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Effects of Administered Ethanol and Methamphetamine on Glial Glutamate Transporters in Rat Striatum and Hippocampus

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Abstract

Exposure to ethanol (EtOH) or methamphetamine (MA) can lead to increase in extracellular glutamate concentration in the brain. Although studies from ours showed the effects of EtOH exposure on key glial glutamate transporters, there is less known about the effects of sequential exposure to EtOH and MA or MA alone on certain glial glutamate transporters. In this study, we investigated the effects of sequential exposure to EtOH and MA on the expression of the major glutamate transporter, glutamate transporter 1 (GLT-1), as well as cystine/glutamate antiporter (xCT) and glutamate aspartate transporter (GLAST) in striatum and hippocampus. We also tested the effects of ceftriaxone (CEF), known to upregulate GLT-1, in animals administered EtOH and MA. Wistar rats were orally gavaged with EtOH (6 g/kg) or water for seven days. On the following day (Day 8), rats received four intraperitoneal (i.p.) injections of MA (10 mg/kg) or saline (vehicle) occurring every 2 hours. Rats were then treated with CEF (200 mg/kg/day, i.p.) or saline on Day 8, 9 and 10. EtOH or MA exposure caused a significant downregulation of GLT-1 expression as compared to control groups in striatum and hippocampus. Furthermore, sequential exposure of EtOH and MA caused a significant downregulation of GLT-1 expression as compared to either drug administered alone in both brain regions. Importantly, GLT-1 expression was restored following CEF treatment. These findings demonstrated that sequential exposure to EtOH and MA has synergistic effect in downregulation of GLT-1 and this effect can be attenuated by CEF treatment.

Keywords

GLT-1; methamphetamine; ethanol; glutamate; xCT; GLAST; gavage

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Author Contributions

FSA participated in study design and conceptualization, drafted and revised the manuscript, collected and analyzed the data. YSA collected the data and helped with the editing of manuscript. YS conceptualized and designed the study, critically revised the manuscript for intellectual content, and approved the final version of the manuscript.

Disclosure Statements

The authors declare no conflict of interest.

INTRODUCTION

Evidence demonstrated increases in ethanol (EtOH) intake with methamphetamine (MA) exposure, which was associated with elevation on the rewarding effects when compared to either drug administered alone (Kirkpatrick et al., 2012; Bujarski et al., 2014). Concomitant exposure to EtOH and MA increased the reward effects and decreased impairments in sleep and performance (Kirkpatrick et al., 2012), and stimulated the release of dopamine in striatum (Nishiguchi et al., 2002; Yamauchi et al., 2000). Thus, concomitant exposure to EtOH and MA can lead to clinical challenges for diagnosis and treatment because of their interactive effects (Sepehrmanesh et al., 2014).

Several studies focused on the role of glutamate in MA abuse (Abekawa et al., 1994; Nash and Yamamoto, 1992; Qi et al., 2009). MA increased the extracellular glutamate concentrations in striatum (Nash and Yamamoto, 1992) and hippocampus (Raudensky and Yamamoto, 2007). It has been shown that repeated high dose treatment with MA (10 mg/kg every 2 hrs \times 4) can lead to damage of dopaminergic terminals and increases of extracellular glutamate concentration (Thomas et al., 2004; Halpin et al., 2013; Bowyer et al., 1994). In addition, studies reported that repeated MA exposure can lead to increase in both extracellular concentrations of dopamine and glutamate in the striatum (Nash and Yamamoto, 1992) and hippocampus (Rocher and Gardier, 2001). Although, it is now well known about the effects of MA exposure in the increase of extracellular glutamate in specific brain regions, there is little known about its effects on glutamate transporters after MA administration or MA administered sequentially with EtOH.

Moreover, it has been demonstrated that EtOH exposure can decrease glutamate uptake and increase extracellular glutamate concentrations in nucleus accumbens (Das et al., 2015; Melendez et al., 2005). Also, chronic EtOH consumption downregulated the expression of major glutamate transporters such as glutamate transporter 1 (GLT-1, its homolog is excitatory amino acid 2) and cystine/glutamate exchanger (xCT) (Rao and Sari, 2014; Sari and Sreemantula, 2012; Aal-Aaboda et al., 2015; Alasmari et al., 2015; Alhaddad et al., 2014; Das et al., 2015). In addition, binge EtOH gavage (4 g/kg, 3 to 4 times a day) decreases the expression of GLT-1 and produces withdrawal signs upon cessation to EtOH exposure (Abulseoud et al., 2014; Das et al., 2016). Glutamate homeostasis is regulated by a number of transporters, including GLT-1, xCT, and glutamate aspartate transporter (GLAST). The present study was aimed to examine the effects of sequential exposure of EtOH and MA as well as the drug alone on GLT-1, xCT, and GLAST expression. We suggested that hyperglutamatergic state induced by MA or EtOH might be associated with deficit in glutamate transporters. We hypothesized that CEF, known GLT-1 upregulator, would normalize the expression of these glutamate transporters after exposure to sequential EtOH and MA in both striatum and hippocampus.

Materials and Methods

Drugs

(+) MA hydrochloride was purchased from Sigma-Aldrich (St. Louis, MO). CEF (Sandoz Inc., Princeton, NJ) was purchased from The University of Toledo Medical Center

Pharmacy. Both drugs were dissolved in saline solution (0.9% NaCl). EtOH (190 proof, Decon Lab) was diluted with water for oral gavage.

Animals

Male Wistar rats were used in this study to examine the effects of sequential EtOH and MA exposure on selective glutamate transporters. Wistar rats were chosen due to their higher EtOH threshold effect compared to other rats (Abulseoud et al., 2014). Wistar rats were received from Harlan Laboratories. Rats were housed in standard plastic tube with free access to food and water throughout the experiments. Rats were kept in 21°C with relative humidity of 50% on a regular 12 hrs light/dark cycle. The experiments and housing procedures were approved by the Institutional Animal Care and Use committee at The University of Toledo with guidelines followed by the Institutional Animal Care and Use Committee of the National Institutes of Health and the Guide for the Care and Use of Laboratory.

EtOH and MA exposure as well as CEF treatment

Timeline of the experimental design is illustrated in Table 1. Wistar rats were divided randomly into six groups: (a) Water gavaged group received i.p saline (i.p.) then saline (i.p.); (b) Water gavaged group received MA (i.p.) then saline (i.p.); (c) Water gavaged group received MA (i.p.) then CEF (i.p.); (d) EtOH gavaged group received saline (i.p.) then saline (i.p.); (e) EtOH gavaged group received MA (i.p.) then saline (i.p.); (f) EtOH gavaged group received MA (i.p.) then CEF (i.p.). Rats received oral gavage of water (control) or EtOH (6 g/kg) for seven days. At Day 8, rats received total of four injections of saline or MA (10 mg/kg) every 2 hrs; and saline or CEF (200 mg/kg, i.p.) was administered 30 minutes following the last MA i.p. injection. At Day 9, rats received saline or CEF 24 hrs following the last MA i.p. injections. At Day 10, rats received last saline or CEF dose, and then rats were euthanized with CO₂ inhalation followed by decapitation.

Brain tissue dissection

Dorsal striatum and Hippocampus were isolated using a cryostat (Leica CM1950) with guidance of rat brain atlas (Paxinos, 2006). Brain regions were kept at -80°C for immunoblotting to detect the selected protein targets.

Immunoblotting procedures

Immunoblotting procedures were done as previously described (Sari et al., 2010; Sari et al., 2009). In brief, striatum and hippocampus samples were lysed in lysis buffer. Quantified proteins were separated through 10% polyacrylamide gels in 1× laemmli buffer. Proteins were then transferred onto PVDF membrane (Bio-Rad Laboratories). Membranes were further blocked with 3 % blocking buffer (fat free milk, LabScientific) in 1× TBST. The membranes were then incubated with primary antibodies (GLT-1, xCT or GLAST) overnight at 4°C. β-tubulin was used as a loading control. Membranes were incubated with chemiluminescent substrate (Super Signal West Pico, Thermo Scientific) and exposed to autoradiography films (Denville Scientific Inc.). The membranes were then developed using

X-Ray film processor (Konica SRX101A – Tabletop). Images of immunoblots were quantified using MCID Digital Imaging Software.

Statistical analysis

Analyses of the effects of MA, EtOH, and CEF on GLT-1, xCT, and GLAST expression were performed using one-way analysis of variance (ANOVA) followed by Newman-Keuls multiple comparison tests. An independent t-test was performed for further comparison between individual groups. All statistical analyses were done using p value less than 0.05 ($p < 0.05$) as level of significance.

Results

Effects of MA and CEF on GLT-1 expression in striatum and hippocampus in water or EtOH oral gavaged rats

We investigated the effects of CEF on GLT-1 expression in striatum and hippocampus in rats that received water (WT) or EtOH through oral gavage procedure and challenged with repeated high dose of MA. One-way ANOVA revealed a significant main effect among water saline-saline (WT-SA-SA), water methamphetamine-saline (WT-MA-SA), and water methamphetamine-ceftriaxone (WT-MA-CEF) groups in striatum [$F(2, 15) = 7.587, p = 0.0053$] and hippocampus [$F(2, 15) = 9.662, p = 0.002$]. Newman-Keuls multiple comparison post-hoc test demonstrated a significant increase in the expression of GLT-1 in WT-MA-CEF treated groups compared to WT-MA-SA treated groups in striatum and hippocampus ($p < 0.01$; Fig. 1A, B). Alternatively, quantitative analysis showed a significant downregulation of GLT-1 expression in WT-MA-SA treated groups as compared to control groups in striatum and hippocampus ($p < 0.05$; Fig. 1A, B). Statistical analysis revealed no significant effects between WT-SA-SA and WT-MA-CEF-treated groups rats in striatum and hippocampus. Furthermore, one-way ANOVA revealed a significant effect between ethanol-saline-saline (EtOH-SA-SA), ethanol-methamphetamine-saline (EtOH-MA-SA), and ethanol-methamphetamine-ceftriaxone (EtOH-MA-CEF) in striatum [$F(2, 15) = 6.092, p = 0.0116$] and hippocampus [$F(2, 15) = 5.467, p = 0.0165$]. Newman-Keuls multiple comparison post-hoc test revealed a significant increase in the expression of GLT-1 in EtOH-MA-CEF treated groups compared to EtOH-MA-SA in striatum and hippocampus ($p < 0.05$, Fig. 2A, B). However, no significant effects were found between EtOH-SA-SA and EtOH-MA-CEF treated groups in striatum and hippocampus (Fig. 2A, B).

Effects of MA and CEF on xCT expression in striatum and hippocampus in water or EtOH oral gavaged rats

We examined the effects of CEF on xCT expression in striatum and hippocampus in rats received water or EtOH through oral gavage and challenged with repeated high dose of MA. One-way ANOVA followed by Newman-Keuls multiple comparison post-hoc test showed no significant effects between WT-SA-SA, WT-MA-SA, and WT-MA-CEF groups in striatum [$F(2, 15) = 0.2770, p = 0.7619$, Fig. 1C, D] and hippocampus [$F(2, 15) = 1.724, p = 0.2119$, Fig. 1C, D]. One-way ANOVA followed by Newman-Keuls multiple comparison post-hoc test demonstrated no significant changes in the expression of xCT between EtOH-SA-SA,

EtOH-MA-SA, and EtOH-MA-CEF in striatum [$F(2, 15) = 0.3416$, $p=0.3416$, Fig. 2 C, D] and hippocampus [$F(2, 15) = 0.03487$, $p=0.9658$, Fig. 2 C, D].

Effects of MA and CEF on GLAST expression in striatum and hippocampus in water or EtOH gavaged rats

GLAST expression was investigated to evaluate the effects of CEF in striatum and hippocampus in rats received water or EtOH through oral gavage and challenged with repeated high dose of MA. One-way ANOVA followed by Newman-Keuls multiple comparison post-hoc test revealed no significant changes in the expression of GLAST between WT-SA-SA, WT-MA-SA, and WT-MA-CEF in both striatum [$F(2, 15) = 0.1772$, $p=0.8393$, Fig. 1 E, F] and hippocampus [$F(2, 18) = 0.1153$, $p=0.8917$, Fig. 1 E, F]. One-way ANOVA followed by Newman-Keuls multiple comparison post-hoc test revealed no significant changes in the expression of GLAST between EtOH-SA-SA, EtOH-MA-SA, and EtOH-MA-CEF [$F(2, 15) = 2.151$, $p=0.1509$, Fig. 2 F] and hippocampus [$F(2, 15) = 0.08096$, $p=0.9226$, Fig. 2 E, F].

Effects of exposure of EtOH alone or with MA on GLT-1 expression in striatum and hippocampus

The effects of sequential exposure of EtOH and MA on the expression of GLT-1 in were examined in both striatum and hippocampus. An Independent t-test showed a significant decrease in the expression of GLT-1 in EtOH-SA-SA groups compared to WT-SA-SA in striatum ($p=0.0285$, Fig. 3A, B and hippocampus ($p=0.0432$, Fig. 3A, B. The effects of EtOH and MA on the expression of GLT-1 were examined in both striatum and hippocampus. An Independent t-test showed a significant decrease of GLT-1 expression in EtOH-MA-SA groups compared to WT-MA-SA in striatum ($p<0.0255$, Fig. 3C, D and hippocampus ($p<0.0399$, Fig. 3C, D).

Discussion

Several studies have reported the negative effects of MA and EtOH exposure in the brain (Kirkpatrick et al., 2012; Kim et al., 2006). Concomitant exposure of EtOH and MA might be lethal, as MA can produce stimulating effects that counteract the depressing effects of EtOH and consequently increases EtOH seeking behavior (Bujarski et al., 2014). The rewards effects have been reported to be greater when MA is consumed with EtOH (Kirkpatrick et al., 2012). Studies from our laboratory reported that chronic EtOH consumption disturbs glutamate homeostasis via downregulation of GLT-1 and xCT (Aal-Aaboda et al., 2015; Rao and Sari, 2014; Sari and Sreemantula, 2012; Sari et al., 2013; Das et al., 2015). However, little is known about GLT-1 and the other glutamate transporters (xCT and GLAST) following MA exposure. Importantly, the present study revealed for the first time that repeated high dose MA (10 mg/kg every 2 hrs, $\times 4$ i.p.) downregulated GLT-1 expression in both the striatum and hippocampus, which might be associated with increases in extracellular glutamate levels as reported in previous studies (Nash and Yamamoto, 1992; Halpin et al., 2013; Bowyer et al., 1994). However, it has been reported that MA at lower dose (2.0 mg/kg) can increase GLT-1 expression in prefrontal cortex in mice, but not in

hippocampus (Qi et al., 2012). We suggest that downregulation of GLT-1 expression may occur only when repeated high dose of MA is applied.

Oral gavage of EtOH alone was found to downregulate GLT-1 expression in striatum and hippocampus. In contrast, the use of low EtOH dose (1g/kg) for 7 days has been reported to decrease glutamate uptake but did not show any changes in the expression of GLT-1 or GLAST (Melendez et al., 2005). In the present study, however, rats were gavaged with EtOH (6 g/kg) for 7 days, which represent the repeated binge dosing paradigm of EtOH (Faingold, 2008). In addition, it has been shown that rats administered EtOH using a binge gavage protocol (4 g/kg, 3 to 4 times a day) reduced GLT-1 expression in the nucleus accumbens, medial prefrontal cortex and striatum (Das et al., 2016; Abulseoud et al., 2014).

Interestingly, MA and EtOH exposure has synergistic effect in the downregulation of GLT-1 expression as compared to either drug administered alone. GLT-1 is responsible for the majority of glutamate uptake in the brain (Danbolt, 2001). Thus, inhibition of GLT-1 synthesis or blockade of GLT-1 can lead to increase glutamate extracellular concentration (Rothstein et al., 1996; Das et al., 2015). In fact, GLT-1 has been linked to many neurodegenerative diseases such as Alzheimer's, Huntington's, Amyotrophic lateral sclerosis (ALS) and epilepsy (Tanaka et al., 1997; Lievens et al., 2001; Lauderback et al., 2001; Rothstein et al., 1995). The elevation in glutamate extracellular concentration also activates post synaptic receptors such as N-methyl-D-aspartate receptor (NMDA), α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA), and Kainate receptors (KA) (Choi, 1992; Meldrum, 2000; Sattler and Tymianski, 2001). Excessive extracellular glutamate concentrations can lead to overstimulation of postsynaptic receptors, which is pivotal in neurodegenerative diseases and neural death (Maragos et al., 1987; Scott et al., 2002; Howland et al., 2002; Gao et al., 2000). This suggests that MA and EtOH may exacerbate glutamatergic excitotoxicity as compared to either drug administered alone.

CEF is known to upregulate GLT-1 expression in different brain regions, including both striatum (Sari et al., 2010) and hippocampus (Aal-Aaboda et al., 2015). It is important to note that several β -lactam antibiotics have been reported to upregulate GLT-1 (Rothstein et al., 2005); and CEF, in particular, was able to attenuate cocaine seeking through normalizing GLT-1 and restoring glutamate homeostasis (Knackstedt et al., 2010). In addition, it has also been found that CEF reduced cocaine seeking in animal model through upregulation of GLT-1 in nucleus accumbens and prefrontal cortex (Sari et al., 2009). In addition, CEF attenuated the hyperactivity and behavioral changes associated with amphetamine (Rasmussen et al., 2011). Furthermore, studies from our laboratory have demonstrated the ability of CEF to decrease EtOH consumption and normalize GLT-1 expression (Sari et al., 2013; Rao and Sari, 2014). Therefore, CEF was one of the best medications in attenuating glutamatergic excitotoxicity that might be associated with EtOH and MA co-abuse. Therefore, we examined the ability of CEF on restoring GLT-1 expression following MA and EtOH. Remarkably, CEF successfully restored GLT-1 expression following MA exposure in all tested water and EtOH groups in both striatum and hippocampus.

The expression of xCT and GLAST following METH and EtOH were also investigated; however, no significant changes were found in either striatum or hippocampus. It is well known that xCT plays an important role in regulating extracellular glutamate concentration

in striatum (Baker et al., 2002) and drug seeking [for review see Ref. (Reissner and Kalivas, 2010)]. It is noteworthy, that we did not find any significant changes in xCT expression in EtOH compared with water group which is consistent with our previous findings that xCT was not downregulated in binge EtOH drinking (Das et al., 2016). There is possibility that EtOH or MA may induce dysfunction of xCT without affecting its expression. Studies are warranted to investigate the effects of MA and EtOH in the function of xCT in specific areas of the brain. Furthermore, GLAST is also responsible for clearing extracellular glutamate concentration; however, this transporter is mainly expressed in other brain regions such as cerebellum and inner ear (Lehre and Danbolt, 1998). Studies have shown that GLAST was not altered in chronic EtOH exposure (Alhaddad et al., 2014) and ALS neurodegenerative disease (Rothstein et al., 1995). However, these studies demonstrated that GLT-1 was downregulated, but GLAST was not affected. Together, we suggest that GLT-1 was more vulnerable to the insults of EtOH and MA exposure as compared to xCT and GLAST.

In conclusion, this study revealed for the first time that repeated high dose of MA downregulated GLT-1 expression in striatum and hippocampus, which may provide a possible pharmacological mechanism in the increase of extracellular glutamate concentrations in specific brain regions. This study also revealed for the first time the effects of sequential EtOH and MA administration on downregulation of GLT-1 but not xCT and GLAST expression in striatum and hippocampus. In addition, there was a synergistic effect of EtOH and MA exposure in downregulation of GLT-1 in striatum and hippocampus as compared to drug administered alone. Importantly, CEF was able to restore GLT-1 expression in MA administered alone or sequentially administered with EtOH. These findings suggest that GLT-1 might be a potential target in EtOH and MA co-abuse, and CEF might alleviate EtOH and MA induced hyperglutamatergic state through the upregulation of GLT-1 expression.

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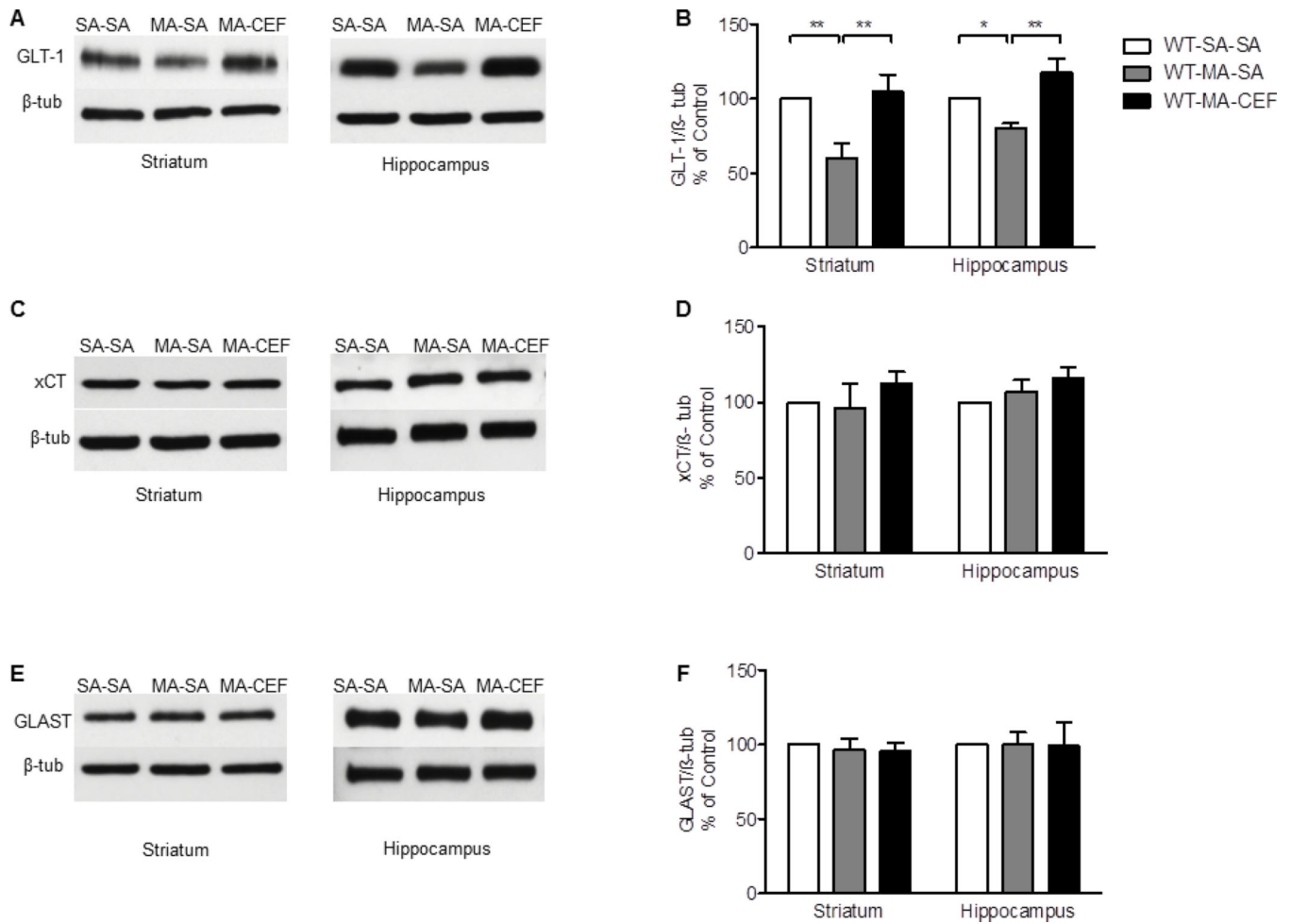


Figure 1. Effects of MA (10 mg/kg i.p. every 2 hrs ×4) and CEF (200 mg/kg) on GLT-1, xCT and GLAST expression in water groups in both striatum and hippocampus. (A, C, and E) Immunoblots for GLT-1, xCT and GLAST, respectively. (B) Quantitative analysis showed a significant increase in the ratio of GLT-1/β-tubulin in WT-MA-CEF groups compared to WT-MA-SA groups in striatum and hippocampus. Significant downregulation of GLT-1 expression was observed in WT-MA-SA groups compared to control WT-SA-SA groups in striatum and hippocampus. No significant difference in GLT-1 expression was found in WT-MA-CEF groups compared to WT-SA-SA group in both brain regions. (D) Quantitative analysis showed no significant difference in the ratio of xCT/β-tubulin between all tested groups in striatum and hippocampus. (F) Quantitative analysis showed no significant difference in the ratio of GLAST/β-tubulin between all tested groups in striatum and hippocampus. Values shown as means ± S.E.M (*p < 0.05 **p < 0.01) (n = 6 for each group).

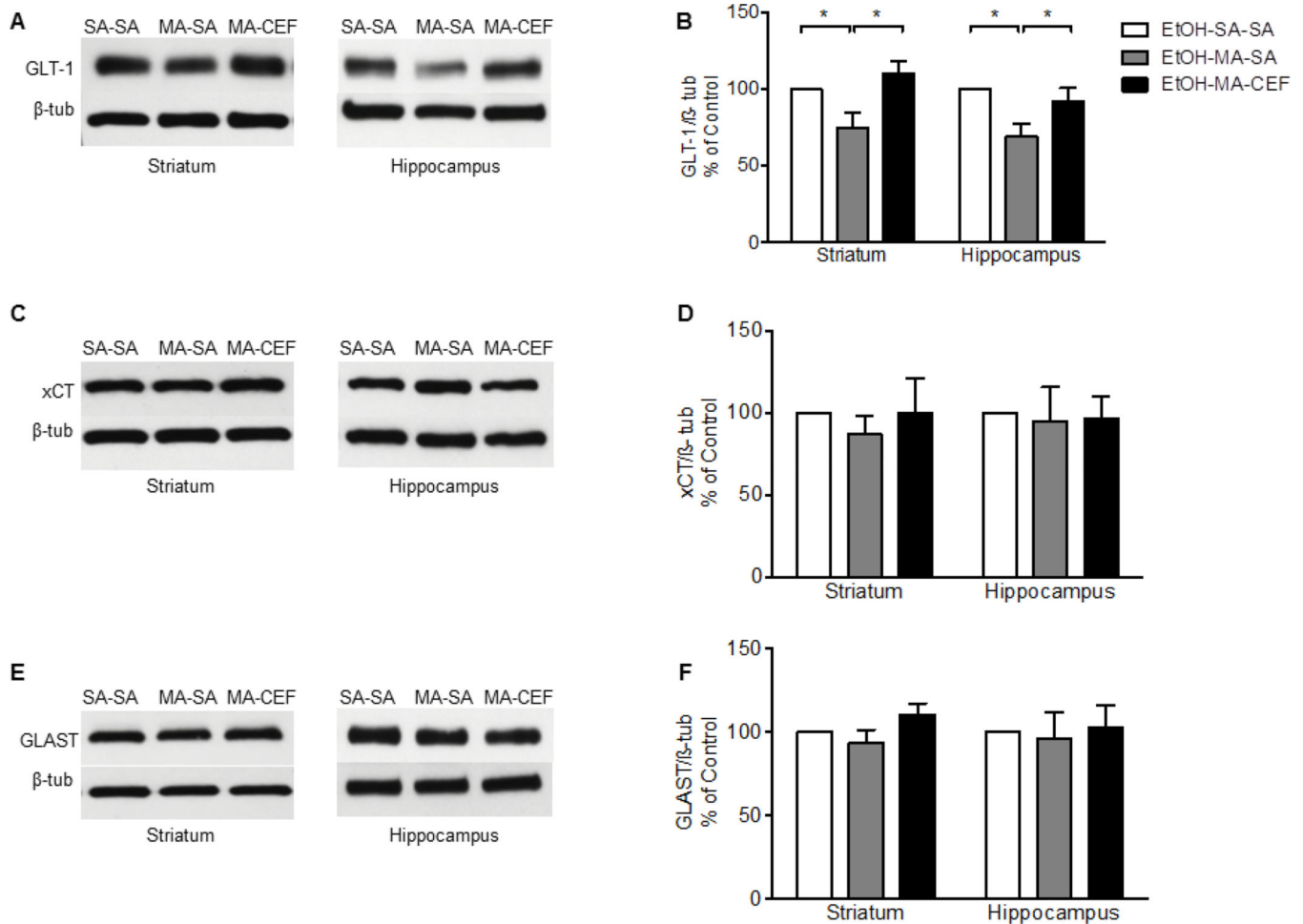


Figure 2.

Effects of MA (10 mg/kg i.p. every 2 hrs \times 4), EtOH (6 g/kg), and CEF (200 mg/kg) on GLT-1, xCT and GLAST in EtOH groups in both striatum and hippocampus. (A, C, and E) Immunoblots for GLT-1, xCT and GLAST, respectively. (B) Quantitative analysis showed a significant increase in the ratio of GLT-1/ β -tubulin in EtOH-MA-CEF groups compared to EtOH-MA-SA group in striatum and hippocampus. A significant downregulation of GLT-1 expression was found in EtOH-MA-SA groups compared to control EtOH-SA-SA groups in both brain regions. No significant difference in GLT-1 expression was found in EtOH-MA-CEF group compared to EtOH-SA control group. (D) Quantitative analysis did not reveal any significant difference in the ratio of xCT/ β -tubulin between all tested groups in both brain regions. (F) Quantitative analysis did not reveal any significant difference in the ratio of GLAST/ β -tubulin between all tested groups in both brain regions. Values shown as means \pm S.E.M (* $p < 0.05$) ($n=6-7$ for each group).

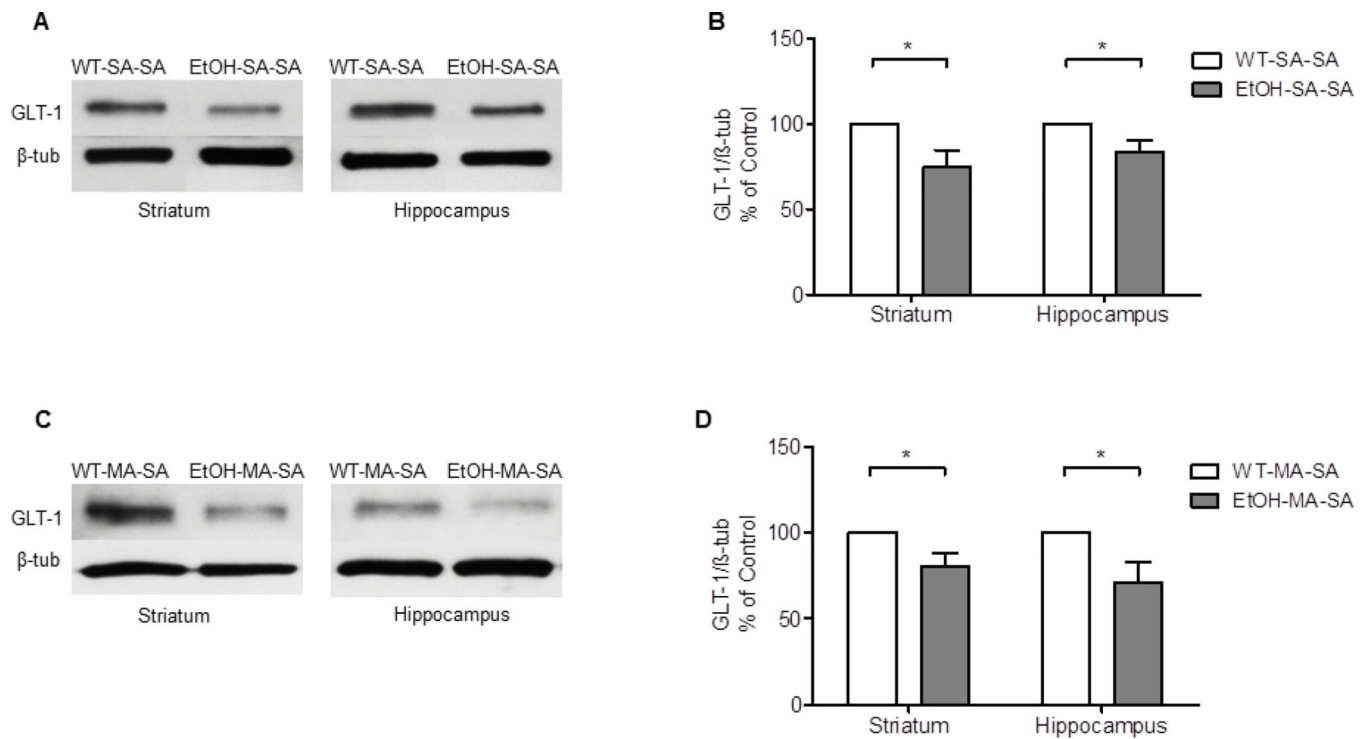


Figure 3.

Effects of MA (10 mg/kg i.p. every 2 hrs \times 4) and EtOH (6 g/kg) on GLT-1 expression in striatum and hippocampus. (A, C) Immunoblots of GLT-1 in WT-SA-SA and EtOH-SA-SA groups as well as in WT-MA-SA and EtOH-MA-SA groups, respectively. (B) Quantitative analysis revealed a significant downregulation of GLT-1 expression in EtOH-SA-SA groups compared to WT-SA-SA groups in striatum and hippocampus, respectively. (D) Quantitative analysis revealed a significant downregulation of GLT-1 expression in EtOH-MA-SA groups compared to WT-MA-SA groups in striatum and hippocampus, respectively. Values shown as means \pm S.E.M (* $p < 0.05$) ($n = 6$ for each group).

Table 1

Timeline of experimental design

Days	Days 1 – 7	Day 8	Day 9 and 10
Treatment	EtOH (6 g/kg) or water oral gavage	MA (10 mg/kg, i.p) or saline every 2 hrs, total 4 doses	CEF (200 mg/kg, i.p) or saline
		CEF (200 mg/kg, i.p) or saline	

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