Impaired cortico-striatal excitatory transmission triggers epilepsy

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STXBP1 and SCN2A gene mutations are observed in patients with epilepsies, although the circuit basis remains elusive. Here, we show that mice with haplodeficiency for these genes exhibit absence seizures with spike-and-wave discharges (SWDs) initiated by reduced cortical excitatory transmission into the striatum. Mice deficient for Stxbp1 or Scn2a in cortico-striatal but not cortico-thalamic neurons reproduce SWDs. In Stxbp1 haplodeficient mice, there is a reduction in excitatory transmission from the neocortex to striatal fast-spiking interneurons (FSIs). FSI activity transiently decreases at SWD onset, and pharmacological potentiation of AMPA receptors in the striatum but not in the thalamus suppresses SWDs. Furthermore, in wild-type mice, pharmacological inhibition of cortico-striatal FSI excitatory transmission triggers absence and convulsive seizures in a dose-dependent manner. These findings suggest that impaired cortico-striatal excitatory transmission is a plausible mechanism that triggers epilepsy in Stxbp1 and Scn2a haplodeficient mice.
Mutations in STXBP1, which encodes Munc18-1, a pre-synaptic protein essential for neurotransmitter release, and SCN2A, which encodes Nav1.2, a voltage-gated sodium channel alpha II subunit, are common in patients with a wide spectrum of neurological disorders, including epilepsy, intellectual disability, autism, and schizophrenia1-7. In particular, STXBP1 and SCN2A mutations are common in patients with early-infantile epileptic encephalopathy (Ohtahara syndrome), West syndrome and Lennox-Gastaut syndrome8,9,10, suggesting a potentially overlapping pathological mechanism.

Stxbp1-haplo-deficient (Stxbp1+/−) mice display emotional11 and spatial12 learning and memory deficits and enhanced aggression11. Additionally, Scn2a-haplo-deficient (Scn2a+/−) mice exhibit deficits in spatial learning and memory13. Therefore, these behavioral phenotypes are consistent with the cognate human syndrome. Moreover, Stxbp1+/− mice14 and Scn2a+/− mice15 show spike-and-wave discharges (SWDs) on electrocorticograms (ECoG).

Slow SWDs (1.5–2.5 Hz) are observed in patients with Ohtahara syndrome16 and Lennox-Gastaut syndrome17, which show de
thalamocortical circuits26 and ganglia as merely a modulator of SWDs primarily produced by mechanisms. Contrary to the previous proposal of the basal into development and conditional knockout lines of both generalization tonic, tonic-clonic, myoclonic, atypical absence and thalamic or thalamo-cortical neurons in generating SWDs within debate continues concerning the critical contributions of cortico-

In the present study, we observed involuntary twitches (5–8 times/h) and jumps (3–5 times/h) during sleep (Supplementary Video 2). However, SWDs, which had negative peaks, did not concur with the twitches or jumps, but rather with behavioral quiescence [91.3%; 73 out of 80 SWD episodes, N = 4 mice] (Fig. 1a). The twitches or jumps occurred with positive deflections in the ECoG recordings (Supplementary Fig. 3). We observed SWDs not only during quiet waking, but also during non-rapid eye movement (REM) and REM sleep in Stxbp1+/− mice (Supplementary Fig. 4).

Striatum as a critical node for epilepsy. We investigated the neural circuits required to generate SWDs in the mutant mice. Local injection of muscimol, a GABA<sub>A</sub> receptor agonist, into the SSC, CPu, or Thal but not into the mPFC or hippocampal CA1 region suppressed SWDs in SSC ECoG recordings in Stxbp1+/− mice (Fig. 2a, left; Supplementary fig. 5a). In Stxbp1+/− mice receiving CPu injection, SWDs were well suppressed not only in the SSC but also in the mPFC and CPu (Fig. 2a, right) where strong SWDs were observed before injection (Fig. 1c). These results demonstrate that neural activities in the SSC, CPu, and Thal are required for the generation or maintenance of SWDs in Stxbp1+/− mice. Although the SSC and thalamus have been well recognized as critical nodes for SWD generation, these results indicate that the CPu is also crucial; thereafter it became a focus of our subsequent experiments. In contrast to muscimol, micro-injection of bicuculline, a GABA<sub>A</sub> receptor antagonist, into the CPu of Stxbp1+/− mice induced myoclonic and subsequent generalized convulsive seizures (Supplementary Fig. 5b).

These data suggest that the CPu is involved in the generation of both absence (non-convulsive) and convulsive seizures. Indeed, short duration monophasic electrical stimulation (single pulse) applied to the CPu of one hemisphere of the brain in adult Stxbp1+/− mice using a depth electrode, triggered generalized SWDs in the SSC, mPFC, CPu, and Thal (Fig. 2b left), and we observed similarities between spontaneous (Supplementary Fig. 2a) and evoked SWDs (Supplementary Fig. 6) in their waveforms and phase relationships. Additionally, brain regions with a higher occurrence of spontaneous SWDs (Fig. 1c) also showed a higher occurrence of evoked SWDs (Fig. 2b, right). These results suggest that brief and local activation of the CPu is sufficient to generate SWDs in Stxbp1+/− mice.

Impaired cortico-striatal excitatory inputs causes epilepsy. To evaluate the comparative impact of Stxbp1 deletion in excitatory and inhibitory neurons on absence seizures, we generated Stxbp1 conditional knockout mice using Emx1-Cre recombinase (Emx1-Cre)11,31,32-mediated dorsal-telencephalic (i.e., the cerebral cortex, hippocampus, and amygdala, but excluding the striatum, globus pallidus, and thalamus) excitatory neuron-specific deletion or Vgat-Cre11,32-mediated global inhibitory neuron-specific deletion. Notably, Stxbp1<sup>flox/−/Emx1-Cre (Stxbp1)<sup>fl/−/Emx</sup></sup> mice reproduced SWDs with negative peaks during behavioral quiescence (Fig. 3a) but did not show twitches or jumps. In contrast,
we observed that Stxbp1fl/fl/Vgat-Cre (Stxbp1fl/+Vgat) mice showed twitches (~4 times over 6 h) and jumps (3–5 times over 6 h) (Supplementary Video 3) coinciding with ECoG-positive deflections (Supplementary Fig. 3b) but not with SWDs (Fig. 3a, right) or any other epileptic phenotypes. Although mice with a conditional haplo-deletion of Stxbp1 in inhibitory neurons using a Gad2-Cre driver (Gad2-Stxbp1fl/+Vgat) have been reported to show severe epileptic phenotypes and a low survival rate, we observed that Stxbp1fl/fl/Vgat-Cre (Stxbp1fl/+Vgat) mice showed a normal survival rate, normal growth and locomotor ability. Our results clearly indicate that Stxbp1 haploinsufficiency in dorsal-terencephalic excitatory neurons is responsible for SWDs during behavioral quiescence, while the same condition in GABAergic neurons is responsible for the twitches/jumps.

Additionally, we found that Scn2afl/+ and Scn2afl/fl/Emx mice but not Scn2afl/fl/Vgat mice showed SWDs during behavioral quiescence, although these were milder than those in Stxbp1fl/+ mice, suggesting an overlapping pathologcal circuit for absence seizures in Stxbp1fl/+ and Scn2afl/+ mice. Similarly to Stxbp1fl/+ mice, muscimol injections into the CPu or Thal suppressed SWDs in Scn2afl/fl/Emx mice (Supplementary Fig. 7).

Microdialysis analysis revealed that basal glutamate release, but not GABA release (normalized against high K+–evoked maximal release), was significantly lower in the CPu of behaving Stxbp1fl/+ mice than in wild-type (WT) mice (Fig. 3b, c). Ampakine (CX516) potentiates postsynaptic AMPA receptors in the presence of glutamate. Intraperitoneal administration (Fig. 3d) or local injection of ampakine into the CPu, but not into the thalamus, significantly reduced SWDs (Fig. 3e). These results indicate that impaired cortico-striatal excitatory synaptic transmission is responsible for the generation of SWDs and epileptic seizures in Stxbp1fl/+ mice.

To confirm the specific contribution of cortico- striatal synaptic transmission to SWD generation, we engineered mice with a conditional deletion of Stxbp1 or Scn2a using a Trpc4-Cre driver mouse line expressing Cre-recombinase in cortical layer 5 pyramidal neurons, which are a major source of excitatory inputs to the striatum (Fig. 4a left; Supplementary Fig. 8a, b), or a Ntsr1-Cre mouse expressing Cre-recombinase in cortical layer 6 neurons, which project to the thalamus (Fig. 4a right). Notably, Stxbp1fl/fl/lox/lox/Trpc4-Cre (Stxbp1fl/lox/Trpc4) mice, but not Stxbp1fl/fl/lox/lox/Ntsr1-Cre (Stxbp1fl/lox/Ntsr) mice, displayed SWDs (Fig. 4b). Similarly, Scn2afl/lox/lox/Trpc4-Cre mice, but not Scn2afl/lox/Ntsr mice, displayed SWDs (Fig. 4c, Supplementary Fig. 8c). By contrast, Ntsr1-Cre-dependent deletion of Cacna1a gene encoding a P/Q-type voltage-gated calcium channel a subunit was reported to produce SWDs in mice. These data suggest that different gene deletions exhibit different effects on downstream circuitry for SWDs (i.e. cortico-thalamic vs. cortico-striatal).
We then used the NeuRet system35,36 to generate mice with an Stxbp1 deletion restricted to cortico-striatal projection neurons (Supplementary Fig. 9). A retrograde lentivirus containing a flippase (FLP) gene was injected into the CPU of adult WT mice (>2 months) and was taken up by axons terminating in the CPU (Fig. 4d). Subsequent injection of an adenovirus-associated virus (AAV) containing a FLP-dependent double-inverted-orientation Cre (DIO-Cre) gene into the SSC allowed SSC neurons projecting to the CPU to express the Cre gene. The floxed Stxbp1 genes in cortico-striatal projecting neurons were then excised (Supplementary Fig. 9). As expected, the lentivirus and AAV-injected Stxbp1fl/fl mice exhibited SWDs, whereas the virus-injected Stxbp1fl/+ or WT mice did not (Fig. 4e). These data indicate that Stxbp1 deletion in cortico-striatal projection neurons, even in the adult stage, is sufficient to cause SWDs.

**Striatal fast-spiking interneurons control epilepsy.** In the CPU, both striatal medium spiny neurons (MSNs) and fast-spiking interneurons (FSIs) receive excitatory inputs from neocortical pyramidal neurons, and the cortex exerts potent feed-forward inhibition on MSNs via FSIs37. We measured synaptic drive to MSNs and striatal FSIs in whole-cell recordings of cortico-striatal brain slices from Stxbp1fl/fl mice. After electrophysiological identification of MSNs and FSIs (Supplementary Fig. 10a, b), excitatory postsynaptic currents (EPSCs) were evoked by electrical stimulation in the SSC neocortical layer 5/6 (Fig. 5a). FSIs can be reliably identified by their characteristic fast-spiking pattern (>200 Hz) with short spike width (<1 ms), clear after-hyperpolarization, and minimal firing adaptations38 (Supplementary Fig. 10a). Notably, we found a significantly faster rundown of EPSCs in the putative FSIs but not in the MSNs of Stxbp1fl/fl mice, at 10- to 40-Hz stimulation as compared with that observed in WT mice (Fig. 5b–d, Supplementary Fig. 11a). The absolute amplitude of the initial EPSCs, the first EPSCs evoked by current injection, in WT and Stxbp1fl/fl mice did not differ significantly, and the amplitudes of asynchronous miniature EPSCs in FSIs and MSNs before and after stimulation also did not change, confirming the presence of unaltered postsynaptic sensitivity (Supplementary Fig. 11b, c). These results indicate that excitatory presynaptic transmission was predominantly impaired in neocortical–striatal FSI connections. The decrease in EPSCs in the FSIs of Scn2afl/fl mice was not significant (Supplementary Fig. 11d, e), likely due to the direct involvement of sodium channels in action-potential generation rather than in synaptic transmission itself, which might reflect the lower SWD occurrence in Scn2afl/fl mice15 relative to that in Stxbp1fl/fl mice. Additionally, the estimated size of the readily releasable pool (RRP) of synaptic vesicles, i.e., the assembly of synaptic vesicles filled with neurotransmitters that are docked, primed, and ready for exocytosis, did not differ between WT and Stxbp1fl/fl mice (Supplementary Fig. 12a–c). We found that the size of the RRPs in FSIs was significantly larger than those in MSNs in both WT and Stxbp1fl/fl mice. Munc18-1 haploinsufficiency might limit the replenishment of synaptic vesicles from reserve or recycling pools to the RRP39 or affect a downstream step of vesicle priming in the RRP40; however, the reason for the dominant impairment in neocortical-striatal FSI connections remains unknown.

To mimic impaired cortical excitatory input to striatal FSIs and reduce their activity in vivo, we injected 1-naphthyl acetyl spermine (NASPM), a selective blocker of calcium-permeable AMPA receptors and abundantly expressed in striatal FSIs but not in MSNs41–43, into the CPU of WT mice (Fig. 6a). At a low dose (5 mM, 0.2 μl, bilateral), SWDs appeared in the CPU and mPFC (Fig. 6b, c) and those with longer durations occasionally appeared in SSC-ECOG recordings (Supplementary Fig. 13a), with
this finding consistent with that in a previous study. Similar to previous observations of dyskinetic movements in mice with selective inhibition of striatal FSIs, NASPM sequentially induced behavioral quiescence, myoclonic, dyskinesia-like tonic, clonic, and generalized tonic-clonic seizures within 1 h of administration and in a dose-dependent manner (Fig. 6b, d, Supplementary Video 4). Epileptic activities in the ECoG and LFP occurred concurrently with generalized seizures (Fig. 6b, right). Long SWDs in ECoG recordings and epileptic convulsive seizures were also observed when NASPM was injected into the reticular thalamic nucleus of WT mice, where abundant calcium-permeable AMPA receptors exist (Supplementary Fig. 13b).

We further examined whether upregulation of striatal FSI activity suppressed SWDs in Stxbp1<sup>+/−</sup> mice using the designer receptors exclusively activated by designer drugs (DREADD) system. Because FSIs are parvalbumin (PV)-positive, we injected AAV-DIO-hM3D (Gq)-mCherry (Supplementary Fig. 14a) into the CPu of Stxbp1<sup>+/−</sup> mice crossed with PV-Cre driver mice, which led to the expression of DREADD (Gq) receptors in striatal FSI by viral injection into the CPu (Fig. 6e, f), or intraperitoneal injection of clozapine-N-oxide (CNO), both effectively suppressed the emergence of SWDs in Stxbp1<sup>+/−</sup> mice (Supplementary Fig. 14b). As expected, activation of AMPA receptors in the CPu suppressed SWDs in Stxbp1<sup>+/−</sup> mice (Top) Representative SSC-ECoG after intraperitoneal injection of the ampakine, CX516. Tick marks represent SWDs. (Bottom) SWDs were suppressed in the first, but not in the second, 60 min following CX516 intraperitoneal injection. Stxbp1<sup>+/−</sup> mice (N = 4), vehicle vs. CX516, significant in the first but not the second 60 min period (Mann-Whitney U test, vehicle vs. CX516, 0-60 min: *P = 0.0268; 60-120 min: *P = 0.4857). e CX516 injection into the CPu, but not the thalamus, suppressed SWDs (3000 s recording after injection, normalized, see Methods), Mann-Whitney U test, vehicle vs. CX516, CPu: *P = 0.0317; Thal: *P = 0.4357). Calculation using unnormalized data in CPu had an tendency of decrease in SWDs but did not reach to a statistical significance. Mean ± SEM (a). *P < 0.05. Numbers of mice are shown in parentheses.

**Fig. 3** Impaired cortico-striatal excitatory transmission underlies SWDs in Stxbp1<sup>+/−</sup> mice. a SWDs appeared in Stxbp1<sup>+/−</sup>/Emx mouse. (Right) Representative SWDs in an Stxbp1<sup>+/−</sup>/Emx mouse. (Left) Number of SSC SWDs (24 h recording). Stxbp1<sup>+/−</sup>/Emx (N = 5), littermate controls (1 WT, 2 Stxbp1<sup>+/−</sup>/, 1 Stxbp1<sup>+/−</sup>/Emx, N = 4); Stxbp1<sup>+/−</sup>/Vgat (N = 5), littermate controls (1 WT, 2 Stxbp1<sup>+/−</sup>/, N = 3). Mann-Whitney U test, control vs. Stxbp1<sup>+/−</sup>/Emx: *P = 0.0286; control vs. Stxbp1<sup>+/−</sup>/Vgat: *P = 0.8255. b Glutamate and GABA release in the CPu of freely behaving WT (N = 6) and Stxbp1<sup>+/−</sup> (N = 6) mice with high K<sup>+</sup> stimulation. c Basal glutamate release (normalized by high K<sup>+</sup>-evoked release), but not GABA release, was significantly lower in Stxbp1<sup>+/−</sup> mice. Mann-Whitney U test, WT vs. Stxbp1<sup>+/−</sup>, glutamate: *P = 0.026; GABA *P = 0.5887. d Potentiation of AMPA receptors in the CPu suppressed SWDs in Stxbp1<sup>+/−</sup> mice. (Top) Representative SSC-ECoG after intraperitoneal injection of the ampakine, CX516. Tick marks represent SWDs. (Bottom) SWDs were suppressed in the first, but not in the second, 60 min following CX516 intraperitoneal injection. Stxbp1<sup>+/−</sup> mice (N = 4), vehicle vs. CX516, significant in the first but not the second 60 min period (Mann-Whitney U test, vehicle vs. CX516, 0-60 min: *P = 0.0268; 60-120 min: *P = 0.4857). e CX516 injection into the CPu, but not the thalamus, suppressed SWDs (3000 s recording after injection, normalized, see Methods), Mann-Whitney U test, vehicle vs. CX516, CPu: *P = 0.0317; Thal: *P = 0.4357). Calculation using unnormalized data in CPu had an tendency of decrease in SWDs but did not reach to a statistical significance. Mean ± SEM (a). *P < 0.05. Numbers of mice are shown in parentheses.
Stxbp1 deletions in cortico-striatal but not cortico-thalamic projection neurons causes SWDs. (Left) Tpc4-Cre dependent tdTomato (red) expression in neocortical layer 5 and the striatum (coronal section). (Right) Ntsr1-Cre dependent tdTomato expression (red) in neocortical layer 6 and the thalamus (coronal section). Scale bars: 500 μm. b Stxbp1fl/fl/Tpc, but not Stxbp1+/−/Ntsr, mice showed SWDs. (Left) Representative recordings. (Right) SSC SWDs numbers (3 h recording). Mann–Whitney U test, Stxbp1fl/fl/Tpc (N = 5) vs. control mice (3 Stxbp1+/−/Tpc, 1 Stxbp1+/−, N = 4), *P = 0.0195; Stxbp1fl/fl/Tpc (N = 5) vs. Stxbp1fl/fl/Tpc (N = 3), *P = 0.0357; Kruskal–Wallis test, *P = 0.0156; Dunn’s multiple comparison test, Stxbp1fl/fl/Tpc (N = 5) vs. Stxbp1fl/fl/Tpc (N = 3), *P < 0.05. However, neither Stxbp1fl/fl/Ntsr (N = 5) nor Stxbp1fl/fl/Ntsr (N = 3) mice increased SWDs, comparable to control mice (1 Stxbp1+/+, 1 Stxbp1+/−/Ntsr, 1 Stxbp1+/+, N = 3). Kruskal–Wallis test, P = 0.3962. e Scn2aflox/fl/Tpc, but not Scn2aflox/fl/Ntsr, mice showed SWDs (3 h recording). Scn2aflox/fl/Tpc (N = 5) vs. Scn2aflox/fl/Tpc (N = 4), Mann–Whitney test, *P = 0.0317. d Injected NeuRret vector (Lenti-FLP) in the striatum is retrogradely transported to the SSC, where FLP activates the Cre recombinase of the injected AAV5-EF1α-fDIO-Cre in the SSC. The Cre recombinase deletes floxed-Stxbp1 and activates tdTomato genes (red). Scale bar: 500 μm. e SWDs appeared in mice with NeuRret-dependent Stxbp1 deletion in cortico-striatal projection neurons (3 h recording). Unpaired t test with Welch’s correction, Stxbp1fl/fl (N = 6) vs. Stxbp1fl/fl (N = 3), t3 = 3.027, *P = 0.0292; Stxbp1fl/fl (N = 6) vs. Stxbp1+/− (N = 3), t3 = 3.027, *P = 0.0292. Numbers of mice are shown in parentheses. *P < 0.05

characteristics (Fig. 7a, Supplementary Fig. 15a–d) and firing rates44. pFSIs displayed a narrower action potential spike width with a higher firing rate, whereas pMSNs displayed a broader spike width with a slow decay and lower firing rate45. Although FSIs supposedly constitute < 5% of total striatal neurons38,42, we obtained ratios of pFSIs to the total number of the recorded neurons in Stxbp1 mice of 15.9%, which was comparable to other studies (18.5%45 or 10%16). This preferential detection of FSIs is presumably due to their characteristic short spike width and high firing rate. Notably, we frequently observed that pFSAI activity dropped at the onset of cortical SWDs (t = 0) in behaving Stxbp1+/− mice (Fig. 7b, top row). Group data confirmed that pFSIs significantly decreased their neuronal activity at the onset of SWDs as compared with that observed at baseline, whereas pMSNs did not (Fig. 7c, d, Supplementary Fig. 15e–g). A significant increase in pMSN activity was not detected, possibly because only a subpopulation of MSNs might contribute to SWD generation. Furthermore, pFSIs occasionally displayed oscillatory spiking activity in phase with the oscillation observed in ECoG recordings during SWDs (Supplementary Fig. 15h).

To investigate whether impaired cortico-striatal excitatory transmission is also observed in animal models of typical absence epilepsy, we tested Genetic Absence Epilepsy Rats from Strasbourg (GAERS) rats, a well-established rat strain showing robust and spontaneous SWDs47. Occurrence frequency and duration of SWDs in GAERS rats (Fig. 8a) were larger than those in Stxbp1+/− mice (Fig. 1a, Supplementary Fig. 1d). Notably, microinjection of CXS16 into the CPUs of GAERS rats significantly reduced the number of SWDs (Fig. 8b), whereas NASPM microinjections increased the number of SWDs (Fig. 8c). These data might suggest the generality of our hypothesis that impaired excitatory inputs onto striatal FSIs leads to epilepsy (Fig. 9; see Discussion).

Discussion

Thalamocortical circuits are widely recognized as the main generators of SWDs19,20, whereas the basal ganglia (e.g., the striatum) have been proposed as merely modifying or suppressing SWDs26,28,48–50, however, a causal relationship between the basal ganglia and SWDs remains obscure. Studies that succeeded in
triggering SWDs by selective manipulations in control (non-epileptic) animals.\textsuperscript{23,51,52} were performed in the thalamus; however, such tests were not reported in the basal ganglia. In this study, we discovered that impairments of the cortico-striatal pathway caused SWDs. The causal role of the striatum in epileptogenesis sharply contrasts with the traditional concepts of the basal ganglia as merely a modulator. Furthermore, we unraveled a critical role for striatal FSIs in SWD generation. Our results provide strong evidence that a pathologic decrease in cortico-striatal excitatory transmission onto FSIs due to genetic mutations represents a causal driver of SWDs linked to epileptogenic phenotypes.

Gittis et al.\textsuperscript{42} reported that the application of a calcium-permeable AMPA receptor blocker to the striatum decreased excitatory postsynaptic currents in cholinergic neurons (~45%) and FSIs (~73%), although the firing rates of only FSIs, and not cholinergic neurons, were selectively decreased. However, we still cannot exclude the possibility that epileptic activity caused by NASPM was partially mediated by cholinergic interneurons.

Pharmacological suppression,\textsuperscript{42} cell ablation,\textsuperscript{53} and optogenetic suppression of striatal FSIs,\textsuperscript{46} have been performed in mice. Neither SWDs nor epileptic seizures were described in these reports; however, Klaus and Plenz\textsuperscript{43} found SWD-like cortical LFP changes followed by pharmacological FSIs inhibition, which is similar to our observation. In particular, non-convulsive seizures such as absence seizure are very difficult to detect without electroencephalographic recordings. Even $Stxbp1^{+/−}$ mice, mice, and GAERS rats showing frequent SWDs appear outwardly normal. It is likely that in previous studies not designed to detect epilepsies, SWDs, or other epileptic brain activity, these may have been overlooked.

In accordance with this finding, we propose a novel cortico-striatal-thalamic neural circuit for epilepsy that traverses the basal ganglia via an indirect pathway (Fig. 9a, b). In this model, impaired cortico-striatal excitatory neurotransmission diminishes FSI activity, which disinhibits MSNs in the GPUs. Activated MSNs then sequentially over-suppress the globus pallidus externus (GPe), resulting in disinhibition of the subthalamic nucleus (STN), activation of the globus pallidus internus/substantia nigra pars reticulata (Gpi/SNr), and over-suppression of the thalamus. Consequently, the conventional model of hyperpolarized thalamic relay neurons produces rebound firing by de-inactivating Cav3.1 T-type Ca$^{2+}$ channel\textsuperscript{39} to further generate SWD and seizures.

Cortico-striatal inputs predominantly activate enkephalin-positive MSNs in the indirect pathway,\textsuperscript{54} suggesting MSN-GPe as a major route for the seizures. Previous results obtained using the GAERS absence epilepsy rat model are consistent with our pro-epileptic cortico-striatal circuit. Specifically, blockade of GABA inhibition in the GPe,\textsuperscript{27} glutamate receptors in the SNR,\textsuperscript{28} and muscimol inactivation of the STN\textsuperscript{26} or SNR\textsuperscript{26,55} suppressed SWDs, whereas blockade of GABA$_A$ receptors in the SNR aggravates SWDs.\textsuperscript{27} Moreover, STN membrane excitability was enhanced in an absence epilepsy mouse model.\textsuperscript{50} However, because there are reciprocal connections between direct and indirect pathway neurons, it also remains to be determined how the disinhibition of FSIs preferentially affects SWDs via the indirect pathway. Direct/indirect pathway-selective manipulation and a more detailed analysis of neuronal interactions among the cortex, basal ganglia, and thalamus are required in future studies to confirm our model.

In $Stxbp1^{+/−}$ mice, in vivo FSI activity decreases before or at the occurrence of SWDs, leading to disinhibition of MSNs. Potential mechanisms might include a faster rundown of excitatory transmission onto FSIs but not MSNs in $Stxbp1^{+/−}$ mice or a higher responsiveness of FSIs to cortical inputs relative to con...
**Fig. 6** Blockade of cortico-striatal FSI excitatory transmission causes SWDs and activation of FSI suppresses SWDs. a Local blockade of excitatory inputs to striatal FSIs by NASPM. b SSC-ECoG and LFP recordings at low (0.2 µl) and high (0.5 µl) doses of NASPM microinjection into the CPu of a WT mouse. SWDs in the mPFC and CPu (0.2 µl) (left) and convulsive epileptic discharges in the SSC, mPFC, and CPu (0.5 µl) (right). c Dose-dependent striatal SWDs in WT mice, caused by NASPM injections (3 h recording after injection). Vehicle (N = 6) vs. low NASPM (N = 6), Mann-Whitney U test, **P = 0.0081. d Dose-dependent convulsive seizures caused by NASPM (3 h recording after injection). Mouse numbers are shown in parenthesis. Vehicle (N = 6) vs. NASPM (1.0 µl, N = 4), Fisher’s exact test, **P = 0.0048. e The majority of PV (green)-positive FSIs in the CPu expressed mCherry (magenta) after AAV injections, indicating expression of the DREADD receptors (arrows: double positive cells, including those with less dense PV signals). Coronal section, scale bars 500 µm (left), 100 µm (right). f SWDs were suppressed by CNO microinjection into the CPu of Stxbp1+/−/PV Gq mice. Number of SWDs (right, 3 h recording). Stxbp1+/−/PV Gq, vehicle vs. CNO, Mann-Whitney U test, **P = 0.0079. Mean ± SEM. **P < 0.01

**Fig. 7** Temporal down-regulation of striatal FSIs at SWD onset in Stxbp1+/− mice. a Averaged spike waveforms of single units from a pFSI and a pMSN (FSI, 52,478 spikes; MSN 1,369 spikes). b Representative single unit activities of one pFSI and two pMSNs (raster plot, top), simultaneously recorded SSC-ECoG, EMG, and CPu LFP around the onset of cortical SWD (arrow). c Averaged peri-event time histograms (500 ms bins) of pFSI (18 cells) and pMSNs (95 cells) from 10 Stxbp1+/− mice. The timings of the SWD onset were aligned at 0. s. d pFSIs activity at the onset of SWDs (−0.5 to +0.5 s) significantly decreased compared to baseline (−5 to −4 s), while pMSNs did not. pFSIs: baseline vs. onset, Mann-Whitney U test, *P = 0.0279, pMSNs: P = 0.5831. Mean ± SEM. *P < 0.05
to that of MSNs\textsuperscript{37,54} (Fig. 9b). Additionally, we observed that the effects of Scn2a haploinsufficiency on corticostriatal transmission were minor (Supplementary Fig. 11d, e). Because Scn2a haploinsufficiency results in the broadening of action potentials\textsuperscript{13}, this could lead to excessive glutamate release followed by a depletion upon repetitive activity. Reduced glutamate transmission in the cortico-striatal pathway is also a possible mechanism in Scn2a\textsuperscript{+/−} mice.

Our results including the reproductions of SWDs by Trpc4-Cre- (corticostriatal neuron-specific) but not Ntr1-Cre- (thalamocortical neuron-specific) dependent Stxbp1 or Scn2a deletions or by corticostriatal projection neurons-specific deletion of Stxbp1 (NeuRet) and the induction of SWDs by brief electrical stimulation of the CPu in Stxbp1 mice, support the corticostriatal pathway as the initial site causally responsible for the seizures and subsequent activation of thalamo-cortical circuits at least in Stxbp1\textsuperscript{+/−} and Scn2a\textsuperscript{+/−} mice, although the corticostriatal-thalamic loop and cortico-thalamic loop are not mutually exclusive and they might influence SWDs cooperatively.

STXBPI and Scn2a mutations have been described in patients with epileptic encephalopathies who show myoclonic, atomic, and atypical absence seizures\textsuperscript{1,5,7,8}, although our model (Fig. 9b) might also cover typical absence epilepsy, as described. Behavioral quiescence, myoclonia, dyskinesia-like tonic or clonic seizures, and tonic-clonic seizures appeared sequentially in a NASPM dose-dependent manner, which was consistent with the presence of dyskinesia in mice following pharmacological suppression of striatal FSIs\textsuperscript{42}. Choreoathetoid movements have also been described in some patients with STXBPI\textsuperscript{6} and Scn2A\textsuperscript{36} mutations. These data suggest that these features are derived from overlapping pathological circuits, and that impaired cortico-striatal excitatory transmission contributes not only to absence (i.e., non-convulsive) seizures, but also to convulsive seizures.

In summary, this study fills an important gap in the seizure literature by addressing how impaired excitatory transmission is frequently observed in models of absence epilepsy\textsuperscript{19,20,57}. Future studies will address this pro-epileptogenic striatal neuronal circuit in the context of excitatory balance in neurodevelopmental disorders\textsuperscript{58,59} and why autism and epilepsy have a high rate of association\textsuperscript{60}.

**Methods**

**Animals.** All animal experimental protocols were approved by the Animal Experiment Committee of the RIKEN Center for Brain Science. Mice and rats were handled in accordance with the guidelines of the RIKEN Center for Brain Science Animal Experiment Committee. Food and water were available ad libitum, and cages (of less than 5 animals) were kept at 23 °C and 55% humidity on a 12-h/12-h light/dark cycle, with the lights off at 20:00. Genetic absence epilepsy rat from Strasbourg (GAERS/Mave, NRPB Rat No. 0285) rats were obtained from the National BioResource Project - Rat, Kyoto University (Kyoto, Japan). Both sexes, over 16 weeks of age.

**Stxbp1 and Scn2a conditional knockout mice.** Stxbp1 floxed mice, which have exon 3 flanked by loxp sites, were maintained on a C57BL/6 N background\textsuperscript{11}. To generate Stxbp1 conditional knockout mice, heterozygous Stxbp1 floxed (Stxbp1\textsuperscript{+/−}) or homozygous Stxbp1 floxed (Stxbp1\textsuperscript{−/−}) mice were mated with Cre transgenic mice. The Empty spiracles homolog 1 (Emx1)-Cre knock-in mice\textsuperscript{1,3,2,12}, vesicular GABA transporter (Vgat)-Cre BAC transgenic mice\textsuperscript{1,2}, short transient receptor potential channel 4 (Trpc4)-Cre transgenic mice\textsuperscript{2}, neurotensin receptor 1 (Ntr1)-Cre transgenic mice (Tg(Ntr1-cre)G220Sat/Mmdc, GENSAT), MMRBC, parvalbumin (PV)-Cre transgenic mice\textsuperscript{32}, and loxp flanked transcription terminator cassette CAG promoter driven tdTomato transgenic (B6.Cg-Gtk(ROSA)26Sortm14(CAG-tdTomato)Hze, Stock No: 007914, The Jackson Laboratory, USA) mice were all maintained on a C57BL/6 J background. Scn2a floxed mice, which have exon 2 flanked by loxp sites, were maintained on a C57BL/6 J background. Scn2a\textsuperscript{+/−} mice, support the cortico-striatal hypothesis. Scn2a conditional knockout mice, Heterozygous Scn2a floxed (Scn2a\textsuperscript{+/−}) or homozygous Scn2a floxed (Scn2a\textsuperscript{−/−}) mice were mated with Cre transgenic mice.

**Somatosensory cortex (SSC) electrocorticogram (ECoG) and electromyogram (EMG) recordings.** Adult mice (>8 weeks, both sexes) were used in this study. ECoG was used to directly monitor cortical activity using stainless steel screw electrodes embedded in the skull. Stainless steel screws (1.1 mm diameter) serving as ECoG electrodes were placed over the bilateral somatosensory cortex (+1.5 mm lateral to midline, 1.0 mm posterior to bregma) under 1–1.5% isoflurane anesthesia or 1.5% halothane anesthesia with N\textsubscript{2}O/O\textsubscript{2} (3:2) ventilation. A reference screw
electrode was implanted on the cerebellum (at midline, 1.5 mm posterior to lambda). A stainless steel wire (100 μm) bipolar electrode was inserted in the cervical region of mice for EMG. Beginning at least one week after surgery, recordings were performed for a week, sampled at 256 Hz and then analyzed off-line (SleepSign, Kissay, Japan). Each animal’s behavior was continuously monitored using an infrared camera.

**Simultaneous ECoG and Local field potential (LFP) recordings from behaving mice** 

Simultaneous ECoG and LFP recordings were recorded from behaving mice (both sexes) using stainless steel wires (1.1 mm diameter) serving as ECoG electrodes were placed over the right somatosensory cortex (1.0 mm posterior to bregma, 1.5 mm to the midline) under 1–1.5 % iso-flurane anesthesia, or Nembutal (50 mg/kg body weight) and 1.5% halothane anesthesia with Na2O3 (3:2) ventilation11. A stainless-steel screw, serving as both a reference and ground electrode, was placed on the cerebellum. A stainless-steel wire was inserted in the posterior region of the trapezius muscle for EMG. To record monopolar LFP recordings of brain regions, isolated stainless steel wires (200-μm diameter) with beveled tips were stereotaxically implanted contralateral to the ECoG electrode, according to the following coordinates (anterior-posterior, medial-lateral, and depth from the cortical surface, mm): medial prefrontal cortex (1.9, 0.3, 1.4), caudate-putamen (0.0, 2.4, 2.5), basolateral amygdala (–1.4, 2.9, 3.7), ventroposterior thalamus (–1.8, 1.5, 3.2), hippocampus CA1 region (–2.5, 2.2, 1.1), and visual cortex binocular zone (–3.4, 3.0, 0.4). Contacts between the electrode and brain surface were covered with a small amount of petroleum jelly and secured with dental acrylic. An antibiotic (ampicillin) was used during surgery.

Following at least one week of recovery from the implant surgery, the animals were tethered to a 16-channel commutator (Plexon, Dallas, TX) and allowed to move freely during recording in an electrically shielded cage with food and water available ad libitum. Each day, microdialysis was performed for 3 h in the daytime. Ringer’s solution (147 mM NaC1, 4.0 mM KCl, 4.5 mM CaCl2) was perfused (2.0 μl/min) and samples were collected every 20 min using a fraction collector (CMA 140). After an equilibration phase (120 min), six fractions were sampled as baseline, then the Ringer’s solution was changed to a high K+ solution (140 mM NaC1, 1 mM NaC1, 2.3 mM CaCl2), using a liquid swivel, for 60 min, and then returned to Ringer’s solution for 60 min. Dialysates in aliquots were immediately stored at −80 °C until measurement. Glutamate and GABA in dialysates were quantified using high-performance liquid chromatography (Eicom) with fluorescence detection (excitation, 340 nm; emission, 440 nm). Amino acids, after on-line polyethylene derivatization with 2-mercaptoethanol, were separated on a 250×4.6 mm, μBondapak C18 column (at 36 °C, Nacalai Tesque, Japan) with the column-switching technique (EAS-20, Eicom). The components of the mobile phases were as follows: 88% 0.1 M citrate buffer/0.2 M phosphate buffer (pH 6.2) and 12% acetonitrile, for glutamate; 75% 0.1 M citrate buffer/0.2 M phosphate buffer (pH 5.4) and 25% acetonitrile, for GABA64.

**Neuron-specific retrograde transport vector (Neurant) system.** 

For construction of diphosphate (FLP) expression lentivirus vectors (Supplementary Fig. 9a, b), an FLP recombinase gene fragment and the pCGL20c Mp vector were digested with BglII and NotI and then ligated. The FLP gene was amplified using the following primers: 5′-TAGACCCGTTCACACCCATGGTCGCTCAGAAGGGAGG-3′ and 5′-GATCGCGGCCGCTTATATGCGTCTATTTATGTAGGATG-3′. Following amplification, PCR products were purified (using a gel purification kit, TOYOBO) and then ligated. The FLP gene fragment was inserted into the NotI site, 3′ of the FLP gene, that was filled by the PrimeSTARHS DNA polymerase (TAKARA). The FLP-EGFP fragment was amplified from the pRS25-EGFP vector (Clontech) by PCR using the following primers: 5′-CAATAGACCGCCTACACACCCATGGTCGCTCAGAAGGGAGG-3′ and 5′-GATCGCGGCCGCTTATATGCGTCTATTTATGTAGGATG-3′.

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microelectrode (IMS-20, Narishige, Japan) (0.1 µl/min). One week after NeuJet vector injections, the mice were injected with the AAV5 vector (CAG promoter/DIO-mCherry: 8.8 × 109 viral genomes/µl, >2 months of age), with the following stereotaxic coordinates: (0.7, ± 2.0, 2.2 mm, 1.0 µl/min). One week after AAV vector injections, the mice were implanted with electrodes for ECoG, and the mPFC and CPUs with bilateral cannulae aimed at the CPu for clonapine-N-oxide (CNO) microinjection. At least 4 weeks after the AAV injections, we commenced ECoG and LFP recordings. The virus titers were as follows: AAV5-EF1a-DIO-hM3D(Gq)-mCherry: 8.8 × 109 viral genomes/µl, and AAV5-EF1a-DIO-mCherry: 9.5 × 109 viral genomes/µl. Clonapine-N-oxide (CNO) (2.9 mM in 0.5% DMSO saline) and vehicle (0.2 µl of vehicle solution (0.5% DMSO saline) and slowly (>2 min) injected bilaterally with a micro-syringe via a polyethylene tube connected to the injection cannula. The injection cannula was withdrawn 30 s after the injection.

**Immunohistochemistry and fluorescence histochemistry.** Mice were deeply anesthetized and perfused transcardially with 4% paraformaldehyde in phosphate-buffered saline (PBS)32. The brains were then removed, post-fixed in 4% paraformaldehyde in PBS, and cryoprotected. Frozen brain sections (30 µm) were thaw-mounted on the cerebellum. A stainless-steel wire monopolar electrode was inserted in the thalamus and slowly (>2 min) injected bilaterally. The electrode tip was marked by electrolytic lesion (50 µA for 10 s positive to the electrode, negative to ground) and verified histologically using hematoxylin and eosin staining.

**In vitro slice electrophysiology.** Sagittal brain slices (350-µm thickness) collected from the mice at postnatal day 24 to 32 (P24–32) were cut on a vibratome (PRO 7, DOSAKA EM, Kyoto, Japan) under isoflurane anesthesia and incubated for at least 1 h in warm (34°C) and equilibrated (95% O2, 5% CO2) artificial cerebrospinal fluid (ACSF) containing (in mM) 125 NaCl, 2.5 KCl, 1 MgSO4, 2 CaCl2, 1.25 NaH2PO4, 26 NaHCO3, 11 glucose, 3 Na-pyruvate, and 1 Na-L-ascorbic acid, to maintain brain slices at 30–31 °C to obtain stable recordings. Data were obtained with a MultiClamp 700B amplifier, filtered (1 kHz), digitized (10 kHz), stored, and analyzed using pCLAMP 10.3 software (Molecular Devices, Foster city, CA). Spike sorting/cell identification. Single units were isolated of neuronal activity. Signals from each electrode were band-pass filtered (1.5–9 kHz) and digitized at 40 kHz (MAP system, Plexon) whenever the amplitude of the signal exceeded a preset voltage threshold (usually > 4 SD of peak height distribution). Stainless steel screw electrodes were implanted as above for ECoG/EMG sampled at 1 kHz. Tetrodes were slowly lowered incrementally to obtain stable recordings. To obtain reliable spike clustering and estimation of neuronal correlates to SWSs, recording time was set typically over 100 min to collect a sufficient number of spikes (usually at least > 1000 spikes). Once the recording session was completed, the electrodes were further advanced over 160 µm to avoid overlap of sampled neurons. Upon completion of the experiment, the electrode tip position was marked by electrolytic lesion (50 µA for 10 s positive to the electrode, negative to ground) and verified histologically using hematoxylin and eosin staining.

**Spiking activity.** Single units were isolated offline using a multi-dimensional cluster cutting software (Offline sort, Plexon). Clusters of spikes were attributed to a single unit based on waveform characteristics (peak-valley < 1 µV). A signal was masked and 1% of the waveform was used to discriminate artifacts by checking spike waveforms. Clusters containing spikes with short inter-spike intervals (< 2 ms) exceeding 0.1% were discarded. Only well-isolated clusters with an ellipsoidal shape in multi-dimensional feature space, with a clear refractory and >2 ms were included in the analysis. Single units stably recorded throughout several sleep/wake cycles were performed using NeuroExplorer.

**Statistics.** The data are presented as mean ± SEM. Statistical analyses (two-sided) were performed using Prism 5 (GraphPad Software, La Jolla, CA, USA).
Comparisons between two genotype groups were performed using a Student’s t test (unpaired), unless otherwise described. When the variance of the data set was significantly different, we used a nonparametric statistical analysis, Mann–Whitney U test followed by t-test with Welch’s correction. P values smaller than 0.05 were considered significant.

**Reporting summary.** Further information on research design is available in the Nature Research Reporting Summary linked to this article.

**Data availability**

A reporting summary for this Article is available as a Supplementary Information file. The source data underlying Figs. 1a–c, 2a, b, 3a–c, 4a, b, 6c, 7c, d, 8d, 9c, and Supplementary Figs 1c, 1b, 4, 7, 11a, b, d, 12b, c, 14c and 15d-g are provided as a Source Data file. The data in this study are available from the corresponding author upon reasonable request.

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**References**

1. Kamiya, K. et al. A nonsense mutation of the sodium channel gene SCN2A in a patient with intractable epilepsy and mental decline. *J. Neurosci.* **24**, 2690–2698 (2004).
2. Saito, H. et al. De novo mutations in the gene encoding STXBP1 (MUNC18-1) cause early infantile epileptic encephalopathy. *Nat. Genet.* **40**, 782–788 (2008).
3. Ogawa, I. et al. De novo mutations of voltage-gated sodium channel alpha gene SCN2A in intractable epilepsies. *Neurology* **73**, 1046–1053 (2009).
4. Rauch, A. et al. Range of genetic mutations associated with severe non-synaptic sporadic intellectual disability: an exome sequencing study. *Lancet Neurol.* **10**, 1674–1682 (2012).
5. Hoischen, A., Krumm, N. & Eichler, E. E. Prioritization of gene SCN2A in intractable epilepsies. *Am. J. Hum. Genet.* **82**, 1316–1336 (2008).
6. Otsuka, M. et al. STXBP1 mutations cause not only Ohtahara syndrome but also West syndrome in a patient with intractable epilepsy and mental decline. *J. Neurosci. Res.* **91**, 1316–1336 (2013).
7. Nakamura, K. et al. Clinical spectrum of SCN2A mutations expanding to Ohtahara syndrome. *Neurology* **81**, 992–998 (2013).
8. Wolf, M. et al. Genetic and phenotypic heterogeneity suggest therapeutic implications in SCN2A-related disorders. *Brain* **140**, 1316–1336 (2017).
9. Otsuka, M. et al. STXBP1 mutations cause not only Ohtahara syndrome but also West syndrome–result of Japanese cohort study. *Epilepsia* **51**, 2449–2452 (2010).
10. Miyamoto, H. et al. Potentiation of excitatory synaptic transmission ameliorates aggression in mice with Stxbp1 haploinsufficiency. *Hum. Mol. Genet.* **26**, 4961–4974 (2017).
11. Orock, A., Logan, S. & Deak, F. Munc18-1 haploinsufficiency of a patient with intractable epilepsy and mental decline. *J. Neurosci. Res.* **27**, 1480–1581 (2016).
12. McCafferty, C. et al. Cortical drive and thalamic feed-forward inhibition control thalamic output synchrony during absence seizures. *Nat. Neurosci.* **21**, 744–758 (2018).
13. Bomben, V. C. et al. Isolated P/Q calcium channel deletion in layer vi corticothalamic neurons generates absence epilepsy. *J. Neurosci.* **36**, 405–418 (2016).
14. Meeran, H. K., Pijn, J. P., Van Luijtenelaar, E. L., Coenen, A. M. & Lopes da Silva, F. F. Cortical focus driven thalamic corticothalamic networks during spontaneous absence seizures in rats. *J. Neurosci.* **22**, 1480–1495 (2002).
15. Polack, P. O. et al. Deep layer somatosensory cortical neurons initiate spike-and-wave discharges in a genetic model of absence seizures. *J. Neurosci.* **27**, 6590–6599 (2007).
16. Deransart, C., Marescaux, C. & Depaulis, A. Involvement of nigral glutamatergic inputs in the control of seizures in a genetic model of absence epilepsy in the rat. *Neuroscience* **71**, 721–728 (1996).
17. Deransart, C. et al. Evidence for the involvement of the pallidum in the modulation of seizures in a genetic model of absence epilepsy in the rat. *Neurosci. Lett.* **265**, 131–134 (1999).
18. Paz, J. T., Chavez, M., Sallet, S., Deniau, J. M. & Chaperon, S. Activity of ventral medial thalamic neurons during absence seizures and modulation of cortical paroxysms by the nirogaloid pathway. *J. Neurosci.* **27**, 929–941 (2007).
19. Kim, D. et al. Lack of the burst firing of thalamocortical relay neurons and resistance to absence seizures in mice lacking alpha(1G) T-type Ca(2+) channel. *Neuron* **31**, 35–45 (2001).
20. Manning, J. P., Richards, D. A., Leresche, N., Crunelli, V. & Bowery, N. G. Cortical-area-specific block of genetically determined absence seizures by ethosuximide. *Neuroscience* **123**, 5–9 (2004).
21. lwasa, T. et al. Cortex-restricted disruption of NMDAR1 impairments neuronal patterns in the barrel cortex. *Nature* **406**, 726–731 (2000).
22. Ogiwara, I. et al. Nav1.1 haploinsufficiency in excitatory neurons ameliorates seizure-associated sudden death in a mouse model of Dravet syndrome. *Hum. Mol. Genet.* **22**, 4784–4804 (2013).
23. Lynch, G., Cox, C. D. & Gall, C. M. Pharmacological enhancement of memory and cognition in normal subjects. *Front. Syst. Neurosci.* **8**, 90 (2014).
24. Okuyama, T., Kitamura, T., Roy, D. S., Ithara, S. & Togesawa, S. Ventral CA1 neurons store social memory. *Science* **353**, 1536–1541 (2016).
25. Kato, S., Kobayashi, K. & Kobayashi, K. Improved transduction efficiency of a lentiviral vector for neuron-specific retrograde gene transfer by optimizing the junction of fusion envelope glycoprotein. *J. Neurosci. Methods* **227**, 151–158 (2014).
26. Kobayashi, K. et al. Survival of corticostriatal neurons by Rho/Rho-kinase signaling pathway. *Neurosci. Lett.* **630**, 45–52 (2016).
27. Mallet, N., Le Moine, C., Chaperon, S. & Gonon, F. Feedforward inhibition of projection neurons by fast-spiking GABA interneurons in the rat striatum in vivo. *J. Neurosci.* **25**, 3857–3869 (2005).
28. Koos, T. & Teppner, J. M. Inhibitory control of neostriatal projection neurons in vivo. *J. Neurosci. Res.* **65**, 4784–4804 (2001).
29. Toonen, R. F. et al. Munc18-1 expression levels control synapse recovery by fast-spiking GABA interneurons in the rat striatum. *J. Neurosci.* **31**, 2677–2687 (2011).
30. Klaus, A. & Plenz, D. A low-correlation resting state of the striatum during associative striatum. *J. Neurosci.* **33**, 167–192 (2007).
31. Gittis, A. H. et al. Selective inhibition of striatal fast-spiking interneurons causes dyskininesia. *J. Neurosci.* **31**, 15727–15731 (2011).
32. Deng, Y. P. et al. Differential localization of the GluR1 and GluR2 subunits of the AMPA-type glutamate receptor among striatal neuron types in rats. *J. Chem. Neuroanat.* **33**, 167–192 (2007).
33. Mallick, A. & Plenz, D. A low-correlation resting state of the striatum during cortical avalanches and its role in movement suppression. *PLoS Biol.* **14**, e1002582 (2016).
34. Kath, C. A. & Graybiel, A. M. Differential entrainment and learning-related dynamics of spike and local field potential activity in the sensorimotor and associative striatum. *J. Neurosci.* **34**, 2845–2859 (2014).
35. Berke, J. D., Okatan, M., Skurski, J. & Eichenbaum, H. B. Oscillatory entrainment of striatal neurons in freely moving rats. *Neuron* **43**, 883–896 (2004).
36. Lee, K. et al. Parvalbumin interneurons modulate striatal output and enhance performance during associative learning. *Neuron* **93**, 1451–1463.e1454 (2017).
37. Danobe, L., Deransart, C., Depaulis, A., Vergnes, M. & Marescaux, C. Pathophysiological mechanisms of genetic absence epilepsy in the rat. *Prog. Neurobiol.* **55**, 27–57 (1998).
38. Arakaki, T., Mahon, S., Chaperon, S., Lebois, A. & Hansel, D. The role of striatal feedforward inhibition in the maintenance of absence seizures. *J. Neurosci.* **36**, 9618–9632 (2016).
39. Arakaki, T. et al. The basal ganglia, the deep prepyriform cortex, and seizure spread: bicuculline is anticonvulsant in the rat striatum. *Proc. Natl Acad. Sci. USA* **86**, 1694–1697 (1989).
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Author contributions
H.M. and K.Y. designed the project. H.M. and T.T performed in vivo electrophysiology experiments. T.T. performed acute slice experiments. H.M., A.S., T.Y., K.A., E.M., M.R., A.O.-A., and T.K.H., performed genetic, histological, biochemical, and pharmacological analyses. I.O., S.I., and K.S. generated mouse lines. K.K, K.K, T.Y., and T.S. prepared virus vectors. H.M. and K.Y. wrote the paper.

Additional information
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