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Review

REVIEW

Glutamate Transport: A New Bench to Bedside Mechanism for Treating Drug Abuse

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Abstract

Drug addiction has often been described as a "hijacking" of the brain circuits involved in learning and memory. Glutamate is the principal excitatory neurotransmitter in the brain, and its contribution to synaptic plasticity and learning processes is well established in animal models. Likewise, over the past 20 years the addiction field has ascribed a critical role for glutamatergic transmission in the development of addiction. Chronic drug use produces enduring neuroadaptations in corticostriatal projections that are believed to contribute to a maladaptive deficit in inhibitory control over behavior. Much of this research focuses on the role played by ionotropic glutamate receptors directly involved in long-term potentiation and depression or metabotropic receptors indirectly modulating synaptic plasticity. Importantly, the balance between glutamate release and clearance tightly regulates the patterned activation of these glutamate receptors, emphasizing an important role for glutamate transporters in maintaining extracellular glutamate levels. Five excitatory amino acid transporters participate in active glutamate reuptake. Recent evidence suggests that these glutamate transporters can be modulated by chronic drug use at a variety of levels. In this review, we synopsize the evidence and mechanisms associated with drug-induced dysregulation of glutamate transport. We then summarize the preclinical and clinical data suggesting that glutamate transporters offer an effective target for the treatment of drug addiction. In particular, we focus on the role that altered glutamate transporters have in causing drug cues and contexts to develop an intrusive quality that guides maladaptive drug seeking behaviors.

Keywords: glutamate transporters, addiction, relapse, cellular redox, n-acetylcysteine

Introduction

Substance abuse disorders represent a major public health problem costing the United States over $740 billion annually in healthcare-related and indirect costs (NIDA, 2017). Addiction is marked by an enduring propensity to relapse. Repeated drug use produces persistent neuroadaptions in key brain circuits that promote this susceptibility to return to drug use following abstinence. Chronic drug use prominently disrupts glutamate homeostasis in nucleus accumbens (NAc). Differential effects on basal extracellular glutamate levels are observed in NAc depending on drug class (e.g., increased by alcohol, decreased by cocaine, unchanged by heroin), but reinstatement initiated by drug-associated cues triggers a large increase in extracellular glutamate across drugs (Scofield et al., 2016); thus, all drugs tested disrupt glutamate homeostasis, leading to increased synaptic glutamate spillover during a drug-seeking event. Likewise, in human drug addicts there is an increase in blood-oxygen-level dependent contrast imaging response to drug-related cues in prefrontal cortex (PFC) associated with later relapse (Goldstein and Volkow, 2011). Moreover, pharmacological interventions that restore glutamate homeostasis have demonstrated efficacy in reducing drug seeking (Reissner and Kalivas, 2010; Scofield et al., 2016).

Glutamate is a nonessential amino acid neurotransmitter responsible for most excitatory synaptic transmission in the central nervous system. Arguably, glutamate is the most important neurotransmitter for normal brain function as the cellular substrate of learning and memory (Citri and Malenka, 2007).

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Most evoked and spontaneous glutamate release is vesicular, occurring directly at the synapse and mediated by voltage-gated calcium channels (Ermolyuk et al., 2013). Synaptic glutamate release rapidly increases extracellular glutamate approaching millimolar concentrations within the synaptic cleft (Moussawi et al., 2011a). These elevated levels typically decline within milliseconds due to diffusion and reuptake. Glutamate transporters control signal transmission by removing glutamate from the synaptic cleft, thereby limiting its time in the extracellular space and access to the extrasynaptic compartment. Thus, under physiological conditions, glutamate transmission is temporally and spatially restricted, acting on glutamate receptors within or on the annulus of the synaptic cleft.

This review emphasizes the role of excitatory amino acid transporters (EAATs), which are ultimately responsible for controlling extracellular glutamate levels, in regulating the motivation to seek drugs. Systematic literature searches were performed using PubMed with search criteria consisting of "EAATX," "EAATX and localization/regulation/trafficking," and "EAATX + drug of abuse," (e.g, EAAT1 and alcohol). Preclinical drug studies include noncontingent and contingent drug administration protocols, although for EAAT2 we mainly limit our discussion to self-administration models due to the surfeit of reports on this transporter subtype (Reissner and Kalivas, 2010; Roberts-Wolfe and Kalivas, 2015). We discuss the normal distribution and expression of glutamate transporters and how drugs of abuse affect them inclusive of *in vitro* and *in vivo* studies. Finally, we address the potential of EAATs as therapeutic targets for addiction and other psychiatric diseases.

EAATS

Glutamate transporters control glutamate homeostasis in the central nervous system and their presence, or lack thereof, creates micro-domains of varying extracellular glutamate concentrations. There are 5 sodium-dependent glutamate transporters or EAATs comprising the solute carrier 1 family (SLC1): EAAT1/GLAST (*SLC1A3*), EAAT2/GLT-1 (*SLC1A2*), EAAT3/EAAC1 (*SLC1A1*), EAAT4 (*SLC1A6*), and EAAT5 (*SLC1A7*). The gene products are designated by the SLC1XX nomenclature. The human proteins use a homogeneous classification system of EAAT1-5, but 3 EAATs initially cloned from rat brain and rabbit intestine were given nonstandard names still used in animal model literature: Glutamate Aspartate Transporter 1 (GLAST) for EAAT1, Glutamate transporter 1 (GLT-1) for EAAT2, and Excitatory Amino Acid Carrier 1 (EAAC1) for EAAT3 (Jensen et al., 2015; Martinez-Lozada et al., 2016). The EAATs take up glutamate against its concentration gradient driven by cotransport with 3 Na^+ and 1 H^+ alongside the export of 1 K^+ (Figure 1) (Grewer et al., 2008). Disrupted glutamate transporter function is linked to a variety of excitotoxicity-related diseases, including Alzheimer's, Parkinson's, amyotrophic lateral sclerosis, epilepsy, multiple sclerosis, and stroke, and more recently psychiatric diseases like schizophrenia (Nakagawa and Kaneko, 2013).

EAAT1/GLAST

Localization/Distribution

Immunocytochemistry detects moderate to strong expression of GLAST in cerebellum, hippocampus, striatum, thalamus, brainstem, and spinal cord, and low to moderate expression in neocortex, amygdala, and hypothalamus (Lehre et al., 1995; Schmitt et al., 1997) (Figure 2). In human postmortem cortical tissue, EAAT1 protein is detected in astrocytic processes bordering glutamatergic synapses and unexpectedly in the soma, axons, and dendritic spines of neurons (Roberts et al., 2014). Neuronal EAAT1 expression was suggested to be due to a truncated EAAT1/GLAST splice variant that is expressed in unhealthy neurons during hypoxia (Sullivan et al., 2007; Roberts et al., 2014). In rat hippocampal cultures GLAST and GLT1 are expressed in neurons up to 7 days *in vitro*, but disappear with astrocyte maturation (Plachez et al., 2004). Adding astrocyte-conditioned medium to neuronal cultures suppresses neuronal GLAST expression, demonstrating the importance of glia-neuron communication.

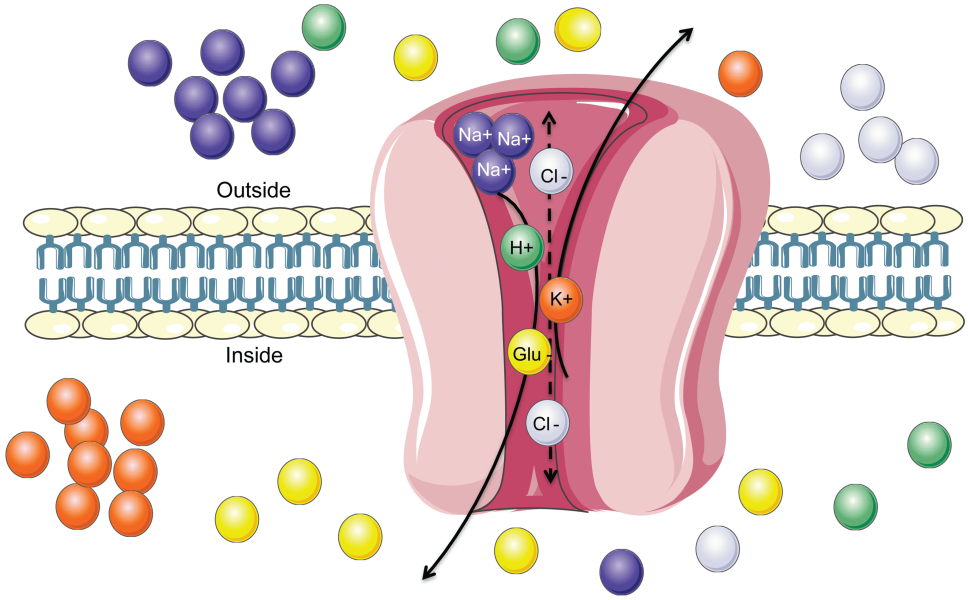

Figure 1. Diagram of a glutamate transporter. The transport of glutamate is coupled with cotransport of 3 sodium (Na+), 1 hydrogen (H+), and 1 potassium (K+) ion along their concentration gradient. The stoichiometry of coupling has been determined for excitatory amino acid transporter (EAAT)1–4; however, the order of ion binding is not completely resolved. EAATs 1–3 compared with EAAT4 transport glutamate with considerably different kinetics and voltage dependence despite a similar uptake mechanism. Additionally, EAATs perform an uncoupled flux of chloride (Cl-) cations. This latter function is most predominant in EAAT4 and EAAT5, and nearly absent in EAAT2.

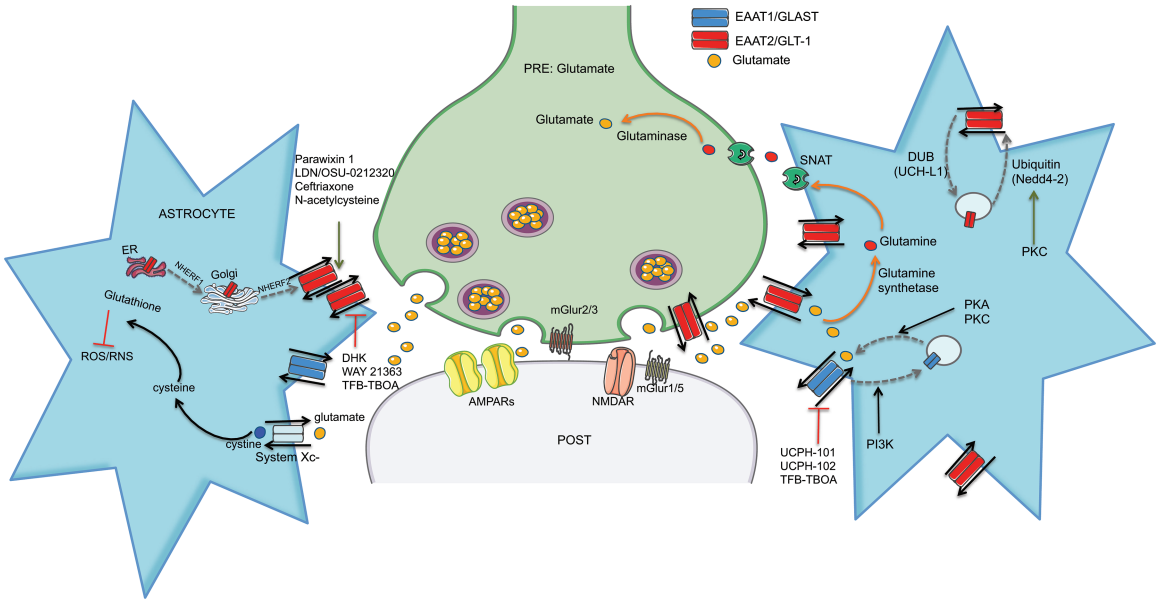

Figure 2. Glial glutamate transporters. Excitatory amino acid transporter EAAT1/GLAST (blue) is located exclusively on astrocytes and other glial cells. EAAT2/GLT-1 (red) is likewise predominately restricted to astrocytes. Orange arrows depict the glutamate-glutamine cycle associated with glutamate uptake: glutamine synthetase converts up taken glutamate to glutamine, glutamine is transported back to the glutamatergic neuron through sodium coupled amino acid transporters (SNATs), and glutamine is converted back to glutamate by glutaminase. The gray dashed arrows depict regulation of EAAT trafficking. Endosomal trafficking of EAAT1 depends on sodium-hydrogen exchanger regulatory factor 1 and 2 (NHERF1 and 2). Rapid cell surface expression of EAATs is also prominently modulated by kinase activity. Protein Kinase A (PKA) and Protein Kinase C (PKC) inhibitors decrease EAAT1 surface expression, while Phosphoinositide 3-Kinase (PI3K) inhibitors promote surface expression. EAAT2 trafficking is regulated both constitutively and inducibly by ubiquitination/deubiquitination cycles. PKC-dependent activation of Neural precursor cell-expressed developmentally downregulated gene 4-2 (Nedd4-2) ubiquitin ligase targets EAAT2 for proteasomal degradation. Specific pharmacological activators (green arrows) and inhibitors (red bar-line) of each transporter are shown. UCPH-101 and UCPH-102 are the first selective EAAT1 inhibitors. TFB-TBOA ((3S)-3-[[3-[[4-(Trifluoromethyl)benzoyl]amino]phenyl]methoxy]-L-aspartic acid) is more selective for EAAT1 and EAAT2 but has low efficacy at EAAT3 as well. Dihydrokainate (DHK) blocks EAAT2 function. WAY-213613 is a competitive inhibitor with higher selectivity and potency for EAAT2 over EAAT1 and EAAT3. Parawixin 1, purified from the venom of spider *Parawixia bistriata*, selectively increases EAAT2 activity. Pyradizine analogs, including LDN/OSU-0212320 increases EAAT2 function through transactivation. Ceftriaxone and other beta-lactam antibiotics increase EAAT2 expression and function. The antioxidant pro-drug N-acetylcysteine (NAC) increases EAAT2 function but may interact with the glutamate transport system at multiple levels.

Reciprocally, astrocytes grown without neurons show reduced GLAST and GLT1 levels (Gegelashvili et al., 1997).

Regulation of Expression/Trafficking

There is little sequence homology between rat and human GLAST/EAAT1 promoters, although they share many predicted transcription factor binding sites (e.g., nuclear factor kappa B [NF-κB], YY1, cocaine- and amphetamine-regulated transcript [CART], specificity protein 1 [SP1]) (Unger et al., 2012). Yin Yang 1 (YY1) is an important negative regulator of EAAT1 expression, while epidermal growth factor (EGF)-dependent transactivation of EAAT1 depends on NF-κB binding (Karki et al., 2015). Reporter assays directly compared the transcriptional regulation of human and rat EAAT1 with known activators of the rat gene (dibutyryl-cyclic adenosine monophosphate [dbCAMP], pituitary adenylate cyclase-activating peptide [PACAP], epidermal growth factor [EGF], and transforming growth factor alpha [TGFa]), revealing an important species-specific regulatory role for the 3'-untranslated region. The 3'-untranslated region represses constitutive transcription in humans but is an enhancer in rodents (Unger et al., 2012). Despite this interesting difference and sequence dissimilarity, the limited studies largely confirm conserved regulatory mechanisms controlling EAAT1 expression across species.

EAAT1 function is regulated at the protein level via post-translational modifications and trafficking. Glutamate itself can rapidly increase GLAST activity by increasing surface expression (Duan et al., 1999). Calcium/calmodulin (CAMKII)-dependent EAAT1 phosphorylation regulates constitutive transporter activity (Chawla et al., 2017), and consensus sequences for Protein Kinase A (PKA), Protein Kinase C (PKC), and Phosphoinositide 3-Kinase (PI3K) suggest that phosphorylation may also rapidly modulate EAAT1 activity. PKA or PKC inhibitors reduce cell surface EAAT1 expression, while a PI3K inhibitor increases surface protein in primary cortical cultures (Guillet et al., 2005).

EAAT1 and Drugs of Abuse

GLAST has been most well studied relating to alcohol addiction. We showed that repeated ethanol exposure (7 days, i.p. 1 g/kg) elevates basal extracellular glutamate levels and reduces glutamate uptake without changing GLT-1 or GLAST protein in NAc 24 hours after the last injection (Melendez et al., 2005) (Table 1). Alcoholism is considered a hyperglutamatergic disease, but postmortem brains of human alcoholics (Flatscher-Bader and Wilce, 2008) and chronic intermittent ethanol-exposed rats (Rimondini, 2002) show increased EAAT1/GLAST in frontal cortex. These increased GLAST levels may represent compensatory increases in protein to offset elevated extracellular glutamate. In contrast, a recent study using a free-choice ethanol-drinking model in male alcohol-preferring rats (P rats) reported no change in GLAST expression in PFC or NAc (Hakami et al., 2016). Chronic ethanol consumption in female P rats increases extracellular glutamate, decreases uptake, and reduces EAAT1 protein in ventral tegmental area (VTA) and NAc shell, with the protein deficit enduring for 2 weeks following ethanol deprivation (Ding et al., 2013). These results are consistent with another previously reported sex-specific ethanol withdrawal effect on glutamate

Table 1. Addictive Drug-Induced Glutamate Transporter Adaptations

	Brain Region	Effect on Expression and	Species	Treatment Protocol
	Ethanol			
EAAT1/ GLAST	NAc core	⇔ Melendez et al., 2005	Rats	1 g/kg i.p. x 7 days
	PFC	⇑ mRNA Flatscher-Bader and Wilce, 2008	humans, postmortem	Alcoholics
	PFC	⇑ mRNA Rimondini et al., 2002	Rats	Chronic intermittent ethanol vapor
	PFC/NAc	⇔ Hakami et al., 2016	P rats	Two-bottle choice
	VTA/NAc shell	⇓ Ding et al., 2013	*Female P rats	Two-bottle choice
	Cannabinoids			
	Hippocampus	⇓ Castaldo et al., 2010	Rat	Perinatal THC
	Cerebellum	⇓ Suarez et al., 2004	Rat	Perinatal THC
EAAT2/ GLT-1	**Cocaine**			
	NAc core	⇓ Knackstedt et al., 2010	Rat	Short access SA and extinction
	NAc core	⇓ Trantham-Davidson et al., 2012	Rat	Short access SA and extinction
	NAc core	⇓ Reissner et al., 2015	Rat	Short access SA and extinction
	NAc core/shell	⇓/⇔Fischer-Smith et al., 2012	Rat	Short access SA and acute withdrawal
	NAc core/shell	⇓⇓/⇓ Fischer-Smith et al., 2012	Rat	Short access SA and long withdrawal
	NAc core/shell	⇓⇓⇓/⇓⇓ Fischer-Smith et al., 2012	Rat	Long access SA and acute withdrawal
	NAc core/shell	⇓⇓⇓⇓/⇓⇓ Fischer-Smith et al., 2012	Rat	Long access SA and long withdrawal
	NAc core	mRNA ⇓ Kim et al., 2016b	Rat	Extended access SA and extended withdrawal
	Amphetamine/Methamphetamine			
	DStr	⇑ Shirai et al., 1996	Rat	Methamphetamine sensitization
	NAc core/shell	⇔ Szumlinski et al., 2016	Mice	Methamphetamine sensitization
	midbrain, NAc, Str, PFC	⇔ Sidiripoulou et al, 2001	Rat	Amphetamine sensitization
	Str and hippocampus	⇓ Althobaiti et al., 2016	Rat	Methamphetamine high 10 mg/kg x 4, every 2 h
	Ethanol			
	NAc core	⇔ Melendez et al., 2005;	Rat	1 g/kg i.p. x 7 days
	NAc core/shell	⇓ Sari et al., 2013	P rats	Chronic drinking
	PFC and Str	⇓ Abulseoud et al., 2014	P Rats/ Wistars	Chronic drinking followed by oral gavage
	VTA/NAc shell	⇔ Ding et al., 2013	*Female P rats	Two-bottle choice
	NAc core	⇔ Griffin et al., 2015	Mice	Chronic intermittent alcohol
	*white blood cells	⇑ Ozsoy et al., 2016	Human	Alcoholics at d 1 and d 28 withdrawal
	Nicotine			
	NAc core	⇓ Knackstedt et al., 2009	Rat	SA and extinction
	NAc core	⇓ Gipson et al., 2013	Rat	SA and extinction
	Opiates			
	NAc core	⇓ Shen et al., 2014	Rat	Heroin SA and extinction
	Cannabinoids			
	Hippocampus	⇓ Castaldo et al., 2010	Rat	Perinatal THC or WIN 55,212-2
EAAT3/ EAAC1	**Amphetamine/Methamphetamine**			
	Str	⇓ Kerdsan et al., 2012	Rat	Meth: acute (8 mg/kg, ip 1x) or chronic (4 mg/kg, ip 14 days)
	PFC	⇓ Kerdsan et al., 2012	Rat	Meth chronic (4 mg/kg, ip 14 days)
	Hippocampus	⇓ Kerdsan et al., 2012	Rat	Meth chronic (4 mg/kg, ip 14 days)
	PFC	⇓/⇔ Lominac et al., 2016	Mice	Chronic meth (10 mg/kg x 10 days) + 21-d withdrawal
	Midbrain, NAc, DStr, PFC	no change Sidiripoulou et al., 2001	Rat	Amphetamine sensitization
	NAc	⇓ Szumlinski et al., 2016	High meth drinking mice	naïve
	NAc	⇓ Szumlinski et al., 2016	Mice	Chronic meth (10 mg/kg x 10 days) + 21 day withdrawal
	NAc	⇔ Szumlinski et al., 2016	Mice	Meth CPP
	Opiates			
	PFC, NAc, VTA/HP	⇑ surface/⇔Wan et al., 2016	Mice	Morphine CPP
	PFC	⇓ Wu et al., 2013	Mice	Morphine-induced reinstatement of CPP
	Cannabinoids			
	Cerebellum	⇓ Suarez et al., 2004	Rat	Perinatal THC

Represents protein level changes except where indicated. Downward arrows indicate a decrease, upward arrows indicate an increase, and sideways arrows indicate no change.

transporter levels (Alele and Devaud, 2005). Other variability likely reflects differences in ethanol administration protocols, drug exposure periods, withdrawal periods, and brain region-dependent effects. Moreover, these data indicate that ethanol can alter GLAST function, likely through increased membrane trafficking, without affecting transcription or translation. In line with this interpretation, ethanol exposure dose-dependently modifies GLAST distribution, promoting a shift from the cytoplasm to the surface in rat astrocyte cultures (Sery et al., 2015). A GLAST knockout mouse model was used to directly examine the GLAST contribution to alcohol addiction and reinforcement with the logical hypothesis that GLAST deletion would promote alcohol intake and reward. Surprisingly, GLAST-/- show lower alcohol consumption and no ethanol preference in the conditioned place preference (CPP) paradigm (Karlsson et al., 2012); however, interpretation of these results is unclear, because endocannabinoid signaling is altered in these knockouts. Thus, more targeted genetic or pharmacologic manipulations are required to fully evaluate this question.

Data pertaining to other drugs is lacking. Nicotine increases GLAST expression through a α7 nicotinic acetylcholine receptor and fibroblast growth factor-2-dependent pathway in rat cortical cultures (Morioka et al., 2014; Morioka et al., 2015). Perinatal delta-9-tetrahydrocannabinol (THC) exposure decreases GLT-1 and GLAST protein and reduces glutamate uptake in hippocampus (Castaldo et al., 2010) and produces an enduring deficit in GLAST levels in cerebellum (Suarez et al., 2004). Repeated intermittent 3,4-methylenedioxymethamphetamine (MDMA) exposure increases GLAST expression in cortex (Kindlundh-Hogberg et al., 2008). Finally, other studies suggest that GLAST contributes to morphine tolerance and antinociceptive effects in spinal cord (Mao et al., 2002; Tai et al., 2007; Eidson et al., 2017).

EAAT2/GLT-1

Localization/Distribution

GLT-1 is one of the most abundant proteins ubiquitously expressed throughout the brain (Takahashi et al., 2015) and is responsible for ~90% of glutamate uptake. EAAT2 is the human homologue of GLT-1, and in postmortem cortical tissue EAAT2 is detected robustly in astrocytic processes and much less frequently in neuronal profiles, including within the postsynaptic density at asymmetric synapses (Roberts et al., 2014). Most GLT-1 immunoreactivity colocalizes with presynaptic marker synaptophysin (within 0.2 μm^2) and is thus considered the synaptic pool, but ~40% of GLT-1 is located distally from the synapse, constituting an extrasynaptic pool (Figure 2) (Minelli et al., 2001). There are at least 3 different GLT-1 isoforms that vary at their C terminus: GLT1a, GLT1b, and GLT1c. These isoforms differ in relative abundance (for example: 90%, 6%, and 1%, respectively, in hippocampus) (Holmseth et al., 2009). There are no data to suggest that these variants alter transport function per se, but they likely contribute to differential protein expression, localization, and/or trafficking (Holmseth et al., 2009). For example, GLT1a but not GLT1b is detected in neurons, and GLT-1b contains a PDZ domain absent in GLT1a (Holmseth et al., 2009; Sogaard et al., 2013).

Regulation of Expression/Trafficking

The rat and human promoters of EAAT2/GLT-1 have many conserved sequences, including binding sites for transcription factors NF-κB, cAMP response element binding protein (CREB), and SP-1 (Sitcheran et al., 2005; Grewer et al., 2014). These sequences and their interacting transcription factors control both constitutive and/or inducible GLT-1 expression. NF-κB, for example, is involved in both basal and inducible regulation of GLT-1 expression. NF-κB mediates GLT-1 induction via cAMP, TGFa, and EGF, and is also required for TNFa-dependent GLT-1 repression in human H4 glioma cells (Sitcheran et al., 2005). Comparisons of the human and rat promoters in reporter assays reveal remarkable similarity but not complete overlap between gene regulation of GLT-1 and EAAT2. For instance, TNFa-dependent inhibition occurs through distinct mechanisms, but EGF, TGFa, and PACAP-dependent transactivation is similar between species (Allritz et al., 2010).

The 3' untranslated region of GLT-1 has predicted binding sites for microRNAs miR-128, miR182, and miR200b, representing another potential means of transcriptional regulation, but these interactions are untested (Lauriat and McInnes, 2007). Surprisingly, miR-124a, the most abundant miR in vertebrate brain, increases GLT-1 protein without altering gene expression denoting translational rather than transcriptional regulation (Morel et al., 2013). This effect is mediated via novel exosomal transfer of neuronal miR-124a into astrocytes. Other translational activators of EAAT2 include corticosterone and retinol through an interaction with the 5' untranslated region (Tian et al., 2007). Conversely, excessive glutamate or oxidative stress significantly inhibits EAAT2 translation (Tian et al., 2007).

EAAT2 expression is additionally regulated at the epigenetic level. There is an CpG island before GLT-1 exon 1 that displays both enrichment of repressive trimethyl-histone H3 alongside decreased acetyl-histone H4 in cerebellum compared with cortex (Perisic et al., 2012). Interestingly, EAAT2 expression in cortex but not the hypermethylated form in cerebellum is inducible by dexamethasone and HDAC inhibitors (Perisic et al., 2010, 2012). This distinction in region-specific methylation might contribute to the parallel difference in protein distribution. Likewise, lower methylation of the EAAT2 promoter is observed in astrocyte-neuron co-cultures compared with astrocytes cultured alone in accordance with higher EAAT2 expression in co-cultures (Yang et al., 2010).

Posttranslational modifications of GLT-1 regulate transporter expression, function, and trafficking. A fraction of EAAT2 is constitutively sumoylated in vivo with this form preferentially retained intracellularly (Foran et al., 2014). GLT-1 palmitoylation at cysteine 38 is required for basal glutamate uptake but does not impact expression or trafficking (Huang et al., 2010). Finally, PKC-dependent phosphorylation of Nedd4-2 ubiquitin ligase promotes the inducible internalization and degradation of GLT-1 (García-Tardón et al., 2012), while constitutive GLT-1 recycling utilizes clathrin-dependent internalization but also involves a ubiquitination cycle (Martinez-Villarreal et al., 2012).

EAAT2 and Drugs of Abuse

Cocaine

In a standard short-access cocaine self-administration and extinction model, GLT-1 is downregulated in NAc core (Knackstedt et al., 2010). GLT-1 protein in NAc core is sensitive to both increasing cocaine intake (extended 6–8 h/d self-administration compared with limited access) and increasing abstinent withdrawal period (1 vs 45 days) (Fischer-Smith et al., 2012). In NAc shell, GLT-1 expression is reduced by limited access with long withdrawal and by extended access with short or long withdrawal. Overall, NAc shell does not display the same progressive decrease in protein levels observed in core and the magnitude of change is lower (Fischer-Smith et al., 2012). Indeed, GLT-1 expression in NAc core but not shell displays a significant negative correlation with cue-induced cocaine seeking. Interestingly, while GLT-1 protein levels

are altered even following short-access and limited withdrawal in NAc core, GLT-1 mRNA is only decreased following prolonged withdrawal from long access self-administration (R. Kim et al., 2016b). This decrease in GLT-1 expression is associated with hypermethylation, suggesting differential engagement of transcriptional and translational mechanisms depending on addiction state.

Amphetamine/Methamphetamine

One study shows methamphetamine sensitization increasing GLT-1 in striatum (Shirai et al., 1996), but a recent study finds no change in GLT-1 in NAc core or shell following another sensitization protocol (Szumlinski et al., 2016). Likewise, no change in GLT-1 in midbrain, NAc, dorsal striatum, or PFC is reported following amphetamine sensitization (Sidiropoulou et al., 2001). Repeated high doses of methamphetamine (10 mg/kg x 4, every 2 hours) decrease GLT-1 in striatum and hippocampus (Althobaiti et al., 2016). The disparity between these results likely reflects differences in dosing and treatment regimens. Indeed, we know that treatment protocol is important for methamphetamine-dependent glutamate dynamics, because methamphetamine self-administration with extinction training reduces basal glutamate levels in dmPFC and NAc (Parsegian and See, 2014), but extracellular glutamate is increased in NAc following withdrawal without extinction (Lominac et al., 2012). Unfortunately, the effect of amphetamine/methamphetamine self-administration on GLT-1 protein is unknown.

Ethanol

The effects of ethanol administration on GLT-1 levels and function are not fully consistent. Sari and colleagues (Sari et al., 2013) report reduced GLT-1 in NAc core and shell in male P rats following chronic drinking. In an ethanol withdrawal study that incorporated 2-bottle choice drinking followed by forced oral gavage in male P rats and outbred Wistar rats, a similar decrease in GLT-1 was observed in PFC and striatum (Abulseoud et al., 2014). Interestingly, in female P rats, EAAT1 rather than EAAT2 levels (discussed above) are reduced in the NAc shell and VTA (Ding et al., 2013). No differences in EAAT2 expression are observed in NAc following noncontingent sensitizing ethanol injections in rats (Melendez et al., 2005) or chronic intermittent ethanol vapor in mice (Griffin et al., 2015). Overall, the reduction in GLT-1 protein following ethanol consumption/exposure is most reliably reproduced in studies utilizing alcohol preferring P rats compared with outbred rats or mice. Therefore, it would be interesting to determine whether basal differences in GLT-1 expression or function exist between P rats and other outbred strains or if they emerge due to significant differences in ethanol consumption. Furthermore, it is also probable that exposure type (daily vs intermittent; choice vs noncontingent) and withdrawal period influence GLT-1 expression and function, and generally more variable results are obtained in noncontingent ethanol exposure models.

There is also clinical evidence for alcohol-dependent EAAT2 regulation. In inpatient alcoholics, EAAT2 and EAAT3 mRNA is increased in white blood cells during early (day 1) and late (day 28) withdrawal compared with healthy controls (Ozsoy et al., 2016). Proton magnetic resonance spectroscopy shows elevated glutamate levels in NAc in alcohol-dependent patients during early withdrawal compared with controls, and craving positively correlates with combined glutamate and glutamine levels in NAc and anterior cingulate cortex (Bauer et al., 2013).

Nicotine, Opiates, and Cannabinoids

Nicotine self-administration in rats decreases GLT-1 protein expression in NAc (Knackstedt et al., 2009; Gipson et al., 2013). Heroin self-administration produces functional deficits in glutamate uptake along with decreased GLT-1 surface expression in NAc (Shen et al., 2014). In a noncontingent mouse model of opiate reward, GLT-1 overexpression in NAc shell reduces morphine CPP (Fujio et al., 2005). A single study links perinatal exposure to THC or WIN 55,212-2 to decreases in GLT-1 protein and glutamate uptake in hippocampus (Castaldo et al., 2010).

EAAT3

Localization/Distribution

In the brain, EAAT3/EAAC1 expression is primarily neuronal (Velaz-Faircloth et al., 1996). EAAT3 is relatively ubiquitous, but higher densities are observed in cortex, hippocampus, basal ganglia, and cerebellum, and it is also found widely in peripheral tissues (Velaz-Faircloth et al., 1996). In hippocampus and cerebellum, synapses are less likely to be bounded by astrocytic processes, perhaps magnifying the importance of neuronal EAATs. EAAT3/EAAC1 is present in all neuronal subcompartments both pre- and postsynaptically (Figure 3) (Nieoullon et al., 2006). The majority of EAAT3/EAAC1 protein resides intracellularly (Nieoullon et al., 2006). As such, it is theorized that EAAT3/EAAC1 is important for diverse functions not necessarily related to glutamate uptake and recycling. For example, glutamate taken up by EAAT3 in gamma amino-butyric acid (GABA) neurons provides a precursor for further GABA production (Bjorn-Yoshimoto and Underhill, 2016). EAAT3 also displays high affinity for L-cysteine and thus serves as the primary source of intracellular precursor for production of the important antioxidant glutathione (GSH) (Watts et al., 2014).

Regulation of Expression

The EAAT3 promoter contains a unique consensus binding site, not shared by EAAT1 or EAAT2, for transcription factor regulatory factor x-1, which positively regulates EAAT3 expression (Ma et al., 2006). Nuclear factor erythroid 2-related factor 2 interacts with a conserved ARE-related sequence in the EAAT3 promoter to induce EAAT3 expression (Escartin et al., 2011). This pathway is activated by oxidative stress to increase EAAT3 expression and augment GSH levels (Escartin et al., 2011). EAAT3 gene expression is also increased by nuclear factor retinoic acid receptor beta (Bianchi et al., 2009).

Three alternative isoforms are reported for human EAAT3 protein. Two isoforms result from skipping of exon 2 or 11 and have significant implications for predicted protein structure (Bjorn-Yoshimoto and Underhill, 2016). The third isoform, identified in humans and mice, is a result of a secondary internal promoter producing an N-terminal truncated protein (Porton et al., 2013). In humans, these alternative isoforms display differential expression in obsessive-compulsive disorder (OCD) patients, and certain variants are associated with disease transmission (Arnold et al., 2006; Porton et al., 2013). This is noteworthy given that OCD, like addiction, is a psychiatric disease related to obsessive thoughts and impulse control deficits.

Given the primarily intracellular EAAT3 localization, rapid regulation of trafficking by posttranslational modifications may be more important than transcriptional mechanisms for controlling function. EAAT3/EAAC1 is constitutively internalized via clathrin and dynamin-dependent mechanisms and recycled back to the plasma membrane via Rab11-dependent trafficking (González et al., 2007). Kinases like PKCα and PI3K participate in activity-dependent EAAT3 trafficking (Bjorn-Yoshimoto and Underhill, 2016).

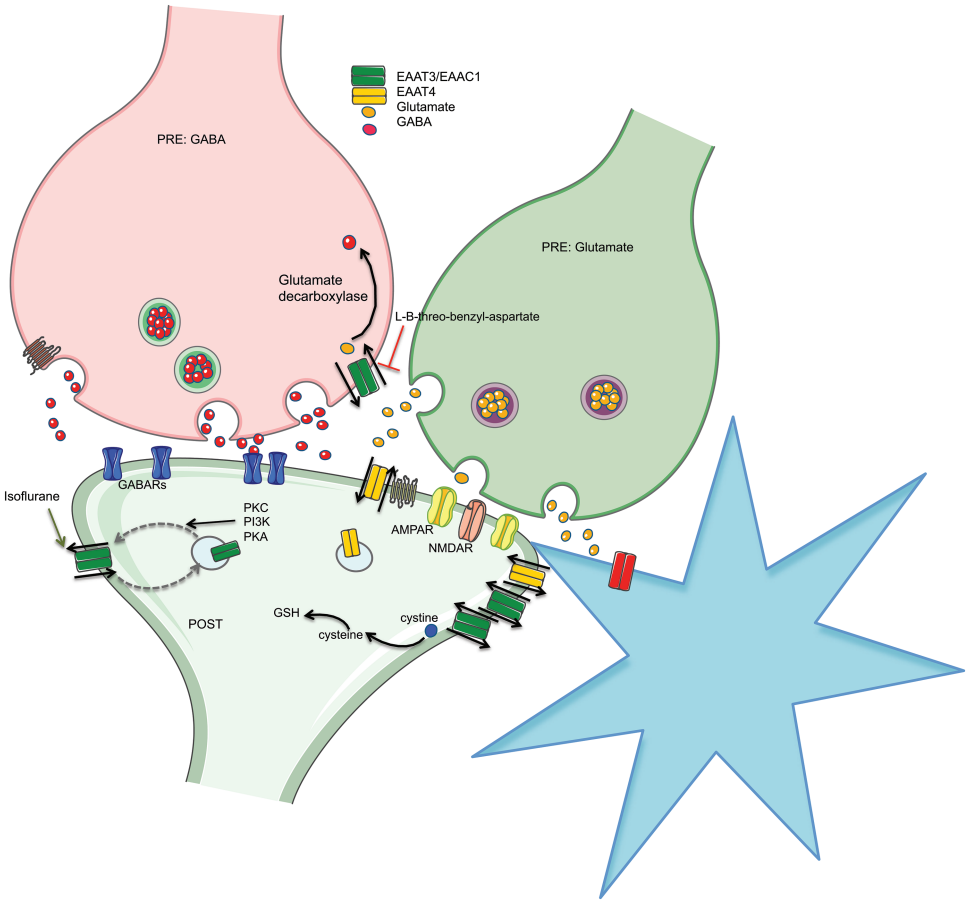

Figure 3. Neuronal glutamate transporters. Neuronal excitatory amino acid transporter EAAT3/EAAC1 (green) is located both and pre- and postsynaptically throughout the brain. EAAT4 (yellow) is likewise a neuronal EAAT found mainly in the cerebellum but with some forebrain and midbrain expression. EAAT3 expressed presynaptically on GABAergic neurons participates in supplying glutamate precursor for GABA synthesis (orange arrow). EAAT3 also more effectively transports L-cysteine compared with the other EAATs, thus directly supplying precursor for glutathione (GSH) synthesis. Rapid regulation of cell surface expression of EAAT3 is important, because the majority of the protein normally resides intracellularly. Protein Kinase A (PKA), Protein Kinase C (PKC), and Phosphoinositide 3-Kinase (PI3K) inhibitors decrease EAAT3 surface expression, demonstrating positive regulation by these kinases. Likewise, isoflurane (and other anesthetics) promotes insertion of EAAT3 in the plasma membrane also through purported kinase-dependent mechanisms (green arrow). The elimination of the ether oxygen in the nonselective EAAT inhibitor threo-beta-hydroxyaspartate (TBOA) to yield L-B-threo-benzyl-aspartate (L-B-BA) produces an inhibitor with 10-fold preference for EAAT3 over EAAT1 and EAAT2 (red bar-line).

EAAT3 and Drugs of Abuse

There is accumulating evidence for some EAAT3 involvement in amphetamine/methamphetamine addiction, but EAAT3 levels have never been measured following self-administration. In striatum, acute (8 mg/kg i.p., 1 day) and repeated (4 mg/kg i.p., 14 days) methamphetamine injections decrease EAAT3 levels, but in frontal cortex this effect is only significant with repeated injections (Kerdsan et al., 2012). Conversely, repeated methamphetamine increases EAAT3 levels in hippocampus, perhaps as compensatory mechanism to counter excitotoxicity in this brain region (Kerdsan et al., 2012). Chronic methamphetamine treatment (10 days, 2 mg/kg i.p.) followed by 21 days of withdrawal reduces EAAT3 levels in NAc (Szumlinski et al., 2016) but not PFC (Lominac et al., 2016). Repeated amphetamine injections fail to alter EAAT3 expression across a number of brain regions (Sidiropoulou et al., 2001). In mice bred for high vs low methamphetamine preference, high-drinking mice have lower EAAT3 levels in NAc core along with elevated extracellular glutamate basally and upon methamphetamine challenge (Szumlinski et al., 2016). The EAAT3 phenotype fails to correlate with methamphetamine place preference in outbred C57BL/6J mice, but the hyperglutamatergic profile observed in NAc of drug-naive methamphetamine high-drinking mice is phenocopied by outbred mice withdrawn from a sensitizing methamphetamine regimen, suggesting that this molecular profile represents both a cause and consequence of methamphetamine addiction (Szumlinski et al., 2016).

There is evidence that EAAT3 trafficking and not just expression can be altered by amphetamines, perhaps contributing to inconsistent results observed with total protein. In midbrain dopamine cells, acute amphetamine treatment decreases EAAT3-specific glutamate uptake via Rho-A-dependent EAAT3 endocytosis, leading to potentiated glutamatergic synaptic transmission (Underhill et al., 2014). These researchers went on to demonstrate that acute methamphetamine similarly augments glutamatergic signaling (Li et al., 2016). These studies implicate immediate changes in EAAT3 function for acute amphetamine effects that could have implications for long-term adaptations downstream contributing to the transition to addiction.

Morphine place conditioning increases membrane EAAT3 levels in mPFC, NAc, and VTA 24 hours after the final injection in mice, although expression is unchanged in hippocampus (Wan et al., 2016). Morphine-induced CPP reinstatement in rats decreases EAAT3 in PFC measured 30 minutes after the

priming injection (Wu et al., 2013). Interestingly, constitutive EAAT3 knockout does not affect acquisition, expression, or reinstatement of morphine preference, but instead morphine CPP extinguishes faster in EAAT3-/- mice, perhaps suggesting that increased EAAT3 following conditioning is a compensatory mechanism to limit reward-related behavior. Cell culture studies validate a dynamic regulation of EAAT3 expression by morphine with dose- and time-dependent effects upon initial exposure, recovery with withdrawal, and increased sensitivity upon reexposure (Wu et al., 2013), which may also go towards explaining opposing changes reported in rat vs mouse CPP studies above.

There is limited data linking EAAT3 to other drugs. Acute cocaine (10 mg/kg i.p.) administered at P15 transiently reduces D-aspartate uptake for 60 minutes associated with increased serine phosphorylation of EAAT1, EAAT2, and EAAT3 in PFC (Sathler et al., 2016). Perinatal THC exposure produces an enduring deficit in EAAT3 levels in cerebellar Purkinje neurons (Suarez et al., 2004).

Translational Studies on EAAT Pharmacotherapies for Addiction

Preclinical Models

The preclinical research linking EAAT dysfunction to drug addiction has primarily focused on identifying compounds that regulate EAAT2/GLT-1 given that GLT-1 mediates most glutamate uptake, but few drugs specifically target one EAAT over the others. Function of other EAATs may be affected or alternative mechanisms may be engaged by these manipulations. This caveat should be kept in mind when considering studies summarized below, as even the "specific" GLT-1 inhibitor dihydrokainate (DHK) can have off-target effects.

The antioxidant cystine pro-drug N-acetylcysteine (NAC) is the best-studied GLT-1 modulating drug. The over-the-counter dietary supplement is commonly indicated for acetaminophen overdose. NAC can suppress NF-κB signaling and prevent downstream activation of proinflammatory cytokines (Oka et al., 2000). NF-κB interacts with the GLT-1 promoter at multiple sites and has been implicated in transcriptional repression and activation (Sitcheran et al., 2005). Furthermore, NAC provides cystine for GSH synthesis and can interact directly with reactive oxygen species (Badisa et al., 2013). Unfortunately, as an oral medication NAC has low bioavailability, thereby requiring large doses. NAC amide (AD4, NAC-amide) is purported to display better bioavailability properties (Jastrzębska et al., 2016).

In rodent self-administration models, NAC inhibits cue- and cocaine-induced reinstatement of cocaine seeking and restores GLT-1 expression (Baker et al., 2003a, 2003b; Madayag et al., 2007; Knackstedt et al., 2010; Amen et al., 2011), and this effect is long lasting following daily NAC administration prior to extinction sessions (Moussawi et al., 2011b; Reichel et al., 2011). A single study using NAC-amide similarly shows reduced cue-induced and cocaine-primed reinstatement (Jastrzębska et al., 2016). A vivo-morpholino anti-sense strategy was used to show that NAC's ability to decrease cue- and cocaine-primed reinstatement depends on increased GLT-1 (Reissner et al., 2015). Daily NAC also reduces extinction responding and cue-induced reinstatement for heroin and nicotine seeking (Zhou and Kalivas, 2008; Ramirez-Nino et al., 2013). Surprisingly, NAC has no effect on cue-induced reinstatement of ethanol seeking (Weiland et al., 2015), but blocks behavioral sensitization to ethanol and associated brain changes in ΔFosB in PFC and NAc (Morais-Silva et al., 2016).

Compared with the overall positive NAC treatment effects on abstinent drug seeking above, the effects on drug reward during ongoing intake are equivocal. NAC does not alter the acute rewarding effects of cocaine during short access (1–2 h/d) self-administration or the acute locomotor stimulating effects (Madayag et al., 2007). However, chronic NAC treatment prevents locomotor sensitization to cocaine (Madayag et al., 2007). One unique study used a second-order schedule to assess effects of NAC on cocaine seeking vs taking within the same experiment, and NAC dose-dependently reduces drug seeking but not taking at acquisition and maintenance stages (Murray et al., 2012). Yet, in the long access self-administration model that produces escalated drug intake, NAC blunts this escalation (Madayag et al., 2007); however, if escalated cocaine intake is preestablished, NAC treatment is ineffective at reducing escalation progression or modifying motivation to seek a drug under a progressive ratio schedule (Ducret et al., 2016). NAC increases sensitivity to punishment in a "voluntary abstinence" model in long access rats only, and in NAC-treated rats, cocaine intake never returns to control levels following punished suppression (Ducret et al., 2016). Taken together, these studies point to a state-dependent NAC treatment effect on measures of cocaine addiction and bring to mind the progressive adaptations in GLT-1 protein and gene expression observed in NAc core with increasing cocaine exposure and withdrawal length (R. Kim et al., 2016b). This state dependence is further supported by the fact that NAC does not increase GLT-1 levels in controls (Knackstedt et al., 2010). Additionally, NAC may be more effective as a relapse prevention therapy for cocaine addiction delivered during abstinence rather than active use, an idea supported by the clinical research. NAC may offer more promise as a suppressant of active drug intake for other substances. Both acute and chronic NAC reduce nicotine self-administration (Ramirez-Nino et al., 2013). Chronic NAC reduces ethanol intake during maintenance drinking while having no effect during acquisition (Quintanilla et al., 2016).

Beta-lactam antibiotics stimulate GLT1 expression and function (Rothstein et al., 2005). These include ceftriaxone, cefazolin, amoxicillin, ampicillin, and cefoperazone, of which ceftriaxone has been best studied. As indicated by their nomenclature, these antibiotics contain a beta-lactam ring conferring their glutamate-regulating activity. Like NAC, the purported mechanism for ceftriaxone to increase GLT-1 expression involves NF-κB-dependent transactivation (Seok-Geun Lee et al., 2008). Repeated dosing is required for optimal effects on behavior and changes in GLT-1 (Rao et al., 2015a).

Ceftriaxone inhibits cue- and cocaine-induced reinstatement of cocaine seeking and restores GLT-1 expression (Sari et al., 2009; Knackstedt et al., 2010; Sondheimer and Knackstedt, 2011). Similarly, ceftriaxone inhibits cue-induced reinstatement of heroin seeking and reverses deficits in GLT-1 expression and glutamate uptake (Shen et al., 2014). Intra-NAc DHK and DL-threo-benzyloxyaspartic acid (DL-TBOA) microinfusions block the effects of ceftriaxone on cocaine seeking (Fischer et al., 2013), and an anti-sense morpholino strategy confirmed GLT-1 dependence of ceftriaxone's effects on heroin seeking (Shen et al., 2014). Ceftriaxone reduces drug-induced reinstatement of methamphetamine and nicotine CPP (Abulseoud, 2012) (Alajaji et al., 2013). Likewise, ceftriaxone reduces physical, somatic, and affective nicotine withdrawal signs in ICR mice (Alajaji et al., 2013) and nicotine intake in female P rats (Sari et al., 2016). Subthreshold doses of MK-801, an NMDAR antagonist, plus ceftriaxone reduce the acquisition of morphine CPP and block extinction and reinstatement of preference (Fan et al., 2012). Therapeutic doses of ceftriaxone

alone given during extinction block reinstatement of morphine CPP and normalize plasticity in NAc shell medium spiny neurons (Hearing et al., 2016). Repeated ceftriaxone treatment reduces alcohol drinking in male and female P rats coincident with an increase in GLT-1 levels in PFC and NAc (Sari et al., 2011, 2016). Other beta-lactams, ampicillin, cefazolin, cefoperazone, and amoxicillin, decrease ethanol intake and increase GLT-1 expression in PFC and NAc (Rao et al., 2015b; Hakami et al., 2016). Further, ceftriaxone alleviates alcohol withdrawal syndrome symptoms (Abulseoud et al., 2014) and reduces relapse-like drinking behavior modeled by restoration of drinking following a 2-week withdrawal (Qrunfleh et al., 2013; Rao and Sari, 2014). Finally, cefazolin and ceftriaxone reduce cue-induced reinstatement to ethanol (Weiland et al., 2015).

Several other agents with glutamate uptake-regulating capabilities have been investigated to a lesser extent. Clavulanic acid, a structural beta-lactam analog with some beta-lactamase inhibitory activity, has greater oral availability and brain penetrability compared with ceftriaxone and negligible antibiotic activity (J. Kim et al., 2016a). Chronic treatment with clavulanic acid increases GLT-1 expression in NAc and reduces motivation for cocaine under a progressive ratio schedule (J. Kim et al., 2016a). Clavulanic acid also reduces morphine CPP and locomotor sensitization (Schroeder et al., 2014). Riluzole, a neuroprotective drug approved for treating amyotrophic lateral sclerosis, increases GLT-1 transcription through a supposed heat shock factor 1-dependent mechanism (Liu et al., 2011) and augments GLT-1 protein levels and function (Carbone et al., 2012). Riluzole reduces expression of locomotor sensitization to methamphetamine (Itzhak and Martin, 2000) and blocks morphine and amphetamine CPP, although on its own it produces a preference (Tzschentke and Schmidt, 1998). Acute and chronic riluzole treatment reduces ethanol self-administration in mice and withdrawal-induced convulsions (Besheer et al., 2009). MS-153 [(R)-(-)-5-methyl-l-nicotinoyl-2-pyrazoline] is a synthetic pyrazoline analog that enhances glutamate uptake (Shimada, 1999). MS-153 blocks the development of amphetamine and morphine CPP (Nakagawa, 2005). It also reduces alcohol intake and increases GLT-1 in NAc, hippocampus, and amygdala in association with increased NF-κB65 and decreased IκB-α in P rats (Alhaddad et al., 2014; Aal-Aaboda et al., 2015). GPI-1046 is a neuroimmunophilin that reduces ethanol consumption and increases GLT-1 levels in PFC and NAc and enhances GSH synthesis in striatum (Tanaka et al., 2002; Sari and Sreemantula, 2012). Propentofylline is a methylxanthine derivative that reduces astroglial activation (Sweitzer and De Leo, 2011) and increases GLT-1 levels in NAc of cocaine withdrawn rats. Accordingly, 5 daily injections of propentyphylline reduce reinstated cocaine seeking (Reissner et al., 2014).

PRECLINICAL MODELS

Clinical trials using NAC to treat drug addiction have been systematically reviewed recently (Deepmala et al., 2015; Nocito Echevarria et al., 2017). There are 5 NAC clinical trials for cocaine dependence. In the largest double-blind placebo-controlled study, NAC does not differ from placebo in self-reported cocaine use or drug-negative urines; however, in a subset of patients that were abstinent at study initiation, their days to relapse increased (LaRowe et al., 2013). In a smaller trial, NAC decreased self-reported use and money spent on cocaine (Mardikian et al., 2007). Proof of principle laboratory trials show decreases in self-reported craving and attentional bias in response to cocaine cues or drug infusion (LaRowe et al., 2007; Amen et al., 2011). These results are in line with NAC acting more as a relapse prevention medication rather than cocaine cessation treatment, which is supported by rodent studies where NAC is largely ineffective at modifying cocaine intake under short access self-administration conditions but reliably reduces reinstatement when given during extinction (Baker et al., 2003a; Madayag et al., 2007; Reissner et al., 2015). In 2 trials conducted for methamphetamine dependence, results are equivocal. Methamphetamine-dependent individuals receiving NAC + naltrexone received no benefit over placebo (Grant et al., 2010); however, a more recent double-blind crossover trial shows NAC reduced craving (Mousavi et al., 2015).

There are 3 clinical trials for NAC with cannabis dependence and 6 for nicotine use or dependence. A small open-label trial shows decreased self-reported cannabis use and craving in adolescents (Gray et al., 2010), although there was no difference between NAC treatment and control in the follow-up study (Roten et al., 2013). However, in a larger, double-blind, placebo-controlled study conducted in adolescent and young adult cannabis-dependent patients, NAC increased drug-negative urines (Gray et al., 2012). A large, multi-site, clinical study through the National Institute on Drug Abuse Clinical Trials Network titled Achieving Cannabis Cessation – Evaluating NAC treatment was established to replicate positive findings in a larger sample (McClure et al., 2014). The results of nicotine clinical trials with NAC vary. This may be due in part to complications associated with high comorbidity between smoking with other drug use and psychiatric disorders. For example, in a study on substance use in bipolar patients, nicotine use was not reduced by NAC (Bernardo et al., 2009), while in a study of pathological gamblers, NAC temporarily reduced nicotine dependence measures (Grant et al., 2014). In a double-blind, placebo-controlled, pilot study of heavy smokers, NAC did not reduce craving or withdrawal symptoms, but the first cigarette smoked after brief abstinence reportedly felt less rewarding (Schmaal et al., 2011). Dependent smokers treated with chronic NAC self-reported reduced daily cigarettes smoked, although actual carbon monoxide levels, craving, and withdrawal systems were unaffected (Knackstedt et al., 2009). Recently, an open-label pilot trial of NAC plus varenicline reported a modest reduction in daily cigarettes smoked (McClure et al., 2015), and a 12-week, double-blind, placebo-controlled pilot of NAC in patients with therapy-resistant tobacco use disorder showed a reduction in daily cigarette use, exhaled carbon monoxide, and depression scores (Prado et al., 2015). Ongoing clinical trials will clarify effects of NAC on substance use and craving.

The clinical data is lacking as it concerns other glutamate uptake modulators in addiction. A single, multi-arm, modified blinded, placebo-controlled efficacy screening trial of cocaine-dependent individuals found riluzole ineffective at reducing drug-negative urines or decreasing craving beyond placebo (Ciraulo et al., 2005). There is currently one double-blind placebo-controlled study recruiting cocaine-dependent participants to assess safety and drug interactions between clavulanic acid and cocaine (NCT02563769).

PERSPECTIVES

Is GLT1 the Whole Story?

Recent research from Underhill and colleagues (Underhill et al., 2014) has ignited interest in neuronal transporter EAAT3 in relation to amphetamine/methamphetamine addiction, but this transporter might be similarly affected by other

psychostimulants. AMPH reduces EAAT3 function in midbrain dopamine neurons, resulting in potentiated glutamatergic transmission (Underhill et al., 2014; Li et al., 2016). Acute administration of cocaine similarly induces long-term potentiation in VTA dopamine cells (Ungless et al., 2001), and it is hypothesized that this early synaptic potentiation contributes to the transition to compulsive drug use (Lüscher, 2013). Thus, it would be interesting to determine if EAAT3 dysfunction contributes to this effect for cocaine and other drugs. Moreover, outside of addiction, it has been shown that EAAT3 plays a critical role in learning and memory. Fear conditioning rapidly increases surface EAAT3 expression in hippocampus, and EAAT3-/- mice display deficits in context and cue-related learning and memory (Wang et al., 2014). These data could have implications for cue and context reward associations during drug self-administration and reinstatement if drugs of abuse modify EAAT3. Interestingly, learning and memory deficits in EAAT3-/- are rescued by NAC treatment likely due to restoration of redox status (see below) (Cao et al., 2012).

We have not discussed EAAT4 or EAAT5. EAAT5 is restricted to retina and thus irrelevant to this discussion. However, EAAT4 is a neuronal glutamate transporter expressed abundantly in cerebellar Purkinje cells, but also at lower levels throughout the forebrain and midbrain with the highest levels in substantia nigra pars compacta, VTA, habenula-interpedunclar system, supraoptic nucleus, lateral posterior thalamic nucleus, and subiculum (Massie et al., 2008). EAAT4 can act as a glutamate-gated chloride channel adding to the ways this transporter might regulate synaptic transmission (Furuta et al., 1997). Little is known about the potential role of EAAT4 as a substrate for drug addiction, but given that EAAT4 protein is detected in midbrain and forebrain regions, the study of drug effects on this transporter should be expanded. This is further supported by evidence of antipsychotic treatments impacting EAAT4 expression (Huerta, 2006; Segnitz, 2009), and the demonstration that learned helpless rats display decreased levels of EAAT4 in the hippocampus and cerebral cortex given the connection between substance abuse and other affective disorders (Zink et al., 2010).

How Much Does Regulation of Redox State Contribute to NAC Treatment Efficacy?

Alcohol, psychostimulants, and opiates affect cellular redox status and can lead to the formation of oxidative or nitrosative stress (ROS and RNS). Redox-sensitive posttranslational modifications on cysteine residues, such as S-glutathionylation and S-nitrosylation, can influence the function of many addiction-related signaling proteins, and there is direct evidence for the involvement of these processes in cocaine addiction. GSH is one of the most important cellular antioxidants, and it is modified to GSH disulfide under conditions of oxidative stress. The GSH:GSH disulfide ratio is a common measure of intracellular redox status. Acute cocaine increases redox potential and S-glutathionylation of proteins in NAc (Uys et al., 2011). Currently, studies are underway to determine which proteins are modified as a result of chronic drug use. In addition to its ability to increase GLT-1, NAC is a precursor for GSH. NAC treatment reverses GSH depletion in rat astroglial cells following cocaine-induced oxidative stress (Badisa et al., 2013).

Nitric oxide is another important molecule regulated by redox state. Cocaine increases nitric oxide levels in dorsal striatum (D. K. Lee et al., 2011). Cue-induced reinstatement of cocaine seeking increases s-nitrosylation and enzymatic activity of extracellular matrix enzymes matrix metalloproteinases (MMPs) in NAc core (Smith et al., 2017). This activation of matrix metalloproteinases during cue-induced reinstatement generalizes across abused drugs and is required for cue-reinstated drug seeking (Smith et al., 2014). In vitro studies indicate that NAC can block activation of pro-MMP-9 and inhibit enzymatic function of activated protein (Pei et al., 2006), thus another parallel mechanism through which NAC could regulate drug seeking.

Targeting Glutamate Transport in Other Psychiatric Diseases

An emerging hypothesis states that addiction is one disease exemplary of a class of disorders that carry intrusive thinking as a symptomatic endophenotype, including OCD, major depression, and schizophrenia (Kalivas and Kalivas, 2016). Each of these diseases has a suggested etiology involving dysregulated glutamatergic transmission with varying degrees of evidence demonstrating EAAT involvement. As mentioned before, the EAAT3 genetic locus is associated with OCD risk (Arnold et al., 2006; Porton et al., 2013), and glutamate-modulating drugs including NAC, riluzole, and minocycline have garnered interest as alternative therapies for resistant OCD with several clinical trials at various stages of completion (Pittenger, 2015). In human postmortem studies, deficits in EAAT protein and RNA are reported in frontal cortex and striatum of depressed patients (McCullumsmith and Meador-Woodruff, 2002; Miguel-Hidalgo et al., 2010). Likewise, a chronic unpredictable stress rat model of depression markedly downregulates EAAT2 and EAAT3 in the hippocampus (Zhu et al., 2017). Also, acute stress in rats causes an enduring reduction of GLT-1 in the NAc (Garcia-Keller et al., 2016), and a recent double-blind clinical trial in veterans comorbid for posttraumatic stress disorder and substance use disorder revealed that NAC reduced both drug craving and symptoms of posttraumatic stress disorder (Back et al., 2016). Sub-anesthetic ketamine, an off-label treatment for refractory depression increasingly used in the clinic, rescued this deficit and the associated behavior (Zhu et al., 2017). Similar results were reported with decreased GLT-1 levels in hippocampus of depressed rats ameliorated by the traditional antidepressant fluoxetine, suggesting that normalization of these proteins may be important for antidepressant efficacy (Chen et al., 2014). Accordingly, double-blind studies in depressed patients show that NAC reduces symptoms of depression (Fernandes et al., 2016; however, see Berk et al., 2014). For schizophrenia, the glutamate hypotheses originated from observations that NMDAR antagonists like phencyclidine and ketamine produce schizophrenia-like symptoms. Examination of human postmortem brains reveals consistent alterations at the level of morphology of glutamatergic neurons and synapses, but alterations of mRNA and proteins related to glutamate transmission, including EAATs, are varied (Hu et al., 2015). Nonetheless, therapies targeting glutamate transmission at multiple levels are being investigated as adjunctive and stand-alone schizophrenia treatments, and NAC in combination with antipsychotic medication significantly improves symptoms of schizophrenia over control (Berk et al., 2008). Altogether, these examples in no way constitute a comprehensive analysis of this literature, as that is beyond the scope of this review, but these data are highlighted to illustrate remarkable mechanistic overlap between addiction and other often comorbid mental diseases. Thus, EAAT-modulating drugs may have translational relevance across compulsive psychiatric disorders.

Conclusions

Disruptions in glutamate homeostasis following chronic drug use are a well-conserved neuropathology contributing to the lasting vulnerability to relapse characteristic of addiction, and

we posit that contributions of glutamate transporters to this phenomenon warrant additional investigation. This requires developing more specific pharmacological tools and additional examination of mechanisms underlying existing drugs. We introduce an emergent role for cellular redox status in response to chronic drugs. We propose that this dual and interactive role between glutamate transporters and redox status be kept in mind as addiction pharmacotherapies are developed and tested. Finally, based on positive clinical trials with NAC that restores GLT-1 levels in animal models of addiction, we suggest that glutamate transport may offer a therapeutic target extending beyond addiction to other psychiatric disorders characterized by intrusive thoughts.

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Statement of Interest

None.

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