Collectively, these results demonstrate that (i) lethal synergy of influenza virus and bacterial coinfection can result from loss of tolerance to infection-induced tissue damage, (ii) morbidity and mortality of coinfection can be independent of pathogen burden or excessive inflammatory response, and (iii) promoting tissue repair can, in principle, rescue coinfected animals from morbidity and mortality, even without affecting pathogen burden. Finally, our influenza–L. pneumophila coinfection model demonstrates the distinction between resistance and tolerance as separate host defense strategies that can both contribute to morbidity and mortality of infectious disease.

References and Notes

Reprinted Cortico-Striatal Stimulation Generates Persistent OCD-Like Behavior

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Although corticostriato-thalamo-cortical (CSTC) circuit dysregulation is correlated with obsessive compulsive disorder (OCD), causation cannot be tested in humans. We used optogenetics in mice to simulate CSTC hyperactivation observed in OCD patients. Whereas acute orbitofrontal cortex (OFC)–ventromedial striatum (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, a first-line OCD treatment. Brief but repeated episodes of abnormal circuit activity may thus set the stage for the development of persistent psychopathology.

OCD is characterized by intrusive distressing thoughts (obsessions) and/or repetitive mental or behavioral acts (compulsions) and is a leading cause of illness-related disability (1, 2). Although the pathophysiology underlying OCD is unclear, multiple lines of evidence implicate dysregulation within cortico-striato-thalamo-cortical (CSTC) circuits (3–6). Specifically, functional imaging studies suggest that hyperactivity in orbitofrontal cortex (OFC) and ventromedial striatum (VMS) is associated with OCD pathology (5, 7, 8). Furthermore, successful treatments are associated with reductions in hyperactivity (9, 10). However, it is not known if OFC-VMS hyperactivity can directly cause OCD symptoms, because increased activity could represent adaptive, homeostatic, or unrelated processes compensating for other primary abnormalities. We therefore used an optogenetic strategy to directly test whether hyperstimulation of glutamatergic OFC-VMS projections leads to OCD-like behaviors in mice.

A Cre-inducible adenovirus-associated vector (AAV) carrying the gene encoding channelrhodopsin (ChR2) fused to enhanced yellow fluorescent protein (EYFP) (pAAV-Ef1a-DIO-ChR2 (H134R)-EYFP; referred to as DIO-ChR2) (11) was stereotactically injected into OFC of EMX-Cre transgenic mice to ensure specific ChR2 expression in cortical glutamatergic neurons (Fig. 1A) (12). Cortical Cre expression led to sustained expression of ChR2-EYFP (Fig. 1B). Unilateral 473-nm stimulation through chronic fiber-optic implants in OFC yielded lateralized increased activation of the immediate early gene c-fos (P < 0.009) (Fig. 1C and D), which demonstrated in vivo cellular activation by laser stimulation. Two weeks postinjection, EYFP staining was seen in OFC cell bodies and axons projecting to VMS (Fig. 1E), which indicated targeting of OFC-VMS projections. In vitro recordings in corticostriatal slices demonstrated VMS field responses after 473-nm laser stimulation of OFC axon terminals in striatum (Fig. 1F). To verify adequate stimulation of ChR2-expressing OFC-VMS terminals in vivo, we implanted stereo opto-electrodes (optrodes) into VMS that permit combined fiber-optic stimulation and 32-channel simultaneous recording of multiple single units (Fig. 1G). In awake behaving mice, in vivo recordings demonstrated robust VMS field responses after 473-nm laser stimulation of OFC axon terminals in striatum (Fig. 1, H and I), which showed
feasibility of activation of specific cortical-VMS projections.

Because patients with OCD have hyperactivity in OFC-striatal circuits (3–6), we predicted that direct elevation of OFC-VMS activity would lead to increases in OCD-related behaviors including grooming, anxiety, and prepulse inhibition (PPI) deficits (13). We injected DIO-ChR2 into the left OFC of EMX-Cre mice and implanted fiber-optic probes unilaterally in left VMS (Fig. 2A). After waiting 3 to 4 weeks for surgical recovery and stable viral expression, we habituated mice to the open field and fiber-optic stimulation apparatus. We then repeatedly elevated activity in OFC-VMS projections by stimulating for 5 min at 10 Hz for five consecutive days (10 ms, 1 to 5 mW) (14). Grooming behavior was recorded with digital video and scored by blind raters for 5 min before (Pre), during (Stim), and after (Post) stimulation (Fig. 2B). Whereas acute OFC-VMS stimulation did not produce grooming, a small but significant progressive increase in grooming time was noted during the prestimulation period on consecutive days (Fig. 2C) (repeated measures analysis of variance (ANOVA), main effect: $P < 0.048$; $F = 4.43$; Fisher’s protected least significant difference (PLSD): for day $3$, $P < 0.03$; for day $5$, $P < 0.047$). Because the prestimulation measurement on days 2 to 5 served as a 24-hour time stamp for effects of stimulation the day before, this suggested that repeated stimulation led to chronic circuit changes that ultimately resulted in sustained, stimulation-independent OCD-like behavior. Although it is possible that stress from handling contributed to the grooming increase in the prestimulation period, stress was minimized by habituation to fiber-optic tethering daily for a week before data collection and was identical for controls and ChR2+ mice. To resolve the time-course of the grooming increase, we examined a new cohort an hour after stimulation (Groom1hour post) (Fig. 2D). We observed a dramatic progressive increase in grooming over consecutive days using this measure (main effect: $P < 0.02$; $F = 7.32$) (Fig. 2E). Although total grooming time increased, there was not a significant increase in stereotyped syntactic grooming chains in ChR2+ animals on day 5 of stimulation (table S1). No differences in grooming time were observed between controls and ChR2+ animals on day 1 of stimulation, which indicated that ChR2 expression without laser stimulation did not lead to an increase in grooming. Notably, increased grooming persisted even

![Image](https://www.sciencemag.org/content/340/6128/1235/F1.large.jpg)
in the absence of stimulation up to 2 weeks later \((P < 0.03)\) (Fig. 2F).

Acute OFC-VMS stimulation led immediately to a large but transient increase in locomotion compared with controls; no differences were observed pre- or poststimulation (fig. S1A). To ensure that increased grooming was not simply a consequence of increased locomotion, we injected DIO-ChR2 and implanted fiber optics in motor cortex (M2) of EMX-Cre mice (fig. S1B). The 5-day stimulation paradigm described above led to increased locomotion (fig. S1C) but not to increased grooming (fig. S1D), which suggested that increased grooming was not simply a side effect of overall increased activity. To determine the specificity of OFC-VMS pathway hyperactivation in induction of persistent grooming, we injected AAV-ChR2 into infralimbic and prelimbic cortex (IL/PrL) (fig. S2A) and stimulated IL/PrL–VMS projections using our 5-day stimulation paradigm. Repeated stimulation of IL/PrL–VMS projections did not lead to a progressive increase in grooming behavior (fig. S2, B to D).

Other OCD-associated behavioral measures were tested after completion of the 5-day stimulation paradigm. No differences were seen in PPI \((13)\) or anxiety levels (in open field and elevated plus maze) compared with testing before stimulation (fig. S3); in addition, no changes in open field anxiety were observed during acute stimulation (fig. S4). Together, these results suggest that repeated stimulation of OFC-VMS projections led to specific induction of repetitive behavior.

We next examined electrophysiologic changes correlated with the progressive grooming increase using VMS stereo-optrodes (Fig. 3A and fig. S5). In awake behaving mice, recordings of multiple single units in the VMS were obtained during the 15-min stimulation protocol and 1 hour postgrooming assessment (Fig. 3B). Light-evoked responses were observed in individual units in response to 10 Hz stimuli (Stim) or 0.1 Hz probe pulses (used to measure light-evoked activity during pre-, post-, and 1 hour poststimulation) over 5 days of repeated stimulation. Cells displayed a range of responses to light pulses, including activation (Fig. 3C), suppression (Fig. 3D), and no effect (Fig. 3E); we therefore used each cell’s stimulation-induced change in firing rate expressed as a Z-score; see supplementary methods) to assess responsiveness to afferent stimulation. Across 5 days of stimulation, mean responses increased both during stimulation and 1 hour poststimulation (Fig. 3F; \(R = 0.21\) and 0.28; \(P < 0.002\) and < 0.001, respectively). Z-scores were greater on day 5 than on day 1, which indicated increased evoked firing (at 10 Hz: \(P < 0.02\); at 0.1 Hz: \(P < 0.004\)). Thus, repeated hyperstimulation led to a marked progressive increase in light-evoked firing paralleling the increase in repetitive behavior.

Finally, we determined whether a medication regimen used to treat OCD would reverse the increases in repetitive behavior and evoked VMS activity. After 7-day grooming induction, we initiated fluoxetine treatment (18 mg/kg weight per day) while continuing daily stimulation (Fig. 4A). Fluoxetine was chosen because serotonin reuptake inhibitors (SRIs) are the only proven monotherapy for OCD \((15)\). Although there was no effect of fluoxetine after 1 week, 2 weeks of treatment led to a reversal of grooming behavior to control levels (Fig. 4B) (main effect: \(P < 0.009, F = 9.53\); Fisher’s PLSD: baseline versus week 2,
Repeated hyperactivation of OFC-VMS projections generates a progressive increase in grooming, temporally linked to a cumulative increase in VMS light-evoked firing. Acute stimulation of the OFC-VMS pathway was not sufficient to produce OCD-relevant excessive grooming (3, 16–18). The behavioral change was persistent, becoming stimulation-independent within 6 days. Although classic theories suggest that abnormal repetitive behaviors, including OCD symptoms, directly result from hyperactivity in CSTC loops (19–21), causation has been difficult to prove. Though genetic and pharmacologic manipulations of norepinephrine and dopamine can lead to transient increases in repetitive behaviors (22), the interventions were not limited to specific circuits, and associated electrophysiologic changes were observed in multiple brain regions. Our optogenetic system permits activation of specific cortico-striatal circuits and genetic definition of the activated cell-type as cortical glutamatergic projection neurons.

Our in vivo electrophysiology data suggest a circuit-based mechanism for establishment of repetitive behaviors. Repeated hyperstimulation led to a marked progressive increase in light-evoked firing paralleling the increase in grooming, suggesting plasticity at OFC-VMS synapses that builds over consecutive days. We speculate that brief episodes of light-induced activity lead to long-lasting changes that prime OFC-VMS synapses, decreasing the activation threshold during subsequent bouts of stimulation. In turn, increased activity at OFC-VMS synapses may transmit information through the CSTC circuit (23–25) and lead to multiple downstream events that ultimately reinforce repetitive behaviors, including (i) plasticity in downstream structures such as thalamus and prefrontal cortex (26), and (ii) increased motivational saliency mediated by the ventral tegmental area (22). This mechanism would be consistent with the observed fluoxetine effects, since selective SRIs have been shown to reduce primary reward processing (27, 28).

OCD is a heterogeneous disorder. Our study therefore may have greater relevance for particular OCD subtypes. For example, dimensional models of OCD have been proposed in which different types of obsessions and compulsions are associated with different circuits (29, 30). Because our results suggest that repeated stimulation of OFC-VMS projections led to specific induction of repetitive grooming, our model may be of particular importance for OCD patients with predominant contamination concerns.

Our findings yield new insight into how psychopathology could develop. Only 5 min of stimulation per day was sufficient to lead to sustained significant behavioral effects. This raises the possibility that pathological changes, including compulsions in OCD, may result from small but repeated bursts of abnormal neuronal activity and also offers suggestions for new treatment approaches or refinements of existing therapies for disorders characterized by repetitive behaviors. For example, our data are consistent with recent clinical studies demonstrating efficacy of ventral capsule–ventral striatum deep brain stimulation in OCD (31, 32), which is thought to act via inhibition of OFC hyperactivity. Optogenetic approaches could be used to dissect circuit mechanisms underlying deep brain stimulation and other treatments, with a goal of identifying new treatment targets.
**Fig. 4.** Perseverative grooming and elevated evoked firing rate are resolved by chronic, but not acute, fluoxetine treatment. (A) Experimental time line for fluoxetine wash-out experiment. (B) Two weeks of fluoxetine treatment reduced grooming to level of controls. Main effect: $P < 0.009; F = 9.53$; Fisher’s PLSD: baseline versus week 2, ***$P < 0.003$. Increased grooming was reestablished after a 1-week fluoxetine wash-out. Main effect: $P < 0.09; F = 3.58$. n values: ChR2 mice = 8; controls = 7. (C) Experimental time line for fluoxetine versus vehicle experiment. (D) Two weeks of fluoxetine treatment reduced grooming to levels of vehicle-treated animals. Main effect: $P < 0.14; F = 2.59$; Fisher’s PLSD: baseline versus week 2, *$P < 0.04$. Fluoxetine: n = 7; vehicle: n = 6. (E) (Left) In stereoptrode–implanted animals, peristimulus Z-scores for 10 Hz stimuli normalized after 2 weeks of fluoxetine ($P < 0.028$); after 2-week wash-out, Z-scores returned to pretreatment levels. (Right) Peristimulus Z-scores for 0.1 Hz probe pulses showed a nonsignificant decrease after fluoxetine treatment, which returned to pretreatment levels after wash-out.

**References and Notes**


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Geniculocortical Input Drives Genetic Distinctions Between Primary and Higher-Order Visual Areas

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Studies of area patterning of the neocortex have focused on primary areas, concluding that the primary visual area, V1, is specified by transcription factors (TFs) expressed by progenitors. Mechanisms that determine higher-order visual areas (VH0) and distinguish them from V1 are unknown. We demonstrated a requirement for thalamocortical axon (TCA) input by genetically deleting geniculocortical TCAs and showed that they drive differentiation of patterned gene expression that distinguishes V1 and VH0. Our findings suggest a multistage process for area patterning: TFs expressed by progenitors specify an occipital visual cortical field that differentiates into V1 and VH0; this latter phase requires geniculocortical TCA input to the nascent V1 that determines genetic distinctions between V1 and VH0 for all layers and ultimately determines their area-specific functional properties.

The neocortex is patterned into functionally distinct fields that include primary sensory areas, which receive modality-specific sensory input from thalamocortical axons (TCAs) that originate from the principal sensory nuclei of the dorsal thalamus (dTTH), and higher-order sensory areas that are connected with the primary areas through intracortical projections (I). Studies of mechanisms that pattern the neocortex into areas, known as arealization, have focused on primary areas and have led to the prevailing model that genetic mechanisms intrinsic to the neocortex are predominant in arealization (2). Transcription factors (TFs) expressed in neocortical progenitors determine the size and position of primary areas (2–5) and regulate guidance information that governs the area-specific targeting of TCAs (6). However, roles for TCAs in arealization remain vague (7–10), and important features of arealization, such as differential gene expression in the embryonic neocortex that relates to nascent areas, develop independently of TCA input (9, 10).

Higher-order areas outnumber primary areas by roughly 10-fold; for example, in mouse, nine higher-order visual areas (VH0) are positioned around the primary visual area (V1) within the occipital neocortex (I1). However, mechanisms that specify and regulate differentiation of the particular properties of higher-order areas and distinguish them from primary areas have yet to be explored (I1, 12). To perform genetic manipulations of dTH neurons required for these studies, we created RORα-ires-Cre mice (RORαCre; fig. S1, A and B) with RORα function intact and expression of Cre recombinase driven by RORα regulatory elements (13). Crossing this RORαCre mouse to conditional reporter lines (fig. S1) revealed Cre-mediated recombination in neurons of the principal sensory nuclei in dTH at embryonic day 14.5 (E14.5), shortly after they become postmitotic (14), with robust recombination in the dorsal lateral geniculate nucleus (dLG) (fig. S1, C to K), which forms the geniculocortical TCA projection that relays visual information from the eyes selectively to V1. Little or no recombination was detected in the neocortex through the end of the first postnatal week, encompassing the differentiation of cortical areas and the time frame of our study (fig. S1, C to K).

We crossed RORαCre mice to mice in which the third exon of the COUP-TF1 gene is flanked by loxp sites, i.e., floxed (fl) COUP-TF1 [COUP-TF1fl/fl is described in (5)], because COUP-TF1 is strongly expressed in dLG, COUP-TF1 deletion diminishes axon growth (15), and most TCAs fail to reach the cortex in COUP-TF1−/− mice (16). COUP-TF1−/− mice are not useful for our studies because of viability issues and defects in cortical development (16). In contrast, the conditional knockout (cKO) mice (RORαCre+/− or RORαCreCre, COUP-TF1fl/fl) were viable and retained robust COUP-TF1 expression in the neocortex (fig. S2, A and B), but COUP-TF1 was deleted from dLG by E15.5 (fig. S2, A and B), and dLG size in cKO mice progressively decreased from the wild-type (WT) size embryonically to virtually absent by postnatal day 7 (P7) (figs. S2, C and D, and S3).

To visualize TCA projections in the cortex, we first used serotonin [5-hydroxytryptamine (5-HT)] immunostaining on tangential sections of flattened P7 cortices. In P7 WT mice, 5-HT staining revealed the geniculocortical TCA projection from dLG to V1, as well as TCA projections from the ventroposterior nucleus (VP) to the primary somatosensory area (S1) and from the medial geniculate nucleus (MG) to the primary auditory area (A1) (fig. 1A). In P7 cKO mice, 5-HT staining showed that TCA projections to S1 and A1 were intact, but the geniculocortical TCA projection to V1 was absent (fig. 1A). The loss of geniculocortical input to V1 in P7 cKO mice was confirmed by anterograde and retrograde axon tracing from dLG and V1 (fig. S4, A and B) and by crossing the cKO mice to a ROSA26-GAP43-eGFP reporter line that labels TCAs by RORαCre reporter activation (fig. S5A). Thus, conditional deletion of COUP-TF1 from dLG using the RORαCre reporter line resulted in deletion of the geniculocortical TCA projection by P7, but COUP-TF1 remained intact in the cortex.

To determine the time course of the geniculocortical TCA projection in cKO mice as compared to WT mice, we bred RORαCre mice on either a WT (COUP-TF1fl/fl; RORαCreCre) or cKO (COUP-TF1fl/fl, RORαCreCre) or COUP-TF1fl/fl, RORαCreCre background, to a conditional reporter line (A1I4 tdTomato) (17). Activation of the tdTomato reporter with the RORαCre line labeled, at high resolution, geniculocortical TCAs from the dLG and TCAs from VP and MG projecting to S1 and A1 (fig. 1B). Geniculocortical TCAs extend tangentially in the subplate and underlie the cortical plate (CP) of nascent V1 by E16.5, invade after birth the overlying V1 CP, and over the first postnatal week arborize in V1 layer 4, their predominant target layer (18). At E16.5, before TCAs invade the CP, the appearance of tdTomato-labeled TCAs was indistinguishable between WT and cKO mice, with
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