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Effects of MS-153 on glutamate transporter 1 and cysteine/glutamate exchanger as well as ethanol drinking behavior in male P rats

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Abstract

Alcohol consumption is largely associated with alterations in the extracellular glutamate concentrations in several brain reward regions. We have recently found that glutamate transporter 1 (GLT-1) is downregulated following chronic exposure to ethanol for five weeks in alcohol-preferring rats, and upregulation of the GLT-1 levels in nucleus accumbens and prefrontal cortex resulted, in part, in attenuating ethanol consumption. Cysteine glutamate antiporter (xCT) was also found to be downregulated after chronic ethanol exposure in P rats, and its upregulation could be valuable in attenuating ethanol drinking. In this study, we examined the effect of a synthetic compound, (R)-(−)-5-methyl-1-nicotinoyl-2-pyrazoline (MS-153), on ethanol drinking and expression of GLT-1 and xCT in the amygdala and hippocampus of P rats. P rats were exposed to continuous free-choice access to water, 15% and 30% ethanol, and food for five weeks, and then after which they received treatments of MS-153 or vehicle for five days. The results showed that MS-153 treatment significantly reduced ethanol consumption in P rats. It was revealed that GLT-1 and xCT expressions were downregulated in both the amygdala and hippocampus of ethanol-vehicle treated rats (ethanol vehicle group) as compared to water control animals. Importantly, MS-153 treatment upregulated GLT-1 and xCT expression in these brain regions. These findings provide important role of MS-153 on these glutamate transporters for the attenuation of ethanol drinking behavior.

Keywords

MS-153; glutamate; GLT-1; amygdala; hippocampus

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Conflict of Interest

The authors declare no conflict of interest. The views expressed in this manuscript are strictly those of the authors and do not necessarily represent those held by the National Institutes of Health (NIH) or NIAAA.

Introduction

Extracellular glutamate concentration is maintained through the uptake mechanism by astrocytes (Parpura and Verkhratsky, 2012). Glutamate uptake by these astrocytes occurs through several types of glutamate transporters [for review see (Danbolt, 2001)]. The most predominant transporter is glutamate transporter 1 (GLT-1, its human homolog termed excitatory amino acid transporter 2, EAAT2), which is responsible for clearing more than 90% of extracellular glutamate (Danbolt, 2001, Mitani and Tanaka, 2003). The import of glutamate into astrocytes through GLT-1 is required for subsequent release by the cysteine glutamate antiporter (xCT), which is responsible for releasing glutamate in exchange for cystine (Warr et al., 1999, Melendez et al., 2005). This results in the regulation of synaptic glutamate release (Moussawi and Kalivas, 2010).

It has become increasingly apparent that glutamate neurotransmission in the nucleus accumbens (NAc) mediates drug-seeking behavior, and the changes in glutamate transmission in this brain region are assumed to mediate the switch from intermittent use of drugs to dependence (Gipson et al., 2014). It is noteworthy to mention that glutamatergic input into the NAc from other brain regions has a key role in regulating addictive behavior. Importantly, it is well known that the NAc receives glutamatergic afferents from the prefrontal cortex (PFC) (Papp et al., 2012, Stefanik et al., 2013), amygdala, particularly the basolateral amygdala (BLA) (Stuber et al., 2011, Papp et al., 2012), and the ventral hippocampus (Britt et al., 2012, Papp et al., 2012). Each of these glutamatergic projections has a role in addictive behavior. For instance, glutamatergic projections from the PFC to the NAc have been implicated in goal-directed behaviors and in executing an adaptive behavioral response (Gipson et al., 2014). Alternatively, activation of glutamatergic projections from the BLA to the NAc also promotes motivated behavioral response (Stuber et al., 2011). Additionally, activation of glutamatergic projections from the hippocampus (Hipp) promotes addiction-like behavior and relapse-like to cocaine seeking behavior (Vorel et al., 2001).

Amygdala has been extensively examined for its role in addiction (Di Ciano and Everitt, 2004), anxiety, memory (particularly aversive learning), and emotional behavior (Lalumiere, 2014). Additionally, both sensitization and drug seeking behaviors require glutamate release into the NAc and the source of this glutamate is from the amygdala and PFC (Kalivas et al., 2009). Alternatively, more attention has been paid recently to the Hipp because it has an important role in reward learning and drug-context memory (Fuchs et al., 2005, Adcock et al., 2006, Hernandez-Rabaza et al., 2008, Delgado and Dickerson, 2012). The Hipp is an important brain region in terms of addiction and drug-context memory (Adcock et al., 2006, Meyers et al., 2006, Shen et al., 2006, Hernandez-Rabaza et al., 2008) and relapse to drug abuse (Vorel et al., 2001, Fuchs et al., 2005). Alternatively, other studies have shown that chronic ethanol exposure is associated with impairment of hippocampal neurogenesis (Herrera et al., 2003, He et al., 2005). Reduction in hippocampal neurogenesis has been linked to cocaine addiction (Noonan et al., 2010). It is well known that astrocytic xCT is an important source of glutathione that can protect against oxidative damage and neurodegeneration (Griffith, 1999, Bridges et al., 2012, Lewerenz et al., 2012). Additionally, it has been shown that glutamate excitotoxicity involves the inhibition of cystine exchange

and eventually neuronal cell death via oxidative stress (Murphy et al., 1989). These studies indirectly showed impact of chronic ethanol exposure and the role of xCT in regulating glutamate level in the Hipp.

Alcohol consumption is associated with alterations in the extracellular glutamate concentrations in various brain reward regions [reviewed by (Rao and Sari, 2012)]. Additionally, studies have reported increases in glutamate concentrations in ethanol withdrawal and binge ethanol-drinking paradigms (Dahchour and De Witte, 1999, Ward et al., 2009), as well as in hippocampal rat slides (Roberto et al., 2004). We have recently found that GLT-1 is downregulated in P rats following exposure to ethanol for five weeks in the NAc but not in the PFC (Sari et al., 2013). Recent studies from our laboratory have shown that ceftriaxone treatment in P rats, known to upregulate GLT-1 expression (Rothstein et al., 2005), resulted in attenuating ethanol consumption and this effect was associated, in part, with upregulation of GLT-1 expression in the NAc and PFC (Sari et al., 2011) as well as in the amygdala (Rao and Sari, 2014).

In this study, we tested MS-153 [(R)-(−)-5-methyl-1-nicotinoyl-2-pyrazoline] as a compound known to enhance glutamate uptake (Shimada et al., 1999). This compound has been shown to prevent release of glutamate in case of ischemia, probably by inhibiting high voltage-gated calcium channels via interactions with protein kinase C (PKC) (Uenishi et al., 1999). Interestingly, MS-153 was effective in inhibiting the development of tolerance and physical dependence to morphine (Nakagawa et al., 2001). Additionally, the conditioned rewarding effects of morphine, methamphetamine and cocaine were also inhibited with MS-153 treatment (Nakagawa et al., 2005). Importantly, we recently reported that MS-153 treatment (at a dose of 50 mg/kg/day i.p.) attenuated ethanol intake in P rats, and that this effect was associated, at least in part, with upregulation of GLT-1 expression in the NAc but not in the PFC. Additionally, we showed that GLT-1 upregulation, in the NAc of MS-153 treated rats, was associated with upregulation of the nuclear NF-kB level and downregulation of the cytoplasmic IkBα level as a possible pathway underlying the GLT-1 upregulatory effect of MS-153 (Alhaddad et al., 2014b). Based upon this evidence, we aimed in this study to examine the effects of MS-153 treatment in modulating the expression of GLT-1 and, importantly, xCT in the amygdala and Hipp of P rats exposed to a continuous five-week ethanol-drinking paradigm.

Material and Methods

Animals

Male alcohol-preferring (P) rats, an established model for alcoholism (Sari et al., 2006), were used in this study to test the effect of MS-153 on ethanol drinking as well as the expressions of GLT-1 and xCT in both the amygdala and Hipp. P rats were received from Indiana University School of Medicine (Indianapolis, USA) at the age of 21–30 Days. In the Department of Laboratory Animal Resources (The University of Toledo, Health Science Campus), P rats were individually housed in bedded plastic tubs. All animals had ad lib access to food and water. P rats were accustomed to a temperature of 25°C, 50% humidity, and a 12-hour light-dark cycle. All experimental and animal housing procedures mentioned were approved by the Institutional Animal Care and Use Committee of The University of

Toledo in accordance with the guidelines of the Institutional Animal Care and Use Committee of the National Institutes of Health and the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, Commission on Life Sciences, 1996). At the beginning of this study, all animals were 90 days old. Three experimental groups were used. (1) The ethanol-naïve vehicle group had free access to food and water only (n=5) and received i.p. injections of vehicle solution (1% DMSO in PBS); (2) the ethanol vehicle group received i.p. injections of the same vehicle solution (n=10); and (3) the ethanol MS-153 group received i.p. injections of MS-153 (50 mg/kg body weight) (n=5). During the entire study, the last two groups (ethanol vehicle group and ethanol MS-153 group) had continuous free-choice access to water, 15% and 30% ethanol and food.

Behavioral drinking paradigms

At the age of 90 days, both the ethanol vehicle and ethanol MS-153 groups were exposed to uninterrupted, free-choice access to water, 15% and 30% ethanol, and food for five weeks, as described recently (Sari et al., 2011). During the last two weeks, we measured body weight, ethanol intake and water intake three times per week. Both ethanol and water intake were measured by subtracting the weight of the bottle containing ethanol or water from its initial weight. Using the densitometry formula, ethanol intake measurements were converted into the grams of ethanol consumed per kilogram of animal body weight per day. The average measurements of ethanol consumption, water intake and body weight during the last two weeks of the five-week drinking paradigm were used as a baseline. All animals that drank less than 4 g of ethanol/kg/day were excluded from the study. On week 6, both the ethanol naïve vehicle and ethanol vehicle groups were i.p. injected with 1% DMSO in PBS. However, the ethanol MS-153 group received i.p injections of MS-153 (50 mg/kg body weight) in 1% DMSO in PBS. All animals were i.p. injected for five consecutive days. Ethanol intake, water intake and body weight were measured every day on which i.p. injections were administered. Furthermore, all animals were euthanized and then decapitated 24 hours after the last injection.

Brain tissue harvesting

After animals were decapitated using a guillotine, brains were immediately dissected out and stored at −70°C. Brain regions were then extracted, in accordance with Paxinos and Watson Atlas for the rat brain (Paxinos and Watson, 2007), in the cryostat machine, which was maintained at −20°C to keep the brain tissues frozen. Extracted brain regions were then kept at −70° C for Western blot analysis to examine protein expression.

Western blot analysis

Western blot was performed in the amygdala and Hipp to determine changes in GLT-1 and xCT expressions, as described recently (Sari et al., 2009, Sari et al., 2011, Sari et al., 2013, Alhaddad et al., 2014a). In brief, the amygdala and Hipp samples were homogenized using lysis buffer with phosphatase and protease inhibitors. Samples were quantified using Bio-Rad quantification reagents (Bio-Rad, Hercules, CA, USA), and equal amounts of proteins from all the groups were loaded on either 10–20% glycine gel (Invitrogen) or Mini-PROTEAN® Precast gels (Bio-Rad). Proteins were transferred electrophoretically onto a

PVDF membrane. Non-specific bands were blocked by incubating the PVDF membrane in blocking buffer containing non-fat dry milk in TBST (50 mM Tris HCl; 150 mM NaCl, pH7.4; 0.1% Tween20) for 30 minutes to 1 hr at room temperature. Subsequently, the membrane was incubated overnight at 4°C after adding either Guinea pig anti-GLT-1 (Millipore; 1:5000 dilution) or rabbit anti-xCT antibody (Novus; 1:1000 dilution). The membranes were washed in TBST, and horseradish peroxidase labeled anti-guinea pig or anti-rabbit secondary antibody (1:5000 dilution) was used to detect the proteins. After washing the membranes, chemiluminescent substrate was used to detect the proteins (SuperSignal® West Pico). Kodak BioMax MR Films (Thermo Fisher Scientific) were used to capture the signals from HRP and a SRX-101A machine was used to develop the films. β-tubulin was used as a loading control. Subsequently, the bands on the films were digitized and analyzed using the MCID system. Finally, the data were reported as GLT-1/β-tubulin or xCT/β-tubulin ratios.

Statistical analysis

General Linear Model (GLM) repeated measures were used to analyze the behavioral data related to ethanol consumption, water intake and body weight, followed by an independent *t*-test to determine the daily effect of treatment. Additionally, one-way ANOVA followed by Newman-Keuls multiple comparisons post-hoc test was used to analyze Western blot data (GLT-1/β-tubulin and xCT/β-tubulin ratio) for comparisons between ethanol-naïve, ethanol vehicle, and ethanol MS-153 treatment groups. All statistical tests results were based on $p<0.05$ level of significance.

Results

Effects of MS-153 on ethanol consumption, water intake, total fluid intake and body weight of P rats

GLM repeated measures demonstrated a significant main effect of day [$F(1, 5) = 11.436$, $p<0.0001$] and a significant day × treatment interaction [$F(1, 5) = 9.239$, $p<0.0001$] of ethanol consumption. An independent *t*-test revealed a statistically significant reduction in ethanol consumption in MS-153-treated rats from 24 hrs after the first injection ($p<0.001$) through the end of the study (Fig. 1A). Furthermore, GLM revealed a significant main effect of day on water intake [$F(1, 5) = 3.558$, $p<0.01$] and a significant day × treatment interaction [$F(1, 5) = 2.704$, $p<0.05$]. An independent *t*-test demonstrated a significant increase in water intake on days1, 3 and 4 of treatment ($p<0.05$) (Fig. 1B). Regarding body weight, statistical analyses using GLM revealed a significant main effect of day [$F(1,5)=3.777$, $p<0.01$] and a significant day × treatment interaction[$F(1,5)=3.451$, $p<0.01$]. Independent *t*-test did not reveal any statistically significant difference in body weight between ethanol vehicle- and ethanol MS-153-treated groups ($p>0.05$) (Fig. 1C). Furthermore, statistical analysis of total fluid intake data demonstrated a significant main effect of day [$F(1, 5) = 2.837$, $p<0.05$] and a non-significant day × treatment interaction [$F(1, 5) = 1.747$, $p > 0.05$]. However, independent *t*-test revealed a significant increase in total fluid intake in MS-153 treated P rats, on days 3 and 4, as compared to vehicle treated rats ($p<0.05$) (Fig. 1D).

Effect of MS-153 on GLT-1 and xCT expression in Amygdala

One-way ANOVA analysis of GLT-1 data revealed a significant difference among all treatment groups [F (2,12)=11.56, p=0.0016]. Additionally, Newman-Keuls multiple-comparisons post-hoc test demonstrated a significant upregulation of GLT-1 expression in MS-153-treated animals compared to vehicle-treated rats ($p<0.01$). Alternatively, GLT-1 expression was found significantly downregulated in the ethanol-vehicle group compared to the naïve-vehicle group ($p<0.01$) (Fig. 2).

Furthermore, one-way ANOVA analysis of Western blot data demonstrated a significant difference between the ethanol-naïve, ethanol vehicle and ethanol MS-15 treatment groups [F (2,12) =9.698, p=0.0031). Newman-Keuls multiple-comparisons post-hoc test demonstrated a significant upregulation of xCT expression in the MS-153-treated group compared to the ethanol vehicle-treated group ($p<0.01$). Furthermore, Newman-Keuls post-hoc test revealed a significant downregulation of xCT expression in ethanol vehicle animals compared to ethanol-naïve vehicle animals ($p<0.05$). However, statistical analysis did not show any significant difference in xCT expression between ethanol-naïve vehicle- and MS-153-treated groups (Fig. 3).

Effect of MS-153 on GLT-1 and xCT expression in the Hippocampus

One-way ANOVA demonstrated a significant difference in GLT-1 expression among all treatment groups [F(2,12)=12.83, p=0.001). Further, Newman-Keuls post-hoc test revealed a significant upregulation of GLT-1 expression in MS-153-treated rats compared to vehicle-treated rats ($p<0.01$). Additionally, GLT-1 expression was found to be significantly downregulated in the ethanol vehicle group as compared to the naïve vehicle group ($p<0.05$). However, no significant difference was found between naïve and MS-153 treatment groups (Fig. 4).

We further explored the effect of MS-153 on xCT expression in the Hipp (Fig. 5). One-way ANOVA demonstrated a significant difference among the ethanol naïve, ethanol vehicle and ethanol MS-153 treatment groups [F (2,12)=7.167, p=0.009). Newman-Keuls, multiple-comparisons, post-hoc test revealed a significant upregulation of xCT expression in the MS-153-treated group compared to the ethanol vehicle-treated group ($p<0.01$). Additionally, xCT was significantly downregulated in the ethanol vehicle group compared to the ethanol naïve vehicle group ($p<0.05$). Statistical analysis did not show a significant difference in xCT expression between the ethanol naïve vehicle and MS-153 treatment groups.

Discussion

In this study, we report that MS-153 treatment is associated with a significant attenuation in daily ethanol consumption starting 24 hours after the first dose of MS-153 compared to vehicle-treated animals. Additionally, water intake in MS-153- treated rats was found to be significantly higher than in vehicle-treated rats. Statistical analyses did not show any significant effect on body weight of the MS-153-treated animals during the study. It is noteworthy that water intake in the MS-153-treated group was significantly higher compared to control animals on days 1, 3 and 4, which could be explained as a behavioral

compensatory mechanism due to reduction in ethanol intake. This is in agreement with recent studies from our lab using ceftriaxone as a GLT-1 upregulator (Sari et al., 2011, Sari and Sreemantula, 2012, Rao and Sari, 2014). We also found that there is increase in total fluid in MS-153 treated group. This was due to increase in water intake in MS-153 treated group as compared to control group.

A reduction in ethanol intake was found, along with significant upregulation of xCT and GLT-1 expressions in the amygdala and Hipp. Elevated extracellular glutamate concentrations have been reported after ethanol exposure in the amygdala and Hipp (Dahchour and De Witte, 1999, Roberto et al., 2004, Chefer et al., 2011). We found a downregulation of GLT-1 in the amygdala and Hipp of ethanol vehicle-treated animals, which can partially explain the elevated glutamate concentrations. Furthermore, upregulation of GLT-1 expression following treatment with MS-153 may be associated in part with the reduction in ethanol intake in this drug-treated group. In this study, we also found significant downregulation of xCT expression in the amygdala and Hipp of P rats exposed to five weeks of continuous ethanol exposure compared to ethanol naïve rats. It is well known that the reduction in xCT expression causes a decrease in extracellular, non-synaptic glutamate concentration, and consequently the loss of glutamatergic tone on presynaptic mGLU2/3 receptors, thereby causing an increase in synaptic glutamate release (Moran et al., 2005, Javitt et al., 2011). Moran and colleagues have found that restoring xCT activity by N-acetylcysteine prevented cocaine-seeking behavior (Moran et al., 2005). Alternatively, activating mGLU2/3 receptors was shown to be effective in attenuating cue-induced ethanol seeking (Zhao et al., 2006). Additionally, chronic exposure to ethanol induced an inhibition of mGLU2/3 receptor function (Moussawi and Kalivas, 2010). It is noteworthy that modafinil requires xCT action to attenuate cocaine reinstatement by activating the mGLU2/3 receptor (Mahler et al., 2014). In our present study, we found that MS-153 treatment for five days caused an upregulation in xCT expression. Restoring the xCT level would eventually lead to a reduction in the extracellular glutamate level and therefore may reduce ethanol drinking.

The Hipp is an important brain region in terms of addiction and drug-context memory (Adcock et al., 2006, Meyers et al., 2006, Shen et al., 2006, Hernandez-Rabaza et al., 2008) and relapse to drug abuse (Vorel et al., 2001, Fuchs et al., 2005). Alternatively, other studies have shown that chronic alcohol exposure is associated with impairment of hippocampal neurogenesis (Herrera et al., 2003, He et al., 2005). Reduction in hippocampal neurogenesis has been linked to cocaine addiction (Noonan et al., 2010). It is well known that astrocytic xCT is an important source of glutathione that can protect against oxidative damage and neurodegeneration (Griffith, 1999, Bridges et al., 2012, Lewerenz et al., 2012). Additionally, it has been shown that glutamate excitotoxicity involves the inhibition of cystine exchange and eventually neuronal cell death via oxidative stress (Murphy et al., 1989). These studies indirectly support the idea that upregulation of xCT is important to decrease extracellular glutamate and possibly decrease the neuronal loss associated with chronic ethanol exposure.

Several studies have focused on the importance of the amygdala in addiction and drug reinforcement (Baxter and Murray, 2002, Sinclair et al., 2012, Christian et al., 2013). Chronic ethanol exposure was found associated with increased presynaptic glutamate release

(Roberto et al., 2004). Alternatively, elevated extracellular glutamate and neuronal loss were found associated with chronic ethanol exposure in the Hipp. These findings further support our suggestions regarding the downregulation of xCT. Accordingly, upregulating xCT expression using MS-153 might be important for targeting the elevated extracellular glutamate concentrations in the amygdala and consequently reducing ethanol consumption.

In summary, the present study demonstrates that MS-153 is effective in attenuating ethanol consumption in male P rats after challenging them with five weeks of a free-choice ethanol-drinking paradigm. The reduction in ethanol intake was found to be associated, at least in part, with upregulation of both xCT and GLT-1 expression in the amygdala and Hipp. Based on these findings, we conclude that MS-153 is a drug target that may normalize GLT-1 and xCT expression and consequently reduce alcohol dependence.

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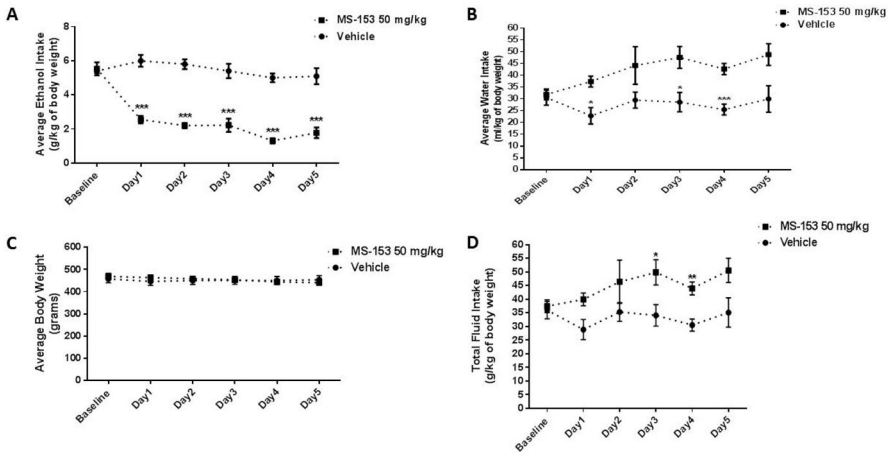

Figure 1.

(A) Effects of MS-153 treatment on average daily ethanol intake (g/kg/day) in male P rats exposed to five weeks of continuous free choice of ethanol and water. Statistical analyses demonstrated a significant difference between MS-153 treated group (n=5) and ethanol vehicle group (n=10). Additionally, independent *t*-test revealed a significant decrease in ethanol intake with MS-153 (50 mg/kg, i.p.) treated group from Day 1 (24 hrs after the first i.p. injection) through Day 5 as compared to ethanol vehicle group. (B) Effects of MS-153 treatment on average daily water intake (ml/kg/day) in male P rats exposed to five weeks of continuous free choice of ethanol and water. Statistical analysis revealed a significant increase in water consumption in MS-153-treated P rats as compared to ethanol vehicle-treated P rats, on days 1, 3 and 4. (C) Effects of MS-153 treatment on body weight (grams) of male P rats exposed to five weeks of continuous free choice access to ethanol and water. Statistical analysis of animals' body weight data revealed no significant difference in body weight between the ethanol MS-153-treated group and the ethanol vehicle-treated group during the entire period of the study. (D) Effect of MS-153 treatment on total fluid intake of male P rats exposed to continuous free choice access to ethanol and water. Statistical analysis showed significant increase in total fluid intake in MS-153 treated group, on days 3 and 4, as compared to vehicle treated group. Data are shown as mean ± SEM. (*p<0.05; **p<0.01; **p<0.001).

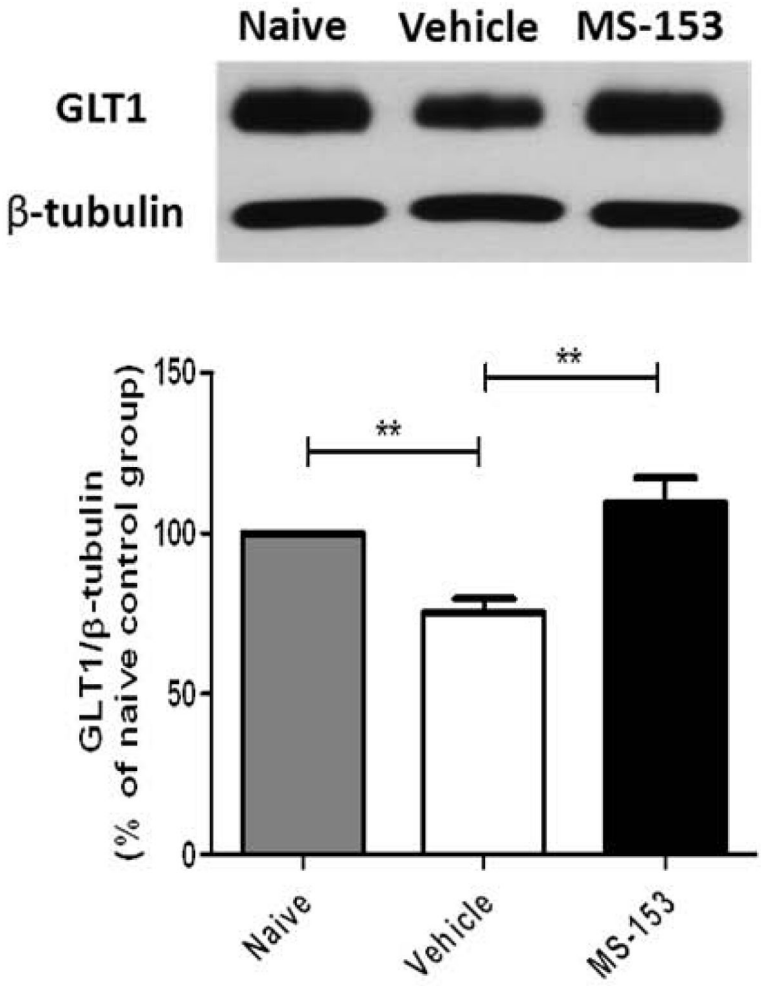

Figure 2.

Effect of MS-153 on GLT-1 expression in the amygdala. Upper panel: Representative immunoblots of GLT-1 and β-tubulin, a loading control, in the amygdala. Lower panel: Quantitative analysis of the immunoblots demonstrated significant upregulation of GLT-1 in the MS-153-treated group (50 mg/kg, i.p.; n=5) as compared to the ethanol vehicle-treated group (n=5). In addition, statistical analysis revealed a significant downregulation of GLT-1 in the ethanol vehicle group as compared to the ethanol naïve vehicle group. Data are shown as mean ± SEM. (**p<0.01).

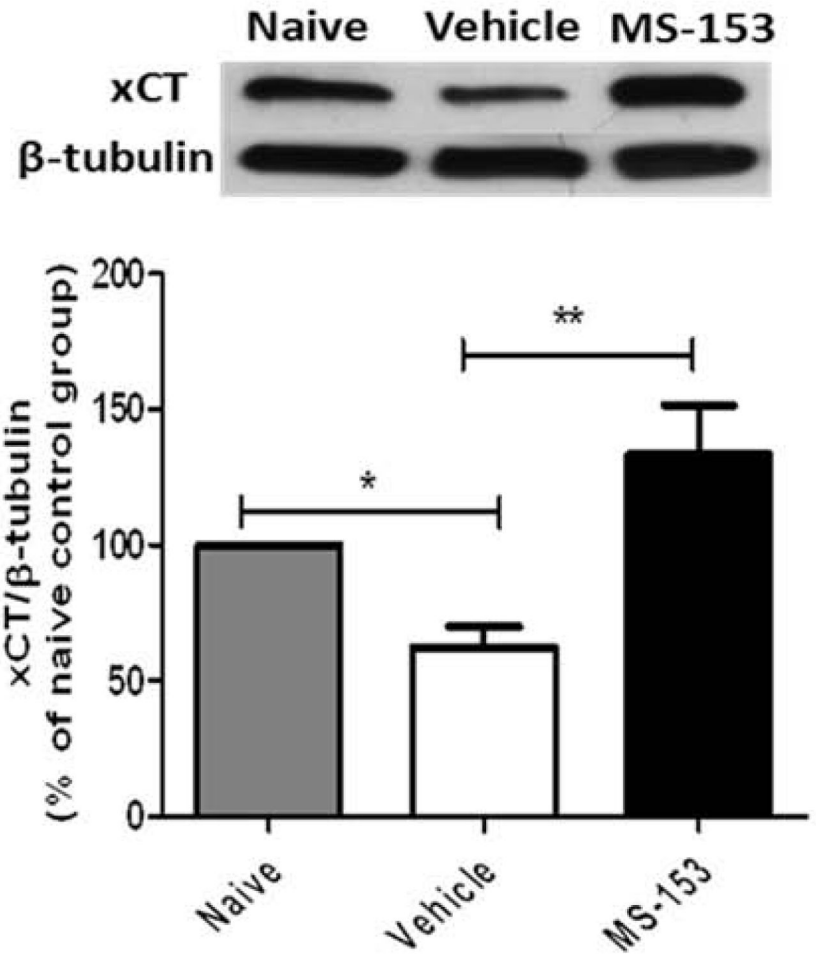

Figure 3.

Effect of MS-153 on xCT expression in the amygdala. Upper panel: Representative immunoblots of xCT and β-tubulin, a loading control, in the amygdala. Lower panel: Quantitative analysis of the immunoblots demonstrated significant upregulation of xCT in the MS-153 treatment group (50 mg/kg, i.p.; n=5) as compared to the ethanol vehicle group (n=5). Alternatively, statistical analysis revealed a significant downregulation of xCT in the ethanol vehicle group as compared to the ethanol naïve vehicle group. Data are shown as mean ± SEM. (*p<0.05; **p<0.01).

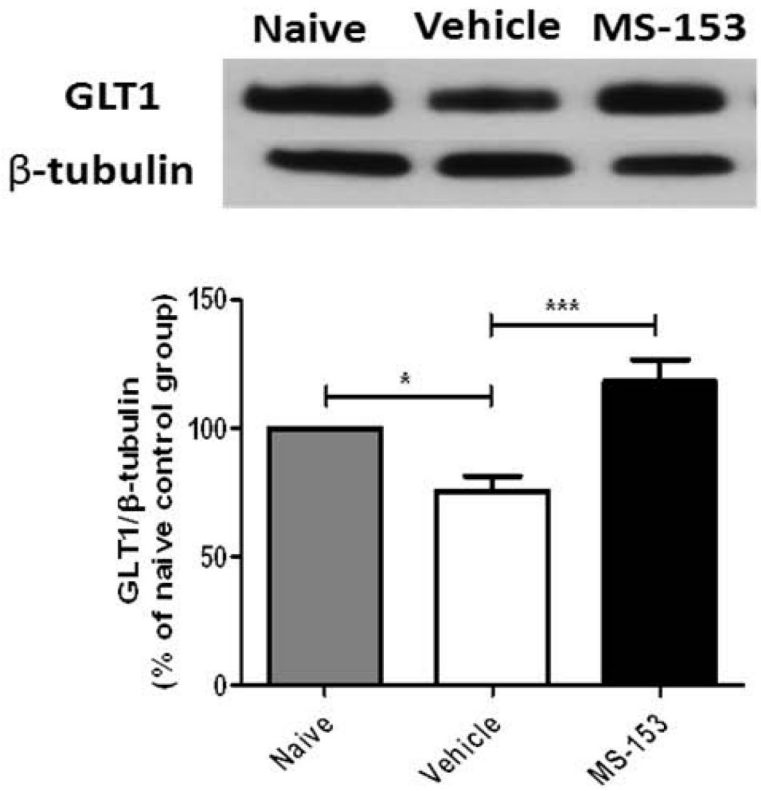

Figure 4.

Effect of MS-153 on GLT-1 expression in the hippocampus. Upper panel: Representative immunoblots of GLT-1 and β-tubulin, a loading control, in the Hipp. Lower panel: Quantitative analysis of the immunoblots demonstrated significant upregulation of GLT-1 in the MS-153 treatment group (50 mg/kg, i.p.; n=5) as compared to the ethanol vehicle group (n=5). Alternatively, statistical analysis revealed a significant downregulation of GLT-1 in the ethanol vehicle group as compared to the ethanol naïve vehicle group. Data are shown as mean ± SEM. (*$p<0.05$; ***$p<0.01$).

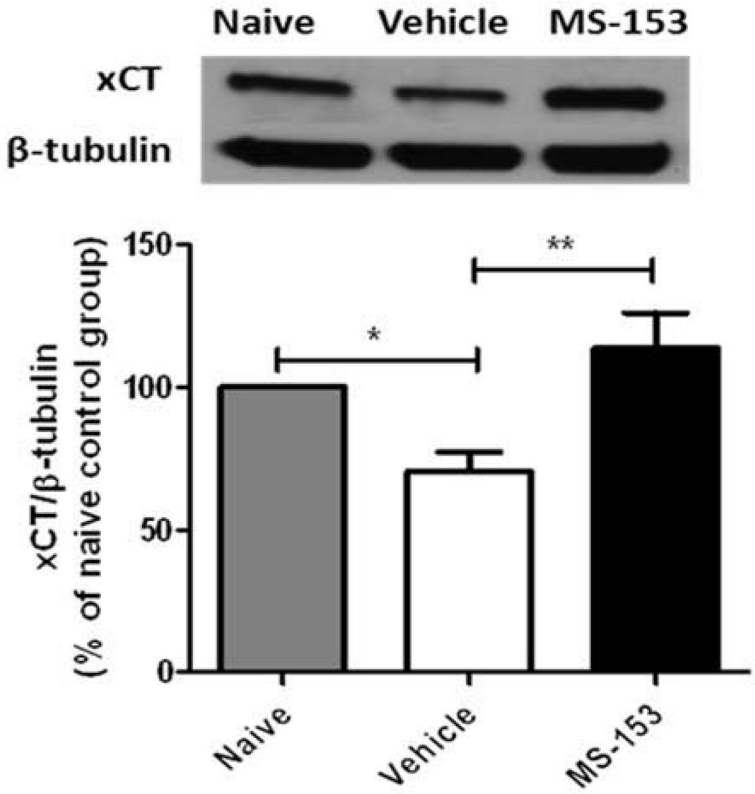

Figure 5.

Effect of MS-153 on xCT expression in hippocampus. Upper panel: Representative immunoblots of xCT and β-tubulin, a loading control, in the Hipp. Lower panel: Quantitative analysis of the immunoblots demonstrated significant upregulation of xCT in the MS-153 treatment group (50 mg/kg, i.p.; n=5) as compared to the ethanol vehicle group (n=5). Alternatively, statistical analysis showed a significant downregulation of xCT in the ethanol vehicle group as compared to the ethanol naïve vehicle group. Data are shown as mean ± SEM. (*$p<0.05$; **$p<0.01$).