The zona incerta modulates spontaneous spike-wave discharges in the rat

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1Department of Psychology, National Cheng Kung University, Tainan, Taiwan; 2Biomimetic Systems Research Center, National Chiao Tung University, Hsinchu, Taiwan; 3Institute of Medical Sciences, Tzu Chi University, Hualien, Taiwan; 4Department of Life Science, National Taiwan University, Taipei, Taiwan; and 5Department of Neurobiology and Anatomical Sciences, University of Mississippi Medical Center, Jackson, Mississippi

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Shaw FZ, Liao YF, Chen RF, Huang YH, Lin RC. The zona incerta modulates spontaneous spike-wave discharges in the rat. J Neurophysiol 109: 2505–2516, 2013. First published February 27, 2013; doi:10.1152/jn.00750.2011.—The contribution of the zona incerta (ZI) of the thalamus on spike-wave discharges (SWDs) was investigated. Chronic recordings of bilateral cortices, bilateral vibrissa muscle, and unilateral ZI were performed in Long-Evans rats to examine the functional role of SWDs. Rhythmic ZI activity appeared at the beginning of SWD and was accompanied by higher-oscillation frequencies and larger spike magnitudes. Bilateral lidocaine injections into the mystacial pads led to a decreased oscillation frequency of SWDs, but the phenomenon of ZI-related spike magnitude enhancement was preserved. Moreover, 800-Hz ZI microstimulation terminates most of the SWDs and whisker twitching (WT; >80%). In contrast, 200-Hz ZI microstimulation selectively stops WTs but not SWDs. Stimulation of the thalamic ventroposteromedial nucleus showed no obvious effect on terminating SWDs. A unilateral ZI lesion resulted in a significant reduction of 7- to 12-Hz power of both the ipsilateral cortical and contralateral vibrissae muscle activities during SWDs. Intraineccral microinfusion of muscimol showed a significant inhibition on SWDs. Our present data suggest that the ZI actively modulates the SWD magnitude and WT behavior.

zona incerta; absence seizure; spike-wave discharge; tremor; deep brain stimulation

IT IS WELL-KNOWN THAT ABSENCE epilepsy is characterized by an abrupt and brief loss of awareness in coincidence with generalized spike-wave discharges (SWDs). The cortex and thalamus, often referred to as the thalamocortical network, appear to play an essential role in the generation of paroxysmal absence seizures (Timofeev and Steriade 2004; van Luijtelaar and Sitnikova 2006). In particular, the lateral sensorimotor area is believed to be the neural site for the generation of absence seizures in numerous animal studies (Chen et al. 2011; Meeren et al. 2002; van Luijtelaar and Sitnikova 2006). During a typical absence seizure, an ongoing motor activity is interrupted during the occurrence of a SWD, but there are minor myoclonic jerks of the eyes or perioral automatism appearing in a number of human cases (Bogacz et al. 2000). In addition, myoclonus only appears in some but not all cases, and it exists in a portion but not the entire episode of a SWD, particularly in the initial period. Thus a complex network seems to involve in the generation of absence seizures and seizure-related myoclonus. However, the precise involvement of thalamocortical network with SWD remains largely unknown.

The zona incerta (ZI) is a part of the ventral thalamus, and most neurons in the ZI are GABAergic (Mitrofanis 2005). In the past two decades, anatomic tracing evidence has indicated that incertal neurons densely connect with various brain regions, including the superior colliculus (Kim et al. 1992; Nicolelis et al. 1992), dorsal thalamus (Bartho et al. 2002; Lavallee et al. 2005), cerebral cortex (Lin et al. 1990; Nicolelis et al. 1992), and the ZI itself (Mitrofanis 2005). The superior colliculus is also known to innervate the facial motor nucleus for controlling facial motor activity (van Luijtelaar and Sitnikova 2006). Previous studies have shown that electrical or chemical stimulation of the ZI results in changes of behavior or posture (Dybdal and Gale 2000; Périer et al. 2002; Supko et al. 1991). Furthermore, stimulation of whisker region of the motor cortex suppresses vibrissal response of the ZI (Urbain and Deschenes 2007). Clinically, ZI stimulation ameliorates Parkinsonian symptoms (Guehl et al. 2008; Phaha et al. 2006; Voges et al. 2002) and central pain (Masri et al. 2009). Moreover, the ZI displays susceptibility to generalized seizures (Brudzynski et al. 1995; Hamani et al. 2002). Recently, ZI stimulation has been demonstrated to be able to stop spontaneous SWDs or pentylenetetrazol-induced SWDs in rats (Liang et al. 2011; Young et al. 2011), suggesting incertal neurons may modulate absence seizures and/or seizure-related myoclonus.

An important fact is the Long-Evans rats often display spontaneous 7- to 12-Hz rhythms or SWDs coincident with whisker twitching (WT) behavior (Nicolelis et al. 1995; Sembra and Komisaruk 1984; Shaw and Liao 2005). Spontaneous SWDs noted in Long-Evans rats have been suggested as phasic events that are the counterparts of absence seizures (Polack and Charpier 2006; Shaw 2004), task preparation/attention (Wiest and Nicolelis 2003), and idling behaviors (Fontanini and Katz 2005). Furthermore, three distinctive 7- to 12-Hz rhythms are known to be produced by the same corticothalamic networks during sensory responses in awake rats (Tort et al. 2010). Interestingly, flash stimulation primarily elicits µ-like α-rhythms of small magnitude but no SWDs in the sensorimotor cortical region of Long-Evans rats and Wistar rats (Shaw 2007). The phenomena of flash-induced responses in rats are analogous to the fact of flash-induced µ-rhythm noted in humans (Pfurtscheller 2003). The occurrence frequency of spontaneous SWDs is significantly reduced with the application of ethosuximide, valproic acid, and diazepam but increased with carbamazepine in a dose-dependent manner (Shaw 2007). Both ethosuximide and lamotrigine significantly not only reduce the occurrence frequency of SWDs, but also ameliorate comorbid anxiety and depression behaviors (Huang...
et al. 2012; Shaw et al. 2009). Although ZI neurons display different firing patterns during various cortical oscillations (including paroxysmal 5- to 9-Hz oscillations) in anesthetized rats (Bartho et al. 2007), the involvement of incertal currents during SWDs, however, is currently unknown in freely moving rats. To clarify further the contribution of ZI to SWDs and accompanying WT in freely moving rats, five experimental procedures, i.e., a natural condition, peripheral block using lidocaine injections into the bilateral whisker pads, micro-stimulation of the ZI or thalamus at three stimulation rates, a unilateral lesion of the ZI, and muscimol infusion into the ZI, were conducted.

MATERIALS AND METHODS

Animal preparation. Adult Long-Evans rats (9 females and 14 males) were used in the study. Animals were kept in a sound-attenuated room under a 12:12-h light-dark cycle with food and water provided ad libitum. All surgical and experimental procedures were reviewed and approved by the Institutional Animal Care and Use Committee of National Cheng Kung University, and all experiments comply with U.S. National Institutes of Health recommended guidelines on the ethical use of animals.

Rats were anesthetized with sodium pentobarbital (50 mg/kg ip). Subsequently, the dorsal surface of an animal’s head was shaved and placed in a standard stereotaxic apparatus. A midline incision was made, and then several concavities were drilled for placing stainless steel screws and microwire electrodes. Screw electrodes were bilaterally implanted over the area of the barrel cortex (AP -2.0 mm, ML 5.0–5.5 mm). An eight-microwire bundle, each made of Teflon-insulated stainless steel microwires (cat. no. 7079; A-M Systems, Sequim, WA), was used to record local field potentials (LFPs) on one side (typically the right) of the ZI (AP -4.0 mm, ML 2.5 mm, and depth 6.7–7.2 mm) or thalamic ventroposteriomedial (VPM) nucleus (AP -4.0 mm, ML 2.5 mm, and depth 5.5–6.0 mm). Several criteria were used to identify the ZI during electrode implantation (Liang et al. 2011): for instance, a compatible depth reading from a stereotaxic apparatus (Paxinos and Watson 1986), a spontaneous firing rate without barbiturate spindelike bursts, a wide receptive field in response to whisker/facial stimulation (Nicolelis et al. 1992), and a narrow portrait of the action potential. Teflon-coated tungsten microwires (cat. no. 7955; A-M Systems) were implanted and used to record vibrissa muscle activities (Shaw and Liao 2005). A 25-gauge syringe needle containing two electrodes (the tips of them were separated by approximately 2–3 mm) was inserted below the incised skin and through the soft tissue of the mastioid pouch. The final location for implanting the wires was determined by an obvious whisker protraction with 0.5-ms and 0.4–mA current electrical stimulation. A ground electrode was implanted 2 mm caudal to the lambda. Surgical procedure and electrode placement for ZI were same to those described in a previous paragraph. Two stainless-steel guide cannulae (cat. no. 842800; A-M Systems) were bilaterally implanted into the ZI (AP -4.0 mm, ML ± 2.5 mm, and depth 6.7–7.0 mm from the cortical surface). Dummy inner cannulae were used to prevent the capillary action in the tube. Dental cement was applied to fasten the connection socket to the surface of the skull. Following suturing to complete the surgery, animals were given antibiotics (chlorotetracycline) and housed individually in cages for recovery.

Muscimol preparation and treatment. Muscimol (Sigma, St. Louis, MO) was dissolved in saline (200 or 1,000 mM per hemisphere). Either saline or muscimol was randomly injected into the ZI unilaterally or bilaterally. A minimum of 7 days elapsed time between the two injections was conducted. For intracranial microinflation, typically 1 μl of saline (0.9% NaCl) or muscimol (pH 7.2–7.4) was administered with a Hamilton syringe at a flow rate of 200 nl/min.

Experimental procedures. Two weeks after surgery, each animal was placed in the recording environment at least 2 times (1 h/day) before testing to allow them habituating to the experimental apparatus. All recordings were performed between 1000 and 1400. After a 30-min acclimation period on the test day, a 1-h spontaneous recording was made to characterize seizure frequency and to identify rats with spontaneous WTs in SWDs. The next day, lidocaine was bilaterally injected (8 mg in 0.4 ml of saline) into mystacial pads of rats with WTs under a short period of halothane anesthesia. A 1-h recording was conducted for 30 min after lidocaine administration.

One week later, ZI microstimulation was performed. Monophasic currents (0.2 ms, 20–50 μA) of 0.5 s was applied through implanted microwires when SWDs occurred by online continuous visual inspection of the brain activity with a real-time oscilloscope. The 0.5-s pulse train used in ZI stimulation is based on the development of a real-time closed-loop seizure controller (Liang et al. 2011; Young et al. 2011). The intensity of electrical stimulation was determined in a subthreshold condition to elicit whisker deflection. Three stimulation frequencies, i.e., 800 Hz (high-frequency stimulation, HFS), 200 Hz (middle-frequency stimulation, MFS), and 20 Hz (low-frequency stimulation, LFS), were used. HFS is functionally associated with the effect of a depolarization block of ZI neurons (Liu et al. 2008). MFS approximates a therapeutic frequency of deep brain stimulation used in Parkinson patients (Garcia et al. 2005; Plaha et al. 2006; Voges et al. 2002). LFS approximates a therapeutic frequency of cortical stimulation in epileptic treatments (Theodore and Fisher 2004). Each stimulation frequency was employed 50 times. These 3 stimulation rates were randomly applied with a counterbalance sequence. All 3 stimulation paradigms were performed on the same day. The proportions of terminating SWDs, WTs, or both in each individual rat during 50 ZI stimulations were calculated as an index to assess the efficiency of ZI stimulation.

Finally, 20-s, 0.5-mA direct currents were passed through all pairs of eight-channel ZI microwire bundles to make a lesion under pentobarbital anesthesia (50 mg/kg ip). The same lesion-producing process was performed again the next day. Behavioral responses during the process of creating the ZI lesion were noted. Five days later, a 1-h recording was performed again in the same time window (between 1000 and 1400).

In the experiment of intracranial drug administration, all recording sessions were performed also between 1000 and 1400. A 1-h spontaneous recording was made to characterize neuronal activity after muscimol drug infusion. The onset of SWDs after saline or muscimol injection, SWD number, cumulative SWD duration, and mean SWD duration in the 1-h time period were collected and analyzed.

After completing the experiment, the animal was deeply anesthetized with sodium pentobarbital (60 mg/kg ip). A transcathodic perfusion with 0.9% saline followed by 3.5% parafomaldehyde in 0.1 M phosphate buffer at pH 7.4 was perfused. The brain was then removed and fixed in the same fixative with 20% sucrose overnight at 4°C. Brain sections were cut at 40- or 80-μm thicknesses in the coronal plane. Sections were counterstained with Nissl. The electrode tracks and the sites of lesion from each animals were reconstructed from histologically stained sections.

Data analysis. Field potentials (0.3–1,000 Hz) and vibrissae muscle activities (100–500 Hz) were simultaneously recorded using a multichannel amplifier (Shaw et al. 2002). Both field potentials and muscle activities were digitized at 2 kHz through a 64-channel analog-to-digital board (PCI-6071E; National Instruments, Austin, TX). A grounded plate was placed under the recording chamber to reduce electromagnetic interference (Shaw et al. 2003). SWDS were characterized by a barrage of large sharp spike discharges with negative polarity (Shaw et al. 2006). The power spectra of SWDs displayed a dominant frequency peak of around 6–10 Hz accompanied by several harmonics (Shaw 2004, 2007). Accordingly, the number of SWDs in a 1-h recording period could be measured. In an attempt to evaluate the correlations among SWDs, WTs, and ZI activities during a SWD,
spike-triggered average was then used (Shaw and Liao 2005). Vibri- sae muscle activity was full-wave-rectified and smoothed by a 40- point moving average (~50 Hz). Two steps were essential for detecting each individual spike within a SWD episode. Selected SWD segments were first passed through a fourth-order high-pass Bessel filter (with a cutoff frequency of 0.5 Hz) to reduce low-frequency drift. Then, spikes were extracted from the filtered data segment by an interactive window discriminator, and subsequently the peaks of selected spikes were identified by searching for the minimum. According to the time stamps of individual spikes of a SWD, selected spikes, coincident LFPs of the ZI, and muscle activities were subsequently averaged. The oscillation frequency of SWDs was calculated according to the time stamps of individual spikes of a SWD, selected spikes/WT activity in coincidence with SWDs. The number (35 ± 10, range, 4–76) and duration (6.3 ± 0.5 s, range, 1.5–10.4 s) of SWDs in a 1-h period were measured. Two typical examples are shown in Fig. 1: one with WTs (Fig. 1A) and the other without WTs (Fig. 1B). During SWDs, the ZI always displayed rhythmic activity (100 ± 0% of 320 SWDs in 8 rats), but the proportion of WT occurrence during SWDs was 66.4 ± 11.9% (218 SWDs in 5 rats). The rhythmic activities of the ZI appeared at the beginning of SWDs, and they were not noted in the entire period of SWD. Rhythmic ZI activity was displayed in 72.2 ± 5.7% of SWD durations. Interestingly, the onset of ZI rhythmic activity always lagged behind that of SWDs (0.16 ± 0.02 s). The ZI also showed rhythmic activity that phase-locked to SWDs using a spike-triggered average (Fig. 1, A and B, right column). The \( \tau_{ZI} \) with regard to SWD spikes was 1.66 ± 0.33 ms.

With respect to temporal time frame, spike magnitude of SWDs was obviously higher when the ZI displayed rhythmic activity (Fig. 1, A and B). The 7- to 12-Hz powers of SWDs during ZI rhythm (1.278.9 ± 172.3 \( \mu V^2 \)) were significantly higher than those of SWDs following termination of ZI rhythm (731.0 ± 155.3 \( \mu V^2 \); \( P = 0.005 \)). In particular, SWD spike magnitude during ZI rhythm was consistently higher than those of SWD segments following termination of ZI rhythm in all experimental animals (Fig. 1C). On average, spike magnitude of SWD segments during ZI rhythm (0.47 ± 0.03 mV) was significantly higher than those of segments with no ZI rhythm (0.22 ± 0.02 mV; \( P < 0.001 \) by paired \( t \)-test; \( n = 8 \)). Additionally, the oscillation frequency of SWD segments when the ZI showed rhythmic activity was significantly higher in all animals except the sixth rat (Fig. 1D). On average, the oscillation frequencies of SWD segments during ZI rhythm (8.32 ± 0.27 Hz) were significantly higher than those of SWD segments following termination of ZI rhythms (7.71 ± 0.27 Hz; \( P < 0.05; n = 8 \)).

WTs tended to appear at the beginning of SWDs (Fig. 1A), but they were not lasting the entire period of SWD. WTs (3.90 ± 0.56 s, range, 2.62–5.77 s) only occurred in 45.5 ± 0.6% of SWD duration. WT duration was significantly shorter than that of ZI rhythmic activities during SWDs (\( P < 0.001 \)). The oscillation frequency of SWDs with WTs (8.98 ± 0.24 Hz) was significantly higher than that of ZI rhythmic activities during SWDs (\( P < 0.001 \)).

Peripheral vibrissa stimulation and SWDs. A typical example with the absence of vibrissa muscle activity after lidocaine injection into the mystacial pads is shown in Fig. 2A. No corresponding activity was observed in the cortex and ZI during the stimulation of vibrissa muscle. Notably, rhythmic ZI activity appeared in all SWDs (100 ± 0% of 235 SWDs in 5 rats). The oscillation frequency of nature/native condition (54.0 ± 7.3) did not significantly differ from that under lidocaine applications (56.2 ± 5.1; \( P = 0.865 \) by paired \( t \)-test; \( n = 5 \)). The durations (nature vs. lidocaine, 62.1 ± 1.9 vs. 64.7 ± 6.6%; \( P = 0.65 \)) and amplitudes (0.21 ± 0.05 vs. 0.23 ± 0.04 mV; \( P = 0.305 \)) of the rhythmic ZI activities in the two conditions were not significantly different. The phenomenon of a higher 7- to 12-Hz power of SWDs during ZI rhythm in a natural condition (1,311.7 ± 249.8 vs. 782.6 ± 251.4 \( \mu V^2 \); \( P = 0.022 \)) was observed under lidocaine condition (1,245.8 ± 291.8 vs. 720.0 ± 263.1 \( \mu V^2 \); \( P = 0.029 \)). The phenomenon of a higher SWD spike magnitude during ZI rhythm in a natural condition (0.45 ± 0.03 vs. 0.24 ± 0.07 mV; \( P < 0.001 \)) also existed after lidocaine administration (0.53 ± 0.08 vs. 0.26 ± 0.06 mV; \( P < 0.05 \); Fig. 2B). Lidocaine application significantly reduced oscillation frequency of SWDs (nature vs. lidocaine, 8.42 ± 0.12 vs. 7.58 ± 0.13 Hz; \( P < 0.0001 \)). Specifically, oscillation frequencies of SWD segments during rhythmic ZI activities and following
termination of ZI rhythms was significantly different in the nature and lidocaine conditions (nature, 8.76 ± 0.17 vs. 8.11 ± 0.32 Hz; lidocaine, 7.63 ± 0.26 vs. 7.35 ± 0.13 Hz; P = 0.041 by repeated-measures ANOVA; Fig. 2C). Moreover, τZI became significantly longer after the lidocaine injection (nature vs. lidocaine, 2.03 ± 1.20 vs. 4.35 ± 1.04 ms; P < 0.005).

**ZI electrical stimulation and SWDs.** With HFS (Fig. 3A), both SWDs and WTs were bilaterally terminated after 0.5-s unilateral ZI stimulation. During HFS, the rat displayed minor whisker retraction of the contralateral side in some cases. After HFS ZI stimulation, cortical activity became low-amplitude, high-frequency pattern, and activity of vibrissae muscle revealed phasic discharges, which was not phase-locked to cortical activity. By contrast, MFS displayed an obvious effect on the obstruction of seizure-locked WTs (1-to-1 phase-lock pattern), but it did not terminate the SWDs (Fig. 3B). In most cases (55.8 ± 9.2%), the magnitude of SWDs became smaller and then progressively increased right after the 0.5-s MFS. MFS in the ZI seemed to selectively alter vibrissae muscles rather than cortical activities. No obvious behavioral reaction was observed during MFS or LFS ZI stimulation. No SWD was elicited by the three ZI stimulations under the state of asynchronous brain activity. The ratio of terminating SWDs by ZI stimulation with three different stimulation frequencies revealed a significant differential effects (Fig. 3C; HFS, 84.7 ± 6.0%; MFS, 22.2 ± 9.6%; LFS, 0 ± 0%; P < 0.001 by repeated-measures ANOVA; n = 8). Moreover, a significant WT termination after ZI stimulation was also observed (Fig. 3D; HFS, 90.0 ± 4.3%; MFS, 60.8 ± 10.3%; LFS, 0 ± 0%; P < 0.001). The oscillation frequencies per hour during three ZI stimulations were not different (HFS, 43.2 ± 8.2; MFS, 40.1 ± 7.6; LFS, 42.3 ± 9.2; P = 0.364), and they were not different from that of nature condition (P = 0.542). To explore fluctuation of SWDs during ZI stimulation, 7- to 12-Hz power of a SWD segment before each stimulation was analyzed. The 7- to 12-Hz powers during three ZI stimulations were not different (HFS, 1,300.7 ± 262.8 μV^2; MFS, 1,298.7 ± 246.4 μV^2; LFS, 1,306.2 ± 254.3 μV^2; P = 0.861).

**The effect of ZI lesion on SWDs.** When electrical current was applied through the first pair of ZI microwires, a transient whisker movement of the contralateral side was noted. Such movement was similar to that observed during ZI HFS. The neural damage made by electrical current primarily located in ZI in all rats with some cases extended dorsally into the VPM (Fig. 4). However, none of them extended ventrally into the subthalamic nucleus. Lesion ranges of eight rats were 1.24 ±

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**Fig. 1.** Relation of activities of the right-ride cortex (Cortex_R), right-side zona incerta (ZI_R), and bilateral vibrissae muscles (VEMG_R and VEMG_L) during spike-wave discharges (SWDs; n = 8). Representative examples of SWD are shown: SWD with whisker tremors (WTs; A) and SWD without WTs (B). Rhythmic incertal activities (bars with diagonal lines) appear in a portion of an SWD, and they are phase-locked to the spikes of the SWDs by a spike-triggered average (solid line on the right). SWD waveforms with nonrhythmic incertal activities were shown by dotted lines on the right. WTs also show a similar phase-locked pattern to the ZI during SWDs. C: spike magnitudes of SWDs with rhythmic ZI activities (Rhythm) are significantly higher than those following termination of ZI rhythms (No Rhythm) in 8 rats. *P < 0.05. D: oscillation frequencies of SWDs during and after rhythmic ZI activities. *P < 0.05.
Five days after the ZI lesion, a total of 461 SWDs \((n = 8)\) were recorded in 1-h recording sessions. The firing patterns from bilateral cortices and vibrissae muscles before (Fig. 5A) and after (Fig. 5B) ZI lesion revealed remarkable differences. In both temporal and spectral patterns, activities of the bilateral cortices and vibrissae muscles showed almost identical patterns before the ZI lesion. In contrast, the ipsilateral cortical spike magnitudes and power of 7–12 Hz from the ZI lesion animals were smaller than those from the contralateral side of cortex. During SWDs, the power of 7–12 Hz after the ZI lesion (79.3 ± 2.7%) was significantly smaller than that before the ZI lesion (100.7 ± 5.4%; Fig. 5C; \(P = 0.004\) by paired \(t\)-test). Additionally, the magnitude and power of 7–12 Hz from the contralateral vibrissae muscle were smaller than those of the ipsilateral side. On average, the power of 7–12 Hz after the ZI lesion (39.5 ± 5.7%) was significantly smaller than that before the ZI lesion (71.6 ± 8.6%; Fig. 5C; \(P = 0.011\)). SWD frequency after the ZI lesion (76.38 ± 18.91) was significantly higher than that before the ZI lesion (42.13 ± 9.19; Fig. 5D; \(P = 0.025\)). However, the numbers of WTs before and after the ZI lesion were not significantly different \((P = 0.347)\). Similarly, the durations of SWDs \((P = 0.67)\) and WTs \((P = 0.194)\) revealed no changes after the ZI lesion (Fig. 5E). The oscillation frequencies of SWDs before \((8.35 ± 0.23\) Hz) and after \((8.41 ± 0.21\) Hz) the ZI lesion were also not different \((P = 0.52)\).

**Neuronal activities of the ZI and VPM during SWDs.** Recordings of LFP and individual neuronal activity of the ZI were also performed in 2 additional animals (Fig. 6A). Our data again showed that ZI rhythmic activity displayed in 78.4 ± 9.2% of SWD durations (52 SWDs in 2 rats). More specifically, ZI neurons revealed a burstlike discharge pattern during high-amplitude rhythmic ZI and LFPs accompanied by non-phase-locked spontaneous neuronal firing. The burstlike discharge, however, was not obvious when the amplitude of the ZI rhythms became smaller or faded out. The ratio of terminating SWDs after ZI stimulation at three stimulation frequencies was rather similar as described in the earlier experimentation (HFS, 88.2 ± 4.3%; MFS, 18.9 ± 9.2%; LFS, 0 ± 0%).

To study further whether such modulatory effect is restricted to ZI but not other thalamic neurons such as VPM, a representative example of recording of LFP and individual neuronal activity was shown in Fig. 6A. An obvious VPM rhythm was found and occurred in 96.3 ± 2.1% of SWD durations (183 SWDs in 5 rats). Similarly, burst discharges were often seen in single-unit neuronal activity during SWDs. In contrast, the ratio of terminating SWDs after VPM stimulation at three different stimulation frequencies (Fig. 7; HFS, 19.5 ± 0.8%; MFS, 16.3 ± 1.3%; LFS, 1.5 ± 1.0%; \(P < 0.001\)) revealed a very different pattern compared with ZI. In particular, the ratio of terminating SWDs with HFS in the VPM was significantly lower than that of ZI HFS \((P < 0.001)\).

**Intraincertal muscimol treatment on SWDs.** Figure 8 shows effect of intraincertal saline or muscimol infusion on SWDs \((n = 7)\). The factor of infusion route (unilateral vs. bilateral)
was associated with significantly different SWD onset latency ($F_{1,41} = 103.609, P < 0.001$), SWD number ($F_{1,41} = 11.382, P = 0.015$), and cumulative SWD duration ($F_{1,41} = 15.553, P = 0.008$). The treatment (muscimol vs. saline) demonstrated a significant effect on SWD onset latency ($F_{2,41} = 103.609, P < 0.001$), SWD number ($F_{2,41} = 30.25, P < 0.001$), total SWD duration ($F_{2,41} = 30.988, P < 0.001$), and mean SWD duration ($F_{2,41} = 10.173, P = 0.003$). Interaction between infusion route and treatment had a significant effect on SWD number ($F_{2,41} = 5.85, P = 0.017$). In particular, the group treated with muscimol of 1,000 mM showed significantly longest onset latency and lowest SWD number and cumulative SWD duration compared with those of the other two groups (Fig. 8).

DISCUSSION

The major findings of this study are as follows. 1) SWDs were always accompanied by ZI rhythm, but rhythmic ZI activity did not last the entire SWDs. However, WTs were not observed in SWD bouts, and they only appeared in a portion of the duration of SWDs. Both the oscillation frequency and spike magnitude of SWDs were significantly increased during the occurrence of either ZI rhythm or WTs. 2) Bilateral lidocaine injections into the mystacial pads led to a decrease in the oscillation frequency of SWDs but showed no effect on ZI-related spike magnitude enhancement. 3) Most SWDs and WTs (>80%) were significantly terminated by 800-Hz ZI stimulation. In contrast, 200-Hz ZI stimulation selectively terminated WTs rather than SWDs, and 20-Hz ZI stimulation showed no effect on either SWDs or WTs. Thalamic VPM stimulation with these 3 stimulation rates had minimum effects on SWD termination. 4) Unilateral ZI lesion resulted in a reduction in 7- to 12-Hz power of both the ipsilateral cortical and contralateral vibrissae muscle activities during SWDs. Last, infusion of muscimol into the ZI had significant inhibitory effect on SWDs.

The relationship between ZI and SWDs. The major contribution of the present study is the utilization of continuous
measuring electrical activities of several brain regions and peripheral vibrissa muscles with behavioral recording under various conditions. Our findings provide evidence of rhythmic incertal activities only occurring in a part of SWD duration, which is strikingly different from the VPM (Meeren et al. 2002; Nicolelis et al. 1995). Furthermore, the delayed and phase-locked ZI rhythmic activity with regard to SWDs was observed in both the nature and lidocaine treatment conditions. ZI rhythm during SWDs was associated with an enhancement of the SWD spike magnitude and 7- to 12-Hz powers of SWDs during the nature or lidocaine conditions. A unilateral lesion of the ZI led to a decrease in 7- to 12-Hz powers of SWDs in the ipsilateral cortex. Higher SWD spike is associated with synchrony of cortical neuronal depolarization in a local area (Timofeev and Steriade 2004). Membrane hyperpolarization due to the intensive inhibitory GABAergic actions is beneficial for maintaining brain rhythms and synchrony of a large network that is greatly associated with activation of T-type calcium channels (Timofeev and Steriade 2004; van Luijtelaar and Sitnikova 2006). Accordingly, the delayed rhythmic incertal activity may help to develop membrane hyperpolarization that builds up sustained high-voltage cortical rhythms through GABAergic incertofugal pathways.

Spontaneous SWDs occurred in coincidence with a sudden arrest of ongoing behavior or immobile behavior in Long-Evans rats (Nicolelis et al. 1995; Shaw 2004). Interestingly, absence seizures consist of a sudden arrest of ongoing behavior and impairment of consciousness and are associated with abrupt occurrence of bilaterally synchronous SWDs over wide cortical areas (Snead et al. 1999). Absence seizures are usually
brief duration, and they may often go unrecognized until patients experience a tonic-clonic seizure. Video EEG provides important information to identify absence seizure events in the clinic. Thus continuous recording of brain activity accompanied by behavioral monitoring is crucial for animal model of absence seizures.

Numerous studies have reported activities of the thalamocortical network during seizures, especially in the VPM (Fansenlow et al. 2001; Meeren et al. 2002; Nicolelis et al. 1995). In the present study, HFS of VPM terminated only 19.5% of SWDs. Previous studies have shown that infusion of ethosuximide into the thalamus, including VPM, has little effect to stop spontaneous SWDs in Long-Evans rats (Chen et al. 2011) and GAERS rats (Richards et al. 2003). However, local infusion of ethosuximide or muscimol into the barrel cortex and neighboring area induces significant inhibition of SWDs in Long-Evans rats.

Fig. 5. Effects of a right-side ZI lesion on SWDs and WTs (n = 8). Examples of temporal and spectral activities of bilateral cortices and vibrissae muscles under the nature (A) and after the ZI lesion (B) are shown. In a natural condition, almost-identical patterns of temporal activities and power spectra appeared in the cortex and vibrissae muscles on both sides. After the ZI lesion, obvious reductions in the spike magnitude and power of 7–12 Hz were found in the primarily ZI-innervated side, i.e., the right-side cortex and left-side vibrissae muscle. The right shows power spectra of the cortical and vibrissae muscle activities before and after the ZI lesion. C: comparison of 7- to 12-Hz powers of SWDs and WTs in the nature and ZI lesion conditions. The relative power was calculated by a normalization of the dominant and nondominant sides of the ZI, i.e., right-side cortex/left-side cortex and left-side VEMG/right-side VEMG. Numbers of SWDs and WTs (D) and durations of SWDs and WTs (E) are shown under the nature and ZI lesion conditions. *P < 0.05 vs. nature. PSD, power spectral density.
rats (Chen et al. 2011; Fanselow et al. 2001) and other strains (Blumenfeld et al. 2008; Manning et al. 2004). Since it is well-known that VPM neurons often reveal precise and restricted projection to the barrel cortex, activation or deactivation of VPM thalamus through either electrical stimulation or drug microinfusion may not be able to produce a widespread cortical area to break down paroxysmal epileptiform discharges or SWDs.

In contrast, the ZI neurons innervated various brain regions, including the dorsal thalamus (Bartho et al. 2002; Lavallee et al. 2005), cerebral cortex (Lin et al. 1990; Nicolelis et al. 1992), and the ZI itself (Mitrofanis 2005). Thus stimulation of GABAergic incertal neurons using ultrahigh frequency stimulation or muscimol infusion may result in a transient disinhibition of incertal-projecting brain areas and lead to altering the stereotypical oscillation of SWDs or attenuate the SWD spike magnitude. Likewise, 800-Hz ZI stimulation, which caused momentary pause of the ZI neurons (depolarization block; Liu et al. 2008), blocked >80% of SWDs. Application of muscimol delayed SWD onset and reduced SWD number within a 1-h period. After 0.5-s 200-Hz ZI stimulation, SWD spike magnitude showed a pattern of an initial slight decline and then recovery in ~60% of cases. A small proportion of SWDs (~20%) was terminated after 200-Hz ZI stimulation, and such a finding was also observed in GAERS rats (Vercueil et al. 1998). In brief, these lines of information suggest that the ZI modulates both the amplitude and genesis of SWDs.

Our present findings also suggest that three intracertal stimulation frequencies revealed rather distinctive effects on SWDs. Electrical stimulation with the ultrahigh frequency showed a significant efficiency in terminating SWDs (Liang et al. 2011; Young et al. 2011). Since it is known that electrical current is able to activate not only the incertal neurons, but also the fiber of passages through the ZI, to address this issue, intracertal infusion of muscimol was used to activate exclusively GABAergic receptors of incertal neurons, and such application resulted in reduction of SWD number within a 1-h period. These results strengthen the conclusion that incertal neurons are indeed modulating SWDs.

Intracertal modulation through the electrical stimulation or drug administration had a transient inhibition on SWDs. How-
ever, SWD occurrence frequency was elevated 5 days after ZI lesion. Since lesion of the ZI and its neighboring area produce long-lasting permanent change, it is very likely that neural network may have an adaptive response after ZI injury. The outcome of ZI lesion may cause less inhibition on numerous thalamic and cortical areas that result in an imbalance of excitatory and inhibitory contribution on the seizure-related networks. Therefore, elevated seizure frequency may be observed after the ZI lesion.

ZI rhythm and WT during SWDs. An interesting fact is that oscillation frequency of SWDs tends to be higher at the beginning of a rhythmic bout, and the spike magnitudes of the SWDs decline toward the end of a seizure (Blumenfeld 2005; Shaw 2004). Some but not all subjects show minor oculomotor rhythmic activity or facial tremors in synchrony with absence epileptic activities (Bogacz et al. 2000; Nicolelis et al. 1995; Semma and Komisaruk 1984; Shaw and Liao 2005). The lateral somatosensory cortex, including the barrel cortex, appears to play a crucial role in the generation of SWDs in rats (Chen et al. 2011; Fanselow et al. 2001; Meeren et al. 2002; Polack et al. 2007). Vibrissae myoclonus does not occur in all SWDs (Nicolelis et al. 1995; Shaw and Liao 2005). Various frontal or parietal loci have also been reported in patients with childhood absence (Holmes et al. 2004; Westmijse et al. 2009). Thus the generation of SWDs without WTs may arise from the nonbarrel cortex.

In the present study, we extended the knowledge regarding the relationship between the peripheral myoclonus and oscillation frequency of absence seizures (Bogacz et al. 2000; Shaw and Liao 2005). For example, oscillation frequency of SWD segments in coincidence with ZI rhythm was significantly higher than that following termination of ZI rhythm in a natural condition (Fig. 1D). WTs were associated with a significant elevation of the SWD oscillation frequency. After lidocaine injections into the bilateral mystacial pads, the phenomenon of the higher-oscillation frequency of SWD segments with rhythmic ZI activity seemed not to exist. A unilateral lesion of ZI showed no effect in oscillation frequency of SWDs. These data suggest that peripheral inputs rather than the ZI may have a greater influence in elevating oscillation frequency of SWDs (Shaw and Liao 2005). In addition, time lag between SWD spike and ZI rhythmic peak was significantly changed, but there was no change in duration and amplitude of ZI rhythms after lidocaine injection. These data imply that peripheral inputs possess a phasic modulation on the incertocortical networks during SWDs.

Numerous studies have also indicated that electrical or chemical stimulation of the ZI induces changes in behavior or
Thalamocortical network action and SWDs. The thalamocortical network and basal ganglia are believed to be the main sites involved in the generation of spontaneous SWDs (Danober et al. 1998; Snead et al. 1999; van Luijtelaar and Sitnikova 2006). In particular, the perioral sensorimotor cortical area is a minimal neural substrate for the generation of absence seizures and myoclonus (Chen et al. 2011; Fanselow et al. 2001; Semb and Komisaruk 1984). The concept of focal cortical abnormality can be further supported by the elevation of interhemispheric functional connectivity of the lateral orbitofrontal cortex of patients with childhood absence epilepsy (Bai et al. 2011). Rhythmic activities of the cortex and thalamic VPM are continuous throughout a SWD, and cortical neurons are phase ahead of the VPM during SWDs (Meeren et al. 2002; Nicolelis et al. 1995; Pinault et al. 2006). In the present study, rhythmic incertal activity is always lagged behind the onset of cortical rhythm during a SWD. Thus the ZI may be recruited by the thalamocortical network of SWDs since ZI only displayed rhythm during a SWD. Additionaly, the advantage of ZI stimulation in Parkinsonian tremors and motor disabilities has also been reported (Guehl et al. 2008; Nandi et al. 2002; Plaha et al. 2006; Voges et al. 2002). In the present study, we demonstrated the potential benefits of ZI stimulation in WT behavioral modulation. Moreover, deactivation of the ZI using AMPA/KAknic acid glutamate receptor antagonists produces inhibition of amphetamine-induced stereotypical activity but not locomotor activity (Supko et al. 1992). The end product of deactivation by glutamate receptor antagonists may be comparable with the effects of an 800-Hz microstimulation-induced neuronal silence. Thus our present data and prior knowledge suggest that the ZI modulates stereotypical rhythmic behaviors, including WTs. Furthermore, ZI has a dense GABAergic projection to the superior colliculus (Kim et al. 1992; Mitrofanis 2005; Nicolelis et al. 1992), which is an important site for the control of facial motor activities (van Luijtelaar and Sitnikova 2006). Accordingly, inhibition of the incertotectal pathway using depolarization-blocking stimulation may contribute to resetting vibrissa muscle activities. However, why 200-Hz ZI stimulation was able to block ~60% of WTs remains largely unknown. Interestingly, how and why 100- to 200-Hz stimulation effectively relieves Parkinsonian symptoms also remains to be elucidated (Garcia et al. 2005).

Thalamocortical network action and SWDs. The thalamocortical network and basal ganglia are believed to be the main sites involved in the generation of spontaneous SWDs (Danober et al. 1998; Snead et al. 1999; van Luijtelaar and Sitnikova 2006). In particular, the perioral sensorimotor cortical area is a minimal neural substrate for the generation of absence seizures and myoclonus (Chen et al. 2011; Fanselow et al. 2001; Semb and Komisaruk 1984). The concept of focal cortical abnormality can be further supported by the elevation of interhemispheric functional connectivity of the lateral orbitofrontal cortex of patients with childhood absence epilepsy (Bai et al. 2011). Rhythmic activities of the cortex and thalamic VPM are continuous throughout a SWD, and cortical neurons are phase ahead of the VPM during SWDs (Meeren et al. 2002; Nicolelis et al. 1995; Pinault et al. 2006). In the present study, rhythmic incertal activity is always lagged behind the onset of cortical rhythm during a SWD. Thus the ZI may be recruited by the thalamocortical network of SWDs since ZI only displayed rhythm during ~70% of a SWD episode and rhythmic incertal activity was associated with higher spike magnitude and 7- to 12-Hz power of SWDs. Furthermore, lesion of ZI reduced 7- to 12-Hz power of SWDs of the corresponding site. Accordingly, it is very likely that incertal neurons are recruited to develop and/or maintain a high-amplitude paroxysm. If it is a case, a transient interruption of the rhythmic incertal activity may influence SWDs. The present study suggested that intracortical interference of electrical stimulation with ultrahigh frequency or muscimol treatment significantly inhibited SWDs. The intracortical muscimol-induced inhibition on SWDs is smaller than those of intrabarrel drug-treated inhibition of SWDs (Chen et al. 2011; Fanselow et al. 2001). Therefore, the ZI appears to play an important role in the development rather than initiation of a paroxysmal SWD.
Some certainty for the “zone of uncertainty”? Exploring the function of the zona incerta.