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Stress-induced reinstatement of alcohol-seeking in rats is selectively suppressed by the neurokinin 1 (NK1) antagonist L822429

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Abstract

Rationale—Genetic inactivation or pharmacological antagonism of neurokinin 1 (NK1) receptors blocks morphine and alcohol reward in rodents, while NK1 antagonism decreases alcohol craving in humans. The role of the NK1 system for relapse-like behavior has not previously been examined.

Objective—Divergence between human and rodent NK1 receptors has limited the utility of NK1 antagonists developed for the human receptor species for preclinical studies of addiction-related behaviors in rats. Here we used L822429, an NK1 antagonist specifically engineered to bind at high affinity to the rat receptor, to assess the effects of NK1 receptor antagonism on alcohol-seeking behaviors in rats.

Methods—L822429 (15 and 30 mg/kg) was used to examine effects of NK1 receptor antagonism on operant self-administration of 10% alcohol in 30-min daily sessions, as well as intermittent footshock stress- and cue-induced reinstatement of alcohol-seeking after extinction of lever responding.

Results—At the doses used, L822429 did not significantly affect alcohol self-administration or cue-induced reinstatement, but potently and dose dependently suppressed stress-induced reinstatement of alcohol seeking, with an essentially complete suppression at the highest dose. The effect of L822429 on stress-induced reinstatement was behaviorally specific. The drug had no effect on conditioned suppression of operant responding following fear conditioning, locomotor activity, or self-administration of a sucrose solution.

Conclusions—To the degree that the reinstatement model provides a model of drug relapse, the results provide support for NK1 antagonism as a promising mechanism for pharmacotherapy of alcoholism, acting through suppression of stress-induced craving and relapse.

Keywords

Substance P; Neurokinin; Alcohol; Stress; Relapse; Reinstatement; Fear conditioning

Introduction

Substance P (SP) and its preferred neurokinin 1 (NK1) receptor play a significant role in stress- and anxiety-related behaviors (Ebner and Singewald 2006). For example, SP delivered intracranially to rats increases anxiety-like behavior in the elevated plus maze, an effect that is reversed by specific NK1 antagonists (Ebner et al. 2004). A role of endogenous SP in mediating stress responses is further suggested by anxiolytic-like properties of NK1 antagonists (Ebner et al. 2008). The SP/NK1 system interacts with several monoamine and neuropeptide systems involved in stress and anxiety responses, including serotonin (Conley et al. 2002; Guiard et al. 2007; Santarelli et al. 2001), norepinephrine (Gobbi et al. 2007; Maubach et al. 2002), dopamine (Hutson et al. 2004; Renoldi and Invernizzi 2006), and corticotropin-releasing hormone (CRH) (Hamke et al. 2006).

While effects of NK1 antagonists on stress responses are well-documented, less is known about their ability to influence drug reward and addiction-related behaviors. Early studies with genetically modified mice suggested a role of the SP/NK1 system in the rewarding effect of morphine, but not that of cocaine. Specifically, null mutants for the NK1 receptor gene (NK1^{-/-} mice) did not express conditioned place preference (CPP) for morphine (Murtra et al. 2000), and this impairment was accompanied by decreased rates of morphine self-administration compared to wild-type mice (Ripley et al. 2002). In contrast, no differences were found in cocaine CPP or self-administration. Furthermore, lesions using neurotoxins specifically targeting NK1 receptor-containing neurons prevented the development of CPP for morphine, but not cocaine (Gadd et al. 2003). This effect appeared to be specific for NK1-expressing neurons in the amygdala, and not for those of the nucleus accumbens.

Recently, we showed that alcohol-related behaviors can be sensitive to genetic deletion or pharmacological blockade of the NK1 receptor. For example, alcohol intake in a two bottle

choice model was decreased in NK1 $-/-$ mice (George et al. 2008), and this was accompanied by a lack of CPP for alcohol, a measure of drug reward (Thorsell et al. 2010). NK1 antagonism using L703,606 replicated the decrease in voluntary alcohol consumption observed in NK1 $-/-$ mice (Thorsell et al. 2010). Consistent with these observations, microRNA silencing of NK1 receptor expression using intracerebroventricular injections decreased alcohol consumption in rats (Baek et al. 2010). Finally, Steensland and colleagues showed a suppression of alcohol self-administration and two bottle choice voluntary consumption following NK1 antagonism with ezlopitant in rats (Steensland et al. 2010). Thus, both pharmacological blockade of NK1 receptors and transient knockdown of their expression mimic the effects of constitutive NK1 receptor gene inactivation. It is therefore unlikely that decreased alcohol consumption in NK1 $-/-$ mice is caused by developmental effects, a possibility that is critical to exclude in order to assess the viability of NK1 antagonism as a potential pharmacotherapy for alcohol addiction.

Preclinical findings with NK1 receptor deletion and antagonism may translate to the human situation. In support of this possibility, we have found that administration of an NK1 antagonist to alcoholics decrease alcohol craving during early abstinence, as measured both under unprovoked conditions, and in response to a challenge that comprised of combination of a social stressor and presentation of alcohol-associated cues. These effects were accompanied by a blockade of anterior insula activity in response to aversive visual stimuli, as measured by functional magnetic resonance imaging (George et al. 2008). Finally, genetic analyses suggest an association of specific NK1 gene haplotypes with increased risk for alcohol dependence (Seneviratne et al. 2009). Taken together, these results implicate the SP/NK1 system in alcohol-related behaviors, and suggest that drugs targeting this system may be effective alcoholism treatments. However, it remains to be established whether NK1 antagonism would suppress ongoing excessive alcohol use, prevent relapse, or both. Furthermore, to the extent that suppression of alcohol craving predicts efficacy to prevent relapse, it remains unknown whether NK1 antagonism would primarily prevent relapse triggered by stress or by alcohol-associated cues, two major categories of relapse-provoking stimuli observed both in the clinic and in animal models (Brownell et al. 1986; Epstein et al. 2006; Le and Shaham 2002; Weiss 2005).

NK1 receptors display considerable divergence between man and rat, potentially complicating the interpretation of results obtained in rat models using antagonists developed for the human receptor. L822429, an NK1 antagonist specifically engineered to have a high affinity for the rat NK1 receptor, is brain penetrant upon peripheral administration and has an anxiolytic-like profile (Ebner et al. 2004; Ebner et al. 2008; Singewald et al. 2008). Here, we resynthesized L822429 and examined its effects on operant self-administration of alcohol, and reinstatement of alcohol seeking induced by intermittent footshock stress (Le et al. 1998) as well as alcohol-associated cues. To further assess the behavioral profile of L822429, we assessed the drug's effect on locomotor activity, oral sucrose self-administration, and conditioned fear after extended training (Pickens et al. 2009, 2010), as assessed in the conditioned suppression procedure (Annau and Kamin 1961; Estes and Skinner 1941).

Methods

Drugs

L822429 was synthesized in the laboratory of Dr. K. Rice at the NIDA Intramural Research Program. L822429 was dissolved in 45% w/v 2-hydroxypropyl β -cyclodextrin and pH was adjusted as necessary. The compound was injected at a volume of 1.5–3 ml/kg.

Subjects

For alcohol studies, male Wistar rats weighing approximately 175–225 g at time of arrival were obtained from Charles River (Wilmington, MA). Rats were allowed at least 1 week to habituate to the housing facility and were handled daily during the following week prior to the start of experimentation. Rats were housed in a reversed light cycle (lights on 20:30, lights off 08:30), and all testing took place during the dark phase. Food and water were available ad libitum, except where explicitly stated. Wistar rats were used for these studies because alcohol-related behaviors in this strain have been well-defined in our laboratory. For fear conditioning, male Long-Evans rats (Charles River, Raleigh, NC, 310–450 g on first day of deprivation) were individually housed in a colony room under a reverse 12-h:12-h light–dark cycle with lights off at 9 AM. We excluded two rats due to experimenter error. Food given per day was the total of pellets earned in training and home cage food chow (weight of food pellets earned in the operant chambers was subtracted from their daily food ration). Long-Evans rats were used for fear conditioning because fear incubation has been established in this strain in our previous work (Pickens et al. 2009). All procedures used were in accordance with the NIH Guide for the Care and Use of Laboratory Animals and were approved by the NIH Animal Care and Use Committee.

Alcohol self-administration training and testing

Self-administration training and testing were carried out as described previously (Cippitelli et al. 2010). In brief, saccharin fading was used and rats were trained on operant self-administration of 10% alcohol in water using procedures based on previous studies (Grant and Samson 1986; Samson 1986). Alcohol solution was delivered into a drinking receptacle in a 0.1-ml volume, on a fixed ratio 1 (FR1) reinforcement schedule, during 30-min sessions that were run 5–6 days/week. Each alcohol delivery was followed by a 5-s timeout interval during which responses were recorded but not reinforced. For cue-induced reinstatement experiments, orange scent was also present in the operant chambers during alcohol sessions. After stable response rates were reached for at least three consecutive days after approximately 3 weeks, rats ($n=11$ –12 per group) were injected with L822429 in a between subjects design (0, 15, or 30 mg/kg; i.p. at 2 ml/kg) 1 h prior to the next operant self-administration session.

Extinction and reinstatement

Reinstatement experiments were carried out as previously described (Cippitelli et al. 2010). After 14–16 days of alcohol self-administration, lever pressing was extinguished over a minimum of 15 sessions. Rats that at that point fulfilled an extinction criterion of less than 20 responses in the 30-min session were used for reinstatement testing. Rats that had not at that point reached the criterion received additional extinction sessions, up to a total of 19. Rats that did not reach the criterion at session 19 were discarded (3/43 in the stress-induced reinstatement experiment and 2/39 in the cue-induced reinstatement experiment). For cue-induced reinstatement, the house light and the orange scent were removed during the extinction phase.

In the stress-induced reinstatement experiment ($n=13$ –14 per group), 15 min of intermittent footshock (0.5 s shock, 0.6 mA, mean intershock interval 40 s) was delivered in the chambers immediately before the reinstatement session. The shock parameters are based on previous studies (Le et al. 1998; Shaham and Stewart 1995). In the cue-induced reinstatement experiment (12–13 per group), the rats were exposed to the orange scent that previously predicted alcohol availability and lever press resulted in contingent presentations of the cue light previously paired with alcohol deliveries. Rats were injected with L822429 (0, 15, or 30 mg/kg) 1 h prior to reinstatement testing. Each rat received only one injection, in a between-subjects design with drug dose as a factor (0, 15, or 30 mg/kg).

Locomotor activity

Rats ($n=6$ per group) were injected, in a between-subjects design, with L822429 (0, 15, or 30 mg/kg), 1 h prior to the start of the locomotor activity session, which lasted for 1 h. Data were recorded in 10-min bins.

Sucrose self-administration

Rats ($n=7$ per group) were water restricted for 22 h for the first 3 days of training, as in operant self-administration training described above, and were given access to 10% sucrose solution (dissolved in tap water) on an FR1 reinforcement schedule during the initial operant self-administration sessions. After achieving consistent response rates (after 4–5 days of training on the FR1 reinforcement schedule), the rats were kept on the FR1 schedule, but a 20-s timeout was imposed following sucrose delivery. These conditions were in place for 10 days, after which time baseline responding reached a stable level. All operant self-administration sessions were 30 min in duration and were run once daily, 5–6 days/week. Rats were then injected with L822429 in a between-subjects design (0 or 30 mg/kg) 1 h prior to the next operant self-administration session.

Fear conditioning

Each Med Associates operant chamber had two levers 9 cm above the floor, but only one lever (“active” retractable lever) activated the pellet dispenser, delivering 45-mg food pellets (# F00021, Bioserv, Frenchtown, NJ). The chambers’ grid floors were connected to electric shock generators. We used a conditioned suppression of food-reinforced responding protocol described in detail in Pickens et al. (2010) and consisting of seven phases: food cup training (one session), operant training (five sessions, final reinforcement schedule, variable interval-60, fear conditioning (ten sessions), incubation period (31 ± 2 days, hereafter referred to as 1 month), baseline session (one session), test for tone-induced conditioned fear (one session), and test for extinction retention (one session). The food-restricted rats were trained during the dark cycle. Sessions began with extension of the active lever and illumination of a red house light. Rats were weighed and fed after the daily sessions. Fear conditioning training occurred over ten 90-min sessions. During each session, the rats were given five 360-s tones (2,900 Hz, 20 dB above background), ranging from 6 to 18 min apart and each containing two electric shocks (0.5-s, 0.5-mA scrambled, shock intensity adjusted for inter-chamber variability) at random times during the tones while earning pellets on a VI-60 schedule. Each tone contained one shock in the first 180 s and one shock in the second 180 s. Conditioned suppression of lever pressing for food pellets was our measure of fear (Annau and Kamin 1961). Lever presses/min were recorded during the 2 min prior to the tone (pre cue) and during the 6-min tone presentation (cue), and were converted into a suppression ratio: suppression ratio = [(pre cue response rate minus cue response rate)/(pre cue response rate plus cue response rate)]. A value of 1 indicates total conditioned suppression of lever pressing during tone presentation (high fear). A value of 0 reflects no lever-press suppression during tone presentation (no fear). The rats assigned to the different treatments were matched for their pre cue (CS) lever pressing and suppression ratios during training.

Conditioned fear to the tone was tested 1 month after fear conditioning training. One h before the start of the test, the three groups of rats ($n=14$ –16 per dose) were injected with 0, 15, or 30 mg/kg L822429 (s.c.) at a concentration of 10 mg/ml (1.5 ml/kg in the 15 mg/kg group, 3 ml/kg in the vehicle, and 30 mg/kg groups). A single 360-s tone was presented without shock, 9 min into the 35-min session.

Finally, we used a prolonged (360 s) CS, because Bouton and colleagues (Waddell et al. 2006) and Davis and colleagues (Walker and Koob 2008; Walker et al. 2009) have suggested that prolonged duration CSs (>1 min) paired with predictable or unpredictable

shocks cause an emotional state similar to anxiety, with different neuronal substrates than those observed with the short-duration fear CSs with predictable shocks used in our previous studies (Pickens et al. 2009).

Statistical analysis

All analyses were carried out using the general linear model module of Statistica 9.0 (StatSoft Inc., Tulsa, OK). Data were evaluated for homogeneity of variance and if deviations from this criterion were detected, additional non-parametric tests were carried out to establish robustness of results. In the self-administration and reinstatement experiments, data were analyzed using a one-way analysis of variance (ANOVA) with L822429 dose as the between-subjects factor. When available, the contribution of pretreatment baseline responding (average of three last pretreatment sessions in self-administration experiments; last extinction session responding in the reinstatement experiments) was in each case evaluated a potential covariate (Vickers and Altman 2001). The covariate was kept in the model if it yielded a significant contribution or reduced residual variance, or was otherwise dropped from analysis. In the locomotor activity experiment, data were analyzed as a one-way ANOVA (for total session activity) with treatment as factor, and also as a two-way ANOVA (for activity broken down into 6 consecutive 10 min bins) with L822429 dose as a between-subjects and bin number, a measure of time as a within-subjects factor. The statistical analysis for the fear conditioning experiment included the between-subjects factors of L822429 dose (0, 15, or 30 mg/kg, s.c.) and the within-subjects factor of test minute (the 6 min of the tone presentation).

Results

L822429 does not suppress operant alcohol self-administration

Table 1 presents data for number of alcohol deliveries, amount of alcohol obtained/kg body weight, active lever presses, and inactive lever presses following injections of L822429 (0, 15, or 30 mg/kg). The number of alcohol deliveries was the primary outcome variable. In the statistical analysis of this measure, pretreatment responding was a highly significant covariate ($F[1, 31]=16.6, p<0.001$), and was therefore kept in the model. One way ANOVA revealed that there was no effect of L822429 dose on the number of alcohol deliveries ($F[2,31]=1.14, p=0.33$). Responding for alcohol at the 15-mg/kg dose was virtually identical to vehicle, while mean responding at the 30-mg/kg dose was numerically decreased by ~20%.

L822429 dose dependently blocks intermittent footshock stress-induced reinstatement

A reinstatement effect was detected as a significantly higher lever responding on testing following exposure to intermittent footshock compared to the non-shock condition in the vehicle-treated group (repeated measures ANOVA: $F[1,13]=15.9, p<0.001$; Fig. 1). Because variance was larger during reinstatement testing than extinction testing, this analysis was repeated using Wilcoxon's matched pairs test, which yielded an essentially identical result ($p<0.05$). Inactive lever responding was not significantly affected by intermittent footshock stress, although there was a trend for an increase ($F[1,13]=4.1, p=0.061$; Fig. 1), likely reflecting response generalization (Shalev et al. 2002).

Extinction responding was not a significant covariate in the analysis of reinstatement responding on the alcohol-associated lever, nor did inclusion of this variable reduce the residual variance; extinction responding was therefore dropped from the model. There was a main effect of L822429 dose on responses on the previously alcohol-associated lever ($F[2,37]=3.8, p=0.03$). Post hoc analysis (Dunnett's test) showed that the 30-mg/kg dose resulted in a significant suppression ($p=0.02$). Dose dependence was evaluated using a

linear regression of response rates vs dose, and was strongly supported ($F[1,38]=7.8$; $p=0.008$). Responses on the inactive lever were not affected by L822429 dose ($F[2,37]=0.45$, $p=0.64$).

L822429 has no effect on cue-induced reinstatement

A reinstatement effect was detected as significantly higher response rates on testing following cue exposure than under the non-cued condition in the vehicle-treated group (repeated measures ANOVA: $F[1,12]=10.5$, $p=0.007$; similar results were obtained using Wilcoxon's matched pairs test). Inactive lever responding was also increased by reexposure to the alcohol-associated cues ($F[1,12]=10.0$, $p=0.008$; Fig. 2).

Extinction responding was not a significant covariate in the analysis of reinstatement responding on the alcohol-associated lever in this experiment, nor did inclusion of this variable reduce the residual variance; extinction responding was therefore dropped from the model. There was no effect of L822429 dose on responses on the alcohol-associated lever ($F[2,34]=0.06$, $p=0.94$). Responses on the inactive lever were also unaffected by L822429 dose, although a trend was observed ($F[2,34]=3.1$, $p=0.06$).

L822429 does not affect locomotor activity or self-administration of 10% sucrose

L822429 did not affect total locomotor activity over 1 h as measured by distance traveled ($F[2,17]=0.03$, $p=0.97$). When data were broken down into 10-min bins, there was a main effect of bin ($F[5,75]=98.13$, $p<0.0001$), reflecting that rats introduced to the novel environment showed a high initial level of exploratory activity that gradually decreased over time. There was, however no main L822429 dose effect ($F[2,75]=0.031$, $p=0.97$) or bin x treatment interaction ($F[10,75]=0.8$, $p=0.66$).

L822429 also did not affect operant responding for sucrose. In this experiment, baseline responding (average of last three pretreatment sessions) only contributed to the model at a trend level ($F[1,11]=3.4$, $p=0.09$), but its inclusion reduced the residual variance and it was therefore retained in the model. There was no effect of L822429 dose on sucrose self-administration ($n=7$ /group; $F[1,11]=0.03$, $p=0.87$; Table 1).

L822429 does not affect conditioned fear

A summary of the experimental timeline for the fear conditioning protocol is shown in Fig. 3a. During training, the rats showed an initial increase in conditioned suppression that was followed by a decrease as training continued (training session: $F[9,387]=17.9$, $p<0.01$) (post hoc comparisons are indicated in Fig. 3b).

Systemic L822429 injections had no effect on conditioned suppression of lever pressing or baseline lever pressing 1 month after extended fear conditioning. The statistical analysis included the between-subjects factors of L822429 dose (0, 15, or 30 mg/kg, s.c.) and the within-subjects factor of test minute (the 6 min of the tone presentation). This analysis revealed a significant effect of test minute ($F[5,215]=24.3$, $p<0.01$), but no effect of, or interaction with L822429 dose ($F[2,43]=0.39$, $p=0.68$) or L822429 dose X test minute interaction ($F[10,215]=0.33$, $p=0.97$). The pre-cue lever-press rates (lever press/min) were: vehicle, 18.8 ± 2.4 ; 15 mg/kg L822429: 20.7 ± 2.9 ; 30 mg/kg L822429: 21.2 ± 2.4 . There was no effect of L822429 dose on pre-cue lever pressing ($F[2,43]=0.27$, $p=0.76$). There was also no effect or interactions of the L822429 dose on the suppression ratios or pre-cue lever-press rates on the extinction retention test given the following day without injections (data not shown, all p values >0.10).

Discussion

We found that stress-induced reinstatement of alcohol seeking is dose dependently suppressed and, at the highest dose, fully blocked by the NK1 receptor antagonist L822429. This effect appears to be behaviorally specific: L822429 had no effect on ongoing alcohol self-administration, reinstatement of alcohol seeking induced by alcohol-associated cues, locomotor activity in a novel environment, or conditioned suppression of food-reinforced responding by a fear cue. This pattern of results indicates that non-specific drug effects are unlikely to confound effects of L822429 on stress-induced reinstatement of alcohol seeking. To our knowledge, this is the first study to report effects of NK1 antagonism on relapse-like behavior.

While we did not find effects of L822429 on alcohol or sucrose self-administration in Wistar rats using an FR1 reinforcement schedule, a recent study (Steensland et al. 2010) reported that self-administration of both these reinforcers was suppressed following treatment with the NK1 antagonist ezlopitant when Long-Evans rats and an FR3 reinforcement schedule were used. The choice of rat strain as well as reinforcement schedule might account for these results. However, the two datasets are consistent, in that they both provide evidence against specific effects of NK1 antagonists to suppress alcohol self-administration in rats. Although ezlopitant appears to have been more potent than L822429, Steensland and colleagues found suppression of alcohol self-administration only at doses that also suppressed sucrose self-administration. In contrast, our highest dose of L822429, 30 mg/kg, had no effect on sucrose self-administration or food pellet self-administration in food-restricted rats in the fear-conditioning experiment.

On the other hand, these findings in rats are in contrast to recent observations in NK1 $-/-$ mice (George et al. 2008), or wild-type mice treated with the NK1 antagonist L-703,606 (Thorsell et al. 2010), where selective reduction of alcohol consumption was observed. Species differences between mice and rats cannot be excluded as a cause of this divergence, but we believe another interpretation is more likely. Escalation of drug intake is a hallmark of addiction. A range of compounds with anti-stress mechanisms, with CRH receptor antagonists as the prototype, selectively suppress escalated alcohol intake that results from a history of dependence or genetic factors, but leave basal rates of alcohol self-administration unaffected (Heilig and Koob 2007). Our mouse findings, both in NK1 $-/-$ animals and with pharmacological NK1 receptor blockade, were obtained in C57/BL6 mice, which have the highest alcohol preference among inbred mouse lines (Belknap et al. 1993). Furthermore, both sets of results were obtained following procedures that lead to further escalation of alcohol intake. Our prediction is therefore that escalated alcohol intake in genetically selected alcohol-preferring rats or in rats with a prolonged history of alcohol dependence will be sensitive to NK1 antagonism.

Because we observed a potent effect on footshock stress-induced reinstatement of alcohol seeking, we assessed the effects of L822429 on fear conditioning, another stress-related behavior that potentially involves the NK1/SP system. Although the procedure used resulted in robust expression of conditioned fear, measured as suppression of operant responding, no effect of NK1 antagonism by L822429 was found in this model. This dissociation may be related to prior observations that conditioned fear stimuli are generally insufficient to reinstate drug seeking (Shaham et al. 2000). L822429 has previously shown anxiolytic-like properties in other animal models such as the elevated plus-maze, suggesting that unconditioned stress responses may be more sensitive to NK1 antagonism than the conditioned fear suppression model used in the present study. It will be important in future studies to explore the effects of L822429 on behaviors that lie at the intersection of alcohol exposure and anxiety, such as acute alcohol withdrawal-induced anxiety.

Several monoamine and neuropeptide systems may be influenced by, or interact with, the NK1 receptor to mediate effects on alcohol seeking. For example, NK1 antagonists increase serotonin function (Conley et al. 2002; Guiard et al. 2007; Santarelli et al. 2001), while serotonergic median raphe neurons have been implicated in stress-induced relapse to alcohol seeking (Le et al. 2002). Furthermore, NK1 antagonists modulate firing patterns of brainstem noradrenergic nuclei (Maubach et al. 2002), and prevent stress-induced increases in noradrenaline and dopamine in the frontal cortex of rats (Hutson et al. 2004; Renoldi and Invernizzi 2006). It has also been demonstrated that activation of NK1 receptors by SP can induce expression of CRH1 receptors (Hamke et al. 2006). This finding is of potentially high relevance in light of the fact that CRH1 antagonists (Bruijnzeel et al. 2009; Le et al. 2000; Shaham et al. 1998) and NK1 receptor antagonists (present report) appear to have a similar behavioral profile on stress-induced reinstatement.

In conclusion, the findings reported here support a potential utility of the NK1 as a target for alcoholism pharmacotherapy. Our preclinical evaluation indicates an ability of NK1 antagonism to selectively inhibit stress-induced reinstatement, in the absence of activity in a range of other behavioral models. This blockade of stress-induced reinstatement did not appear to be contingent on anxiolytic-like activity as assessed by the conditioned fear-suppression model, although other models, such as the elevated plus-maze might be more sensitive and reveal anxiolytic-like actions at the doses that suppressed stress-induced reinstatement. To the extent the reinstatement model can predict clinical efficacy (Epstein et al. 2006), our results suggest the possibility that relapse induced by stressful life events could be inhibited by NK1 antagonists. Indirect support for this possibility is provided by our prior observation that an NK1 antagonist, when given to alcoholics, suppressed stress-induced alcohol cravings (George et al. 2008); although not a direct measure of efficacy, reduction of craving is a promising surrogate efficacy marker that is likely to predict clinical activity (Sinha 2009).

Finally, relapse to excessive alcohol consumption following extended intervals of abstinence is a hallmark of alcoholism, and its prevention is a critical objective for novel therapies (Heilig and Egli 2006; McLellan et al. 2000). Naltrexone, currently on the market for alcoholism, selectively inhibits cue- or alcohol priming-induced reinstatement, but not stress-induced reinstatement of alcohol seeking in rats (Le et al. 1999; Liu and Weiss 2002). In contrast, no currently approved medication inhibits stress-induced relapse. The possibility that NK1 antagonists might be able to do so suggests a potential for using them in combination with naltrexone to achieve additive effects.

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Fig. 1.

L822429 dose dependently blocks intermittent footshock stress-induced reinstatement. The data presented are the number (mean±SEM) of previously active lever presses exhibited over 30 min during extinction sessions (for vehicle treated group only) and during reinstatement sessions that followed 15 min of intermittent footshock. Drug injections were given 1 h prior to footshock on reinstatement test day. Vehicle-treated group shown in *black bars*, 15 mg/kg L822429 group in *gray bars*, and 30 mg/kg L822429 in *white bars*. ### $p < 0.001$ vs vehicle group reinstatement responding; * $p < 0.05$ vs vehicle group reinstatement responding. For detailed statistics, see “Results”

Fig. 2. L822429 has no effect on cue-induced reinstatement. The data presented are the number (mean±SEM) of previously active lever presses exhibited over 30 min during extinction sessions (for vehicle-treated group only) and during reinstatement sessions where alcohol-paired odor and light cues were represented. Drug injections were given 1 h prior to reinstatement sessions on test day. Vehicle-treated group shown in *black bars*, 15 mg/kg L822429 group in *gray bars*, and 30 mg/kg L822429 in *white bars*. ^{##} $p < 0.01$ vs vehicle group reinstatement responding. For detailed statistics, see “Results”

Fig. 3.

NK1 receptor blockade had no effect on conditioned fear: **a** timeline of the fear conditioning protocol. **b** Acquisition of fear conditioning: suppression ratios across ten sessions of tone presentations. *Different from session 1, $p < 0.05$; # different from session 10, $p < 0.05$. **c** Mean ($\pm$ SEM) test suppression ratios (minute by minute time course across the 6-min test trial) after systemic injections of vehicle or L822429 in rats given 50 tones presented with shocks over 10 days and tested for fear conditioning 1 month after training (14–16 per dose)

Table 1

Alcohol (10% v/v) and sucrose (10% w/v) operant self-administration data. The data presented are the number of rewards obtained, active and inactive lever presses, and intake expressed as g/kg. Data are means $\pm$ SEM

	Alcohol (10% v/v)			Sucrose (10% w/v)		
	L822429 dose			L822429 dose		
	0 mg/kg	15 mg/kg	30 mg/kg	0 mg/kg	30 mg/kg	30 mg/kg
Rewards	44.73 (5.14)	46.33 (6.48)	36.25 (7.24)	80.29 (1.69)	74.29 (5.97)	
Intake (g/kg)	0.83 (0.09)	0.88 (0.12)	0.64 (0.13)	1.58 (0.06)	1.53 (0.13)	
Active lever responses	56.09 (6.75)	64.00 (10.14)	46.50 (9.27)	228.7 (23.40)	224.3 (43.34)	
Inactive lever responses	4.82 (1.10)	5.08 (1.39)	7.42 (3.66)	9.43 (2.29)	5.86 (3.07)	