Roles of the subthalamic nucleus and subthalamic HCN channels in absence seizures

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Absence epilepsy is a generalized, nonconvulsive type of epilepsy with multifactorial origins (Crunelli and Leresche 2002). Absence seizures consist of a brief and sudden impairment of consciousness, which results from bilateral synchronized spike and wave discharges (SWDs) in the electroencephalogram (EEG) over the wide cortical areas of the brain. SWDs may occur frequently and last as long as tens of seconds. The occurrence and duration of SWDs may be bidirectionally altered by ictal activity and the output nuclei of the basal ganglia, and it shows rhythmic activities that are well synchronized with tremors in patients with Parkinson’s disease (Levy et al. 2002). Therefore, the STN is one of the major sites for deep brain stimulation (DBS) in treating Parkinson’s disease. The rhythmic activities of the STN seemingly involve the pacemaker activity of voltage-gated channels, including the low-voltage-activated calcium channel, the slowly inactivating sodium channel, the delayed-rectifier potassium channel, and the hyperpolarization-activated cyclic nucleotide-gated cation (HCN) channel (Gillies and Willshaw 2004 and references therein).

Previous studies using GAERS showed that the cortico-STN-SN pathway is necessary in generating SWDs (Deransart et al. 1996; Paz et al. 2005; Slaght et al. 2004). However, the precise role of this pathway and the molecular mechanism underlying this role are poorly understood. To address this issue, in this study, we characterized the absence seizures of tottering (tg) mice, a well-established model of absence epilepsy, using both in vivo and in vitro electrophysiological techniques. tg mice are Caβ2.1 (P/Q type) calcium channel mutant mice that exhibit absence seizures, ataxia, and dystonia-like intermittent motor dysfunctions (Fletcher et al. 1996; Noebels and Sidman 1979). Furthermore, previous studies have reported altered synaptic properties in tg mice (Matsushita et al. 2002; Qian and Noebels 2000; Sasaki et al. 2006). In this study, we examined the involvement of the cortico-STN-SN pathway in SWD generation in tg mice in vivo. In summary, we found that the membrane excitability of STN neurons was enhanced, at least partly, by a reduction of the HCN channel current in tg mice in vitro. Pharmacological blockade and activation of the HCN channel activity of STN neurons in vivo bidirectionally altered the mean duration of the
SWDs but did not affect the occurrence of SWDs. Furthermore, modulation of STN activity by DBS in vivo resulted in bidirectional modulations of the mean duration and number of SWDs. These results indicate that the basal ganglia pathway in tg mice plays a positive role in SWD generation through enhanced STN activity, and they imply that the HCN channel in the STN contributes to the maintenance system of epileptic rhythms originated in the thalamocortical loops.

MATERIALS AND METHODS

Animals. The C57BL/6tg strain of tg mice was obtained from the Jackson Laboratory (Bar Harbor, ME). Littermate mice were used as the wild-type (wt) control. Mice were provided with a commercial diet (CE-2; Nihon Clea, Tokyo, Japan) and water ad libitum under specific pathogen-free conditions with controlled temperature (22 ± 2°C), humidity (55 ± 5%), and lighting (12:12-h light-dark cycle with lights on at 6 AM). Genotyping of tg mice was performed with PCR as described previously (Sasaki et al. 2006). Mice were maintained and propagated by mating between heterozygous pairs in the Center for Experimental Animals, National Institutes of Natural Sciences. All animal studies were reviewed and approved by the Institutional Animal Care and Use Committee of the National Institutes of Natural Sciences and were performed in accordance with institutional guidelines for animal experiments.

Surgical procedure. Some experiments were performed in tg mice (both sexes, 2–4 mo of age). Because absence seizures do not occur under anesthetic conditions, experiments were performed in the awake condition by the recording method described by Chiken et al. (2008). An animal was initially anesthetized with xylazine hydrochloride [4 mg/kg body wt intraperitoneally injected (ip); Sigma, St. Louis, MO] and ketamine hydrochloride (100 mg/kg body wt ip; Sankyo, Tokyo, Japan). Subsequently, the animal was placed in a stereotaxic frame. Wounds and pressure points were infiltrated with 2% lidocaine (Sigma). After removal of the scalp, small screws were placed on the skull as anchors. A U-shaped plastic frame for holding the head was cemented to the skull via the small screws in the skull. Body temperature was maintained (38°C, rectal) with a homeothermic blanket (Bio Research Center, Nagoya, Japan) during the surgical procedure. A recovery period of >24 h was provided for the mouse after this procedure. During in vivo recording, the head was secured to a stereotaxic frame via the plastic frame. After the SWD occurrence was checked, the mouse was held to a stereotaxic frame under light anesthesia with ketamine hydrochloride (50 mg/kg body wt ip). A part of the skull in one hemisphere was removed to access the motor cortex, STN, and SN. Bipolar stimulating electrodes (tip distance 300–400 μm) made of Teflon-coated tungsten wires (50-μm diameter; Inter Medical, Nagoya, Japan) were inserted into the forelimb region of the motor cortex. The stimulation site was confirmed by the observation of limb movements evoked by intracortical microstimulation (train of 10 pulses at 333 Hz, 200-μs duration, up to 40 μA). Subsequently, stimulating electrodes were cemented to the skull.

Electroencephalogram recording. EEG recordings were performed to monitor the occurrence of SWDs in mice. Handmade screw electrodes were permanently implanted in the epidermal space of the frontal cortex. The corresponding stereotaxic coordinates were 1.9–2.1 mm anterior to the bregma and 0.9–1.1 mm lateral to the midline. Reference electrodes were fixed on the interparietal bone. EEG recordings were obtained with a dual-channel MEG-2100 amplifier (Nihon Kohden, Tokyo, Japan). In some experiments, EEGs were recorded for both the left and right hemispheres to determine whether SWDs occurred synchronously in both hemispheres. To detect the occurrence of SWDs, we used modified procedures described previously by Song et al. (2004). SWD occurrence was detected by the increment of the power spectrum of an EEG from 5 to 9 Hz. The initiation and termination of an SWD event were determined by the first and last spike parts of the SWD, respectively. Only SWDs with a minimum spike number of 4 were included in analyses, and SWDs separated by <1 s were regarded as a single SWD event. All processes to determine SWD initiation and termination were performed by blind tests to avoid inclusion of arbitrary artifacts.

Blocker microinjection. Pharmacological blockades were introduced by local microinjection via a glass micropipette (tip diameter 25–35 μm) connected to a 2-μl Hamilton syringe. Glutamatergic transmission was blocked by kynurenic acid, a nonselective antagonist for the N-methyl-d-aspartic acid (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) kainate receptors (20–40 nl, 50 mM; MP Biomedicals, Solon, OH). Kynurenic acid was prepared by dissolving it in 0.9% NaCl and 0.1% bromophenol blue (Wako Pure Chemical Industries, Osaka, Japan), and this solution was adjusted to pH 7.3–7.4 with 1 M NaOH. The HCN channel was blocked with ZD7288 (20 nl, 100 μM; Tocris Cookson, Avonmouth, UK). The voltage dependence of the HCN channel was modified with lamotrigine (LTG; 20 nl, 50 mM; Tocris Cookson). ZD7288 and LTG were prepared individually by dissolving each in 0.01 M PBS (pH 7.4). Saline (0.9% NaCl adjusted to pH 7.3 with 1 M NaOH; 40 nl) and PBS (pH 7.4) were also injected as negative controls. Bromophenol blue was added to confirm the injection site. Because the color of bromophenol blue became fainter when ZD7288 was added in the same solution, bromophenol blue was not co-injected with ZD7288 or LTG. Thus, when these drugs were injected, the kynurenic acid solution including bromophenol blue (prepared together as described above) was injected at the same coordinate as the drug at the end of the experiment. Specifically, injection into the SN was performed at the following coordinates: 1.0–1.1 mm anterior to the lambda, 1.5–1.6 mm lateral to the midline, and 4.2–4.3 mm below the cortical surface. Injection into the STN was performed at 1.9–2.1 mm posterior to the bregma, 1.5–1.7 mm lateral to the midline, and 3.9–4.6 mm below the cortical surface. Injection was performed slowly to avoid diffusion of the injectant into neighboring structures. The effects of the injection were examined beginning 5 min after the injection. At the end of the experiments, mice under deep general anesthesia with halothane (Takeda Chemical Industries, Osaka, Japan) were killed by decapitation. Their brains were removed immediately and were embedded in OCT compound (Sakura Finetechnical, Tokyo, Japan), which is an embedding medium used for frozen tissue specimens to ensure that an optimal cutting temperature is achieved. Subsequently, the brains were frozen quickly to confirm the injection site and diffusion of the injectant. In all experiments, EEG recordings were simultaneously performed.

Extracellular recording. Extracellular recordings were obtained in the STN and the SN with the use of an Axoclamp-2A amplifier in the bridge mode (Molecular Devices, Sunnyvale, CA) and a DAM-80 amplifier (World Precision Instruments, Sarasota, FL). For extracellular recordings, glass electrodes were used (Narishige, Tokyo, Japan). Pipette resistance was 4–8 MΩ for the STN and 1–3 MΩ for the SN when filled with 1 M NaCl. Extracellular recordings were obtained at the following coordinates: 1.8–2.1 mm posterior to the bregma, 1.5–1.7 mm lateral to the midline, and 4.1–4.6 mm below the cortical surface in the STN and 0.9–1.5 mm anterior to the lambda, 1.4–1.7 mm lateral to the midline, and 4.0–4.6 mm below the cortical surface in the SN. The recording electrode was advanced vertically with either a step motor-actuated manipulator (SM-21; Narishige) or an oil hydraulic-actuated manipulator (MO-10; Narishige). In all in vivo experiments, EEG recordings were performed simultaneously. STN neurons were identified by triphasic responses to cortical stimulation (200-μs-duration single pulse; 50–70-μA intensity) through the electrode implanted in the motor cortex (1.4–1.8 mm anterior to the bregma and 1.4–1.8 mm lateral to the midline). The triphasic response consists of early and late phases of excitation, which were interrupted by a brief period of inhibition (Chiken et al. 2008; Nambu et al. 2000).

Deep brain stimulation. Biphasic DBSs (200 ns each, ±30 μA, 7–190 Hz) were applied to the STN in tg mice through a concentric bipolar electrode (IMB-9008; Inter Medical) inserted into the STN.
Stimulus trains were made by a stimulator (SEN-7203; Nihon Kohden), and constant currents were generated by an isolator (SS-2033; Nihon Kohden).

**Data acquisition for in vivo recordings.** EEG data were band-pass filtered from 1.5 Hz to 1 kHz and digitized at 1 kHz, and extracellular signals were band-pass filtered from 300 Hz to 10 kHz and digitized at 20 kHz, with the CED 1401-plus interface (Cambridge Electronic Design, Cambridge, UK) or the Power1401 mk 2 interface (Cambridge Electronic Design). Data acquisition was performed with Spike2 software (Cambridge Electronic Design). To evaluate the effect of blocker microinjection into the brain, the number of SWDs, the total duration of SWDs, and the mean duration of SWDs were compared between before and after the injection. Unit activities were isolated from extracellular activities if a 2:1 or better signal-to-noise ratio was obtained. The amplitude of noise was estimated by fitting the amplitude histogram of baseline signals with the Gaussian equation. The upper 95th percentile of the amplitude was used as the maximum noise amplitude. Responses to the cortical stimulation were examined by constructing peristimulus time histograms (bin width of 1 ms) for 100 stimulus trials.

**Slice preparation.** Some experiments were performed in vitro with patch-clamp methods in tg and wt mice (both sexes, P30–57). Basal ganglia slices were prepared as reported by Beurrier et al. (2006). Mice under deep halothane anesthesia were killed by decapitation. Their brains were removed quickly and put into ice-cold cutting ganglia slices were prepared as reported by Beurrier et al. (2006). The tissue was saturated with carbogen (95% O2 and 5% CO2). The tissue was cut into 350-μm-thick slices with a vibratome (DTK-1000; Dosaka, Kyoto, Japan). Subsequently, these slices were incubated at 32°C for 30 min and then at room temperature for at least 30 min in artificial cerebrospinal fluid (ACSF), which contained (in mM) 126 NaCl, 3.5 KCl, 1.2 NaH2PO4, 25 NaHCO3, 1.3 MgCl2, 2 CaCl2, and 11 glucose and was saturated with carbogen (95% O2 and 5% CO2). The tissue was bubbled with carbogen. After the recovery period, individual slices were transferred to a submerged recording chamber and continuously perfused with the ACSF maintained at 30–32°C. The neurons were visualized by infrared differential interference contrast (IR-DIC) microscopy (BX51WI; Olympus Optical, Tokyo, Japan) and an IR-DIC video system (C2400-79H; Hamamatsu Photonics, Hamamatsu, Japan).

**Data acquisition of in vitro recordings.** Whole cell voltage-clamp recordings were made of STN neurons. Patch pipettes were made of borosilicate capillaries (Hilgenberg, Malsfeld, Germany). Pipette resistances were 2–4 MΩ for whole cell recording of HCN channel current), 4–6 MΩ (for cell-attached recording of HCN channel current), and 3–5 MΩ (for whole cell recording of T-type calcium channel current). Pipette solutions contained the following (in mM): for whole cell recording of the HCN channel current [liquid junction potential (2.5 mV) was not compensated], 130 K-methanesulfonate, 6 NaCl, 10 HEPES, 0.2 EGTA, 4 Mg-ATP, 0.3 Na-GTP, and 10 phosphocreatine, adjusted to pH 7.3 with KOH; for cell-attached recording of the HCN channel current [liquid junction potential (19.0 mV) was compensated], 120 KCl, 20 TEA-Cl, 10 HEPES, 5 4-AP, 2 CaCl2, 1 MgCl2, and 1 BaCl2, adjusted to pH 7.3 with KOH; and for whole cell recording of T-type calcium channel current [liquid junction potential (16.5 mV) was compensated], 120 Cs-methanesulfonate, 20 TEA-Cl, 10 HEPES, 5 4-AP, 10 EGTA, 4 Mg-ATP, 0.3 Na-GTP, and 5 QX-314, adjusted to pH 7.3 with CsOH. Currents were recorded with an EPC10 patch-clamp amplifier (HEKA Elektronik, Lambrecht, Germany). The access resistance was <20 MΩ and compensated by 50%. The access resistance was checked several times during the recording with compensation turned off. Cells were rejected if access resistance increased above 20 MΩ. Data acquisition was performed with the PULSE (version 8.78; HEKA Elektronik) and PATCHMASTER (version 2.52; HEKA Elektronik) programs. The current signals were low-pass filtered at 10 kHz and digitized at 20 kHz.

In whole cell current-clamp recordings, membrane potentials were recorded with an Axoclamp-2B amplifier (Molecular Devices). Patch pipettes were filled with the same intracellular solution as that used for voltage-clamp recordings. The pipette resistances were 3–5 MΩ when filled with the intracellular solution. Electrical signals were low-pass filtered at 3 kHz and then digitized at 10 kHz with an ITC-16 interface (Instrutech, Port Washington, NY) that was controlled by Igor Pro (Wavemetrics, Lake Oswego, OR). In some whole cell current-clamp recordings, membrane potentials were amplified with an EPC10 patch-clamp amplifier and recorded by the PATCHMASTER program. Compensation of the bridge balance was performed and electrical signals were low-pass filtered at 3 kHz and then digitized at 10 kHz with PATCHMASTER. The patch pipettes, intracellular solution, and extracellular solution used in these experiments were the same as those used for whole cell recording of the HCN channel current. Membrane capacitances were calculated from the total amount of charges that was required to clamp membrane when a step pulse (10 mV) was applied.

**Whole cell HCN channel currents.** To record the HCN channel-mediated currents in voltage-clamp mode, we modified the procedures described by Chen et al. (2005) and Kuisle et al. (2006). Tail currents were used to obtain the activation curve, which was fitted with the Boltzmann equation.

**Cell-attached HCN channel currents.** To examine the effect of LTG, we recorded the HCN channel currents by cell-attached voltage-clamp recording, as described by Poolos et al. (2002). HCN channel-mediated currents were examined as steady-state currents during 1.4–1.5 s of test potentials.

**T-type voltage-gated Ca2+ channel current.** Ca3v (T-type) calcium channel currents were recorded in voltage-clamp mode. We modified the procedures described by Powell et al. (2009). To prevent spike generation and contamination of the HCN channel currents, 500 nM tetrodotoxin (Wako Pure Chemical Industries) and 5 mM CsCl were added to the ACSF.

**Pharmacological modulation of HCN channel activity.** To modulate the HCN channel activity, 10 μM ZD7288 or 100 μM LTG was added to ACSF to block or activate the HCN channel, respectively. In cell-attached recording of the HCN channel currents, LTG was added to the pipette solution. The effect of LTG was examined 5 min after the formation of the gigaohm seal. In whole cell recordings of the HCN channel currents, control data were obtained 5 min after establishing the whole cell mode. Subsequently, drugs were applied to the bath solution. The effects of pharmacological modulations were examined 5 min after the application.

**Data analyses and statistics.** Data analyses were performed with Spike2 and Igor Pro. Frequency analysis of EEG was performed with Igor Pro. For statistical analyses, unless stated otherwise, pooled data are shown as means ± standard error (SE). Unless stated otherwise, we used two-tailed, unequal-paired t-tests for comparisons between different experimental data sets. In all cases, significance was set at \( P < 0.05 \). Statistical analyses were performed with Excel 2003 (Microsoft, Redmond, WA).

**Simulation of the STN neuron.** Simulation was performed with the NEURON simulator (version 7.1) (Hines and Carnevale 2006). In particular, we used the rat STN neuron model developed by Gillies and Willshaw (2006). Because the HCN channel current density of the original STN neuron model was much lower than the experimental value we recorded in wt mice, we increased the conductance of the original STN neuron model was much lower than the experimental value we recorded in wt mice, we increased the conductance of the HCN channel current. In whole cell recordings of the HCN channel currents, control data were obtained 5 min after establishing the conductive sheath. In whole cell recordings of the HCN channel currents, control data were obtained 5 min after establishing the whole cell mode. Subsequently, drugs were applied to the bath solution. The effects of pharmacological modulations were examined 5 min after the application.

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RESULTS

Properties of cortical SWDs. tg mice are known as model mice for examining absence epilepsy (Noebels and Sidman 1979). Previous studies showed that tg mice around 3–4 wk of age start to develop SWDs, which occur only in the awake condition (Noebels and Sidman 1979; Song et al. 2004). In this study, we first examined the prevalence and occurrence of absence seizures in tg mice. Of the 102 tg mice examined, 69 exhibited SWDs under the awake recording condition described in MATERIALS AND METHODS. In the following in vivo experiments, we used only tg mice that had at least 6 SWDs every 10 min. Simultaneous EEG recordings were performed to monitor the occurrence of SWDs in the mice. These EEG recordings showed that the SWDs from 32 tg mice had a mean duration of 1.8 s [0.4 standard deviation (SD), range from 0.5 to 5.7 s] and occurred an average of 17.3 times (8.1 SD) every 10 min. The intra-SWD frequency, which was revealed by spectral analysis of the EEG signal, ranged from 6 to 7 Hz. In some experiments, EEG recordings were performed bilaterally to check the synchronous occurrence of SWDs in the right and left hemispheres. In all of the bilaterally recorded SWDs (83/83 SWDs in 8 tg mice), SWDs were synchronized in both hemispheres. These temporal properties of SWDs in tg mice were consistent with those previously described (Song et al. 2004).

Firings of SN neurons are synchronized with SWDs. The basal ganglia receive glutamatergic excitatory inputs from the cerebral cortex in the striatum and the STN. The basal ganglia send GABAergic outputs from the SNr and the entopeduncular nucleus to the thalamus, the superior colliculus, and the brain stem nuclei (Cebrían et al. 2005). To determine whether SWD-related cortical activities of tg mice were transmitted in and output from the basal ganglia, we recorded the extracellular activities, and simultaneously obtained EEGs, in eight SN neurons in eight tg mice. This examination revealed that SN neurons increased the firing rate in synchronization with SWDs (Fig. 1A). To analyze the firing rate, 80 sets of extracellular unit activities during 85 ms before and after the negative spike peaks of the SWDs (Fig. 1B) were averaged for each SN neuron. The frequency of the SWD-spike-trigger-averaged unit activities is shown in Fig. 1C. This mean frequency increased around $\Delta t = 0$, where $\Delta t$ is the time for the negative peak of the SWD spikes. These results indicate that the SWD-related cortical activities were both transmitted in the basal ganglia circuit and output from the basal ganglia.

**Fig. 1.** Subthalamic neuron (STN)-substantia nigra (SN) pathway is involved in spike and wave discharge (SWD) generation. A: example of simultaneous EEG (top) and extracellular unit recording from an SN neuron (bottom). An episode of SWD is marked with a line. B: example of analysis of frequency of SWD-spike-trigger-averaged action potentials. Top: a spike in the SWD in an expanded time scale. Bottom: example of unit recording relative to the spike. The negative peak of the spike is set to $\Delta t = 0$. C: mean frequency of SWD-spike-trigger-averaged action potentials (APs) in 640 SWDs in 8 cells (bin width 5 ms) (error bars: SD). Dashed line with a gray band indicates mean ± SE firing rate during the interictal phase. D: typical image of sagittal section of the brain frozen in OCT compound. Injection site was confirmed by bromophenol blue after the injection of kynurenic acid into the SN. E: EEG recordings before (top) and after (bottom) injection of kynurenic acid (2 nmol in 40 nl of saline) into the SN. SWDs are shown with vertical bars above each EEG trace. Inset: SWD marked with a filled triangle is expanded. F: effect of injection into the SN on the total duration of SWDs in 10 min. Left: saline injection ($n = 5$). Right: kynurenic acid injection ($n = 5$) (mean ± SE; ***$p < 0.001$, 2-tailed paired t-test).
SWDs are blocked by injection of kynurenic acid in the SN. Although extracellular recordings suggested that the SWD-related activities were output from the SN, the SN activity may be unnecessary for SWD generation. To test the necessity of SWD-related outputs from the SN, we examined the effect of the unilateral blockade of glutamatergic transmission on SWD generation by injection of kynurenic acid (2 nmol in 40 nl of saline). Bromophenol blue was dissolved in kynurenic acid solution to confirm the injection site after the recording (Fig. 1D). SWD generation was dramatically inhibited by kynurenic acid injection ($n = 5$; Fig. 1E). The total SWD duration before and after the injection was 20.7 ± 5.6 s/10 min and 0.9 ± 0.4 s/10 min, respectively. In contrast, when 40 nl of saline was injected into the SN, SWD generation was unaffected ($n = 5$; data not shown). Specifically, the total SWD duration before and after the injection was 22.7 ± 3.7 s/10 min and 23.7 ± 3.7 s/10 min, respectively. To quantitatively assess the effects of the kynurenic acid injection and the saline injection, we compared the total SWD durations in the 10 min before and after the injections. Kynurenic acid injection significantly decreased the SWD duration ($P < 0.001$, 2-tailed paired $t$-test), whereas saline injection did not affect the duration (Fig. 1F). This result suggests that the SWD-related outputs from the SN were necessary in SWD generation. This finding is consistent with previous studies of genetic absence epilepsy rats (Deransart et al. 1996; Paz et al. 2007).

Firings of STN neurons are synchronized with SWDs. The SN receives the main excitatory input from the STN. To examine whether SWD-related cortical activities of tg mice were transmitted through the STN, we recorded extracellular activities, along with simultaneously obtained EEGs, in eight STN neurons in four tg mice. STN neurons were identified by the triphasic responses to the cortical stimulation (Chiken et al. 2008) (Fig. 2A). These results showed that STN neurons increased the firing rate in synchronization with the SWDs (Fig. 2B). The firing rates of STN neurons were analyzed in the same way as for SN neurons. The mean frequency of the SWD-spike-triggered unit activities in eight STN neurons is shown in Fig. 2C. This frequency increased around $\Delta t = 0$, where $\Delta t$ is the time for the negative peak of the SWD spikes. These results indicate that the SWD-related cortical activities were transmitted through the STN.

SWDs are blocked by injection of kynurenic acid in the STN. The STN receives glutamatergic excitatory inputs directly from the cerebral cortex (Afsharpour 1985; Beurrier et al. 2006; McGeorge and Faull 1989), in addition to GABAergic inhib-
ity inputs from the globus pallidus (Beurrier et al. 2006; van der Kooy et al. 1981). To determine whether the cortico-subthalamic pathway is involved in SWD generation, kynurenic acid was used to block the glutamatergic transmission in the STN. Bromophenol blue dissolved in kynurenic acid solution confirmed the injection site after the recording (Fig. 2D). SWD generation was almost completely inhibited by kynurenic acid injection (n = 9; Fig. 2E). The total SWD duration before and after the injection was 30.6 ± 9.9 s/10 min and 2.5 ± 0.8 s/10 min, respectively. When PBS (0.05 M, pH 7.3) was injected into the STN, SWD generation was unaffected (n = 9; data not shown). The total SWD duration before and after the injection was 35.8 ± 7.0 s/10 min and 38.0 ± 8.8 s/10 min, respectively. To examine the effects of kynurenic acid injection and PBS injection, we compared the total SWD duration within 10 min after the injection to the duration before the injection. Kynurenic acid injection significantly decreased the SWD duration (P < 0.001, 2-tailed paired t-test), whereas PBS injection did not affect the duration (Fig. 2F). Our results indicate that STN neurons not only show synchronization with SWDs (Paz et al. 2005) but also play an essential role in SWD generation.

Membrane excitability is enhanced in STN neurons in tg mice. The in vivo studies described above demonstrated that glutamatergic transmission through the STN was necessary for SWD generation. We therefore further focused on the STN and examined whether it played a positive role in SWD generation. First, we compared the intrinsic excitability of STN neurons between wt mice and tg mice under a current-clamp condition. The initial resting potential of the recorded neurons was set to −65 mV by hyperpolarizing current injection to suppress spontaneous firing and to minimize the cell-to-cell variability. When the depolarizing step currents were injected into the STN neurons, those in the tg mice generated more action potentials than those in the wt mice (Fig. 3A). The mean numbers of action potentials were significantly larger in tg mice than in wt mice for both 25-pA and 50-pA injections (P < 0.01; Fig. 3B). However, the threshold membrane potentials for spike generation in wt mice (−47.3 ± 0.9 mV; n = 12) and tg mice (−48.3 ± 0.6 mV; n = 12) were not significantly different (P = 0.36). To analyze the sensitivity of the membrane potential to the current injection, hyperpolarizing step currents (25 pA) were injected into STN neurons in both tg and wt mice. The STN neurons in tg mice showed larger voltage deflection than those in wt mice (Fig. 3C), and the amplitude of the voltage deflection was significantly larger in tg mice than in wt mice (P < 0.01; Fig. 3D). These results indicate that the sensitivity of the membrane potential to the current injection, namely the membrane excitability, was higher in tg mice than in wt mice, and they suggest that the increased excitability of the STN neurons resulted from a higher membrane resistance in tg mice than in wt mice.

HCN channel activity is reduced in STN neurons in tg mice. The results of the current-clamp recordings (Fig. 3, A–D) implied that membrane resistance was higher in tg mice than in wt mice. To examine this possibility, voltage-clamp recordings were also performed in STN neurons of both types of mice. We found that the membrane resistance was significantly higher in tg mice (263.1 ± 25.9 MΩ; n = 20) than in wt mice (164.4 ± 12.7 MΩ; n = 20) (P < 0.01). Because the HCN channel is one of the ion channels that controls the membrane resistance at resting and hyperpolarizing potentials (Robinson and Siegelbaum 2003; Santoro and Baram 2003), we also examined the properties of the HCN channel in this study. In addition, we examined Ca,3 (T-type) calcium channel currents because previous investigation revealed that neuronal T-type currents underlie burst firings and oscillatory behaviors associated with absence seizures (Huguenard and Prince 1992; Perez-Reyes 2003). The HCN channel is activated by hyperpolarized potentials negative to −60 mV, which is similar to the resting potential of most neurons. The HCN channel-mediated currents were recorded from STN neurons in wt and tg mice under voltage-clamp conditions (Fig. 3E), which revealed that the tail currents of the HCN channel were smaller in tg mice than in wt mice. The relationship between the peak amplitudes of the tail currents and test potentials is shown in Fig. 3F.

Furthermore, we analyzed five kinds of factors in both tg and wt mice: the peak amplitude of the tail currents, the half-activation voltage, the slope factor of the normalized current-voltage (I-V) curve, the time constant at the activation phase, and the current density of the HCN channel currents evoked at −120 mV. First, we compared the peak amplitude of the tail currents at −120 mV. Because the HCN channel is fully activated at −120 mV, the tail currents at −120 mV indicate the amount of the HCN channel expressed in the neuron. This analysis revealed that the tail current amplitude activated at −120 mV in tg mice was significantly less than that in wt mice (P < 0.01; Fig. 3G). Second, the voltage dependence of the HCN channel was obtained by plotting the normalized tail current amplitude against the test potential. STN neurons in tg and wt mice showed almost superimposing voltage dependence of activation (Fig. 3F, inset). The plot was fitted with the Boltzmann equation. The half-activation voltage (P = 0.67; Fig. 3H) and the slope factor (P = 0.82; Fig. 3I) were not significantly different across the tg and wt mice. Next, we examined the gating kinetics of the activation phase. The HCN channel opens slowly in response to voltage hyperpolarization. These kinetics are affected by the subunit combination of the HCN channel (Jung et al. 2007). The activation phase of the current trace evoked at −80 mV was fitted with a single exponential function, which revealed that the mean time constant was not significantly different between wt and tg mice (P = 0.99; Fig. 3J). These results indicate that the STN neurons in tg mice had smaller HCN channel currents than those in wt mice, but the voltage dependence and kinetic properties were similar. Finally, we examined the current density of the HCN channel current evoked at −120 mV, which revealed that the current density of tg mice was significantly smaller than that of wt mice [−9.4 ± 0.4 pA/pF (n = 18) for wt, −8.1 ± 0.4 pA/pF (n = 19) for tg; P < 0.05].

Next, we examined T-type calcium channel currents in the STN of both tg and wt mice. Representative traces of T-type currents evoked at −30 mV in mice are depicted in Fig. 4A. The current density of T-type current evoked at −40 mV was not significantly different among the groups (P = 0.17; Fig. 4B). This result indicates that T-type currents of STN neurons in tg mice were not significantly different from those in wt mice.

We also checked the membrane capacitance of tg and wt mice. The capacitances of tg mice were mildly, but significantly, smaller than those of the wt mice (P < 0.05; Fig. 3K). Finally, we simulated the effect of a mild reduction of HCN
in HCN channel activity to the membrane excitability, the STN neurons. Thus, to examine the contribution of the change in HCN channel activity could affect the intrinsic membrane excitability of the STN neurons.

It was unclear whether such a modest change in HCN channel activity in the STN neurons of wt mice was partially blocked by 10 μM ZD7288 or activated by 10 μM LTG and the membrane excitability levels before and after the applications were compared under a current-clamp condition. LTG is an anticonvulsant and is known as both an activator of the HCN channel (Poolos et al. 2002) and a use-dependent blocker of some sodium channels (Kuo and Lu 1997).

Bidirectional modulations of HCN channel affect membrane excitability in STN neurons. The results described above demonstrated that HCN channel activity was mildly reduced in the STN neurons of tg mice. In addition, the neural simulation implied that reduction of HCN channel activity enhances membrane excitability. However, on the basis of these results, it was unclear whether such a modest change in HCN channel activity could affect the intrinsic membrane excitability of the STN neurons. Thus, to examine the contribution of the change in HCN channel activity to the membrane excitability, the HCN channel in the STN neurons of wt mice was partially blocked by 10 μM ZD7288 or activated by 10 μM LTG and the membrane excitability levels before and after the applications were compared under a current-clamp condition. LTG is an anticonvulsant and is known as both an activator of the HCN channel (Poolos et al. 2002) and a use-dependent blocker of some sodium channels (Kuo and Lu 1997).

First, we examined the effect of 10 μM ZD7288 on the HCN channel, using a whole cell voltage-clamp recording method (Fig. 5A). Application of ZD7288 partially, but significantly, decreased the peak amplitude of the HCN channel current evoked at −120 mV (P < 0.01, 2-tailed paired t-test; Fig. 5B) and increased the membrane resistance [184.0 ± 22.0 MΩ (n = 5) for control, 250.1 ± 13.9 MΩ (n = 5) after the application; P < 0.05, 2-tailed paired t-test]. However, the membrane capacitance [48.8 ± 5.0 pF (n = 5) for control, 51.4 ± 7.1 pF (n = 5) after the application] by 10.2 ± 3.5 on June 25, 2017 http://jn.physiology.org/ Downloaded from.
(n = 5) after the application; *P* = 0.28, 2-tailed paired *t*-test], the holding current [−69.5 ± 8.6 pA (n = 5) for control, −114.0 ± 13.1 pA (n = 5) after the application; *P* = 0.08, 2-tailed paired *t*-test], and the access resistance [14.4 ± 0.6 MΩ (n = 5) for control, 14.9 ± 0.9 MΩ (n = 5) after the application; *P* = 0.30, 2-tailed paired *t*-test] were not significantly changed.

Next, we examined the effect of partial blockade of the HCN channel current on the membrane excitability of the STN neurons, using a whole cell current-clamp recording method. The initial resting potential of the recorded neurons was set to −65 mV by injection of hyperpolarizing currents. After blocking the HCN channel with ZD7288, we found that injection of depolarizing step currents generated more action potentials than before the blockade (Fig. 5C). The mean number of action potentials was increased after the blockade, when 25 pA and 50 pA of depolarizing currents were injected (*P* < 0.01, 2-tailed paired *t*-test; Fig. 5J). When 25 pA of hyperpolarizing step currents were injected, the STN neurons exhibited a larger voltage deflection after the blockade compared with that before (Fig. 5D). The mean amplitude of the voltage deflection was significantly larger after the blockade with ZD7288 compared with that before (*P* < 0.01, 2-tailed paired *t*-test; Fig. 5J). These results indicate that reducing HCN channel activity enhanced membrane excitability in the STN neurons.

Next, we examined the effect of HCN channel activation by LTG on the membrane excitability of the STN neurons. The effect of LTG application on the HCN channel was examined by cell-attached voltage-clamp recording as previously reported (Poolos et al. 2002). A typical trace recorded by this method is shown in Fig. 5E. LTG partially, but significantly, shifted the half-activation voltage (*P* < 0.05, 2-tailed paired *t*-test; Fig. 5F), whereas the resting membrane potentials were not significantly different (*P* = 0.45, 2-tailed paired *t*-test; Fig. 5F). We also examined the effect of LTG on the membrane excitability of STN neurons, using whole cell current-clamp recording. The initial resting potential of the recorded neurons was set to −65 mV by injection of hyperpolarizing currents. After LTG was used to activate the HCN channel, the injection of depolarizing step currents generated fewer APs than before the activation (Fig. 5G). The mean number of action potentials was smaller after activation (n = 7) than before activation by LTG (n = 7) when either 25 pA or 50 pA of depolarizing currents was injected (*P* < 0.01, 2-tailed paired *t*-test; Fig. 5I). When 25 pA of hyperpolarizing step currents was injected, the STN neurons showed a smaller voltage deflection after the activation by LTG compared with before activation (Fig. 5H). The mean amplitude of voltage deflection was significantly smaller after activation compared with before activation by LTG (*P* < 0.01, 2-tailed paired *t*-test; Fig. 5J). These results
Modulation of HCN channel activity in the STN affects mean duration of SWD. The series of in vitro experiments described above suggested that the intrinsic membrane excitability of STN neurons was increased in tg mice and that this increment, at least partly, resulted from the reduction of the HCN channel activity. However, these experiments did not reveal the effect of the enhanced excitability of the STN neurons on SWD generation. Therefore, we next examined the effects of bidirectional HCN channel modulation on SWD generation in the STN in tg mice. Specifically, we examined the effects of blockade and activation of the HCN channel activity by ZD7288 injection and LTG injection in the STN, respectively. First, the HCN channel in the STN was blocked in vivo by ZD7288 injection (2 pmol in 20 nl of PBS). Representative examples of EEG traces before and after the injection are shown in Fig. 6A. Before injection, the total number of SWD occurrences and the mean duration of the SWDs were 38.4 ± 4.0 s/10 min and 1.9 ± 0.2 s, respectively. The respective values after ZD7288 injection were 31.9 ± 3.2 s/10 min and 2.2 ± 0.2 s. Furthermore, the total number of SWD occurrences and the mean duration of the SWDs were 41.2 ± 5.3 s/10 min and 1.9 ± 0.1 s, respectively, before PBS injection and 40.2 ± 5.1 s/10 min and 1.9 ± 0.1 s after PBS injection. The effect of the blocker injection was evaluated first by comparing the SWD occurrence and mean duration before and after the injection and then by comparing the effect of ZD7288 injection to that of the PBS injection. The effects of ZD7288 and PBS injections on the number of SWD occurrences were not significantly different (P = 0.25, 2-tailed paired t-test; data
not shown). In contrast, the effects of these injections on the mean duration of SWDs were statistically different; ZD7288 injection prolonged the mean duration \( (P < 0.01, \text{2-tailed paired } t\text{-test}; \text{Fig. 6C, left}) \). The cumulative distributions of the SWD duration were obtained for pooled data from all mice used in this experiment. After ZD7288 injection, the cumulative distribution was shifted to higher values (Fig. 6D). These results indicate that blocking the HCN channel in the STN prolonged the mean duration of SWDs in \( \text{tg} \) mice.

Next, we examined the effect of pharmacological upregulation of the HCN channel activity by LTG injection (1 nmol in 20 nl PBS) on SWD generation. Representative examples of EEG traces before and after the injection are shown in Fig. 6B. Before LTG injection, the total number of SWD occurrences and the mean duration of the SWDs were 42.2 \( \pm \) 5.8 s/10 min and 2.1 \( \pm \) 0.1 s, respectively. After injection, the respective values were 36.9 \( \pm \) 5.7 s/10 min and 1.8 \( \pm \) 0.1 s. Before PBS injection, the total number of SWD occurrences and the mean duration of the SWDs were 36.4 \( \pm \) 5.2 s/10 min and 2.0 \( \pm \) 0.1 s, respectively; after injection, the values were 32.8 \( \pm \) 4.4 s/10 min and 2.1 \( \pm \) 0.1 s. We analyzed the effect of LTG injection using the same method as that used to study the effect of ZD7288 injection. The effects of LTG and PBS injections on the number of the SWD occurrences were not significantly different (\( n = 9 \); \( P = 0.52, \text{2-tailed paired } t\text{-test}; \text{data not shown})). In contrast, the effects of the injections on the mean duration of the SWDs were statistically different; LTG injection shortened the mean duration (\( P < 0.01, \text{2-tailed paired } t\text{-test}, \text{Fig. 6C, right}) \). After LTG injection, the cumulative distribution of the SWD duration was shifted to smaller values (Fig. 6E). These results indicate that LTG injection in the STN shortened the mean duration of SWDs in \( \text{tg} \) mice. To check the effect of use-dependent blockade of sodium channels by LTG on SWD generation, we also injected lidocaine (2 nmol in 20 nl PBS) into the STN. The mean duration of the SWDs was unaffected (2.2 \( \pm \) 0.1 s before the injection, 2.1 \( \pm \) 0.1 s after the injection), whereas the firing rate was reduced by lidocaine (data not shown). Furthermore, the effect of lidocaine on the mean duration of the SWDs (94.1 \( \pm \) 4.4%; \( n = 9 \)) was not significantly different from the effect of PBS injection (\( P = 0.13, \text{2-tailed paired } t\text{-test}; \text{data not shown})). Although intracellularly applied QX-314 (a quaternary lidocaine derivative) can block HCN currents (Perkins and Wong 1995), and extracellularly applied lidocaine can partially block HCN currents in vitro (Meng et al. 2011), lidocaine injection did not alter the mean SWD duration. This result implies that lidocaine at this
dose in vivo did not block the HCN channel, at least, so potently as ZD7288, which caused prolongation of the mean SWD duration. The result of lidocaine injection also indicates that use-dependent blockade of the sodium channel was not involved in the action of LTG to shorten the mean SWD duration. These results suggest that the HCN channel in the STN is involved in maintaining the rhythmic activity of SWDs.

Deep brain stimulation modulation of STN activity affects SWD generation. Bidirectional pharmacological modulations of HCN channel activity resulted in bidirectionally altered mean duration of the SWDs. Because these drugs are known to have some side effects (Chen 2004; Sánchez-Alonso et al. 2008), we tested the involvement of the STN in maintaining the rhythmic activity of SWDs using another method to modulate STN activity. In this experiment, we modulated STN activity with DBS and examined its effect on SWD generation (Grill et al. 2004). We stimulated the STN with a concentric bipolar electrode (30 μA) to result in an informational lesion (Grill et al. 2004). We stimulated high-frequency stimulations to mask the intrinsic activity and neurons and the transmission of the neural activity, whereas superposed to enhance both the intrinsic activity of STN (Limousin et al. 1995). Briefly, low-frequency stimulations are dependent on the stimulus frequency employed (Grill et al. 2004; Sanchez-Alonso et al. 2004). We tested the involvement of the STN in maintaining the rhythmic activity of SWDs using another method to modulate STN activity. In this experiment, we modulated STN activity with DBS and examined its effect on SWD generation (Grill et al. 2004). We stimulated the STN with a concentric bipolar electrode (30 μA, 7–190 Hz of stimulus frequency) and evaluated the effect of the stimulation by comparing the number and mean durations of SWD occurrences before and during the stimulation. We compared the effect of 30-μA stimulation with that of sham stimulation. A representative EEG trace is shown in Fig. 7A, top, which clearly indicates that SWD occurrence was enhanced during low-frequency stimulations and suppressed during high-frequency stimulations. To exclude the possibility that DBS directly affects the rhythm of SWDs frequency analyses were performed, which revealed that the strong spectrum component of SWDs remained around 6–7 Hz even during DBS at 10 Hz (Fig. 7A, bottom). These data imply that the rhythm of the SWDs was unaffected by DBS. On the other hand, DBS affected the number and mean duration of SWDs. Analyses of the SWDs demonstrated that the mean duration of the SWDs during 30-μA stimulation was significantly longer at low-frequency stimulations (7 and 10 Hz, \( P < 0.01 \)) and shorter at high-frequency stimulations (130 and 190 Hz, \( P < 0.05 \)) than the mean duration during sham stimulations (2-tailed paired t-test except for 190 Hz; 2-tailed unpaired t-test for 190 Hz) (Fig. 7B). Similarly, the number of SWD occurrences during 30-μA stimulation was significantly larger at low-frequency stimulations (7 and 10 Hz, \( P < 0.05 \)) and smaller at high-frequency stimulations (130 and 190 Hz, \( P < 0.01 \)) than the number during sham stimulations (Fig. 7C). These results show that DBS from low to high stimulus frequencies generated frequency-dependent bidirectional modulations of the mean duration and the number of SWD occurrences. These results suggest that the STN is involved in maintaining the rhythmic activity of SWDs. Also of note, these results are consistent with the results of pharmacological modulation of the HCN channel in the STN in tg mice (Fig. 6).

Fig. 7. Frequency-dependent effects of deep brain stimulation (DBS) in STN on SWDs in tg mice. A: EEG recordings during DBS in the STN at various stimulus frequencies (top). Periods of DBS are shown by bars above the trace. Stimulus frequency was incrementally increased (7, 10, 30, 70, 130, and 190 Hz from left). EEG waveforms around the gray lines were expanded (middle; open triangle for control, gray triangle for 7 Hz, black triangle for 10 Hz), and power spectra are shown for each (bottom). B: effect of DBS on the mean duration of SWDs. Effects are calculated by dividing the mean SWD duration during DBS by that before the DBS. Open bars, sham stimulation (n = 6); filled bars, effect of DBS at 30 μA (n = 6 except for 190 Hz, n = 5 for 190 Hz; \( **P < 0.01, *P < 0.05 \), 2-tailed paired t-test except for 190 Hz, 2-tailed unpaired t-test for 190 Hz). C: DBS effects on the number of SWDs. Effects are calculated by dividing the number of SWDs during DBS by that before the DBS. Open bars, sham stimulation; filled bars, effect of DBS at 30 μA (n = 6; \( **P < 0.01, *P < 0.05 \), 2-tailed paired t-test).
DISCUSSION

In this study, we investigated the role of the basal ganglia in rhythm generation of absence seizures in vivo and in vitro measurements of tg mice. We found that the STN is located in a strategic position for transmitting SWD-related activities through the basal ganglia and that membrane excitability of STN neurons is enhanced in tg mice to play a positive role in SWD generation. The enhanced STN excitability in tg mice was, at least partly, caused by reduced HCN channel activity. Pharmacological modulations of the HCN channel activity altered the mean duration of the SWDs bidirectionally. Moreover, electrical modulations of STN activity altered SWD generation bidirectionally in a stimulus frequency-dependent manner. These bidirectional modulations of the SWD mean duration indicate that the STN is involved in the maintenance system of SWDs.

Involvement of basal ganglia in SWD generation. The present study showed that SN neurons exhibited an increased firing rate in synchronization with SWDs in tg mice. The increased synchronous firing in the SN indicates that the basal ganglia receive SWD-related activities from the cerebral cortex, transmit them within the basal ganglia, and output them from the SN. Furthermore, SN neurons receive glutamatergic inputs from the STN. Our finding that kynurenic acid injection into the SN almost completely abolished SWDs suggests that the increased firing rate was caused by excitatory inputs from the STN. This idea is consistent with previous studies that used GAERS and other generalized convulsive epilepsy models (Depaulis et al. 1988, 1989). In turn, the STN receives monosynaptic glutamatergic inputs directly from the cerebral cortex. Furthermore, the finding that SWDs were blocked by kynurenic acid injection into the STN suggests that the direct excitatory inputs from the cortex play a dominant role in increasing the firing rate of STN neurons. Collectively, these results demonstrate that the SWD-related cortical activities are transmitted by the cortico- STN-SN pathway in tg mice.

Enhanced membrane excitability in STN neurons of tg mice. Although the involvement of the basal ganglia in SWD generation was demonstrated previously by in vivo studies, these studies did not reveal the molecular and cellular bases of the involvement. In this study, we found that the membrane excitability in STN neurons is enhanced in tg mice. This result implies that the membrane capacitance and resistance in STN neurons are decreased. In line with our finding that modulations of HCN channel activity altered membrane excitability (Fig. 5), previous studies have shown that the HCN channel plays a critical role in the regulation of membrane excitability through controlling the membrane resistance (Robinson and Siegelbaum 2003; Santoro and Baram 2003). Whole cell voltage-clamp recordings showed that the HCN channel currents were smaller in tg mice than in wt mice, whereas the voltage dependence of activation and the activation time constant remained unaffected. Epileptogenic effects of reducing the HCN channel activity have previously been reported in seizure models (Kole et al. 2007; Shah et al. 2004) and in knockout and mutant mice (Chung et al. 2009; Ludwig et al. 2003), whereas epileptogenic effects of increasing the HCN channel expression in the thalamus have been also reported in the rat absence epilepsy model (Budde et al. 2005). On the other hand, it has been reported that the expression pattern of the HCN channel isoforms (HCN1 and HCN2) is changed in some seizure models (Brewster et al. 2002; Budde et al. 2005; Richichi et al. 2008). Furthermore, the seizure-dependent downregulation of HCN channel gating by altered phosphorylation signaling has also been reported (Jung et al. 2010). In our present study, the finding that no changes occurred in the activation time constant or voltage dependence suggests that the expression pattern and phosphorylation signaling are unaltered in tg mice.

Whole cell voltage-clamp recordings also showed that the membrane capacitance of STN neurons was smaller in tg mice than in wt mice. Although the data did not show significant differences, previous studies reported a similar trend of smaller capacitance in neurons of CaV2.1 (P/Q type) calcium channel mutant mice: layer IV and V pyramidal neurons of tg mice (Sasaki et al. 2006) and Purkinje cells of rocker mice (Kodama et al. 2006). Furthermore, it has been shown that leainer mice have significantly smaller membrane capacitance and enhanced membrane excitability in their Purkinje cells (Ovsepian and Friel 2008). These studies suggest that defects in the function of CaV2.1 calcium channels generally lead to smaller capacitances of neurons. In tg mice, smaller capacitances of STN neurons may be involved in the enhanced membrane excitability.

Effect of pharmacological modulation of HCN channel activity in the STN. We also evaluated the effect of modulating HCN channel activity in the STN on SWD generation. First, we injected ZD7288 into the STN in tg mice, and this blockade of the HCN channels prolonged the mean duration of individual SWDs, whereas the number of SWDs was unchanged. Conversely, injection of LTG shortened the mean duration. Because ZD7288 has nonspecific effects (Chen 2004; Sánchez-Alonso et al. 2008), the T-type calcium channel current and glutamatergic transmission might be partially blocked in this experiment. However, because blockade of either the T-type calcium channel or glutamatergic transmission generally inhibits absence epilepsy, we conclude that the effect of ZD7288 in prolonging the mean duration of the SWDs is mediated by the HCN channel. Therefore, our data indicate that bidirectional modulations of the HCN channel by ZD7288 and LTG in the STN result in bidirectional modulation of the mean duration of SWDs.

In the present study, in vitro experiments showed the decrement of HCN channel activity in STN neurons of tg mice, in addition to the enhancement of membrane excitability by blockade of the HCN channel in STN neurons of wt mice. Collectively, these results suggest that, in tg mice, the basal ganglia play an active role in SWD generation by increasing the membrane excitability in STN neurons, at least partly as a result of decreasing their HCN channel activity. Because the number of SWD occurrences was unchanged after block or activation of the HCN channel, the cortico-basal ganglia circuit may not be involved in the initiation system of SWDs. However, the altered mean duration implies that the cortex-basal ganglia circuit is involved in the maintenance mechanism of epileptic rhythms in tg mice.

Effect of electrical modulation of STN activity by DBS. To evaluate the functional role of the STN in SWD generation, we applied DBS to the STN at different stimulus frequencies in tg mice. Low-frequency DBS (7 and 10 Hz) prolonged the mean SWD duration and increased the number of SWD occurrences, whereas high-frequency DBS (130 and 190 Hz) shortened the
mean SWD duration and decreased the number of SWD occurrences. These results support the idea that the STN is involved in the maintenance mechanism of epileptic rhythms in

REFERENCES


Inherited cortical HCN1 channel loss

Kole MH, Bräuer AU, Stuart GJ.

Inherited epilepsy: spike-wave and focal motor

Meng QT, Xia ZY, Liu J, Bayliss DA, Chen X.

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Matsushita K, Wakamori M, Rhyu IJ, Arii T, Oda S, Mori Y, Imoto K.

Molecular physiology of low-voltage-activated T-type calcium channels.


H11001 channels in rat hippocampal neurones.


Kuo CC, Lu L.

Monakow KH, Akert K, Kunzle H.


Kha HT, Finkelstein DI, Tomás D, Drago J, Pow DV, Horne MK.


Ovsepian SV, Friell DD. The leaner P/Q-type calcium channel mutation renders cerebellar Purkinje neurons hyper-excitable and eliminates Ca\textsuperscript{2+} -Na\textsuperscript{+} spike bursts. Eur J Neurosci 27: 93–103, 2008.


