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Neuronal excitatory amino acid transporter EAAT3: Emerging functions in health and disease

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1. Introduction

Plasma membrane neurotransmitter transporters maintain extracellular concentrations of neurotransmitters by facilitating transport into the cytosol. This regulation of extracellular neurotransmitters limits binding to receptors and activation of downstream signaling pathways. In addition to this critical function, transporters modulate neuronal activity via direct gating of transporter-associated ion channels and indirectly through trafficking of transporters to and from the plasma membrane. These functions are dependent on diverse expression patterns and levels of the transporters throughout the brain. This diversity is highlighted within the excitatory amino acid transporter family, which consists of five excitatory amino acid transporters found in the mammalian central nervous system (CNS). EAAT1 (GLAST) and EAAT2 (GLT-1) are primarily expressed in astrocytes while EAAT3 expression is mainly observed in many neurons throughout the brain (Holmseth et al., 2012). In contrast, EAAT4 is most prominently expressed in cerebellar Purkinje neurons and EAAT5 is exclusively found in the retina. In general, the astroglial transporters are highly expressed in the brain; EAAT2 is the most abundant, followed by EAAT1 with approximately a 4-fold lower expression (Holmseth et al., 2012). High expression levels of these glial transporters are consistent with their role in glutamate clearance (Lehre and Danbolt, 1998; Rothstein et al., 1996; Tanaka et al., 1997).

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Conflicts of interest

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The role of the neuronal transporter EAAT3 in brain has been more difficult to elucidate. Levels of EAAT3 are approximately 100-fold lower than EAAT2 (Holmseth et al., 2012) but EAAT3 expression is observed throughout the CNS, with enriched expression in the cerebral cortex, hippocampus, cerebellum and basal ganglia (Rothstein et al., 1994; Shashidharan et al., 1997). Given the lack of selective EAAT3 inhibitors, studies have relied on EAAT3 transporters expressed in various cells and endogenous transporters expressed in cultured hippocampal neurons (Diamond and Jahr, 1997; Grewer et al., 2000; Wadiche et al., 1995b), as well as EAAT3 knockout mice (Scimemi et al., 2009) to determine the physiological functions of EAAT3. These studies determined that the time course of glutamate in the synaptic cleft is a function of the binding of glutamate to EAATs and that transport of glutamate does not significantly contribute to the amplitude or kinetics of synaptic responses due to the relatively slow transport cycle (Diamond and Jahr, 1997; Tong and Jahr, 1994; Wadiche and von Gersdorff, 2006). Interestingly, EAAT3 knockout mice exhibit few behavioral deficits (Peghini et al., 1997), and antisense oligonucleotide knockdown in the striatum results in minimal elevation of extracellular glutamate levels or neurodegeneration, in contrast to knockdown of EAATs 1 and 2 (Rothstein et al., 1996). The lack of neurodegeneration is particularly surprising given that EAAT3 also serves as a cysteine transporter (Aoyama et al., 2006; Watts et al., 2014; Zerangue and Kavanaugh, 1996). Cysteine is the rate-limiting substrate for the synthesis of the antioxidant glutathione and its extracellular depletion is hypothesized to contribute to neurodegeneration. EAAT3 is also the dominant glutamate transporter in the intestines and provides nutrient absorption from the diet (Hu et al., 2018), but knockout animals appear to grow at a comparable rate to their litter mates (Peghini et al., 1997). The most remarkable initial observation from EAAT3 knockout mice was aminoaciduria due to the absence of EAAT3 in the kidneys (Peghini et al., 1997). The reported lack of overt behavioral abnormalities in the EAAT3 knockout mice suggest that either EAAT3 is not integral to regulation of glutamatergic signaling in the brain or that substantial developmental compensatory changes are induced in these mice. Human genetic studies and constitutive deletion mouse models have now provided evidence that EAAT3 has important roles in regulating neuronal signaling.

2. Human genetic findings at *SLC1A1*/EAAT3

Interest in the EAAT3 gene (*SLC1A1*) has emerged in relation to a number of disorders (Table 1), with the clearest findings emerging in dicarboxylic aminoaciduria. In 2011, Bailey and colleagues described three individuals (one singleton and two siblings) with two different mutations in *SLC1A1* leading to complete loss of EAAT3 function (Bailey et al., 2011). All three cases showed aminoaciduria, elevated glutamate and aspartate levels in the urine, reflecting the role of EAAT3 in the reuptake of these amino acids from the glomerular filtrate (Bailey et al., 2011). The two siblings were noted to have a history of kidney stones. In addition, one of them was described as having compulsive-like behavior, but a full psychiatric evaluation was not possible (Bailey et al., 2011). The singleton had no known medical history and was discovered on the basis of population screening. To our knowledge, these are the only three individuals with complete loss of EAAT3 function who have been described in the literature.

A heterozygous *SLC1A1* deletion that includes the promoter region and first exon was also identified in an extended Palauan family with multiple generations affected with schizophrenia or schizoaffective disorders (Myles-Worsley et al., 2013). While some *SLC1A1* gene expression was preserved, the resulting truncated protein did not localize to the membrane, and glutamate transport was lost in transfected cell lines (Afshari et al., 2015). A case-control study of *SLC1A1* in schizophrenia, with a nested replication, found association with a single nucleotide polymorphism (SNP; rs7022369), but the implicated allele was associated with *increased* expression in postmortem samples (Horiuchi et al., 2012). Because this gene was not identified in a genome-wide association in schizophrenia (Pardinas et al., 2018), and the functional impacts of the deletion and the associated SNP appear to manifest in opposite directions, the role of *SLC1A1* to schizophrenia/schizoaffective disorders is yet unclear.

There has also been great interest in the potential role of *SLC1A1* in obsessive-compulsive disorder (OCD). The first genome-wide linkage scan of OCD, which focused on extended families with pediatric-onset OCD probands, found suggestive linkage to the chromosome 9p24 region containing *SLC1A1* (Hanna et al., 2002). Another group then replicated a nearly identical pattern of linkage in extended families with onset before adulthood (Willour et al., 2004). Multiple subsequent family-based studies found association at a number of single nucleotide polymorphisms, with stronger evidence in males and in early-onset OCD, and most findings clustered in the 3' region of the gene (Arnold et al., 2006; Dickel et al., 2006; Kwon et al., 2009; Samuels et al., 2011; Shugart et al., 2009; Stewart et al., 2007; Wendland et al., 2009). A pooled analysis across published studies found only nominally significant association in the overall group of patients with OCD, although this signal was again stronger in males, and pediatric-onset OCD was not analyzed separately (Stewart et al., 2013). Efforts to screen for coding variants in OCD indicate that they are very rare (1/1400 OCD subjects screened), and the only identified variant has at most minimal impact on EAAT3 function (Veenstra-VanderWeele et al., 2001; Veenstra-VanderWeele et al., 2012; Wang et al., 2009). However, using cell models, one study has suggested that common non-coding SNPs associated with OCD may lead to increased *SLC1A1* expression (Wendland et al., 2009), serving as a potential entry point for studying the impact of *SLC1A1* expression on behaviors relevant to OCD.

3. The impact of EAAT3 on learning and behavior in mouse models

The initial description of mice lacking *Slc1a1*/EAAT3 reported a significant decrease in spontaneous locomotor behavior in the open field, and no impairment on Morris water maze or rotarod (Peghini et al., 1997). More recent studies did not find a change in baseline locomotor activity and confirmed that loss of EAAT3 does not appear to confer changes in spontaneous behaviors into early adulthood (Aoyama et al., 2006; Zike et al., 2017b), although one study found changes in activity and anxiety-like behavior prior to adulthood (Bellini et al., 2018). However, further studies indicate age-related changes in older adult mice, as well as changes in learning, pain-sensitivity, and drug response in younger animals, as described further below.

3.1. Neurodegeneration and aging

Aoyama and colleagues first identified a learning phenotype in *Slc1a1*/EAAT3 null mice related to EAAT3's role in uptake of cysteine for neuronal synthesis of glutathione, the primary antioxidant in the brain (Aoyama et al., 2006). They found no behavioral deficits in young adult *Slc1a1* null mice, but older adult mice (11 months) showed significant impairments in the Morris Water Maze, a hippocampus-dependent test of spatial learning. They observed neurodegeneration due to oxidative stress in the hippocampus of these animals, which could be rescued by N-acetylcysteine, an alternative substrate for glutathione synthesis that bypasses EAAT3 (Aoyama et al., 2006). Loss of hippocampal neurons due to transient cerebral ischemia is also increased in mice lacking *Slc1a1*, again due to decreased glutathione levels that can be rescued with N-acetylcysteine (Won et al., 2010). Recent work by Berman and colleagues has demonstrated that EAAT3 also has an important role in preventing oxidative stress-mediated loss of dopaminergic neurons (Berman et al., 2011). In addition to age-dependent loss of dopaminergic neurons, they also found impairment in the pole test of balance and coordination at 12 months, which could again be rescued with N-acetylcysteine. Together, these findings support an important role for EAAT3 in preventing oxidative stress-induced neurodegeneration.

3.2. EAAT3 in development and adulthood

More recent studies suggest learning deficits in *Slc1a1* null mice that are not dependent upon age-related neuronal loss. For example, Wang and colleagues showed diminished fear conditioning in *Slc1a1* null mice as early as 7 weeks of age, when neuronal loss is not evident (Wang et al., 2014). Biochemical changes associated with fear conditioning were likewise attenuated in the EAAT3 null animals, including immediate early gene activation and changes in EAAT2 and glutamate receptor subunit GluR1 (Wang et al., 2014). A separate study observed a decrement in context-dependent fear conditioning, supporting an impact of EAAT3 constitutive deletion on learning; however, this was only observed following treatment with isoflurane, which enhances EAAT3 trafficking to the membrane (Cao et al., 2014b). These results implicate EAAT3 in synaptic plasticity associated with learning, although further work will be necessary to understand the breadth of its impact on cognitive function.

EAAT3 also has important functions in the basal ganglia that are not age-dependent. Using mice that contain an excise-able STOP cassette that interferes with *Slc1a1* transcription (*Slc1a1*-STOP mice), Zike and colleagues demonstrate that mice with ablated expression of *Slc1a1* have decreased sensitivity to amphetamine-induced locomotion. Further, in comparison to wildtype littermate controls, these mice have diminished amphetamine-induced stereotypies and dopamine receptor D₁ agonist-induced grooming (Zike et al., 2017b), two types of repetitive behavior that may have relevance to OCD and Tourette syndrome (Castellan Baldan et al., 2014; Zike et al., 2017a). These animals showed no loss of dopaminergic neurons at the ages tested (< 4 months), but they did display a decrease in both pre- and post-amphetamine dopamine levels in the dorsal striatum, suggesting a baseline change in dopaminergic system function that is exacerbated by amphetamine treatment. Decreased dopamine receptor D₁ expression as assessed by radioligand membrane binding and diminished immediate early gene activation in the dorsal striatum

compared to ventral striatum were also evident in the *Slc1a1*-STOP mice (Zike et al., 2017b).

In adult *Slc1a1*-STOP animals, viral Cre-mediated restoration of EAAT3 expression in the midbrain, but not in the striatum, led to a partial rescue of the amphetamine response but not the D₁ agonist response (Zike et al., 2017b). The lack of complete rescue suggests that EAAT3's activity in other brain regions or during development is also necessary for normal basal ganglia function. Overall, these findings suggest that EAAT3 may directly contribute to dopamine-dependent, striatally-mediated repetitive behavior, but comparable experiments examining increased EAAT3 expression are ongoing.

In parallel with this work in the *Slc1a1*-STOP animals, Bellini and colleagues recently reported alterations in behavior and dopaminergic system function in prepubertal *Slc1a1* null mice (Bellini et al., 2018). They focused on a time period around weaning, from postnatal day 14–35 (P14–P35), finding that *Slc1a1* null weanlings spent less time immobile in the open field, instead showing increased fine, “restless” movements, despite ambulating a similar amount to wildtype controls. The null weanlings also have more anxiety-like behavior, as indexed by spending more time in the closed arms of the elevated plus maze, as well as burying more marbles on the marble burying test. In addition, unlike the adult *Slc1a1*-STOP animals, the *Slc1a1* null weanlings had more bouts of grooming with a disrupted syntactic grooming chain with more time spent licking their paws, but no difference in length of grooming bouts, in comparison to the wildtype control animals (Bellini et al., 2018). The difference in observed behaviors between the *Slc1a1* null weanlings and the adult *Slc1a1*-STOP mice could be due to differences in developmental time point assessed or controls used (e.g. age- and sex-matched wildtype C57BL/6 animals in Bellini et al., 2018 instead of wild-type littermates as in Zike et al., 2017b).

Bellini and colleagues also described a decrease in dopamine receptor D₁ expression, but not D₂ expression, in the *Slc1a1* null striatum, paralleling the radioligand binding findings in *Slc1a1*-STOP mice (Bellini et al., 2018). Their data show enhanced baseline metabotropic glutamate receptor mGluR1-mediated G_q-protein signaling in the striatum of *Slc1a1* null mice, which leads to downregulation of dopamine receptor D₁ expression. These results suggest that loss of EAAT3 contributes to activation of mGluR1 receptors that are typically located extra-synaptically. Using viral-mediated expression of Designer Receptors Exclusively Activated by Designer Drugs (DREADDs), they then showed that decreasing G_q signaling in striatal D₁-expressing medium spiny neurons in the dorsolateral striatum restored D₁ expression in *Slc1a1* null mice. Conversely, increasing G_q signaling in these neurons in wildtype mice led to increased frequency of grooming bouts without changing their duration (Bellini et al., 2018).

Of note, behavioral profiling of the *Slc1a1* null and STOP mice has primarily focused on comparing homozygous mutant animals with either wildtype littermate controls or age- and sex-matched controls, without extensive characterization of heterozygous animals demonstrating 50% loss of expression. Two groups have recently reported behavioral profiling of animals heterozygous for *Slc1a1* and found no change in locomotor activity but reported different results regarding anxiety-like behavior compared to wildtype littermate

controls (Afshari et al., 2015; Gonzalez et al., 2017). Heterozygotes also had no response to amphetamine and no change in tissue dopamine levels (Gonzalez et al., 2017). Overall, the behavioral data across these different studies is not directly comparable because of the use of different mechanisms (null versus STOP versus heterozygote), ages (from weanlings to adults), and controls (littermates versus matched wildtypes). Regardless, all of these model systems point to the involvement of EAAT3 in dopamine-mediated, striatally-dependent behaviors. The neuronal and molecular mechanisms underlying EAAT3's role in basal ganglia circuits and in dopamine neurons in particular are discussed in more detail below.

3.3. EAAT3 and pain

The EAATs have also been implicated in the etiology of chronic pain. Several studies have documented decreased expression levels of EAATs 1–3 in the spinal cord in different chronic pain models (Sung et al., 2003). Similarly, chronic morphine treatment and morphine tolerance are associated with decreased EAAT1–3 expression (Mao et al., 2002; Nakagawa et al., 2001). Both hyperalgesia and morphine tolerance are reversed by positive regulators of glutamate transporters and the NMDA receptor blocker MK-801, suggesting that regulation of extracellular glutamate concentrations is critical in these pathological conditions. Although decreased EAAT3 transporters were documented in these studies, nonselective transport blockers and activators were used so the specific contribution of EAAT3 to the NMDA-mediated changes in pain sensitivity is not known. However, studies showing that specific knockdown of EAAT3 and intracellular administration of the EAAT inhibitor TBOA in neuronal recordings affect extrasynaptic NMDA receptor signaling (Li et al., 2017; Scimemi et al., 2009) suggest that EAAT3 may play an important role in changes observed in chronic pain states and morphine tolerance.

4. Impact of EAAT3 on neuronal activity

4.1. EAAT3 regulation of synaptic responses

The human genetic studies and the recent data with EAAT3 knockout mouse models point to EAAT3 regulation of brain circuits, especially in disease states. The effect of EAAT3 transporters on synaptic activity is subtle compared to the astroglial transporters EAAT1 and EAAT2 despite their peri-synaptic localization on the postsynaptic aspect of glutamate terminals (He et al., 2000) because of their low comparative expression levels and the fact that EAAT3 transporters are not key regulators of synaptic glutamate concentrations (Diamond and Jahr, 1997; Tong and Jahr, 1994; Wadiche and von Gersdorff, 2006). EAAT3 does, however, regulate responses of extrasynaptic receptors, including NMDA receptors containing the NR2B subunit (Li et al., 2017; Scimemi et al., 2009) and metabotropic glutamate receptors (Otis et al., 2004; Wadiche and Jahr, 2005) (Fig. 1). Extrasynaptic NMDA receptors containing the NR2B subunit are activated in the EAAT3 knockout mice and in studies using intracellular TBOA in the patch pipette during whole-cell patch-clamp recordings from midbrain dopamine neurons, a method for selectively inhibiting EAAT3 transporters with a nonselective glutamate transport blocker (Li et al., 2017). These findings are significant because extrasynaptic NMDA receptors are implicated in the modulation of LTP induction threshold (Yang et al., 2017) and psychostimulant-induced locomotor behavior (Li et al., 2017; Liu et al., 2005; Mao et al., 2009; Shen et al., 2011).

Another mechanism by which EAAT3 controls neural activity is by providing the substrate for synthesis of GABA in GABAergic terminals (Mathews and Diamond, 2003). Blockade of EAATs reduced miniature inhibitory postsynaptic currents while increasing glutamate uptake increased GABA synthesis in the hippocampus indicating that glutamate transport into GABA neurons is critical for the activity of glutamic acid decarboxylase and synthesis of GABA. Thus, neuronal glutamate transporters can potentiate inhibitory, GABAergic synapses and this mechanism may be an important negative feedback regulation of excitatory neurotransmission.

4.2. Uncoupled conductances

Many plasma membrane transporters exhibit uncoupled ion conductances in addition to their transport capabilities. All of the EAATs have uncoupled anion conductances. These conductances are gated by substrates of the transporters and can both facilitate and inhibit neuronal excitability based on the ion gradients in specific cell types (Ingram et al., 2002; Melzer et al., 2005). For example, an uncoupled anion conductance associated with the dopamine transporter (DAT) can be activated by DAT substrates, including amphetamine and methamphetamine, DAT substrates, and excites dopamine neurons (Beckstead and Williams, 2007; Ingram et al., 2002). The neuronal EAATs 3–5 have a larger ratio of anion conductance to transport compared to the glial transporters EAATs 1 and 2 (Wadiche et al., 1995a). In fact, although the transport rates for EAAT3 and EAAT4 differ substantially, they exhibit similar unitary current amplitudes and similar absolute open probabilities (Torres-Salazar and Fahlke, 2007). EAAT4 has the largest anion conductance compared to transport and has largely been assumed to be a glutamate-gated anion channel necessary for regulation of Purkinje cell excitability (Melzer et al., 2005). In retinal rod bipolar cells, the chloride conductance mediated by EAAT5 is sufficient to hyperpolarize the presynaptic terminal and suppress transmitter release (Veruki et al., 2006), essentially behaving as a pre-synaptic inhibitory receptor (Wersinger et al., 2006). However, physiological consequences of the anion conductance for the other EAATs have not yet been reported and further studies are necessary to understand how these conductances contribute to the normal control of neuronal excitability.

5. Mechanisms of EAAT3 modulation and trafficking

Mutations in EAAT3 associated with disease states can result in disruption of various aspects of EAAT3 function. Plasma membrane transporters are modulated via changes in expression levels, degradation and trafficking. The mechanisms of genetic regulation of EAAT3 have been reviewed elsewhere (Bianchi et al., 2014); we will therefore focus on recent advances in understanding the modulation of EAAT3 through trafficking.

Large intracellular pools of EAAT3 have been documented in several brain areas (Conti et al., 1998; Furuta et al., 1997; Gonzalez et al., 2002; He et al., 2000; Holmseth et al., 2012; Rothstein et al., 1994; Sheldon et al., 2006; Sims et al., 2000; Yang and Kilberg, 2002), and the fact that glutamate transporters buffer extracellular glutamate concentrations through binding suggests that the number of transporters on the cell surface is a key determinant of buffering efficiency. EAAT3 rapidly cycles to and from the plasma membrane and

intracellular compartments under baseline conditions with a 5–7 min half-life for EAAT3 (Fournier et al., 2004). Thus, the large intracellular pool of EAAT3 transporters may represent transporters that are available for trafficking to the plasma membrane in response to stimulation.

5.1. EAAT3 trafficking

Early studies indicated that activation of protein kinase C (PKC) increases the membrane localization of EAAT3 (Davis et al., 1998; Dowd and Robinson, 1996). Interestingly, this is in contrast to the actions of PKC on the dopamine transporter (DAT) (Daniels and Amara, 1999; Melikian and Buckley, 1999; Vaughan et al., 1997; Zhu et al., 1997), the norepinephrine transporter (NET) (Apparsundaram et al., 1998a, 1998b), the GABA transporter (GAT) (Beckman et al., 1999), and other members of the glutamate transporter family (Kalandadze et al., 2002; Susarla et al., 2004), in which activation of PKC decreases the plasma membrane expression of the carriers. Multiple physiological stimuli initiate PKC-mediated trafficking of EAAT3. One example is isoflurane, an anesthetic that leads to insertion of EAAT3 to the plasma membrane (Huang and Zuo, 2003). This trafficking is dependent on PKC phosphorylation of EAAT3 at serine 465 (Huang et al., 2006) and has been shown to also regulate trafficking of GluR1 (Cao et al., 2014a) and play a role in context-related learning and memory (Cao et al., 2014b).

To date, several other pathways have also been identified that modulate EAAT3 through trafficking including activation of other kinases (e.g. PI3K, PKA) (Davis et al., 1998; Guillet et al., 2005) as well as specific receptors that can mediate these cascades (e.g. platelet-derived growth factor receptors that stimulate a PI3K-mediated insertion pathway (Fournier et al., 2004; Sims et al., 2000). Interestingly, there is also a close relationship between regulation of EAAT3 transporters and glutamate receptors. Activation of NMDA receptors in models of NMDA-dependent long-term depression (LTD) or potentiation (LTP) modulate EAAT3 uptake through trafficking (Levenson et al., 2002; Waxman et al., 2007). NMDA-dependent long-term potentiation (LTP) in the hippocampus induced by high-frequency stimulation of Shaeffer collaterals increases trafficking of EAAC1 to the plasma membrane in a PKC-dependent process (Levenson et al., 2002). An LTD model in which a brief (5 min) application of NMDA to hippocampal neurons decreases membrane localization of EAAT3 (EAAC1) in a calcium and clathrin-dependent mechanism (Waxman et al., 2007). Interestingly, LTD in adult cortex is more readily acquired when glutamate transporters are blocked, suggesting a role for the internalization of EAAT3 in the initiation of LTD (Massey et al., 2004).

In addition, trafficking of EAAT3/EAAC1 transporters control activation of extrasynaptic NMDA and mGluR receptors. In the cerebellum, glutamatergic synapses display mGluR-dependent plasticity that is dependent on glutamate transporters (Otis et al., 2004) and selective blockade of EAAT3 at parallel fiber synapses in the cerebellum enhance mGluR-mediated excitatory postsynaptic potentials (Brasnjo and Otis, 2001).

5.2. Amphetamine-mediated trafficking of EAAT3

The mechanism of action of amphetamine has largely been attributed to modulation of the dopaminergic system. Indeed, DAT trafficking by amphetamine has been recognized as an important component of amphetamine-associated behavioral responses. However, more recently it was discovered that amphetamine also stimulates internalization of EAAT3 (Underhill et al., 2014). EAAT3 internalization results in potentiation of glutamate receptor responses in midbrain dopamine neurons primarily due to activation of NMDA receptors containing NR2B subunits that are likely localized to extrasynaptic sites (Li et al., 2017). These data are consistent with the changes in amphetamine-induced locomotion and stereotypies observed in the Slc1a1-STOP animals (Zike et al., 2017b), suggesting that amphetamine-mediated trafficking of EAAT3 is an important mechanism underlying the behavioral effects of amphetamine.

While examining the basic cell biology of this phenomenon, we found that, similar to its effects on DAT, amphetamine-induced internalization of EAAT3 is dependent on dynamin and activation of the small GTP-ase RhoA, but independent of clathrin (Underhill et al., 2014; Wheeler et al., 2015). DAT transport of amphetamine and its entry into the cell is required for trafficking of both DAT and EAAT3 (Kahlig et al., 2006; Wheeler et al., 2015), implying that amphetamine acts at an intracellular target to stimulate Rho activation (Fig. 2). One candidate receptor is the trace amine-associated receptor 1 (TAAR1), which is localized to intracellular compartments and is activated by dopamine and amphetamine (Borowsky et al., 2001; Bunzow et al., 2001). Indeed, Xie and Miller found that DAT trafficking in response to amphetamine was dependent upon TAAR1 expression and absent in TAAR1 knockout animals (Xie and Miller, 2009). Further studies examining the potential for TAAR1 to regulate EAAT3 trafficking in response to amphetamine are ongoing.

Intracellular RhoA signaling is negatively regulated by PKA phosphorylation, that can also be stimulated by amphetamine (Fig. 2 (Borowsky et al., 2001; Bunzow et al., 2001; Xie and Miller, 2009)). Exogenous stimulation of G_s-coupled D5 receptors before exposure to amphetamine in dopamine neurons induced PKA-mediated phosphorylation/inactivation of RhoA resulting in decreased amphetamine-induced hyperlocomotion, implicating this pathway in regulating behavioral outcomes of amphetamine treatment. RhoA activation by stimuli other than amphetamine may also lead to EAAT3 internalization. For example, we have evidence that stimulation of LPA receptors by lysophosphatidic acid in HEK293 cells is sufficient to stimulate EAAT3 internalization. Conversely, EAAT3 trafficking to the membrane may be mediated directly through PKC that directly stimulates insertion of EAAT3 into the membrane, or by activation of PKA that inhibits the actions of RhoA. This complex trafficking pathway could be modulated by a myriad of receptors known to activate RhoA, PKA and PKC and contribute to regulation synaptic activity of glutamatergic synapses.

6. Conclusions

Current research supports a significant role for EAAT3 in glutamatergic synapses in the midbrain, GABAergic synapses in the hippocampus, as well as cellular survival mediated by the cysteine transport capacity of EAAT3 in midbrain and hippocampal neurons. While

EAAT3 is traditionally described as the neuronal carrier in glutamatergic synapses, this view is currently being challenged and it is now generally agreed that EAAT3 is found in most neurons throughout the brain in glutamatergic, dopaminergic and GABAergic neurons. It is clear that there are intricate reciprocal interactions between EAAT3 transporters and postsynaptic glutamate receptors that are required for synaptic transmission and plasticity. The physiological relevance of these interactions will become more defined as we focus on specific circuits with more sophisticated pharmacological and genetic tools for manipulating EAAT3.

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Fig. 1. Schematic of glutamatergic signaling onto a midbrain dopamine neuron.

The presynaptic neuron forms a glutamatergic synapse with the dopamine neuron. The pre-synaptic neuron may be modulated by dopamine through the dopamine D1 receptor. Dopamine may affect the dopamine neuron through dopamine D2 autoreceptor stimulation or interaction with the dopamine transporter (DAT). Glutamate released by the pre-synaptic neuron stimulates post-synaptic AMPA and NMDA receptors, extrasynaptic NMDA-NR2B receptors as well as metabotropic glutamate receptors (mGluRs). Synaptic glutamate is buffered by the glutamate transporters and is subsequently moved out of the extracellular space.

Fig. 2. Amphetamine-induced EAAT3 internalization pathway.

Amphetamine enters the post-synaptic, dopamine neuron through the dopamine transporters (DAT). Once inside the cell, amphetamine can stimulate activation of the small GTPase RhoA. Activated RhoA leads to cytoskeletal rearrangements that internalize the neuronal glutamate transporter, EAAT3 (as well as DAT). Glutamate spillover and/or internalization of EAAT3 contribute to stimulation of extrasynaptic NMDA and metabotropic glutamate receptors (mGluR) receptors.

Table 1

EAAT3 mutations associated with neuropathologies.

Disorder	Type of Evidence	Functional Impact	References
Dicarboxylic Aminoaciduria	Missense <i>SLC1A1</i> single nucleotide variant in two brothers	Both variants led to loss of EAAT3 function in transfected cell line.	Bailey et al., 2011
Schizophrenia/ Schizoaffective Disorder	3 base-pair <i>SLC1A1</i> exonic deletion variant in a singleton Both variants were inherited from parents without dicarboxylic aminoaciduria.		
	Deletion on 9p24 including <i>SLC1A1</i> promoter and exon 1 in extended family with multiple generations segregating schizophrenia and schizoaffective disorder.	Truncated protein does not localize to cell membrane and EAAT3 function lost in transfected cell line.	Myles-Worsley et al., 2013; Afshari et al., 2015
Obsessive- Compulsive Disorder	Case-control <i>SLC1A1</i> association study with nested replication.	Associated allele also associated with increased expression in postmortem brain.	Horiuchi et al., 2012
	Replicated linkage to chromosome 9p24 in extended pedigrees with early-onset OCD		Hanna et al., 2002; Willour et al., 2004
	Association to <i>SLC1A1</i> common single nucleotide polymorphisms in males, particularly in early-onset OCD	Associated haplotype leads to increased <i>SLC1A1</i> expression in cell lines and is associated with increased expression in postmortem brain.	Arnold et al., 2006; Dickel et al., 2006; Wendland et al., 2009