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 D_1 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 midbrain 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 D_1 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. 1*A*). As expected, *Slc1a1* mRNA was reduced in ST mice relative to WT littermate controls (Fig. 1*B*) via quantitative RT-PCR (qRT-PCR) of the dorsal striatum (unpaired *t* test; $P < 0.0001$, $n = 5$ per genotype). Immunoblots of whole-striatum synaptosomes also demonstrated ablated EAAT3 protein expression in ST mice compared with WT littermate controls ($P = 0.0001$, $n = 6$ per genotype) (Fig. 1*C*).

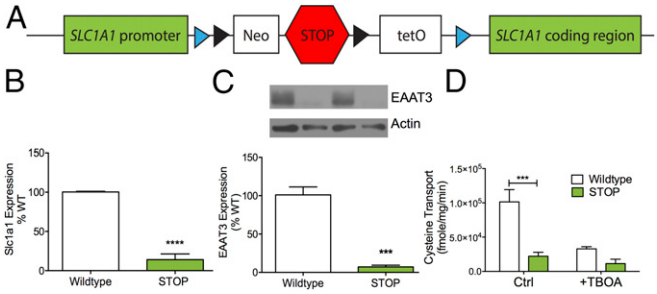

Fig. 1. ST mice have reduced *Slc1a1*/EAAT3 protein expression and function. (*A*) Schematic of the *Slc1a1*-STOP construct. Black triangles represent flippase recognition target sequences, and blue triangles represent LoxP sites. Neo, PGK-EM7-NEO minigene; STOP, Stop signal; tetO, tetracycline operon. (*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.

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), [^{35}S]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. 1*D*). 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. S1*A*).

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. 2*A*). A moderate dose (3.0 mg/kg) further accentuated this difference [curve–fit analysis; $F(4,496) = 13.32$, $P < 0.0001$] (Fig. 2*B*). At the highest amphetamine dose tested (8.0 mg/kg), at which

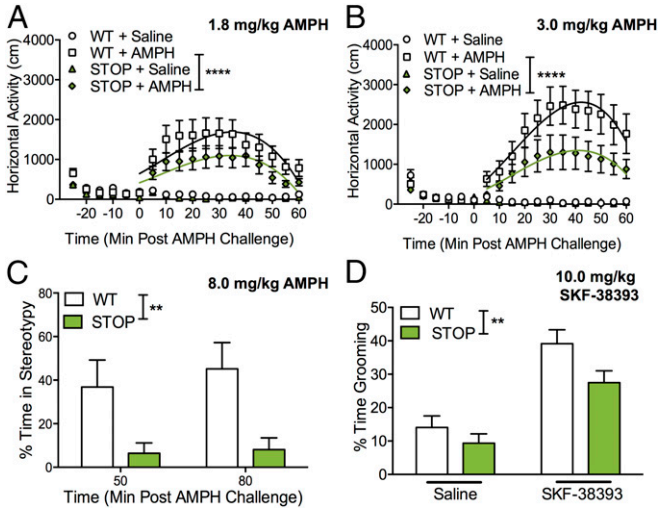

Fig. 2. ST mice have attenuated locomotor response to low- and moderate-dose amphetamine challenge. (*A* and *B*) Following acute D-amphetamine challenge (1.8 and 3.0 mg/kg), WT and ST mice demonstrate locomotor hyperactivity, which was attenuated in ST mice at both 1.8 mg/kg (*A*) [curve–fit analysis, $t = 0$–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 × 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. S3*A*). 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. 2*C* and Movie S1). Mice did not exhibit stereotypic behavior following saline challenge (Fig. S3*B*).

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 D_1 receptor (D_1) 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. 2*D*).

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 × 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. 3*A* and Fig. S4*A*). A similar but less robust main effect of amphetamine was observed in the NAc core [two-way ANOVA; drug × genotype; $F(1,12) = 1.55$, $P = 0.24$; drug; $F(1,12) = 17.52$, $P = 0.001$; $n = 4$ per genotype] (Fig. 3*B* and Fig. S4*B*) and shell [two-way ANOVA; drug × 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. 3*C* and Fig. S4*C*).

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 D_1 antagonist, [^{3}H]-SCH-23390. D_1 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. 3*D*); however, binding estimates of D_1 density in the ventral striatum were not affected (Fig. S5*A*). We also measured dorsal striatal membrane binding of the D_2 receptor using a D_2 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. S5*B*).

Decreased Striatal Dopaminergic Transmission in ST Mice. To corroborate a previous report of no change in dopamine neuron numbers or morphology in *Slc1a1*-null mice at 3 mo of age (47), we performed dopamine transporter (DAT) immunohistochemistry

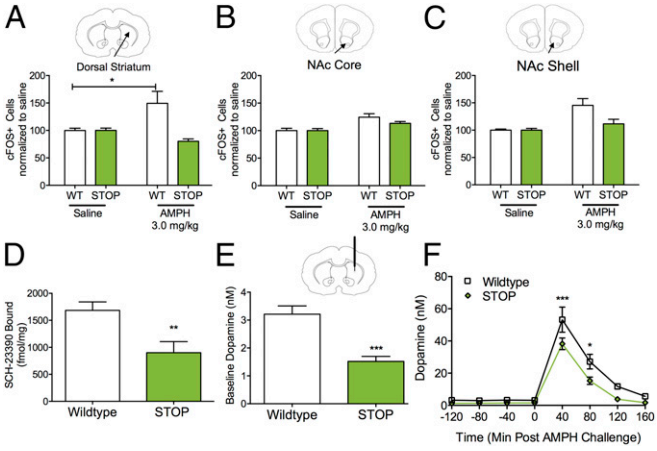

Fig. 3. EAAT3 loss affects amphetamine-induced *cFos* expression, dopamine receptor membrane density, and extracellular dopamine concentrations in the dorsal striatum. (*A*–*C*) Quantification of *cFos*$^+$ cells was performed in the dorsal striatum (*A*), NAc core (*B*), and NAc shell (*C*). (*A*) Staining 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 × 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 × 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 D_1 receptor density estimated with [^{3}H]-SCH-23390 binding in dorsal striatum membrane preparations (unpaired *t* test; $t = 3.1$, $^{**}P = 0.008$, $n = 8$ WT, $n = 6$ STOP). (*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 × 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. 3*E*). No differences were observed in dopamine clearance as measured by the slope of the no-net-flux regression line (Fig. S8*A*). 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 × 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. 3*F*). 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. 4*A*). 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. 4*B*); 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. 4*C*). No difference in perseverative grooming was observed between ST:GFP and ST:Cre mice after injection of the D_1 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. 4*D*). 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).

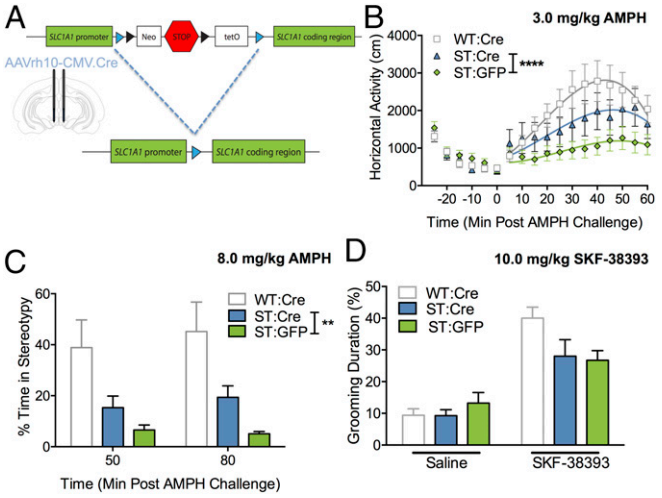

Fig. 4. Viral Cre-mediated rescue of midbrain *Slc1a1*/EAAT3 expression rescues amphetamine but not SKF-38393 phenotypes in ST mice. (*A*) Schematic of Cre-mediated excision of the neo-STOP-tetO cassette in ST mice, leading to endogenous *Slc1a1*/EAAT3 expression. Blue triangles represent LoxP sites. 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].

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 D_1 receptor agonist, we were able to probe the effects of EAAT3 ablation on basal ganglia-mediated behavior. As hypothesized, 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. 3*A*), 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 D_1 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 striatally 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. 1*A* 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 Neuroanalytical 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 μL 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 ± SEM.

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1. Koran LM, Hanna GL, Hollander E, Nestadt G, Simpson HB (2007) Practice guideline for the treatment of patients with obsessive-compulsive disorder. *Am J Psychiatry* 164(7, Suppl):5–53.
2. Michael S, Ritsner AGA (2007) Quality of life impairment in schizophrenia, mood and anxiety disorders. *New Perspectives on Research and Treatment* (Springer International Publishing, Cham, Switzerland).
3. Dougherty DD, Rauch SL, Jenike MA (2004) Pharmacotherapy for obsessive-compulsive disorder. *J Clin Psychol* 60:1195–1202.
4. Simpson HB, et al. (2013) Cognitive-behavioral therapy vs risperidone for augmenting serotonin reuptake inhibitors in obsessive-compulsive disorder: A randomized clinical trial. *JAMA Psychiatry* 70:1190–1199.
5. Sarris J, et al. (2015) N-acetyl cysteine (NAC) in the treatment of obsessive-compulsive disorder: A 16-week, double-blind, randomised, placebo-controlled study. *CNS Drugs* 29:801–809.
6. Aouizerate B, et al. (2004) Deep brain stimulation of the ventral caudate nucleus in the treatment of obsessive-compulsive disorder and major depression. Case report. *J Neurosurg* 101:682–686.
7. Fontaine D, et al. (2004) Effect of subthalamic nucleus stimulation on obsessive-compulsive disorder in a patient with Parkinson disease. Case report. *J Neurosurg* 100:1084–1086.
8. Radua J, Mataix-Cols D (2009) Voxel-wise meta-analysis of grey matter changes in obsessive-compulsive disorder. *Br J Psychiatry* 195:393–402.
9. Rosenberg DR, et al. (1997) Frontostriatal measurement in treatment-naive children with obsessive-compulsive disorder. *Arch Gen Psychiatry* 54:824–830.

10. Menzies L, et al. (2008) Integrating evidence from neuroimaging and neuropsychological studies of obsessive-compulsive disorder: The orbitofronto-striatal model revisited. *Neurosci Biobehav Rev* 32:525–549.
11. Starck G, et al. (2008) A 1H magnetic resonance spectroscopy study in adults with obsessive compulsive disorder: Relationship between metabolite concentrations and symptom severity. *J Neural Trans* 115:1051–1062.
12. Whiteside SP, Port JD, Deacon BJ, Abramowitz JS (2006) A magnetic resonance spectroscopy investigation of obsessive-compulsive disorder and anxiety. *Psychiatry Res* 146:137–147.
13. Welch JM, et al. (2007) Cortico-striatal synaptic defects and OCD-like behaviours in Sapap3-mutant mice. *Nature* 448:894–900.
14. Shmelkov SV, et al. (2010) Slitrk5 deficiency impairs corticostriatal circuitry and leads to obsessive-compulsive-like behaviors in mice. *Nat Med* 16:598–602, 591p following 602.
15. Ahmari SE, et al. (2013) Repeated cortico-striatal stimulation generates persistent OCD-like behavior. *Science* 340:1234–1239.
16. Burguiere E, Monteiro P, Feng G, Graybiel AM (2013) Optogenetic stimulation of lateral orbitofronto-striatal pathway suppresses compulsive behaviors. *Science* 340: 1243–1246.
17. Pauls DL (2008) The genetics of obsessive compulsive disorder: A review of the evidence. *Am J Med Genet C Semin Med Genet* 148C:133–139.
18. Hanna GL, et al. (2002) Genome-wide linkage analysis of families with obsessive-compulsive disorder ascertained through pediatric probands. *Am J Med Genet* 114: 541–552.
19. Willour VL, et al. (2004) Replication study supports evidence for linkage to 9p24 in obsessive-compulsive disorder. *Am J Hum Genet* 75:508–513.
20. Veenstra-VanderWeele J, et al. (2001) Genomic organization of the SLC1A1/EAAC1 gene and mutation screening in early-onset obsessive-compulsive disorder. *Mol Psychiatry* 6:160–167.
21. Arnold PD, Sicard T, Burroughs E, Richter MA, Kennedy JL (2006) Glutamate transporter gene SLC1A1 associated with obsessive-compulsive disorder. *Arch Gen Psychiatry* 63:769–776.
22. Dickel DE, et al. (2006) Association testing of the positional and functional candidate gene SLC1A1/EAAC1 in early-onset obsessive-compulsive disorder. *Arch Gen Psychiatry* 63:778–785.
23. Stewart SE, et al. (2007) Association of the SLC1A1 glutamate transporter gene and obsessive-compulsive disorder. *Am J Med Gen* 144B:1027–1033.
24. Shugart YY, et al. (2009) A family-based association study of the glutamate transporter gene SLC1A1 in obsessive-compulsive disorder in 378 families. *Am J Med Gen* 150B:886–892.
25. Wendland JR, et al. (2009) A haplotype containing quantitative trait loci for SLC1A1 gene expression and its association with obsessive-compulsive disorder. *Arch Gen Psychiatry* 66:408–416.
26. Stewart SE, et al. (2013) Meta-analysis of association between obsessive-compulsive disorder and the 3′ region of neuronal glutamate transporter gene SLC1A1. *Am J Med Genet B Neuropsychiatr Genet* 162B:367–379.
27. Afshari P, et al. (2015) Characterization of a novel mutation in SLC1A1 associated with schizophrenia. *Mol Neuropsychiatry* 1:125–144.
28. Myles-Worsley M, et al. (2013) Deletion at the SLC1A1 glutamate transporter gene cosegregates with schizophrenia and bipolar schizoaffective disorder in a 5-generation family. *Am J Med Genet B Neuropsychiatr Genet* 162B:87–95.
29. Horiuchi Y, et al. (2012) Association of SNPs linked to increased expression of SLC1A1 with schizophrenia. *Am J Med Genet B Neuropsychiatr Genet* 159B:30–37.
30. Underhill SM, et al. (2014) Amphetamine modulates excitatory neurotransmission through endocytosis of the glutamate transporter EAAT3 in dopamine neurons. *Neuron* 83:404–416.
31. Holmseth S, et al. (2012) The density of EAAC1 (EAAT3) glutamate transporters expressed by neurons in the mammalian CNS. *J Neurosci* 32:6000–6013.
32. Nieoullon A, et al. (2006) The neuronal excitatory amino acid transporter EAAC1/EAAT3: Does it represent a major actor at the brain excitatory synapse? *J Neurochem* 98:1007–1018.
33. Bjorn-Yoshimoto W, Underhill SM (2016) The importance of the excitatory amino acid transporter 3 (EAAT3). *Neurochem Int*.
34. Otis TS, Brasnjo G, Dzubay JA, Pratap M (2004) Interactions between glutamate transporters and metabotropic glutamate receptors at excitatory synapses in the cerebellar cortex. *Neurochem Int* 45:537–544.
35. Mathews GC, Diamond JS (2003) Neuronal glutamate uptake Contributes to GABA synthesis and inhibitory synaptic strength. *J Neurosci* 23:2040–2048.
36. Aoyama K, et al. (2006) Neuronal glutathione deficiency and age-dependent neurodegeneration in the EAAC1 deficient mouse. *Nat Neurosci* 9:119–126.
37. Watts SD, Torres-Salazar D, Divito CB, Amara SG (2014) Cysteine transport through excitatory amino acid transporter 3 (EAAT3). *PLoS One* 9:e109245.
38. Scimemi A, Tian H, Diamond JS (2009) Neuronal transporters regulate glutamate clearance, NMDA receptor activation, and synaptic plasticity in the hippocampus. *J Neurosci* 29:14581–14595.
39. Li MH, et al. (2017) Amphetamine and methamphetamine increase NMDAR-GluN2B synaptic currents in midbrain dopamine neurons. *Neuropsychopharmacol*.
40. Tanaka KF, et al. (2010) Flexible accelerated STOP tetracycline operator-knockin (FAST): A versatile and efficient new gene modulating system. *Biol Psychiatry* 67: 770–773.
41. Ahmari SE, Risbrough VB, Geyer MA, Simpson HB (2012) Impaired sensorimotor gating in unmedicated adults with obsessive-compulsive disorder. *Neuropsychopharmacol* 37:1216–1223.
42. Castellan Baldan L, et al. (2014) Histidine decarboxylase deficiency causes tourette syndrome: Parallel findings in humans and mice. *Neuron* 81:77–90.
43. Taylor JL, Rajbhandari AK, Berridge KC, Aldridge JW (2010) Dopamine receptor modulation of repetitive grooming actions in the rat: Potential relevance for Tourette syndrome. *Brain Res* 1322:92–101.
44. Starr BS, Starr MS (1986) Grooming in the mouse is stimulated by the dopamine D1 agonist SKF 38393 and by low doses of the D1 antagonist SCH 23390, but is inhibited by dopamine D2 agonists, D2 antagonists and high doses of SCH 23390. *Pharmacol Biochem Behav* 24:837–839.
45. Rebec GV, White IM, Puotz JK (1997) Responses of neurons in dorsal striatum during amphetamine-induced focused stereotypy. *Psychopharmacology (Berl)* 130:343–351.
46. Ikemoto S (2002) Ventral striatal anatomy of locomotor activity induced by cocaine, D-amphetamine, dopamine and D1/D2 agonists. *Neuroscience* 113:939–955.
47. Berman AE, et al. (2011) N-acetylcysteine prevents loss of dopaminergic neurons in the EAAC1–/– mouse. *Ann Neurol* 69:509–520.
48. Chefer VI, Zapata A, Shippenberg TS, Bungay PM (2006) Quantitative no-net-flux microdialysis permits detection of increases and decreases in dopamine uptake in mouse nucleus accumbens. *J Neurosci Methods* 155:187–193.
49. Veenstra-VanderWeele J, et al. (2012) Functional studies and rare variant screening of SLC1A1/EAAC1 in males with obsessive-compulsive disorder. *Psychiatr Genet* 22: 256–260.
50. Samuels J, et al. (2011) Comprehensive family-based association study of the glutamate transporter gene SLC1A1 in obsessive-compulsive disorder. *Am J Med Gen* 156B: 472–477.
51. Varley CK, Vincent J, Varley P, Calderon R (2001) Emergence of tics in children with attention deficit hyperactivity disorder treated with stimulant medications. *Compr Psychiatry* 42:228–233.
52. Madruga-Garrido M, Mir P (2013) Tics and other stereotyped movements as side effects of pharmacological treatment. *Int Rev Neurobiol* 112:481–494.
53. Parraga HC, Parraga MI, Harris DK (2007) Tic exacerbation and precipitation during atomoxetine treatment in two children with attention-deficit hyperactivity disorder. *Int J Psychiatry Med* 37:415–424.
54. Voon V, et al. (2009) Chronic dopaminergic stimulation in Parkinson's disease: From dyskinesias to impulse control disorders. *Lancet Neurol* 8:1140–1149.
55. Kwon JS, et al. (2009) Association of the glutamate transporter gene SLC1A1 with atypical antipsychotics-induced obsessive-compulsive symptoms. *Arch Gen Psychiatry* 66:1233–1241.
56. Cai J, et al. (2013) Influence of polymorphisms in genes SLC1A1, GRIN2B, and GRIK2 on clozapine-induced obsessive-compulsive symptoms. *Psychopharmacology (Berl)* 230:49–55.
57. Peghini P, Janzen J, Stoffel W (1997) Glutamate transporter EAAC-1-deficient mice develop dicarboxylic aminoaciduria and behavioral abnormalities but no neurodegeneration. *EMBO J* 16:3822–3832.
58. Porton B, et al. (2013) Isoforms of the neuronal glutamate transporter gene, SLC1A1/EAAC1, negatively modulate glutamate uptake: Relevance to obsessive-compulsive disorder. *Transl Psychiatry* 3:e259.
59. Murray RM, Paparelli A, Morrison PD, Marconi A, Di Forti M (2013) What can we learn about schizophrenia from studying the human model, drug-induced psychosis? *Am J Med Genet B Neuropsychiatr Genet* 162B:661–670.
60. Krebs G, Heyman I (2015) Obsessive-compulsive disorder in children and adolescents. *Arch Dis Child* 100:495–499.
61. Dittrich WH, Johansen T (2013) Cognitive deficits of executive functions and decision-making in obsessive-compulsive disorder. *Scand J Psychol* 54:393–400.
62. Franklin KBJ, Paxinos G (2013) *Paxinos and Franklin's The Mouse Brain in Stereotaxic Coordinates* (Academic, an imprint of Elsevier, Amsterdam), 4th Ed, Vol 1 (unpaged).
63. National Research Council (2011) *Guide for the Care and Use of Laboratory Animals* (National Academies Press, Washington, DC), 8th Ed.
64. Furness DN, et al. (2008) A quantitative assessment of glutamate uptake into hippocampal synaptic terminals and astrocytes: New insights into a neuronal role for excitatory amino acid transporter 2 (EAAT2). *Neuroscience* 157:80–94.
65. Pakkenberg B, Gundersen HJ (1989) New stereological method for obtaining unbiased and efficient estimates of total nerve cell number in human brain areas. Exemplified by the mediodorsal thalamic nucleus in schizophrenics. *APMIS* 97:677–681.
66. Gondre-Lewis MC, Darius PJ, Wang H, Allard JS (2016) Stereological analyses of reward system nuclei in maternally deprived/separated alcohol drinking rats. *J Chem Neuroanat* 76:122–132.