




Lower excitatory synaptic gene expression in orbitofrontal cortex and striatum in an initial study of subjects with obsessive compulsive disorder

Sean C. Piantadosi^{1,2} · Brittany L. Chamberlain² · Jill R. Glausier² · David A. Lewis^{1,2}  · Susanne E. Ahmari^{1,2}

Received: 7 November 2018 / Revised: 4 March 2019 / Accepted: 3 April 2019 / Published online: 5 June 2019
© The Author(s), under exclusive licence to Springer Nature Limited 2019

Abstract

Obsessive compulsive disorder (OCD) is a severe illness that affects 2–3% of people worldwide. OCD neuroimaging studies have consistently shown abnormal activity in brain regions involved in decision-making (orbitofrontal cortex [OFC]) and action selection (striatum). However, little is known regarding molecular changes that may contribute to abnormal function. We therefore examined expression of synaptic genes in post-mortem human brain samples of these regions from eight pairs of unaffected comparison and OCD subjects. Total grey matter tissue samples were obtained from medial OFC (BA11), lateral OFC (BA47), head of caudate, and nucleus accumbens (NAc). Quantitative polymerase chain reaction (qPCR) was then performed on a panel of transcripts encoding proteins related to excitatory synaptic structure, excitatory synaptic receptors/transporters, and GABA synapses. Relative to unaffected comparison subjects, OCD subjects had significantly lower levels of several transcripts related to excitatory signaling in both cortical and striatal regions. However, a majority of transcripts encoding excitatory synaptic proteins were lower in OFC but not significantly different in striatum of OCD subjects. Composite transcript level measures supported these findings by revealing that reductions in transcripts encoding excitatory synaptic structure proteins and excitatory synaptic receptors/transporters occurred primarily in OFC of OCD subjects. In contrast, transcripts associated with inhibitory synaptic neurotransmission showed minor differences between groups. The observed lower levels of multiple glutamatergic transcripts across both medial and lateral OFC may suggest an upstream causal event. Together, these data provide the first evidence of molecular abnormalities in brain regions consistently implicated in OCD human imaging studies.

Introduction

Obsessive compulsive disorder (OCD) affects 2–3% of people worldwide, and has been identified by the World Health Organization (WHO) as a leading cause of illness related disability [1] due to its chronic and relapsing course. The substantial societal burden of OCD—an estimated \$10

billion annual cost in the U.S [2]—stems in part from sub-optimal pharmacotherapeutic treatments. First-line monotherapy with selective serotonin reuptake inhibitors (SSRIs) leads to remission in only 10–20% of patients, with 25% failing to experience any symptom improvement [3, 4]. Development of more effective treatments has been limited by a lack of knowledge regarding the molecular pathology of the disorder.

Neuroimaging studies have consistently identified cortical and striatal abnormalities in OCD [5]. Specifically, structural MRI studies suggest that anatomical abnormalities in the orbitofrontal cortex (OFC) and striatum may contribute to OCD pathogenesis [6]. Functional neuroimaging studies have also identified OFC and striatum as key loci of dysfunction in OCD, with most evidence suggesting hyperactivity in, and increased connectivity between, these regions [7–11]. Furthermore, hyperactivity in these critical regions is increased during symptom provocation and decreased in response to successful SSRI therapy [7, 12],

Supplementary information The online version of this article (<https://doi.org/10.1038/s41380-019-0431-3>) contains supplementary material, which is available to authorized users.

✉ Susanne E. Ahmari
ahmarise@upmc.edu

¹ Center for Neuroscience, University of Pittsburgh, Pittsburgh, PA, USA

² Department of Psychiatry, University of Pittsburgh, Pittsburgh, PA, USA

suggesting this abnormal activity may be involved in OCD symptomatology. Further evidence for a causal relationship between activity in cortico-striatal circuits and OCD pathophysiology comes from studies indicating that deep brain stimulation of ventral striatal targets both relieves OCD symptoms and reverses cortico-striatal hyperactivity [13, 14]. Finally, a causal relationship between OFC-striatal activity and OCD-relevant behaviors has been demonstrated using rodent models and optogenetics [15, 16]. Thus, current knowledge regarding circuit level disruptions in OCD is consistent across multiple lines of evidence and highlights abnormalities in OFC and striatum.

Although the etiology of OCD is unknown, twin studies support a genetic contribution. A recent meta-analysis including 37 samples from 14 twin studies of obsessive-compulsive symptoms found that genetic factors accounted for 40% of the phenotypic variance; no significant contribution from shared environmental factors was noted [17]. Unbiased genome-wide association studies (GWAS) indicate that genes encoding proteins found at or around excitatory synapses may account for this genetic contribution [18, 19]. Specifically, significant associations were observed with *DLGAP1* (discs large-associated protein 1), a post-synaptic density scaffolding protein family member, and *ISMI*, which is associated with expression of multiple genes related to glutamatergic signaling including members of the *DLGAP* family and glutamate receptors [18]. In a second OCD GWAS study, the most strongly associated marker was observed near the gene encoding *PTPRD* (protein tyrosine phosphatase delta), which promotes the differentiation of glutamatergic synapses [20] and interacts with the post-synaptic adhesion molecule *SLITRK3* (SLIT and NTRK-like protein 3) [19]. Excitatory synapse involvement is also supported by the well-replicated finding of an association between OCD and *SLC1A1*, which encodes the primary neuronal glutamate transporter [21–27]. Finally, studies in transgenic mice also indicate that dysregulation of glutamatergic synapses may lead to OCD-relevant cortico-striatal dysfunction. Specifically, both *Sapap3* (also known as *Dlgap3*) and *Slitrk5* constitutive knockout mice display OCD-relevant compulsive grooming phenotypes and abnormal cortico-striatal activity that can be rescued with chronic fluoxetine treatment [16, 28, 29].

Because alterations in excitatory neurotransmission are commonly associated with homeostatic changes in inhibitory neurotransmission, prior work has also examined γ -amino butyric acid (GABA) signaling in OCD. A small literature in humans supports a possible role for GABAergic dysfunction by demonstrating decreased mPFC GABA levels via magnetic resonance spectroscopy in subjects with OCD [30], and increased mPFC GABA levels following a single ketamine infusion [31]. In addition, transcranial magnetic stimulation (TMS)-based

neurophysiological indices suggest dysregulated GABA-signaling in OCD patients [32]. Tourette syndrome, which is highly comorbid with OCD [33], has also been associated with reduced numbers of striatal GABAergic interneurons (both parvalbumin- and choline acetyltransferase-expressing subtypes) in human post-mortem studies [34, 35]. Preclinical evidence likewise supports a potential role for GABAergic alterations in repetitive behavior. For example, dysfunction in striatal parvalbumin positive interneurons has been linked to compulsive grooming behavior in *Sapap3* knockout mice [16]. In addition, simulation of post-mortem findings from Tourette syndrome in mice via ablation of parvalbumin positive interneurons in the dorsal striatum leads to an increase in stress-induced grooming and anxiety [36].

Although the potential roles of glutamatergic and GABAergic signaling in OCD have been broadly investigated in human neuroimaging studies, only one prior study has directly explored the possible underlying molecular substrates of these signaling changes by quantifying gene expression in human post-mortem brain tissue [37]. This study examined the dorsolateral prefrontal cortex (DLPFC) via microarray analyses in a group of subjects that had OCD, obsessive-compulsive personality disorder (OCPD), and/or tic disorder diagnoses. Although decreased parvalbumin levels (*PVALB*) were observed in OCD subjects compared to unaffected comparison subjects, gene set analyses did not reveal either glutamatergic or GABAergic neurotransmission as a top altered pathway. However, this study did not examine the OFC or striatum, which have been more commonly implicated in the OCD disease process [38, 39] than the DLPFC [10]. To assess whether glutamatergic and/or GABAergic signaling is altered in these brain regions, we quantified the expression of 16 genes whose protein products are critical for normal excitatory and inhibitory neurotransmission (Table 1) in post-mortem tissue samples from the medial OFC, lateral OFC, caudate, and nucleus accumbens (NAc) of subjects with OCD and unaffected comparison subjects.

Materials and methods

Human postmortem subjects

Brain specimens ($N = 16$) were obtained through the University of Pittsburgh Brain Tissue Donation Program during autopsies conducted by the Allegheny County Medical Examiner's Office (Pittsburgh, PA) after consent was given by next-of-kin. An independent panel of experienced clinicians examined clinical records, toxicology, psychological autopsy data, and structured interviews with family members to make consensus DSM-IV diagnoses. Unaffected

Table 1 Target transcripts and their reported involvement in OCD and related disorders

Gene	Transcript grouping ^a	Encoded protein function	OCD and OCD-related disorder references
<i>DLGAP1</i>	Excitatory structure	Post-synaptic scaffolding	Two SNPs located within <i>DLGAP1</i> with strong associations with OCD, though not genome wide significant [18]
<i>DLGAP2</i>	Excitatory structure	Post-synaptic scaffolding	Two SNPs (rs6558484 and rs7014992) associated with reduced white-matter volume in pediatric OCD [52]
<i>DLGAP3</i>	Excitatory structure	Post-synaptic scaffolding	<i>Dlgap3</i> (<i>Sapap3</i>)-knockout mice display compulsive grooming phenotype. In humans, <i>DLGAP3</i> mutations are associated with pathological grooming/ trichotillomania [28, 44, 46]
<i>DLGAP4</i>	Excitatory structure	Post-synaptic scaffolding	N/A
<i>SLITRK1</i>	Excitatory structure	Transmembrane and signaling protein	Mutations associated with Tourette Syndrome and trichotillomania [53, 57]
<i>SLITRK3</i>	Excitatory structure	Transmembrane and signaling protein	<i>SLITRK3</i> protein interacts with PTPRD, which harbors the OCD-associated SNP that is closest to genome-wide significance [19]
<i>GRIA1</i>	Excitatory receptors & transporters	Ionotropic AMPA receptor subunit	Two polymorphisms (rs707176 and rs2963944) associated with pediatric OCD [54]
<i>GRIN2B</i>	Excitatory receptors & transporters	Ionotropic NMDA receptor subunit	Positive association between 5072 T/G polymorphism and OCD using a family-based design [58]
<i>SLC1A1</i>	Excitatory receptors & transporters	Neuronal glutamate transporter	Multiple variants associated with OCD [21–26]
<i>SLC17A7</i>	Excitatory receptors & transporters	Vesicular glutamate transporter	N/A
<i>GABARAP</i>	GABA synapse	GABA-receptor clustering in membrane	N/A
<i>PVALB</i>	GABA synapse	Calcium binding protein found in inhibitory interneurons	Reduction in transcript expression in DLPFC of OCD subjects and subjects with eating disorders [37]
<i>CALBI</i>	GABA synapse	Calcium binding protein found in inhibitory interneurons	N/A
<i>GADI</i>	GABA synapse	GABA synthesizing enzyme	Down-regulation of <i>GADI</i> transcript expression in caudate and putamen of Tourette Syndrome subjects [55]
<i>GAD2</i>	GABA synapse	GABA synthesizing enzyme	Significant association of two polymorphisms (rs8190748 and rs92990) in early-onset OCD [56]
<i>SLC32A1</i>	GABA synapse	Vesicular GABA transporter	N/A

^aTranscript grouping used for calculation of composite measures (see Methods)

Table 2 Summary of demographic and postmortem characteristics of human subjects

	Comparison subjects	OCD subjects	Statistics
Number of subjects (<i>n</i>)	8	8	
Sex (F/M)	4/4	4/4	
Mean age (\pm SD)	43.6 (14.9)	45.3 (14.9)	$t_{14} = 0.2$, $p = 0.83$
Range	20–65	20–69	
PMI (\pm SD)	16.1 (5.1)	18.2 (7.8)	$t_{14} = 0.6$, $p = 0.54$
Brain pH (\pm SD)	6.6 (0.2)	6.7 (0.2)	$t_{14} = 1.6$, $p = 0.13$
RNA ratio	1.6 (0.2)	1.6 (0.2)	$t_{14} = 0.3$, $p = 0.75$
RNA integrity number	7.8 (0.6)	7.9 (0.5)	$t_{14} = 0.2$, $p = 0.87$
Suicide, <i>n</i> (%)	0 (0%)	3 (38%)	
Antidepressants ATOD, <i>n</i> (%)	0 (0%)	5 (63%)	

Demographic and postmortem characteristics for eight subjects with OCD and eight unaffected comparison subjects. *PMI* post-mortem interval, *ATOD* at time of death, *SD* standard deviation

comparison subjects underwent identical assessments and were determined to be free of any lifetime psychiatric illnesses. All procedures were approved by the University of Pittsburgh's Committee for the Oversight of Research and Clinical Training Involving Decedents and Institutional Review Board for Biomedical Research. To reduce biological variance, each subject with OCD ($n = 8$) was matched for sex, and as closely as possible for age and RNA integrity number (RIN), with one unaffected comparison subject. Each subject pair was processed together through all experimental procedures to reduce experimental variance. Biological replication was not performed due to the small number of available subjects and scarcity of available tissue. Subject groups did not differ in mean age ($t_{14} = 0.2$, $p = .83$), post-mortem interval (PMI; $t_{14} = 0.6$, $p = .54$), brain pH ($t_{14} = 1.6$, $p = .13$), RNA ratio ($t_{14} = 0.3$, $p = .75$), or RIN ($t_{14} = 0.2$, $p = .87$), (Table 2).

Tissue collection & RNA extraction

Standardized amounts (50 mm³) of gray matter were collected via a cryostat from four separate brain regions identified cytoarchitectonically: medial orbitofrontal cortex (mOFC, BA11), lateral orbitofrontal cortex (lOFC, BA47), head of the caudate nucleus, and the nucleus accumbens core. RNA was extracted using an RNeasy Plus Mini kit (QIAGEN, Valencia, CA). RIN values were obtained using

the Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA). Each subject had a RIN value ≥ 7 , indicative of high-quality, intact RNA.

Selection of transcripts for expression analysis

Using qPCR we examined the expression of 16 excitatory and inhibitory synaptic genes, the majority of which (11/16) have been genetically linked to OCD and OCD-related disorders (e.g., grooming disorders, skin picking, Tourette syndrome) (Table 1). Transcripts were broadly grouped into three categories based on the function of their protein product: (1) excitatory synaptic structure: contains transcripts in the *DLGAP* and *SLITRK* families, both of which have established roles in synapse formation and stabilization; (2) excitatory synaptic receptors and transporters: contains two transcripts encoding ionotropic glutamate receptors (*GRI1A* and *GRIN2B*), the vesicular glutamate transporter *SLC17A7*, and the excitatory amino acid transporter *SLC1A1*; and (3) GABA synapse: contains the GABAergic synapse scaffolding protein *GABARAP*, two major GABA synthesizing enzymes (*GAD1* and *GAD2*), two calcium binding proteins that characterize two functionally different classes of inhibitory interneurons found in the striatum and cortex (*PVALB* and *CALB1*), and the vesicular GABA transporter (*SLC32A1*).

Quantitative PCR (qPCR)

Complementary DNA (cDNA) was generated using 90 ng of RNA per sample and qScript cDNA SuperMix (Quanta BioSciences, Gaithersburg, MD) following universal cycling conditions (65–59 °C touchdown and 40 cycles of 10 s at 95 °C, 10 s at 59 °C, and 10 s at 72 °C). PCR products were amplified in triplicate to generate three technical replicates on a Bio-Rad CFX96 Real-Time PCR system (Bio-Rad, Hercules, California USA) using SYBR Green I nucleic acid gel stain. Subject pairs were run on the same qPCR plate to control for technical variance. Two housekeeping genes (β -Actin [*ACTB*] and Cyclophilin-A [*PPIA*]) were used to normalize target mRNA levels. Primer sequences for all housekeeping and target genes, as well as the transcript variants of each transcript amplified (identified using NCBI Primer-BLAST), can be found in Supplemental Table 1. The difference in cycle threshold (dCT) for each target transcript was calculated by subtracting the geometric mean CT for the two reference genes (each run in triplicate) from the mean CT of the target transcript (mean of the three technical replicates). There were no significant differences in the geometric mean of CTs of the two housekeeping genes across all four brain regions (Supplemental Fig. 1). Because dCT represents the log₂-transformed expression ratio of each target transcript to the reference genes, the

relative level of the target transcript for each subject is reported as $2^{-\text{dCT}}$ [40].

Statistical analysis

To determine the OCD-associated expression patterns of transcripts, we employed a mixed-design analysis of covariance (ANCOVA). Brain region was considered a within-subjects variable with 4 levels (BA11, BA47, caudate nucleus, NAc), and OCD diagnosis was the between subjects factor. Main effects of OCD diagnosis and brain region, as well as the interaction between diagnosis and brain region, were tested using F tests for each individual transcript. Covariates included in each model were age, sex, PMI, and RIN. Data from all 16 subjects were analyzed in all regions unless noted in the text in cases of low transcript expression. In a minority of cases (6 total transcripts), data from the caudate and NAc of subject pair 8 were not collected due to low RNA yield (see Supplemental Table 2 for detailed information about each transcript). If significant interactions were observed between diagnosis and brain region, post-hoc pairwise comparisons were conducted using Sidak tests to control for overall type I error. Multiple comparison correction was conducted using the Benjamini-Hochberg procedure and a false discovery rate of 0.05 [41]. For main effects and interactions, correction was made across 16 transcripts. For pairwise comparisons conducted only after a significant interaction, correction was made for 22 comparisons. For individual transcript comparisons, uncorrected p -values are reported in the main text and corrected p -values are reported in Supplemental Table 4.

Composite scores of three groups of transcripts grouped broadly by the function of their encoded proteins (excitatory synaptic structure; excitatory synaptic receptors and transporters; GABA synapse) were computed. First, normalized (Z scored) expression levels were calculated for each transcript using $(Z = \frac{X - \mu}{\sigma})$, where μ is mean of expression ratios for the unaffected comparison subjects for a given transcript, σ is standard deviation of expression ratio for the unaffected comparison subjects for a given transcript, and X is the individual transcript measure from an unaffected comparison or OCD subject. Z -scores were then summed for all excitatory synaptic structure, excitatory synaptic receptors and transporters, and GABA synapse measures (see Table 1: Transcript grouping) within a given diagnostic group for each brain region. Post-hoc pairwise comparisons were conducted using Sidak tests to control for overall type I error. Multiple comparison adjustments were again made using the Benjamini-Hochberg analysis with a false discovery rate of 0.05. Both corrected and uncorrected comparisons are

presented for composite scores in the main text, and full statistics can be found in Supplemental Table 4.

Individual t -tests were performed with the Holm-Sidak multiple comparison correction and an alpha of 0.05 to analyze the effect of (1) antidepressants at time of death, (2) presence of benzodiazepines at time of death, (3) comorbid MDD diagnosis, and (4) presence of tobacco use at time of death on expression of transcripts in OCD subjects (Supplemental Fig. 2–5). Statistical analyses were performed using SPSS (SPSS, Inc.; Chicago, IL).

Results

Effect of OCD diagnosis on expression of excitatory synaptic structure transcripts in cortical and striatal regions

Regional patterns of expression of excitatory structural transcripts implicated in OCD and grooming disorders were first examined (Fig. 1). Several of these transcripts displayed lower expression in OCD subjects compared to unaffected comparison subjects selectively in OFC and not striatum. First, although expression of the scaffolding protein transcript *DLGAP1* was unaffected by diagnosis ($F_{1,10} = 2.8$, $p = .13$) or region ($F_{3,30} = 1.8$, $p = .17$), the interaction between these two factors was significant ($F_{3,30} = 3.5$, $p = .03$). Post-hoc comparisons revealed that mean *DLGAP1* levels were significantly lower in BA47 (–48%, $F_{1,10} = 5.1$, $p = .05$) but not different in BA11, caudate, and NAc ($p > .05$) (Fig. 1a). In addition, expression of another *DLGAP* family member, *DLGAP2*, significantly differed by diagnosis ($F_{1,10} = 34.4$, $p = .0001$) and brain region ($F_{3,30} = 3.5$, $p = .03$), with a significant interaction ($F_{3,30} = 5.4$, $p = .004$). Post-hoc pairwise comparisons of simple main effects showed lower *DLGAP2* in OCD subjects selectively in BA11 (–43%: $F_{1,10} = 27.7$, $p = .0004$) and BA47 (–41%: $F_{1,10} = 15.8$, $p = .003$) [expression was not significantly different in caudate or NAc ($p > .05$)] (Fig. 1b). A third transcript, *SLITRK3* (Fig. 1f), showed a similar pattern, with expression significantly affected by diagnosis ($F_{1,8} = 21.7$, $p = .002$), a trend towards a brain region effect ($F_{2,16} = 2.9$, $p = .08$), and a significant interaction between diagnosis and brain region ($F_{2,16} = 4.2$, $p = .03$). Post-hoc pairwise comparisons indicated lower *SLITRK3* levels in BA11 (–42%: $F_{1,8} = 34.3$, $p = .0004$) and BA47 (–32%: $F_{1,8} = 7.8$, $p = .02$), but not in caudate (–24%: $F_{1,8} = 2.3$, $p = .17$; too low to detect in NAc).

Several excitatory synaptic structural transcripts also showed lower expression in both OFC and striatum of OCD subjects compared to unaffected comparison subjects. First, although ANCOVA revealed a significant effect of

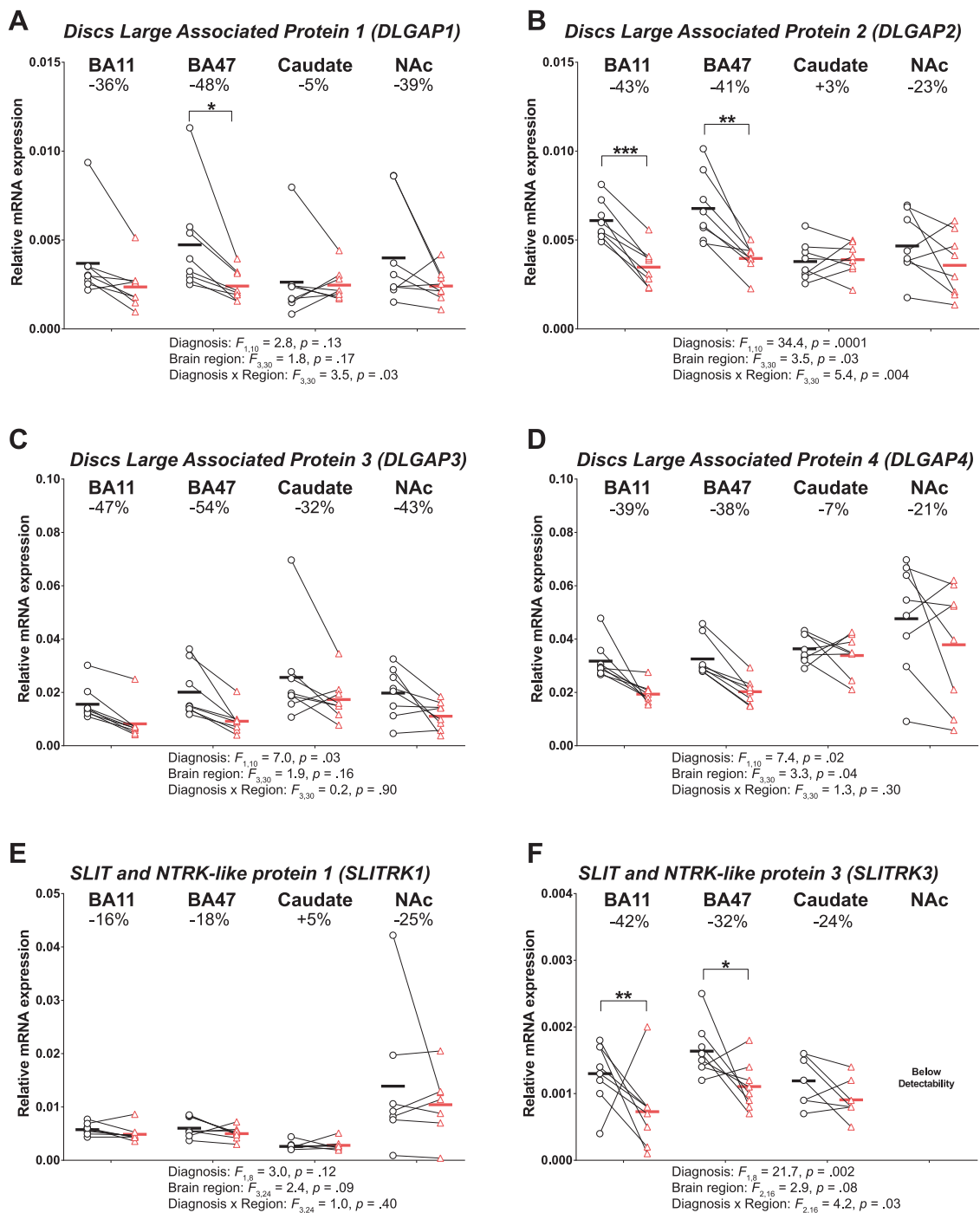


Fig. 1 Expression of excitatory synaptic structure transcripts in OFC and striatum of OCD subjects and unaffected comparison subjects. qPCR analysis of excitatory synaptic structure transcripts across two orbitofrontal (BA11 and BA47) and two striatal (caudate and NAc) brain regions in OCD. Unaffected comparison subjects and OCD subjects are represented by black open circles and red open triangles,

respectively. A line drawn between a circle and a triangle indicates a matched pair. Horizontal bars indicate group means. Percentages indicate the percentage difference in transcript level of the OCD subject relative to the unaffected comparison subject. Post-hoc significance following significant interactions is indicated by brackets; *** $p < .001$; ** $p < 0.01$; * $p < 0.05$

diagnosis on *DLGAP3* expression ($F_{1,10} = 7.0, p = .03$), there was no effect of brain region ($F_{3,30} = 1.9, p = .16$) or interaction between diagnosis and region ($F_{3,30} = 0.2, p = .90$) (Fig. 1c). Similarly, levels of *DLGAP4* (Fig. 1d)

were lower in both OFC and striatum [significant effect of diagnosis ($F_{1,10} = 7.4, p = .02$) and brain region ($F_{3,30} = 3.3, p = .04$); no interaction ($F_{3,30} = 1.3, p = .30$)]. Notably, *SLITRK1* was the only excitatory synaptic structure

transcript tested which showed no differences in expression related to diagnosis (Fig. 1e) ($F_{1,8} = 3.0, p = .12$) or brain region ($F_{3,24} = 2.4, p = .09$), and no interaction between the two factors ($F_{3,24} = 1.0, p = .40$).

Effect of OCD diagnosis on expression of transcripts encoding excitatory synaptic receptors and transporters in cortical and striatal regions

We next assessed levels of transcripts encoding glutamate receptors and transporters (Fig. 2). Expression of the transcript *GRIA1*, which encodes the GluR1 subunit of the AMPA receptor, was lower in OFC and striatum of OCD subjects (Fig. 2a) [effect of diagnosis ($F_{1,8} = 9.0, p = .02$); no effect of region ($F_{3,24} = 1.0, p = .39$) or interaction ($F_{3,24} = 1.6, p = .21$)]. Similarly, expression of *GRIN2B*, which encodes the NR2B subunit of the NMDA receptor, was lower in OCD subjects (Fig. 2b) [main effect of

diagnosis ($F_{1,8} = 6.9, p = .03$); no effect of region ($F_{3,24} = 1.8, p = .17$) or interaction ($F_{3,24} = 1.1, p = .36$)]. A significant main effect of diagnosis on expression of *SLC1A1*, a glutamate transporter that has been associated with OCD, was also observed ($F_{1,10} = 5.0, p = .05$) (Fig. 2c). This transcript also displayed a significant effect of brain region ($F_{3,30} = 3.2, p = .04$) and an interaction ($F_{3,30} = 3.7, p = .02$); subsequent pairwise comparisons indicated *SLC1A1* in OCD subjects was lower only in BA47 (-34%: $F_{1,10} = 7.9, p = .03$), although a trend was also observed in BA11 (-37%: $F_{1,10} = 3.9, p = .08$). Finally, we measured levels of *SLC17A7*, which encodes the pre-synaptic vesicular glutamate transporter 1 (VGLUT1). Consistent with previous work [42], *SLC17A7* was undetectable in striatum; however, levels were lower in both OFC regions in OCD subjects [significant effect of diagnosis ($F_{1,10} = 12.4, p = .005$), no effect of brain region ($F_{1,10} = 0.02, p = .89$), and no interaction ($F_{1,10} = 0.02, p = .89$) (Fig. 2d)].

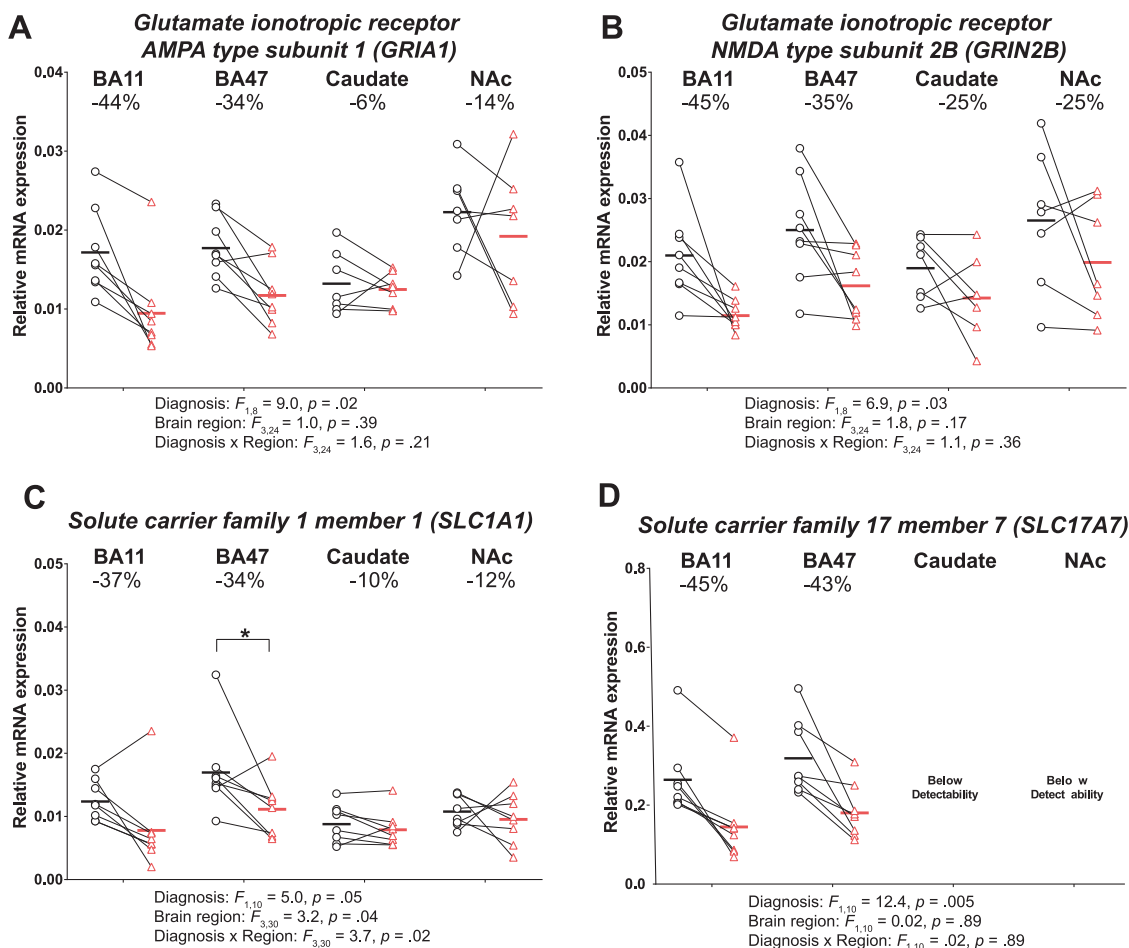


Fig. 2 Expression of transcripts encoding excitatory synaptic receptors and transporters in OFC and striatum of OCD subjects and unaffected comparison subjects. qPCR analysis of transcripts encoding excitatory synaptic receptors and transporters across two orbitofrontal (BA11 and BA47) and two striatal (caudate and NAc) brain regions in OCD. Unaffected comparison subjects and OCD subjects are represented by

black open circles and red open triangles, respectively. Lines represent matched pairs. Horizontal bars indicate group means. Percentages indicate the percentage difference in transcript level of the OCD subject relative to the unaffected comparison subject. Post-hoc significance following significant interactions is indicated by brackets; * $p < 0.05$

Effect of OCD diagnosis on GABA synapse transcripts in cortical and striatal regions

To determine if OCD was associated with alterations in GABAergic transcript levels in OFC and striatum, we first quantified levels of the two major GABA synthesizing enzymes, *GAD1* (Fig. 3a; GAD_{67}) and *GAD2* (Fig. 3b; GAD_{65}). ANCOVA revealed a significant effect of diagnosis ($F_{1,10} = 6.9$, $p = .03$) and brain region ($F_{3,30} = 4.3$, $p = .01$) on *GAD1* levels, with an interaction between the two factors ($F_{3,30} = 7.2$, $p = .001$). Post-hoc comparisons revealed that OCD subjects had significantly elevated *GAD1* in BA11 (+99%: $F_{1,10} = 9.5$, $p = .01$), with no other region differing by diagnosis ($p > .05$). In contrast, there was no effect of diagnosis on *GAD2* levels ($F_{1,10} = 2.6$, $p = .14$), although we did observe a significant effect of brain region ($F_{3,30} = 3.9$, $p = .02$) and a trend toward a significant interaction ($F_{3,30} = 2.9$, $p = .052$).

We also examined transcripts encoding parvalbumin and calbindin (*PVALB* and *CALB1*), calcium-binding proteins used as markers of cortical and striatal interneuron subtypes. Although diagnosis had no detectable effect on *PVALB* levels ($F_{1,10} = 2.8$, $p = .13$), an effect of brain region ($F_{2,20} = 5.6$, $p = .01$) and an interaction between region and diagnosis ($F_{2,20} = 4.9$, $p = .02$) were observed [note; *PVALB* expression was too low to detect in the NAc, consistent with previous studies [43]]. Pairwise comparisons suggested increased levels of *PVALB* in BA11 of OCD subjects, although the effect was not significant (+41%: $F_{1,10} = 2.9$, $p = .11$). Similarly, no effect of diagnosis ($F_{1,10} = .84$, $p = .38$) and a significant effect of brain region ($F_{3,30} = 24.7$, $p = .0001$) were observed on *CALB1* levels; however, there was no significant interaction ($F_{3,30} = 1.3$, $p = .27$).

Given the lower levels of transcripts that encode excitatory post-synaptic structural proteins in OCD (Fig. 1), we also examined *GABARAP*, which encodes a protein involved in GABA receptor scaffolding (Fig. 3e). In contrast to the excitatory transcripts, no effect of OCD diagnosis ($F_{1,8} = 2.8$, $p = .13$), brain region ($F_{3,24} = 2.3$, $p = .10$), or interaction ($F_{3,24} = 1.3$, $p = .30$) was observed for *GABARAP*. Levels of *SLC32A1*, which encodes the vesicular inhibitory amino acid transporter, were also not significantly affected by diagnosis (Fig. 3f; $F_{1,8} = .004$, $p = .95$) or brain region ($F_{3,24} = 2.0$, $p = .14$), and no interaction was detected ($F_{3,24} = 2.2$, $p = .10$).

Composite measures of transcripts across cortical and striatal regions in OCD

To determine the relative contribution of each brain region to the observed changes in transcript levels, we computed composite summary scores from the normalized expression ratios of the transcripts grouped into three categories:

excitatory synaptic structure, excitatory synaptic receptors and transporters, and GABA synapse-related (Fig. 4). ANCOVA showed a significant main effect of OCD diagnosis on the excitatory synaptic structure transcript composite measure ($F_{1,10} = 26.3$, $p = .0004$; corrected $p = .0012$), a trend towards a main effect of region ($F_{3,30} = 2.7$, $p = .06$; corrected $p = .06$), and a significant diagnosis-by-region interaction ($F_{3,30} = 4.0$, $p = .02$; corrected $p = .02$). Pairwise comparisons revealed significantly reduced levels of excitatory synaptic structure transcripts in BA11 ($F_{1,10} = 13.5$, $p = .004$; corrected $p = .02$) and BA47 ($F_{1,10} = 25.6$, $p = .0005$; corrected $p = .006$) of OCD subjects, with no changes in caudate and NAc ($p > .05$) (Fig. 4a). Similarly, the excitatory synaptic receptor/transporter composite measure showed significant reductions in BA11 and BA47 of OCD subjects, but no changes in caudate or NAc [main effect of diagnosis ($F_{1,10} = 10.4$, $p = .009$; corrected $p = .01$); significant effect of region ($F_{3,30} = 4.0$, $p = .02$; corrected $p = .02$); significant interaction ($F_{3,30} = 4.9$, $p = .007$; corrected $p = .01$); BA11 post-hoc ($F_{1,10} = 10.1$, $p = .01$; corrected $p = .02$); BA47 post-hoc ($F_{1,10} = 16.5$, $p = .002$; corrected $p = .01$); caudate and NAc ($p > .05$)] (Fig. 4b). However, the GABA synapse composite measure indicated that GABA-related transcript levels in OCD subjects were elevated in BA11 compared to unaffected comparison subjects, ($F_{1,10} = 11.4$, $p = .007$; corrected $p = .02$), but unchanged in BA47, caudate, and NAc ($p > .05$) [main effect of diagnosis ($F_{1,10} = 6.1$, $p = .03$; corrected $p = .03$); main effect of region ($F_{3,30} = 4.9$, $p = .007$; corrected $p = .02$); significant interaction ($F_{3,30} = 8.7$, $p = .0003$; corrected $p = .0009$)] (Fig. 4c).

Discussion

Glutamatergic synaptic dysfunction in cortical and striatal brain regions has been suggested as a contributor to the pathophysiology of OCD, but evidence to date has been indirect. Using qPCR in human post-mortem tissue, we now support this hypothesis by demonstrating decreased levels of transcripts encoding excitatory synaptic proteins in OFC and striatal regions in OCD subjects (Figs. 1, 2). Composite scores in OCD subjects revealed that these significant reductions in both the excitatory synaptic structure and excitatory synaptic receptors/transporters transcript groupings were preferentially localized to OFC (BA11 and BA47) and not detected in striatum (caudate and NAc) (Fig. 4a, b). More subtle increases in GABA synapse transcripts were also identified in OFC, but these were observed selectively in BA11 (Figs. 3, 4c). Together, these data provide the first evidence of potential molecular dysfunction in brain regions consistently implicated in human imaging studies of OCD [6, 8].

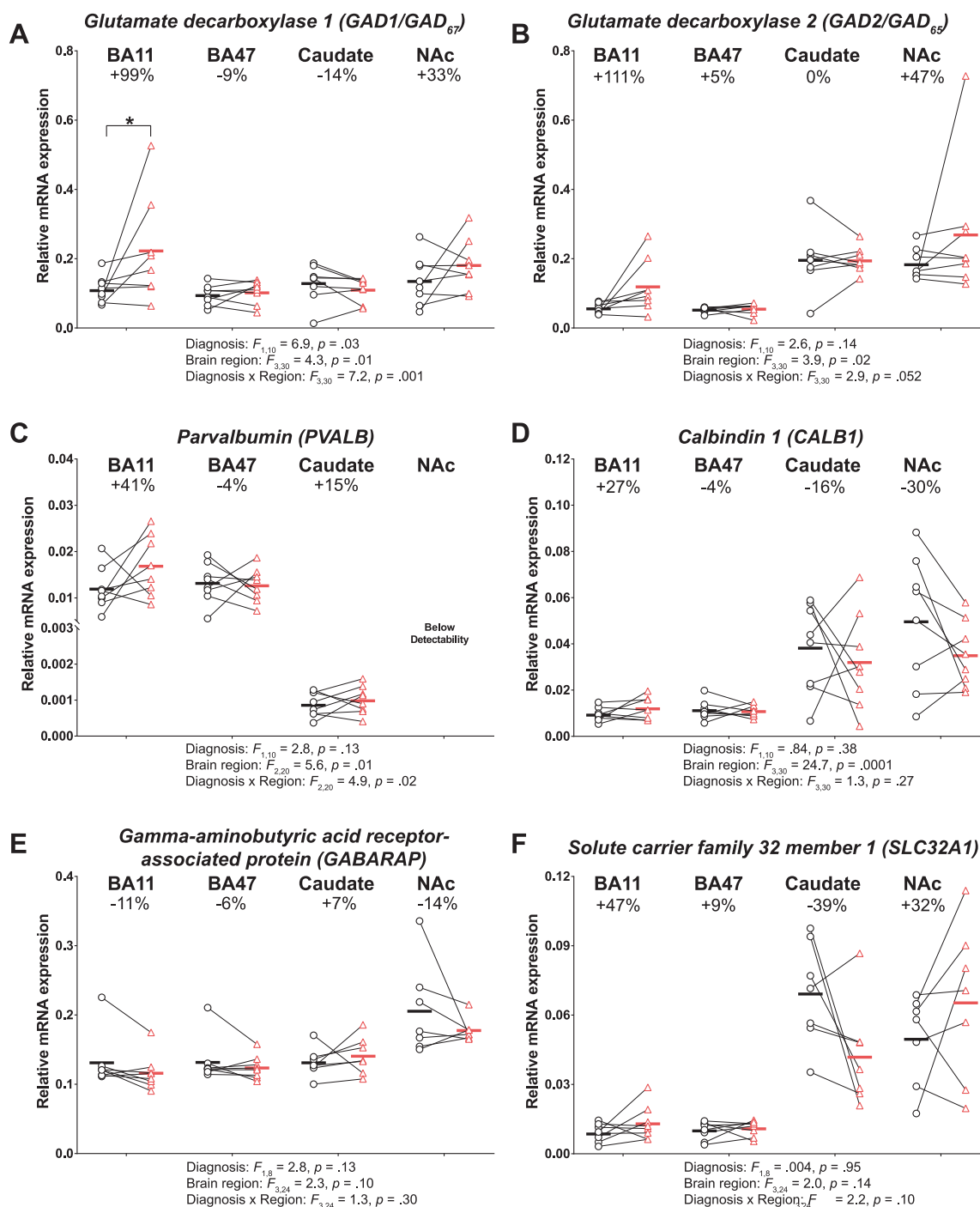


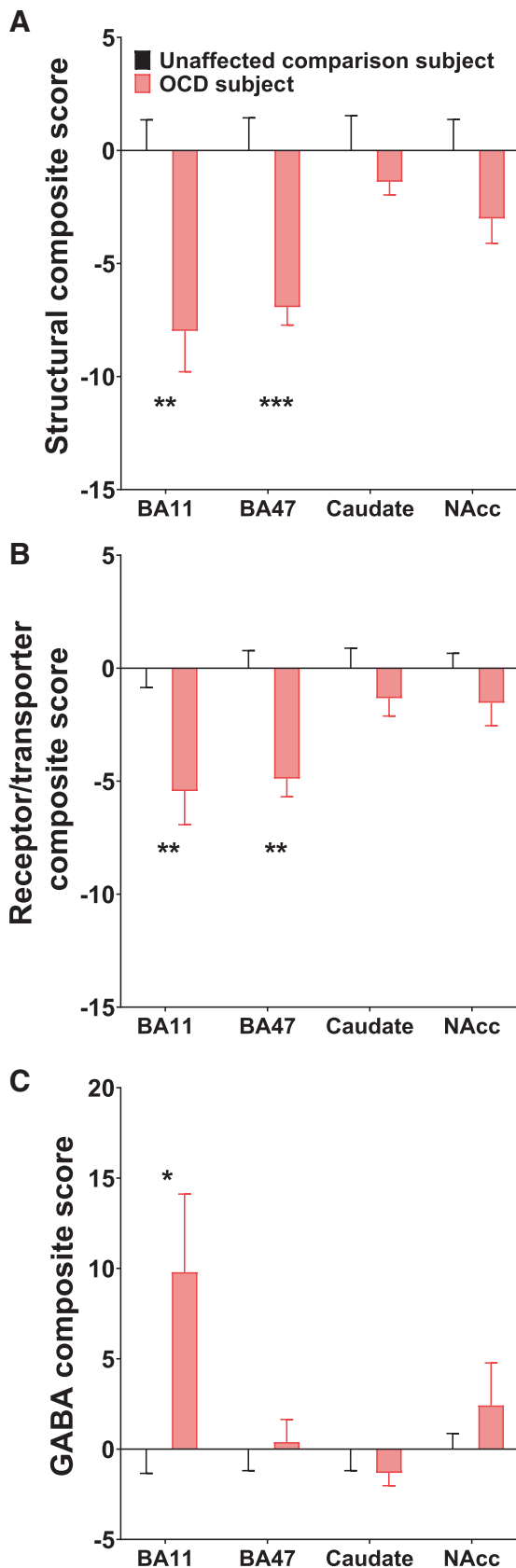
Fig. 3 Expression of GABA synapse transcripts in OFC and striatum of OCD subjects and unaffected comparison subjects. qPCR analysis of GABA synapse transcripts across two orbitofrontal (BA11 and BA47) and two striatal (caudate and NAc) brain regions in OCD. Unaffected comparison subjects and OCD subjects are represented by black open

circles and red open triangles, respectively. Lines represent matched pairs. Horizontal bars indicate group means. Percentages indicate the percentage difference in transcript level of the OCD subject relative to the unaffected comparison subject. Post-hoc significance following significant interactions is indicated by brackets; * $p < 0.05$

Decreased levels of three key excitatory synaptic structure transcripts are observed in OFC and striatum

Several of the observed down-regulated transcripts have previously been linked to OCD [7, 18], including *DLGAP3*.

This gene encodes the post-synaptic scaffolding protein most commonly known as SAPAP3, and has been linked to OCD and associated disorders through genetic studies [44–46] [though see [45] for caveats]. In human postmortem tissue from OCD subjects, we found that *DLGAP3* expression was



◀ **Fig. 4** Composite measures of excitatory synaptic structure, excitatory synaptic receptors and transporters, and GABA synapse transcripts across cortical and striatal regions in OCD. **a** In OCD subjects, composite measures of excitatory structural transcripts were lower in BA11 and BA47 relative to unaffected comparison subjects. No differences were observed in either the caudate or NAcc. **b** Composite scores of excitatory receptors and transporters were reduced in BA11 and BA47 and unchanged in caudate and NAcc in OCD subjects. **c** GABA composite scores were elevated in BA11 and unchanged in BA47, caudate, and NAcc of OCD subjects (c). Post-hoc significance following significant interactions is indicated by asterisks; *** $p < .001$; ** $p < 0.01$; * $p < 0.05$

significantly decreased across all four cortical and striatal regions tested (Fig. 1a–d). This finding is consistent with the fact that *Sapap3* knockout mice, which constitutively lack the *Sapap3* gene, display compulsive behavior, and provides further support for the hypothesis that decreases in SAPAP3 protein are involved in the generation of compulsive behavior in humans [8, 16, 28]. In addition, the same expression pattern was seen with *DLGAP4*. Interestingly, other *DLGAP* family members showed decreased levels specifically in OFC. Expression of *DGLAP1*, which has recently been identified as the locus of two of the most strongly associated single nucleotide polymorphisms (SNPs) in case-control OCD GWAS [18, 19], was selectively decreased only in OFC and not striatum (Fig. 1a); the same pattern was observed for *DLGAP2* (Fig. 1b). These data raise the possibility that different *DLGAP* family members could differentially impact particular OCD symptom domains based on the regional localization of their alterations in expression.

In addition, in OFC of OCD subjects, we observed a selective reduction in expression of another gene that has been associated with OCD: *SLC1A1* (Fig. 2c), which encodes the neuronal glutamate transporter EAAT3. *SLC1A1* was first implicated in OCD pathophysiology by two reports identifying genetic variants in two independent patient populations [21, 22]. Subsequent studies have replicated these findings, although identified variants have not always been consistent [24–26]. Interestingly, our finding of reduced levels of *SLC1A1* in OFC of OCD subjects relative to unaffected comparison subjects is not aligned with the fact that the most consistently associated SNP in OCD is predicted to result in increased expression of *SLC1A1* [23, 27]. To address this discrepancy, future post-mortem brain studies could examine expression levels of recently-identified *SLC1A1* variants that may serve as negative regulators of SLC1A1/EAAT3 function in cortex and striatum [23].

Downregulation of multiple excitatory synaptic transcripts in OFC suggests an upstream causal event

As discussed above, our data can be used to make inferences about the potential role of particular genes in OCD pathology.

However, the most striking finding is the simultaneous downregulation of multiple excitatory synapse-related transcripts in both medial (BA11) and lateral OFC (BA47) (Fig. 4a, b). Although it is possible that each transcript change is an independently occurring event, this pattern of expression suggests the possibility of an upstream factor leading to abnormal coordinated excitatory synapse gene regulation across the OFC. One potential explanation is altered expression of an upstream “master regulator” gene that influences expression of transcripts associated with post-synaptic excitatory synapse function. Alternatively, due to the diversity of transcripts that were down-regulated, these changes may reflect an overall decrease in number or size of synaptic contacts or dendritic spines in OFC. In turn, this would fit with one of the most consistent findings in structural imaging studies of OCD: a reduction in volume of both left and right OFC [6, 47, 48]. Precedent for this is observed in other neuropsychiatric illnesses such as schizophrenia, in which dendritic spine loss is one of the greatest indices of gray matter loss [49–51].

Synthesis of downregulation of glutamatergic transcripts with existing literature

As discussed above, our findings appear consistent with the structural imaging literature. What becomes more challenging is integrating these data with the existing functional imaging literature demonstrating hyperactivity in OFC at baseline and with symptom provocation in OCD patients [10]. At face value, the results seem contradictory, since one might expect that downregulation of excitatory transcripts in OFC would be consistent with overall decreases in OFC activity. This apparent discrepancy could be explained in several ways. First, there are examples in which knockout of excitatory post-synaptic density proteins leads (potentially counter-intuitively) to both increased neural activity and compulsive grooming behavior: 1) Constitutive knockout of *Sapap3* leads to striatal hyperactivity; 2) Constitutive knockout of *Slitrk5* results in OFC hyperactivity [16, 28, 29]. However, causal links between *Sapap3* and *Slitrk5* down-regulation in specific brain regions and hyperactivity have not yet been demonstrated. Second, our observed decreased levels of excitatory synaptic transcripts could be compensating for changes in neural activity in upstream structures. For example, functional imaging studies demonstrate hyperactivity and increased gray matter volume in the thalamus of OCD subjects [6]. Increased thalamo-cortical drive in OCD patients could therefore be the primary driver of OFC hyperactivity, and lead to compensatory down-regulation of excitatory post-synaptic proteins at thalamo-cortical synapses. In this scenario, OFC hyperactivity could still be observed if this compensation is not sufficient to counteract the increased thalamic drive. If

this is the case, we would predict that our observed decrease in excitatory transcript levels in OCD subjects is localized to cortical neurons receiving projections from thalamus, and not neurons in cortical output layers. Thus, our observations from the post-mortem OCD brain lend themselves to directly testable novel hypotheses regarding OCD pathophysiology. Our data also highlight the importance of moving beyond the targeted set of transcripts tested here, which were mostly selected due to their previous genetic links to OCD and related disorders, and investigating the molecular pathology of OCD on a larger scale—i.e., the transcriptome or proteome.

Potential caveats

When interpreting this study, it is important to note several limitations. First, the sample size is small, with eight pairs of OCD and unaffected comparison subjects. In order to maximize our statistical power to detect differences related to brain region and OCD diagnosis, we have matched our subjects as closely as possible for factors that can affect expression of mRNA (e.g., age, sex, and RIN). No significant effects of these or other post mortem factors were observed in our cohort (Table 2). Other limitations include our current inability to rule out the possibility that comorbid diagnoses other than MDD (Fig.S4; see Supplemental table 3) may be affecting mRNA expression due to a lack of statistical power. In particular, this dataset does not allow us to determine if comorbid anxiety disorders in subjects with OCD are contributing to the observed decreases in excitatory synaptic transcript expression. Future studies with a comparator group matched for anxiety disorders will be necessary to make this determination. Similarly, due to lack of power, we are limited in our ability to determine whether medications or other substances contribute to the observed differences in gene expression, although exploratory analyses did not identify any significant impact of antidepressant use, benzodiazepine use, or tobacco use at time of death in OCD subjects (Fig. S2,S3,S5). It is also important to note that the use of targeted qPCR to conserve statistical power in our small sample prevented us from exploring whether subjects with OCD have different levels of expression of alternatively spliced transcripts compared to unaffected subjects. Although the primary protein coding isoform was always amplified, primer pairs for several transcripts also amplified multiple splice variants (see Supplemental Table 1 for details). Our targeted qPCR approach was unable to determine if expression of these transcript variants differs by diagnosis or region. It is therefore possible that the observed excitatory transcript downregulation in orbitofrontal cortex could be accompanied by compensatory upregulation of alternative transcripts not studied here, which could limit the functional

impact of the observed transcriptional changes and affect interpretation of our results. This possibility could be addressed in future studies using RNA sequencing (RNA-seq). Finally, although some of the effects in Figs. 1–3 would not survive Benjamini-Hochberg correction for multiple comparisons due to our limited sample size and power (see Supplemental Table 4 for uncorrected and corrected p -values for all comparisons), this is mitigated by survival of the significant effects of the aggregate analysis following correction (Fig. 4). Thus, the composite analysis demonstrates that differential expression of transcripts was most prominent in orbitofrontal regions in subjects with OCD.

Despite these potential caveats, this report is, to the authors' knowledge, the first identification of molecular dysfunction in either orbitofrontal or striatal brain regions in OCD. This targeted examination will therefore serve as the foundation for future better-powered studies that will allow us to examine the potential impact of comorbidities, medication status, and disease heterogeneity on expression of excitatory synaptic transcripts.

Acknowledgements We would first like to thank the subjects and their families for the generous gift of brain donation. Tissue from some subjects was obtained from the NIH NeuroBioBank at the University of Pittsburgh Brain Tissue Donation Program. We would also like to thank Kelly Rogers for her assistance with subject selection and tissue blocking, and Dr. Sue Johnston for her assistance with clinical assessments of post-mortem subjects. Funding support was provided by the Brain Research Foundation Fay and Frank Seed Grant (SEA, SCP).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

Publisher's note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

References

1. Kessler RC, Berglund P, Demler O, Jin R, Merikangas KR, Walters EE. Lifetime prevalence and age-of-onset distributions of DSM-IV disorders in the National Comorbidity Survey Replication. *Archiv Gen Psychiatry*. 2005;62:593–602.
2. Eaton WW, Martins SS, Nestadt G, Bienvenu OJ, Clarke D, Alexandre P. The burden of mental disorders. *Epidemiol Rev*. 2008;30:1–14.
3. Bollini P, Pampallona S, Tibaldi G, Kupelnick B, Munizza C. Effectiveness of antidepressants. Meta-analysis of dose-effect relationships in randomised clinical trials. *Br J Psychiatry*. 1999;174:297–303.
4. Pigott TA, Seay SM. A review of the efficacy of selective serotonin reuptake inhibitors in obsessive-compulsive disorder. *J Clin Psychiatry*. 1999;60:101–6.
5. Graybiel AM, Rauch SL. Toward a neurobiology of obsessive-compulsive disorder. *Neuron*. 2000;28:343–7.

6. Rotge JY, Guehl D, Dilharreguy B, Tignol J, Bioulac B, Allard M, et al. Meta-analysis of brain volume changes in obsessive-compulsive disorder. *Biol Psychiatry*. 2009;65:75–83.
7. Pauls DL, Abramovitch A, Rauch SL, Geller DA. Obsessive-compulsive disorder: an integrative genetic and neurobiological perspective. *Nat Rev Neurosci*. 2014;15:410–24.
8. Harrison BJ, Soriano-Mas C, Pujol J, Ortiz H, Lopez-Sola M, Hernandez-Ribas R, et al. Altered corticostriatal functional connectivity in obsessive-compulsive disorder. *Arch Gen Psychiatry*. 2009;66:1189–200.
9. Fitzgerald KD, Welsh RC, Stern ER, Angstadt M, Hanna GL, Abelson JL, et al. Developmental alterations of frontal-striatal-thalamic connectivity in obsessive-compulsive disorder. *J Am Acad Child Adolesc Psychiatry*. 2011;50:938–48 e3.
10. Menzies L, Chamberlain SR, Laird AR, Thelen SM, Sahakian BJ, Bullmore ET. Integrating evidence from neuroimaging and neuropsychological studies of obsessive-compulsive disorder: the orbitofronto-striatal model revisited. *Neurosci Biobehav Rev*. 2008;32:525–49.
11. Whiteside SP, Port JD, Abramowitz JS. A meta-analysis of functional neuroimaging in obsessive-compulsive disorder. *Psychiatry Res*. 2004;132:69–79.
12. Rauch SL, Shin LM, Dougherty DD, Alpert NM, Fischman AJ, Jenike MA. Predictors of fluvoxamine response in contamination-related obsessive compulsive disorder: a PET symptom provocation study. *Neuropsychopharmacology*. 2002;27:782–91.
13. Rauch SL, Dougherty DD, Malone D, Rezaei A, Friehs G, Fischman AJ, et al. A functional neuroimaging investigation of deep brain stimulation in patients with obsessive-compulsive disorder. *J Neurosurg*. 2006;104:558–65.
14. Bourne SK, Eckhardt CA, Sheth SA, Eskandar EN. Mechanisms of deep brain stimulation for obsessive compulsive disorder: effects upon cells and circuits. *Front Integr Neurosci*. 2012; 6:29.
15. Ahmari SE, Spellman T, Douglass NL, Kheirbek MA, Simpson HB, Deisseroth K, et al. Repeated cortico-striatal stimulation generates persistent OCD-like behavior. *Science*. 2013;340:1234–9.
16. Burguiere E, Monteiro P, Feng G, Graybiel AM. Optogenetic stimulation of lateral orbitofronto-striatal pathway suppresses compulsive behaviors. *Science*. 2013;340:1243–6.
17. Taylor S. Etiology of obsessions and compulsions: a meta-analysis and narrative review of twin studies. *Clin Psychol Rev*. 2011;31:1361–72.
18. Stewart SE, Yu D, Scharf JM, Neale BM, Fagermess JA, Mathews CA, et al. Genome-wide association study of obsessive-compulsive disorder. *Mol Psychiatry*. 2013;18:788–98.
19. Mattheisen M, Samuels JF, Wang Y, Greenberg BD, Fyer AJ, McCracken JT, et al. Genome-wide association study in obsessive-compulsive disorder: results from the OCGAS. *Mol Psychiatry*. 2015;20:337–44.
20. Takahashi H, Craig AM. Protein tyrosine phosphatases PTPdelta, PTPsigma, and LAR: presynaptic hubs for synapse organization. *Trends Neurosci*. 2013;36:522–34.
21. Dickel DE, Veenstra-VanderWeele J, Cox NJ, Wu X, Fischer DJ, Van Etten-Lee M, et al. Association testing of the positional and functional candidate gene SLC1A1/EAAC1 in early-onset obsessive-compulsive disorder. *Arch Gen Psychiatry*. 2006;63:778–85.
22. Arnold PD, Sicard T, Burroughs E, Richter MA, Kennedy JL. Glutamate transporter gene SLC1A1 associated with obsessive-compulsive disorder. *Arch Gen Psychiatry*. 2006;63:769–76.
23. Porton B, Greenberg BD, Askland K, Serra LM, Gesmonde J, Rudnick G, et al. Isoforms of the neuronal glutamate transporter gene, SLC1A1/EAAC1, negatively modulate glutamate uptake:

- relevance to obsessive-compulsive disorder. *Transl Psychiatry*. 2013;3:e259.
24. Samuels J, Wang Y, Riddle MA, Greenberg BD, Fyer AJ, McCracken JT, et al. Comprehensive family-based association study of the glutamate transporter gene SLC1A1 in obsessive-compulsive disorder. *Am J Med Genet B Neuropsychiatr Genet*. 2011;156B:472–7.
 25. Shugart YY, Wang Y, Samuels JF, Grados MA, Greenberg BD, Knowles JA, et al. A family-based association study of the glutamate transporter gene SLC1A1 in obsessive-compulsive disorder in 378 families. *Am J Med Genet B Neuropsychiatr Genet*. 2009;150B:886–92.
 26. Stewart SE, Fagerness JA, Platko J, Smoller JW, Scharf JM, Illmann C, et al. Association of the SLC1A1 glutamate transporter gene and obsessive-compulsive disorder. *Am J Med Genet B Neuropsychiatr Genet*. 2007;144B:1027–33.
 27. Zike ID, Chohan MO, Kopelman JM, Krasnow EN, Flicker D, Nautiyal KM, et al. OCD candidate gene SLC1A1/EAAT3 impacts basal ganglia-mediated activity and stereotypic behavior. *Proc Natl Acad Sci USA*. 2017;114:5719–24.
 28. Welch JM, Lu J, Rodriguiz RM, Trotta NC, Peca J, Ding J-DD, et al. Cortico-striatal synaptic defects and OCD-like behaviours in Sapap3-mutant mice. *Nature*. 2007;448:894–900.
 29. Shmelkov SV, Hormigo A, Jing D, Proenca CC, Bath KG, Milde T, et al. Slitrk5 deficiency impairs corticostriatal circuitry and leads to obsessive-compulsive-like behaviors in mice. *Nat Med*. 2010;16:598–602.
 30. Simpson HB, Shungu DC, Bender J, Mao X, Xu X, Slifstein M, et al. Investigation of cortical glutamate-glutamine and gamma-aminobutyric acid in obsessive-compulsive disorder by proton magnetic resonance spectroscopy. *Neuropsychopharmacology*. 2012;37:2684–92.
 31. Rodriguez CI, Kegeles LS, Levinson A, Ogden RT, Mao X, Milak MS, et al. In vivo effects of ketamine on glutamate-glutamine and gamma-aminobutyric acid in obsessive-compulsive disorder: Proof of concept. *Psychiatry Res*. 2015;233:141–7.
 32. Richter MA, de Jesus DR, Hoppenbrouwers S, Daigle M, Deluce J, Ravindran LN, et al. Evidence for cortical inhibitory and excitatory dysfunction in obsessive compulsive disorder. *Neuropsychopharmacology*. 2012;37:1144–51.
 33. Du JC, Chiu TF, Lee KM, Wu HL, Yang YC, Hsu SY, et al. Tourette syndrome in children: an updated review. *Pediatr Neonatol*. 2010;51:255–64.
 34. Kalanithi PS, Zheng W, Kataoka Y, DiFiglia M, Grantz H, Saper CB, et al. Altered parvalbumin-positive neuron distribution in basal ganglia of individuals with Tourette syndrome. *Proc Natl Acad Sci USA*. 2005;102:13307–12.
 35. Kataoka Y, Kalanithi PS, Grantz H, Schwartz ML, Saper C, Leckman JF, et al. Decreased number of parvalbumin and cholinergic interneurons in the striatum of individuals with Tourette syndrome. *J Comp Neurol*. 2010;518:277–91.
 36. Xu M, Li L, Pittenger C. Ablation of fast-spiking interneurons in the dorsal striatum, recapitulating abnormalities seen post-mortem in Tourette syndrome, produces anxiety and elevated grooming. *Neuroscience*. 2016;324:321–9.
 37. Jaffe AE, Deep-Soboslay A, Tao R, Hauptman DT, Kaye WH, Arango V, et al. Genetic neuropathology of obsessive psychiatric syndromes. *Transl Psychiatry*. 2014;4:e432.
 38. Breiter HC, Rauch SL, Kwong KK, Baker JR, Weisskoff RM, Kennedy DN, et al. Functional magnetic resonance imaging of symptom provocation in obsessive-compulsive disorder. *Arch Gen Psychiatry*. 1996;53:595–606.
 39. Rauch SL, Savage CR, Alpert NM, Fischman AJ, Jenike MA. The functional neuroanatomy of anxiety: a study of three disorders using positron emission tomography and symptom provocation. *Biol Psychiatry*. 1997;42:446–52.
 40. Vandesompele J, De Preter K, Pattyn F, Poppe B, Van Roy N, De Paepe A, et al. Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. *Genome Biol*. 2002;3:RESEARCH0034.
 41. Benjamini YHY. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J Royal Statist Soc Series B*. 1995;57:289–300.
 42. Vigneault E, Poirel O, Riad M, Prud'homme J, Dumas S, Turecki G, et al. Distribution of vesicular glutamate transporters in the human brain. *Front Neuroanat*. 2015;9:23.
 43. Bernacer J, Prensa L, Gimenez-Amaya JM. Distribution of GABAergic interneurons and dopaminergic cells in the functional territories of the human striatum. *PLoS ONE*. 2012;7:e30504.
 44. Bienvenu OJ, Wang Y, Shugart YY, Welch JM, Grados MA, Fyer AJ, et al. Sapap3 and pathological grooming in humans: Results from the OCD collaborative genetics study. *Am J Med Genet B Neuropsychiatr Genet*. 2009;150B:710–20.
 45. Boardman L, van der Merwe L, Lochner C, Kinnear CJ, Seedat S, Stein DJ, et al. Investigating SAPAP3 variants in the etiology of obsessive-compulsive disorder and trichotillomania in the South African white population. *Compr Psychiatry*. 2011;52:181–7.
 46. Zuchner S, Wendland JR, Ashley-Koch AE, Collins AL, Tran-Viet KN, Quinn K, et al. Multiple rare SAPAP3 missense variants in trichotillomania and OCD. *Mol Psychiatry*. 2009;14:6–9.
 47. Atmaca M, Yildirim BH, Ozdemir BH, Aydin BA, Tezcan AE, Ozler AS. Volumetric MRI assessment of brain regions in patients with refractory obsessive-compulsive disorder. *Prog Neuropsychopharmacol Biol Psychiatry*. 2006;30:1051–7.
 48. Atmaca M, Yildirim H, Ozdemir H, Tezcan E, Poyraz AK. Volumetric MRI study of key brain regions implicated in obsessive-compulsive disorder. *Prog Neuropsychopharmacol Biol Psychiatry*. 2007;31:46–52.
 49. Selemon LD, Goldman-Rakic PS. The reduced neuropil hypothesis: a circuit based model of schizophrenia. *Biol Psychiatry*. 1999;45:17–25.
 50. Penzes P, Cahill ME, Jones KA, VanLeeuwen JE, Woolfrey KM. Dendritic spine pathology in neuropsychiatric disorders. *Nat Neurosci*. 2011;14:285–93.
 51. Glausier JR, Lewis DA. Dendritic spine pathology in schizophrenia. *Neuroscience*. 2013;251:90–107.
 52. Wu K, Hanna GL, Easter P, Kennedy JL, Rosenberg DR, Arnold PD. Glutamate system genes and brain volume alterations in pediatric obsessive-compulsive disorder: A preliminary study. *Psychiatry Res Neuroimaging*. 2013;211:214–20.
 53. Abelson JF. Sequence Variants in SLITRK1 Are Associated with Tourette's Syndrome. *Science*. 2005;310:317–20.
 54. Ortiz AE, Gassó P, Mas S, Falcon C, Bargalló N, Lafuente A, et al. Association between genetic variants of serotonergic and glutamatergic pathways and the concentration of neurometabolites of the anterior cingulate cortex in paediatric patients with obsessive-compulsive disorder. *World J Biol Psychiatry*. 2015;17:394–404.
 55. Lenington JB, Coppola G, Kataoka-Sasaki Y, Fernandez TV, Palejev D, Li Y, et al. Transcriptome Analysis of the Human Striatum in Tourette Syndrome. *Biol Psychiatry*. 2016;79:372–82.
 56. Mas S, Pagerols M, Gassó P, Ortiz A, Rodriguez N, Morer A, et al. Role of and genes in early-onset obsessive-compulsive disorder: results from transmission disequilibrium study. *Genes Brain Behav*. 2014;13:409–17.
 57. Zuchner S, Cuccaro ML, Tran-Viet KN, Cope H, Krishnan RR, Pericak-Vance MA, et al. SLITRK1 mutations in Trichotillomania. *Mol Psychiatry*. 2006;11:888–9.
 58. Arnold PD, Rosenberg DR, Mundo E, Tharmalingam S, Kennedy JL, Richter MA. Association of a glutamate (NMDA) subunit receptor gene (GRIN2B) with obsessive-compulsive disorder: a preliminary study. *Psychopharmacology*. 2004;174:530–8.