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REVIEW

Neurobiology of Obsessive–Compulsive Disorder from Genes to Circuits: Insights from Animal Models

Ying-Dan Zhang[1] · Dong-Dong Shi[1,2] · Zhen Wang[1,2,3]

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Abstract Obsessive-compulsive disorder (OCD) is a chronic, severe psychiatric disorder that has been ranked by the World Health Organization as one of the leading causes of illness-related disability, and first-line interventions are limited in efficacy and have side-effect issues. However, the exact pathophysiology underlying this complex, heterogeneous disorder remains unknown. This scenario is now rapidly changing due to the advancement of powerful technologies that can be used to verify the function of the specific gene and dissect the neural circuits underlying the neurobiology of OCD in rodents. Genetic and circuit-specific manipulation in rodents has provided important insights into the neurobiology of OCD by identifying the molecular, cellular, and circuit events that induce OCD-like behaviors. This review will highlight recent progress specifically toward classic genetic animal models and advanced neural circuit findings, which provide theoretical evidence for targeted intervention on specific molecular, cellular, and neural circuit events.

Keywords Obsessive-compulsive disorder (OCD) · Animal models · Genes · Circuits · Neurobiology

✉ Dong-Dong Shi
laural.dong.shi@gmail.com

✉ Zhen Wang
wangzhen@smhc.org.cn

[1] Shanghai Mental Health Center, Shanghai Jiao Tong University School of Medicine, Shanghai 200030, China

[2] Shanghai Key Laboratory of Psychotic Disorders, Shanghai Mental Health Center, Shanghai Jiao Tong University School of Medicine, Shanghai 201108, China

[3] Shanghai Intelligent Psychological Evaluation and Intervention Engineering Technology Research Center, Shanghai 200030, China

Introduction

Obsessive-compulsive disorder (OCD) is a chronic, debilitating psychiatric disorder characterized by intrusive thoughts and compulsive repetition [1, 2]. Currently, approved first-line interventions including cognitive behavioral therapy (CBT) and selective serotonin reuptake inhibitors (SSRIs) only partially alleviate symptoms, with 30%–40% of patients being resistant to treatment [3, 4]. Furthermore, last-resort invasive interventions such as deep brain stimulation or surgical procedures have had mixed success in alleviating severe symptoms of patients suffering from treatment-refractory conditions, and these interventions were largely based on empirical evidence that is far from being mastered [5–7]. Thus, substantial challenges remain in the field of OCD etiology and therapeutics, and further research is needed to deeply understand the potential pathophysiology that underlies obsessions and compulsions.

Though clinical studies can provide insight into disease processes from genetic, brain imaging, and neurobiochemical perspectives [8, 9], studies in humans are inherently limited in their ability to dissect pathologic processes due to their non-invasive nature. Animal models of OCD have become indispensable tools that have the potential to compensate for such limitations and help to understand the biological bases of complex neuropsychiatric diseases by providing means to test biological causality [10]. During the last decades, there have been many attempts to develop animal models of OCD, which may provide a route for furthering our understanding and treatment of OCD. Ideally, a valid animal model of OCD should have three validities (Fig. 1): face validity (phenomenological similarity), predictive validity (pharmacological response), and construct validity (etiologic theory) [11–13]. Specifically, face validity indicates that the model recapitulates specific symptoms of the human

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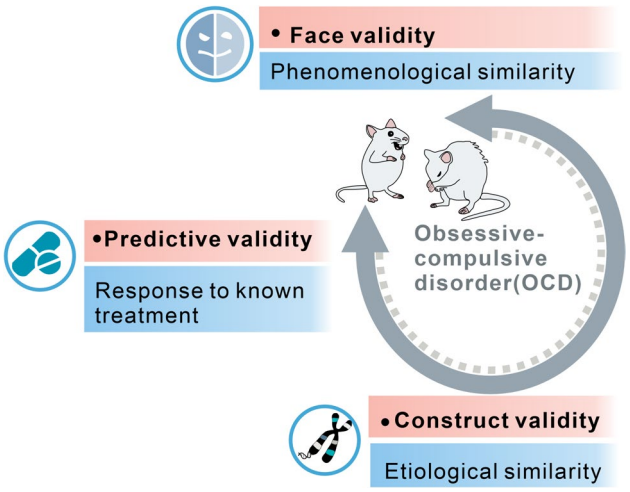

Fig. 1 Validities of OCD to evaluate rodent models. The validity of the model can be established by studying three validities: face validity, construct validity, and predictive validity. When the animal model can recapitulate some behavioral characteristics similar to compulsions in being repetitive, excessive, and inappropriate, it is considered to have face validity. Predictive validity signifies that the model mimics pharmacological treatment in humans, that is to say, the symptoms induced in the model should be reversed by first-line treatment such as chronic SSRIs administration. The construct validity of the model is based on the hypothesis of the physiopathology or etiology of OCD. The neural features underlying OCD observed in animals are similar to those known to be implicated in humans.

condition of OCD. The animals showing repetitive and/or rigid, excessive, and inappropriate compulsive-like behavior (e.g., excessive grooming, checking) are generally considered to have good face validity. Predictive validity signifies that the model responds to treatments that predict the effects of those treatments in OCD patients in a way, which is most often assessed with respect to responsiveness to SSRIs in animals. Construct validity refers to the similarity between the mechanisms underlying abnormal behavior observed in animals and the currently known potential etiology of OCD in humans [14, 15]. However, OCD is a heterogeneous disorder, and a specific animal model is unlikely to mirror the full extent of OCD, thus researchers should choose appropriate animal models depending on their research goals.

Non-human primates are particularly valuable models due to their greater similarity to humans, but their use is greatly limited by animal ethics, costs, maneuverability, and lack of tools [16]. Thus, rodents are the most widely used animal model to study OCD. Rodent models of OCD have been generated through diverse means, including genetic engineering, circuit manipulation, pharmacologically induced, natural occurrence, and neurodevelopmental intervention [10, 13, 14, 17–30] (Table 1). Comprehensive reviews on the various animal models relevant to OCD-like behaviors have been covered elsewhere previously [13, 14, 17, 21, 31]. In the last decade, the ability to study cellular physiology

Table 1 Approaches to constructing animal models of OCD.

General approach	Specific method	Strengths	Weaknesses	References
Genetics	Knockouts (*Hoxb8*, *Sapap3*, *Slitrk5*); overexpression (*Eaat3*)	Recapitulates genetic abnormality in human disorder; focus on gene of interest	Variable penetrance of genetic abnormality in rodents, some models exhibit additional behavioral and neural abnormalities not related to OCD; Human relevance of phenotype may be difficult to establish	[14, 17, 18]
Circuit manipulation	Optogenetic stimulation/chemogenetic manipulation	Spatial and temporal control over neural circuit function; may recapitulate some findings in humans with DBS	Current limitations in knowledge of neural circuit abnormalities in human disorder	[10, 19, 20]
Pharmacological	Administration of neurotransmitter agonist or antagonist (8-OHDPAT, Quinpirole, mCPP, RU24969)	Focus on the neurotransmitter system of interest	Lack of evidence that OCD involves selective lesions of a single neurotransmitter system	[13, 14, 21]
Immunological	Streptococcal antigen exposure	Focus on neuroinflammatory effects	Lack of specificity for OCD	[22–25]
Neurodevelopmental	Neonatal clomipramine	Easy to administer	Lack of specificity for OCD	[26, 27]
Behavioral	Naturally occurrence; induced by experimental manipulation	Focus on phenotypes of interest	Stereotypic and repetitive behaviors are present in many neuropsychiatric conditions other than OCD	[28–30]

OCD, obsessive compulsive disorder; 8-OHDPAT, 8-hydroxy-2-(di-n-propilamino) tetralin; mCPP, meta-chlorophenylpiperazine; DBS, deep brain stimulation.

using photosensitive, genetically encoded molecules has profoundly transformed neuroscience. Transgenic and optogenetic techniques have given researchers unprecedented access to the function of specific genes and discrete neural circuit elements and have been instrumental in the identification of novel brain pathways that become dysregulated in neuropsychiatric diseases[32], and manipulations of specific genes or circuits promise a useful new approach to generate animal model. Importantly, the combination of genetic and circuit-specific manipulation technology in recent studies allows us to deeply identify the molecular and circuit events underlying abnormal repetitive behaviors relevant to OCD in rodents. This review will focus on the most recent progress specifically toward classic genetic animal models and advanced neural circuit findings, which help to better understand biological mechanisms underlying OCD from a genetic to circuit-level perspective and provide direction for ongoing research on this disorder.

Genetics in OCD

Genetic Basis Relevant to OCD

Common compulsive behavior in OCD patients includes actions such as hand washing, checking, and ordering. Indeed, these themes do not occur randomly, and OCD patients with different cultural and social backgrounds worldwide have been preoccupied with certain themes consistently, which increases the possibility of a common genetic basis [33]. The strongest evidence for a heritable component of OCD derives from twin and family studies that have demonstrated that OCD is familial and the familiality is partly due to genetic factors [34]. As described in the review across twin studies using a dimensional approach, OCD symptoms are heritable, with genetic influences in the range of 45–65% in childhood-onset OCD and 27–47% in adults-onset OCD [35]. In general, the heritability of OCD is approximately 50% on the basis of concordance rates in monozygotic and dizygotic twin studies [8]. Given this, researchers have been searching for the specific genes that create a risk for developing OCD, and genome-wide association studies (GWAS) and identification of de novo mutations (DNMs) are mainly used strategies to further explore genetic mechanisms.

The neuronal glutamate transporter gene *SLC1A1* has been a very promising candidate gene for OCD based on linkage studies and convergent evidence implicating glutamate in OCD pathophysiology [36–38]. A meta-analysis incorporated previously associated *SLC1A1* single-nucleotide polymorphisms (SNPs) and showed only modest associations that were not significant after multiple-test correction [39]. Notably, the results do not undermine the potential contribution of glutamatergic dysregulation to OCD pathology and demonstrate the need for next-generation sequencing and larger collaborative samples. The 2 published GWASs of OCD have identified SNPs with roles in glutamate signaling and excitatory synaptic functions [39, 40], though have not yet reached genome-wide significance in a meta-analysis of the two consortia [41]. Then Burton *et al.* used pediatric obsessive-compulsive trait phenotypes and identified a genome-wide significant region in the genome that included the *PTPRD* gene [42] mediating synapse adhesion and the development of excitatory synapses, which had been previously highlighted in the OCD Collaborative Genetics Association Study (OCGAS) [40]. Using obsessive-compulsive symptoms rather than a clinical diagnosis, a study of adult twins identified a genome-wide significant SNP in *MEF2B* [43]. Strom *et al.* enrolling 14140 individuals diagnosed with OCD have provided evidence of a new genome-wide significant locus on chromosome 3p21.1 implicated in OCD [44], which has added new genome-wide significant regions to our current findings. In addition, two whole-exome sequencing (WES) studies of parent-offspring OCD trios conducted by Cappi *et al.* have identified genes associated with the pathology of OCD, such as *CHD8* and *SCUBE1*, which have provided compelling evidence for the role of de novo mutations (DNMs) in OCD [45, 46]. Furthermore, whole-genome sequencing (WGS) has been considered the preferred genomic platform due to more classes and sizes of mutations than WES. Then Lin *et al.* applied WGS and identified three high-confidence chromatin modifiers (*SETD5*, *KDM3B,* and *ASXL3*) as OCD candidate risk genes, which are likely to be upstream regulators of neurotransmitter system expression and control necessary neurocognitive functions [47].

To date, while there does not appear to be a specific "OCD gene", there is evidence that particular versions or alleles of certain genes may signal greater vulnerability. That said, it is far from clear how these genes influence the development of OCD, and there is plenty of research that still needs to be done. Larger sample sizes and next-generation sequencing are needed to identify the potential role of genes in future studies.

Genetically Manipulated Animals

There has been a common strategy using transgenic technology to establish animal models of neuropsychiatric disorders, due to the increasing sophistication of available techniques [48–50]. These strategies allow investigators to upregulate or downregulate genes of interest in specific brain regions at particular developmental timepoints, with temporal and spatial precision that has not been achievable previously [51]. Integration of genetics with complementary methodologies (e.g., activity imaging, electrophysiology,

and anatomical methods) provides a glimpse of highly selective means to control specific cell types in brain regions of interest in animals [52, 53], which provide support for performing cell-type specific interventions in humans. Thus, the circuit-specific function of candidate genes identified in human studies can now be directly assessed in mice. However, the generation of targeted transgenics relevant to OCD is still in its infancy, largely due to a lack of reproducibility in human genetic studies when identifying candidate genes. The current genetic models of OCD are mainly not based on a known mutation related to OCD in humans. Rather, they are based on behavioral similarities, like repetitive, compulsive-like behaviors and anxiety-like behavior (Table 2), which have been proposed to be similar to specific OCD symptoms [17, 18]. The mouse genetic models could deepen our understanding of the role of certain genes in compulsive behavior, and shed light on the molecular and cellular mechanisms underlying the pathogenesis of OCD.

Hoxb8 Mutant Mice

One of the first transgenic models reported to be associated with OCD was the *Hoxb8* knockout (KO) mice, which was generated by the Capecchi lab in 2002[54]. Then the investigations focused on *Hoxb8* mutant mice over the past decades have provided unexpected discoveries and striking insights concerning the causes of compulsive grooming in mice. This was unexpected given that HOXB8 is a member of a large family of transcription factors well known for their important roles in establishing body patterning during development. *Hoxb8* KO mice do not exhibit changes in body morphology but rather show severe coat loss due to excessive grooming [54]. Through close observation of a large number of *Hoxb8* KO mice, Tränkner *et al.* revealed a strong female sex bias. The females, but not the males, consistently show anxiety-like behavior in addition to excessive-grooming. Notably, the severity of symptoms in males and females separates at the beginning of sexual maturity, which can be attenuated by lowering female sex hormone levels [55]. *Hoxb8* KO mice display corticostriatal circuit defects with pre- and postsynaptic structural and function alteration, which suggests that the *Hoxb8* gene appears to play an important role in maintaining brain homeostasis including regulating corticostriatal circuit function and behavioral output [56]. Long-term treatment with fluoxetine can reduce behavioral impairments, supporting the potential clinical relevance of this model [56]. Thus, the *Hoxb8* model is promising in that excessive grooming has face similarity to symptoms observed in OC spectrum disorders and may involve neural systems similar to those involved in compulsive behavior in patients, furthermore, it currently has predictive validity in terms of SRRI treatment.

HOXB8 is widely expressed in the olfactory bulb, orbital cortex, hippocampus, caudate-putamen, and brainstem in mice brain [57], and cortical expression (orbitofrontal cortex and anterior cingulate cortex) is strongest in critical brain regions implicated in the pathophysiology of OCD. HOXB8 is expressed early during the embryonic developmental period [54] and its expression is maintained in the subset of cells broadly distributed in the brain, an important question to ask is which cell type is critical to the development of pathological grooming in *Hoxb8* KO mice [17]. In 2010, Chen *et al.* first determined that the expression of *Hoxb8* in the brain originated from bone marrow-derived microglia that migrated into the brain during the postnatal period. Cell-type specific deletion of *Hoxb8* restricted to a subset of the microglia precursors fully recapitulated hair removal behavior. Normal bone marrow transplantation into *Hoxb8* KO mice could efficiently rescue the excessive grooming phenotypes [58]. Furthermore, direct selective ablation of the *Hoxb8* microglia subpopulation is sufficient to induce excessive grooming and anxiety-like behavior [55], which suggests that *Hoxb8*-lineage microglia function mediates the pathophysiology of grooming phenotypes. Nagarajan *et al.* have directly demonstrated the connection between outputs from optogenetically stimulated *Hoxb8* microglia and the activation of neurons and neural circuits responsible for inducing grooming and anxiety-like behaviors [59]. Thus, pathological grooming behavior observed in *Hoxb8* KO mice may originate from defective microglia within specific regions of the brain, and the *Hoxb8* model provides a much deeper insight into the mechanism of OCD at genetic and cellular levels. Furthermore, immunological abnormalities have been widely linked to many psychiatric disorders [60], the *Hoxb8* mouse model may provide evidence to support a link between cells (microglia expressing *Hoxb8*) involved in immune response, brain function, and pathological grooming.

Sapap3 Mutant Mice

SAPAP3 (known as DLGAP3/GKAP3) is a post-synaptic scaffolding protein gene expressed in corticostriatal circuits, particularly highly in the striatum. Welch *et al.* reported that the *Sapap3* KO mice displayed several OCD-like behavioral phenotypes, including anxiety-like behaviors, and excessive auto-grooming, ameliorated by treatment with SSRIs [61]. Consistently, Soto *et al.* reproduced OCD-like behaviors in *Sapap3* KO mice and further revealed that SAPAP3 is expressed in astrocytes and neurons of the striatum, and both cell types made contributions to OCD-like phenotypes in mice. Importantly, SAPAP3 rescue in astrocytes or neurons displayed different degrees of rescue for self-grooming and anxiety-like behaviors [62]. The *Sapap3* KOs display defects in cortico-striatal synapses in structural,

Table 2 Evaluation of genetic animal models of OCD.

Gene target	Organism	Behavioral phenotype	CSTC circuit expression	Neuropathophysiology	Response to SSRIs	References
Hoxb8	KO mouse	Excessive grooming; female sex bias (increased anxiety, more excessive grooming)	HOXB8 is expressed in bone-marrow-derived microglia that migrate into brain OFC, cingulate cortex, and basal ganglia regions	Corticostriatal circuit impairments (excess dendritic spines, pre- and postsynaptic structural defects, long-term potentiation, and miniature postsynaptic current defects)	Response to fluoxetine	[54–59]
Sapap3	KO mouse	Excessive grooming; increased anxiety; impaired reversal learning and behavioral flexibility	SAPAP3 is mainly expressed in the neocortex, striatum, hippocampus, and thalamus	Impaired corticostriatal function (reduced fEPSP); increased baseline firing rates of MSN in striatum; decreased power of LFP oscillations in OFC; increased neural activity in mPFC; astrocytes and neurons in striatum make contributions to OCD-like phenotypes	Response to fluoxetine	[61–66, 68–71]
Slitrk5	KO mouse	Excessive grooming; increased anxiety	SLITRK5 mainly expressed in the neocortex, striatum, and hippocampus	Injured corticostriatal function (reduced fEPSP); increased FosB expression in OFC; decreased MSN dendritic arbor complexity; decreased striatal volume	Response to fluoxetine	[76]
Eaat3/ Slc1a1	OE mouse	Excessive grooming	SLC1A1 is mainly expressed in the human cortex, thalamus, and striatum.	Corticostriatal synapses indicated alterations in NMDA receptor composition/ function and impaired synaptic plasticity	Response to fluoxetine	[82]
5-HT2c	KO mouse	Compulsive-like behaviors (non-nutritive chewing, increased head-dipping); anxiolytic phenotype	Involvement of serotonin signaling in the CSTC circuit	Disruption of serotonin signaling; decreased level of corticotrophin hormone response to stimulation	–	[85, 86]
DAT	Knockdown mouse	Exhibit more stereotyped and predictable syntactic grooming chains	DAT is the membrane transport protein of the dopaminergic synapse located in the presynaptic neurons and regulates extracellular dopamine levels	Elevated (170%) levels of extracellular dopamine in the neostriatum; altered corticostriatal excitatory transmission	–	[87]

Table 2 (continued)

Gene target	Organism	Behavioral phenotype	CSTC circuit expression	Neuropathophysiology	Response to SSRIs	References
Vmat2	aVMAT2cKO mouse	Excessive grooming, anxiety-like behaviors	VMAT2 is expressed in PFC astrocytes	Increased MAOB activity; A significant reduction of mPFC DA levels; Strengthening of PFC-Striatum transmission in medium spiny neurons	–	[88]
Cbln2	KO mouse	Compulsive behaviors (stereotypic pattern running, marble burying, explosive jumping, and excessive nest building)	Largely expressed in distinct subsets of excitatory cortical neurons	Exhibit decreased brain serotonin levels	Response to fluoxetine	[89]

KO, knockout; OE, overexpression; CSTC, cortico-striato-thalamo-cortical circuit; SSRIs, selective serotonin reuptake inhibitors; MSN, medium spiny neuron; LFP, local field potential; fEPSP, field excitatory post-synaptic potential; OFC, orbitofrontal cortex; mPFC, medial prefrontal cortex; NMDA, N-methyl-D-aspartate receptor; MAOB, monoamine oxidase B; DA, dopamine.

electrophysiological, and biochemical studies [61, 63]. There is a subtle structural defect in the postsynaptic complex of the striatum, with a significant reduction in the thickness of the dense layer in *Sapap3* KO mice. Functional defects seem to parallel structural defects. The researchers examined synaptic transmission in the striatum using electrophysiological recordings and found that field excitatory postsynaptic potentials (fEPSPs) were significantly reduced in *Sapap3* KOs. Viral rescue of SAPAP3 expression in the striatum of *Sapap3* KO mice could prevent behavioral abnormalities and reverse the striatal neurotransmission defects [61]. Later studies have further revealed that thalamostriatal synaptic activity was unaffected by *Sapap3* deletion, in contrast to corticostriatal synapses [64], suggesting an important role for *Sapap3* in postsynaptic glutamatergic synaptic function at cortico-striatal synaptic transmission [61]. *In vivo* electrophysiological recordings showed significant elevation in baseline firing rates of putative medium spiny neurons (MSNs) in the striatum of KOs, and optogenetic stimulation of lateral orbitofrontal cortex (lOFC) terminals in the central striatum reduced striatal MSNs firing rates and alleviated compulsive grooming in *Sapap3* Kos [63], suggesting a direct relationship between abnormal cortico-striatal signaling and compulsive behavior. Furthermore, striatal MSNs showed an increased response to the *in vitro* optogenetic activation of secondary motor area (M2) terminals in the striatum of the *Sapap3* KO mice, supporting a potential role for M2-striatal circuit may contribute to compulsive behaviors [65]. These findings demonstrate a link between molecular changes at cortico-striatal synapses and repetitive pathological behaviors in *Sapap3* KO mice. Together, *Sapap3* KO mice achieve face validity, construct validity, and predictive validity in the assessment of behaviors and neuropathophysiology implicated in OCD.

Some authors have further attributed this abnormal behavior and brain function to alterations in metabotropic glutamate receptor 5 (mGluR5) signaling, a receptor that is highly expressed in the striatum [66]. Notably, a recent genetic analysis of post-mortem brains demonstrated reduced expression of the SAPAP3 protein in the striatum of OCD patients, and variants of the *SAPAP3* gene have been reported to be associated with early-onset OCD and trichotillomania, another compulsive disorder previously [67]. Together, these data support the hypothesis that alterations in striatal activity patterns contribute to the generation of compulsive episodes. Besides, several studies in *Sapap3* KO mice have corroborated the potential implication of the frontal cortex, which may contribute to impaired behavioral flexibility [68, 69] and the imbalance of habitual and goal-directed behavior in *Sapap3* KO mice [70]. Manning *et al.* revealed that impaired instrumental reversal learning was associated with increased neural activity in the medial prefrontal cortex [68]. In addition, the lateral OFC exhibited

network dysfunction in *Sapap3* knockout mice, demonstrated by alterations in local field potential (LFP) oscillations and increased burst firing in lOFC [71], extending our understanding of the underlying neuropathophysiology in OCD.

Slitrk5 Mutant Mice

Shortly after the initial characterization of the *Sapap3* KO strain, SLIT and NTRK-like protein-5 (SLITRK5), another synaptic protein, was implicated in OCD-relevant behaviors. SLITRK5 is predominantly expressed in the central nervous system (CNS) and contains two important conservative domains consisting of leucine repeats (LRRs) located at the amino-terminal in the extracellular region and tyrosine residues (Tyr) located at the carboxyl-terminal in the intracellular domains. These special structures make SLITRK5 play an important role in the pathological process of the CNS and participate in many essential steps of central nervous system development including neuronal process outgrowth, and synaptogenesis [72]. Mutations in *SLITRK5* genes have been implicated in mental disorders, such as Tourette syndrome, autism spectrum disorders (ASDs), Parkinson's disease (PD), and attention-deficit/hyperactivity disorder (ADHD) [73, 74]. In human samples, a burden of *SLITRK5* coding variants that influence synapse formation *in vitro* has previously been described in OCD cases relative to controls [75].

In 2010, a genetic study provided direct evidence for the role of SLITRK5 in the development of OCD-like behaviors. Shmelkov *et al.* described that the loss of *Slitrk5* led to OCD-like behaviors in mice. From 3 months, these knockout mice showed increased anxiety-like and excessive grooming behaviors, causing hair loss and skin lesions, which was alleviated by chronic fluoxetine treatment [76]. *Slitrk5* was detected to localize to the postsynaptic zone, *Slitrk5* KO mice displayed anatomical defects and deficiency in corticostriatal glutamatergic transmission mediated by changes in glutamate receptor composition. In addition, *Slitrk5* KOs had reduced striatal volume, complexity of dendritic arbors in striatal medium spiny neurons, and the expression of glutamate receptor subunits NR2A, NR2B, GluR1, and GluR2 were decreased in the striatum. Investigation of the neural circuit abnormalities underlying these behavioral findings revealed that FosB expression was specifically higher in OFC of *Slitrk5* Kos [76]. In all, this evidence suggested that cortico-striatal dysfunction may be responsible for the observed behavioral abnormalities in *Slitrk5* KOs.

Eaat3/ Slc1a1 Overexpression Mice

Excitatory amino acid transporters (EAATs) are glutamate transporters in the solute carrier 1A (SLC1A) family [77], which is fairly ubiquitously expressed in the brain. It is important in maintaining low local concentrations of glutamate, where its predominant post-synaptic localization can buffer nearby glutamate receptors and modulate excitatory neurotransmission and synaptic plasticity [78]. Several mouse models completely or partially deficient in *Eaat3* have shown no change in anxiety-like or repetitive behaviors [79–81]. Importantly, Delgado-Acevedo *et al.* generated a transgenic mouse with conditional *Eaat3* overexpression in the forebrain and showed that the *Eaat3* overexpression mice displayed increased anxiety-like and repetitive behaviors, which were both restored by chronic treatment with fluoxetine. Electrophysiological and molecular analyses at corticostriatal synapses indicated alterations in NMDA receptor composition/ function and impaired synaptic plasticity, highlighting the impact of EAAT3 on regulating these synapses and suggesting they may contribute to the observed behavioral alterations [82]. Intriguingly, the rs301430C allele, a *SLC1A1* polymorphism highly replicated in human OCD research was related to increased transcript levels [83], which suggested that overexpression may contribute to susceptibility to OCD. Consistently, genetic linkage and association evidence of OCD point to *SLC1A1* [84], which is prominently expressed in the cortical-striatal-thalamic-cortical circuit. Although genome-wide screens have shown a correlation between OCD and *EAAT3*, it is only in recent years that work has emerged showing an altered function of *EAAT3* in relation to OCD phenotypes. Perturbations in the expression or function of EAAT3 can likely add to the risk of OCD-like behavior, though it is probably part of a large and complex interwoven system.

Other

Other transgenic models, such as *5-HT2c* KO mice [85, 86], and *DAT* KD mice [87], show a number of behavioral abnormalities that may be related to several basal ganglia- and dopamine-related disorders. Recently, Petrelli *et al.* produced a conditional deletion of the vesicular monoamine transporter 2 (*Vmat2*) specifically in astrocytes (aVMAT2cKO mice) and found excessive grooming and anxiety-like behavior in mice. They have also detected alterations in mPFC-to-dorsomedial striatum synapses. Importantly, behavioral and synaptic changes were rescued by re-expression of mPFC VMAT2 and L-DOPA treatment [88]. In addition, Seigneur *et al.* have recently reported that constitutive *Cbln2* KO mice, but not *Cbln1* KO mice, display robust compulsive behaviors, including stereotypic pattern running, marble burying, explosive jumping, and excessive nest building, and exhibit decreased brain serotonin levels, which can be alleviated by fluoxetine treatment. Injection of recombinant CBLN2 protein into the dorsal raphe of *Cbln2* KO mice largely reversed their compulsive behaviors [89],

suggesting that *Cbln2* controls compulsive behaviors by regulating serotonergic circuits in the dorsal raphe.

The mouse genetic tools, such as conditional knockout mice, BAC transgenesis, neuronal cell-type-specific gene expression profiling, and optogenetics, can be readily applied to precisely interrogate the roles of genes, cell types, and neuronal activities within a given circuit in the pathogenesis of OCD-like behaviors in mice. Thus, the emergence of genetic models exhibiting multiple OCD-like behaviors, particularly excessive and often self-injurious grooming, has begun to provide novel insights into the neurobiological basis of such pathological behaviors, studying the neural mechanisms of super-stereotypy in these models may further our understanding of the neural mechanisms of compulsive behaviors [90]. Currently, rapid advances in human genetics, particularly the increasing availability of powerful sequencing technologies, provide an opportunity to search for candidate risk genes that may be causal in OCD in unprecedented ways. If such candidate genes could be found, the introduction of critical genes into genetically engineered mice may help to establish novel OCD mouse models with construct validity. In sum, the crosstalk between the study of animal models with precise gene-editing tools for mechanistic dissection and the study of human models with true disease validity is needed to advance biological understanding and therapeutics for OCD.

Neural Circuitry of OCD

Neuroanatomy and Neural Circuits Associated with OCD

Neuroimaging findings from humans with OCD support a cortico-striato-thalamo-cortical (CSTC) circuitry model focused on a network of brain regions involving the frontal cortex, striatum, and thalamus, which is widely considered to be the neuroanatomical substrates of OCD [91]. The CSTC circuits are aberrant during both resting periods and episodes of symptom provocation in OCD individuals [92], which return to normal levels in patients responding to first-line intervention treatments [93–95]. Furthermore, the neuromodulation technology for treatment-refractory patients including deep brain stimulation (DBS) [96], transcranial direct current stimulation (tDCS), and repetitive transcranial magnetic stimulation (rTMS) have been reported with a relevant beneficial effect by modulating underlying disturbances in CSTC neural circuit, and may act at a distance [97, 98]. Thus, to have an idea of how brain malfunctions can give rise to obsessions and compulsions, further understanding of CSTC networks is helpful.

The CSTC pathway is a multi-synaptic neuronal circuit that connects the cortex, striatum, and thalamus [99]. The prefrontal cortex is the most dorsal portion of the frontal lobe, viewed as the highest integration center for emotional processing and cognitive function. The striatum is the information processing hub in the middle of the brain and receives inputs from other brain regions like the cortex to the basal ganglia. The thalamus, part of the diencephalon, acts as a relay station of limbic information, sensory information, and motor information [7]. Briefly, unprocessed signals of these neuronal circuits run from specific cortical areas, through the striatum and globus pallidus, where habitual behaviors and conditioned responses are re-enforced, to the thalamus which is a sensory and motor relay and regulates alertness, and then back to the cortical areas (Fig. 2A). The typical conceptualization of CSTC circuitry entails a direct and indirect pathway, which is defined as a positive-feedback and negative-feedback loop respectively. The direct pathway (accelerator) with the net effect of excitation on the thalamus involves direct projections from the striatum to the globus pallidus interna (GPi) [8]. The indirect pathway (brake) with the net effect of inhibition on the thalamus involves indirect projections from the striatum to GPi *via* Gpe. In healthy individuals, the excitatory, direct pathway is modulated by the indirect pathway's inhibitory function [8]. In OCD patients, an imbalance of activity between the direct and the indirect loop results in excess tone in the former over the latter, which is thought to underlie the manifestation of OCD (Fig. 2B) [8].

With subsequent work on the neurobiology of OCD came to light, accumulating evidence pointed out that OCD is mediated by parallel, partly segregated, CSTC circuits that are involved in sensorimotor, cognitive, and affective processes [4, 91, 100]. Van den Heuvel *et al.* have integrated data and proposed a revision of the classical CSTC model composed of five parallel neurocircuits that functionally link the frontocortical and subcortical areas in OCD [101]. Then Shephard *et al.* expanded on van den Heuvel *et al.*'s model to propose several ''clinical profiles'' that reflect different phenotypes of OCD (executive function, sensory phenomena, response inhibition, reward processing, fear regulation) (Fig. 3) [91]. Detailly, the sensorimotor circuit (green) is involved in stimulus–response-based habitual behavior. The dorsal cognitive circuit (blue) is involved in emotion regulation and executive functions such as planning and working memory. The ventral cognitive circuit (purple) is involved in response inhibition. The ventral affective circuit (yellow) is involved in processing and reward responsiveness. The frontolimbic circuit (red) is involved in emotional responses like anxiety and fear extinction. Consistently, brain imaging studies have reported that in OCD, the nodes of these networks display abnormal activity at rest and during symptom provocation [100], although there are inconsistencies in the directionality of findings across studies [102, 103]. This issue could be due to heterogeneity in the OCD samples

selected, differences in imaging methods, or both factors. In addition, structural and functional imaging data supported that the alterations in frontolimbic, frontoparietal, and cerebellar networks likely be implicated in the OCD pathology [94, 104, 105], which suggests that the neurobiological OCD model continuously extends from the classical CSTC circuit to a more complex neural circuit integrated into whole brain network. These are, however, correlations; it is highly impracticable and almost impossible to identify a direct causal relationship in humans between symptoms of OCD and the observed neural abnormalities. This issue has spurred the development of experimental animal systems by manipulating specific circuits to deeply clarify the molecular and circuit events underlying OCD, given key aspects of OCD-related brain regions are evolutionally conserved between humans and rodent species.

Manipulation of Neural Circuitry Implicated Compulsive-like Behaviors

Molecular pathways affect the function of neurons and synapses, and hence neuronal connectivity and circuits,

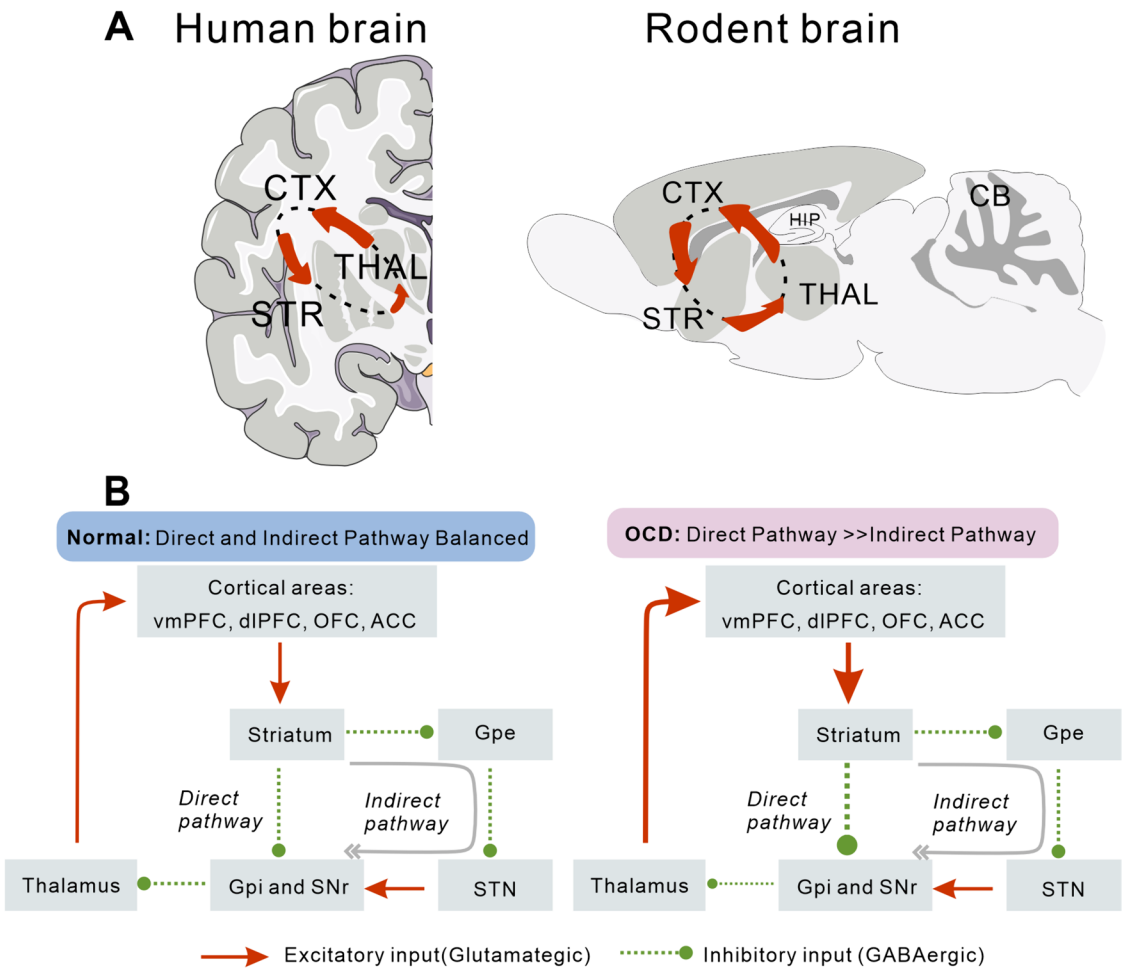

Fig. 2 The cortico–striato–thalamo–cortical (CSTC) circuitry. **A** Simplified neuroanatomical model of the CSTC circuitry in human and rodent brain. Left panel, Diagram of a human brain section (coronal view) illustrating the major brain regions composing the CSTC circuitry. Right panel, Diagram of a rodent brain section (sagittal view) illustrating the equivalent CSTC circuitry in the corresponding rodent brain structures. All brain regions depicted here are representative of a schematic brain diagram and are not intended to provide exact anatomical locations. CTX, cortex; STR, striatum; THAL, thalamus; HIP, hippocampus; CB, cerebellum. **B** Descriptive visualization of direct and indirect pathways within CSTC circuitry of healthy subjects (left panel) and patients with OCD (right panel). The direct pathway (accelerator) with the net effect of excitation on the thalamus involves direct projections from the striatum to Globus Pallidus interna (GPi). The indirect pathway (brake) with the net effect of inhibition on the thalamus involves indirect projections from Striatum to GPi via Gpe. In healthy individuals, the excitatory, direct pathway is modulated by the indirect pathway's inhibitory function. In OCD patients, an imbalance of activity between the direct and the indirect loop results in excess tone in the former over the latter. Solid arrows depict excitatory inputs whereas dashed dots indicate an inhibitory input. Line thickness represents the strength of the excitation/inhibition. vmPFC, ventromedial prefrontal cortex; dlPFC, dorsolateral prefrontal cortex; OFC, orbitofrontal cortex; ACC, anterior cingulate cortex; GPi, globus pallidus interna; SNr, substantia nigra pars reticulata; GPe, globus pallidus externa; STN, subthalamic nucleus.

to modify brain function. The ever-expanding genetic and imaging studies suggest abnormalities in specific brain regions, which seem to converge toward CSTC synaptic dysfunction in OCD pathology [9, 18, 106]. However, a single model is insufficient to elucidate OCD pathophysiology, because OCD is highly heterogeneous. Integration of other brain structures beyond the CSTC circuits may also be required to establish causal links in OCD pathophysiology. The next generation of research in OCD needs to address the neural circuitry underlying the behavioral symptoms, the cell types playing critical roles in these circuits, and common intercellular signaling pathways in animals. The researchers have therefore turned to animal models to test the causal role of specific circuits in the generation and relief of OCD-like symptoms; and determine precise localization of neurochemical abnormalities that lead to abnormal repetitive behaviors. In the late 2000s, the advent of optogenetics technology allows precise modulation of neural circuit activity in the generation of behavior, this technique takes advantage of restricted expression of light-activated ion channels in particular neural populations to allow spatially and temporally specific reversible stimulation in awake behaving animals. Through tissue-specific expression and local stimulation of light-activated proteins, distinct neural circuits can therefore be rapidly activated or inhibited without affecting neighboring cells [53, 107]. Using optogenetic tools, researchers are now able to selectively isolate distinct neural circuits that contribute to these disorders and perturb these circuits *in vivo*, which in turn may lead to the normalization of maladaptive behavior. Recently, several studies have integrated optogenetics with complementary technologies to validate circuitry models by directly stimulating or inhibiting

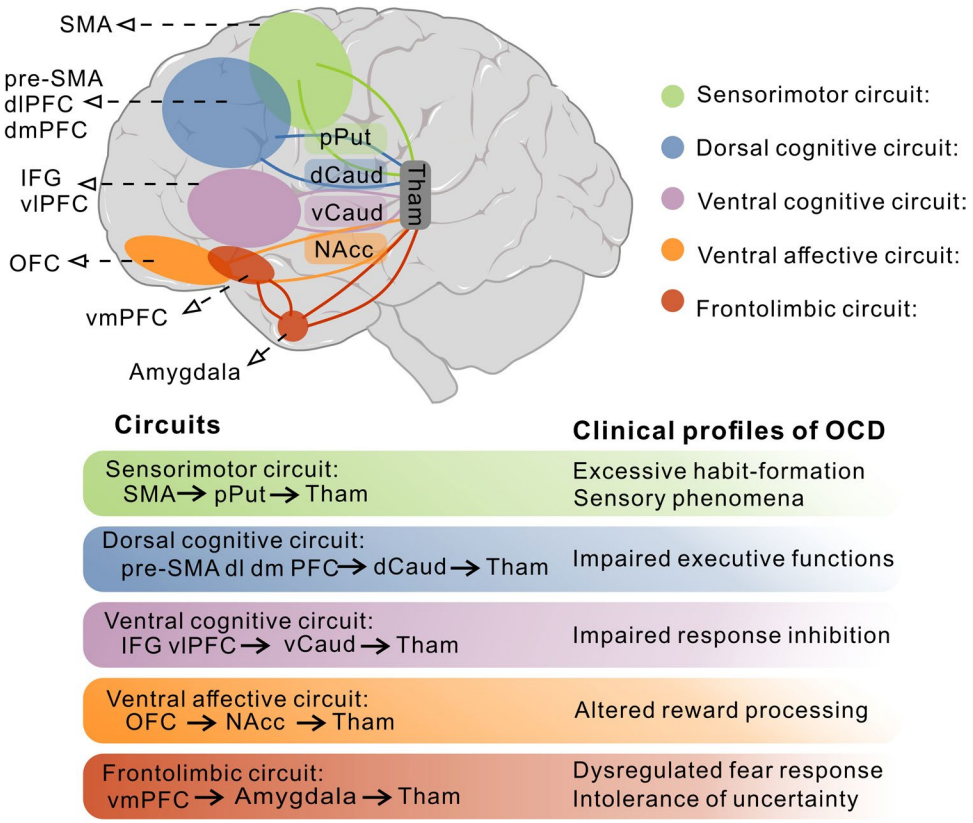

Fig. 3 Overview of the circuits involved in OCD. There are five neurocircuits involved in the CSTC model in OCD proposed by van den Heuvel *et al.* [101]. Then Shephard *et al.* expanded on van den Heuvel *et al.*'s model to propose several ''clinical profiles'' that reflect different phenotypes of OCD [91]. The model is mediated by parallel, partly segregated neurocircuits implicated in sensorimotor, cognitive, affective, and motivational processes. Detailly, the sensorimotor circuit (green) is involved in stimulus–response-based habitual behavior. The dorsal cognitive circuit (blue) is involved in emotion regulation and executive functions such as planning and working memory. The ventral cognitive circuit (purple) is involved in response inhibition. The ventral affective circuit (yellow) is involved in processing and reward responsiveness. The frontolimbic circuit (red) is involved in emotional responses like anxiety and fear extinction. Notably, the model also emphasizes the significance of crosstalk between the neurocircuits, such as the ventral and dorsal cognitive circuits exerting top-down regulation on emotion-related systems mediated by affective and the front-limbic circuits. SMA, supplementary motor area; pPut, posterior part of putamen; Tham, thalamus; pre-SMA, pre-supplementary motor area; dl dmPFC, dorsolateral, dorsomedial prefrontal cortex; dCaud, dorsal part of caudate nucleus; IFG, inferior frontal gyrus; vlPFC, ventrolateral prefrontal cortex; vCaud, ventral part of caudate nucleus; OFC, orbitofrontal cortex; NAcc, nucleus accumbens; vmPFC, ventromedial prefrontal cortex.

components of neural networks [32, 107], and further determined specific circuits necessary and/or sufficient to either generate or alleviate OCD-like symptoms in mice (Table 3). In this section, we will review recent studies about behavioral output involved in repetitive behaviors under circuit-based manipulation.

Corticostriatal System

The technical advance of optogenetic manipulation was initially applied to the study of OCD pathology and treatment in two back-to-back studies. Ahmari *et al.* used optogenetics to directly generate hyperactivity in mOFC-ventral striatal projections and directly tested whether hyperstimulation of glutamatergic OFC-ventromedial striatum (VMS) projections led to OCD-like behaviors in mice [108]. Whereas acute OFC-VMS stimulation did not produce repetitive behaviors, repeated hyperactivation over multiple days generated a progressive increase in grooming, a mouse behavior related to OCD. Increased grooming persisted for 2 weeks after stimulation cessation. The grooming increase was temporally coupled with a progressive increase in light-evoked firing of postsynaptic VMS cells. Both increased grooming and evoked firing were reversed by chronic fluoxetine. Then Xue *et al.* established a mice model with OCD-like excessive self-grooming using repeated stimulations [109] as Ahmari *et al.* reported. In parallel, a study by Burguiere *et al.* [63] used optogenetics to probe OFC-striatal circuits in *Sapap3* KO mice to treat compulsive behavior. The activation of the lOFC-striatal circuit compensated for impaired fast-spiking neuron striatal microcircuits restored MSN tone-response inhibition, and ameliorated compulsive grooming of *Sapap3* KO mice. The two separate studies suggest that whether used in conjunction with previously validated transgenic models or on their own, optogenetic tools may revolutionize the study of disease-relevant circuits in animal models of OCD. Then, Corbit *et al.* adapted optogenetics to dissect neural circuits underlying OCD-related phenotypes and demonstrated that strengthened secondary motor area (M2) inputs in the ventral striatum of *Sapap3* KOs likely contributed to striatal hyperactivity and compulsive behaviors, supporting a potential role for supplementary and pre-supplementary motor cortex in the pathology and treatment of OCD [65]. Together, these studies implicate the striatum's role in the grooming state of rodents, potentially through balancing the activity of the corticostriatal circuit.

The striatum comprises several subdivisions, each with differential neural circuitry and function. Ventral striatal islands of Calleja (IC) neurons are evolutionally conserved across many species, predominantly in the olfactory tubercle (OT). Zhang *et al.* have revealed that optogenetic activation of OT D3 neurons robustly induced self-grooming in mice in competition with other ongoing behaviors. Conversely, the inactivation of these neurons halted ongoing grooming. *In vivo,* calcium signal recordings from subpopulations of OT D3 neurons revealed elevated neuronal activity before and during grooming. The local striatal output was regulated by synaptic bonds with neighboring OT neurons (mainly spiny projection neurons), whose firing rates displayed grooming-related modulation [110]. This study uncovers a surprising role of the striatal microcircuitry network in regulating motor output and has important implications for the neural control of grooming. Thus, this evidence has implicated corticostriatal circuits as critical brain regions controlling levels of anxiety and OCD-like behaviors.

Limbic System

Amygdala The amygdala consists of the basolateral amygdala (BLA), medial amygdala (MeA), and the central nucleus of the amygdala (CeA), which interact with CSTC pathways in the processing of cognition and emotional regulation[111]. Imaging studies have revealed alterations in the volume and activity of the amygdala in OCD patients [112, 113], which is considered highly relevant to the pathophysiology of OCD. Paul *et al.* have shown abnormal amygdala-prefrontal connectivity during the appraisal of symptom-related stimuli [114], which is consistent with the involvement of affective circuits in the functional neuroanatomy of OCD. To identify the neural circuitry controlling OCD-checking behaviors, Sun *et al.* established a quinpirole-treated mouse model of OCD-like checking and anxiety-like behavior. This model displayed increased excitability of mPFC-projecting BLA^{Glu} neurons controlling OCD-like checking behavior. Optical activation of BLA^{Glu} terminals in the mPFC accelerated the process of the quinpirole-induced OCD-like checking behavior. Conversely, optical inhibition in the mPFC restored the checking behaviors induced by quinpirole. These findings suggest that the BLA^{Glu}-mPFC pathway plays an important role in the development of OCD-like checking behaviors and may be an upstream input to the CSTC circuitry, which is a vital complementary part of the BLA-CSTC model in the pathophysiology of OCD [115].

MeA is a critical center for modulating innate emotional behaviors. Glutamatergic neurons and GABAergic neurons in the input and output circuits of the posterior dorsal subdivision of MeA (MeApd) participate in grooming behavior. Hong *et al.* used cell-type specific functional manipulations of distinct neuronal populations within MeApd and suggested that glutamatergic neurons in MeApd promoted repetitive self-grooming. Conversely, GABAergic MeApd neurons suppressed self-grooming. This work provided a novel framework for understanding circuit-level mechanisms underlying repetitive grooming behavior [116].

Table 3 Manipulation of OCD-related regions implicated compulsive-like behaviors.

Optogenetic stimulation	Specific circuit or cell	OCD-relevant characteristics	Putative mechanisms of neuropathophysiology	Response to SSRIs	References
Corticostriatal system	OFC-VMS repeated stimulation in WT mice	A progressive increase in grooming	Increase in the light-evoked firing of postsynaptic VMS cells	Response to fluoxetine	[108, 109]
	lOFC-CS optogenetic stimulation in Sapap3-KOs	Alleviates compulsive grooming	Enhances feed-forward inhibition in striatal microcircuits	–	[63]
	Ventral striatal the islands of Calleja (IC) neurons	Robustly induced self-grooming in mice	Elevated neuronal activity before and during grooming	–	[110]
Limbic system-Amygdala	BLA^{Glu}-mPFC	Accelerated OCD-like checking behavior	Increased excitability of mPFC-projecting BLA^{Glu} neurons in the quinpirole-induced model	–	[115]
	The posterior dorsal subdivision of MeA (MeApd)	Glutamatergic neurons promote repetitive self-grooming; GABAergic neurons suppress self-grooming	The circuit-level control of grooming behaviors by the MeApd	–	[116]
Limbic system- Lateral septum	VS-LSv-Tu	Triggers delayed but robust excessive grooming behavior	LSv is implicated in the manifestation of repetitive grooming behavior	–	[118]
Limbic system-Hypothalamus	LH-PVH circuit	The activation of glutamatergic LH→PVH terminals promotes self-grooming behavior; The activation of GABAergic LH→PVH terminals disrupted repetitive grooming induced by water spray and promoted feeding behavior	Parallel LH→PVH circuit as a potentially important brain mechanism linking compulsive and feeding behaviors	–	[125]
Brain stem system	SNc-lOFC-VMS	Optogenetic inhibition of SNc-VMS projections and activation of SNc-lOFC projections could alleviate excessive self-grooming in both OCD-like mice and Sapap3 KO mice	SNc-VMS and SNc-lOFC circuits mediate SNc dopaminergic regulation of self-grooming through their actions on postsynaptic D1R in VMS PV cells and presynaptic D2Rs in lOFC-targeting DANs, respectively	_	[109]
	CeA-MPL^{SST}-VTA^{DA} circuit	The activation of the MPL^{SST} neurons induced self-grooming	The signal from the CeA specifically triggers the MPL-mediated self-grooming	Response to fluoxetine	[134]

Table 3 (continued)

Optogenetic stimulation	Specific circuit or cell	OCD-relevant characteristics	Putative mechanisms of neuropathophysiology	Response to SSRIs	References
Spinal system	$Cbln2^+$ Sp5C to spinal neural circuit	Activation of $Cbln2^+$ Sp5C neurons evoked long-lasting repetitive orofacial self-grooming, and activation of spinal-projecting $Cbln2^+Sp5C$ neurons evoked long-lasting repetitive grooming	Increased GCaMP fluorescence of spinal-projecting $Cbln2^+Sp5C$ neurons during oil-induced orofacial self-grooming	Response to fluoxetine	[137]

OFC, orbitofrontal cortex; VMS, ventromedial striatum; lOFC, lateral orbitofrontal cortex; CS, central striatum; BLA, basolateral amygdala; mPFC, medial prefrontal cortex; VS, the hippocampal ventral subiculum; LSv, ventral lateral septum; Tu, hypothalamus tuberal nucleus; LH, lateral hypothalamus; PVH, paraventricular hypothalamus; SNc, substantia nigra pars compacta; CeA, central amygdala; MPL, medial paralemniscal nucleus; VTA, ventral tegmental area; Cbln2, Cerebelline-2; Sp5C, spinal trigeminal nucleus; MeApd, the posterior dorsal subdivision of medial amygdala; DANs, midbrain dopamine neurons.

The lateral septum (LS) With abundant inputs from neocortical and all cortical regions, LS is an ideal site for integrating perception and experience signals in order to modulate the activity of hypothalamic and midbrain nuclei that regulate motivated behaviors [117]. The ventral division of the lateral septum (LSv) is a limbic structure long known to be associated with emotional processes and stress responses. Mu *et al.* reported that optogenetic activation of LSv triggered robust grooming behavior, suggesting that LSv is implicated in the manifestation of repetitive grooming behavior [118]. By mapping the upstream and downstream areas of LSv contributing to self-grooming, they identified hippocampal-septal-hypothalamus (VS→LSv→Tu) circuitry in the limbic system linking hippocampal ventral subiculum to the ventral lateral septum (LSv) and then lateral hypothalamus tuberal nucleus. Optogenetic activation of this circuit triggered delayed but robust excessive grooming with patterns closely resembling those evoked by emotional stress. Conversely, inhibition of this circuit significantly suppressed grooming triggered by emotional stress. In addition, Xu *et al.* have revealed that LSv received emotional state-related signals from the PVN, and triggered stress-related grooming [119]. These results uncover a previously unknown limbic circuitry involved in regulating stress-induced grooming behavior and pinpoint a critical role of LSv in this ethologically important behavior [118].

Hypothalamus The hypothalamus, the initial part of the hypothalamic-pituitary-adrenal (HPA) axis, plays a vital role in regulating stress response in the CNS. Clinically, the dysfunction of the HPA axis has been implicated in the pathogenesis of OCD [120, 121]. Notably, lesions, such as suprasellar tumors [122] or a primary hypothalamic dysfunction [123] in the hypothalamus likely lead to obsessive-compulsive symptoms. The increased c-fos expression in the hypothalamus was observed in two OCD mouse models induced by pharmacological reagents (8-hydroxy-DPAT hydrobromide (8-OH-DPAT) and RU24969) [124]. The lateral hypothalamus (LH), a part of the posterior hypothalamus, functions as a vital center for modulating vertebrate behavior including stress, energy balance, reward, and motivated behavior. The input of LH GABA and glutamate neurons targets a common subset of paraventricular hypothalamus (PVH) neurons, revealing LH→PVH circuit is likely to be implicated in these behaviors. Optogenetically manipulating the activity of LH glutamatergic and GABAergic inputs targeting the PVH differentially promoted either feeding or repetitive self-grooming. The activation of glutamatergic LH→PVH terminals promoted robust, repetitive self-grooming behavior, suggesting a high level of compulsivity. Strikingly, optogenetic activation of GABAergic LH→PVH terminals disrupted repetitive grooming induced by water spray and promoted feeding behavior, which pro-

vides a framework for parallel LH→PVH circuit as a potentially important brain mechanism linking compulsive and feeding behaviors [125].

Brain Stem System

Midbrain The midbrain dopaminergic neurons play a critical role in the control of cognitive and motor behaviors and have been implicated in OCD-like repetitive stereotyped, which are predominantly located in two nuclei: substantia nigra pars compacta (SNc), ventral tegmental area (VTA) [126]. Studies have demonstrated that pharmacological disruption of midbrain dopamine signaling in animals elevated compulsive-like behaviors [127] as well as similar dopaminergic effects on stereotypy in vocalizations or grooming behavior [128, 129]. Pagliaccio *et al.* have utilized neuromelanin-sensitive MRI as a non-invasive proxy measure of midbrain dopamine function among children with OCD and identified that neuromelanin-MRI signal was higher within both the SNc and VTA among children with OCD [130]. Using an OCD animal model via OFC-VMS repeated stimulation in WT mice as previously reported, Xue *et al.* revealed that SNc dopaminergic neurons modulated grooming behavior via a dual gating mechanism from cortical and striatal projections. Detailly, optogenetic inhibition of SNc-VMS projections and activation of SNc-lOFC projections could alleviate excessive self-grooming, which are consistent with the results in *Sapap3* KO mice. Collectively, these results identify the hub role of SNc in regulating OCD-like behaviors via SNc-lOFC-VMS "detour" [109].

Pons The pons is the portion of the brainstem, located inferior to the midbrain, superior to the medulla oblongata, and anterior to the cerebellum [131]. In OCD, one study reported gray matter (GM) volume reduction in bilateral pons [132]. Luisa *et al.* reported that OCD patients with a predominant contamination/washing dimension showed significantly increased mean diffusivity (MD), axial diffusivity (AD), and radial diffusivity (RD) values in white matter (WM) region of pons [133], which suggest that OCD pathophysiology may be at least partly underpinned by abnormal microstructure of pons. In the rostral pons, the medial paralemniscal nucleus (MPL) is a cone shape of the nucleus, the lateral border of which is the auditory relay nuclei of the lateral lemniscus. The excitatory somatostatin-positive neurons in MPL (MPL^{SST} neurons) in mice have been reported to mediate grooming behavior, and the activity of MPL^{SST} neurons is associated with the initiation and maintenance of grooming behavior [134]. Optogenetic or chemogenetic activation of the MPL^{SST} neurons induced robust self-grooming, which was fluoxetine sensitive. Furthermore, MPL^{SST} neurons-mediated grooming behavior was triggered by the input from the CeA, and the VTA^{DA} neurons received monosynaptic inputs from MPL^{SST} neurons. Sun *et al.* have identified a CeA-MPL^{SST}-VTA^{DA} circuit controlling self-grooming and post-stress anxiety alleviation in mice, the signal from the CeA specifically triggered the MPL-mediated self-grooming, while the output to the VTA occupied a central position in mediating the impact of MPL-mediated post-stress anxiety regulation [134]. These results provide novel insights into the function and circuitry of MPL^{SST} neurons in both the initiation and maintenance of repetitive brain-to-spinal neural circuits.

Spinal System

Most research on brain circuits for self-grooming has been focused on forebrain areas in animals. Clinically, some studies have shown corticospinal tract alterations in adult and pediatric OCD patients [135, 136]. Currently, some researchers have proposed that brain-to-spinal neural circuits are critical for rhythmic movements associated with repetitive self-grooming, that is to say, that the brain coordinates with the spinal cord to generate repetitive movements [137]. Cerebelline-2 (*Cbln2*) is the marker gene defining the mechanosensory dorsal horn in the spinal cord and is also robustly expressed in specific layers of the spinal trigeminal nucleus (Sp5C), which has been reported to regulate compulsive behaviors [89]. Xie *et al.* have reported that Cbln2 expressing neurons in the caudal part of the Sp5C form a neural circuit to the cervical spinal cord to maintain repetitive orofacial grooming behavior in mice, suggesting a brain-to-spinal sensorimotor loop for repetitive behavior. Green fluorescent protein (GFP) fused with a calmodulin (CaM) protein and the CaM-binding peptide (later condensed to GCaMP) is a commonly used calcium indicator for optical imaging of neural activity [138]. The increased GCaMP fluorescence of spinal-projecting $Cbln2^+$ Sp5C neurons was observed in mice during oil-induced orofacial self-grooming. Chemogenetic activation of spinal-projecting $Cbln2^+$ Sp5C neurons evoked long-lasting grooming-like repetitive forelimb movements. Conversely, inhibition of these neurons reduced the time spent on stress-induced orofacial grooming behavior [137]. We believed that the spinal projection of $Cbln2^+$ Sp5C neurons may provide clues on brain-to-spinal neural circuits underlying grooming behavior.

Conclusion

Animal models are essential to enhance our understanding of OCD pathogenesis and to perform preclinical testing of novel therapeutics *in vivo*, allowing general toxicity testing of new treatments. Given the heterogeneity and etiological complexity of OCD, many animal models have been generated in the last decades to explore different aspects

associated with OCD through diverse strategies in rodents (Fig. 4). Although nonhuman primates are evolutionarily closer to humans than rodents, unlike research in rodent models, extensive tools for exquisite capability for circuit and genetic manipulation are not yet fully available. Recently, Zhai *et al.* first reported a group of single-caged rhesus monkeys, which exhibited spontaneous and persistent sequential motor behaviors (SMBs) closely resembling human OCD rituals and similar patterns of response to SSRIs. These rhesus monkeys carried damaging variants in genes and showed alterations in neurocircuitry associated with OCD, providing a spontaneous animal model for investigating the neurobiology of OCD [139].

It is exceptionally important to acknowledge that a single model cannot recapitulate the entirety of OCD in humans, likely corresponding to a subset of the disorder. One fact that cannot be ignored is that intrusive thoughts often accompanied by compulsive behavior are exceedingly difficult to quantify in animals. Thus, it is feasible to focus on robust and easily quantified behaviors like grooming, and compulsive checking behavior to probe the underlying neural mechanism. Every model has its strengths and weaknesses, which should be taken into consideration for determining the needs it can serve.

Transgenic models are likely to be particularly helpful for the development and screening of anti-compulsive drugs, because of convincing validities and the ability to rapidly generate large phenotypically-stable cohorts. The genetic mouse models displayed a striking degree of overlap in the endogenous expression patterns throughout the brain, which is strongly implicated in the CSTC circuit with synaptic dysfunction. However, the current genetic models of OCD are mainly not based on a known mutation related to OCD in humans, rather than based on behavioral similarity, thus it may be difficult to explain the true relevance in humans. Genetic manipulation provided an important platform for carrying out further functional validation on the impacts of candidate gene mutations identified from human genetic studies of OCD. The establishment of animal genetic models should not be a "fishing expedition", but could focus on specific genes thought to be involved in OCD. Animal research needs to closely follow advances in the clinical literature that provide relevant endophenotypes and biomarkers [140]. As the genetic studies evolve and sample sizes increase, we expect that more reliable and robust results will provide critical insights into the underlying biological pathways that will inform new transgenic animal models and guide drug repositioning or development toward compounds targeting disturbed biological pathways. Moreover, the advent of CRISPR/Cas technology enables targeted genome editing and allows for the rapid generation of transgenic animal models. Notably, restoring normalized gene expression in patients seems to be an attractive strategy, with several recently developed methods holding great promise, including direct expression of a gene or minigene-variant with split vectors for larger constructs, antisense oligonucleotides, transcriptional activators or repressors and excision or replacement of pathogenic fragments [141].

In contrast, the circuit-manipulation models look more convincing in terms of construct validity and may have promise for the development and refinement of circuit-based treatment approaches, including DBS and transcranial magnetic stimulation (TMS), despite the lack of relevant studies on predictive validity. Increasingly, psychiatric disorders including OCD are becoming understood as disorders of specific neural circuits [10]. Circuit manipulation tools (optogenetics, chemogenetics) in animal models have led to our rapidly growing understanding of how circuits are altered to produce maladaptive behaviors. Given the advances in our understanding of neural circuitry in OCD, it is natural to ask whether these discoveries could offer therapeutic promise. Over the past decade, neuromodulation strategies have evolved and become an increasingly attractive treatment alternative across psychiatric disorders via modulating circuit function. Notably, optogenetics likely be used as blueprints for the novel DBS protocols *in vivo*. Creed *et al.* have adapted insight obtained from optogenetic manipulations *in vivo* to propose a novel DBS protocol, acute low-frequency DBS not classical high-frequency DBS, emulating optogenetic mGluR-dependent normalization of synaptic transmission [142]. In addition, Valverde *et al.* applied a combination of optogenetics, *in vivo* electrophysiology, behavioral tasks, and mathematical modeling and demonstrated that cortical somatostatin interneurons may constitute a promising and less invasive target for stimulation [143]. As such, it is conceivable that, in the future, circuit-manipulation in animal models can help us optimize DBS protocols in OCD by carefully choosing the stimulation site and with a clear aim about which circuit alteration needs to be restored [144]. The surgical invasiveness of DBS may mean that this therapy would be reserved for only treatment-refractory OCD cases. Notably, TMS, a non-invasive neuromodulation technique, has been widely used in the treatment of OCD. However, key mechanisms supporting the efficiency of TMS remain unclear and there is still no consensus about the stimulation target and optimal stimulation parameters. We expect future research on circuit manipulation in animal models will provide useful clues for translational study. Furthermore, optogenetic activation of the lOFC-striatal circuit compensated for impaired fast-spiking neuron striatal microcircuits ameliorated compulsive grooming of *Sapap3* KO mice, offering the basis for potential therapeutic target in OCD by suggesting how circuits can be targeted to restore normal function [63]. However, the translation of optogenetic interventions to humans is a promising but far-fetched research avenue [145]. One of the many problems in the

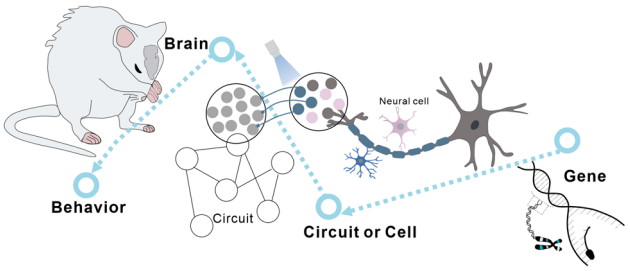

Fig. 4 Manipulation of genes or neural circuits implicated compulsive-like behaviors in rodents. The strategies were employed to study OCD in experimental animal systems by manipulating specific genes, neural circuits, or cells in the brain to deeply clarify the molecular and circuit events underlying OCD. Genetic manipulation provided an important platform for carrying out further functional validation on the impacts of candidate gene mutations identified from human genetic studies of OCD. In circuit models, researchers could evaluate whether manipulation of specific circuits using optogenetic or chemogenetic techniques could generate compulsive-like behavior. Stimulation of particular cell types with circuits could potentially contribute to fewer side effects and superior efficacy of gross regional neuromodulation.

optogenetic manipulation of human brain circuits is that visible light cannot penetrate deep inside brain tissue. Owing to this shortcoming, Chen *et al.* have focused on developing an elegant technological solution using up-conversion nanoparticles that could convert highly penetrative infrared light into visible light within the brain [146]. Thus, future innovation in OCD research will require fostering interactions between neuroscientists, physicians, and engineers to optimize safe transfection in humans and more focus on the conserved neural circuits across species to translate neurobiological findings in patients.

As cumulative literature investigating the basic neurobiology of core neural processes in OCD, we can gain an improved understanding of circuit dysfunction. More work will be needed to better understand the stimulus pattern, subregion, and cell type specificity in relation to compulsive-like behavior in rodents. These may help delineate the specific circuit-based mechanisms underlying the therapeutic efficacy of TMS or DBS and provide evidence for performing cell-type specific interventions in humans.

Here, building on the fine-grained gene and circuit-level insights afforded by animal models, we gain a better understanding of specific circuits and cell pathology in OCD. Despite the limitations in using animal models to study psychiatric disorders, these findings in the evolutionally conserved gene and circuitry provide promising avenues for future therapeutic discovery and might help to guide future translational studies.

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Conflict of interest The authors declare no conflict of interest.

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