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# Glutamate transporter GLT-1 as a therapeutic target for substance use disorders

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## Abstract

The development of new treatments for substance use disorders requires identification of targetable molecular mechanisms. Pathology in glutamatergic neurotransmission system in brain reward circuitry has been implicated in relapse to multiple classes of drugs. Glutamate transporter 1 (GLT-1) crucially regulates glutamatergic signaling by removing excess glutamate from the extrasynaptic space. The purpose of this review is to highlight the effects of addictive drug use on GLT-1 and glutamate uptake, and using GLT-1 as a target in addiction pharmacotherapy. Cocaine, opioids, ethanol, nicotine, amphetamines, and cannabinoids each affect GLT-1 expression and glutamate uptake, and restoring GLT-1 expression with N-acetylcysteine or ceftriaxone shows promise in correcting pre-clinical and clinical manifestations of drug addiction.

### Keywords

glutamate transport; glutamate transporter 1; excitatory amino acid transporter 2; accumbens; addiction; glutamate; N-acetylcysteine; ceftriaxone

## Introduction

Use of addictive substances represents one of the largest public health burdens to our society (1). Although some replacement medications have been developed treating substance use disorders (2), the long-term prognosis for abstinence in drug-dependent individuals remains poor (3). An understanding of the fundamental neurobiology underlying substance use disorders promises to unveil new treatments for addiction that target the underlying disease neuropathology.

One of the most heavily studied neurobiological mechanisms underlying substance use disorders is glutamate neurotransmission and the brain circuits utilizing glutamate, in particular glutamatergic synapses in the corticostriatal system (4). The neurotransmitter glutamate interacts with metabotropic receptors (5, 6), N-methyl-D-aspartate (NMDA) receptors (7), and alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors (8), each of which has been implicated in animal models of substance use

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disorders. Yet human clinical trials aimed at modulation of these various molecular partners of glutamate for treatment of substance use disorders have shown mixed results at best (9–11). One alternative strategy to targeting glutamate receptors is to pharmacologically modulate the proteins responsible for maintaining homeostatic glutamate concentrations at the synapse (12). Multiple classes of addictive drugs disrupt glutamate homeostasis, resulting in excessive release of glutamate, which is thought to trigger relapse to drug seeking (13, 14). Glutamate transporter 1 (GLT-1; also referred to as excitatory amino acid transporter, EAAT2) is responsible for the majority of glutamate uptake in brain areas implicated in addiction (15), and is thus critically involved in addiction related glutamate homeostasis.

The purpose of this review is to highlight literature demonstrating the effects of drugs of abuse to reduce GLT-1, and to describe the largely ameliorating effects of restoring GLT-1 on substance use disorders in pre-clinical and clinical research. We discuss the literature relevant to each general class of addictive drug and studies that have been conducted with compounds that restore the function of GLT-1. In particular, two compounds have been studied extensively in this regard, N-acetylcysteine (NAC) and ceftriaxone.

N-acetylcysteine was developed as mucolytic agent for treating cystic fibrosis, and is also used to treat acetaminophen poisoning (16), where it acts as a procystine drug to increase glutathione synthesis and help offset the potentially lethal redox imbalance created by acetaminophen (17). Ceftriaxone is a third generation cephalosporin antibiotic with a beta lactam core. However, it has been identified as a potent upregulator of GLT-1 with protective effects in mouse models of amyotrophic lateral sclerosis (ALS) (18), and is now in clinical trials for ALS (19). Several other beta lactam containing drugs were also shown to increase GLT-1 transcription via nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) (20).

## Cocaine

The regulation of GLT-1 in the nucleus accumbens, dorsal striatum, and prefrontal cortex has been examined in nine pre-clinical studies as outlined in table 1(21–29). In contrast to measurements in the core subcompartment of the nucleus accumbens (NAcore), these studies have demonstrated no changes in GLT-1 expression in prefrontal cortex (PFC) or dorsal striatum; although, GLT-1 activity is decreased in dorsal striatum after experimenter-administered cocaine. The majority of these studies have employed cocaine self-administration. Typically, cocaine delivery is paired with cues, for example a simultaneous presentation of a light and a tone, and these cues are capable of reinstating drug seeking (an animal model of relapse) after an extended period (1 week to 90 days) of drug unavailability. Drug seeking can also be reinstated by re-exposure to the context previously paired with drug or an experimenter-delivered priming injection of cocaine.

All but one of 6 studies employing cocaine self-administration showed that GLT-1 protein and/or sodium-dependent glutamate uptake was reduced in NAcore. One of these studies extensively characterized the effects of self-administered cocaine on GLT-1 by varying the length of cocaine access (2 hours vs. 6 hours) and withdrawal (2 days vs. 45 days) on GLT-1

protein levels in the NAcore and neighboring accumbens subcompartment NAshell. This study found that the NAcore exhibits a more profound cocaine-induced loss of GLT-1 protein and a stronger correlation between GLT-1 downregulation and length of cocaine access and withdrawal. GLT-1 downregulation becomes increasingly pronounced with longer periods of abstinence from cocaine. Interestingly, the more profound the loss of GLT-1, the more profound its restoration by ceftriaxone (24), suggesting a homeostatic restoration of GLT-1. The possibility of homeostatic normalization (i.e. restoring GLT-1 to physiological levels, and not further increasing past physiological levels) is supported by the fact that neither ceftriaxone nor NAC raise levels of GLT-1 in NAcore in control animals (21).

In total there are 12 studies (21–24, 26–33) characterizing the normalization of GLT-1 in cocaine treated animals by systemic or intra-accumbens NAC, ceftriaxone, or the methylxanthine, propentofylline (reviewed in table 2). All studies examining the reinstatement of cocaine seeking discovered that these therapies prevent reinstatement by context, cue, or cocaine injection, with the exception that cocaine reinstatement after two days of withdrawal is not prevented by ceftriaxone (24). Prevention of reinstatement depends on upregulating GLT-1 in NAcore and not NAshell (24). Selective inhibition of the restoration of GLT-1 in NAcore by local microinjection of GLT-1 antisense prevented NAC from inhibiting reinstated cocaine seeking (23). This knock-down study also revealed that another target for NAC, the cystine-glutamate exchanger, was not a factor in cue-induced reinstatement. Thus, the relapse prevention conferred by NAC was not impaired by antisense knockdown of cystine glutamate exchanger in NAcore. This study conclusively demonstrated that GLT-1, not cystine-glutamate exchange is the primary target for chronic NAC in ameliorating reinstated cocaine seeking. As an important translational step in evaluating NAC, daily injections of NAC for two weeks during extinction training produced an enduring reduction in reinstated cocaine seeking even 2–3 weeks after the last NAC injection (32, 33). NAC also blocked the locomotor sensitization to repeated experimenter administered injections of cocaine, and prevented the escalation of cocaine seeking in animals with extended access (6 hour daily sessions instead of 2 hour daily sessions) (30). In contrast to escalated cocaine self-administration, a number of studies found that neither NAC nor ceftriaxone decreased the maintenance level of cocaine intake during short access cocaine self-administration (28, 30, 31).

These pre-clinical data are largely consistent with the results of a recent double-blind placebo controlled trial of NAC for treating cocaine dependence (34). In this trial, NAC showed no difference from placebo in self-reported cocaine use or cocaine positive urines. However, an exploratory analysis of 13 patients that were abstinent at the start of the trial showed a significant prolongation of time to relapse. Thus, both pre-clinical and clinical evidence suggests that NAC may not be an effective medication for cocaine cessation, but rather is an effective treatment for relapse prevention in cocaine abstinent individuals. The capacity of NAC to protect against relapse without inhibiting active use is consistent with the aforementioned preclinical studies showing that NAC inhibits reinstated cocaine seeking, but not cocaine self-administration. The possibility that NAC may selectively block the motivation to seek drug (craving) rather than the rewarding properties of cocaine is further indicated by two small double-blind crossover trials in cocaine dependent individuals

showing NAC reduced the desire to use cocaine initiated by either cocaine-associated cues (35) or an IV infusion of cocaine (31). Additional clinical trials are reviewed in table 3 (36, 37)

One final preclinical study determined that the self-administration of either cocaine or sucrose produced equivalent reductions of GLT-1 in NAcore (29). This raises the possibility that self-administration of a reinforcer, whether drug related or not, can decrease the levels of GLT-1 in the NAcore. However, in this study there was no yoked-saline control group for comparison, so it is unknown if the levels of GLT-1 were reduced by sucrose or cocaine, or if both treatments were ineffective relative to a proper control group.

## Opioids

The one study employing heroin self-administration showed marked decreases in GLT-1 protein and glutamate uptake in NAcore (38). Research on the relationship between GLT-1 and opioids using experimenter-administered morphine are more mixed. Noncontingent morphine administration decreases GLT-1 protein levels in the dorsal horn of the spinal cord (39), cerebellum, cortex (40), thalamus and striatum (41). Withdrawal from morphine, in contrast, increases GLT-1 protein in striatum (41) and in hippocampus (40). Surprisingly, the increased hippocampal levels of GLT-1 are localized strictly to synaptic terminals of neurons despite the fact that GLT-1 (normally restricted to astrocytes) was virtually absent from these terminals in control animals.

Pharmacologically manipulating GLT-1 affects the motivational (i.e. reinstatement) and somatic (e.g. tolerance and withdrawal) effects of opioid use, with the former arising from on GLT-1 upregulation in nucleus accumbens (38, 42), and the latter depending on GLT-1 upregulation in locus coeruleus (43). NAC restores glutamate uptake after heroin self-administration, and thereby prevents glutamate overflow and reinstated heroin seeking (38). The protection against heroin reinstatement endures up to 40 days after discontinuing daily NAC (44). Conditioned place preference, a model in which an animal chooses to spend time in an environment previously paired with experimenter-administered drug, is prevented by MS-153 (45) (a GLT-1 “agonist”) and promoted by TBOA (46) (a glutamate uptake antagonist).

GLT-1 manipulation also decreases morphine tolerance and withdrawal (reviewed in table 2) (47–49). Interestingly, this effect partly depends on GLT-1 expression in the dorsal horn of the spinal cord (39). Amitriptyline prevents morphine tolerance (defined as loss of the anti-nociceptive property of acute morphine) and this is associated with a reversal of the spinal glutamate overflow induced by an acute morphine injection in chronically morphine treated animals. Both effects of amitriptyline depend on upregulating GLT-1, which in turn depends on NFkB signaling, indicating that amitryptiline and ceftriaxone (20) may share a common mechanism in upregulating GLT-1.

To date, no clinical studies have investigated GLT-1 manipulation as a treatment for opioid use disorders in humans.

## Ethanol

While noncontingent administration of ethanol reduces glutamate uptake (without affecting GLT-1 levels) in the nucleus accumbens (50), the evidence from ethanol self-administration animal models shows mixed effects on GLT-1 mediated glutamate uptake. One study, comparing alcohol preferring cAA rats with and without 20 months of ethanol consumption under a two-bottle choice paradigm, demonstrated decreased glutamate uptake in cortex (51). Another study, comparing Sprague-Dawley rats consuming dextrose or 6–7.5% ethanol in their diet for two weeks found no change in protein expression levels or uptake in hippocampus, cortex, or hypothalamus (52). A third study, in which P (alcohol preferring) rats had free access to two concentrations of ethanol or water for five weeks showed decreased GLT-1 protein in nucleus accumbens but not PFC (53). In contrast to these mixed results on GLT-1 protein, a number of studies (reviewed in table 2) demonstrate that following ethanol self-administration, treatment with ceftriaxone to increase GLT-1 and glutamate transport in the accumbens, PFC and/or amygdala reduces ethanol use or reinstated ethanol seeking (53–56). The decrease in ethanol self-administration produced by increasing GLT-1 protein is contrary to cocaine and opioids where restoration of GLT-1 did not affect the rates of self-administration.

No clinical trials have specifically investigated the effect of NAC or other compounds that increase GLT-1 synthesis for treatment of alcohol dependence. However, one group conducted a post-hoc analysis of patients with bipolar disorder whose depressive symptoms were successfully treated with NAC (57) but found no decrease in alcohol drinking (58). Other trials have investigated the effects of NAC on liver disease associated with alcohol consumption (59, 60), and post-hoc analyses of these data sets would be interesting in light of the pre-clinical effects of NAC in decreasing ethanol self-administration.

## Nicotine

Three studies have examined the effects of nicotine on GLT-1 protein levels. Nicotine self-administration decreases GLT-1 in NAcore (61, 62) but experimenter administered nicotine does not (62). In addition, self-administered nicotine reduces GLT-1 only in the accumbens, and does not affect GLT-1 in PFC, amygdala, or ventral tegmental area (VTA) (62). Interestingly, GLT-1 protein is upregulated in cerebellum both by pre-natal and post-natal exposure to nicotine (63). Ceftriaxone administration in pre-clinical models prevents tolerance (64) and withdrawal (65) symptoms from experimenter-administered nicotine. Ceftriaxone also prevents reinstatement, but not the acquisition or extinction of conditioned place preference (65). NAC decreases nicotine self-administration, and was also found to transiently decrease food self-administration in one study (66).

A number of clinical trials have examined the effects of NAC on human cigarette consumption. A small, short term double blind trial demonstrated no reduction in self-reported craving, and a mild reduction in self-reported withdrawal symptoms. However, this group found a strong reduction in self-reported rewarding effects of the first cigarette smoked after an abstinent period (67), which is consistent with pre-clinical data showing decreases in self-administration due to NAC. Another study revealed a reduction in the

number of cigarettes smoked after 4 weeks of NAC treatment, without affecting estimates of nicotine craving or withdrawal (62). Clinical trials failed to show decreased use of incidental nicotine in cannabis dependent adolescents (68), patients with bipolar disorder (58), and smokers being evaluated for the effects of NAC on mutagenic markers (69) (although this group was specifically instructed not to modulate their smoking habits during the trial). In contrast, NAC showed transient benefits for nicotine dependence over placebo in patients with comorbid pathological gambling (70). A promising new avenue of research is a combination therapy of varenicline and NAC, which has shown tolerability in an open-label trial (71).

## Amphetamine/methamphetamine

No self-administration studies have examined the link between amphetamine use disorder and GLT-1. However, three studies have examined effects of experimenter-administered amphetamine on GLT-1. One study found increased GLT-1 protein in dorsal striatum (72), while another showed only a non-significant trend in this direction (73). Interestingly, amphetamine appears to reverse GLT-1 transport of glutamate in cerebral cortex (74). Experimenter administered amphetamine increased glutamate levels and this effect was blocked by administration of dihydrokainate (DHK) or l-trans-2,4-pyrrolidine dicarboxylate (PDC) (GLT-1 inhibitors that normally increase extracellular glutamate). Amphetamine has established roles in reversing other neurotransmitter transporters, such as the dopamine transporter (DAT) and the vesicular monoamine transporter (VMAT) (75), although the authors propose a non-specific effect on glutamate uptake transporters due to hypoxia secondary to amphetamine-induced vasoconstriction. Ceftriaxone prevents locomotor sensitization following repeated amphetamine treatment (76), and blocks reinstatement but not the acquisition of conditioned place preference (77). However, GLT-1 activation by MS-153 (45), or GLT-1 viral overexpression in NAshell (42) are both capable of preventing the acquisition of conditioned place preference to amphetamine.

One clinical trial investigated the use of NAC in combination with naltrexone for methamphetamine use disorders, with negative results (78). It is difficult to interpret this finding in light of the complete lack of pre-clinical studies examining a link between self-administered amphetamine/methamphetamine and GLT-1.

## Cannabinoids

One study determined that WIN (a cannabinoid CB1 receptor agonist) or delta-9-THC administered to pregnant dams resulted in offspring with increased cerebral GLT-1 protein and glutamate uptake (79). An additional study determined that ceftriaxone diminishes some aspects of cannabinoid tolerance (80).

Despite the scarcity of pre-clinical research on the link between GLT-1 and cannabinoids, clinical trials of NAC for cannabis use disorders are extensive and highly promising. NAC is well tolerated and may appears to decrease craving for cannabis (81), although one open-label trial found negative results (82). Interestingly, despite a lack of self-reported decreases in days of use, an 8-week, 116 subject double blind RCT examining the effect of NAC in cannabis dependent adolescents demonstrated a large effect for decreasing cannabis positive

urines in NAC vs. placebo (83), leading to the initiation of a multi-site phase 3 trial for treatment of cannabis dependent adults (84). This is the clearest example of efficacy yet demonstrated for a GLT-1 modulating therapy in substance use disorders.

## Other disorders of compulsive behavior

In addition to the effects on substance abuse listed above, NAC has demonstrated efficacy for treatment of pathological gambling (70, 85), and demonstrated a large effect size for treatment of trichotillomania (86). Compulsivity has been suggested as a late phase development in substance use disorders (87). Substance use disorders, unlike trichotillomania and other disorders of the obsessive-compulsive spectrum (88), involve a distinct rewarding, pleasurable quality. NAC, which reduces craving but not rewarding properties (31) may be less effective at curbing substance use disorders than it is at curbing compulsive disorders that are more strictly negatively reinforcing. However, the role of GLT-1 in modulating the negative and positively reinforcing properties of compulsive, pathological behaviors remains an open question that will require further clinical and laboratory trials with humans.

## Unanswered Questions

### What are the relationships between drug use model and GLT-1 effects?

We have discussed various models of drug use which vary by drug class and administration paradigm (e.g. experimenter administered vs. self-administered). The relationship of drug use model to GLT-1 effects appear to depend partly on both of these factors. While most of the studies employing a self-administration paradigm demonstrate decreases in GLT-1 expression, those employing experimenter-administered drug show no changes in protein levels for nicotine, ethanol, or cocaine, and either no change or increased protein levels in the case of amphetamine/methamphetamine. However, experimenter administered drug can modulate GLT-1 function in the absence of protein level changes. This modulation takes the form of a decrease in the case of ethanol and cocaine, and a reversal of function in the case of amphetamine. In contrast, experimenter administered opiates consistently downregulate GLT-1 protein. This finding may be partly explained by a study demonstrating that astrocytes cultured in the presence of delta opioid receptor agonists or the absence of glutamate have reduced GLT-1 expression(89). Thus, opiates may directly downregulate GLT-1 protein via delta opioid signaling, while other drugs may depend on drug-induced changes in extracellular glutamate in NAcore.

### Is GLT-1 modulation a necessary mechanism for all effective substance use disorder treatments?

As outlined above, restoring GLT-1 function ameliorates drug use in animal models and clinical trials. Here we ask the question whether restoring GLT-1 may be an off-target action of other treatments for substance use disorders, and thus constitute a general mechanism whereby treatments can reduce drug dependence. Recently, levodopa was found to reverse long-access induced escalation of cocaine intake in a subset of rats (90) with decreased dopamine signaling, and demonstrated positive results in a subset of cocaine dependent patients hypothesized to have decreased dopamine synthesis (91). Interestingly, levodopa

treatment of rats with a lesion of the dopamine system produced increases of GLT-1 mRNA in the striatum, while no effects on GLT-1 resulted from levodopa in unlesioned rats (92). This suggests that GLT-1 upregulation may underlie levodopa's ability to treat cocaine use disorders selectively in humans and rats with diminished dopamine signaling. Also along these lines, subanesthetic ketamine administration signals via mammalian target of rapamycin (mTOR) activation (93) and mTOR activation is necessary for the epidermal growth factor (EGF) and insulin mediated upregulation of GLT-1 in cultured astrocytes (94). Subanesthetic ketamine treatment has recently shown preliminary positive effects in a small double-blind cross-over trial, in which cocaine dependent patients reported decreased craving (95). This suggests that sub-anesthetic ketamine may exert anti-craving effects through mTOR-mediated upregulation of GLT-1 in accumbens core astrocytes. Interestingly, a short course of experimenter administered ketamine injections led to decreased GLT-1 expression in hippocampus that persisted for up to six months (96). However, opposite changes in GLT-1 protein levels have been reported in hippocampus versus NAcore following opioid administration (38, 40). Therefore, a decrease in hippocampal GLT-1 following ketamine does not necessarily preclude an increase in NAcore GLT-1.

## What important differences are there among GLT-1 upregulating therapies?

Included in this review are pre-clinical studies using a host of drugs to upregulate GLT-1 as a therapy for substance use disorders. Most of these studies used NAC or ceftriaxone, but GPI-1046, amitryptiline, and propentofylline also upregulated GLT-1. Clavulanic acid, a beta lactam-containing drug (which likely upregulates GLT-1) also prevents several morphine dependent behaviors (97). Several additional drugs exist which are capable of upregulating GLT-1 (98, 99). The variety of drugs available for upregulating GLT-1 may be advantageous, as multiple treatment options with different side effect profiles can be offered to patients based on their particular desires or concerns. These drugs may also differ in their efficacy due to mechanism for regulating GLT-1 or bioavailability to the brain. For example, although both propentofylline and NAC are capable of preventing relapse to substance use disorders in pre-clinical models, the relapse prevention ability of NAC persists for a long time after discontinuation of NAC (44), whereas relapse prevention by propentofylline is gone by six days after discontinuating daily propentofylline (27). The necessity of GLT-1 upregulation for the therapeutic effects of NAC does not preclude the possibility of NAC having other complementary mechanisms, such as normalizing redox imbalances associated with drug abuse (100).

A second, related question regards the mechanism underlying GLT-1 upregulation by these various therapies. Ceftriaxone's mechanism of GLT-1 upregulation is dependent on NFkB-induced increased transcription (20), and there is evidence that amitryptiline shares this mechanism (39). In contrast, NAC *decreases* NFkB signaling in multiple cell lines (101, 102), and the mechanism responsible for NAC-induced GLT-1 upregulation remains undetermined. GLT-1 can be therapeutically upregulated at the level of translation (98) rather than transcription, although the relative effects of GLT-1 upregulation via these two pathways have not been determined in pre-clinical substance use disorder models.

### What side effects may be expected from upregulating GLT-1?

GLT-1 is an important modulator of the glutamatergic system. Glutamate, as a highly abundant neurotransmitter, plays a role in almost every brain function, raising concerns about off-target effects might occur from restoring or upregulating GLT-1. Surprisingly little research has investigated this obvious question. GLT-1 upregulation may slightly delay motor skill re-learning after stroke (103), and may or may not interfere with cognitive functions such as object recognition (104) and spatial navigation (105). GLT-1 upregulation effectively treats substance use disorders via restoration of normal GLT-1 levels in areas such as nucleus accumbens where GLT-1 has been decreased by exposure to drugs of abuse. But GLT-1 upregulation by these therapies may result in supra-normal expression levels in brain areas without substance-induced GLT-1 downregulation. In addition to non-specific effects on the glutamate system, all therapies that decrease drug seeking in animal models are potentially concerning for their effects on non-pathological motivated behaviors (e.g. food seeking) and other dopamine related functions (e.g. locomotion). Only one study reviewed here demonstrated that NAC transiently decreased food consumption (66). As the acute effects of NAC depend partly on indirect stimulation of pre-synaptic mGluR2/3 receptors (106), and stimulation of these receptors decreases motivation to consume food (107), it is likely that these transient effects were not due to GLT-1 upregulation. In addition, multiple studies reviewed here found no effects of GLT-1 manipulation on locomotion (27, 30).

## Conclusion

Despite several remaining questions concerning mechanisms of GLT-1 regulation and therapeutic efficacy, the majority of pre-clinical and clinical research reviewed here suggests that GLT-1 upregulation is a promising strategy for treating substance use disorders and other disorders characterized in part by compulsive behavior. As the translational gap between initial discovery (108) and phase 3 clinical trials (84) is closed, therapies targeting GLT-1 upregulation may offer new solutions to some of our most crippling public health problems.

ABBREVIATIONS

| | | |
|---|---|---|
| GLT1 | glutamate transporter 1 | aka EAAT2 (excitatory amino acid transporter 2) |
| NAC | N-acetylcysteine | |
| NAcore | nucleus accumbens core | |
| NAshell | nucleus accumbens shell | |
| PFC | prefrontal cortex | |
| VTA | ventral tegmental area | |
| CPP | conditioned place preference | |
| CPA | conditioned place aversion | |
| PDC | l-trans-pyrrolidine-2,4- dicarboxylic acid | high affinity glutamate transporter inhibitor |
| DHK | dihydrokainic acid | GLT-1 inhibitor |

| | | |
|---|---|---|
| GPI-1046 | | a neuroimmunophilin which upregulates GLT1 |
| ND | not done | |

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## TABLE 1

EFFECTS OF DRUGS OF ABUSE ON GLT-1 AND GLUTAMATE UPTAKE

| Citation | Drug of abuse | Drug administration route | Changes in GLT-1 expression | Changes in glutamate uptake |
|---|---|---|---|---|
| (74) | Amphetamine | Experimenter administration | ND (not done) | Reversal of function - increased glutamate in dialysate following amphetamine injection, can be blocked by administering GLT-1 blockers |
| (73) | Amphetamine | Experimenter administration | No change in protein levels in VTA, substantia nigra, nucleus accumbens, PFC, or dorsal striatum (although this showed a trend) | ND |
| (79) | Cannabinoid receptor CB1 agonist (WIN) | Experimenter administration to pregnant dams | Increased protein levels but not mRNA levels in PFC of offspring | Increased in PFC of offspring |
| (21) | Cocaine | Self administration | Decreased protein in nucleus accumbens | Decreased in nucleus accumbens |
| (23) | Cocaine | Self administration | Decreased protein in NAcore | ND (sodium independent only) |
| (24) | Cocaine | Self administration | Decreased protein in NAcore correlates strongly with length of access and length of withdrawal, decreased protein in NAshell correlates weakly with both measures | ND |
| (22) | Cocaine | Self administration | ND | Decreased in NAcore |
| (25) | Cocaine | Self administration | Decreases are greater in NAcore than NAshell in long-access animals | ND |
| (26) | Cocaine | Experimenter administration | No change in dorsal striatum | Decreased in dorsal striatum |
| (29) | Cocaine | Self administration | No change in accumbens relative to food trained animals | ND |
| (27) | Cocaine | Self administration | Decreased protein in NAcore but not dorsomedial PFC | ND |
| (28) | Cocaine | Self administration | Decreased protein in NAcore | ND |
| (53) | Ethanol | Self administration | Decreased in accumbens but not PFC | ND |
| (52) | Ethanol | Self administration | No change in hippocampus, hypothalamus, or cortex | No change in hippocampus, cortex, or hypothalamus |
| (51) | Ethanol | Self administration | ND | Decreased in cerebral cortex |
| (50) | Ethanol | Experimenter administration | No change in protein in NAcore | Decreased glutamate uptake in NAcore |
| (38) | Heroin | Self administration | Decreased protein in NAcore | Decreased in NAcore |
| (96) | Ketamine | Experimenter administration | Decreased protein in hippocampus persists for up to 6 months after 12 days of administration | ND |
| (72) | Methamphetamine | Experimenter administration | Increased protein in dorsal striatum, no changes in cortex, thalamus, hippocampus, amygadala | ND |
| (40) | Morphine | Experimenter administration (assessed during withdrawal) | Increased protein in hippocampus (specifically in neuron terminals); no change in mRNA; no changes in astrocytes | Decreased in cerebellum and cortex, increased in hippocampus |

| Citation | Drug of abuse | Drug administration route | Changes in GLT-1 expression | Changes in glutamate uptake |
|---|---|---|---|---|
| (39) | Morphine | Experimenter administration | Decreased protein in dorsal horn of spinal cord | ND |
| (41) | Morphine, naloxone | Experimenter administration | Decreased protein levels in thalamus and striatum following morphine, abundant increase in protein levels in striatum after naloxone administration to dependent animals | ND |
| (62) | Nicotine | Self administration and experimenter administration | Decreased protein in NAcore for self-administration group but not experimenter-administration group; no changes in VTA, PFC, or amygdala | ND |
| (61) | Nicotine | Self administration | Decreased protein in NAcore | ND |
| (63) | Nicotine | Experimenter administration to pregnant dams and w/or w/o additional administration to offspring | Increased protein in cerebellum, higher increases in offspring who receive additional nicotine | ND |

## TABLE 2

EFFECTS OF GLT-1 MANIPULATION ON PRE-CLINICAL MODELS OF SUBSTANCE USE DISORDERS

| Reference | Drug of abuse | GLT-1 pharmacotherapy | Effects on drug abuse behavior | Role of GLT-1 |
|---|---|---|---|---|
| (76) | Amphetamine | Ceftriaxone | prevents locomotor sensitization of amphetamine | ND |
| (80) | Cannabinoids | Ceftriaxone | prevents some aspects of tolerance | ND |
| (21) | Cocaine | Ceftriaxone, NAC | prevents cue- and cocaine-induced reinstatement; no locomotor effects | restores protein levels in NAcore, no effects on protein levels in PFC, no effects in naïve rats |
| (30) | Cocaine | NAC | prevents escalation in long access model, prevents behavioral sensitization, prevents cocaine-induced reinstatement | ND |
| (31) | Cocaine | NAC | decreases cocaine-induced reinstatement but not cocaine self-administration | ND |
| (32) | Cocaine | NAC | prevents cocaine, context, or cue-induced reinstatement after abstinence or extinction, up to two weeks after discontinuation of NAC | ND |
| (23) | Cocaine | NAC, RNA anti-sense morpholinos against GLT1 | NAC prevents cue-induced reinstatement | restores protein levels in NAcore; NAC in combination with GLT1 knockdown increases cue-induced reinstatement to levels beyond vehicle |
| (29) | Cocaine | Ceftriaxone | prevents cue-induced reinstatement but not reinstatement to food seeking | increased protein levels in accumbens and PFC |
| (24) | Cocaine | Ceftriaxone, DHK or TBOA (GLT-1 antagonists) | prevents reinstatement after extended withdrawal but not short term withdrawal | restores protein levels proportionally to their cocaine-induced decrease in NAcore and NAshell; behavioral effect depends on GLT-1 activity in NAcore but not NAshell |
| (22) | Cocaine | Ceftriaxone | no effects on drug seeking during extinction | restores uptake in accumbens |
| (33) | Cocaine | NAC | prevents cocaine and cue induced reinstatement of cocaine seeking, effect persists for two weeks after discontinuation of NAC | ND |

| Reference | Drug of abuse | GLT-1 pharmacotherapy | Effects on drug abuse behavior | Role of GLT-1 |
|---|---|---|---|---|
| (26) | Cocaine | Ceftriaxone | ND | No change in dorsal striatum |
| (27) | Cocaine | Propentofylline, morpholinos against GLT-1 in NAcore | Transiently prevents cue and cocaine primed reinstatement | Restores GLT-1 in NAcore; knockdown of GLT1 in NAcore abolishes reinstatement-prevention effects |
| (28) | Cocaine | Ceftriaxone | enduring prevention of cocaine reinstatement, no effects on self-admin; prevents locomotor sensitization | restores protein in accumbens |
| (55) | Ethanol | Ceftriaxone | decreased ethanol drinking | increased protein in PFC, accumbens, and amygdala |
| (54) | Ethanol | Ceftriaxone | prevents reinstatement to ethanol without affecting reinstatement for sucrose | increased protein levels in accumbens and PFC |
| (56) | Ethanol | Ceftriaxone | decreased ethanol drinking | increased protein levels in accumbens and PFC |
| (53) | Ethanol | GPI-1046 | decreased ethanol drinking | increase protein levels in PFC and restores protein levels in accumbens |
| (38) | Heroin | Ceftriaxone, morpholinos against GLT-1 in accumbens core | prevents cue-induced reinstatement | restores glutamate uptake; relapse prevention is abolished by GLT-1 knockdown in NAcore |
| (44) | Heroin | NAC | decreases drug seeking during extinction and prevents reinstatement, an effect persisting up to 40 days after disconinuation of NAC | ND |
| (77) | Methamphetamine | Ceftriaxone | prevents reinstatement of methamphetamine CPP but not acquisition | Increased mRNA in PFC but not accumbens |
| (45) | Methamphetamine, cocaine, morphine | MS-153 (GLT1 “agonist”) | prevents CPP for all classes of drugs without affecting their acute locomotor effects | ND |
| (42) | Methamphetamine, morphine | Viral over-expression | Blocks acquisition of methamphetamine and morphine CPP without affecting withdrawal symptoms | Increased protein levels in NAshell |
| (39) | Morphine | Amitryptyline | Prevents tolerance to anti-nociceptive effects of acute morphine | Restores/ overexpresses protein in dorsal horn of spinal cord, prevents glutamate overflow due to |

| Reference | Drug of abuse | GLT-1 pharmacotherapy | Effects on drug abuse behavior | Role of GLT-1 |
|---|---|---|---|---|
| | | | | acute morphine administration in morphine exposed animals |
| (47) | Morphine | Ceftriaxone | prevents naloxone induced withdrawal symptoms in morphine dependent rats | ND |
| (49) | Morphine | Ceftriaxone, DHK | prevents morphine tolerance to anti-nociceptive effects | morphine tolerance prevention requires GLT-1 (is blocked by DHK) |
| (48) | Morphine | Ceftriaxone, TBOA | prevents morphine induced hyperthermia | Effect depends on glutmate uptake (blocked by TBOA) |
| (43) | Morphine | Viral over-expression | prevents naloxone induced withdrawal symptoms in morphine dependent rats | Increases glutamate uptake and protein levels |
| (46) | Morphine, naloxone | TBOA | facilitates naloxone-induced CPA and morphine CPP | ND |
| (65) | Nicotine | Ceftriaxone | prevents reinstatement of CPP but not acquisition or extinction; decreases nicotine withdrawal | ND |
| (66) | Nicotine | NAC | decreases self-administration of nicotine, transiently decreases self-administration of food | ND |
| (64) | Nicotine | Ceftriaxone | decreases tolerance to nicotine's anti-nociceptive effects | ND |

**TABLE 3**

CLINICAL TRIALS OF GLT-1 MODULATION FOR SUBSTANCE USE/COMPULSIVITY

| Reference | Patient population | Pharmacotherapy | Study design | Study length | Number of subjects | Results of primary outcome | Results of secondary outcome |
|---|---|---|---|---|---|---|---|
| (58) | Bipolar disorder with incidental alcohol or tobacco use | NAC | Double blind RCT | 20 weeks plus 4 week washout | 75 | Decreased depressive symptoms (published in Berk, Copolov et al. 2008) | No decrease in incidental tobacco or alcohol use |
| (82) | Cannabis dependent adolescents | NAC | Double blind RCT | 8 weeks | 89 | No difference in self-reported craving | |
| (81) | Cannabis dependent adolescents | NAC | Open-label | 4 weeks | 24 | NAC is well-tolerated with few adverse effects that are mild or moderate | Decreased self-reported use and craving; no change in urine metabolites |
| (83) | Cannabis dependent adolescents | NAC | Double blind RCT | 8 weeks | 116 | Decreased cannabinoid-positive urines | |
| (68) | Cannabis dependent adolescents who also smoked cigarettes | NAC | Double blind RCT | 8 weeks | 68 | No differences in cigarettes smoked | Placebo group showed poorer cannabis outcomes for cigarette smokers than non-smokers |
| (34) | Cocaine dependence | NAC | Double blind RCT | 8 weeks | 111 | No differences in cocaine-negative urines or self-reported cocaine use | Increased delay to relapse for patients abstinent at beginning of trial in dose-dependent fashion (High dose NAC >Low dose NAC > Placebo) |
| (36) | Cocaine dependence | NAC | Double blind cross-over | 3 days inpatient for each condition, separated by one week | 13 | NAC is well-tolerated with few adverse effects that are mild or moderate | Decreased self-reported craving during study for NAC participants but was not different from placebo |
| (35) | Cocaine dependence | NAC | Double blind cross-over | 3 days inpatient for each condition, separated by one week | 15 | Decreased viewing time, self-reported interest and self-reported craving when viewing cocaine slides in NAC vs. placebo | No changes in physiological measures |
| (37) | Cocaine dependence | NAC | Open-label dose finding | 4 weeks | 23 | NAC is well-tolerated up to 3600 mg/day | Decreased self-reported use, craving, and |

| Reference | Patient population | Pharmacotherapy | Study design | Study length | Number of subjects | Results of primary outcome | Results of secondary outcome |
|---|---|---|---|---|---|---|---|
| | | | | | | | money spent on cocaine; money spent on cocaine |
| (31) | Cocaine dependence | NAC vs. baclofen | Double blind cross-over (no placebo) | 4 days per treatment separated by one week | 6 | NAC decreases self-reported craving induced by an IV infusion of cocaine, but does not decrease the “high” or “rush” associated with the cocaine | |
| (70) | Comorbid nicotine dependence and pathological gambling | NAC | Double blind RCT | 12 weeks w/ additional 3 month follow up | 28 | For NAC vs. placebo: Early, transient decrease in nicotine dependence; delayed decrease in gambling dependence severity | N/A |
| (78) | Methamphetamine dependence | NAC + naltrexone | Double blind RCT w/ dose escalation; NAC group also received naltrexone | 8 weeks | 31 | No difference in self-reported craving | No difference in self-reported use or methamphetamine-negative urines |
| (71) | Nicotine dependence | NAC + varenicline | Open label | 4 weeks | 19 | Tolerable and safe, with mainly mild side effects and high medication adherence | Reduction in cigarettes per day |
| (67) | Nicotine dependence (mild) | NAC | Double blind RCT | 3 days | 22 | NAC decreases self-reported rewarding effects of first cigarette smoked after a period of abstinence, and slightly reduces withdrawal symptoms but does not decrease craving | |
| (69) | Nicotine users w/o regard to dependence status | NAC | Double blind RCT | 6 monts | 41 | Decreased mutagenicity in bronchoalveolar lavage samples | No change in smoking but participants were specifically instructed not to decrease their levels of smoking |
| (62) | Nicotine dependence | NAC | Double blind RCT | 4 weeks | 29 | Decrease in number of cigarettes smoked, after exclusion of two heavy alcohol drinkers | No changes in carbon monoxide or cravings |
| (85) | Pathological gambling | NAC | Open label trial with dose escalation to identify responders followed by double blind RCT | 8 weeks (open label), 6 weeks (RCT) | 27 (open label), 13 (RCT) | Open label responders had 30% reduction in gambling symptoms, | 5/6 on NAC remained responders at end of RCT, 2/7 on placebo remained responders at end of RCT |
| (86) | Trichotillomania | NAC | Double blind RCT w/ dose escalation | 12 weeks | 50 | Decreased hair pulling symptoms for NAC (large effect size) | |