Rhythmic Bursting in the Cortico-Subthalamo-Pallidal Network during Spontaneous Genetically Determined Spike and Wave Discharges

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Absence seizures are characterized by impairment of consciousness associated with bilaterally synchronous spike-and-wave discharges (SWDs) in the electroencephalogram (EEG), which reflect paroxysmal oscillations in thalamocortical networks. Although recent studies suggest that the subthalamic nucleus (STN) provides an endogenous control system that influences the occurrence of absence seizures, the mechanisms of propagation of cortical epileptic discharges in the STN have never been explored. The present study provides the first description of the electrophysiological activity in the cortico-subthalamo-pallidal network during absence seizures in the genetic absence epilepsy rats from Strasbourg, a well established model of absence epilepsy. In corticostriatal neurons, the SWDs were associated with repetitive suprathreshold depolarizations correlated with EEG spikes. These cortical paroxysms were reflected in the STN by synchronized, rhythmic, high-frequency bursts of action potentials. Intracellular recordings revealed that the intraburst pattern in STN neurons was sculpted by an early depolarizing synaptic potential, followed by a short hyperpolarization and a rebound of excitation. The rhythmic hyperpolarizations in STN neurons during SWDs likely originate from a subpopulation of pallidal neurons exhibiting rhythmic bursting temporally correlated with the EEG spikes. The repetitive discharges in STN neurons accompanying absence seizures might convey powerful excitation to basal ganglia output nuclei and, consequently, may participate in the control of thalamocortical SWDs.

Key words: absence epilepsy; basal ganglia; burst firing; corticosubthalamic neurons; in vivo; subthalamic neurons

Introduction
Absence epilepsy is a generalized, nonconvulsive epilepsy of multifactorial genetic origin (Panayiotopoulos, 1997; Crunelli and Lerescu, 2002). Absence seizures consist of a brief, sudden impairment of consciousness concomitant with bilateral synchronized spike-and-wave discharges (SWDs) in the electroencephalogram (EEG) over wide cortical areas (Panayiotopoulos, 1997). Electrophysiological recordings in patients (Williams, 1953) and animal models of absence epilepsy (for review, see Danobe et al., 1998; Crunelli and Lerescu, 2002; Timofeev and Steriade, 2004) show that SWDs result from abnormal synchronized oscillations in thalamocortical networks. A number of studies performed in genetic absence epilepsy rats from Strasbourg (GAERS), a well established model of absence epilepsy (Marescaux et al., 1992; Danobe et al., 1998) suggest that the basal ganglia provide an endogenous control system for absence seizures via a modulation of the glutamatergic subthalamic pathway (for review, see Danobe et al., 1998; Deransart et al., 1998, 2002).

Specifically, alteration of the excitatory synaptic influence of the subthalamic nucleus (STN) on the substantia nigra pars reticulata (SNr), either by bilateral injection of a GABA agonist in the STN or a blockade of nigral glutamatergic NMDA receptors, has been shown to suppress absence seizures (Deransart et al., 1998). Furthermore, either bilateral excitotoxic lesions or high-frequency stimulation of the STN dramatically affects the occurrence of cortical paroxysms in GAERS (Vercueil et al., 1998). Consistent with the antiepileptic effect of an interruption of the excitatory subthalamo-recipient transmission, intranigral injection of GABA receptor antagonists increases the occurrence of SWDs in GAERS (Danobe et al., 1998; Deransart et al., 1998). Together, these findings suggest that the occurrence of absence seizures is modulated by changes in the activity of subthalamic neurons.

STN neurons integrate powerful monosynaptic glutamatergic inputs originating from corticosubthalamic (cSTh) neurons and relay these excitatory signals to the SNr and the globus pallidus (GP) (Deniau et al., 1978; Van Der Kooy and Hattori, 1980; Kitai and Deniau, 1981; Afsharpour, 1985; Rouzaire-Dubois and Scarnati, 1987; Maurice et al., 1998; Magill et al., 2000). GABAergic neurons of the GP shape the activity of STN neurons by a disinhibitory mechanism involving corticostratal and striatopallidal pathways (Maurice et al., 1998; Bevan et al., 2002b) and via reciprocal connections with the STN (Magill et al., 2000; Bevan et al., 2002b). Recent studies indicate that rhythmic activity in the cerebral cortex can promote oscillations in STN and GP, the
phase relationships of which are controlled by the reciprocal excitatory and inhibitory connections between these two nuclei (Plenz and Kitai, 1999; Magill et al., 2000, 2001; Bevan et al., 2002b).

The effect of cortical SWDs on the activity of STN neurons remains unknown, and the electrical events occurring in corticostriatal-subthalamo-pallidal networks during absence seizures have never been studied. Because these questions are crucial to our understanding of how the basal ganglia influence absence seizures, we examined in vivo in GAERS the intracellular and/or extracellular activity of CSth neurons, STN neurons, and GP neurons simultaneously with the spontaneous SWDs occurring in the EEG of the functionally related cortical region.

Materials and Methods

All experiments were performed in accordance with local Ethical Committee and European Union guidelines (directive 86/609/European Economic Community), and every precaution was taken to minimize stress and the number of animals used in each series of experiments.

Animal preparation. Experiments were performed in vivo on 24 adult (3–12 months of age) rats from the GAERS strain. Animals were anesthetized initially with sodium pentobarbital (40 mg/kg, i.p.; Sanofi, Lorraine, France) and ketamine (100 mg/kg, i.m.; Imalgène, Rhone Mérieux, France). A cannula was inserted into the trachea, and the animal was placed in a stereotaxic frame. Wounds and pressure points were maintained (36.5–37.5°C) with a homeothermic blanket. At the end of the experiments, animals received an overdose of sodium pentobarbital (200 mg/kg, i.p.).

Electrophysiological recordings. EEG recordings were obtained with a low-impedance (~60 KΩ) silver electrode placed on the dura above the orofacial motor cortex (12.5 mm anterior to the interaural line; 3.5–4 mm lateral to the midline) (Hall and Lindholm, 1974; Neafsey et al., 1986; Paxinos and Watson, 1986), and the reference electrode was placed in the muscle on the opposite side of the head.

Intracellular recordings were performed using glass micropipettes filled with 2 M potassium acetate (40–70 MΩ). Measurements of apparent membrane input resistance and time constant were based on the linear electrical cable theory applied to an idealized isopotential neuron. The parent input resistance was assessed by measurement of the slope of the extracellular activity of CSth neurons, STN neurons, and GP neurons simultaneously with the spontaneous SWDs occurring in the EEG of the functionally related cortical region.

The waveform. Numerical values are given as means ± SEM unless stated

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Intracellular recordings were performed using glass micropipettes filled with 2 M potassium acetate (40–70 MΩ). Measurements of apparent membrane input resistance and time constant were based on the linear electrical cable theory applied to a neuronal isolate. The membrane potential was computed as a function of the time constant of the exponential decay of the membrane potential to reach 63% of its final value. The values of membrane potential were corrected according to the tip potential recorded extracellularly before and after termination of the intracellular recording. For single-unit extracellular recordings and extracellular labeling (see below), glass electrodes were filled with 0.5 M NaCl and 1.7% neurobiotin (15–20 MΩ; Vector Laboratories, Burlingame, CA).

Cortical cells, located in the orofacial motor cortex, were recorded within 300 μm of the EEG electrode at the following coordinates: 12.5 mm anterior to the interaural line, 3–3.8 mm lateral to the midline, and 1491–2025 μm below the cortical surface. They were electrophysiologically identified as CSth neurons by their antidromic activation by electrical stimulation within the ipsilateral STN (5.1–5.4 mm anterior to the interaural line, 2.5–2.7 mm lateral to the midline, and 7.3–7.6 mm ventral to the brain surface). STN stimuli used to test antidromic activation (200 μA; duration; 2–27 V) were applied with a bipolar concentric electrode (NE-100; Rhode Island Medical Instruments, Woodland Hills, CA). To ensure correct positioning of the stimulating electrode in the STN, multunit responses to whiskers stimulation were recorded through the stimulating electrode (Maurice et al., 2003). The criteria used to identify antidromic action potentials were: (1) a constant latency of antidromic responses despite imposed changes of membrane potential, (2) collision of antidromic spikes with spontaneously occurring orthodromic action potentials, and (3) the all-or-none property of the evoked spikes when the stimulation was just below threshold for antidromic activation.

Intracellular, single-unit and multiunit extracellular recordings in the STN were obtained from the subthalamic projection field of the orofacial motor cortex (Kolomiets et al., 2001). The corresponding stereotaxic coordinates were 4.9–5.4 mm anterior to the interaural line, 2.4–2.8 mm lateral to the midline, and 7042–7962 μm ventral to the brain surface. Extracellular recordings from GP neurons were obtained at the following coordinates: 7.6–8.1 mm anterior to the interaural line, 3.7 mm lateral to the midline, and 5025–6724 μm ventral to the brain surface.

In all experiments, the intracellular or single-unit and multiunit extracellular recordings were simultaneously performed with the corresponding ipsilateral cortical EEG.

Morphological identification. Extracellularly recorded neurons were labeled by juxtaocular injection of neurobiotin (Pinault, 1996; Mailly et al., 2003). Briefly, positive current pulses (1–8 nA; 200 ms) were applied at a frequency of 2.5 Hz through the bridge circuit of the amplifier. The current was slowly increased while the electrode was advanced toward the neuron in 1 μm steps (LSS-1000 Inchworm Motor Positioning System; Burleigh Instruments, Fishers, NY) until the cell discharge was driven by the injected current (see Fig. 3A2, inset). Current pulses were applied for a 10–15 min period to obtain a reliable labeling of neuronal processes. For intracellular recordings, depolarizing current pulses (0.2–1 nA; 100–200 ms) were applied at a frequency of 2.5 Hz at the end of the recording period. The histochemical methods used to reveal the morphology of neurobiotin-filled neurons were described in detail previously (Slaght et al., 2002a). The position of labeled neurons within the structures was confirmed using the atlas of Paxinos and Watson (1986).

Three-dimensional neuronal reconstruction. Soma, dendrites, and axons of some STN neurons were precisely drawn under 25–63× oil immersion objectives and plotted in three dimensions using the video computer software (Image Pro Plus; Media Cybernetics, Silver Spring, MD) and the Atlas Processing System (APPS; MicroBrightField, Colchester, VT). Three-dimensional neuronal reconstruction consisted of segmenting the neuron according to the extracellular labeling, and 3-D models of neurons were visualized using Lightwave software (Newtek, San Antonio, TX) as described previously by Mailly et al. (2003). 3-D reconstructed models of neurons could be rotated around the x-, y-, and z-axes, and the sources of light and camera were adjusted to enhance the 3-D appearance of reconstructed neurons in three-dimensional images (see Fig. 3A2).

Data acquisition and analysis. Intracellular records were obtained under current-clamp conditions using the active bridge mode of an Axoclamp-2B amplifier (Axon Instruments, Union City, CA). Data were stored on-line on a DRA 800 digital tape recorder (Biologic, Clax, France) and then digitized with a sampling rate of 20 kHz (intracellular signals), 10 kHz (extracellular signals), or 300 Hz (EEG) for off-line analysis. To perform spectral analysis of EEG potentials, fast Fourier transforms were applied using Spike 2 (CED Software; Cambridge Electronic Design, Cambridge, UK). Cross-correlograms of the firing between two simultaneously recorded units were obtained by first encoding the position of the peak of the action potentials into separate channels using the memory buffer function of Spike 2; the event correlation function of Spike 2 was then used to produce the cross-correlogram. The amplitude of action potentials was calculated as the potential difference between their voltage threshold, measured as the membrane potential at which the dV/dt exceeded 10 V s−1 (Mahon et al., 2003) and the peak of the waveform. Numerical values are given as means ± SEM unless stated.
Intracellular activity of CSth neurons

We recorded from CSth neurons (n = 7 from six GAERS) located in the orofacial motor cortex. The depth of intracellular recordings, between 1491 and 2025 μm from the cortical surface, suggested that CSth neurons were located in the deep part of layer V (Hall and Lindholm, 1974), a laminar localization that is consistent with previous findings obtained from anterograde and retrograde labeling of rat CSth neurons (Orrieux et al., 2002). CSth neurons were identified electrophysiologically by antidromic activation after electrical stimulation of the ipsilateral STN (Fig. 1A). Antidromic latencies ranged between 1.1 and 3 ms (1.6 ± 0.2 ms; n = 7 cells) (Fig. 1A), consistent with the fast (2–4 ms) orthodromic activation of STN neurons in response to motor cortex stimulation (Kitai and Deniau, 1981; Kolomiets et al., 2001). The passive membrane properties of CSth neurons, measured during interictal periods, included a membrane potential of −61.3 ± 0.7 mV (from −64 to −59.2 mV; n = 7 cells) (Figs. 1B1,C, 2A1,2A2) and a membrane time constant of 13 ± 0.8 ms (range, 9.2–15.8 ms; n = 7 cells) (Fig. 1B1). The apparent input resistance, measured from the slope of the linear V–I curve (Fig. 1B2), was 18.5 ± 1.2 MΩ (from 15 to 24.1 MΩ; n = 7 cells). In five CSth neurons, the firing pattern evoked by intracellular injection of suprathreshold current pulses was characteristic of “regular-spiking” neocortical neurons (Fig. 1B1) (Connors and Gutnick, 1990; Steriade, 2004). The two remaining cells exhibited the firing mode of the “intrinsically bursting” neocortical neurons. The firing frequency–current (F–I) relationship, which followed a sigmoidal function (Fig. 1B2), indicated that CSth neurons reached a maximal firing rate of 78.3 ± 20.4 Hz (SD, from 50 to 100 Hz; n = 6 cells) in response to current pulses of +0.8 nA. In most CSth neurons (six of seven cells), large-amplitude hyperpolarizing current pulses induced a depolarizing “sag” of membrane potential (Fig. 1B1, arrowhead), likely caused by a hyperpolarization-activated inward cationic current (Ih). The negative current pulse was followed immediately by a postinhibitory rebound of depolarization (Fig. 1B1, arrow), possibly caused by the slow kinetics of Ih and/or by a low voltage-activated calcium potential.

Five recordings from CSth neurons (seven) permitted observations on spontaneous transitions between interictal and ictal activities. During SWDs, CSth neurons exhibited suprathreshold rhythmic depolarizations (Figs. 1C, 2B1), which were temporally correlated with spike–wave complexes (Fig. 2B1,C). These repetitive membrane depolarizations were superimposed on a tonic membrane hyperpolarization (dashed line). The power spectrum of the SWD shown in C reveals a dominant frequency of ~1 Hz. The inset shows the spectral analysis of the corresponding interictal EEG, which exhibited a preferential frequency at ~2 Hz. Results depicted in A, C, D, B1, and B2 are from two different CSth neurons. In A, B1, and C, the values of the membrane potential are indicated on the left. In this and all following figures, the top trace in each pair is the EEG, and the bottom trace is the simultaneously recorded extracellular or intracellular voltage (unless stated otherwise). Error bars represent SEM.

Results

Properties of SWDs

The SWDs (n = 1303) recorded in the present study had a mean duration of 5.7 ± 0.2 s (from 0.8 to 129.5 s) and occurred once every 22.6 ± 1.2 s. The intra-SWD frequency, revealed by spectral analysis of the EEG signal, ranged from 7 to 9.7 Hz (7.9 ± 0.1 Hz) (Fig. 1D). These temporal properties of SWDs, as well as the shape of individual spike-wave complexes, are similar to those described previously under analogous experimental conditions (Pinault et al., 1998; Charpier et al., 1999; Slght et al., 2002a,b, 2004; Pinault, 2003) and in freely moving GAERS (Marescaux et al., 1992; Deransart et al., 2003).

Figure 1. Intracellularly recorded activity of corticosthalmic neurons during SWDs. A, CSth neurons were identified by their antidromic activation after electrical stimulation (vertical arrow) of the ipsilateral STN. The antidromic latency, which was measured as indicated by the double-headed arrow, was not affected by DC-induced hyperpolarization (from −59 to −72 mV; IREF = −0.5 nA) (top traces). The superimposed bottom traces show a collision of an antidromic spike with a spontaneous orthodromic action potential (asterisk). B, Electrical membrane properties of GAERS CSth neurons. B1, Voltage responses of a CSth neuron (top traces) to intracellular injection of positive and negative square current pulses (bottom traces). The responses induced by the negative current pulses are an average of six successive trials. The dashed line indicates the membrane potential from which the current steps were applied (−59 mV). Note the sag in membrane voltage (arrowhead) during the −1.1 nA-induced hyperpolarization. At the break of this current pulse, the cell exhibited a rebound response (arrow) eliciting multiple action potentials. B2, Plot of the voltage changes (∆V) (bottom) and of the mean firing frequency (<F>) (top) as a function of current intensity. The apparent input resistance was measured from the linear portion of the V–I curve. Note the membrane rectification (arrowhead) in response to current pulses less than −0.9 nA. The F–I relationship was best fitted by a sigmoidal function (r² = 0.99). C, Spontaneous antidromic activity of a CSth neuron (bottom trace) simultaneously recorded with the EEG (top trace). The occurrence of an SWD in the EEG was accompanied in the CSth cell by rhythmic suprathreshold depolarizations, which were superimposed on a tonic membrane hyperpolarization (dashed line). D, The power spectrum of the SWD shown in C reveals a dominant frequency of ~1 Hz. The inset shows the spectral analysis of the corresponding interictal EEG, which exhibited a preferential frequency at ~2 Hz. Results depicted in A, C, D, B1, and B2 are from two different CSth neurons. In A, B1, and C, the values of the membrane potential are indicated on the left. In this and all following figures, the top trace in each pair is the EEG, and the bottom trace is the simultaneously recorded extracellular or intracellular voltage (unless stated otherwise). Error bars represent SEM.
Changes in the electrical activity of STN neurons during SWDs were examined by extracellular recordings (n = 30 cells from six GAERS animals). Recorded cells were morphologically identified by juxtacellular injection of neurobiotin (see Materials and Methods) (Fig. 3A2). Labeled cells were located in the subthalamic projection field of the orofacial motor cortex (Kolomiets et al., 2001) and exhibited morphological characteristics of STN neurons, with a soma of diameter ~20 μm, four to five primary dendrites, and axonal projections to the GP and the SNr (Beurrier et al., 1999; Bevan and Wilson, 1999) (Fig. 3A2) (see also Fig. 6A).

Extracellularly recorded STN neurons showed various patterns of background firing (Fig. 3A1,B), characterized by either
low-frequency single action potentials (Fig. 3A) or a sustained irregular firing (Fig. 3B). Pooling all cells, the mean firing rate during interictal periods was 12.6 ± 1.6 Hz (from 0.3 to 34.4 Hz; n = 30 cells) (Fig. 3C). During SWDs, the firing frequency of STN neurons reached a mean value of 21.8 ± 0.6 Hz (range, 3–48 Hz; from 292 SWDs; n = 30 cells) (Fig. 3C), corresponding to a probability of discharge in association with individual EEG spikes of 0.88 ± 0.02 (from 0.68 to 1; n = 30 cells). We further characterized the SWDs-induced changes in the STN firing rate by measuring, for each cell, the increase in firing frequency as a function of its interictal value. A clear inverse relationship was found with the percentage increase decaying exponentially as the function of measurement as indicated in Fig. 2C1). In both histograms, the distribution was best fitted by a double Gaussian fit (first action potential, bin size, 1 ms; r² = 0.96; all action potentials, bin size, 1 ms; r² = 0.98). AP, Action potential.

As observed in CSth neurons, the latency of the first action potential in the STN burst was distributed bimodally (Fig. 4E), with a mean value of 16.8 ± 0.1 ms (range, 2.1–75.6 ms; n = 10,510 bursts from 441 SWDs; n = 30 cells) (Fig. 4E). The firing frequency during STN bursts was 206.8 ± 0.9 Hz (range, 58–670 Hz; n = 10,510 bursts from 441 SWDs; n = 30 cells).}

As observed in CSth neurons, the latency of the first action potential in the STN burst was distributed bimodally (Fig. 4E) with respect to the peak negativity of the corresponding EEG spike, with two principal values at −18.7 and −1.9 ms (n = 15643 action potentials from 441 SWDs; n = 30 cells). A similar analysis of all the action potentials in a burst also led to a bimodal distri-
Intracellular recordings were made from STN neurons (membrane properties). Intracellular recordings of STN neurons: electrical membrane time constant of 5.31 ms. Included an interictal membrane potential of 0.2 to −0.8 nA, was linear, indicating the absence of membrane rectification in the hyperpolarizing direction (data not shown). The location and the morphological features of these neurons (Fig. 6A) were similar to those described after juxtacellular dye injection (see Fig. 3A2), including a soma of 25 μm in diameter, four primary dendrites, and a bifurcated axon projecting to both SNr and GP, indicating the identity of extracellularly and intracellularly recorded STN neurons.

Although their spontaneous interictal firing was slow (<35 Hz), STN neurons were capable of firing at rates as high as several hundred Hertz during SWD-associated spontaneous bursting (see above) or in response to depolarizing current pulses (Fig. 6B,D). The current-driven discharge of STN neurons was characterized by a slight spike frequency adaptation and showed a very wide dynamic range, with peak firing rates as high as 500 Hz (Fig. 6C,D). Despite the passage of large depolarizing currents, the membrane potential between action potentials approximated the same voltage range seen during spontaneous firing. Interestingly, high-frequency bursts similar to those discharged during SWDs (Figs. 4A–D) occurred as rebound responses after hyperpolarizing current pulses (Fig. 6B, gray trace).

These membrane properties of the GAERS STN neurons closely resemble those previously described in vitro from non-epileptic rats (Bevan and Wilson, 1999; Beurrier et al., 1999; Bevan et al., 2000; Hallworth et al., 2003). Thus, it is very unlikely that the epileptiform discharges of STN neurons during absence seizures were caused by a specific alteration in their intrinsic excitability (see Discussion).

Intracellular recordings of STN neurons: cellular events associated with SWDs

In five intracellularly recorded STN neurons, spontaneous transitions between interictal and ictal periods were characterized by a switch from single-spike activity to burst firing mode similar to that observed in extracellular records. The rhythmic bursting of STN neurons during SWDs was accompanied by a triphasic sequence of intracellular events (Fig. 7B): (1) an early short-duration depolarizing potential that could generate an action potential, (2) a transient, large membrane hyperpolarization that lasted 16.6 ± 0.1 ms (from 4.2 to 30.5 ms; n = 1801 from five cells), and (3) a postinhibitory rebound of depolarization leading to a burst with a relatively fixed latencies for the first three action potentials. The rhythmic hyperpolarizations were increased in amplitude under DC depolarization (Fig. 7C1) and reversed in polarity for membrane potentials less than −73 mV (Fig. 7C3). These observations strongly suggest that the rhythmic hyperpolarizations in STN neurons during SWDs represent CI-dependant, GABAA-mediated, hyperpolarizing postsynaptic potentials (IPSPs) (see Discussion). These presumed IPSPs started 10.36 ± 0.13 ms (range, −40 to +6.17 ms; n = 1801 IPSPs from five cells) before the spike component of SWDs and reached their peak potential at −2.73 ± 0.13 ms (range, −30 to +14 ms; n = 1801 IPSPs from five cells) (Figs. 7B, 9B).

Extracellular recordings of GP neurons

We made extracellular recordings of GP neurons (n = 13 from five GAERS) to examine whether these neurons were involved in the rhythmic bursting of the STN during SWDs. We found two distinct GP cell populations, in term of firing pattern, without any evidence for a differential topographical distribution within...
the nucleus. The smaller group of GP neurons [called “early discharging” GP (ED-GP) neurons; \( n = 3 \) of 13 cells] was characterized by a slow irregular background firing that shifted, during SWDs, to rhythmic single spikes or short bursts (two to four action potentials) correlated with the EEG spike (Figs. 8A1, A2). This rhythmic firing was maintained throughout the cortical seizure. The mean latency of all action potentials to the peak negativity of the EEG spike was \( -18.67 \pm 0.14 \) ms \((n = 2409\) action potentials from three cells) (Fig. 8A2). Simultaneous recordings \((n = 2\) from two GAERS) of ED-GP neurons with STN neurons showed that their random firing patterns in the absence of SWDs (Fig. 8C1, C2) became highly correlated with each other (Fig. 8D2) and tightly time-locked to the spike-wave complexes (Fig. 8D1) during seizures.

In contrast, the main class of GP neurons, called “pausing” GP (P-GP) neurons \((n = 10\) of 13 cells), displayed a tonic interictal activity. During SWDs, discharges were transformed into a burst firing pattern with interburst periods that coincided with cortical EEG spikes (Fig. 8B1, B2). These periods of electrical silence in P-GP neurons occurred \( 14.76 \pm 0.21\) ms before the EEG spike \((n = 1260\) “pauses” from 10 cells) (Fig. 8B2) and lasted for \( 32.1 \pm 11.5\) ms (SD, range, 15.7–49.2 ms; \( n = 1260\) pauses from 10 cells). The recovery after the electrical silence was systematically associated with a transient increase in the firing rate (Figs. 8B2, inset, 9A, bottom trace).

**Discussion**

The present study provides the first description of electrical activities in the cortico-subthalamo-pallidal networks during absence seizures. Our principal findings are: (1) during SWDs, CSth neurons exhibit rhythmic suprathreshold depolarizations superimposed on a sustained hyperpolarization lasting for the entire cortical paroxysm; (2) STN neurons display synchronized repetitive bursts of action potentials in-phase with EEG spikes; (3) intracellular analysis indicates that rhythmic firing in STN neurons results from an early excitation, followed by a hyperpolarization and then a late excitation, and (4) during SWDs, GP neurons display either a rhythmic bursting synchronous with the cortical paroxysm or a sustained firing momentarily interrupted by EEG spikes. These results indicate that the propagation of cortical paroxysms through the cortico-subthalamo-pallidal pathway generates a bursting pattern in the STN that might produce a powerful phasic synaptic excitation of the basal ganglia output nuclei.

**Rhythmic bursting of CSth neurons during SWDs**

To our knowledge, the present experiments provide the first description of the intracellular activity of identified CSth neurons. The occurrence of SWDs in the EEG was concomitant with a sudden, pronounced modification in the activity of CSth neurons. The interictal, small-amplitude, irregular synaptic activity in CSth neurons, generating an erratic firing pattern, changed, during an SWD, into a step-like behavior with suprathreshold depolarizations in-phase with the EEG spikes. As previously observed in other GAERS cortical neurons (Charpier et al., 1999; Slaght et al., 2002a,b), the SWD-associated rhythmic depolarizations in CSth neurons were concomitant with a tonic membrane hyperpolarization, likely attributable to a synaptic disfacilitation responsible for an increase in membrane resistance (Contreras et al., 1996; Charpier et al., 1999; Slaght et al., 2002b).

Interestingly, the synaptic activity as well as the firing rate of CSth neurons during SWDs differ significantly from those of GAERS corticostriatal neurons (Slaght et al., 2002b, 2004), which exhibit oscillating membrane depolarizations that remain subthreshold or elicit single action potentials during seizures. The increased excitability of CSth neurons could result from interactions between synaptic inputs and specific voltage-gated intrinsic channels. Consistent with this hypothesis, we observed in CSth neurons, in response to hyperpolarizing current injection, a depolarizing sag of membrane potential followed by a postanodal rebound of excitation. These observations provide indirect evidence that CSth neurons may express \( I_h \) and a low-voltage activated calcium current (Fig. 1B1), which act together to promote oscillations and repetitive firing in cortical neurons (for review, see Reyes, 2001; Migliore and Shepherd, 2002).
Origin of the rhythmic excitation and inhibition in STN neurons during SWDs

The GAERS STN neurons, intracellularly recorded in the course of this study, displayed membrane properties including membrane potential, postanodal rebound of excitation, action potential properties, and V–I and F–I relationships similar to those previously described in vitro from normal rats (Beurrier et al., 1999, 2000; Bevan and Wilson, 1999; Bevan et al., 2000, 2002a). These important observations indicate that the increased firing rate in STN neurons accompanying the SWDs does not result from an altered intrinsic excitability.

The rhythmic bursts in STN neurons, observed in extracellular recordings during SWDs, were characterized frequently by an early action potential, usually preceding the corresponding EEG spike, followed by a short electrical silence (<25 ms) and then a high-frequency cluster of action potentials. This pattern of electrical events in the STN is very similar to that observed after electrical stimulation of the motor cortex (Kitai and Deniau, 1981; Kita, 1994; Kolomiets et al., 2001). Intracellular recordings let us characterize membrane and synaptic events underlying this triphasic response. The initial discharge of STN neurons during cortical paroxysms was elicited by a short depolarizing potential likely induced by the early discharge of CSth neurons (Fig. 9), which provide powerful excitatory synaptic inputs to STN neurons (Kitai and Deniau, 1981; Kita, 1994; Magill et al., 2000; Kolomiets et al., 2001). The brief silence within the STN bursts associated with the spike-wave complexes resulted from a transient (~17 ms) hyperpolarization that reversed in polarity at membrane potentials less than ~73 mV (Fig. 7C). This value is very close to the chloride equilibrium potential of $\text{GABA}_A$ current measured in STN neurons from normal rats (Bevan et al., 2000, 2002a).

Thus, rhythmic hyperpolarizations observed during SWDs are probably $\text{GABA}_A$–mediated IPSPs that may be induced by a subpopulation of GABAergic pallidosubthalamic neurons (~25% of the GP neurons described in this study), which generate bursts of action potentials just before (~16 ms) the peak of the STN inhibitory potentials (Fig. 9, ED–GP neurons). The burst activity in ED–GP neurons may well be initiated, at least in part, by a short-latency excitation attributable to the early firing of excitatory subthalamopallidal neurons (Kita and Kitai, 1991; Magill et al., 2000; Nambu et al., 2000) (Fig. 9). The postinhibitory rebound of firing in STN neurons may result from synergistic interactions between active membrane properties and excitatory synaptic inputs. Indeed, STN neurons possess low-threshold calcium channels responsible, at least in part, for the generation of burst activity after membrane hyperpolarization (Beurrier et al., 1999, 2000; Bevan and Wilson, 1999; Bevan et al., 2000; Song et al., 2000). Such intrinsic rebound properties in STN neurons, which were also found in the present study on removal of large-amplitude hyperpolarizing current pulses (Fig. 6B), might contribute to the postinhibitory (late) bursting in STN neurons (Fig. 9). Moreover, in accordance with previous in vitro studies (Bevan et al., 2002b), we found that the repetitive action potentials that followed large-amplitude IPSPs impinging on STN neurons were almost exactly in-phase (Fig. 7B), an intrinsic process that would tend to promote coherent bursting in the STN during absence seizures (Figs. 5B, 4). The delayed firing of CSth neurons would provide an additional excitatory synaptic mechanism promoting the late STN bursting during SWDs (Fig. 9).

The functional impact of P–GP neurons on STN activity re-
Pathophysiology

The main finding of the present study is the synchronized rhythmic bursting of STN neurons during absence seizures. This novel phenomenon, maintain the paroxysmal thalamocortical oscillations. Second, the recovery of the irregular, desynchronized, firing in STN neurons at the end of the SWD, together with the rebound of excitation in striatal projection neurons (Slaght et al., 2004), could initiate the postictal decrease in the activity of nigrothalamic neurons (Deransart et al., 2003) and so participate in the termination of the seizure.

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