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Pharmacological modulation of mGluR7 with AMN082 and MMPIP exerts specific influences on alcohol consumption and preference in rats

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ABSTRACT

Growing evidence supports a role for the central nervous system (CNS) neurotransmitter L-glutamate and its metabotropic receptors (mGluRs) in drug addiction in general and alcohol-use disorders in particular. Alcohol dependence, for instance, has a genetic component, and the recent discovery that variations in the gene coding for mGluR7 modulate alcohol consumption further validates involvement of the L-glutamate system. Consequently, increasing interest emerges in developing L-glutamatergic therapies for the treatment of alcohol abuse and dependence. To this end, we performed a detailed behavioral pharmacology study to investigate the regulation of alcohol consumption and preference following administration of the mGluR7-selective drugs N,N′-dibenzyhydryl-ethane-1,2-diamine dihydrochloride (AMN082) and 6-(4-Methoxyphenyl)-5-methyl-3-(4-pyridinyl)-isoxazolo[4,5-*c*]pyridin-4(5*H*)-one hydrochloride (MMPIP). Upon administration of the allosteric agonist AMN082 (10 mg/kg, i.p.) in rats, there was a significant decrease in ethanol consumption and preference, without affecting ethanol blood metabolism. In contrast, mGluR7 blockade with MMPIP (10 mg/kg, i.p.) showed an increase in alcohol intake and reversed AMN082's effect on ethanol consumption and preference. Both mGluR7-directed pharmacological tools had no effect on total fluid intake, taste preference, or on spontaneous locomotor activity. In conclusion, these findings support a specific regulatory role for mGluR7 on alcohol drinking and preference and provide evidence for the use of AMN082-type drugs as potential new treatments for alcohol-use disorders in man.

Keywords Alcohol, AMN082, L-glutamate, mGluR7, MMPIP, two-bottle choice.

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INTRODUCTION

Alcohol is consumed and abused by a large population worldwide resulting in severe physical and mental health disorders. Alcohol dependence, also referred to as alcoholism, is characterized by compulsive alcohol intake despite severe adverse consequences on body and mind. It is well-established, as for other drugs of abuse, that alcohol induces changes in the mesolimbic dopamine reward system (Carboni *et al.* 2000; Engleman *et al.* 2000). As blood alcohol levels rise, neurons of the ventral tegmental area (VTA) in the midbrain are activated and release dopamine into numerous target areas including the nucleus accumbens (NAc) and prefrontal cortex (Carboni *et al.* 2000; Engleman *et al.* 2000; Weiss & Porrino 2002; Gilman *et al.* 2008; Ding *et al.* 2009; Howard *et al.* 2009). Furthermore, a variety of the physiological, biochemical and behavioral effects of ethanol are known to involve the L-glutamatergic system (Gruol, Parsons & DiJulio 1997; Netzeband *et al.* 1997; Minami *et al.* 1998; Dodd *et al.* 2000; Littleton *et al.* 2001; Simonyi *et al.* 2004). Microdialysis studies have shown that ethanol increases L-glutamate levels in many brain regions including the NAc (Moghaddam & Bolinao 1994; Selim & Bradberry 1996; Szumlinski *et al.* 2007).

L-glutamate is the primary excitatory neurotransmitter in the mammalian central nervous system (CNS) playing an important role in many physiological, behavioral and pathological processes. It has been shown that slower, modulatory actions of L-glutamate are mediated by the eight metabotropic glutamate receptors (mGluR1 to −8) coupled to G-proteins that induce a variety of

intracellular signaling cascades modulating synaptic transmission and neuronal excitability throughout the CNS. In contrast, fast excitatory effects of L-glutamate are mediated by three ionotropic receptor (iGluR) families: *N*-methyl-D-aspartate (NMDA)-, α-amino-3-hydroxi-5-methyl-ioxyzole-4-propionic acid (AMPA)- and kainate (KA)-receptors (Nakanishi *et al.* 1994; Kew 2004; Palucha & Pilc 2005; Gasparini *et al.* 2008). The mGluRs are members of the G-protein-coupled receptor (GPCR) superfamily, the most abundant receptor gene family in the human genome (Luttrell 2006). Based on amino acid sequence similarity, agonist pharmacology, and signal transduction pathways, mGluRs are categorized into three groups: group I (mGluR1 and 5) up-regulate phosphatidylinositol-hydrolysis and Ca^{2+}-mobilization. In contrast, group II (mGluR2 and 3) and group III (mGluR4, −6, −7 and −8) receptors inhibit adenylate cyclase and regulate Ca^{2+}- and K^{+}-channels (Thomas 2002; Wang *et al.* 2004; Rodriguez-Moreno & Sihra 2007; Niswender & Conn 2010). Targeting mGluRs is considered promising for the treatment of alcohol-use disorders. In fact, the mGluR5-selective antagonist 2-methyl-6-(phenylethynyl)pyridine (MPEP) dose-dependently reduced ethanol-reinforced responding during peak periods of behavior occurring during the early hours of the diurnal dark phase (Hodge *et al.* 2006). MPEP also inhibited the discriminative stimulus properties of consumed ethanol during a self-administration test session (Besheer, Stevenson & Hodge 2006). In addition, pre-treatatment of C57BL/6J mice with MPEP or the mGluR1 antagonist 7-(hydroxyimino)cyclopropa[b] chromen-1a-carboxylate ethyl ester (CPCCOEt) dose-dependently reduced measures of ethanol self-administration, blocked the expression of ethanol-induced place conditioning, as well as ethanol consumption under 24 hours free-access conditions (Lominac *et al.* 2006). Furthermore, the mGluR2/3 agonist LY379268 and the mGluR8 agonist (S)-3,4-DCPG attenuated ethanol self-administration and reinstatement (Backstrom & Hyytia 2005).

Although mGluR7 is widely distributed throughout the brain (Okamoto *et al.* 1994; Kinoshita *et al.* 1998), its highest expression was found in neocortex, thalamus, hypothalamus, hippocampus, locus coeruleus and NAc (Saugstad *et al.* 1994; Kinzie *et al.* 1995). Its presynaptic localization within symmetrical and asymmetrical synapses suggested that mGluR7 might serve as heteroreceptor to regulate GABA release as well as an autoreceptor to control L-glutamate release (Bradley *et al.* 1996). Mice with genetic ablation of mGluR7 showed antidepressant- and anxiolytic-like activities in a number of behavioral tests (Cryan *et al.* 2003). These mice have reduced amygdala- and hippocampus-dependent conditioned fear and aversion responses (Masugi *et al.* 1999). Moreover, it has been found that the mGluR7 activator AMN082 induced a dose-dependent decrease in immobility in despair-related tests suggesting antidepressant-like activity (Palucha *et al.* 2007). In addition, AMN082 is known to modulate high frequency synaptic transmission in the rat basolateral amygdala (Ugolini, Large & Corsi 2008) further suggesting that pharmacological interference with mGluR7 may provide novel treatments for psychiatric disorders such as depression, anxiety and possibly drug addiction.

Regarding ethanol-related behaviors, chronic exposure of rats to an ethanol-containing liquid diet decreased mRNA levels for mGluR7 in the CA3 region of the hippocampus (Simonyi *et al.* 2004). To our knowledge, the first study directly linking mGluR7 to alcohol-related behavior was published by Vadasz *et al.* (2007), who identified the mGluR7-locus as a *cis*-regulated gene for alcohol consumption; in this study, mice with a reduction of mGluR7 transcripts were found to consume more alcohol than controls. Interestingly, Li, Gardner & Xi (2008) demonstrated that systemic and intra-NAc administration of the mGluR7 allosteric agonist AMN082 lowered NAc extracellular GABA and increased extracellular glutamate, but had no effect on extracellular DA levels; and a year later, the same group showed that AMN082 treatment in rats inhibited cocaine-induced enhancement of electrical brain-stimulation reward and intravenous cocaine self-administration (Li *et al.* 2009). Taken together, these results clearly indicate a role for mGluR7 in the brain circuitry of reward and drug addiction.

Here, we hypothesized that selective pharmacological modulation of mGluR7 with novel drug tools such as AMN082 and 6-(4-Methoxyphenyl)-5-methyl-3-(4-pyridinyl)-isoxazolo[4,5-*c*]pyridin-4(5*H*)-one hydrochloride (MMPIP) has the potential to exert specific influences on alcohol consumption, preference and possibly addiction without affecting control parameters such as taste preference/neophobia, total fluid intake and locomotor activity (for contrast however, see the work by Salling, Faccidomo & Hodge (2008). To investigate this exciting possibility, we decided to apply well selected dosing regimens of AMN082 and MMPIP in conjunction with the two-bottle free-choice behavioral paradigm in rats, using combinations of different ethanol concentrations with water and adequate control taste substances. Overall, we aim to examine whether mGluR7 functional activity in the CNS exerts specific regulatory roles on alcohol drinking and reinforcing properties in mammals.

MATERIALS AND METHODS

Animals and housing

Male Wistar rats (Charles River, Sulzfeld, Germany; 250–350 g body weight) were housed in standard plexiglas

cages and kept in the animal facilities under standard laboratory conditions (12/12 hours light–dark cycle, lights off at 7 am, 22°C, 55% relative humidity) with free access to water and standard rat chow. Experimental procedures were approved by the local government of the Oberpfalz (Bavaria, Germany) and followed the European Communities Council directive (86/609/EEC). All efforts were made to minimize the number of animals used.

Drugs

Ethanol was obtained from J.T. Baker (Deventer, the Netherlands). The tastants saccharin sodium salt dihydrate was purchased from Merck (Hehenbrunn, Germany) and quinine hemisulfate monohydrate was obtained from AlfaAesar (Karlsruhe, Germany). Ethanol and the tastants were diluted in tap water. AMN082 was synthesized at Novartis Pharma AG (Basel, Switzerland) and MMPIP was purchased from Ascent Scientific (Bristol, UK). Drugs were diluted in 0.5% methylcellulose from AMIMED (Allschwil, Switzerland) for i.p. injections. The volume of injection (1 ml/kg) was adjusted to body weight. Rat doses for AMN082 (5, 10, and 20 mg/kg, i.p.) were chosen based on previously published studies (Mitsukawa *et al.* 2005; Palucha *et al.* 2007; Fendt *et al.* 2008; Siegl, Flor & Fendt 2008; Stachowicz *et al.* 2008; Hikichi *et al.* 2010b); higher doses in rats (30–60 mg/kg) and mice (10–20 mg/kg) induced motor side-effects such as mild ataxia and/or body tremor; these side-effects emerged 12–21 minutes after i.p. administration of AMN082. Moreover, early pharmacokinetic studies conducted at Novartis Pharma AG demonstrated that AMN082 reaches low micromolar brain levels at 0.25–1 hour after systemic administration with significant drug levels still present after 24 hour, although reduced by >90% compared with the 1-hour timepoint. Taken together, *in vivo* pre-application of AMN082 for 30 or 60 minutes, prior to the examination of behavioral or physiological read-outs, seems to be ideal, and the maximum tolerated dose in rats appears to be 20 mg/kg. Dosing and pre-application of MMPIP was conducted based on recent studies where 10–30 mg/kg of MMPIP were used in both mice (Hikichi *et al.* 2010b) and rats (Hikichi *et al.* 2010a).

Ethanol preference drinking: two-bottle free-choice model

Oral alcohol self-administration and preference were studied using a two-bottle choice paradigm (see experimental timeline in Supporting Information Fig. S1) (Choi *et al.* 2004; Kamens, Andersen & Picciotto 2010; Lee *et al.* 2010). Briefly, rats were presented with two bottles filled with water and given 1 week to acclimatize to individual housing conditions and handling in the testing environment. On day 1, one bottle was filled with a 1% ethanol solution. The positions of the bottles were changed every day to control for position preference. The concentration of ethanol was raised every fourth day, increasing from 1 to 3, 6, 10%, and finally to 20% (v/v) ethanol in tap water. Ethanol solutions were made by diluting 95% ethanol with the appropriate volume of water (i.e. volume-by-volume). All fluids were presented in 250 ml graduated plastic cylinders from Eheret (Emmendingen, Germany) with stainless steel drinking spouts from Tecniplast (Varese, Italy) which were securely held through the wire mesh cage lid. The bottles were weighed to the nearest tenth of a gram before they were placed on the cage. Fluid intakes in grams were converted to milliliters with the assumption for all solutions that 1 ml = 1 g. To control for spillage and leakage caused by evaporation or experimenter handling, weekly 'drip' averages (loss of fluid in a cage with no animal present) were subtracted from individual fluid intakes. For each concentration, the average ethanol consumption per day was obtained and used for the analysis. To obtain an accurate measure of ethanol consumption, grams of ethanol consumed per kilogram of body weight per day were calculated for each rat. A measure of the relative ethanol preference ratio was calculated for each ethanol concentration by dividing the total ethanol solution consumption by the total fluid (ethanol plus water) consumption. Intakes from both bottles were summed to obtain total fluid intake.

Tastant drinking preference for quinine and saccharin

After the ethanol self-administration procedure, we examined potential differences in taste preference, which could influence ethanol consumption and preference. Rats were tested for saccharin (sweet) and quinine (bitter) fluid intake and preference in an order-balanced experimental design that can detect taste neophobias (Crabbe *et al.* 1996). Saccharin and quinine solutions were used because of their strong tastes and lack of caloric value. The concentration of saccharin (0.04 and 0.08%) and quinine (0.02 and 0.04 mM) were raised at day 4 and the positions of the bottles were changed every day to control for position preference (see experimental timeline in Supporting Information Fig. S1). Throughout the experiments, fluid intake and body weight were measured every day. Similarly, the relative taste preference ratio was calculated at each concentration by dividing the total saccharin or quinine solution consumption by total fluid consumption.

Pharmacological manipulations

Rats were injected with saline, AMN082 (5, 10 and 20 mg/kg, i.p.) or MMPIP (10 mg/kg, i.p.) to determine

their effects on ethanol consumption and preference for a solution of 3% ethanol versus tap water. Rats were presented with two bottles, one containing 3% ethanol and the other tap water, for 4 days. Starting on day 5, rats received daily saline injections 30 minutes before the dark phase and before presentation of the bottles. Fluid levels were read 4 and 24 hours later.

AMN082 and MMPIP injections started after the rats reached stable ethanol consumption (<10% variation over a 4-day period). Rats were given 7 days of access to food and water after ethanol testing before testing the effect of AMN082 and MMPIP on saccharin and quinine consumption.

Open-field locomotor activity

Spontaneous activity was measured to examine the potential locomotor effects of our effective doses of AMN082 and MMPIP in plexiglas home cages using the Ethovision 3.1 recording system [Noldus Information Technology, VA, USA; (Noldus, Spink & Tegelenbosch 2001; Spink *et al.* 2001; Pham *et al.* 2009]. Rats were i.p. injected with methyl cellulose vehicle (1 ml/kg), AMN082 (10 mg/kg) or MMPIP (10 mg/kg) to examine their pharmacological effects on spontaneous locomotor activity 30 minutes, 4 hours and 24 hours after injection. Rats were observed for 1 hour and horizontal distance traveled (cm) recorded was used as an index of motor function.

Ethanol metabolism

Animals were given a single dose of ethanol (3.5 g/kg; i.p.) together with either vehicle (1 ml/kg) or AMN082 (10 mg/kg). Blood samples were taken from tails 1, 2, 3, 4 and 5 hours after injection. Blood was collected in heparin-treated tubes and centrifuged. Then, ethanol was determined spectrophotometrically (Hu *et al.* 2004; Cai *et al.* 2006). Blood ethanol concentration values were expressed as milligram ethanol per milliliter of blood.

Statistical analysis

Data were expressed as means ± standard error of the mean. Statistical analyses on all experiments were carried out using one-way measures of variance (ANOVA) followed by the Tukey *post hoc* test. The criterion for statistical significance was $P < 0.05$.

RESULTS

Ethanol consumption

Rats were first tested with increasing ethanol concentrations to establish an optimal working dose for our planned pharmacological experiments. There was a significant main effect of ethanol concentration (Fig. 1). Analysis of the initial taste reactivity to ethanol and water has revealed a significantly higher consumption towards the 3% ethanol solution with approximately 2 g/kg of alcohol consumption [Fig 1a, $F_{(4,39)} = 16.248$; $P < 0.001$]. The preference ratio was also significantly higher for the 3% solution [Fig 1b, $F_{(4,39)} = 21.356$; $P < 0.001$] but no difference was observed in total fluid intake [Fig 1c, $F_{(4,39)} = 1.219$; $P = 0.314$].

Preference for non-alcohol tastants

To determine whether differences in ethanol consumption and preference could be due to taste sensitivity, we examined consumption of a sweet (saccharin) and bitter (quinine) tastant in the five groups previously tested with ethanol (see experimental timeline in Supporting Information Fig. S1); the results are summarized in Fig. 2. There were no differences in saccharin consumption (Fig. 2a) or preference (Fig. 2b) in any of the groups. Total fluid intake was not changed as well (Fig. 2c). There

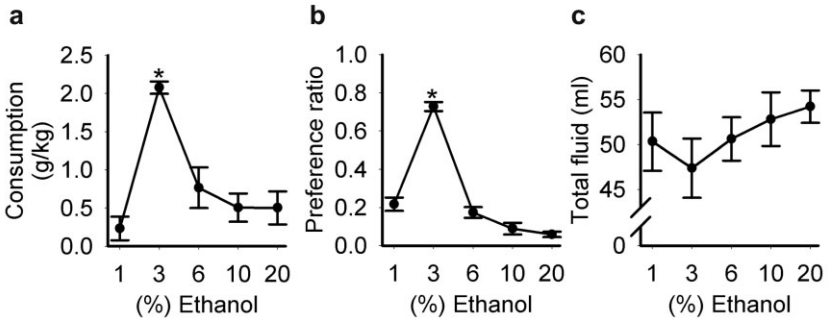

Figure 1 Voluntary alcohol consumption in rats as a function of ethanol concentration. Data represent consumption, preference and total fluid intake as a function of ethanol concentration. Rats were subjected to the two-bottle choice paradigm with one bottle containing tap water and the other containing different ethanol concentrations. (a) Ethanol consumption (g/kg); (b) Ethanol preference ratio and (c) Total fluid intake (ml) in male Wistar rats. Values represent means ± standard error of the mean; $n = 8$ per group; $^*P < 0.05$

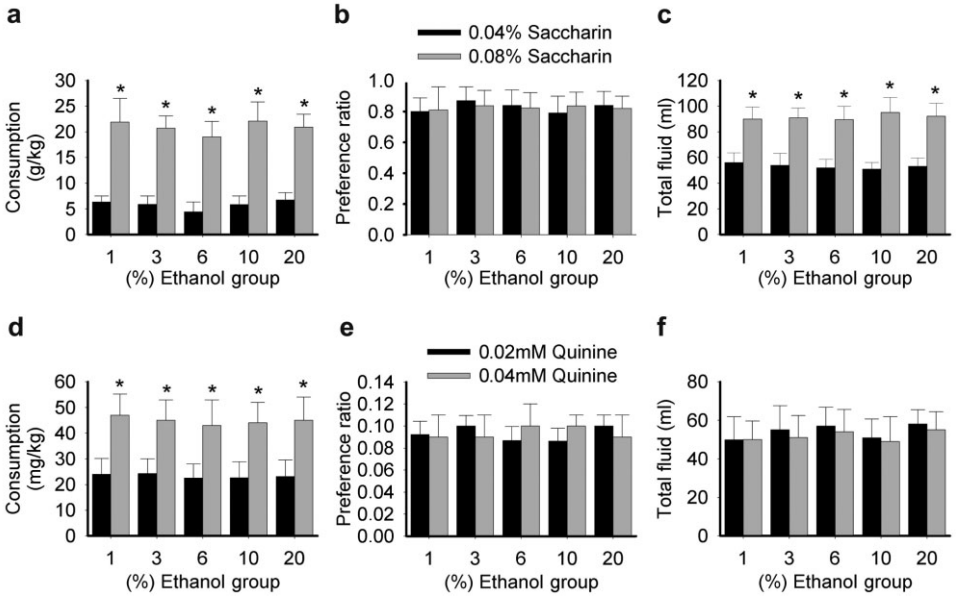

Figure 2 Differential alcohol intake and preference was not associated to any taste neophobia. Data represent consumption, preference and total fluid intake for the tastants saccharin and quinine with continuous access; two bottle choice test. After completion of the ethanol intake experiment (Fig. 1), the same groups of rats were given access to different concentrations of saccharin (a–c) and quinine (d–f) solutions, without receiving further ethanol. a and d, saccharin (g/kg) and quinine consumption (mg/kg); b and e, saccharin and quinine preference ratio; c and f, total volume intake (ml). Values represent means ± standard error of the mean; $n = 8$ per group; $^*P < 0.05$

was a significant main effect of concentration, i.e. rats consumed significantly more saccharin when the 0.08% concentration of saccharin was presented compared with the 0.04% concentration [$F_{(1,39)} = 14.488$; $P < 0.001$]. In addition, there was a significant main effect of saccharin concentration on total fluid consumption. In fact all groups consumed more fluid when the 0.08% concentration was presented compared with the 0.04% solution [$F_{(1,39)} = 27.511$; $P < 0.001$]. However, saccharin concentration had no statistically significant effect on preference ratio [$F_{(1,39)} = 1.411$; $P = 0.377$].

All rat groups consumed a similar amount of the bitter tastant quinine. When consumption was analyzed, there was a significant main effect of concentration [Fig. 2d: $F_{(1,39)} = 13.168$; $P < 0.001$]; more quinine was consumed when the high concentration of quinine (0.04 mM) was presented compared with the low concentration (0.02 mM). However, preference ratio (Fig. 2e) and total fluid intake (Fig. 2f) were not affected by quinine concentration [$F_{(1,39)} = 1.201$; $P = 0.478$ and $F_{(1,39)} = 1.974$; $P = 0.265$, respectively].

Effects of AMN082 and MMPIP on ethanol and tastant consumption and preference in rats

A robust effect of AMN082 on ethanol consumption and ethanol preference is shown in Fig. 3. Using the two-bottle choice test with one bottle containing a 3% ethanol concentration and the other bottle containing water, 10 mg/kg of AMN082 decreased ethanol consumption in rats during the first 4 hours following injection, evident by a significant main effect of dose [Fig 3a, $F_{(3,31)} = 14.452$; $P < 0.001$]. A dose-dependent reduction of the ethanol preference ratio was also observed [Fig 3b, $F_{(3,31)} = 20.564$; $P < 0.001$]. Interestingly, Alcohol consumption and preference were also reduced when the highest dose of 20 mg/kg of AMN082 was used but this reduction was less pronounced compared with 10 mg/kg of AMN082. A possible explanation is that such higher doses of AMN082 may have an opposite effect on mGluR7, as the receptor may get internalized by AMN082 (as demonstrated by Pelkey *et al.* 2007). In contrast, the total fluid intake was not modified [Fig 3c, $F_{(3,31)} = 1.563$; $P = 0.397$]. By 24 hours after drug injection, the effects of AMN082 on ethanol consumption [Fig 3d, $F_{(3,31)} = 0.547$; $P = 0.261$] and preference [Fig 3e, $F_{(3,31)} = 0.687$; $P = 0.247$] were no longer apparent. We also performed the two-bottle choice test with 10% ethanol versus water; here, AMN082 had no significant modulatory effect on ethanol consumption or preference measured either after 4 or 24 hours (see Supporting Information Fig. S1).

Differences in ethanol consumption and preference may be affected by pre-ingestion factors (e.g. taste). To determine if there were general effects of AMN082 treatment on ethanol-unrelated tastants, rats received 10 mg/kg AMN082 injections when given access to saccharin or quinine. In these studies, 4 hours after injection

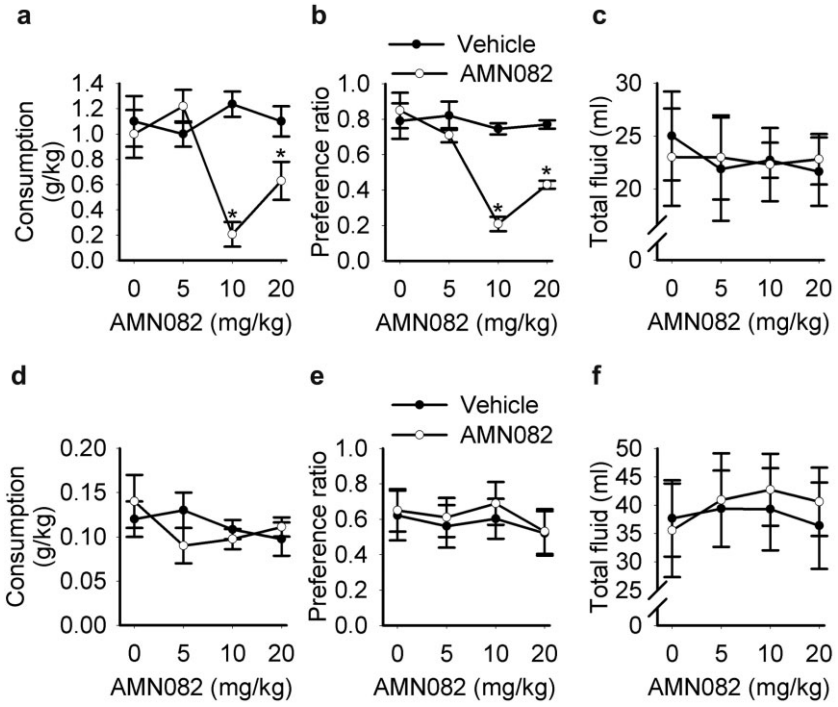

Figure 3 AMN082 treatment in rats decreases ethanol drinking and preference. The effect of treatment with AMN082, using a 3% ethanol solution, on: (a, d) ethanol consumption (g/kg); (b, e) ethanol preference ratio; and (c, f) total fluid intake (ml). Data are represented as means ± standard error of the mean for consumption during the first 4 hours (a–c) and 20 hours later (d–f) of choice administration; $n = 8$ per group; $^*P < 0.05$

there was no difference between AMN082 and vehicle-treated groups (data not shown); based on this, we decided to examine tastant consumption and preference also 24 hours post-injection. The results are summarized in Fig. 4. There were no significant main effects on saccharin consumption after AMN082 administration [$F_{(1,19)} = 1.748$; $P = 0.577$]. AMN082 did not affect the saccharin preference ratio [$F_{(1,19)} = 0.994$; $P = 0.457$] or total fluid intake in these groups [$F_{(1,19)} = 1.277$; $P = 0.284$]. There was a significant main effect of saccharin concentration. Rats consumed more saccharin when the 0.08% concentration of saccharin was presented compared with the 0.04% concentration [$F_{(1,19)} = 22.457$; $P < 0.001$]. However, saccharin concentration had no effect on the preference ratio [$F_{(1,19)} = 0.924$; $P = 0.411$]. In addition, there was a significant main effect of saccharin concentration on total fluid consumption as all the groups consumed more fluid when the 0.08% concentration was presented compared with the lower concentration [$F_{(1,19)} = 20.150$; $P < 0.001$]. Furthermore, for quinine solutions, the ANOVA did not show any effects of AMN082 dosage; see Fig. 4. In summary, these results suggest that decreased consumption of ethanol and decreased ethanol preference following AMN082 administration are not associated with altered taste preference.

The effects of the mGluR7 antagonist MMPIP on ethanol consumption and preference are shown in Fig. 5. Based on a recent study we used MMPIP at 10 mg/kg (Hikichi *et al*. 2010b) and, as for the AMN082 studies, an ethanol concentration of 3%. MMPIP increased ethanol consumption compared with vehicle-treated rats during the first 4 hours after injection [Figs 5a, $F_{(1,19)} = 6.415$; $P = 0.024$]. An increase of ethanol preference ratio was also observed [Fig 5b, $F_{(1,19)} = 5.024$; $P = 0.031$], while the total fluid intake remained unchanged [Fig 5c, $F_{(1,19)} = 0.325$; $P = 0.264$]. As for AMN082, no effects were observed on alcohol consumption and preference 24 hours after MMPIP injection (see Supporting Information Fig. S1).

MMPIP did not affect saccharin consumption [Fig 6a, $F_{(1,19)} = 0.807$; $P = 0.197$] and preference [Fig 6b, $F_{(1,19)} = 1.244$; $P = 0.137$]. However, rats consumed more saccharin when the 0.08% solution was presented compared with 0.04% [$F_{(1,19)} = 15.278$; $P < 0.001$], but the saccharin concentration had no effect on the preference ratio [$F_{(1,19)} = 0.354$; $P = 0.240$]. In addition, there was a main effect of saccharin concentration on total fluid consumption as all the groups consumed more fluid when the 0.08% compared with the 0.04% concentration was presented [$F_{(1,19)} = 26.422$; $P < 0.001$]. As for AMN082, the MMPIP administration had no effect on

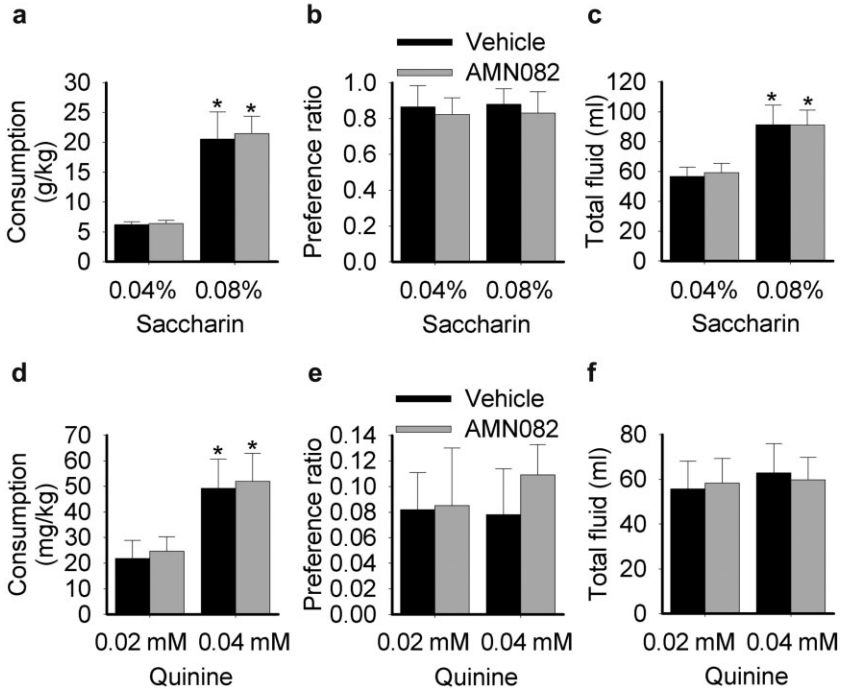

Figure 4 AMN082 treatment in rats did neither alter saccharin nor quinine consumption and preference. The effect of treatment with 10 mg/kg of AMN082 on: (a) saccharin (g/kg) and (d) quinine (mg/kg) consumption; (b) saccharin and (e) quinine preference ratio; (c, f) total fluid intake (ml). Data are represented as means ± standard error of the mean; $n = 10$ per group; $^*P < 0.05$

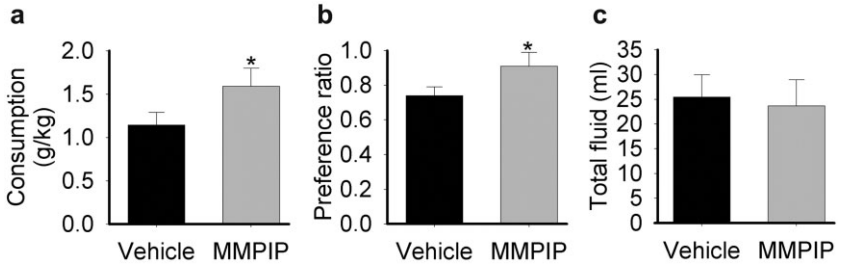

Figure 5 Treatment with 6-(4-methoxyphenyl)-5-methyl-3-pyridin-4-ylisoxazonolo[4,5-c]pyridin-4(5H)-one (MMPIP) increases ethanol drinking and preference. The effect of treatment with 10 mg/kg of MMPIP, using a 3% ethanol solution, on: (a) ethanol consumption (g/kg); (b) ethanol preference ratio; and (c) total fluid intake (ml). Data are represented as means ± standard error of the mean; $n = 10$ per group; * $P < 0.05$

quinine consumption [Fig 6d, $F_{(1,19)} = 0.647$; $P = 0.218$] and preference [Fig 6e, $F_{(1,19)} = 1.647$; $P = 0.211$] demonstrating again that increased consumption of and preference for ethanol upon MMPIP administration is not caused by altered taste preference.

Effects of AMN082 and MMPIP on open field locomotor activity and ethanol metabolism

We examined AMN082 and MMPIP on spontaneous locomotor activity. Both drugs were used at 10 mg/kg (i.p.), as in all the experiments shown above, and the results are presented in Fig. 7a. There were no differences in basal spontaneous locomotion of rats treated with either AMN082 and MMPIP (both at 10 mg/kg, i.p.) or vehicle [$F_{(2,29)} = 0.409$; $P = 0.745$; Fig. 7a]. In addition, there were no differences in metabolism of ethanol (3.5 g/kg; i.p.) between vehicle, AMN082-, and MMPIP-treated rats as shown in Fig. 7b. The slopes of the regression lines were –0.53 ± 0.06, –0.52 ± 0.08 and –0.55 ± 0.05 for vehicle, AMN082 and MMPIP treatment, respectively.

AMN082 effect on ethanol preference challenged with MMPIP

In a further experiment we tested whether the AMN082-induced reduction of ethanol consumption and preference can be blocked by pre-treatment with the

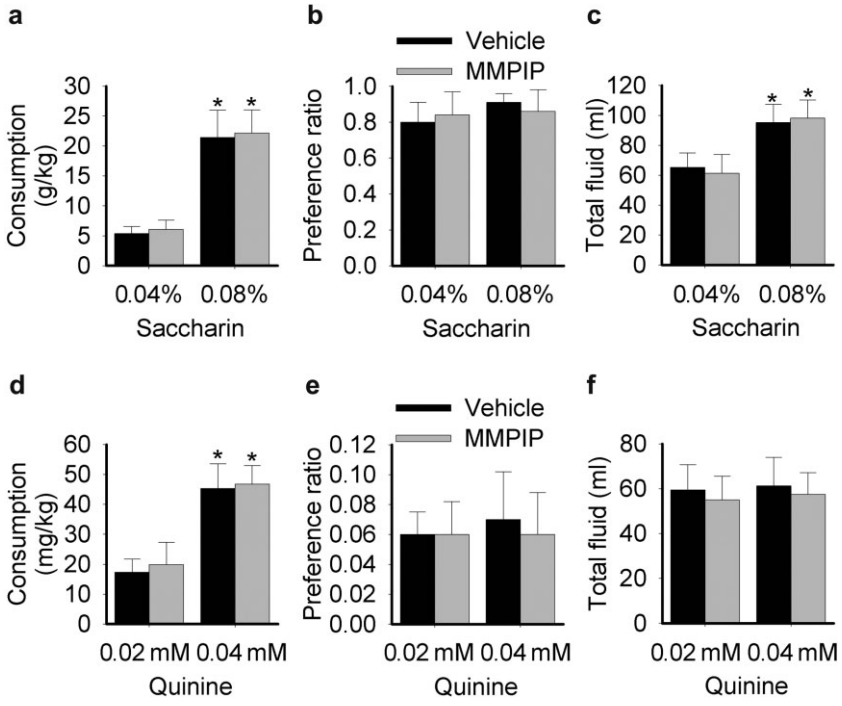

Figure 6 Treatment with 6-(4-methoxyphenyl)-5-methyl-3-pyridin-4-ylisoxazonolo[4,5-c]pyridin-4(5H)-one (MMPIP) did not alter saccharin and quinine consumption and preference. The effect of treatment with 10 mg/kg of MMPIP on: (a) saccharin (g/kg) and (d) quinine (mg/kg) consumption; (b) saccharin and (e) quinine preference ratio; and (c, f) total fluid intake (ml). Data are represented as mean ± standard error of the mean; $n = 10$ per group; $^*P < 0.05$

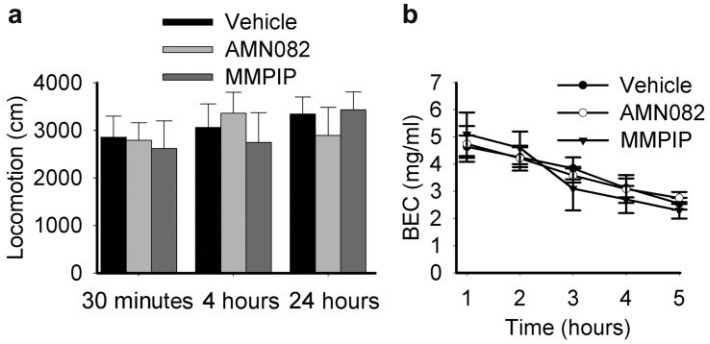

Figure 7 AMN082 and 6-(4-methoxyphenyl)-5-methyl-3-pyridin-4-ylisoxazonolo[4,5-c]pyridin-4(5H)-one (MMPIP) show no effect on spontaneous locomotor activity and blood ethanol metabolism in rats. (a) Three groups of rats were i.p. injected with vehicle (0.5% methylcellulose, 1 ml/kg), 10 mg/kg of AMN082 and 10 mg/kg of MMPIP and locomotor activity was monitored, 30 minutes, 4 and 24 hours after injection, for 60 minutes. (b) Three groups of rats received 3.5 g/kg ethanol (i.p.) and blood ethanol levels were monitored for 5 hours. Data are represented as means ± standard error of the mean; $n = 10$ per group

mGluR7-selective antagonist MMPIP injected 30 minutes before AMN082 was administered (both at 10 mg/kg, i.p.; Fig. 8). Vehicle pre-treated rats, challenged with AMN082, showed reduced alcohol consumption and preference as compared with animals treated with vehicle/vehicle. This AMN082-induced reduction in ethanol consumption and preference was reversed by MMPIP pre-treatment during the first 4 hours following injections, evident by a significant main effect of drug for alcohol consumption [Fig 8a, $F_{(2,29)} = 10.380$; $P < 0.01$] and for alcohol preference [Fig 8b, $F_{(2,29)} = 17.675$; $P < 0.01$]. Importantly, combined AMN082 and MMPIP administration had no effect on total fluid intake [Fig 8c, $F_{(2,29)} = 0.284$; $P = 0.837$].

DISCUSSION

Understanding the molecular and cellular basis of ethanol abuse and addiction may open up novel avenues for the development of therapeutics for alcoholism in

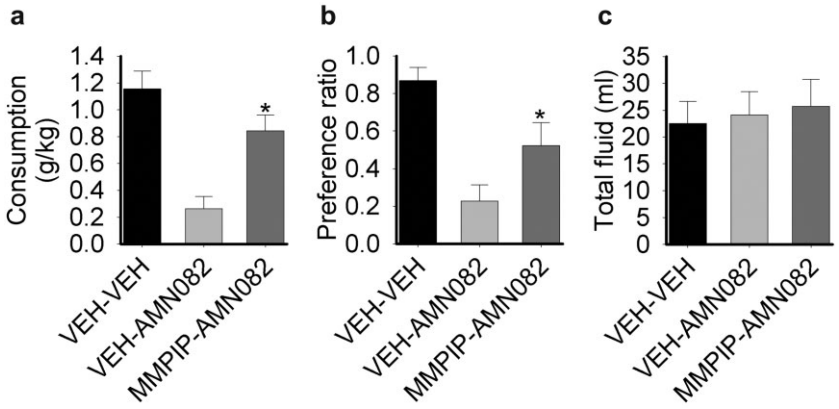

Figure 8 Treatment with 6-(4-methoxyphenyl)-5-methyl-3-pyridin-4-ylisoxazonolo[4,5-c]pyridin-4(5H)-one (MMPIP) blocks AMN082-induced inhibition of alcohol intake and preference. When using a 3% ethanol solution, co-administration of MMPIP significantly blocked AMN082-induced reduction of ethanol consumption (g/kg, a), ethanol preference ratio (b) but did not affect total volume intake (ml, c); both drugs were dosed i.p. at 10 mg/kg; VEH = vehicle. Data are represented as means ± standard error of the mean; $n = 10$ per group; $^*P < 0.05$ compared with VEH-AMN082 group

man. Here, we demonstrate for the first time that mGluR7 activation with AMN082 specifically reduces alcohol intake and preference in rats without affecting taste preference, alcohol metabolism and locomotor activity, whereas mGluR7 blockade with MMPIP enhances alcohol consumption and preference. These findings strongly suggest that functional activity of mGluR7 in mammalian brain exerts control on the development of ethanol reward. In our pharmacological studies we applied the two-bottle choice preference drinking method, a paradigm which has already been used in the seminal genetic studies that identified the mGluR7 gene as a modulatory locus for alcohol drinking (Vadasz *et al.* 2007).

For more than a decade, mGluRs are in the focus of drug discovery for neurological and psychiatric disorders including drug and alcohol addiction. For instance, MPEP-related compounds, i.e. mGluR5 antagonists, and LY379268, an mGluR2/3 agonist, are currently tested in multiple clinical studies. Regarding alcohol-related behavior, MPEP and LY379268 dose-dependently decreased ethanol self-administration in mice and in alcohol preferring rats (Backstrom & Hyytia 2005; Schroeder, Overstreet & Hodge 2005; Hodge *et al.* 2006; Besheer *et al.* 2010) supporting a crucial role for these receptors in the regulation of alcohol intake and reward.

Recently, the group-III receptor mGluR7 was found to represents a very intriguing target for studies on alcohol-related behaviors because of its genetic linkage to ethanol drinking (Vadasz *et al.* 2007), and its proven role in cocaine reward (Li *et al.* 2008; Li *et al.* 2009; Li *et al.* 2010), and due to its highest evolutionary conservation compared with all other mGluRs suggesting significant physiological roles (Flor *et al.* 1997). In a previous study, the mGluR7 allosteric agonist AMN082 was already shown to non-selectively reduce both, sucrose and ethanol administration in mice; in addition, 10 mg/kg of AMN082 (i.p.) in mice also reduced spontaneous locomotor activity (Salling *et al.* 2008). In our study, however, the same dose reduced alcohol intake and preference in rats with no effect on locomotor activity and saccharin consumption. Pharmacokinetic differences between rats and mice are likely to contribute to these discrepancies. In fact, a previous study performed with rats and mice identified lower optimal doses required for mice than for rats (Fendt *et al.* 2008). Furthermore, procedural differences between our study and the one by Salling *et al.* (2008), e.g. operant conditioning (fixed-ratio 4 schedule of reinforcement) versus preference drinking, regular versus sucrose-sweetened ethanol, may also contribute to these contrasting results. In addition, it was reported that administration of a low dose of 6 mg/kg (i.p.) of AMN082 changed spontaneous locomotor activity in C57BL/6J mice (Palucha *et al.* 2007), rendering higher doses in mice difficult to interpret.

Our findings on AMN082 to specifically reduce ethanol drinking in rats compare well with the recent study on mGluR7 and cocaine reward. Here, treatment with AMN082 in rats dose-dependently inhibited cocaine-induced enhancement of electrical brain-stimulation reward and intravenous cocaine self-administration as well as cocaine-induced reinstatement of cocaine-seeking (Li *et al.* 2009; Li *et al.* 2010). Again, AMN082 did not affect basal locomotor activity or sucrose intake (Li *et al.* 2009). AMN082 treatment selectively blocked cocaine-induced changes in extracellular GABA without affecting dopamine, suggesting a central role for mGluR7 in cocaine reinforcement (Li *et al.* 2008; Li *et al.* 2009). Our findings on alcohol consumption and preference suggest an involvement of mGluR7 in the

rewarding effects of ethanol. As the same doses of AMN082 failed to alter oral sucrose consumption, we argue that mGluR7 selectively modulates ethanol's rewarding properties, rather than unspecific appetitive reward. When rats were injected with the mGluR7-selective antagonist MMPIP, ethanol drinking and preference were enhanced suggesting that mGluR7 functional impairment induces an alcoholism-like phenotype. This finding is supported by recently published study by Vadasz *et al*. (2007) who demonstrated that mice carrying mutations that lead to lower mGluR7 mRNA expression drink significantly more alcohol.

Our main finding of the present study that mGluR7 specifically controls the reinforcing properties of alcohol, i.e. functional activation of this presynaptic receptor reduces ethanol preference and consumption, raises important questions regarding the underlying neural circuitry and cellular pathways. Although experimental evidence is lacking, there are at least three mechanistically different alternatives to explain our observations: first, mGluR7 is well known to play a modulatory role in the stress axis of the brain, e.g. systemic AMN082 results in mGluR7-dependent regulation of blood stress hormones (an effect that is absent in mGluR7-deficient mice) (Mitsukawa *et al*. 2005). Moreover, mGluR7 is of critical importance for the glucocorticoid receptor-mediated negative feedback system of the brain stress axis (Mitsukawa *et al*. 2006). Interestingly, life stress and stress hormones such as glucocorticoids are reported to affect human drinking patterns (Cooper *et al*. 1992; Fahlke *et al*. 1994), and hypothalamic and/or hippocampal mGluR7 activation may counteract harmful drinking. Second, mGluR7 shows highest expression in the NAc, amongst a few other brain regions (Kinoshita *et al*. 1998), and local administration of AMN082 reduces GABA-release and increases L-glutamate release in NAc (Kinoshita *et al*. 1998; Li *et al*. 2008). Both effects were reversed by the mGluR7 antagonist MSOP. Interestingly, pharmacological actions of ethanol are mediated, at least in part, by the postsynaptic targets of those neurotransmitters, i.e. $GABA_A$ and NMDA receptors. Thus, the effect of AMN082 on transmitter release could alter agonist-occupancy at those receptors, which eventually may affect ethanol's allosteric modulating efficacy, possibly resulting in reduced ethanol reward mediated by the NAc. Finally, mGluR7 also shows prominent expression in the amygdala, prefrontal cortex and most strongly in the locus coeruleus; three structures that have all been implicated in the reinforcing effects of ethanol (Rodriguez Echandia & Foscolo 1988; Hodge, Chappelle & Samson 1996; Roberts, Cole & Koob 1996; June *et al*. 2001; Besheer, Cox & Hodge 2003; Salling *et al*. 2008). Our future studies will aim to dissect which of those neural structures and circuits are most critical for mGluR7's regulatory role on alcohol reinforcement. Once the responsible brain structures are identified, investigations addressing the subcellular pathways need to follow.

Interestingly, the L-glutamatergic system has a crucial role in alcoholism, as demonstrated by the clinically effective compound acamprosate acting through this system (for review see Spanagel 2003; Spanagel & Kiefer 2008). In addition, a hyperglutamatergic state has been implicated in the etiology of alcohol dependence (Tsai & Coyle 1998; Pulvirenti & Diana 2001; Siggins *et al*. 2003; Spanagel *et al*. 2005). As mGluR7 is known to regulate presynaptic L-glutamatergic transmission, our findings may indicate that dampening of this transmission via the activation of mGluR7 may have the potential to inhibit development of alcohol dependence.

In terms of molecular pharmacological mechanism, AMN082 governs context-dependent receptor activity in the brain, activating selectively certain mGluR7 pathways in specific brain regions and cells, but leaving other mGluR7 pathways untouched. For instance, cAMP metabolism in clonal cell lines, HPA axis-driven stress hormone release to the blood and synaptic plasticity in the amygdala are modulated by AMN082, while certain mGluR7-regulated hippocampal ion channel functions stay unaffected (Ayala *et al*. 2008; Niswender & Conn 2010). Although the underlying physiological basis remains unknown, it seems likely that AMN082 targets mGluR7 such that only a subset of mGluR7's signal transduction pathways are activated, possibly via a novel pharmacological site at the receptor-G protein interface. Interestingly, MMPIP selectively antagonizes AMN082 *in vitro* and *in vivo* (Suzuki *et al*. 2007; Li *et al*. 2008, 2009, 2010), but also acts in a context-dependent manner affecting similar physiological and behavioral parameters as AMN082, but leaving other mGluR7 functions unaffected (Niswender & Conn 2010; Niswender *et al*. 2010).

Taken together, our study demonstrates that mGluR7 functional activity interferes with the rewarding effects of alcohol drinking suggesting that further exploration of mGluR7-directed pharmaceuticals with context-dependent and AMN082-like mechanism may provide future therapeutic value for the treatment of alcohol abuse and dependence in man.

Disclosure/Conflict of Interest

The authors declare no conflict of interests.

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Authors' Contribution

AB was responsible for the study design, data analysis and drafted the paper. AB, KF and MD contributed to the acquisition of animal data. FG provided assistance with pharmacological manipulation. PJF facilitated with study design and interpretation of findings and provided critical revision of data and paper. All authors critically reviewed the content of the paper and approved the final version for publication.

References

Ayala JE, Niswender CM, Luo Q, Banko JL, Conn PJ (2008) Group III mGluR regulation of synaptic transmission at the SC-CA1 synapse is developmentally regulated. Neuropharmacology 54:804–814.

Backstrom P, Hyytia P (2005) Suppression of alcohol self-administration and cue-induced reinstatement of alcohol seeking by the mGlu2/3 receptor agonist LY379268 and the mGlu8 receptor agonist (S)-3,4-DCPG. Eur J Pharmacol 528:110–118.

Besheer J, Cox AA, Hodge CW (2003) Coregulation of ethanol discrimination by the nucleus accumbens and amygdala. Alcohol Clin Exp Res 27:450–456.

Besheer J, Grondin JJ, Cannady R, Sharko AC, Faccidomo S, Hodge CW (2010) Metabotropic glutamate receptor 5 activity in the nucleus accumbens is required for the maintenance of ethanol self-administration in a rat genetic model of high alcohol intake. Biol Psychiatry 67:812–822.

Besheer J, Stevenson RA, Hodge CW (2006) mGlu5 receptors are involved in the discriminative stimulus effects of self-administered ethanol in rats. Eur J Pharmacol 551:71–75.

Bradley SR, Levey AI, Hersch SM, Conn PJ (1996) Immunocytochemical localization of group III metabotropic glutamate receptors in the hippocampus with subtype-specific antibodies. J Neurosci 16:2044–2056.

Cai YQ, Cai GQ, Liu GX, Cai Q, Shi JH, Shi J, Ma SK, Sun X, Sheng ZJ, Mei ZT, Cui D, Guo L, Wang Z, Fei J (2006) Mice with genetically altered GABA transporter subtype I (GAT1) expression show altered behavioral responses to ethanol. J Neurosci Res 84:255–267.

Carboni E, Silvagni A, Rolando MT, Di Chiara G (2000) Stimulation of in vivo dopamine transmission in the bed nucleus of stria terminalis by reinforcing drugs. J Neurosci 20:RC102.

Choi DS, Cascini MG, Mailliard W, Young H, Paredes P, McMahon T, Diamond I, Bonci A, Messing RO (2004) The type 1 equilibrative nucleoside transporter regulates ethanol intoxication and preference. Nat Neurosci 7:855–861.

Cooper ML, Russell M, Skinner JB, Frone MR, Mudar P (1992) Stress and alcohol use: moderating effects of gender, coping, and alcohol expectancies. J Abnorm Psychol 101:139–152.

Crabbe JC, Phillips TJ, Feller DJ, Hen R, Wenger CD, Lessov CN, Schafer GL (1996) Elevated alcohol consumption in null mutant mice lacking 5-HT1B serotonin receptors. Nat Genet 14:98–101.

Cryan JF, Kelly PH, Neijt HC, Sansig G, Flor PJ, van Der Putten H (2003) Antidepressant and anxiolytic-like effects in mice lacking the group III metabotropic glutamate receptor mGluR7. Eur J Neurosci 17:2409–2417.

Ding ZM, Rodd ZA, Engleman EA, McBride WJ (2009) Sensitization of ventral tegmental area dopamine neurons to the stimulating effects of ethanol. Alcohol Clin Exp Res 33:1571–1581.

Dodd PR, Beckmann AM, Davidson MS, Wilce PA (2000) Glutamate-mediated transmission, alcohol, and alcoholism. Neurochem Int 37:509–533.

Engleman EA, McBride WJ, Wilber AA, Shaikh SR, Eha RD, Lumeng L, Li TK, Murphy JM (2000) Reverse microdialysis of a dopamine uptake inhibitor in the nucleus accumbens of alcohol-preferring rats: effects on dialysate dopamine levels and ethanol intake. Alcohol Clin Exp Res 24:795–801.

Fahlke C, Engel JA, Eriksson CJ, Hard E, Soderpalm B (1994) Involvement of corticosterone in the modulation of ethanol consumption in the rat. Alcohol 11:195–202.

Fendt M, Schmid S, Thakker DR, Jacobson LH, Yamamoto R, Mitsukawa K, Maier R, Natt F, Husken D, Kelly PH, McAllister KH, Hoyer D, van der Putten H, Cryan JF, Flor PJ (2008) mGluR7 facilitates extinction of aversive memories and controls amygdala plasticity. Mol Psychiatry 13:970–979.

Flor PJ, Van Der Putten H, Ruegg D, Lukic S, Leonhardt T, Bence M, Sansig G, Knopfel T, Kuhn R (1997) A novel splice variant of a metabotropic glutamate receptor, human mGluR7b. Neuropharmacology 36:153–159.

Gasparini F, Bilbe G, Gomez-Mancilla B, Spooren W (2008) mGluR5 antagonists: discovery, characterization and drug development. Curr Opin Drug Discov Devel 11:655–665.

Gilman JM, Ramchandani VA, Davis MB, Bjork JM, Hommer DW (2008) Why we like to drink: a functional magnetic resonance imaging study of the rewarding and anxiolytic effects of alcohol. J Neurosci 28:4583–4591.

Gruol DL, Parsons KL, DiJulio N (1997) Acute ethanol alters calcium signals elicited by glutamate receptor agonists and K+ depolarization in cultured cerebellar Purkinje neurons. Brain Res 773:82–89.

Hikichi H, Murai T, Okuda S, Maehara S, Satow A, Ise S, Nishino M, Suzuki G, Takehana H, Hata M, Ohta H (2010a) Effects of a novel metabotropic glutamate receptor 7 negative allosteric modulator, 6-(4-methoxyphenyl)–5-methyl-3-pyridin-4-ylisoxazonolo[4,5-c]pyridin-4(5H)-one (MMPIP), on the central nervous system in rodents. Eur J Pharmacol 639:106–114.

Hikichi H, Nishino M, Fukushima M, Satow A, Maehara S, Kawamoto H, Ohta H (2010b) Pharmacological effects of metabotropic glutamate receptor ligands on prepulse inhibition in DBA/2J mice. Eur J Pharmacol.

Hodge CW, Chappelle AM, Samson HH (1996) Dopamine receptors in the medial prefrontal cortex influence ethanol and sucrose-reinforced responding. Alcohol Clin Exp Res 20:1631–1638.

Hodge CW, Miles MF, Sharko AC, Stevenson RA, Hillmann JR, Lepoutre V, Besheer J, Schroeder JP (2006) The mGluR5 antagonist MPEP selectively inhibits the onset and maintenance of ethanol self-administration in C57BL/6J mice. Psychopharmacology (Berl) 183:429–438.

Howard EC, Schier CJ, Wetzel JS, Gonzales RA (2009) The dopamine response in the nucleus accumbens core-shell border differs from that in the core and shell during operant ethanol self-administration. Alcohol Clin Exp Res 33:1355–1365.

Hu JH, Ma YH, Yang N, Mei ZT, Zhang MH, Fei J, Guo LH (2004) Up-regulation of gamma-aminobutyric acid transporter I

mediates ethanol sensitivity in mice. Neuroscience 123:807–812.

June HL, Harvey SC, Foster KL, McKay PF, Cummings R, Garcia M, Mason D, Grey C, McCane S, Williams LS, Johnson TB, He X, Rock S, Cook JM (2001) GABA(A) receptors containing (alpha)5 subunits in the CA1 and CA3 hippocampal fields regulate ethanol-motivated behaviors: an extended ethanol reward circuitry. J Neurosci 21:2166–2177.

Kamens HM, Andersen J, Picciotto MR (2010) Modulation of ethanol consumption by genetic and pharmacological manipulation of nicotinic acetylcholine receptors in mice. Psychopharmacology (Berl) 208:613–626.

Kew JN (2004) Positive and negative allosteric modulation of metabotropic glutamate receptors: emerging therapeutic potential. Pharmacol Ther 104:233–244.

Kinoshita A, Shigemoto R, Ohishi H, van der Putten H, Mizuno N (1998) Immunohistochemical localization of metabotropic glutamate receptors, mGluR7a and mGluR7b, in the central nervous system of the adult rat and mouse: a light and electron microscopic study. J Comp Neurol 393:332–352.

Kinzie JM, Saugstad JA, Westbrook GL, Segerson TP (1995) Distribution of metabotropic glutamate receptor 7 messenger RNA in the developing and adult rat brain. Neuroscience 69:167–176.

Lee MR, Hinton DJ, Song JY, Lee KW, Choo C, Johng H, Unal SS, Richelson E, Choi DS (2010) Neurotensin receptor type 1 regulates ethanol intoxication and consumption in mice. Pharmacol Biochem Behav 95:235–241.

Li X, Gardner EL, Xi ZX (2008) The metabotropic glutamate receptor 7 (mGluR7) allosteric agonist AMN082 modulates nucleus accumbens GABA and glutamate, but not dopamine, in rats. Neuropharmacology 54:542–551.

Li X, Li J, Gardner EL, Xi ZX (2010) Activation of mGluR7s inhibits cocaine-induced reinstatement of drug-seeking behavior by a nucleus accumbens glutamate-mGluR2/3 mechanism in rats. J Neurochem 114:1368–1380.

Li X, Li J, Peng XQ, Spiller K, Gardner EL, Xi ZX (2009) Metabotropic glutamate receptor 7 modulates the rewarding effects of cocaine in rats: involvement of a ventral pallidal GABAergic mechanism. Neuropsychopharmacology 34: 1783–1796.

Littleton JM, Lovinger D, Liljequist S, Ticku R, Matsumoto I, Barron S (2001) Role of polyamines and NMDA receptors in ethanol dependence and withdrawal. Alcohol Clin Exp Res 25:132S–136S.

Lominac KD, Kapasova Z, Hannun RA, Patterson C, Middaugh LD, Szumlinski KK (2006) Behavioral and neurochemical interactions between Group 1 mGluR antagonists and ethanol: potential insight into their anti-addictive properties. Drug Alcohol Depend 85:142–156.

Luttrell LM (2006) Transmembrane signaling by G protein-coupled receptors. Methods Mol Biol 332:3–49.

Masugi M, Yokoi M, Shigemoto R, Muguruma K, Watanabe Y, Sansig G, van der Putten H, Nakanishi S (1999) Metabotropic glutamate receptor subtype 7 ablation causes deficit in fear response and conditioned taste aversion. J Neurosci 19:955–963.

Minami K, Gereau RWT, Minami M, Heinemann SF, Harris RA (1998) Effects of ethanol and anesthetics on type 1 and 5 metabotropic glutamate receptors expressed in Xenopus laevis oocytes. Mol Pharmacol 53:148–156.

Mitsukawa K, Yamamoto R, Ofner S, Nozulak J, Pescott O, Lukic S, Stoehr N, Mombereau C, Kuhn R, McAllister KH, van der Putten H, Cryan JF, Flor PJ (2005) A selective metabotropic glutamate receptor 7 agonist: activation of receptor signaling via an allosteric site modulates stress parameters in vivo. Proc Natl Acad Sci USA 102:18712–18717.

Mitsukawa K, Mombereau C, Lotscher E, Uzunov DP, van der Putten H, Flor PJ, Cryan JF (2006) Metabotropic glutamate receptor subtype 7 ablation causes dysregulation of the HPA axis and increases hippocampal BDNF protein levels: implications for stress-related psychiatric disorders. Neuropsychopharmacology 31:1112–1122.

Moghaddam B, Bolinao ML (1994) Biphasic effect of ethanol on extracellular accumulation of glutamate in the hippocampus and the nucleus accumbens. Neurosci Lett 178:99–102.

Nakanishi S, Masu M, Bessho Y, Nakajima Y, Hayashi Y, Shigemoto R (1994) Molecular diversity of glutamate receptors and their physiological functions. EXS 71:71–80.

Netzeband JG, Parsons KL, Sweeney DD, Gruol DL (1997) Metabotropic glutamate receptor agonists alter neuronal excitability and Ca2+ levels via the phospholipase C transduction pathway in cultured Purkinje neurons. J Neurophysiol 78:63–75.

Niswender CM, Conn PJ (2010) Metabotropic glutamate receptors: physiology, pharmacology, and disease. Annu Rev Pharmacol Toxicol 50:295–322.

Niswender CM, Johnson KA, Miller NR, Ayala JE, Luo Q, Williams R, Saleh S, Orton D, Weaver CD, Conn PJ (2010) Context-dependent pharmacology exhibited by negative allosteric modulators of metabotropic glutamate receptor 7. Mol Pharmacol 77:459–468.

Noldus LP, Spink AJ, Tegelenbosch RA (2001) EthoVision: a versatile video tracking system for automation of behavioral experiments. Behav Res Methods Instrum Comput 33:398–414.

Okamoto N, Hori S, Akazawa C, Hayashi Y, Shigemoto R, Mizuno N, Nakanishi S (1994) Molecular characterization of a new metabotropic glutamate receptor mGluR7 coupled to inhibitory cyclic AMP signal transduction. J Biol Chem 269:1231–1236.

Palucha A, Pilc A (2005) The involvement of glutamate in the pathophysiology of depression. Drug News Perspect 18:262–268.

Palucha A, Klak K, Branski P, van der Putten H, Flor PJ, Pilc A (2007) Activation of the mGlu7 receptor elicits antidepressant-like effects in mice. Psychopharmacology (Berl) 194:555–562.

Pelkey KA, Yuan X, Lavezzari G, Roche KW, McBain CJ (2007) mGluR7 undergoes rapid internalization in response to activation by the allosteric agonist AMN082. Neuropharmacology 52:108–117.

Pham J, Cabrera SM, Sanchis-Segura C, Wood MA (2009) Automated scoring of fear-related behavior using EthoVision software. J Neurosci Methods 178:323–326.

Pulvirenti L, Diana M (2001) Drug dependence as a disorder of neural plasticity: focus on dopamine and glutamate. Rev Neurosci 12:141–158.

Roberts AJ, Cole M, Koob GF (1996) Intra-amygdala muscimol decreases operant ethanol self-administration in dependent rats. Alcohol Clin Exp Res 20:1289–1298.

Rodriguez Echandia EL, Foscolo MR (1988) Effect of lesions of the dorsalis raphe nuclei on the initiation of alcohol preference in rats. Drug Alcohol Depend 21:141–146.

Rodriguez-Moreno A, Sihra TS (2007) Metabotropic actions of kainate receptors in the CNS. J Neurochem 103:2121–2135.

Salling MC, Faccidomo S, Hodge CW (2008) Nonselective suppression of operant ethanol and sucrose self-administration by the mGluR7 positive allosteric modulator AMN082. Pharmacol Biochem Behav 91:14–20.

Saugstad JA, Kinzie JM, Mulvihill ER, Segerson TP, Westbrook GL (1994) Cloning and expression of a new member of the L-2-amino-4-phosphonobutyric acid-sensitive class of metabotropic glutamate receptors. Mol Pharmacol 45:367–372.

Schroeder JP, Overstreet DH, Hodge CW (2005) The mGluR5 antagonist MPEP decreases operant ethanol self-administration during maintenance and after repeated alcohol deprivations in alcohol-preferring (P) rats. Psychopharmacology (Berl) 179:262–270.

Selim M, Bradberry CW (1996) Effect of ethanol on extracellular 5-HT and glutamate in the nucleus accumbens and prefrontal cortex: comparison between the Lewis and Fischer 344 rat strains. Brain Res 716:157–164.

Siegl S, Flor PJ, Fendt M (2008) Amygdaloid metabotropic glutamate receptor subtype 7 is involved in the acquisition of conditioned fear. Neuroreport 19:1147–1150.

Siggins GR, Martin G, Roberto M, Nie Z, Madamba S, De Lecea L (2003) Glutamatergic transmission in opiate and alcohol dependence. Ann N Y Acad Sci 1003:196–211.

Simonyi A, Christian MR, Sun AY, Sun GY (2004) Chronic ethanol-induced subtype- and subregion-specific decrease in the mRNA expression of metabotropic glutamate receptors in rat hippocampus. Alcohol Clin Exp Res 28:1419–1423.

Spanagel R (2003) [The role of the glutamatergic system in alcohol addiction]. Fortschr Neurol Psychiatr 71 (Suppl 1):S33–S35.

Spanagel R, Kiefer F (2008) Drugs for relapse prevention of alcoholism: ten years of progress. Trends Pharmacol Sci 29:109–115.

Spanagel R, Pendyala G, Abarca C, Zghoul T, Sanchis-Segura C, Magnone MC, Lascorz J, Depner M, Holzberg D, Soyka M, Schreiber S, Matsuda F, Lathrop M, Schumann G, Albrecht U (2005) The clock gene Per2 influences the glutamatergic system and modulates alcohol consumption. Nat Med 11:35–42.

Spink AJ, Tegelenbosch RA, Buma MO, Noldus LP (2001) The EthoVision video tracking system—a tool for behavioral phenotyping of transgenic mice. Physiol Behav 73:731–744.

Stachowicz K, Branski P, Klak K, van der Putten H, Cryan JF, Flor PJ, Andrzej P (2008) Selective activation of metabotropic G-protein-coupled glutamate 7 receptor elicits anxiolytic-like effects in mice by modulating GABAergic neurotransmission. Behav Pharmacol 19:597–603.

Suzuki G, Tsukamoto N, Fushiki H, Kawagishi A, Nakamura M, Kurihara H, Mitsuya M, Ohkubo M, Ohta H (2007) In vitro pharmacological characterization of novel isoxazolopyridone derivatives as allosteric metabotropic glutamate receptor 7 antagonists. J Pharmacol Exp Ther 323:147–156.

Szumlinski KK, Diab ME, Friedman R, Henze LM, Lominac KD, Bowers MS (2007) Accumbens neurochemical adaptations produced by binge-like alcohol consumption. Psychopharmacology (Berl) 190:415–431.

Thomas U (2002) Modulation of synaptic signalling complexes by Homer proteins. J Neurochem 81:407–413.

Tsai G, Coyle JT (1998) The role of glutamatergic neurotransmission in the pathophysiology of alcoholism. Annu Rev Med 49:173–184.

Ugolini A, Large CH, Corsi M (2008) AMN082, an allosteric mGluR7 agonist that inhibits afferent glutamatergic transmission in rat basolateral amygdala. Neuropharmacology 55:532–536.

Vadasz C, Saito M, Gyetvai BM, Oros M, Szakall I, Kovacs KM, Prasad VV, Toth R (2007) Glutamate receptor metabotropic 7 is cis-regulated in the mouse brain and modulates alcohol drinking. Genomics 90:690–702.

Wang JQ, Tang Q, Parelkar NK, Liu Z, Samdani S, Choe ES, Yang L, Mao L (2004) Glutamate signaling to Ras-MAPK in striatal neurons: mechanisms for inducible gene expression and plasticity. Mol Neurobiol 29:1–14.

Weiss F, Porrino LJ (2002) Behavioral neurobiology of alcohol addiction: recent advances and challenges. J Neurosci 22:3332–3337.

SUPPORTING INFORMATION

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Figure S1 Experimental design and timetable of procedures.

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