Pharmacological activation of mGlu4 and mGlu7 receptors, by LSP2-9166, reduces ethanol consumption and relapse in rat

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A B S T R A C T
Addiction is a chronic and highly relapsing disorder hypothesized to be produced by an imbalance between excitatory and inhibitory neurotransmission. For more than a decade, emerging evidence indicates that manipulation of glutamatergic neurotransmission, by group III mGlu receptors (mGlu4/7/8), could be a promising approach to develop therapeutic agents for the treatment of addiction. Thus, the aim of the present study is to determine whether LSP2-9166, a mixed mGlu4/mGlu7 orthosteric agonist, could reduce ethanol self-administration, ethanol motivation and reacquisition after protracted abstinence in a preclinical model of excessive ethanol intake. Male Long Evans rats were chronically trained to consume large amount of ethanol in operant cages for several weeks. Once they reached a stable level of consumption (about 1 g of pure ethanol/kg bodyweight/15min), the effect of LSP2-9166 was evaluated on different aspects of the operant self-administration behavior. In this study, we found that the intracerebroventricular infusion of LSP2-9166 dose dependently reduced ethanol consumption, motivation for ethanol and reacquisition of ethanol self-administration after abstinence. Together, these results support recent preclinical findings showing that pharmacological modulation of mGlu receptors may serve as an effective treatment for reducing ethanol consumption and relapse.

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1. Introduction
Alcohol Use Disorder (AUD) is a chronic and highly relapsing disorder, characterized by a loss of control over alcohol consumption and binge drinking episodes that facilitate escalation of alcohol consumption and craving. Given the health risk and psychosocial consequences of AUD, one of the principal aims of fundamental research is to identify new therapeutic targets for the treatment of AUD.

Like many drugs of abuse, alcohol induces neuroadaptations in the mesolimbic dopaminergic reward system which arises from the midbrain ventral tegmental area (VTA) and projects to the forebrain nucleus accumbens (NAc) and the prefrontal cortex (PFC) (Kalivas and Volkow, 2005; Koob and Volkow, 2010). In recent years, a large body of evidence suggests a key role of glutamate in multiple biochemical and behavioral aspects of ethanol (Burnett et al., 2016; Dodd et al., 2000). Numerous studies have found that chronic administration of ethanol increases extracellular glutamate level in many brain areas of the reward system (Szmulinski et al., 2007; Zhu et al., 2007). This hyperglutamatergic state plays a key role in the development and maintenance of dependence since it is implicated in different aspects of drug addiction such as reinforcement, withdrawal and relapse (Kalivas and Volkow, 2005).

Glutamate is the major excitatory amino-acids transmitter in the mammalian brain. It is involved in many physiological, behavioral and pathological processes including learning and memory. The action of glutamate is mediated by both fast-acting ligand-gated ion channels, commonly named « ionotropic glutamate receptors » (iGluR), and slow-acting G-protein coupled receptors also known as metabotropic glutamate (mGlu) receptors (Kew and Kemp, 2005).
Numerous studies have found that the pharmacological blockade of iGluR inhibited both reinforcement and reinstatement of drug intake (cocaine, amphetamine, nicotine, alcohol, etc) (Gass and Olive, 2008) but the therapeutic potential of these receptors is limited because ionotropic receptor modulation is associated with serious adverse effects, including neurotoxicity and psychogenicity (Bisaga et al., 2000).

In contrast to the iGluR, eight metabotropic glutamate (mGlu) receptors, that mediate slower, modulatory glutamatergic transmission, have been identified and are classified into three groups (I, II, and III) depending on their signal transduction pathways, sequence homology, and pharmacological selectivity. On the one hand, group I receptors (mGlu1 and mGlu5) activate the Gαq class of G-proteins which upregulates phosphoinositol hydrolysis and activates intracellular messengers including protein kinase C. On the other hand, Group II (mGlu2/3) or Group III (mGlu4, 6, 7, 8) receptors activate the Gαq class of G-proteins which is negatively coupled to adenylyl cyclase (AC) and therefore decreases intracellular levels of cyclic adenosine monophosphate (cAMP) (D’Souza, 2015). Currently, mGlu receptors are considered as promising targets to develop new treatments for AUD. Indeed, compounds that block Group I mGlu activity are able to limit ethanol self-administration not only in vitro models but in animal models. For example, the mGlu5-selective antagonist 2-methyl-6-[(phenylethyl)methyl]pyridine (MPEP) decreases ethanol self-administration in mice (Hodge et al., 2006) and rats (Schroeder et al., 2005) and reduces motivation and relapse (Bäckström et al., 2004; Besheer et al., 2008). In addition, other studies also support the evidence that Group II mGlu receptors modulate ethanol-related behaviors (Bäckström and Hyttä, 2005; Sidhpura et al., 2010).

The Group III mGlu receptors, and especially the mGlu7 receptors, may be an interesting target to treat AUD. They primarily have a presynaptic localization suggesting that mGlu7 might serve as heteroreceptors to regulate GABA release as well as autoreceptors to control L-glutamate release (Bradley et al., 1996). mGlu7 receptors are widely distributed throughout the central nervous system with the highest concentration in structures implicated in the reinforcing properties of ethanol (hippocampus, amygdala, prefrontal cortex, NAc, etc) (Saugstad et al., 1994). Because of a lack of mGlu7 subtype-selective agents, only a few studies demonstrating the therapeutic potential of mGlu7 receptors in addiction is currently available. Lately, AMN082, a specific and efficacious mGlu7 positive allosteric modulator, has become available (Mitsukawa et al., 2005). Using this new compound, a couple of studies showed that activation of these receptors was able to reduce ethanol self-administration and preference for alcohol (Bahi et al., 2012; Salling et al., 2008). However, in some studies, this effect was nonspecific to the drug (Salling et al., 2008). Moreover, recent data have challenged these results because AMN082 is quickly metabolized into a major metabolite which displays high affinity for monoamine transporters (Sukoff Rizzo et al., 2011).

Recently, LSP-29166, a new mixed mGlu4/mGlu7 orthosteric agonist has become available (Acher et al., 2012). LSP-29166 is more effective on mGlu4 in comparison with mGlu7 with a ED50 of 0.06 mM versus 1.97 mM, respectively (ED50 for mGlu8 is 55.6 μM) (Acher et al., 2012). To our knowledge, this is the most powerful mGlu7 orthosteric agonist synthesized so far. Moreover, these orthosteric compounds have the advantage of being soluble in water; they cross the blood brain barrier and present a high bioavailability (Cajina et al., 2014; Goudet et al., 2012). Regarding the therapeutic potential of group II mGlu receptors, the aim of the present study was to examine whether LSP-29166 could specifically modulate ethanol self-administration in a preclinical model of excessive ethanol intake.

2. Material and methods

2.1. Animals

Male Long Evans rats (weighing 280–300 g at the beginning of the experiment) were obtained from Janvier labs (Le Genest Saint Isle, France). To our knowledge, no study has demonstrated a difference in the expression, functionality or pharmacological modulation of mGlu receptors between male or female rats. Thus in the present work we used only male rats. A total of 19 rats was used in the present study. Animals were individually housed in a controlled environment under a 12-h light/dark cycle (lights on at 8 a.m.) with food and water available ad libitum. All experiments were performed in conformity with the European Community guiding principles for the care and use of animals (2010/63/UE, CE Off. J. 20 October 2010), the French decree n° 2013–118 (French Republic Off. J., 2013) and approved by the local ethics committee [Comité Régional d’Ethique en Matière d’Experimentation Animale de Picardie (CREMEAP), University of Picardie Jules Verne].

A total of 19 rats was used in the present study. Twelve rats were used for the first experiment to carry out the dose response analysis of the LSP2-9166 treatment and were tested in a within-subject and Latin-square counterbalanced design. The remaining group of 7 rats was trained in parallel with the same number of sessions and was injected with artificial cerebrospinal fluid (aCSF). Thereafter, the whole group of 19 rats was used to constitute 2 groups for the reacquisition experiment with 6 rats of the dose response experiment in each group plus 2 and 3 rats for the LSP2-9166 and aCSF groups, respectively. After the reacquisition study, baseline sessions were carried out and rats were randomly assigned to two groups (n = 9 per groups) for the sucrose experiments, one rat was excluded because the baseline sessions were not stable. Finally, two weeks after the end of the sucrose experiments rats were tested for the locomotion study and randomly assigned to the aCSF (n = 10) and the LSP (n = 7) groups, respectively.

2.2. Drugs

LSP2-9166 was synthetized in the laboratory of Francine Acher. For intracerebroventricular (i.c.v.) injection, LSP2-9166 was dissolved to a final concentration of 50 μM, 125 μM, 250 μM, 500 μM in aCSF containing 147 mM of NaCl, 2.7 mM of KCl, 1.2 mM of CaCl2 and 0.85 mM of MgCl2 and was adjusted to pH 6.4 with HCl.

For self-administration experiments, ethanol (VWR, Strasbourg, France) was diluted in tap water at the final concentration of 20% (v/v).

2.3. Surgery

For i.c.v treatment, surgical implantation with a guide cannula was performed as previously described (Jeanblanc et al., 2015). Briefly, rats were continuously anesthetized with isoflurane and placed in a stereotaxic frame (ASI Instrument, Warren, MI, USA). A stainless steel guide cannula was implanted into the right lateral cerebral ventricle of each animal, according to coordinates from the Paxinos and Watson rat brain atlas (1988): Anteroposterior (AP): −0.8 mm relative to bregma; lateral (L): +1.4 mm relative to medial suture; ventral (V): −3 mm relative to skull surface. The cannula were anchored in the skull with screws and dental cement and cleaned twice a week with dental nerve broaches (Micro-Mega, Besancon, France). Subject weights were monitored daily after the surgery to ensure recovery. One week after surgery, subjects returned to self-administration training and were habituated to the microinjection procedure with 3 sham injections. All rats survived the surgical procedure.
For treatments, aCSF or LSP2-9166 (50 μM, 125 μM, 250 μM or 500 μM) were i.c.v. infused using an injection cannula projecting 1 mm beyond the tip of the guide cannula. Two microliters of solution were injected at the rate of 1 μL/min with a microinjection pump (Harvard apparatus, Les Ulis, France). After completion of i.c.v. injection, the cannula was held in place for an additional minute to allow diffusion of the solution.

2.4. Behavioral acquisition of the self-administration task

As previously described (Lebourgeois et al., 2017), the excessive ethanol intake phenotype (binge drinking like phenotype) was induced by a two-step paradigm: First, rats were habituated to the taste and the odor of ethanol during 3 weeks of 20% ethanol intermittent access (Wise, 1973). Thereafter, rats were trained to self-administer 20% ethanol solution during short-session access (15 min) in an operant chamber. The criterion for acquisition of ethanol self-administration was a mean of 30 deliveries per 15 min over 3 consecutive daily self-administration sessions. Once rats reached a high and stable level of intake, we sought to test the effect of LSP2-9166 in a preclinical model of chronic ethanol self-administration.

2.5. Intermittent access to 20% ethanol

After 1 week of acclimation, intermittent access to 20% ethanol was provided to achieve large amounts of self-administered ethanol (>5 g pure ethanol/kg bodyweight/24 h). This procedure was adapted from Simms et al. (2008). Briefly, rats were given access to two bottles, one bottle containing tap water and the other containing 20% ethanol, for 24-h sessions, on Mondays, Wednesdays and Fridays for 3 weeks (a total of 9 drinking sessions). At the end of each session, bottles were weighed to assess ethanol consumption (g of pure ethanol/kg bodyweight) and preference for ethanol (the ratio of ethanol consumed to total fluid intake) and replaced by two bottles of water. The bottle placement in the cage (left or right) was alternated between each session to avoid side preferences.

2.6. Self-administration apparatus and training methods

Ethanol self-administration training was conducted in standard operant chambers connected to the Packwin software (Bioseb, Vitrolles, France), as previously described (Alaux-Cantin et al., 2013; Jeanblanc et al., 2014). Briefly, chambers are equipped with two opposite levers, each of which was located below a light cue and next to a liquid dipper dispenser module. Press on the active lever triggered the associated light cue for 2 s and produced the delivery of 0.1 ml of 20% ethanol solution. The activation of the light cue during 2 s corresponded to a time-out period during which each press was recorded but not reinforced. Responses on the other lever were retained, but produced no consequence (i.e., neither light cue nor delivery). After two overnight sessions (16 h), three 1-h sessions and three 30-min sessions during which a fixed ratio 1 schedule (FR1) was arranged, rats were trained on an FR3 schedule of reinforcement, with the session duration progressively decreased to 15 min. During these experiments, stable level of self-administration was defined as three consecutive sessions with <20% variation in the responding level.

2.7. Effect of LSP2-9166 on 20% ethanol self-administration behavior

Once ethanol consumption was high and stable, the effect of an i.c.v. injection of aCSF or LSP2-9166 (50 μM, 125 μM, 250 μM or 500 μM; 30 min before the test) was tested on the level of ethanol 20% self-administration. The doses and timing of injection were chosen based upon pilot studies. The total number of rats used in this experiment was 12 and a within-subject design was used. Injections were performed according to a Latin-square counter-balanced design with 1 day of washout between each injection.

2.8. Effect of LSP2-9166 on the reinforcing efficacy of the 20% ethanol solution

A progressive ratio (PR) schedule test was also performed to investigate the effect of LSP2-9166 (0 or 250 μM) on the reinforcing efficacy of ethanol in 19 rats. In this experiment, the effort required to obtain a reward (i.e. the number of presses on the active lever) was continuously increased after each new reward delivery (3, 4, 5, 7, 9, 12, 15, 18, 20, 23, 25, 28, 30, 33, 35). During the 15-min session, the breaking point, defined as the maximal response requirement achieved to obtain a single reward of ethanol was measured and may be considered as an index of motivation.

2.9. Effect of LSP2-9166 on relapse like behavior

Once rats have stabilized their responding for 20% ethanol under a FR3 schedule, daily self-administration sessions were stopped and rats were submitted to an extended period of abstinence in home cages. After 2 weeks of protracted abstinence, rats returned in self-administration chambers to induce relapse. A priming delivery of ethanol (0.1 ml of a 20% ethanol solution) was given without any press required at the beginning of the re-acquisition session to provide an olfactory and gustatory cue. Ethanol was then available on an FR3 schedule for the duration of the 15-min session. During this test, aCSF or LSP2-9166 was i.c.v. injected, 30 min before the beginning of the behavioral experimentation, to evaluate the anti-relapse properties of LSP2-9166.

2.10. Effect of LSP2-9166 on sucrose self-administration

In another experiment, we investigated if the effect of LSP2-9166 was specific to ethanol and also not due to side effects. To test this hypothesis, rats were trained to self-administer 4% sucrose (a non-drug rewarding substance) during 15-min sessions under a FR3 schedule of reinforcement. Once sucrose consumption was stable (three consecutive sessions with a variation <20%), the effect of an i.c.v. injection of aCSF or LSP2-9166, 30 min before the test, was evaluated on operant self-administration of sucrose.

2.11. Effect of LSP2-9166 locomotor activity

In another experiment, we investigated if LSP2-9166 has an effect on locomotor activity measured in the open field test. Locomotor activity was recorded for 15 min after an i.c.v. injection of aCSF or LSP2-9166 (250 μM), 30 min before the test. The activity monitoring chamber consists in a 40 x 40 x 30 cm box with opaque acrylic walls, transected with infrared photocell beams 2 cm above the floor at 16 sites along each side. Locomotor chambers were illuminated with indirect white light (20 lux). Horizontal locomotion was measured from photocell beam interruptions using Acti-Track software (Bioseb, Vitrolles, France). Results are expressed as distance travelled (cm) during 3 min intervals.

2.12. Statistical analysis

All data were expressed as mean ± standard errors to the mean. Statistical analyses were performed using one-way ANOVAs or mixed model ANOVA followed by a Tukey’s post-hoc test when it’s
appropriate. Two-way repeated measures ANOVA was used to analyze data on locomotor activity. For simple comparisons (PR schedule and sucrose experiment), data were analyzed with a Student’s t-test. Statistical analyses were performed using the SigmaPlot software (version 11; Systat software, Inc., San Jose, CA USA) and a p ≤ 0.05 was considered significant.

3. Results

3.1. Effect of LSP2-9166 on 20% ethanol self-administration behavior

In a first experiment, we evaluated the effect of an i.c.v. injection of LSP2-9166 (0, 50, 125, 250 or 500 µM; 30 min before the beginning of the test) on ethanol self-administration. The mean numbers of active lever presses were analyzed using a one way ANOVA, which revealed a significant main effect of treatment (F[(4,44) = 6.751, p < 0.001]). Subsequent post-hoc Tukey analyses showed that rats that received an injection of LSP2-9166 at the dose of 250 or 500 µM significantly reduced their number of presses on the active lever in comparison with rats that received aCSF (respectively −60 and −80%) (Fig. 1A). As expected, analogous effects were observed regarding ethanol intake (see Fig. 1B). Rats treated with LSP2-9166 at the dose of 250 or 500 µM consumed significantly less ethanol than aCSF-treated rats (treatment main effect, F[(4,44) = 7.095, p < 0.01]). No treatment effect was observed on the inactive lever presses (F[(4,44) = 0.979, p > 0.05]).

Together, these data indicate that an acute infusion of LSP2-9166 at the dose of 250 or 500 µM, but not at the dose of 50 µM or 25 µM, was able to reduce ethanol consumption. In light of this result, the LSP2-9166 dose of 250 µM was considered as the effective dose and was used for the rest of our study.

3.2. Effect of LSP2-9166 on the reinforcing efficacy of the 20% ethanol solution

To further assess the anti-addictive properties of LSP2-9166, we did a second experiment during which, we used a PR schedule, to evaluate the effect of LSP2-9166 (0 or 250 µM) on the reinforcing efficacy of ethanol. The number of active and inactive lever presses and the breaking point values during the 15-min session are depicted in Fig. 2A and B. Student’s t-test revealed that LSP2-9166-treated rats pressed significantly fewer times (−55%) during the PR session (p < 0.001) and displayed a significantly lower breaking point value (−65%) compared with that of control rats (p < 0.001). There was no statistical difference regarding the number of presses on the inactive lever.

3.3. Effect of LSP2-9166 on relapse like behavior

We also examined the effect of an i.c.v. injection of LSP2-9166 at the dose of 250 µM on relapse after 2 weeks of protracted abstinence (Fig. 3). The mixed-ANOVA revealed a significant effect of abstinence (F[(1,15) = 11.42, p = 0.0041]) a significant effect of treatment (F[(1,15) = 4.28, p = 0.05]) and an interaction between factors (treatment x abstinence; F[(1; 15) = 4.41, p = 0.05]).

Fig. 1. LSP2-9166 dose dependently reduced ethanol self-administration. Effect of an i.c.v. injection of LSP2-9166 (0, 50, 125, 250 or 500 µM; 30 min before the test) on ethanol self-administration. The total number of rats used in this experiment was 12 and a within-subject design was used. (A) Total active lever presses were significantly lower for rats treated with 250 and 500 µM of LSP2-9166 compared with that of aCSF-treated rats, while no statistical effect was found in inactive lever (C). (B) Rats treated with LSP2-9166 250 or 500 µM, consumed significantly less ethanol than aCSF-treated rats. **: p < 0.01, ***: p < 0.001 versus aCSF; +: p < 0.05, ++: p < 0.01 versus the 500 µM dose.
3.4. Effect of LSP2-9166 on sucrose self-administration

In order to assess the effect of LSP2-9166 on a non-drug reward, we tested the properties of an i.c.v. infusion of LSP2-9166 at the dose of 250 μM on sucrose self-administration. LSP2-9166 at the dose of 250 μM did not affect the number of active and inactive presses during the 15-min session (n = 9/group). ***: p < 0.001.

![Fig. 2. LSP2-9166 reduced the reinforcing efficacy of the 20% ethanol solution. (A) LSP2-9166 250 μM treated-rats pressed significantly fewer on the active lever compared to aCSF treated rats, while no statistical effect was found in inactive lever. (B) Similarly LSP2-9166 250 μM treated-rats displayed a significantly lower breaking point compared to controls (n = 19/group). ***p < 0.001.](image1)

![Fig. 3. Effects of an i.c.v. injection of LSP2-9166 on ethanol-induced reacquisition. Active or inactive lever responding during the mean of the two last sessions of ethanol self-administration (Baseline) and during the reacquisition test (Reacquisition) in aCSF (n = 8) or LSP2-9166 250 μM-treated (n = 9) rats. During this test, aCSF-treated rats have the same level of responding before and after abstinence while LSP2-9166-treated rats significantly decreased their reacquisition of operant self-administration behavior. Inactive lever presses were unaffected by either reacquisition protocol or LSP2-9166 treatment. *: p ≤ 0.05.](image2)

![Fig. 4. LSP2-9166 did not impair sucrose self-administration. (A) Effect of an i.c.v. infusion of LSP2-9166 on 4% sucrose self-administration. LSP2-9166 at the dose of 250 μM did not affect the number of active and inactive presses during the 15-min session (n = 9/group). ***: p < 0.001.](image3)

![Fig. 5. LSP2-9166 decreased locomotor activity measured in the open field test. LSP2-9166 injected 30 min before the test at the dose of 250 μM significantly decreased locomotor activity (n = 10 in the Acsf group and n = 7 in the LSP2-9166 group).](image4)

3.5. Effect of LSP2-9166 locomotor activity

The results obtained in the open field are depicted in Fig. 5. The two-way ANOVA revealed a significant decrease of locomotor activity by LSP2-9166 treatment ([F(1,16) = 12.46, p = 0.003]), a significant effect of time ([F(4,60) = 47.75, p < 0.001]) and no significant interaction between factors (treatment x time; [F(4; 60) = 1.63, p = 0.17]).
4. Discussion

Since many years, the pharmacological modulation of mGlu activity has been described as a promising target to treat addictive behaviours. In this study, we have shown for the first time that LSP2-9166, an mGlu4/mGlu7 orthosteric agonist, selectively reduces ethanol consumption, reinforcing efficacy of ethanol and relapse without affecting sucrose self-administration.

Our results add to a growing preclinical literature showing that pharmacological manipulations of mGlu receptors could be useful in many neurological and psychiatric disorders including drug addiction. For more than a decade, considerable evidence indicates that disruption of glutamate homeostasis play a key role in drug consumption and reward (Burnett et al., 2016; Gass and Olive, 2008). Following chronic drug exposure, a decrease in extracellular glutamate concentration was found in the nucleus accumbens (Baker et al., 2003; Madayag et al., 2007). These neuroadaptations result in decreasing the mGlu2/3 tone and, because mGlu2/3 have an inhibitory-release function, lead in turn to enhanced glutamate release (Kalivas, 2009). In therapeutic, restoration of extrasynaptic level of glutamate and thus of glutamate homeostasis is a promising target to treat addiction because an elevation of glutamate may trigger drug craving and LSP2-9166 decreases relapse. Regarding regulation of dopamine levels in the nucleus accumbens by group III mGlu receptors agonists, the results are mixed. A previous study has shown that mGlu7 receptor activation failed to alter extracellular dopamine levels in the nucleus accumbens (Li et al., 2008) while non-selective agonist of group III mGlu receptors has been shown to inhibit dopamine release (Hu et al., 1999). Li et al. (2008) have also shown that the selective mGlu7 receptor agonist, AMN082, into the NAc, dose-dependently decreases extracellular GABA and increases extracellular glutamate. The inhibition of medium spiny GABAergic output neurons in the NAc, predominantly projecting to the ventral pallidum that is necessary for the motivational effect of drug of abuse may explain the decrease in the reinforcing efficacy of ethanol by LSP2-9166 in our study.

By acting on group III mGlu receptors, we expected that an acute injection of LSP2-9166 could reduce this hyperglutamatergic state to limit ethanol consumption and relapse. To test this hypothesis, we used a preclinical model of excessive ethanol intake (i.e. binge drinking like phenotype), assuming that chronic repetition of binge intoxications could also induce the hyperglutamatergic state found in addiction.

First, we demonstrated that an i.c.v. injection of LSP2-9166 (250 μM) reduced ethanol consumption and ethanol breaking point by more than 60%. The PR schedule of reinforcement is used in preclinical studies to assess both motivational and rewarding properties of drugs (Markou et al., 1993). During this test, the “cost” of ethanol is progressively increased after each delivery to determine the maximal effort that the rat will exhibit for a single ethanol reward. By drastically reducing the breaking point, we found that LSP2-9166 is able to strongly reduce the reinforcing efficacy of alcohol predicting its efficiency to reduce craving.

Considering that addiction is a chronic and highly relapsing disorder, one of the principal aims of fundamental research is to identify new treatments to prevent relapse and thus to maintain abstinence or eventually to significantly reduce ethanol intake during resumption of intake (as known for naloxone). So, in a third experiment, we sought to determine if LSP2-9166 could also limit this addiction hallmark. Using the reacquisition model of relapse we have found that LSP2-9166 was able to halve ethanol consumption when rats were re-exposed to the drug after protracted abstinence in home cages. By comparison with relapse after extinction session, reacquisition after abstinence, is a model which more closely mimics the conditions which are inherent to abstinence and relapse in human (Venniro et al., 2016).

Interestingly, we found that the effective dose of LSP2-9166, which was able to reduce ethanol consumption and relapse, failed to alter sucrose consumption. Thus, unlike other pharmacological modulators of mGlu7, such as AMN082 (Salling et al., 2008), it is possible to consider that LSP2-9166 selectively modulates ethanol’s rewarding properties rather than non-drug appetitive reward. Although the lack of effect of LSP2-9166 on sucrose self-administration indicates that it appears devoid of motor side effects, our results obtained in the open field show that it reduces the locomotor activity of animals. Thus we cannot rule out that motor effects of LSP2-9166 may have influenced its efficacy on ethanol operant self-administration. But it is important to note that it had no effect on sucrose intake while we chose the sucrose concentration in such a way to get the same level of responding as for the ethanol solution. Thus, in case of motor side effects of LSP2-9166 on ethanol responding, this should also been observed for sucrose.

According to our results, substantial evidences have already shown that mGlu7 are closely involved in ethanol rewarding properties and addiction. Vadazs et al. have identified mGlu7 as a cis-regulated gene for alcohol consumption. In addition, mice carrying mutations that lead to lower mGlu7 expression consumed more ethanol (Vadasz et al., 2007). In the same way, knock-down of mGlu7, in the nucleus accumbens, is associated with an increase ethanol-induced conditioned place preference and elicits excessive consumption in rats (Bahi, 2013). According to this results, pharmacological activation of mGlu7, with AMN082, decreases ethanol consumption and preference (Bahi et al., 2012) and reduces reinstatement of extinguished ethanol-induced conditioned place preference (Bahi, 2012). In contrast, mGlu7 selective antagonism, with MMPIP, enhanced ethanol intake and reversed the effect of AMN082 (Bahi et al., 2012).

Due to a prolonged lack of selective mGlu4 agonist, the functional role of theses receptors in addiction is less detailed. In mice, deletion of mGlu4, decreased ethanol (2 g/kg)-induced motor stimulation while other ethanol-related behaviors, such as ethanol consumption or withdrawal symptoms, are unaltered (Blednov et al., 2004). More recently, pharmacological modulation of these receptors, by LSP1-2111, has shown that inactivation of these receptors decreases the expression of cocaine sensitization (Zaniewska et al., 2014). However, the efficacy of this agonist on ethanol addiction is currently unknown.

5. Conclusion

In conclusion, the current data demonstrated that pharmacological modulation of mGlu4 and mGlu7, with LSP2-9166, a new orthosteric agonist of these receptors, can reduce ethanol consumption, reinforcing efficacy of ethanol and relapse in a preclinical model of excessive ethanol intake. These results add to a growing number of studies showing that group III mGlu receptors play a key role in alcohol addiction. However, pharmacological mechanisms involved in these effects need to be elucidated, especially by defining the respective role of mGlu4 and mGlu7, using selective antagonists of these receptors.

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evidenced-based actions against addictive disorders.

Conflicts of interest

The authors declare no conflict of interest.

Author’s contribution

MN designed the experiments. SL, JJ and CV contributed to the acquisition of animal data. MN, CV, SL assisted with data analysis. MN, CV, SL, JS, AP and MD contributed to the interpretation of results. JE, ACM and YF contributed with technical support. MD contributed to the interpretation of pharmacological data. SLP, MD, NL, AG, EC, MB, ES, DHM, CC, MA, and CS assisted with data analysis. JE, ACM and YF assisted with the acquisition of animal data. MN designed the experiments. SL, JJ and CV contributed to the acquisition of animal data. MN, CV, SL assisted with data analysis and interpretation of findings. MN and SL wrote the paper. All authors critically reviewed content and approved final version for publication.

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