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The Effects of Acamprosate and Neramexane on Cue-Induced Reinstatement of Ethanol-Seeking Behavior in Rat

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This study examines, for the first time, the effects of acamprosate and the non-competitive *N*-methyl-D-aspartate (NMDA) receptor antagonist neramexane on ethanol-seeking induced by alcohol-related environmental stimuli in an animal model of relapse. Wistar rats were trained to operantly self-administer ethanol (10% w/v) or water on a fixed-ratio 1 schedule in a 30-min daily session. Ethanol availability was signaled by an olfactory discriminative stimulus of orange extract (S^+). In addition, each lever press was accompanied by a 5-s illumination of the operant chamber's house light (CS^+). Water availability was signaled by anise odor (S^-) and 5-s white noise stimulus (CS^-). After completion of the conditioning phase, indicated by stable levels of responding, operant behaviors were extinguished. Prior to reinstatement tests, animals were divided into groups according to either treatment with acamprosate (100, 200 mg/kg given twice), neramexane (1.0, 2.0, 4.0 mg/kg), or vehicle. In vehicle-treated rats, re-exposure to the S^+/CS^+ in the absence of further ethanol availability elicited strong recovery of responding. No effect was observed following presentation of water-paired cues (S^-/CS^-). Acamprosate dose-dependently attenuated recovery of responding elicited by ethanol-paired cues (S^+/CS^+), whereas responding under S^-/CS^- was not modified by drug administration. Treatment with 1.0 and 2.0 mg/kg of neramexane did not significantly modify responding under both S^+/CS^+ and S^-/CS^- conditions. However, a slight reduction of cue-induced reinstatement of alcohol seeking was observed. At the dose of 4.0 mg/kg, neramexane elicited a marked inhibition of responding following presentation of both ethanol- and water-paired cues. In conclusion, acamprosate significantly and selectively reduced alcohol-seeking elicited by environmental stimuli predictive of alcohol availability. Treatment with neramexane that shares part of the pharmacological effects of acamprosate on NMDA receptors, however, resulted in a nonselective reduction of lever responding.
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INTRODUCTION

Alcoholism is a widespread psychiatric pathology and is one of the major causes of health problems in western countries. To date, successful treatment of alcohol craving and relapse remains a problem, although advances have been made with the development of acamprosate (calcium bis-*n*-acetyl homotaurinate, Campral®) and naltrexone, the only two clinically approved medications for the treatment of alcohol abuse and relapse prevention (O'Brien *et al*, 1996; Spanagel and Zieglgänsberger, 1997).

One branch of research on craving and relapse focuses on the glutamatergic system as a major player in the regulation of relapse behavior. This hypothesis is supported by evidence claiming that alcohol consumption may lead to adaptive changes within this neurotransmitter system, resulting in a hyper-glutamatergic state and alterations of glutamate receptors (Littleton, 1995; Spanagel and Zieglgänsberger, 1997; Tsai and Coyle, 1998; De Witte *et al*, 2003). Consistently, inhibition of glutamate neurotransmission leads to a reversal of a number of ethanol actions, modulating its intake, and, as recently described, reducing drug-seeking behavior in animal models of relapse (Siggins *et al*, 2003; Bäckström and Hyytiä, 2004).

The therapeutic efficacy of acamprosate has been attributed to its ability in restoring maladaptive changes of the glutamatergic system resulting from prolonged alcohol use and dependence. Thus, acamprosate acts mainly on a hyper-glutamatergic state, yet having only little effect on a 'normal' glutamatergic state (Dahchour and De Witte, 2000, 2003; Spanagel *et al*, 2005). Acamprosate can modulate hyper-glutamatergic activity by a variety of actions. Specifically, it regulates NMDA receptor subunit composition (Spanagel and Zieglgänsberger, 1997; Rammes *et al*, 2001), binds to a specific spermidine-sensitive site

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modulating the NMDA receptor in a complex way (Al-Qatari *et al*, 1998; Naassila *et al*, 1998), blocks voltage-dependent Ca^{2+}-channels (Littleton, 1995; Allgaier *et al*, 2000), or as recently hypothesized, interacts with group I metabotropic glutamate receptors subtype 5 (mGluR5) (Harris *et al*, 2002, 2003; Bäckström *et al*, 2004).

A critical factor contributing to the relapsing nature of alcohol addiction is conditioning to environmental stimuli that have become associated with the subjective actions of ethanol over the course of an individual's history of alcohol abuse. Exposure to alcohol cues increases the urge to drink and facilitate relapse in detoxified human alcoholics (eg Ludwig *et al*, 1974; McCusker and Brown, 1990, 1991; Monti *et al*, 1993; Staiger and White, 1991). While there is some debate as to the specific mechanism by which alcohol cues elicit alcohol-seeking (eg McCusker and Brown, 1991; Monti *et al*, 2000; Newlin, 1987; O'Brien *et al*, 1998; Tiffany and Conklin, 2000), recent data suggest that the glutamatergic system may be involved in the regulation of this behavior (Bäckström *et al*, 2004). We hypothesize, therefore, that acamprosate, owing to its ability to modulate glutamatergic activity, may, at least in part, function as an anti-craving compound by reducing the impact of environmental conditioning factors on relapse behavior.

In the present study, using a validated animal model of relapse (Katner *et al*, 1999; Ciccocioppo *et al*, 2001), the ability of acamprosate to reduce ethanol-seeking behavior induced by environmental conditioning factors has been investigated. In addition, experiments were conducted to compare the effects of this compound to those produced by neramexane (1-amino-1,3,3,5,5-pentamethyl-cyclohexane; MRZ 2/579), a low-affinity, noncompetitive NMDA receptor antagonist that has been recently shown to reduce the increase of ethanol intake during the alcohol deprivation effect (Hölter *et al*, 2000; Vengeliene and Spanagel, 2004) and that is currently undergoing a phase II trial.

METHODS

Animals

Male Wistar rats (Charles River), weighing between 250 and 300 g at the beginning of the experiments, were employed. Rats were housed in groups of three in a temperature- and humidity-controlled vivarium on a reverse 12 h light/dark cycle (lights off at 0930 h) and were offered free access to tap water and food pellets (4RF18, Mucedola, Settimo Milanese, Italy). All animals were handled once daily for 5 min for 1 week before the beginning of the experiments. All procedures were conducted in adherence to the *European Community Council Directive for Care and Use of Laboratory Animals*.

Drugs

Acamprosate (Lipha, Lyon, France) and neramexane (Merz Pharmaceuticals, Frankfurt a.M., Germany) were dissolved in sterile isotonic saline and injected intraperitoneally (i.p.) at a volume of 1 ml/kg. Injections were given 12 and 2 h (acamprosate) or 30 min (neramexane) before reinstatement tests (Hölter *et al*, 2000; Rammes *et al*, 2001).

Self-Administration Apparatus

The self-administration stations consisted of operant conditioning chambers (MED Associates Inc., St Albans, VT) enclosed in sound-attenuating, ventilated environmental cubicles. Each chamber was equipped with a drinking reservoir (volume capacity: 0.2 ml) positioned 4 cm above the grid floor in the center of the front panel of the chamber, and two retractable levers located 3 cm (one to the right and the other to the left) from the drinking receptacle. An infusion pump was activated only by responses on the right (active) lever independent of the liquid delivered, while responses on the left (inactive) lever were recorded but did not result in an activation of the pump. Activation of the pump resulted in the delivery of 0.1 ml of either saccharin solution (self-administration training), ethanol or water (conditioning phase). Auditory (70 dB white noise) and visual (light cue) stimuli were presented via a speaker and house light located on the front panel. An IBM compatible computer controlled the delivery of fluids, presentation of stimuli, and recording of the behavioral data.

Alcohol Self-Administration Training

Animals were trained to self-administer 10% (w/v) ethanol in a single 30-min daily session on a fixed-ratio 1 (FR 1) schedule of reinforcement where each response resulted in delivery of 0.1 ml of fluid as described previously (Weiss *et al*, 1993). During the first days of training, responses at the lever were reinforced by delivery of 0.2% (w/v) saccharin solution into the drinking receptacle. Following acquisition of saccharin-reinforced responding, rats were trained to self-administer ethanol using a modification of the 'sucrose-fading procedure' (Samson, 1986) that employed saccharin instead of sucrose (Weiss *et al*, 1993). During the first two days of training, responses at the lever were reinforced by a 0.2% saccharin solution containing 5.0% (w/v) ethanol. After acquisition of saccharin-maintained responding, a second but inactive lever was introduced. During all training and testing phases, responses at this lever were recorded as a measure of nonspecific behavioral activation, but had no programmed consequences. Beginning on day three, the concentration of ethanol was gradually increased from 5 to 8% and finally 10% (w/v), while the concentration of saccharin was correspondingly decreased to 0%.

Cue-Induced Reinstatement of Alcohol Seeking Behavior

This experimental procedure consisted of three phases:

Conditioning phase. The purpose of the conditioning phase was to train the rats to discriminate the availability of ethanol (reward) *vs* water (non-reward). This phase started at completion of the saccharin-fading procedure. Discriminative stimuli (S^D) predictive of 10% ethanol *vs* water availability were presented during the single ethanol or water daily self-administration session (one 30-min session/day). An orange flavor extract served as the S^+ for ethanol, whereas water availability was signaled by anise extract

(S^-). The olfactory stimuli were generated by depositing five to six drops of the respective extract into the bedding of the operant chamber immediately before extension of the levers and session initiation, and remained present throughout the 30-min sessions. In addition, each lever press resulting in delivery of ethanol was paired with illumination of the chamber's house light for 5.0 s (CS^+), while lever presses resulting in water delivery were followed by a 5.0 s white noise (CS^-). Concurrently with the presentation of these stimuli, a 5-s time-out period was in effect, during which responses were recorded, but not reinforced. At the end of each session, the bedding of the chamber was changed and bedding trays were thoroughly cleaned. Ethanol and water sessions were given in random order up to a total of 10 ethanol and 10 water sessions. The presentation sequence was: for 10% ethanol days 1, 2, 3, 6, 8, 11, 14, 15, 17, and 19; for water days 4, 5, 7, 9, 10, 12, 13, 16, 18, and 20.

Extinction phase. After completion of the conditioning phase, rats were subjected to 30-min extinction sessions on 17 consecutive days. Extinction sessions began by extension of the levers without presentation of the S^D. Responses at the previously active lever activated the syringe pump but did neither result in the delivery of ethanol or water nor in the presentation of the response-contingent cues (house light or white noise). This phase was introduced to eliminate the capacity of the self-administration chamber and the singe pump to nonspecifically motivate the animals' behavior by leaving the ability of the cues to predict ethanol availability unaltered. For statistical purposes, the mean ($\pm$SEM) of the last three extinction sessions of each individual group was used.

Reinstatement testing. Reinstatement tests began 1 day after the final extinction session and were conducted over 2 consecutive days. In these tests, rats were exposed to the same conditions as during the conditioning phase, except that liquids (alcohol or water) were not made available. Sessions were initiated by extension of both levers and presentation of either the ethanol- (S^+) or water- (S^-) associated discriminative stimuli that remained present throughout the entire 30-min session. Responses at the previously active lever were followed by activation of the syringe pump motor and presentation of the CS^+ ('house light') in the S^+ condition or the CS^- ('white noise') in the S^- condition. Half of the animals was tested under the S^+/CS^+ condition on day 1 and under the S^-/CS^- condition on day 2. Conditions were reversed for the second half of the animals. The number of responses on both the active and the inactive levers was recorded throughout the experiment.

To test the effects of acamprosate and neramexane on cue-induced reinstatement, rats were matched on the basis of their performance during the last three extinction sessions and divided into three groups (acamprosate) or four groups (neramexane) with a similar baseline number of responses during extinction. To familiarize the animals with the administration procedure, they were injected i.p. with saline during the last three extinction sessions.

Experiment 1: effect of i.p. injection of acamprosate on cue-induced reinstatement of alcohol-seeking behavior. For the reinstatement test, one group ($n = 10$) of rats was injected i.p. with isotonic saline (control), while the other two groups ($n = 10$) received 100 and 200 mg/kg of acamprosate given 12 and 2 h before testing. In a half of the rats the effect of acamprosate was tested the day after the last extinction session under the S^+/CS^+ condition and on the following day under the S^-/CS^- condition. In the other half, the drug was first tested under S^-/CS^- and then under S^+/CS^+ conditions. The number of responses on both the active and the inactive levers was recorded throughout the experiment.

Experiment 2: effect of i.p. injection of neramexane on cue-induced reinstatement of alcohol-seeking behavior. For the reinstatement test, one group ($n = 12$) of rats was injected i.p. with isotonic saline (control), while the other three groups ($n = 10$–11) received 1.0, 2.0, and 4.0 mg/kg of neramexane given 30 min before testing. In one half of the rats, the effect of neramexane was tested the day after the last extinction session under the S^+/CS^+ condition and on the following day under the under the S^-/CS^- condition. In the other half, the drug was first tested under S^-/CS^- and then under S^+/CS^+ condition. The number of responses on both the active and the inactive levers was recorded throughout the experiment.

Statistical Analysis

The different experimental phases were analyzed separately. For the reinstatement experiment, differences among responses during discrimination training were analyzed with two-way ANOVA with one factor between (ethanol *vs* water) and one factor within (days). Extinction was analyzed by one-way (time) ANOVA for repeated measures. The reinstatement and the effect of acamprosate and neramexane on reinstatement responses were analyzed by two-way ANOVA, one factor within (reinstatement condition) and one factor between (treatment). ANOVAs were followed by Newman–Keuls *post-hoc* tests. Statistical significance was set to $p < 0.05$.

During the reinstatement test, owing to an electric power failure, data from eight animals (three treated with acamprosate 100 mg/kg, three with acamprosate 200 mg/kg, and two with neramexane 2.0 mg/kg) were irremediably lost. These animals have been excluded from the study and in the experiment with acamprosate statistical analysis was carried on 10 rats treated with vehicle and seven rats per group injected with 100 and 200 mg/kg of the drug. Similarly, in the neramexane experiment, the group injected with 2.0 mg/kg was originally composed of 10 animals but only eight could be used for data analysis.

RESULTS

Experiment 1: Effect of i.p. Injection of Acamprosate on Cue-Induced Reinstatement of Alcohol-Seeking Behavior

At the end of the conditioning phase, the number of ethanol-reinforced responses was significantly higher com-

pared to water-reinforced responses ($F(1,46) = 155.81$; $p < 0.01$). Lever pressing decreased during the 17-day extinction phase and passed from 20.0 ± 2.9 of the first extinction day to 8.1 ± 1.05 of the last day of extinction (Figure 1). For the reinstatement test, analysis of variance revealed a nonsignificant overall effect of treatment ($F(2,21) = 2.4$; NS), but a significant treatment-reinstatement condition interaction ($F(4,42) = 5.66$; $p < 0.01$). Specifically, further *post hoc* tests demonstrated a significant reinstatement of ethanol-seeking in the vehicle-treated group (Figure 1) under the S^+/CS^+ stimulus condition, whereas under S^-/CS^- responding remained at extinction levels. The administration of acamprosate significantly attenuated recovery of responding elicited by ethanol-paired cues ($F(2,21) = 5.96$; $p < 0.05$), whereas drug treatment did not modify responding under the S^-/CS^- condition ($F(2,21) = 0.21$; NS). This difference was confirmed by a Newman–Keuls test that revealed a significant effect of acamprosate 200 mg/kg under the S^+/CS^+ condition compared to vehicle-treated rats ($p < 0.05$). The lower dose of 100 mg/kg showed a clear trend to an inhibition of cue-induced reinstatement of ethanol-related behavior, but statistical values were not significant. Linear regression analysis ($y = a + bx$) revealed a significant correlation (factor $= -0.9$) between acamprosate dose and reinstatement inhibition. Responses at the inactive lever were almost absent throughout all experimental phases and were not affected by drug treatment.

Experiment 2: Effect of i.p. Injection of Neramexane on Cue-Induced Reinstatement of Alcohol-Seeking Behavior

At the end of the conditioning phase, the number of ethanol-reinforced responses was significantly higher compared with water-reinforced responses ($F(1,82) = 237.55$; $p < 0.01$). Lever pressing decreased during the 17-day extinction phase and passed from 19.87 ± 1.8 of the first extinction day to 8.2 ± 1.05 of the last day of extinction (Figure 2). For the reinstatement test, analysis of variance

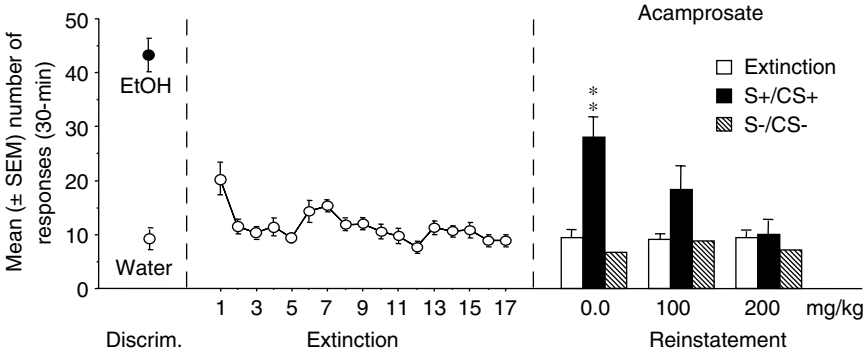

Figure 1 Effects of acamprosate on cue-induced alcohol-seeking behavior. Shown are the mean (±SEM) numbers of lever presses per 30-min session during the different phases of the experiment. Discrimination phase—responses during the last ethanol (filled circle) and water (empty circle) conditioning session, respectively. Extinction phase—number of lever presses during the course of extinction sessions. Reinstatement tests—animals were divided into three groups with similar extinction baseline (extinction bars represent the mean ± SEM from the last three extinction sessions of each individual group) and injected 12 and 2 h before tests with 100, 200 mg/kg of acamprosate or its vehicle, respectively. Half of the animals was tested under the S^+/CS^+ condition on day 1 and under the S^-/CS^- condition on day 2. Conditions were reversed for the second half of the animals. $^{**}p < 0.01$ denotes differences from extinction performance within each respective group.

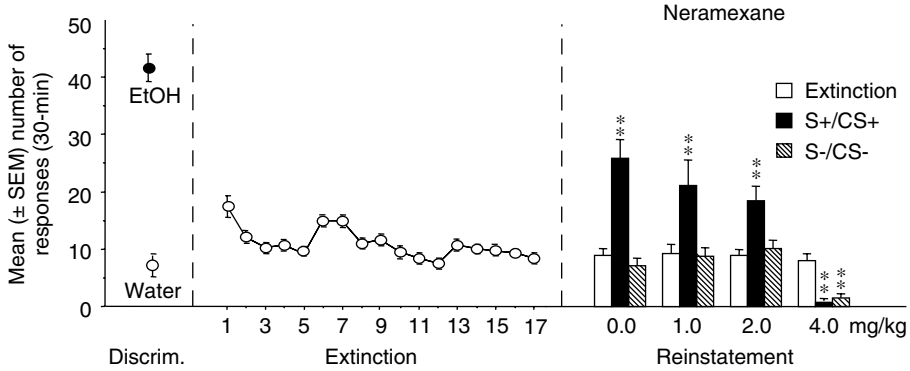

Figure 2 Effects of neramexane on cue-induced alcohol-seeking behavior. Shown are the mean (±SEM) numbers of lever presses per 30 min session during the different phases of the experiment. Discrimination phase—responses during the last ethanol (filled circle) and water (empty circle) conditioning session, respectively. Extinction phase—number of lever presses during the course of extinction sessions. Reinstatement tests—animals were divided into three groups with similar extinction baseline (extinction bars represent the mean ± SEM from the last three extinction sessions of each individual group) and injected 30 min before test with 1.0, 2.0, and 4.0 mg/kg of neramexane or its vehicle. Half of the animals was tested under the S^+/CS^+ condition on day 1 and under the S^-/CS^- condition on day 2. Conditions were reversed for the second half of the animals. $^{**}p < 0.01$ denotes differences from extinction performance within each respective group.

revealed a significant overall effect of treatment ($F(3,38) = 6.86$; $p < 0.01$), and a significant treatment-reinstatement condition interaction [$F(6,76) = 9.24$; $p < 0.01$]. Specifically, further *post hoc* tests demonstrated a significant reinstatement of ethanol-seeking in the vehicle-treated group under the S^+/CS^+ stimulus condition, whereas under S^-/CS^- responding remained at extinction levels (Figure 2). The administration of neramexane significantly attenuated recovery of responding elicited by both ethanol-paired cues (S^+/CS^+) and water-paired cues ($F(3,38) = 9.22$; $p < 0.01$; and $F(3,38) = 6.33$; $p < 0.01$, respectively). This difference was confirmed by a Newman–Keuls test that revealed a significant effect of neramexane at 4.0 mg/kg under both S^+/CS^+ and S^-/CS^- conditions compared to vehicle-treated rats ($p < 0.01$). Lower doses of the drug did not significantly modify lever responding. However, at the dose of 2.0 mg/kg, neramexane showed a trend to an inhibition of the reinstatement of extinguished behavior during presentation of ethanol- but not water-paired cues.

Responses at the inactive lever were almost absent throughout all experimental phases, and statistical analysis revealed the absence of the main effect of treatment. However, a significant treatment-reinstatement condition interaction was observed. *Post hoc* comparisons demonstrated a significant difference between animals treated with vehicle and with 4.0 mg/kg neramexane under both S^+/CS^+ and S^-/CS^- conditions ($p < 0.01$).

DISCUSSION

A number of recent studies have employed operant response-reinstatement models of relapse to examine the significance of conditioned responses to alcohol cues in the initiation and maintenance of ethanol-seeking behavior after extinction (Katner *et al*, 1999; Ciccocioppo *et al*, 2001, 2002, 2003). These studies demonstrate that environmental stimuli that have become associated with the reinforcing actions of ethanol—either by means of classical conditioning, or by acting as discriminative stimuli signalling the availability of ethanol—elicit reliable reinstatement of responding in rats. In the present study, a combination of environmental stimuli has been used in which olfactory cues served as discriminative factors, while the auditory/visual cues were presented contingent to lever pressing. These results demonstrate that the combination of stimuli presented via these two different modalities elicits a marked reinstatement of responding. We suggest, however, that under these conditions the reinstatement behavior is primarily controlled by the overall environmental context rather than being controlled by conditioned stimuli. It is known that the presentation of conditioned stimuli has the ability to initiate a chain of instrumental actions, namely Pavlovian-to-instrumental transfer, leading to drug-seeking responses (Everitt *et al*, 2001). However, in our experiments the same lever has been associated, under a different context, to both water and ethanol availability. We assume, therefore, that if the reinstatement of extinguished responding would have been primarily motivated by instrumental transfer we should have observed, at least in part, an increase of responding not only following presentation of S^+/CS^+ but also under S^-/CS^- condition. Our results instead show that under the S^-/CS^- condition responses on the active lever remained at extinction levels. This supports the view that the reinstatement behavior is context specific and rules out nonspecific arousal as a factor in the response reinstatement.

Acamprosate dose-dependently reduced reinstatement of ethanol-seeking behavior. Responding for cues predictive of water availability was not influenced by acamprosate treatment. The selectivity of the effect of acamprosate over the range of doses used here is consistent with results obtained in previous studies in which this drug was tested on ethanol self-administration or on the alcohol deprivation effect under operant conditions (Hölter *et al*, 1997; Heyser *et al*, 1998, 2003).

From the mechanistic point of view, acamprosate is reported to act as a functional NMDA receptor antagonist—partially through an interplay with mGluR5 receptors (Harris *et al*, 2002, 2003; Bäckström *et al*, 2004)—modulating activity of dopaminergic neurons within the mesolimbic reward system (Olive *et al*, 2002; Cano-Cebrian *et al*, 2003). Since augmented dopaminergic activity is thought to underlie drug-seeking responses (Spanagel and Weiss, 1999; Liu and Weiss, 2002), in reinstatement experiments acamprosate might attenuate ethanol-paired cue effects through an inhibition of glutamatergic activity and a subsequent decrease of dopaminergic function. Importantly, in several other studies it has been shown that acamprosate reduces ethanol-conditioned effects that are known to be under the control of the dopaminergic system (Cole *et al*, 2000; Quertemont *et al*, 2002; McGeehan and Olive, 2003).

In a second series of experiments, we tested neramexane, a compound with cyclohexane structure that has similar characteristics to memantine. Following initial characterization in animal models, it appears to have promising effects on alcohol craving and relapse (Hölter *et al*, 1996, 2000; Parsons *et al*, 2000; Vengeliene and Spanagel, 2004). In the present study, pretreatment with neramexane at doses of 1.0 and 2.0 mg/kg did not significantly reduce cue-induced alcohol-seeking, although a trend to a reduction was observed. At the highest dose (4.0 mg/kg), the animals even pressed significantly less in response to ethanol-associated cues. However, compared to extinction baseline, a significant reduction of responding following presentation of water-paired stimuli and inhibition of responding at the inactive lever was also observed, pointing to a sedative effect of the compound at this dose. This is in accordance with previous studies (Piasecki *et al*, 1998; Bienkowski *et al*, 1999) describing unspecific reduction of lever-pressing behavior and impairment of motor coordination at 4.0 mg/kg or higher doses of the compound.

Assuming that a functional antagonism of the NMDA receptor by acamprosate underlies its anticraving properties, one should expect that the direct blockade of the NMDA receptor channel by neramexane should also interfere with cue-induced reinstatement of alcohol-seeking in the same way. Apparently this is not the case, at least looking at the markedly different selectivity of the two compounds. A possible explanation is that other acamprosate actions (ie reduction of a hyper-glutamatergic state, interaction with mGluR5 receptors, and/or inhibition of voltage-dependent Ca^{2+} activity) may contribute in deli-

neating the beneficial antirelapse profile of this drug. Another reason for this discrepancy is that different drug injection schedules were used. Thus, neramexane was administered once, whereas acamprosate was administered twice given 12 and 2 h before testing. Taking into account that acamprosate as well as noncompetitive NMDA receptor antagonists influence the expression of NMDA receptor subunits (Rammes *et al*, 2001) and that multiple injections are more effective in this respect, one might speculate that two injections of neramexane would have been more effective in cue-induced reinstatement of ethanol-seeking behavior.

Clinical studies, using naltrexone, demonstrated that this drug effectively reduces relapse rates among abstinent alcoholics in treatment (O'Malley *et al*, 1992; Volpicelli *et al*, 1992; O'Brien *et al*, 1996). This effect seems to depend, at least in part, on the ability of naltrexone in reducing the urge to drink, as elicited by the presentation of alcohol cues (Monti *et al*, 1999; Rohsenow *et al*, 2000). Interestingly, it has been shown that a combined naltrexone and acamprosate treatment can result in additive effects and improved therapy outcome (Kiefer *et al*, 2003). Additionally, Mason *et al* (2002) could demonstrate a significant pharmacokinetic interaction of both compounds, which in turn leads to an increased absorption of acamprosate, when co-administered with naltrexone. Such an increased absorption could predict greater efficacy for the combination than for a single treatment with any of the compounds. Based on the results of the present work, however, we speculate that another possibility of interaction between these two drugs may exist at the level of their ability in modulating reactivity to alcohol cues. The results of our study serve in providing further insight aimed at evaluating this hypothesis at the clinical level.

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