kg/hr) and following drug (mean ± SEM g/kg/hr = 1.23 ± 0.11) test sessions demonstrating that AMN082 did not have carryover effects (Figure 1a, middle and right). Furthermore, there were no observations of significant amounts of residual fluid left in the trough following sessions indicating that the mice were drinking the solution. On the day that blood alcohol concentrations (BAC) were measured, the average dose consumed for mice tested (n=15) was 1.1 g/kg/hr which corresponds to a BAC of 48.3 mg/dl per session ($r^2 = 0.95$) measured following a 1 hr ethanol self-administration session, confirming that the mice were consuming the ethanol (Figure 1b).

Mice from the sucrose-only group (n = 12) were trained similarly for approximately four weeks prior to testing. 5 mice did not acquire adequate responding (below 20 active lever responses for last 10 days prior to testing) and were removed from the experiment. Level of sucrose-self administration (as measured by reinforcements delivered) in the remaining mice (n=7) was equivalent to the ethanol group during the last 10 sessions prior to testing (sucrose reinforcements: mean ± SEM = 34.7 ± 5.7; ethanol reinforcements: mean ± SEM, 33.8 ± 3.7).

Effects of AMN082 on Ethanol Self-administration

AMN082 decreased ethanol self-administration. Separate one-way RM ANOVA identified a main effect was found for active ($F(15,60) = 7.872$, $p< 0.001$) and inactive lever presses ($F(15,60) = 7.583$, $p<0.001$). *Post hoc* analyses showed that AMN082 significantly decreased the number of active lever responses at 10 mg/kg and inactive lever responses at 5.6 and 10 mg/kg compared to vehicle suggesting that AMN082's effects were nonselective for alcohol reinforcement at 10 mg/kg (Figure 2a). Additional analysis revealed significant decreases in dose consumed ($F(15,60) = 9.259$, $p< 0.001$) from vehicle at 10 mg/kg only (Figure 2b). Accuracy was not affected by AMN082 as the percentage of active lever responses do not differ significantly among doses (Figure 2c). Similarly, the number of head pokes did not differ among doses (Figure 2d). It is important to note that the majority of mice exhibited observable full-body tremors at 10 mg/kg, occasionally at 5.6 mg/kg, and none at the 3.0 and 1.0 mg/kg dose.

Effects of AMN082 on Sucrose Self-administration

AMN082 decreased sucrose self-administration in a similar manner to the effects on ethanol self-administration. Like ethanol self-administration, there was a significant main effect of AMN082 on total ethanol reinforced lever presses ($F(6,23) = 3.983$, $p< 0.05$) and on inactive lever presses ($F(5,18) = 4.228$, $p< 0.05$). *Post hoc* analyses revealed that AMN082 decreased active and inactive lever presses at 10 mg/kg (Figure 3a). Unlike ethanol self-administration, inactive lever responses did not differ at 5.6 mg/kg. A main effect was found for reinforcements delivered ($F(6,23) = 3.876$, $p< 0.05$) where 10 mg/kg AM082 significantly decreased the number of sucrose reinforcements delivered (Figure 3b). Similar to ethanol self administration, percentage of active lever responses and headpokes were unaltered by drug treatment (Figure 3c and 3d).

Effects of AMN082 on Locomotor Activity

AMN082 significantly reduced locomotor activity in the open field. There was a significant main effect of AMN082 on distance traveled (m/hr) in a 1 hour test session ($F(13,26) = 32.465$, $p < 0.001$). *Post hoc* analysis showed that AMN082 significantly reduced locomotor activity at both the 3.0 and 10 mg/kg doses compared to vehicle (Figure 4a). When locomotor data were divided into 15 minute intervals, two-way repeated measures ANOVA repeated the main effect for AMN082 dose ($F(13,78) = 32.465$, $p < 0.001$) and showed a main effect for time ($F(13,78) = 18.36$, $p < 0.001$) and a significant interaction for dose and time ($F(6,78) = 4.111$, $p < 0.001$). *Post hoc* analysis revealed that the motor inhibiting effects of 10 mg/kg AMN082 were immediate and long-lasting (Figure 4b). By contrast, 3.0 mg/kg, AMN082 significantly decreased activity only for the 1st 30 minutes in the open field (Figure 4b). At 10 mg/kg but not 3.0 mg/kg, tremors appeared to contribute greatly to these locomotor deficits but did not entirely limit ambulation.

Effects of AMNO82 on Corticosterone Levels

AMN082 has been shown to increase stress hormone levels in mice (Mitsukawa et al., 2005), which have been shown to alter ethanol self-administration (Fahlke et al., 1994). To verify bioactivity and determine if AMN082-induced changes in ethanol self-administration are

related to stress hormone levels, we examined the effects of a full dose range of AMN082 (1, 3, 5.6 and 10 mg/kg) on plasma corticosterone levels in ethanol self-administering mice. There was a significant main effect of AMN082 on plasma levels of corticosterone [$F(13,49) = 19.465$ $p < 0.001$). *Post hoc* analysis revealed that AMN082 (5.6 and 10 mg/kg) produced significant increases in plasma corticosterone as compared to vehicle control. Corticosterone levels (mean ± SEM) following AMN082 injection were: vehicle (128.3 ± 14.4 ng/mL); 5.6 mg/kg (257.7 ± 20.6 ng/mL); 10 mg/kg (315.9 + 33.6 ng/mL).

DISCUSSION

The primary purpose of this study was to investigate the effects of the mGluR7 positive modulator AMN082 on ethanol self-administration. At the highest dose tested, AMN082 (10 mg/kg) significantly decreased operant responding for ethanol in C57BL/6J mice. This reduction did not appear to be selective for ethanol since the mGluR7 positive modulator also decreased sucrose self-administration and inactive lever responses under identical reinforcement conditions. Furthermore, AMN082 reduced spontaneous locomotor activity at all doses tested (3.0 and 10 mg/kg) and increased plasma corticosterone levels at higher doses (5.6 and 10 mg/kg). These findings suggest that AMN082 produced multiple functional effects that likely contributed to the reduction in ethanol self-administration.

The results of this study add to a growing preclinical literature showing that pharmacological manipulation of group II and III mGluRs attenuate ethanol self-administration via nonspecific actions. For example, the mGluR2/3 (group II) agonist LY37968, which decreases glutamate transmission by activating presynaptic autoreceptors, attenuates ethanol self-administration and blocks the cue-induced reinstatement of ethanol-seeking behavior (Backstrom and Hyytia, 2005; Zhao et al., 2006). Similarly, the mGluR8 (group III) agonist (S)-3,4-DCPG attenuates ethanol self-administration and reinstatement (Backstrom and Hyytia, 2005). However both of these compounds also profoundly decrease motor activity, which suggests that any effects on ethanol self-administration and relapse may be secondary to motor inhibition. The mGluR7 (group III) agonist AMN082, which was used in the present study, has also been shown to decrease glutamate by a pre-synaptic mechanism (Marabese et al., 2007). Our results show that AMN082 produces a dose-dependent reduction in ethanol self-administration of inbred C57BL/6J mice. However, this reduction is not specific to ethanol as it reduced operant responding for sucrose at the same doses and is likely a result of nonspecific reductions in locomotor activity. Thus, evidence to date suggests that agonists of group II and II mGluRs have no specific effect on ethanol self-administration.

Alternatively, emerging evidence suggests that post-synaptic mGluR5 (group I) may specifically regulate ethanol self-administration in the absence of motor and other nonspecific effects. For example, the non-competitive mGluR5 antagonist MPEP has been shown suppress the reinforcing effects of ethanol in rats and mice (Backstrom et al., 2004; Hodge et al., 2006; Schroeder et al., 2005), attenuate the motivation to self-administer ethanol (Besheer et al., 2007b), block relapse-like behavior in multiple models (Backstrom et al., 2004; Schroeder et al., 2005), and inhibit the discriminative stimulus properties of investigator- and self-administered ethanol (Besheer et al., 2003; Besheer et al., 2006). Similarly, the mGluR5 antagonist MTEP also reduces the reinforcing effects of ethanol in rats and mice (Cowen et al., 2005; Cowen et al., 2007). Importantly, these dose-dependent effects of mGluR5 antagonists on ethanol self-administration are not associated with disruptions in motor performance (Besheer et al., 2007b; Cowen et al., 2007; Hodge et al., 2006). By contrast, other recent data indicate that blockade of post-synaptic mGluR1 (also group I) with the highly potent and brain penetrant antagonist JNJ 16259685 reduces alcohol self-administration and progressive ratio performance via nonspecific effects on motor activity in rats (Besheer et al., 2007a, b). Thus, of all of the mGluR compounds tested to date, the only indication of ethanol-

specificity comes from studies examining low doses of mGluR5 antagonists. It remains to be determined if lower doses of group II or III mGluR compounds, or novel agents, will show specific involvement in ethanol reinforcement.

Consistent with the role of mGluR7 in stress-related responses, the results of this study are in agreement with other data showing that AMN082 increases corticosterone levels and other stress hormones in an mGluR7 dependent manner (Mitsukawa et al., 2005). The high expression of mGlu7 receptors in the paraventricular nucleus (PVN) neurons of the hypothalamus might predict that mGluR7 activation disinhibits PVN neurons that release ACTH, increasing corticosterone levels either by a decreased glutamatergic tone on GABAergic neurons or by direct inhibition via mGluR7 on GABAergic neurons that synapse on PVN neurons (Tasker et al., 1998). We observed that AMN082 (5.6 and 10 mg/kg) significantly increased plasma corticosterone levels at 30 minutes post-injection. Since humans report stress as a factor that precipitates drinking (Cooper et al., 1992) and studies using rats have found that increasing corticosterone levels leads to increased ethanol self-administration (Fahlke et al., 1994), (Fahlke et al., 1996), it might be predicted that these doses of AMN082 would increase operant ethanol self-administration. However, at AMN082 (5.6 mg/kg) did not alter ethanol self-administration despite increasing plasma corticosterone levels by nearly 100%. Therefore, it appears that acute increases in corticosterone through mGluR7 activation do not lead to increased ethanol self-administration.

Although the results of this study suggest that allosteric activation of mGlu7 receptors may not be a good target for therapeutic interventions in alcoholism, this interpretation is complicated by several factors related to drug specificity. First, recent evidence indicates that systemic injections of AMN082 increase rather than decrease glutamate release in the nucleus accumbens (Li et al., 2007). This suggests that AMN082 may have effects that are distinct from its purported selective activation of autoreceptors, which should decrease glutamate release. Second, a major dilemma in interpreting the effects of AMN082 is that comparable doses to those used in the present study were recently shown to produce body tremors and locomotor deficits in mGluR7 knockout mice (Palucha et al., 2007). Thus, despite a very thorough receptor-binding analysis during its initial identification (Mitsukawa et al., 2005) these findings strongly suggest that off-target actions of AMN082 may be responsible for the effects observed in the present study. Overall, the full body tremors observed at higher doses appear to be the predominant behavioral effect that is responsible for the large decrease in sucrose and ethanol self-administration and locomotor activity at 10 mg/kg. It remains to be determined whether other strategies, such as site-specific infusions of AMN082 or a more selective positive modulator, may show specific involvement of this receptor in ethanol self-administration.

In conclusion, modulating glutamate transmission via pharmacological targeting of mGluRs remains a potential therapy for decreasing ethanol consumption. Ideally, a drug therapy must selectively decrease ethanol consumption in the absence of major side effects or reductions in general motivation. The results of this study indicate that the mGluR7 allosteric modulator AMN082 does not meet this fundamental preclinical criterion. The widespread expression of mGlu7 receptors and possible off-target effects of AMN082 make interpreting its effects difficult and call for further characterization of the receptor using more selective approaches.

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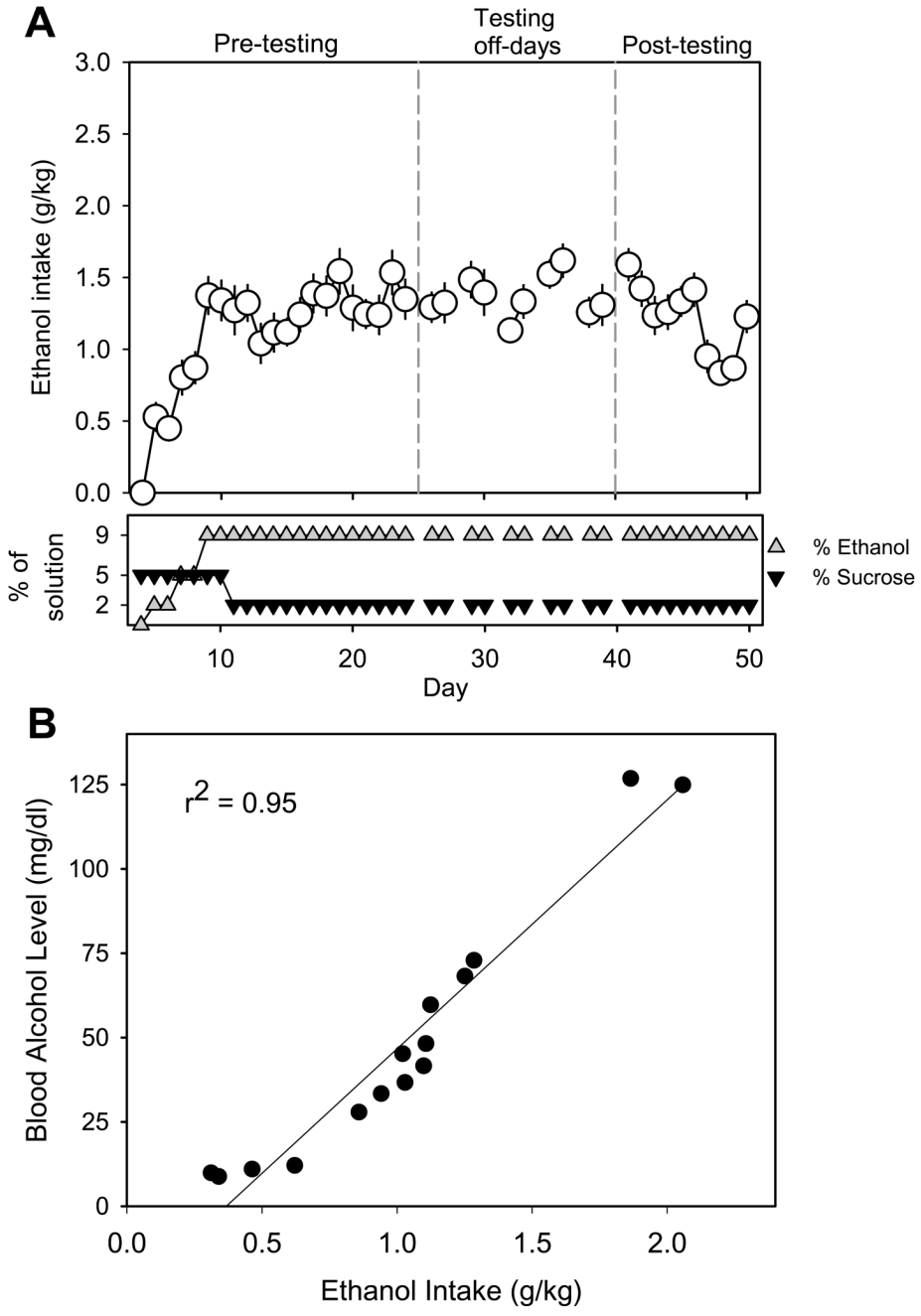

Figure 1.

(**A**) Top panel shows ethanol intake (g/kg) plotted as a function of days during pre-testing, testing off-days, and post-testing phases of the 1-h ethanol (2% sucrose/9% ethanol) self-administration experiment. Values represent mean ±SEM from n=16 mice. Bottom panel shows percentage of ethanol and sucrose in the self-administered solution during each phase. (**B**) Linear regression of blood alcohol concentration (mg/dl) shown as a function of ethanol dose (g/kg) consumed. Blood samples were taken immediately following a single 1-hour self-administration session four days after AMN082 testing. Evaluated using Pearson's correlation (n = 15).

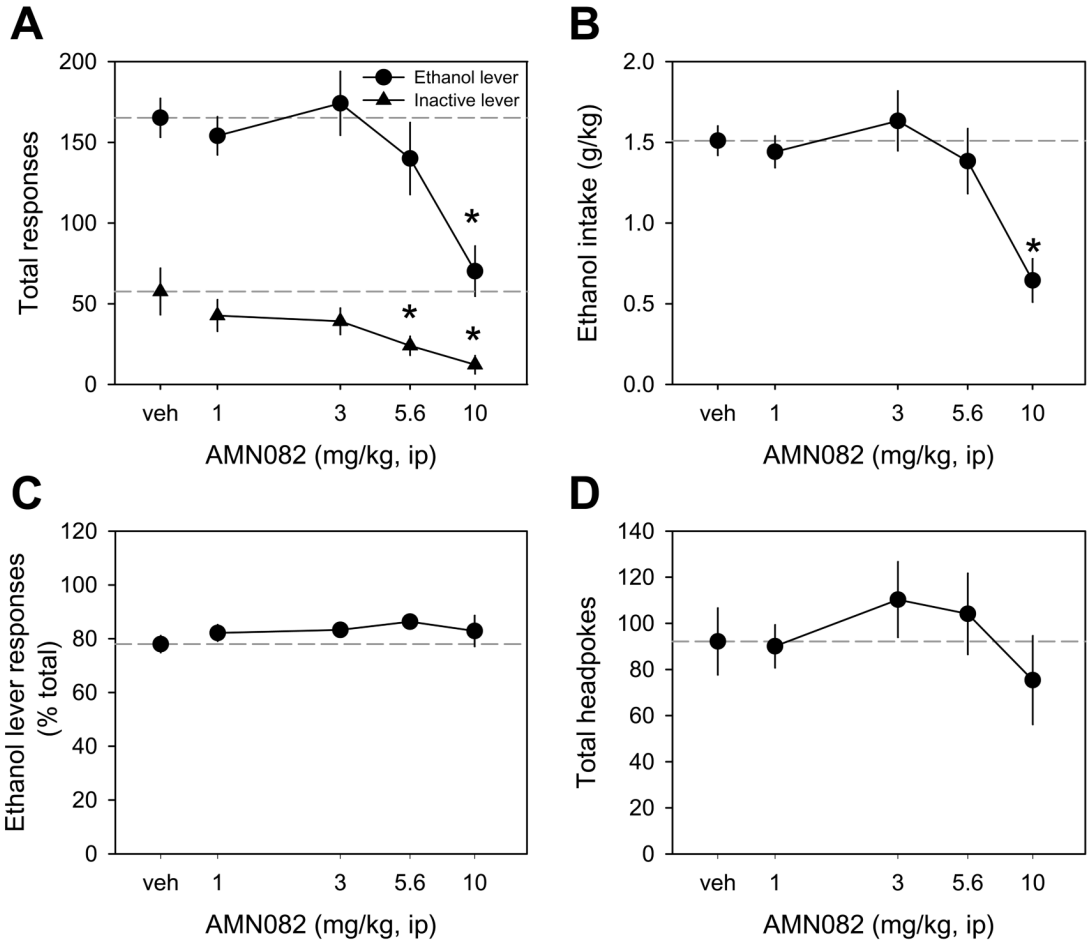

Figure 2. Effects of AMN082 on sweetened ethanol self-administration during 1-h sessions by C57BL/6J mice

(**A**) Total responses on the ethanol and inactive lever, (**B**) ethanol dose consumed (g/kg), (**C**) percentage of responses on the ethanol lever, and (**D**) headpokes in the ethanol well plotted as a function of AMN082 dosage (mg/kg). AMN082 was administered intraperitoneally (ip) 30 minutes prior to testing. Values represent mean ±SEM from n=16 mice; * $p<0.05$ vs vehicle (veh) control (Dunnett's test). Horizontal dashed lines represent mean performance under veh conditions.

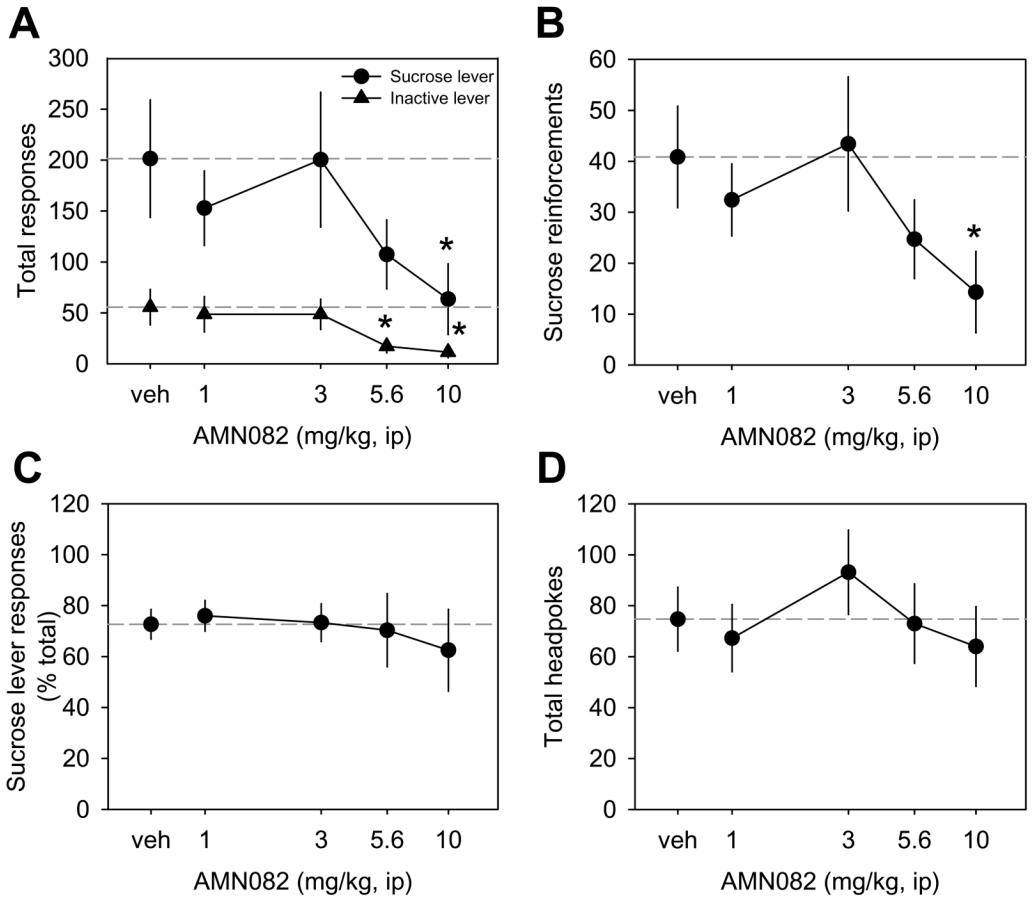

Figure 3. Effects of AMN082 on sucrose self-administration during 1-h sessions by C57BL/6J mice

(**A**) Total responses on the sucrose and inactive lever, (**B**) total number of sucrose reinforcements, (**C**) percentage of responses on the sucrose lever, and (**D**) headpokes in the sucrose well plotted as a function of AMN082 dosage (mg/kg). AMN082 was administered intraperitoneally (ip) 30 minutes prior to testing. Values represent mean ±SEM from n=7 mice; * $p<0.05$ vs vehicle (veh) control (Dunnett's test).

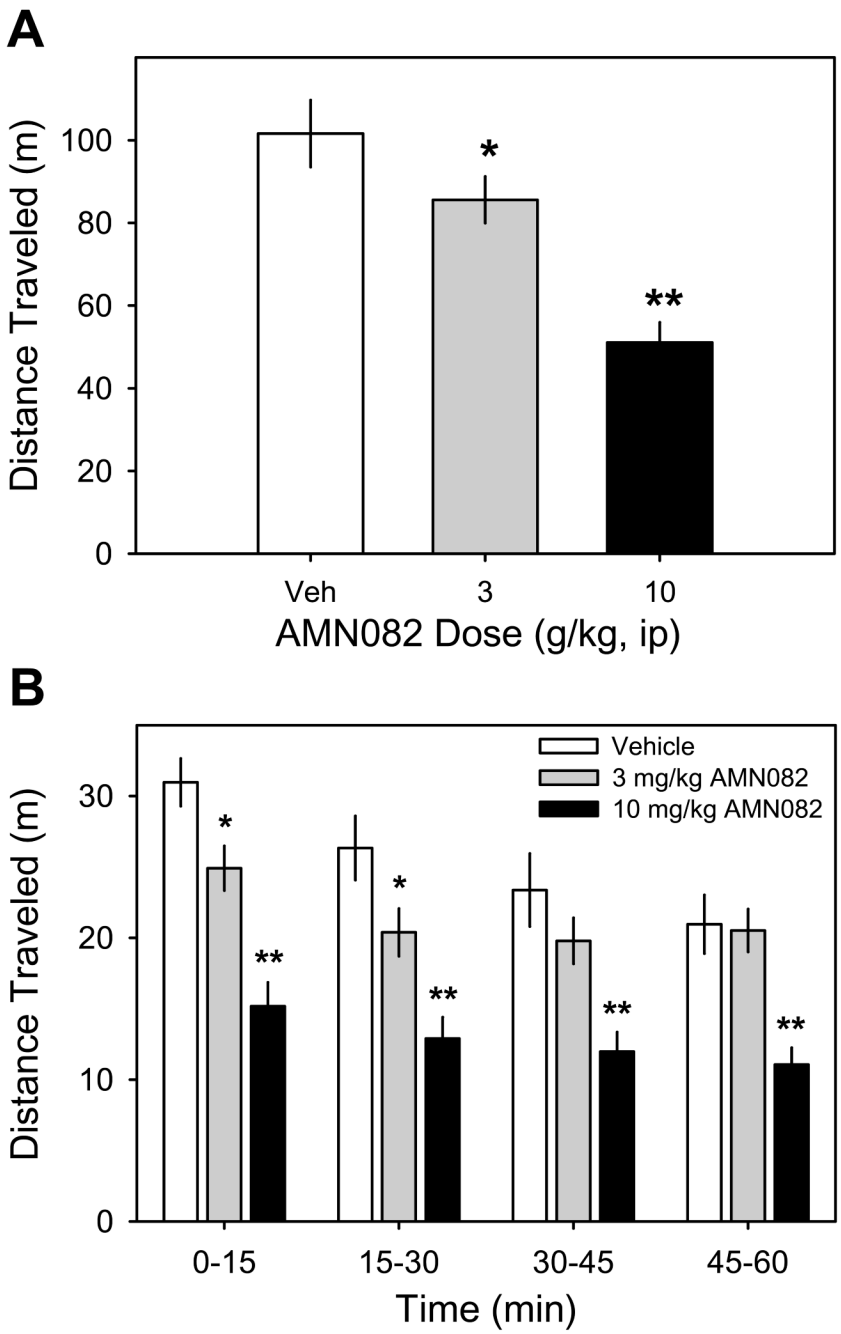

Figure 4. Effects of AMN082 on spontaneous locomotor activity in an open field

Horizontal distance traveled (m) plotted as a function of AMN082 dosage (mg/kg) for the (**A**) total 1-h session or (**B**) plotted as a function of 15-min intervals showing the time-course of AMN082 effects at each dose. AMN082 was administered intraperitoneally (ip) 30 minutes prior to testing. Values represent mean ±SEM for n=14 mice, * $p<0.05$, ** $p<0.01$ vs vehicle (veh) control group (Dunnett's test).