



OCD candidate gene *SLC1A1/EAAT3* impacts basal ganglia-mediated activity and stereotypic behavior

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Obsessive-compulsive disorder (OCD) is a chronic, disabling condition with inadequate treatment options that leave most patients with substantial residual symptoms. Structural, neurochemical, and behavioral findings point to a significant role for basal ganglia circuits and for the glutamate system in OCD. Genetic linkage and association studies in OCD point to *SLC1A1*, which encodes the neuronal glutamate/aspartate/cysteine transporter excitatory amino acid transporter 3 (EAAT3)/excitatory amino acid transporter 1 (EAAC1). However, no previous studies have investigated EAAT3 in basal ganglia circuits or in relation to OCD-related behavior. Here, we report a model of *Slc1a1* loss based on an excisable STOP cassette that yields successful ablation of EAAT3 expression and function. Using amphetamine as a probe, we found that EAAT3 loss prevents expected increases in (i) locomotor activity, (ii) stereotypy, and (iii) immediate early gene induction in the dorsal striatum following amphetamine administration. Further, *Slc1a1*-STOP mice showed diminished grooming in an SKF-38393 challenge experiment, a pharmacologic model of OCD-like grooming behavior. This reduced grooming is accompanied by reduced dopamine D₁ receptor binding in the dorsal striatum of *Slc1a1*-STOP mice. *Slc1a1*-STOP mice also exhibit reduced extracellular dopamine concentrations in the dorsal striatum both at baseline and following amphetamine challenge. Viral-mediated restoration of *Slc1a1/EAAT3* expression in the midbrain but not in the striatum results in partial rescue of amphetamine-induced locomotion and stereotypy in *Slc1a1*-STOP mice, consistent with an impact of EAAT3 loss on presynaptic dopaminergic function. Collectively, these findings indicate that the most consistently associated OCD candidate gene impacts basal ganglia-dependent repetitive behaviors.

obsessive-compulsive disorder | Tourette | basal ganglia | dopamine | EAAC1

Obsessive-compulsive disorder (OCD) is a common neuropsychiatric condition that ranks among the top 10 causes of disability worldwide (1, 2). Primary forms of therapy include serotonin-reuptake inhibitors (SRIs) and cognitive behavioral therapy; however, only 50–60% of patients exhibit adequate response to current treatment approaches, and most patients have clinically significant residual symptoms (1, 3). Other agents, including antipsychotic medications and glutamatergic agents, have been investigated to augment SRIs but have shown limited evidence of efficacy to date (4, 5). Surgical intervention with deep-brain stimulation in the ventral capsule/ventral striatum or subthalamic nucleus shows promise but is reserved for the most severely ill patients (6, 7). New treatments based on greater understanding of pathophysiology are therefore needed.

Multiple lines of evidence indicate that the basal ganglia are critically affected in OCD. Structural neuroimaging studies

demonstrate altered caudate volume in OCD (8, 9), and functional imaging has identified hyperactivity in cortico-striatal circuits, both at baseline and with symptom provocation (10). Interestingly, some reports using magnetic resonance spectroscopy describe elevated striatal glutamatergic signal in the caudate (11, 12), suggesting increased intracellular glutamate and/or GABA. Recent work in genetic and optogenetic mouse models of SRI-sensitive compulsive-like grooming behavior have also implicated cortico-striatal signaling, suggesting the relevance of dysfunctional basal ganglia signaling to repetitive behavior across species (13–16).

Family studies support a significant role for genetics in OCD, with increased heritability in early-onset OCD (17). Suggestive linkage to the chromosome region 9p24, which contains *SLC1A1* in addition to other genes, was initially established in a genome-wide linkage scan of OCD and then was independently replicated

Significance

Genetic linkage and association studies in obsessive-compulsive disorder (OCD) implicate *SLC1A1* (encoding the neuronal glutamate transporter excitatory amino acid transporter 3, EAAT3), and neuroimaging studies demonstrate abnormal basal ganglia circuit function in OCD. However, no previous studies have investigated the role of EAAT3 in these circuits or tested its impact on repetitive behavior. Using a combined genetic and pharmacological challenge approach, we have demonstrated that ablated expression of EAAT3 diminishes basal ganglia-mediated repetitive behavior in mice. Targeted rescue of midbrain expression points to an impact of EAAT3 on dopaminergic neuron function, suggesting a model for synthesizing glutamate and dopamine effects on stereotypic behavior. These findings provide evidence that EAAT3 impacts basal ganglia-dependent repetitive behavior and suggest a potential target for drug development.

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(18, 19). Analysis of *SLC1A1*, which codes for the neuronal glutamate transporter EAAT3 (excitatory amino acid transporter 3), identified significant association in the 3' region, with stronger evidence in males (20–22). Some, but not all, subsequent studies also identified an association with polymorphisms in the 3' gene region, with the greatest evidence for association with the rs301430C allele (21–26), which is linked to elevated *SLC1A1* expression in lymphoblastoid cells, human postmortem brain, and a luciferase reporter assay (25). Taken together, these data suggest that OCD susceptibility may result from elevated *SLC1A1* expression and that decreasing EAAT3 activity therefore could be a therapeutic target. Association findings, gene-expression differences, and deletions of *SLC1A1* have also been reported in schizophrenia and bipolar disorder (27–29), indicating a potential role for EAAT3 in a broader array of neuropsychiatric disorders.

SLC1A1 mRNA and EAAT3 protein are strongly expressed in the cortex and the striatum and in mesolimbic and nigrostriatal dopaminergic neurons (30–33). EAAT3 localizes to peri- and postsynaptic regions (32), where it serves three apparent functions: (i) buffering glutamate concentrations around peri/extrasynaptic NMDA and metabotropic glutamate receptors (34); (ii) taking up glutamate as an intracellular precursor for GABA synthesis (35); and (iii) taking up cysteine for glutathione synthesis and protection from oxidative stress (36, 37).

One logical place for *SLC1A1*/EAAT3 to impact cortico-striatal signaling is in the GABAergic medium spiny neurons (MSNs) of the striatum, which receive glutamatergic inputs from the cortex and provide output to the thalamus via the direct and indirect pathways of the basal ganglia. EAAT3 activity limits NR2B-containing NMDA receptor-dependent signaling at hippocampal glutamatergic synapses (38) and could similarly impact postsynaptic signaling in striatal MSNs. In addition, recent reports suggest that EAAT3 is expressed in dopaminergic neurons projecting from the ventral tegmental area (VTA) and the substantia nigra (SN) to the ventral and dorsal striatum, respectively (30). It therefore is possible that the subpopulation of EAAT3 in mid-brain dopaminergic neurons could impact OCD-implicated basal ganglia circuitry via neuromodulation. This possibility is especially relevant in light of recent evidence that amphetamine elicits endocytosis of EAAT3 and causes elevated signaling at glutamate receptors in dopaminergic neurons (30, 39).

Despite its genetic association with OCD, no studies have addressed the functional impact of *Slc1a1*/EAAT3 on OCD-relevant brain circuits or on OCD-like behaviors. To explore these questions, we used a flexible knockin approach (40) to generate mice with constitutively reduced *Slc1a1*/EAAT3 expression (*Slc1a1*-STOP mice, hereafter “ST mice”). We hypothesized that these animals would show decreased liability to repetitive behaviors, based on the implication of increased *SLC1A1* expression in OCD risk. Beyond assessing spontaneous repetitive behaviors, which occur at low baseline frequency, we examined sensitivity to pharmacologically induced compulsive-like behaviors using amphetamine (which causes dopamine efflux and increased synaptic dopamine levels) and the dopamine D₁ receptor agonist SKF-38393. Our flexible knockin approach also permitted targeted excision of the STOP cassette, allowing us to localize the impact of EAAT3 loss on repetitive behaviors.

Results

Slc1a1/EAAT3 Expression and Transporter Function Are Reduced in ST Mice.

To investigate the functional impact of *Slc1a1*/EAAT3 expression in OCD-relevant behavioral assays, we used the flexible accelerated STOP tetracycline (FAST) operator knockin system (40) to create a knockin mouse with globally reduced *Slc1a1*/EAAT3 expression (Fig. 1A). As expected, *Slc1a1* mRNA was reduced in ST mice relative to WT littermate controls (Fig. 1B) via quantitative RT-PCR (qRT-PCR) of the dorsal striatum (unpaired *t* test; $P < 0.0001$, $n = 5$ per genotype). Immunoblots of whole-striatum

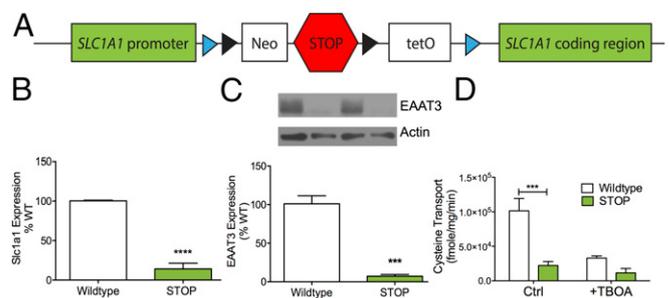


Fig. 1. ST mice have reduced *Slc1a1*/EAAT3 protein expression and function. (A) Schematic of the *Slc1a1*-STOP construct. Black triangles represent flippase recognition target sequences, and blue triangles represent LoxP sites. Neo, PGK-EM7-NEO minigene; STOP, Stop signal; tetO, tetracycline operon. (B) ST mice have reduced *Slc1a1* mRNA expression as measured by qRT-PCR of dorsal striatum (unpaired *t* test; $t = 11.81$, **** $P < 0.0001$, $n = 5$ per genotype). (C) EAAT3 protein expression is reduced in striatal synaptosome preparations from ST mice (lanes 2 and 4) relative to WT mice (lanes 1 and 3) (unpaired *t* test; $t = 8.84$, *** $P = 0.0001$, $n = 6$ per genotype). The figure is representative of three separate experiments. Average protein expression is demonstrated in the bar graph. (D) Na⁺-dependent uptake of L-cysteine (50 μM) is abolished in striatal synaptosome preparations from ST mice. The nonselective EAAT inhibitor threo-β-benzyloxyaspartate (TBOA) (100 μM) reduced WT synaptosome uptake but did not affect uptake from ST synaptosomes [two-way ANOVA; inhibitor × genotype $F(1,20) = 8.361$, $P = 0.009$; inhibitor $F(1,20) = 15.7$, $P = 0.0008$; genotype $F(1,20) = 25.34$, $P < 0.0001$, $n = 6$ per genotype; post hoc Sidak's multiple comparison test, *** $P < 0.05$]. The figure is representative of three separate experiments.

synaptosomes also demonstrated ablated EAAT3 protein expression in ST mice compared with WT littermate controls ($P = 0.0001$, $n = 6$ per genotype) (Fig. 1C).

We next probed the functional consequences of reduced EAAT3 expression using striatal synaptosome transport assays (32). Because EAAT3 is the primary source for neuronal cysteine (37), [³⁵S]cysteine was used as the substrate for EAAT3 synaptosome uptake. Na⁺-dependent uptake of cysteine in synaptosomes prepared from ST mice was ablated relative to WT synaptosomes [two-way ANOVA; inhibitor × genotype $F(1,20) = 8.361$, $P = 0.009$; inhibitor $F(1,20) = 15.7$, $P = 0.0008$; genotype $F(1,20) = 25.34$, $P < 0.0001$, $n = 6$ per genotype; post hoc Sidak's multiple comparison test, $P < 0.05$] (Fig. 1D). As expected from previous reports (31), we were unable to detect a difference in Na⁺-dependent glutamate uptake in striatal synaptosomes from ST mice relative to WT littermate controls in either the presence or absence of the EAAT inhibitor dihydrokainic acid (DHK) (Fig. S1A).

ST Mice Show No Changes in Spontaneous Behavior. ST mice and littermate controls were subjected to a battery of behavioral tasks to determine if baseline behavioral differences were present. No anxiety-like phenotypes, compulsive-like phenotypes, or deficits in sensorimotor gating (41) were observed in ST mice relative to WT littermate controls as measured by changes in open-field activity, time spent in the open arms of the elevated zero maze, light–dark emergence, prepulse inhibition, or spontaneous grooming (Fig. S2).

Pharmacological Probing of Basal Ganglia Circuitry Reveals Reductions in Basal Ganglia-Dependent Repetitive Behavior in ST Mice.

To induce basal ganglia-mediated locomotor and repetitive behaviors, D-amphetamine was administered acutely in ST mice and WT littermate controls. At a low dose (1.8 mg/kg), amphetamine-induced locomotion was significantly attenuated in ST mice relative to controls [curve-fit analysis; $F(4,496) = 6.89$, $P < 0.0001$] (Fig. 2A). A moderate dose (3.0 mg/kg) further accentuated this difference [curve-fit analysis; $F(4,496) = 13.32$, $P < 0.0001$] (Fig. 2B). At the highest amphetamine dose tested (8.0 mg/kg), at which

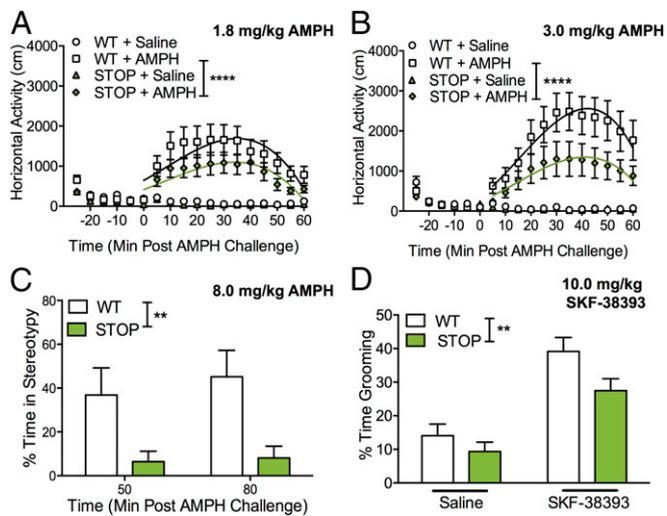


Fig. 2. ST mice have attenuated locomotor response to low- and moderate-dose amphetamine challenge. (A and B) Following acute *D*-amphetamine challenge (1.8 and 3.0 mg/kg), WT and ST mice demonstrate locomotor hyperactivity, which was attenuated in ST mice at both 1.8 mg/kg (A) [curve-fit analysis, $t = 0-60$; $F(4,496) = 6.891$, $****P < 0.0001$, $n = 14$ per genotype] and 3.0 mg/kg (B) [curve-fit analysis, $t = 0-60$; $F(4,496) = 13.32$, $****P < 0.0001$, $n = 14$ per genotype]. (C) A main effect of genotype is observed on stereotypy following high-dose amphetamine challenge (8.0 mg/kg) in ST mice and WT littermate controls. Stereotypic behavior was scored by trained, blinded, independent observers at 50 and 80 min post challenge [two-way ANOVA; genotype $F(1,42) = 12.09$, $**P = 0.001$, $n = 12$ per genotype]. (D) SKF-38393 challenge reveals a main effect of drug and genotype for grooming behavior following agonist challenge in ST mice and WT controls. Grooming behavior was scored by trained, blind, independent observers [two-way repeated-measures ANOVA; drug \times genotype, $F(1,26) = 0.74$, $P = 0.40$; drug, $F(1,26) = 28.5$, $P < 0.0001$; genotype, $F(1,26) = 7.95$, $**P = 0.0091$; $n = 14$ per genotype].

stereotypic behavior dominates over locomotion (42), there were no differences in overall locomotor behavior (Fig. S3A). However, when a separate cohort of animals was tested at this 8.0-mg/kg dose, blinded video scoring of stereotypic behavior at 50 and 80 min postamphetamine revealed a main effect of genotype in ST and WT littermate controls [two-way ANOVA; genotype $F(1,42) = 12.09$, $P = 0.0012$, $n = 12$ per genotype] (Fig. 2C and Movie S1). Mice did not exhibit stereotypic behavior following saline challenge (Fig. S3B).

To examine the impact of ablated EAAT3 expression independent of presynaptic dopamine release triggered by amphetamine administration, we acutely challenged ST mice with the dopamine D₁ receptor (D₁) agonist SKF-38393 (10 mg/kg, i.p.) to induce perseverative grooming (43, 44). Via two-way ANOVA, a main effect of genotype was identified following SKF-38393 challenge in ST mice and controls (two-way repeated-measures ANOVA; drug, $F(1,26) = 28.5$, $P < 0.0001$; genotype, $F(1,26) = 7.95$, $P = 0.0091$, $n = 14$ per genotype) (Fig. 2D).

Amphetamine-Dependent *cFos*⁺ Induction Is Decreased in the Dorsal Striatum of ST Mice. Amphetamine-induced locomotion and stereotypy are dependent on discrete subregions of the striatum (45, 46). We therefore quantified *cFos* immunoreactivity in the dorsal striatum and the nucleus accumbens (NAc) core and shell in response to amphetamine (3.0 mg/kg). In the dorsal striatum, a main effect of genotype was observed in *cFos*⁺ cells, with an amphetamine-induced increase in *cFos*⁺ cells in WT littermate controls that was absent in ST mice [two-way ANOVA; drug \times genotype; $F(1,17) = 9.91$, $P = 0.006$; genotype; $F(1,17) = 9.91$, $P = 0.006$; $n = 5$ or 6 per genotype; post hoc Sidak's multiple comparison test, $P < 0.05$] (Fig. 3A and Fig. S4A). A similar but

less robust main effect of amphetamine was observed in the NAc core [two-way ANOVA; drug \times genotype; $F(1,12) = 1.55$, $P = 0.24$; drug; $F(1,12) = 17.52$, $P = 0.001$; $n = 4$ per genotype] (Fig. 3B and Fig. S4B) and shell [two-way ANOVA; drug \times genotype $F(1,12) = 4.74$, $P = 0.05$; drug $F(1,12) = 13.59$, $P < 0.003$; $n = 4$ per genotype] in WT and ST mice (Fig. 3C and Fig. S4C).

Dopamine Receptor Density Is Reduced in Striatal Membranes of ST Mice. To assess whether the blunted dopamine agonist response in ST mice could be explained by changes in striatal dopamine receptor density, we performed binding experiments in striatal membrane preparations from both dorsal and ventral striatum using a D₁ antagonist, [³H]-SCH-23390. D₁ binding was decreased in membranes isolated from the dorsal striatum of ST mice relative to WT littermate controls (unpaired *t* test; $P = 0.008$, $n = 6$ WT mice, $n = 8$ ST mice) (Fig. 3D); however, binding estimates of D₁ density in the ventral striatum were not affected (Fig. S5A). We also measured dorsal striatal membrane binding of the D₂ receptor using a D₂ antagonist, [³H]-methylspiperone (NMSP) and observed a trend toward decreased binding (unpaired *t* test; $P = 0.058$, $n = 6$ WT mice, $n = 8$ ST mice) (Fig. S5B).

Decreased Striatal Dopaminergic Transmission in ST Mice. To corroborate a previous report of no change in dopamine neuron numbers or morphology in *Slc11l*-null mice at 3 mo of age (47), we performed dopamine transporter (DAT) immunohistochemistry

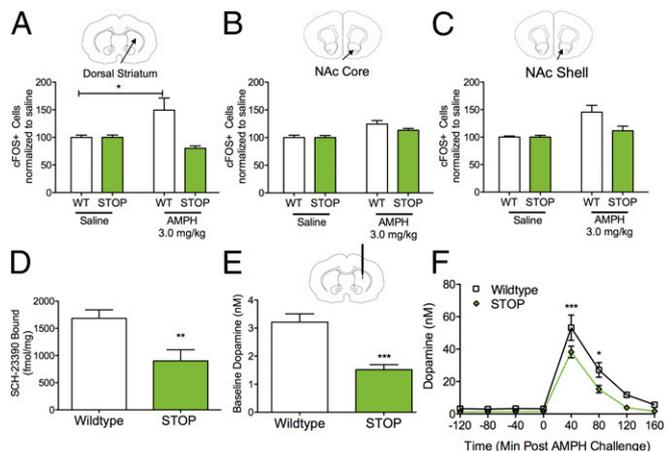


Fig. 3. EAAT3 loss affects amphetamine-induced *cFos* expression, dopamine receptor membrane density, and extracellular dopamine concentrations in the dorsal striatum. (A–C) Quantification of *cFos*⁺ cells was performed in the dorsal striatum (A), NAc core (B), and NAc shell (C). (A) Staining for *cFos*⁺ cells reveals an amphetamine (3.0 mg/kg, i.p.)-dependent increase in the dorsal striatum of WT mice that is absent in ST mice (two-way ANOVA; drug \times genotype; $F(1,17) = 9.91$, $P = 0.006$; genotype; $F(1,17) = 9.91$, $P = 0.006$; $n = 5$ or 6 per genotype; post hoc Sidak's multiple comparison test, $*P < 0.05$). (B) Staining for *cFos*⁺ cells reveals a main effect of amphetamine on *cFos*⁺ cells in the NAc core of WT and ST mice (two-way ANOVA; drug, $F(1,12) = 17.52$, $P < 0.01$; $n = 4$ per genotype). (C) Staining for *cFos*⁺ cells reveals a main effect of amphetamine on *cFos*⁺ cells in the NAc shell of WT and ST mice and a trend-level interaction and genotype effect [two-way ANOVA; drug \times genotype and genotype $F(1,12) = 4.74$, $P = 0.05$; drug $F(1,12) = 13.59$, $P < 0.01$; $n = 4$ per genotype]. (D) Dopamine D₁ receptor density estimated with [³H]-SCH-23390 binding in dorsal striatum membrane preparations (unpaired *t* test; $t = 3.1$, $**P = 0.008$, $n = 8$ WT, $n = 6$ ST). (E) ST mice have significantly lowered dopamine levels at baseline as measured by the extrapolation of linear regression using no-net-flux microdialysis (unpaired *t* test, $t = 4.89$, $***P = 0.0006$, $n = 6$ per genotype). (F) Dorsal striatal dopamine levels are significantly reduced in ST mice relative to WT controls following systemic administration of amphetamine (3 mg/kg, i.p.) [two-way repeated-measures ANOVA; time \times genotype, $F(7,70) = 2.52$, $P = 0.0226$; genotype $F(1,10) = 9.34$, $P = 0.01$; Sidak's multiple comparison; $***P < 0.001$, $*P < 0.05$; $n = 6$ per genotype].

in the midbrain of 10- to 12-wk-old ST mice and littermate controls and found no differences in stereological DAT⁺ cell number estimates (Fig. S6). To explore presynaptic mechanisms that could account for altered response to dopamine agonists in the ST mice, we measured tissue levels of dopamine and its major metabolite, 3,4-Dihydroxyphenylacetic acid (DOPAC), 30 min after amphetamine (3.0 mg/kg i.p.) or saline injection. Via two-way ANOVA, significant drug effects were observed on SN dopamine and DOPAC, VTA DOPAC, and dorsal striatum dopamine (Fig. S7). A main effect of genotype was observed only in SN DOPAC (Fig. S7).

We next investigated if extracellular striatal dopamine levels were altered in ST mice relative to WT controls. Basal, steady-state extracellular dopamine levels were first measured using the quantitative technique of no-net-flux microdialysis (48). In freely moving mice, dorsal striatal extracellular dopamine levels were found to be significantly lower in ST mice than in WT littermate controls (unpaired *t* test; $P = 0.0006$, $n = 6$ per genotype) (Fig. 3E). No differences were observed in dopamine clearance as measured by the slope of the no-net-flux regression line (Fig. S8A). Conventional microdialysis revealed a significant elevation in dopamine levels following amphetamine (3 mg/kg, i.p.) compared with baseline levels in both WT and ST mice. However, absolute levels of dopamine after amphetamine were significantly reduced in ST mice compared with WT controls [repeated-measures two-way ANOVA; time \times genotype, $F(7,70) = 2.52$, $P = 0.0226$; genotype $F(1,10) = 9.34$, $P = 0.01$; Sidak's multiple comparison; $P < 0.001$; $P < 0.05$; $n = 6$ per genotype] (Fig. 3F). No genotypic differences were seen in dopamine metabolite levels at baseline or in response to amphetamine challenge (Fig. S8 B and C).

Viral-Mediated Rescue of *Slc1a1*/EAAT3 in the Midbrain Attenuates Amphetamine-Induced Behavioral Deficits in ST Mice. To test the hypothesis that our findings could be explained by the impact of EAAT3 ablation on midbrain dopaminergic neurons, we took advantage of the FAST construct to restore *Slc1a1*/EAAT3 expression in the midbrain of ST mice via Cre-Lox recombination. ST mice were bilaterally infused with either AAVrh10-CMV.Cre (ST:Cre) or AAVrh10-CMV.eGFP (ST:GFP) in the central midbrain [−3.3 mm anteroposterior (AP), ±0.4 mm mediolateral (ML), −4.5 mm dorsoventral (DV)], and WT littermate controls were bilaterally infused with AAVrh10-CMV.Cre (WT:Cre) (Fig. 4A). After a 2-wk incubation period, we found that ST:Cre animals showed a greater locomotor response to amphetamine (3.0 mg/kg) than ST:GFP control animals [curve-fit analysis; $F(4,478) = 6.84$, $P < 0.0001$, $n = 12$ ST:GFP, $n = 15$ ST:Cre] (Fig. 4B); however, their locomotor response remained less than that of WT animals. We also observed a significant main effect of Cre virus in ST:Cre and ST:GFP controls following high-dose (8.0 mg/kg) amphetamine-induced stereotypy [two-way ANOVA; Cre virus $F(1,46) = 9.45$, $P = 0.0035$] (Fig. 4C). No difference in perseverative grooming was observed between ST:GFP and ST:Cre mice after injection of the D₁ agonist SKF-38393 [two-way ANOVA; drug, $F(1,50) = 19.2$, $P < 0.0001$; virus, $F(1,50) = 0.13$, $P = 0.72$; $n = 12$ ST:GFP mice, $n = 15$ ST:Cre mice] (Fig. 4D). Rescue of EAAT3 expression and viral spread in the midbrain of ST:Cre mice was confirmed via Western blot (Fig. S9) and immunohistochemistry (Fig. S10). To verify the specificity of midbrain rescue, we assessed the impact of viral Cre-mediated restoration of EAAT3 in the dorsal striatum and found no differences in amphetamine- or SKF-38393-mediated repetitive behavior in comparison with GFP controls (Fig. S11).

Discussion

Multiple studies have identified linkage and association of *SLC1A1*/EAAT3 with OCD (20–25, 49, 50); however, this *in vivo* study assesses whether changes in EAAT3 expression affect repetitive behavior. By using amphetamine as well as a dopamine D₁ receptor agonist, we were able to probe the effects of EAAT3 ablation on basal ganglia-mediated behavior. As hypothesized,

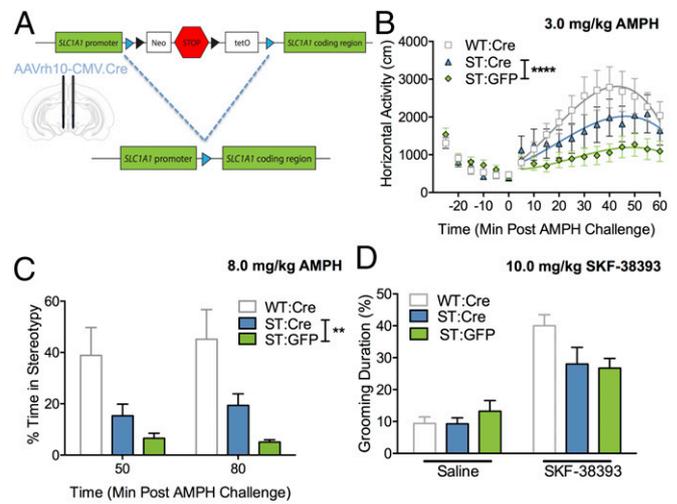


Fig. 4. Viral Cre-mediated rescue of midbrain *Slc1a1*/EAAT3 expression rescues amphetamine but not SKF-38393 phenotypes in ST mice. (A) Schematic of Cre-mediated excision of the neo-STOP-tetO cassette in ST mice, leading to endogenous *Slc1a1*/EAAT3 expression. Blue triangles represent LoxP sites. The drawing indicates the injection position in the central midbrain of ST mice and WT controls (AP −3.3 mm, ML ±0.4 mm, DV −4.5 mm). (B) ST:Cre mice exhibit an increased hyperlocomotor response to 3.0 mg/kg amphetamine in comparison with ST:GFP littermate controls [curve-fit analysis, $t = 0-60$; $F(4,478) = 6.84$, $****P < 0.0001$; $n = 12$ ST:GFP, $n = 15$ ST:Cre]. (C) A main effect of Cre virus is observed on stereotypy following high-dose amphetamine challenge (8.0 mg/kg) in ST:Cre and ST:GFP controls. Stereotypic behavior was scored by trained, blinded, independent observers at 50 and 80 min post challenge [two-way ANOVA; Cre virus, $F(1,46) = 9.453$, $**P = 0.0035$; $n = 12$ ST:GFP, $n = 14$ ST:Cre]. (D) ST:Cre mice showed no difference in stereotyped grooming behavior in response to 10 mg/kg SKF-38393 in comparison with ST:GFP littermate controls [two-way ANOVA; drug, $F(1,50) = 19.2$, $P < 0.0001$; virus, $F(1,50) = 0.13$, $P = 0.72$; $n = 12$ ST:GFP, $n = 15$ ST:Cre].

we detected a decrease in hyperlocomotion, stereotypic behavior, and striatal dopamine concentrations in response to these drugs in ST mice (Figs. 2 and 3). Coupled with our findings of partial Cre-mediated rescue in the midbrain, this decrease in repetitive behavior is consistent with multiple previous lines of evidence supporting a role for dopaminergic pathology in compulsive-like behavior. Most immediately relevant to our findings, the *Hdc*-null mouse model, based upon a family demonstrating a complex neuropsychiatric phenotype including complete penetrance for Tourette syndrome and partial penetrance for OCD, displayed elevated stereotypy in response to amphetamine challenge (42). In humans, dopamine agonists, including amphetamine and the dopamine precursor L-DOPA, are well-known triggers of repetitive behavior, from simple motor movements to frankly compulsive behavior in disorders linked to altered dopamine homeostasis (51–54). Indirect evidence also suggests an interaction between the dopamine system and *SLC1A1* in humans. Atypical antipsychotic medications, which act in part as dopamine receptor antagonists, trigger obsessive-compulsive symptoms in some patients, and recent evidence suggests that polymorphisms in *SLC1A1* moderate susceptibility to this uncommon drug-induced compulsivity (55, 56).

Our data show that EAAT3 ablation leads to decreased immediate early gene activation in dorsal striatal neurons in response to amphetamine (Fig. 3A), in addition to reductions in extracellular dopamine concentrations in the striatum at baseline and following amphetamine challenge (Fig. 3 E–F). These data are consistent with a change in presynaptic dopaminergic neuron function as a result of EAAT3 loss and align with a recent study demonstrating that EAAT3 impacts glutamatergic input onto midbrain dopaminergic neurons (30). Specifically, Underhill and

colleagues (30) found that EAAT3 is internalized in response to amphetamine, resulting in increased glutamate exposure and potentiation of AMPA and NMDA glutamate receptor-mediated synaptic transmission in midbrain dopamine neurons. Based on these convergent data, we hypothesized that chronic increases in perisynaptic glutamate levels at dopaminergic neurons may elicit a homeostatic mechanism in ST mice that underlies the attenuated response to amphetamine. In support of this idea, we found that viral-mediated rescue of midbrain *Slc1a1*/EAAT3 resulted in increased amphetamine-induced locomotor and stereotypy behavior compared with ST mice infused with a control virus (Fig. 4 B and C). This impact of midbrain viral rescue contrasted with rescue of *Slc1a1*/EAAT3 in the striatum of ST mice, which had no impact on amphetamine-induced behavior (Fig. S11). Importantly, the lack of complete rescue of amphetamine response, as well as the lack of change in D₁ agonist response, suggests that EAAT3 ablation in dopaminergic neurons (at least in adult animals) may not be the only mechanism impacting response to dopamine agonists in ST mice. Further study of EAAT3 ablation, restoration, and overexpression will be needed to dissect its importance in specific brain regions, neuronal subtypes, and, importantly, within particular developmental windows.

Of note, the initial published evaluation of *Slc1a1*/EAAT3-null mice reported decreased activity in the open field, although this result was not consistent with a later report, which described no baseline differences but found impaired Morris Water Maze performance in aged animals as the result of oxidative stress-mediated neuronal loss (36, 57). In our baseline assessment of activity, anxiety-like behavior, and compulsive-like behavior, we found no significant changes in the ST animals. This lack of effect on baseline behavior is consistent with some data suggesting that the OCD-associated *SLC1A1* alleles lead to increased, not decreased, expression; however, postmortem studies are needed to clarify the direction of the change in *SLC1A1* expression in OCD (25, 58). Examining the impact of *SLC1A1* overexpression on OCD-relevant behaviors in mice also would assess this hypothesis more directly. These data also may indicate the difficulty of detecting a potential decrease in low levels of spontaneous compulsive-like behavior (25). In addition, one subsequent report in *Slc1a1*/EAAT3-null mice described oxidative stress-mediated loss of dopamine neurons in animals at 12 mo of age but no differences at 3 mo (57). Even though our model does retain some degree of preserved EAAT3 function (Fig. 1), we therefore restricted our work to younger animals and ruled out decreases in DAT immunohistochemistry (Fig. S6) or diminished dopamine or DOPAC levels in the midbrain (Fig. S7). Furthermore, the results of our midbrain viral rescue (Fig. 4) are not consistent with dopamine neuron loss as a mechanism of altered response to amphetamine or SKF-38393.

As with many studies aimed at unraveling pathophysiology in a preclinical context, it is important not to over-interpret these data in relation to the human condition. We therefore believe that the ST mouse should be considered as a putative model of reduced liability to dopamine-induced and basal ganglia-mediated repetitive behaviors (42). Although heterozygous *SLC1A1* deletions have been reported in schizophrenia and schizoaffective disorder (27, 28), our findings do not clearly indicate a psychosis-like phenotype, because of the absence of observed changes in baseline behavior or

prepulse inhibition. In the context of psychotic disorders, our observation of decreased sensitivity to amphetamine could be considered the opposite of what might be expected, because amphetamine can induce psychosis in humans (59). Because of this apparent contradiction, further work using the ST mice, including heterozygous animals, is warranted to understand better the potential contribution of *SLC1A1* deletions to the risk of psychosis.

In summary, we report the evaluation of the OCD candidate gene *Slc1a1*/EAAT3 in relation to OCD-relevant circuitry and behavior in an animal model. Using dopaminergic agonism as a probe, we demonstrate the relevance of EAAT3 to striatal dopaminergic neurotransmission and to repetitive behavior. The partial rescue of dopamine agonist response by restoration of EAAT3 expression in the midbrain demonstrates an *in vivo* functional impact that matches previous cell model and *ex vivo* reports of EAAT3 effects in dopaminergic neurons. More work is needed to examine the effects of manipulating EAAT3 expression in other proposed models of strially mediated repetitive behavior (13, 14) and in cognitive tasks relevant to OCD (60, 61). Our results also suggest that EAAT3 antagonists should be evaluated in relation to dopamine agonist response and, perhaps, more broadly in relation to basal ganglia-mediated repetitive behavior across species.

Materials and Methods

A brief summary of experimental procedures is provided here; additional details are available in *SI Materials and Methods*. Raw counts of *cFos*⁺ cells following saline or amphetamine challenge in the dorsal striatum, NAC, and somatosensory cortex of WT and ST mice are given in Table S1. DAT⁺ stereology sampling parameters are given in Table S2.

ST Mice. All animal care and testing were approved by the New York State Psychiatric Institute (NYSPI) or Vanderbilt Institutional Animal Care and Use Committee and were in accordance with the NIH's *Guide for the Care and Use of Laboratory Animals* (62). Homologous recombination was used to introduce a floxed-NeoSTOP-tetO-*Slc1a1* cassette into the native *Slc1a1* locus (Fig. 1A and *SI Materials and Methods*). Behavioral experiments were performed in 8- to 16-wk-old ST and WT littermate control mice in the NYSPI Rodent Neuro-analytical Core or the Vanderbilt Laboratory for Neurobehavior Core Facility between 1000 and 1600 hours. A preliminary behavioral experiment at Vanderbilt ($n = 6$ ST mice and 6 controls) showed similar behavioral result trends; however because variances and absolute values differed from those tested at Columbia University/NYSPI, these results are not included. Viral rescue experiments used bilateral injections of 0.3 μ L of AAVrh10.CMV.PI.Cre.rBG (University of Pennsylvania Vector Core) or AAVrh10.CMV.PI.eGFP.WPRE.bGH (University of Pennsylvania Vector Core) at a titer of 1×10^{13} genomic copies/mL. Animals recovered at least 2 wk before testing.

Statistical Analysis. Data were analyzed using Prism (GraphPad). Two-tailed, unpaired Student *t* test or two-way ANOVA with Sidak's posttests was used to analyze all primary data except for locomotor data, which were analyzed using nonlinear curve-fit analysis. Specific statistical analyses for each dataset are described in *Results* and in figure legends. In the text and figures, all data are reported and shown as the mean \pm SEM.

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