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mGluR5 Antagonist MPEP Reduces Ethanol-Seeking and Relapse Behavior

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The glutamatergic system plays an important role in mediating neurobehavioral effects of ethanol. Metabotropic glutamate receptors subtype 5 (mGluR5) are modulators of glutamatergic neurotransmission and are abundant in brain regions known to be involved in ethanol self-administration. Here, we studied the effects of 2-methyl-6-(phenylethynyl)-pyridine (MPEP), a highly potent, noncompetitive mGlu5 receptor antagonist, on voluntary ethanol consumption and relapse behavior. For this purpose, we used two models for the measurement of relapse behavior: (i) reinstatement of ethanol-seeking behavior by drug-associated cues and (ii) the alcohol deprivation effect in long-term ethanol-consuming rats. In the first set of experiments, rats were trained to lever press for ethanol in the presence of a distinct set of cues. After extinction, the animals were exposed to the respective cues that initiated reinstatement of responding. A response-contingent ethanol prime further enhanced responding compared to the conditioned cues alone. Under these conditions, MPEP (0, 1, 3, and 10 mg/kg) attenuated ethanol seeking significantly and in a dose-related manner. However, at the highest dose, MPEP also decreased the number of inactive lever responses. In the second set of experiments, rats with 1 year of ethanol experience and repeated deprivation phases were used. A subchronic treatment with MPEP (twice daily; 0, 3, and 10 mg/kg) resulted in a significant and dose-dependent reduction of the alcohol deprivation effect (ADE). Although the same MPEP treatment regimen decreased baseline drinking, this effect was not as pronounced as on the ADE. These results show in two commonly used models of relapse to ethanol that pharmacological targeting of mGlu5 receptors may be a promising approach for the treatment of alcoholism.
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INTRODUCTION

The neurobiological and molecular basis of relapse to ethanol is still not well understood; however, preclinical as well as clinical data imply that relapse can be induced through different pathways (Spanagel and Zieglgänsberger, 1997; Lê and Shaham, 2002; Weiss and Porrino, 2002). One pathway seems to involve the glutamatergic system (Tsai and Coyle, 1998), where chronic alcohol intake leads to compensatory changes. During withdrawal and abstinence, increased glutamatergic excitatory neurotransmission leads to a state of hyperexcitability, which may then be associated with an enhanced risk to relapse (Tsai and Coyle, 1998; Spanagel and Bienkowski, 2002). Diminishing the activity of a hyperexcited glutamatergic system might be a promising therapeutic approach in order to reduce the risk to relapse in abstinent patients. From a pharmacological point of view, this can be best achieved by targeting postsynaptic glutamate receptors. A variety of postsynaptic glutamate receptors exist; however, the metabotropic glutamate receptor subtype 5 (mGluR5) has recently received considerable attention due to its abundance in brain regions known to be involved in drug reinforcement (Shigemoto *et al*, 1993; Romano *et al*, 1995; Lu *et al*, 1999; Wang *et al*, 2003). In addition, the initial report by Chiamulera *et al* (2001) demonstrated the absence of cocaine self-administration in mGluR5 knockout mice and the dose-dependent reduction of cocaine self-administration following the administration of the selective antagonist 2-methyl-6-(phenylethynyl)-pyridine (MPEP) (Gasparini *et al*, 1999). Taking these findings into consideration, we hypothesized that mGluR5 might be a promising pharmacological target for the prevention of relapse to alcohol.

To test this hypothesis, we studied the effects of MPEP in two commonly used models of relapse; namely, the reinstatement model and the alcohol deprivation model (see, for review, Spanagel, 2000; Lê and Shaham, 2002;

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Shaham *et al*, 2003). In the reinstatement paradigm, the animal is trained under operant conditions to self-administer ethanol and is then subjected to extinction. Following extinction, various conditions have been reported to lead to reinstatement of ethanol seeking, including ethanol priming and stress (Lê *et al*, 1998). However, the most reliable reinstatement of ethanol seeking has been obtained after presenting the subjects with stimuli previously associated with ethanol availability (Katner *et al*, 1999; Ciccocioppo *et al*, 2001; Liu and Weiss, 2002).

Another animal model to study relapse-like behavior is the alcohol deprivation effect (ADE) (see, for review, Spanagel and Hölter, 1999). In this model, alcohol-experienced animals show a transient increase in alcohol consumption and alcohol preference after a period of forced abstinence (alcohol deprivation). This robust effect can be observed under home cage drinking and under operant conditions in various species, including monkeys (Sinclair, 1971).

Both models have now been pharmacologically validated with antirelapse compounds that are clinically used for treating alcoholics (Spanagel and Hölter, 2000; Lê and Shaham, 2002). In conclusion, there appears to be a good correspondence between the events that induce relapse in rats and those that provoke relapse in humans.

MATERIALS AND METHODS

Animals

Tests for reinstatement were carried out using 10 male Long–Evans rats (HsdBlu:LE, Harlan Sprague–Dawley, Indianapolis, IN) weighing 200–250 g at the start of experiments. The animals were housed in pairs in Macrolon IV cages in a temperature- and humidity-controlled room under a 12-h light/dark cycle (lights on at noon). Water and pellet food (RM1, SDS, Witham, UK) were available *ad libitum* in the home cage except during initial training (see below). All behavioral testing was carried out during the dark phase of the light/dark cycle between 0800 and 1100, 5 days a week.

For the experiments on the ADE and baseline drinking, 30 male Wistar rats (Martinsried, Germany), weighing 260–300 g at the start of the experiment, were housed individually in standard hanging rodent cages (Ehret, Emmendingen, Germany) on a 12-h light/dark cycle with lights on at 0700. Animals were provided with food (Ssniff, Soest, Germany), tap water, and 5, 10, and 20% (v/v) ethanol solutions *ad libitum* for 12 months.

All experimental procedures were approved by the respective Committees on Animal Care and Use, and carried out following the local Animal Welfare Acts and in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC).

Drugs

MPEP (Alexis Biochemicals, Lausen, Switzerland) was used in all experiments as its hydrochloride salt dissolved in saline (0.9%). To obtain complete effects of MPEP in the home cage experiments (ADE and baseline drinking), rats received two intraperitoneal (i.p.) injections per day. MPEP was administered for a total of 3 days twice daily at 0800 and 1800.

Reinstatement Procedure

Ethanol self-administration. All training and testing procedures took place in operant chambers (Lafayette Instrument, Lafayette, IN) enclosed in ventilated sound-attenuating cubicles. The chambers were equipped with two response levers on each side of the two drinking cups in the center of the front panel. A stimulus light was mounted above the right response lever. Auditory stimuli (2.9 kHz, 65 dB) were delivered from a loudspeaker positioned on top of the self-administration chamber. Responses at the appropriate lever activated a syringe pump that delivered a 0.1 ml drop of fluid into one of the two drinking cups. Rats were trained to self-administer ethanol orally in daily 30-min sessions using a saccharin fading procedure. Briefly, rats were placed on a 12-h water deprivation for 3 consecutive days and were trained to respond for a 0.1 ml drop of 0.2% (w/v) saccharin solution on both levers under a fixed ratio 1 (FR1) schedule of reinforcement. After this initial training, water deprivation was terminated and animals had free access to food and water throughout the subsequent training and testing. During the next three sessions, responses at the right lever resulted in the delivery of 0.1 ml of 5% (w/v) ethanol + 0.2% saccharin solution. Responses at the left lever were recorded but had no programmed consequences. Thereafter, the concentration of ethanol was increased first to 8% and then to 10% (w/v), while the concentration of saccharin was decreased until saccharin was eliminated completely from the drinking solution over a period of 13 sessions.

Conditioning and extinction procedures. During conditioning, discriminative stimuli were established that predicted availability of 10% ethanol and an alternate fluid with low reinforcing properties. Preliminary tests showed that rats also responded for water at relatively high rates. Therefore, 80 μM quinine solution was offered as the nonreinforcing fluid. Beginning with self-administration training with the 10% ethanol concentration, olfactory discriminative stimuli (S^D) predicting either ethanol or 80 μM quinine hydrochloride solution availability were presented during self-administration sessions. Ethanol availability was signaled by anise odor (S^+), whereas quinine availability (ie nonreward) was signaled by grapefruit odor (S^-). The olfactory stimuli were generated by placing a small piece of absorbent paper containing a drop of either anise or grapefruit essential oil next to the self-administration chamber inside the sound-attenuation cubicle immediately before the session. In addition to these discriminative stimuli, each lever press resulting in ethanol delivery was accompanied by a 3-s light stimulus (CS^+), whereas a 3-s auditory stimulus (CS^-) was presented with quinine delivery. During the first week of the 7-week conditioning phase, rats were given ethanol sessions only. Thereafter, ethanol and quinine sessions were given in a random order until rats received a total of 18 ethanol and 18 quinine sessions. Subjects were then subjected to 30-min extinction sessions, during which responding had no programmed consequences and the olfactory stimuli

signaling ethanol or quinine availability were withheld. Extinction sessions continued for a total of 20 sessions.

Reinstatement tests. Reinstatement sessions began on day 1 postextinction. During the first session, rats were presented with S^D predictive of quinine availability (grapefruit odor), and responses on the previously active lever resulted in the 5-s presentation of the auditory stimulus and activation of the syringe pump motor, but not in the delivery of drinking solution. During the next sessions, rats were tested for ethanol-seeking behavior under two reinstatement conditions: with S^+/CS^+ present and with S^+/CS^+ accompanied by response-contingent 0.2 ml oral ethanol priming. Both sessions were initiated by the presentation of the ethanol-associated S^D (anis odor), and active lever responses were followed by the activation of syringe motor and presentation of ethanol-associated CS^+, the 5-s light. In addition, during the session with ethanol priming, the first two lever responses produced a 0.1 ml ethanol solution to the drinking cup. Half of the subjects were tested under the S^+/CS^+ condition on day 2 postextinction and the S^+/CS^+/ethanol priming condition on day 4 postextinction. On intervening days, rats remained in their home cages. The conditions were reversed for the other half of the animals.

Effect of MPEP on reinstatement. The effect of MPEP on ethanol-seeking behavior was examined using the reinstatement procedure with the ethanol priming described above (S^+/CS^+/ethanol priming). Reinstatement testing was conducted twice a week with the rats confined to their home cages on intervening days. MPEP was administered 30 min prior to the sessions in a Latin-square, within-subjects design (0, 1, 3, and 10 mg/kg).

ADE Procedure and Baseline Drinking

Long-term ethanol self-administration. After 1 week of habituation to the animal room, all rats were given continuous access to tap water and to 5, 10, and 20% (v/v) ethanol solutions in their home cages. Spillage and evaporation were minimized by the use of bottle caps with ball bearings (Ehret, Emmendingen, Germany). With this procedure, the ethanol concentration in a given solution stayed constant for at least 1 week (see Hölter *et al*, 1998). All drinking solutions were renewed weekly and at that time, the positions of the four bottles were changed to avoid location preferences. After the initial 8 weeks of continuous ethanol access, every 4 weeks of drinking were followed by a deprivation phase of two weeks. At the end of the eighth deprivation phase the animals were used for the experiments.

ADE measurement. Baseline measures were determined by daily weighing of the bottles and the animals at 1000 for three preabstinence days. Daily ethanol intake, weight changes, total fluid intake, total ethanol preference, and preferences for the three ethanol solutions were calculated from these measurements. After the last day of measurement, the ethanol bottles were removed from the cages leaving the animals with food and tap water *ad libitum*. After 14 days, the ethanol solutions were presented again to the animals at 1000 and the daily weighing routine was reintroduced for 4 postabstinence days to assess the ADE.

Effects of MPEP on baseline drinking and the ADE. The effects of subchronic MPEP treatment on baseline drinking and the ADE were determined in rats with 12 months of ethanol experience in the long-term paradigm described above.

For evaluating the effects of MPEP on baseline drinking, the animals were divided into three groups ($n = 10$ each) and treated with two doses of MPEP (3 and 10 mg/kg, i.p.) and saline for 3 subsequent days. After 4 weeks, the animal groups were subjected to a deprivation phase for 14 days to assess the effects of MPEP on the ADE. The first two injections of MPEP were given prior to the representation of the ethanol bottles. To ensure that previous MPEP treatment during baseline drinking did not influence our ADE measurements animal groups received different treatment during ADE and baseline drinking.

Locomotor Activity

Locomotor activity testing was carried out in 24 Wistar rats that underwent the long-term ethanol self-administration procedure with repeated deprivation phases. The animals were put into a sound-attenuated experimental room 12 h before testing. Animals were divided into three groups ($n = 8$ per group). According to a detailed time schedule, the treatment groups received either two injections of MPEP (10 mg/kg; 12 h and 1 h prior to the activity measurement) + ethanol (0.5 g/kg, 12% v/v i.p. in saline) or two injections of saline + ethanol, respectively. Control animals accordingly received three injections of saline. Measurements started immediately after the injection of ethanol. Activity monitoring was conducted in square-shaped boxes ($49 \times 49 \times 38$ cm ($L \times W \times H$)) and illuminated from above with 25 Lux. Rats were placed individually into the boxes and activity was monitored in 20-min bins for 2 h by a video camera (Sony CCD-IRIS). The recorded data were analyzed using the image processing system EthoVision 3.0 (Noldus Information Technology, Wageningen, The Netherlands).

Data analysis. In the reinstatement experiments, the number of active and inactive lever responses across the reinstatement conditions and MPEP doses was analysed using one-way ANOVA with repeated measures. In experiments using the alcohol deprivation model, treatment effects on postabstinence days were assessed by two-way ANOVA with repeated measures (treatment × days). Locomotor activity experiments were analyzed using one-way ANOVA. The accepted level of significance was $p \leqslant 0.05$. Newman–Keuls test was applied for *post hoc* comparisons when appropriate.

RESULTS

MPEP Dose-Dependently Reduced Cue-Induced Reinstatement of Ethanol-Seeking Behavior

Following the saccharin fading procedure, rats developed stable responding for 10% ethanol during the 18 conditioning sessions. The mean (±SEM) number of responses for

ethanol during the final three sessions was 32.0 ± 3.6, corresponding to an ethanol intake of 0.53 ± 0.05 g/kg, while the number of inactive lever responses was 6.4 ± 1.7. At the same time, the number of quinine responses decreased, reaching the level of 11.9 ± 2.0 responses during the last three sessions. The number of inactive lever responses during these sessions was 9.9 ± 2.2.

Figure 1a shows that exposure of subjects to the S^+/CS^+ stimulus conditions reliably reinstated responding on the previously active lever above the extinction level, while the quinine-associated S^-/CS^- condition failed to alter responding (factor condition $F(3,27) = 29.74$, $p < 0.0001$). Further analyses indicated that the response-contingent 0.2 ml ethanol prime significantly enhanced responding compared to the S^+/CS^+ alone ($F(1,9) = 15.11$, $p = 0.004$). Since this ethanol volume corresponded to an ethanol intake of only 0.04 g/kg in these rats, it is likely that orosensory rather than central effects of ethanol were responsible for enhanced responding.

The mGluR5 antagonist MPEP attenuated ethanol seeking significantly and in a dose-related manner in the S^+/CS^+/ethanol condition ($F(3,27) = 8.56$, $p < 0.0001$). However, at the highest 10 mg/kg dose, MPEP also decreased the number of the inactive lever responses ($F(3,27) = 3.55$, $p = 0.028$) (Figure 1b).

MPEP Dose-Dependently Reduced the ADE

At first, the effect of subchronic MPEP treatment on basal alcohol intake was measured (Figure 2a). Animals consumed on an average 2.9 ± 0.15 g/kg ethanol per day.

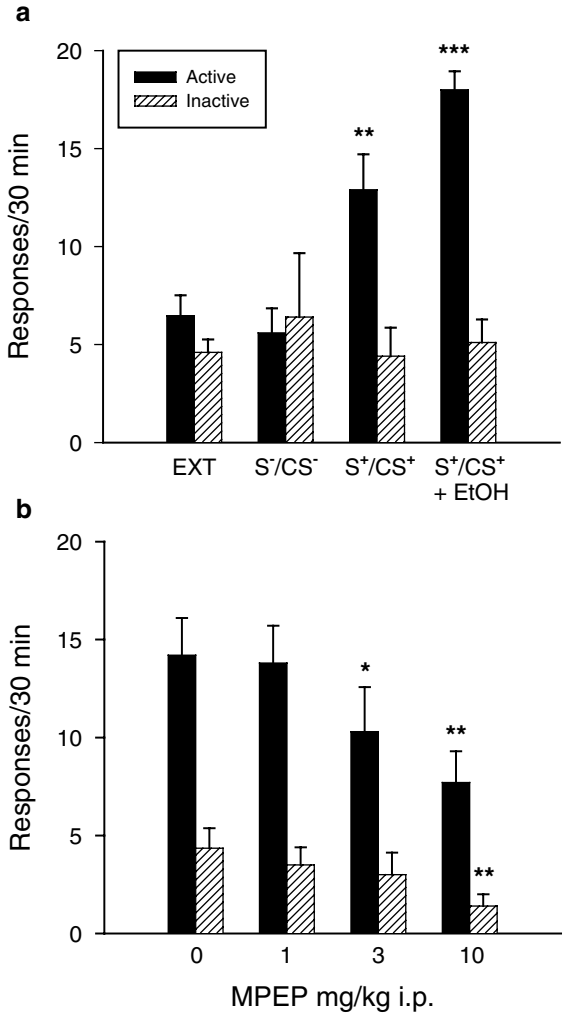

Figure 1 Reinstatement of ethanol responding under stimulus conditions associated with ethanol and quinine availability (a) and modulation of ethanol responding with MPEP (0, 1, 3, and 10 mg/kg; i.p.) (b). *EXT*, responses during the final three extinction sessions; S^-/CS^-, responses during stimuli predictive of quinine availability; S^+/CS^+ and S^+/CS^+/EtOH, responses during stimuli predictive of ethanol availability. The effects of MPEP were tested under the S^+/CS^+/EtOH condition following the first reinstatement sessions. Shown are the mean (±SEM) numbers of responses at the previously active and inactive levers during 30-min sessions in 10 rats. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, significantly different from the baseline or vehicle condition, paired *t*-tests.

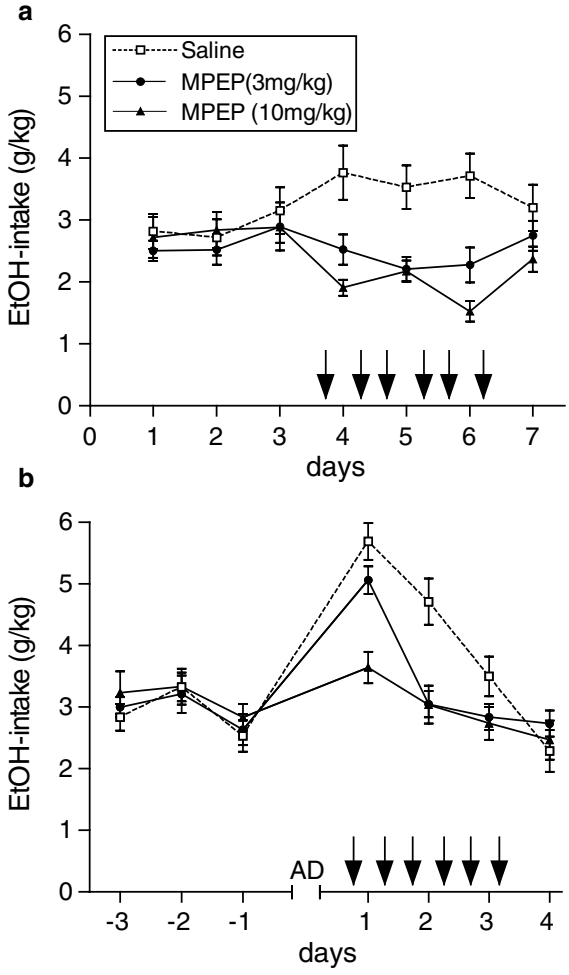

Figure 2 Effects of MPEP (0, 3, and 10 mg/kg; i.p.) on ethanol intake in rats that consumed voluntarily ethanol for 1 year and underwent repeated deprivation phases. In (a) the effects of subchronic MPEP treatment on baseline drinking is shown and in (b) its effects on the ADE are illustrated. The figures show the mean daily alcohol intake in g ethanol/kg bodyweight (±SEM) obtained in 10 rats per group. Arrows indicate MPEP injections. Measurements were taken during the last 3 days before deprivation (AD = alcohol deprivation) and first 4 days after representation of ethanol. Significant differences were found for both treatment groups: on days 4–6 (baseline drinking, a) and for the first 3 postdeprivation days (ADE, b) compared to the saline control group. For reasons of clarity of the figures asterisks are not shown here.

Subchronic treatment of MPEP over 3 days produced a significant attenuation of basal ethanol intake in both treatment groups compared to the control group (treatment: $F(2,27) = 5.337$, $p = 0.011$). However, this effect was mainly due to an increase in the saline control group over days (factor time: $F(6,54) = 3.372$, $p = 0.007$). It is suggested that multiple saline injections were stressful to the animals and that they slightly increased their ethanol intake over days—a phenomenon that we have already observed previously (Spanagel and Hölter, 1999). In contrast, MPEP injections had no significant effect over the time course of the experiment (factor time: $F(6,162) = 1.438$, $p = 0.203$).

After 2 weeks of abstinence, a significant ADE occurred characterized by a transient increase in ethanol intake (factor ADE: $F(6,54) = 30.782$, $p < 0.001$) during the 4 post-abstinence days in comparison with the 3 preabstinence days (Figure 2b). Figure 2b shows further the influence of MPEP (0, 3, and 10 mg/kg; i.p.) given twice daily for 3 days on the ADE. In comparison to the effect of saline in control animals, MPEP dose-dependently diminished alcohol intake on representation after the deprivation period. Two-way analysis of variance with repeated measures over 4 days after withdrawal revealed a significant effect of treatment [$F(2,27) = 5.143$, $p = 0.013$], a significant effect of days [$F(3,81) = 92.983$, $p < 0.001$], and a significant interaction between factors [$F(6,81) = 11.467$, $p < 0.001$]. *Post hoc* analysis (Newman–Keuls test) showed a significant difference between the treatment groups and controls on the 3 postdeprivation days.

Subchronic MPEP treatment had no influence on body weight and water intake: body weight: before treatment 547 ± 10 g, after treatment 548 ± 9 g in the saline group and before treatment 549 ± 6 g, after treatment 545 ± 5 g in the MPEP group (10 mg/kg). Water intake before treatment 8.0 ± 1 g/kg, after treatment 8.6 ± 1 g/kg in the saline group and before treatment 7.3 ± 0.9 g/kg, after treatment 6.7 ± 0.9 g/kg in the MPEP group (10 mg/kg).

MPEP Treatment did not Produce Sedative Effects

Ethanol at the applied low dose (0.5 g/kg) increased locomotor activity for 2 h; however, MPEP treatment (10 mg/kg) decreased the resulting activity to the level of the saline-treated control animals (Figure 3). One-way ANOVA revealed no significant treatment effects following ethanol + MPEP treatment ($F(2,21) = 0.886$; $p = 0.43$).

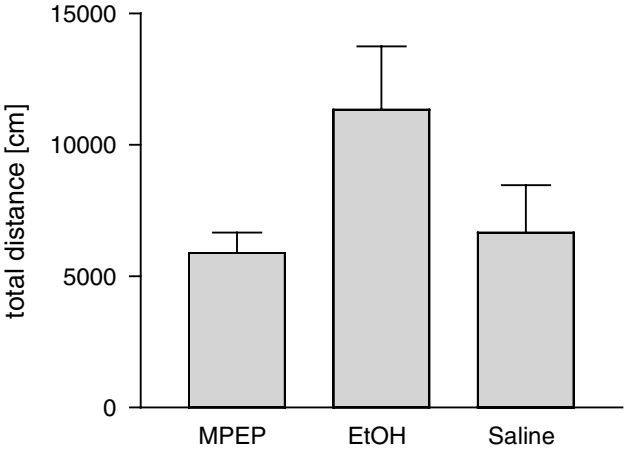

Figure 3 Effects of MPEP and ethanol injections on locomotor activity compared to saline controls in eight rats/group. Treatment groups received two injections of MPEP + EtOH or two injections of saline + EtOH, respectively. Controls were treated with saline. The bars show the total distance (±SEM) moved during 2 h.

DISCUSSION

The effects of the selective mGluR5 antagonist MPEP were tested in two commonly used models of relapse to ethanol drinking. In both models, MPEP significantly reduced relapse-like behavior in a dose-dependent manner. MPEP also affected baseline drinking under home cage conditions; however, this effect was not as pronounced as on relapse-like drinking behavior.

MPEP was first tested in a rat model of cue-induced reinstatement of ethanol-seeking behavior. For this purpose, distinct cues (smell and light) were paired with ethanol responding. Following extinction this set of distinct cues renewed responding on the ethanol lever, an effect that was further augmented by contingent ethanol priming. With ethanol priming, the rats were presented with two additional ethanol-associated cues, the odor and taste of ethanol that were present during conditioning and were not subjected to extinction because quinine conditioning sessions and extinction training were conducted with no ethanol in the fluid delivery system. It is therefore possible that enhanced responding seen after ethanol priming was caused by simultaneous presentation of all constituents of the stimulus complex present during conditioning. However, it has been found previously that presentation of a liquid dipper containing either ethanol or water reinstated responding, suggesting that nonspecific sensory properties of the liquid made available may also contribute to reinstatement (Bienkowski *et al*, 2000). This possibility was not investigated in our study. Furthermore, we do not know whether the small oral priming dose of ethanol alone could have reinstated responding, or whether the environmental cues associated with ethanol availability and the orosensory properties of ethanol acted additively to induce reinstatement. In this context, it is worth noting that although contingent or injected ethanol have been previously reported to reinstate ethanol responding, this effect shows high variability and inconsistency (Chiamulera *et al*, 1995; Lê *et al*, 1998, 1999; Vosler *et al*, 2001; Lê and Shaham, 2002).

Under the described conditions, MPEP significantly reduced reinstatement of ethanol-seeking behavior. As the priming dose of ethanol was very small (on average 0.04 g/kg), it is likely that MPEP reduced the ability of the stimulus complex associated with ethanol (ie the environmental cues and orosensory properties of ethanol) to reinstate lever pressing rather than counteracted the central pharmacological effects of ethanol. Similarly, MPEP has been shown to attenuate the expression of other conditioned behaviors, including conditioned fear, morphine- and cocaine-induced place preference, and taste aversion (Schulz *et al*, 2001; Popik and Wrobel, 2002; McGeehan and Olive, 2003; Schachtman *et al*, 2003).

With the MPEP doses used, no nonspecific effects on behavior were observed, but at a dose of 10 mg/kg, MPEP reduced responding on the inactive lever. Attenuation of inactive lever responses could be interpreted as nonspecific motor impairment by MPEP at the highest dose. However, it is also possible that active and inactive lever responses are not completely independent of each other. For example, we have commonly seen that the number of both active and inactive lever responses decrease during extinction in our behavioral model. Therefore, suppression of responding at the inactive lever by MPEP could also be interpreted as response generalization. This conclusion is further supported by our locomotor activity measurements. Although MPEP reduced ethanol-induced locomotor stimulation, animals were not impaired under these conditions when compared to saline-treated control animals. In other studies MPEP at doses of 2.5–10 mg/kg, delivered i.p., had also no effect on exploratory locomotor activity in rats (Tatarczynska *et al*, 2001; Henry *et al*, 2002; Paterson *et al*, 2003). However, another study indicated that much higher doses of MPEP and ethanol resulted in an additive effect of MPEP on ethanol-induced sedation in mice (Sharko and Hodge, 2003).

The reinstatement model appears to be useful for the study of the impact of conditioned cues on relapse to alcohol drinking; however, the usefulness of the reinstatement model in representing human relapse behavior has two important limitations (Spanagel, 2000). First, researchers to date have not conclusively demonstrated that rats going through a reinstatement procedure are truly ethanol dependent in the sense that they exhibit uncontrolled ethanol responding. Second, it appears that extinction of ethanol-seeking behavior usually plays only a minor role in alcoholic patients trying to achieve and maintain abstinence. Consequently, the reinstatement model may not accurately reflect the situation of abstinent alcoholics experiencing craving and relapse. Other aspects of relapse-like drinking behavior might be better covered by the ADE, which is represented in rats in which long-term ethanol self-administration alternates with repeated ethanol deprivation phases.

In our ADE model, subchronic MPEP treatment also significantly reduced relapse-like drinking behavior in a dose-dependent manner without any observable side effects on body weight or total fluid intake. Although the ADE model has good predictive validity—pharmacological agents that have been shown to attenuate relapse rates in humans (acamprosate, naltrexone, and 5-HT_3-antagonists) also attenuate the ADE (Spanagel and Hölter, 2000; Rodd-Henricks *et al*, 2000)—this model has certain limitations to mimic the human situation. Thus the drinking profile of an ADE does not closely reflect the profile of relapse drinking in alcoholics (Spanagel, 2000) and furthermore little is known about the neuronal circuits involved in the ADE. However, it has been repeatedly shown that noncompetitive NMDA receptor antagonists abolish the ADE (Hölter *et al*, 1996, 2000; Bienkowski *et al*, 2001), suggesting a crucial role of NMDA receptors in mediating the ADE. The fact that synaptic transmission at NMDA receptors is modulated by simultaneous activation of mGluR5 (Sorensen and Conn, 2003) could at least provide a clue for the effects of MPEP on the ADE. This functional coupling could result from the postsynaptic association of NMDA receptors with a complex of proteins, which includes different scaffolding proteins (eg PSD-95, Homer, Shank), but other receptors including mGluR5 are also linked to this complex (Kotecha *et al*, 2003), and activation of mGluR5 can lead to an enhancement of NMDA receptor function through phosphorylation by protein kinase C (Hermans and Challiss, 2001; Schoepp and Conn, 1993). The high affinity of MPEP for mGluR5 receptors, which is more than 1000-fold higher compared to NMDARs, makes it very unlikely that under the used conditions MPEP affects NMDARs directly (Oleary *et al*, 2000; Gubellini *et al*, 2001; Spooren *et al*, 2001; Kozela *et al*, 2003). In summary, the functional coupling of mGluR5 and NMDA receptor suggests that the blockade of mGluR5 by MPEP reduces glutamatergic signaling through NMDA receptors and thereby interacts with ethanol-seeking and relapse behavior.

Having the limitations of the animal models used in the present study in mind, it is proposed that pharmacological targeting of mGluR5 might be a promising therapeutic line to pursue in alcoholic patients. This assumption is supported by three other recent findings: (i) In a preliminary report it has been shown that MPEP decreases operant ethanol self-administration during periods of peak consumption in mice (Sharko *et al*, 2002). (ii) The finding that MPEP evokes anxiolytic- and antidepressant-like effects in rats (Tatarczynska *et al*, 2001; Pilc *et al*, 2002) also has implications for the treatment of alcoholism due to the high comorbidity with these psychiatric disorders. (iii) Furthermore, Harris *et al* (2002) showed that acamprosate exhibits binding and functional characteristics that are consistent with an mGluR5 antagonist. These authors further speculate that acamprosate's ability to reduce relapse rates in alcoholic patients may result from its alterations in glutamatergic neurotransmission through mGluR5s.

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