Is Spontaneous High-Voltage Rhythmic Spike Discharge in Long Evans Rats an Absence-Like Seizure Activity?

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Submitted 21 May 2003; accepted in final form 24 June 2003

Shaw, Fu-Zen. Is spontaneous high-voltage rhythmic spike discharge in Long Evans rats an absence-like seizure activity? J Neurophysiol 91: 63–77, 2004. First published June 25, 2003; 10.1152/jn.00487.2003. A distinct high-voltage rhythmic spike (HVRS) discharge characterized by a barrage of negative spikes oscillating at 5–12 Hz was observed in chronically implanted Long Evans rats. Spontaneous HVRS discharges were exhibited in 90% of 40 Long Evans rats and occurred during sudden arrest of ongoing behavior (immobility) with occasional facial/whisker twitching. However, the function of HVRS discharges in Long Evans rats remains inconclusive to date and has been associated with alpha tremor/mu rhythm, attentive mu wave, and absence seizure. To elucidate the function of HVRS discharges in Long Evans rats, several experiments were performed. In a 6-h recording session (12:00–18:00), HVRS activities primarily occurred in several specific vigilance states, being particularly abundant in a short-lasting period before vigilance changes. Several characteristics, such as durations, oscillatory frequencies, and interspike intervals (ISIs) of HVRS discharges, were altered during wake-sleep states. Oscillatory frequencies were negatively correlated with durations of HVRS segments. In addition, ISIs of a HVRS episode exhibited a crescendo-decrescendo pattern. These variable ISIs could explain why a negative correlation was found between oscillatory frequencies and durations of HVRS episodes. Moreover, HVRS discharges were demonstrated to have widespread and near-synchronous distribution to bilateral cortical areas. In addition, innocuous electrical stimuli were unable to stop ongoing HVRS discharges. By contrast, noxious stimuli elicited behavioral arousal and immediately terminated most HVRS discharges. Cortical-evoked potentials in response to mild electrical stimulation under HVRS discharges were different from those under waking state but resemble those under slow-wave sleep with a smaller magnitude. Moreover, the temporal and spectral characteristics of spontaneous HVRS activities were analogous to those of seizure activities induced by penicillin and pentylentetrazol. The incidence of spontaneous HVRS discharges was significantly decreased by ethosuximide administration. Based on these results, HVRS discharge might not be associated with a voluntary mu-rhythm behavior, instead it behaves as an absence-like seizure activity. These results were also collaborated using other genetic absence-seizure rats, such as WAG/Rij and GAERS rats. Possible mechanisms for the generation and termination of paroxysmal HVRS discharges are also discussed.

INTRODUCTION

Absence seizures or “petit mal” seizures are the most characteristic form of generalized epilepsy. They consist of a sudden arrest of ongoing behavior and impairment of consciousness and are associated with abrupt occurrence of bilaterally synchronous 3-Hz spike-and-wave discharges (SWDs) in the electroencephalogram (EEG) over wide cortical areas (Niedermeyer 1999b). A number of reports have indicated that the occurrence of SWDs is dependent on wake-sleep cycles in humans (Kellaway 1985; Niedermeyer 1999b; Sato et al. 1973; Terzano et al. 1989) and primates (Steriade 1974). Extensive investigation of the feline penicillin generalized epilepsy model has revealed that the generation of SWDs is closely linked to the mechanisms that mediate spindles (Gloor and Fariello 1988; Kostopoulos et al. 1981). However, an artificial drug-induced epileptic model could not substantiate the relationship between absence seizures and natural sleep spindles. Alternatively, it would be better to correlate the occurrence of spontaneous SWDs during natural wake-sleep states.

Several genetic absence-like epileptic models, which exhibit spontaneous paroxysmal high-voltage rhythmic spike (HVRS) discharge oscillating in the range of 5–12 Hz, are found in several rat strains (Buzsáki et al. 1988; Coenen et al. 1991; Danover et al. 1998; Marescaux et al. 1992; Snead et al. 1999), such as WAG/Rij and GAERS rats. The aberrant EEG activities were proposed to be an absence-like seizure because the incidence of HVRS discharges is reduced by the administration of antiepileptic drugs, such as ethosuximide, and the arrest behavior usually coincides with facial twitching during bilateral HVRS discharges (Coenen et al. 1991; Danover et al. 1998; Kandel and Buzsáki 1997; Marescaux et al. 1992; Meeren et al. 2002; Snead et al. 1999). These HVRS discharges are also dependent on vigilance levels like those found in humans (Lannes et al. 1988; Drinkenburg et al. 1991). Previous studies have indicated variable incidences of HVRSs in different vigilance states, but the relationship between occurrences of HVRSs and sleep onset as well as basic characteristics under different vigilance states remain largely unknown. To address these issues, continuous long-term recording to monitor occurrence of HVRS discharges during wake-sleep cycles needs to be performed.

Similar HVRS activities or compatible rhythmic neuronal activities were found in Long Evans rats by several laboratories (Kaplan 1985; Nicolesis and Faselov 2002; Nicolesis et al. 1995; Semba and Komisaruk 1984; Semba et al. 1980; Vanderwolf 2000). However, the function of these HVRS activities in Long Evans rats remains inconclusive to date. Because HVRS discharges often coincide with synchronous whisker movement with a small deflection (whisker twitching), they have been suggested to be associated with a movement disorder, such as alpha tremors (Semb and Komisaruk 1984; Semba et al. 1980). Another interpretation of HVRS oscillations is a dy-
namic process of sensory information from vibrissa under an awake behavioral state (Fanselow et al. 2001; Nicolelis and Fanselow 2002; Nicolelis et al. 1995). A third consideration of HVRS discharges is related to absence epilepsy (Kaplan 1985). The two first functional hypotheses of HVRSs result from emphasis on rhythmic whisker behavior under the waking state. In general, a rhythmic behavior cannot readily be observed during sleep. Therefore evaluation of the occurrence of HVRS discharges during wake-sleep states appears to play an important role in defining their possible functional correlates.

In an attempt to examine the functions of HVRS discharges in Long Evans rats, the incidence of HVRS activities during wake-sleep states was first characterized in chronically implanted rats. A multisite recording was performed to evaluate spatiotemporal characteristics of HVRS discharges. Several basic characteristics of HVRSs, for example, the incidence, duration, oscillatory frequency, and interspike interval, during wake-sleep states were compared. To evaluate the functional processes in the cerebral cortex, electrical stimuli were utilized to correlate the evoked responses under different vigilance levels, including the state of HVRS discharges. In addition, pro- and anticonvulsants were also used to evaluate the function of HVRSs.

**METHODS**

Adult female Long Evans rats (4–6 mo old; 300–350 g) were used in the study. Animals were kept in a sound-attenuated room under a 12:12-h light-dark cycle (07:00–19:00 lights on) with food and water provided ad libitum. The detailed procedures of animal preparation were described in a previous study (Shaw et al. 2002). Briefly, the recording electrodes were implanted under pentobarbital anesthesia (50 mg/kg ip). Ketamine was administered as needed to maintain a proper anesthetic depth during surgery. Subsequently, the rat was placed in a standard stereotaxic apparatus. The dorsal surface of the skull was exposed and cleaned. Six stainless steel screws were driven bilaterally into the skull overlaying the frontal (A +2.0, L 2.0 with reference to the Bregma), parietal (A −2.0, L 2.0), and occipital (A −6.0, L 2.0) regions of the cortex to record cortical activities. The parietal lead was placed over the tail and hindlimb representative area of the primary somatosensory cortex (Shaw et al. 1999a). A ground electrode was implanted 2 mm caudal to lambda. Care was taken to prevent electrodes from penetrating the underlying dura. In addition, two seven-strand stainless steel microwires were bilaterally inserted into the dorsal neck muscles to record electromyographic (EMG) activity. Dental cement was applied to fasten the connection socket to the surface of the skull. After suturing to complete the surgery, animals were given antibiotics and housed individually in cages for recovery.

Two weeks after surgery, animals were placed individually in clear acrylic chambers so that their behaviors could be easily observed. A narrow opening at the top of the chamber permitted unimpeded feeding of the head plug/cable assembly to the skull implant. To allow rats to habituate to the experimental apparatus, each rat was placed in the recording environment at least two times (1 h/day) prior to testing. On the day of the recording, a 30-min period was allowed for the rat to become familiar with the chamber. The experiment was performed during a 6-h recording period per day (12:00–18:00) in conjunction with video tracking of behavioral changes.

After completion of the experiment, the animal was killed with an overdose of pentobarbital sodium. Special attention was given to ascertain that the stainless steel screws had not penetrated the dura. All surgical and experimental procedures were reviewed and approved by the Institutional Animal Care and Use Committee of Tzu Chi University.

EEGs (0.3–70 or 0.3–1,000 Hz) recorded from skull electrodes and the EMG of the nuchal muscle (100–500 Hz) were amplified and digitized (Shaw et al. 2002). A grounded plate was placed under the recording chamber to reduce electromagnetic interference (Shaw et al. 2003). A three-stage classification of vigilance states during wake-sleep cycles, i.e., wakefulness (AW), slow-wave sleep (SWS), and paradoxical sleep (PS), was used in the present study (Fig. 1). The three-stage classification is a popular differentiation for wake-sleep states in rodent studies (Coenen et al. 1991; Danober et al. 1998; Shaw et al. 2002). Vigilance was determined by visual inspection of temporal and spectral characteristics of bioelectric trials as well as video monitoring. AW was characterized by high-frequency cortical activity with higher muscle tone. In the present study, AW consisted of both passive and active wakefulness. Under active wakefulness, theta activity was prominently shown in parietal/occipital areas as well as phasic muscle activity exhibited during voluntary behavior (Fig. 2, **). By contrast, quiescent rest (immobility) was considered "passive wakefulness" (Fig. 2, right part of **). SWS was characterized by both EEG with a high-amplitude oscillation within the delta frequency range (0.5–4 Hz) and low nuchal EMG activity. During SWS, the sleep spindle and/or the K-complex wave were often observed, particularly in the frontal area. PS was identified by EEG with a low-amplitude, high-frequency pattern as well as muscle atonia. In particular, theta-rhythm activity always occurred at the occipital EEG (Fig. 1, inset), and clear cardiac activity (QRS complex of electrocardiogram) was also observed in the EMG trace exhibiting muscle atonia (Fig. 1). These criteria have been well documented in previous studies (Gottesmann 1992; Steriade et al. 1993). In an attempt, an interesting short-lasting period, i.e., an intermediate state (IS), was marked by a transition from SWS to PS or from PS to AW/SWS. A group of high-amplitude spindles was occasionally exhibited in the frontal/parietal regions under an IS state (Gottesmann 1996; Shaw et al. 2002). The IS period was usually in the range of several to tens of seconds (Gottesmann 1996). In the present study, the IS period was included in the PS state for the three-stage vigilance classification.

In an attempt to extract characteristics of HVRS episodes under different vigilance levels, several analyses were performed. Two steps were essential to detect each individual spike within a HVRS episode. Selected HVRS segments were first passed through a second-order high-pass Bessel filter (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 minimal/maximal limitations. Therefore the amplitude of each individual spike could be determined. Furthermore, intervals of consecutive spikes were also able to be calculated. Moreover, the variability of spike intervals during HVRS episodes was also studied, particularly the first five and the last five spike intervals. In addition, Fourier transformation with a Hamming window was utilized to calculate the power spectra of selected HVRS episodes. Consequently, the prominent frequency peak of a selected HVRS episode in the range of 5–14 Hz could be measured. Moreover, cross-correlation was used to measure synchronous oscillations between two cortical sites. The amplifier, data-acquisition system, and entire analysis programs have been described in detail elsewhere (Shaw et al. 1999b, 2002).

To evaluate the cortical function during HVRS discharges, electrical stimulation was applied to rats. Constant electrical current pulses of 0.5-ms duration (Grass 48) were delivered to the middle part of the rat’s tail via multistrand stainless steel wires. The wires were held in place with adhesive tape, and electrode cream was applied to enhance the contact. A variety of stimulus intensity (0.2–1.2 mA) was used to evaluate behavioral responsiveness to the external stimulation. The portion of termination of paroxysmal HVRS discharges by different intensities of electrical stimulation was measured. To correlate cortical evoked responses under AW, SWS, and HVRS discharges, the intensity of the electrical current was set in the range of 0.3–0.5 mA, which could activate low-threshold receptors but did not elicit behav-
FIG. 1. Typical polygraphic recordings during awake (AW), slow-wave sleep (SWS), and paradoxical sleep (PS). Each state shown from top to bottom is electroencephalograms (EEGs) of the frontal, parietal, and occipital cortices and a dorsal neck electromyogram (EMG). A small-amplitude, desynchronous EEG and higher muscle activity were seen under AW state. A high-amplitude, delta-frequency synchronous EEG as well as lower muscle tone were recorded under SWS. A small-amplitude, desynchronous EEG and muscle atonia were observed under PS. Note the clear theta peak in the occipital power spectrum under PS (inset). Obvious cardiac activity (the QRS complex of the electrocardiogram, indicated by /[^H20323/H20323] in the inset) is shown on the EMG tracing during PS.
FIG. 2. A representative example of high-voltage rhythmic spike (HVRS) discharges under AW state. Two HVRS oscillations, primarily exhibited in frontal and parietal areas, were observed in the right part of the top panel. The HVRS was characterized by a cluster of large negative peaks (*). A prominent 8-Hz oscillation in company with 2 harmonics (\(\downarrow\)) was depicted in the power spectrum of the HVRS. The HVRS occurred as behavioral immobility, which was indicated by stable tonic muscle activity. During voluntary movement (**), the EMG showed phasic activities, and occipital/parietal leads exhibited rhythmic activity. This rhythmic activity was in the theta range (●). PSD, power spectral density.
ior indicative of discomfort (Shaw et al. 1999a), such as vocalization. Nonnoxious electrical stimulation at the same intensity was applied to rats under AW, SWS, and HVRS discharges to compare the cortical- evoked responses. Evoked potentials under AW, SWS, and HVRS discharges were selected and then averaged from the responses of ~200, 400, and 600 trials, respectively. Data trials were rejected if they showed a contaminated movement artifact or large baseline drift or did not meet the criteria of a specific vigilance state (Shaw et al. 2002).

Proconvulsants and anticonvulsant were used to evaluate the function of HVRSs. For a proconvulsant, penicillin (PEN; 800,000 U/kg) (Avoli 1980) or pentylentetrazol (PTZ; 20 mg/kg) (Snead et al. 1999) was intraperitoneally administered to induce generalized convulsive seizures in rats with or without inherent spontaneous HVRS discharges. Comparison of temporal morphology between induced epileptic activity and spontaneous HVRSs was performed. Oscillatory frequencies of induced and spontaneous HVRSs were also evaluated. Ethosuximide (25 mg/kg) was intraperitoneally injected into rats to access its anticonvulsant activity. The dosage of ethosuximide has been demonstrated to effectively and significantly reduce the incidence of HVRS discharges in absence-like seizure rats and humans (Hardman and Limbird 2001; Peeters et al. 1988). The incidence of HVRSs was compared hourly before and after ethosuximide injection. Data were expressed as means ± SE in the present study.

RESULTS

Thirty-six (90%) of 40 rats exhibited obvious spontaneous HVRS discharges. We further analyzed the incidence and characteristics of HVRSs under different vigilance states during a 6-h recording period in 15 rats. The durations of AW, SWS, and PS in the 6-h recording session were 2.51 ± 0.29, 2.71 ± 0.2, and 0.77 ± 0.12 h, respectively. In particular, AW (45.2 ± 3.6%) was dominant during the first 2 h, SWS (37 ± 2.5%) during the next 2 h, and PS (50.7 ± 6.2%) during the last 2 h.

Incidence of HVRS during wake-sleep states

HVRS was characterized by a barrage of large spike discharges with negative polarity (Fig. 2. *). The amplitude of HVRS could reach 2 mV (0.2–2 mV). HVRSs were usually found in both frontal and parietal leads, i.e., sensorimotor areas, but seldom occurred in the occipital area (Figs. 2, 3, 7, and 9A). Spontaneous HVRS discharges usually exhibited a wax-and-wane pattern with a single cycle or multiple cycles. In most cases, the magnitude of HVRSs recorded from the frontal area was higher than that of the parietal area. These observations were consistent with previous results reported in either the same strain (Kaplan 1985; Somba and Komisaruk 1984; Somba et al. 1980) or other strains of rats (Buzsáki et al. 1988; Coenen et al. 1991; Danober et al. 1998; Marescaux et al. 1992). The incidence of HVRS discharges in each individual rat was variable during a 6-h recording period (37–562; 289 ± 41). In total, 4,327 HVRS episodes were identified in 15 rats, and HVRS discharges were primarily exhibited in four distinct situations. Under the AW state (Fig. 2), HVRS discharges coincided with behavioral arrest ("passive wakefulness"). Meanwhile, 66% of the HVRS episodes were coincident with obvious vibrissal twitching from video-EEG monitoring. All HVRS discharges were observed before the onset of whisker twitching, but the reverse was never seen. Muscle tone in the neck was sometimes diminished, thereafter inducing a gradual and slight drop of the head (Fig. 2. *). Compared with passive wakefulness, no HVRS was observed during active wakefulness, which was identified by both theta activity of the occipital lead and phasic EMG (Fig. 2. **).

In addition to the AW state, HVRS discharges were also observed during SWS with abundant sleep spindles. HVRSs were often interpolated within sleep spindle/K complex sequences. Although HVRSs occurred during SWS, a large portion of HVRS discharges was observed during the short-lasting period of vigilance changes. For example, HVRS discharges were directly followed by a transition to SWS, and afterward interspersed with sleep spindle/K complex waves (Fig. 3A). Another period of investigational interest was the transitional period into or out of PS states, i.e., the IS state. HVRS discharges occurred for short-lasting periods from SWS to PS (Fig. 3B) or from PS to AW. Occasionally, rats exhibited a sudden body jerk and then were awakened after the occurrence of HVRS activities in the IS state. Besides the occurrence under SWS and IS states, HVRSs were also detected in PS. Collectively, the incidence of HVRSs under AW, SWS, IS, and PS states were 73.8, 9.7, 5.6, and 10.9%, respectively. Moreover, the incidence of HVRSs under different vigilance levels in the three strains of rats was summarized in Table 1.

Basic characteristics of HVRSs during wake-sleep states

Although temporal profiles of HVRSs during wake-sleep states were very similar, several markedly different characteristics were also found. HVRS discharges of variable durations were usually clustered together (Fig. 4). In particular, most groups of HVRSs occurred prominently before changes of vigilance states (Fig. 4), such as the transition from AW to SWS (Fig. 4, *) and IS states (Fig. 4, **). In addition, large differences in the distribution of HVRS durations under different vigilance levels were found. Most HVRS episodes were in the range of 1–5 s, but a large portion of longer HVRS discharges occurred at AW and SWS states (Fig. 5B). Durations of HVRS episodes under different vigilance states were significantly different (AW, 4.47 ± 0.08 s; SWS, 4.38 ± 0.16; IS, 3.84 ± 0.23; PS, 3.51 ± 0.17; P < 0.001 by Kruskal-Wallis 1-way ANOVA on ranks). The durations of HVRS episodes under AW were longer than those of other states (P < 0.05 by Dunn’s test); HVRS durations of SWS were significantly longer than those of the IS and PS states (P < 0.05 by Dunn’s test).

In addition to the observations of variable HVRS durations, oscillatory frequencies of HVRS episodes were also analyzed. For a selected HVRS episode, a clear 8-Hz peak in conjunction with two harmonics was found in its power spectra (Fig. 2, *). The results are corroborated by previous studies (Buzsáki et al. 1988; Coenen et al. 1991; Danober et al. 1998; Marescaux et al. 1992). HVRS segments with a longer duration (>1 s) were selected for frequency analysis to minimize the error due to poor frequency resolution. Prominent frequencies of HVRS oscillations (Fig. 5A) were significantly altered during wake-sleep states (AW, 8.46 ± 0.02 Hz; SWS, 8.16 ± 0.05; IS, 8.06 ± 0.14; PS, 8.05 ± 0.11; P < 0.00003 by Kruskal-Wallis 1-way ANOVA on ranks). In particular, oscillatory frequencies of HVRSs under the AW state were higher than those of other states (P < 0.05 by Dunn’s test). Moreover, oscillatory frequencies of HVRSs showed a small negative
trend with HVRS durations (Fig. 5B), particularly in AW and SWS states with statistical significance.

More interestingly, the interspike intervals (ISIs) were variable during a HVRS episode (Fig. 6A). ISIs exhibited a crescendo-decrescendo pattern in a HVRS episode. During a HVRS discharge, a high-amplitude spike was accompanied by a longer ISI (Fig. 6A). Moreover, a single or oligo high-amplitude spike was usually followed by a group of small-amplitude spikes during a HVRS discharge. In addition, HVRS activities were often terminated after a large number of high-amplitude spikes with longer ISIs (Figs. 2, 3, and 6A). Compared with first and final five ISIs of HVRS episodes (Fig. 6B), they were modulated by vigilance states during both the beginning (AW, 106.5 ± 0.5 ms; SWS, 110.2 ± 0.9; IS, 111.8 ± 1.6; PS, 116.4 ± 1.3; P < 0.000001 by Kruskal-Wallis 1-way ANOVA on ranks) and the end sessions (AW, 123.4 ± 0.6 ms; SWS, 126.1 ± 1; IS, 122.3 ± 2.5; PS, 122.7 ± 1.5; P < 0.001 by Kruskal-Wallis 1-way ANOVA on ranks). Initial ISIs under AW were significantly shorter than those of other states (P < 0.05 by Dunn’s test); initial ISIs under SWS were also significantly shorter than those of PS. On the other hand, ISIs at the end of HVRS segments under SWS were significantly longer than those of AW and PS states (P < 0.05 by Dunn’s test). Moreover, ISIs at the end of HVRS episodes were significantly longer than those of initial intervals under all states (P < 0.0001 by Wilcoxon signed-rank test).

**Functional characteristics of HVRS discharges**

A 12-channel recording of cortical field potentials was performed in three rats. HVRS discharges were widespread in bilateral cortical areas, particular in frontoparietal areas (Fig. 7). It is congruent with previous results (Buzsáki et al. 1988). In most HVRS discharges (>90%), the posterior parietal or occipital site often lagged behind frontoparietal areas with variable cycles (Figs. 3, 7, and 9), but the reverse never was seen. For instance, time lags between CH2 and CH12 and between CH1 and CH11 were ~0.5 s (7 cycles; Fig. 7). Moreover, HVRS discharges exhibited an aspect of synchronous oscillation in the range of 7–10 Hz, but they showed small variable average phase shifts (~4.0–1.5 ms; 0.25 ± 0.57 ms) between channels (CROSS in Fig. 7).

To further evaluate functional processing of the primary somatosensory cortex during HVRS discharges, electrical stimulation was applied to the rat’s tail (n = 5). HVRS discharges were often interrupted by innocuous electrical stimuli (Fig. 8A). In general, innocuous somatic evoked potentials finish within 200 ms under either awake or sleep (Fig. 8C) (Shaw et al. 2002). Therefore if HVRS activity was terminated within 300 ms after electrical stimuli, the stimulation was interrupted.
considered to be effective; otherwise, the remainders were taken as ineffective ones. Most ongoing HVRS activities persisted to mild electrical stimuli but were immediately terminated by a high-intensity current (Fig. 8B; \( P < 0.0000001 \) by 1-way repeated-measures ANOVA). Moreover, HVRS discharges were usually immediately interrupted by unexpected stimulation, such as a clap. Furthermore, temporal morphologies of cortical evoked potentials under AW, SWS, and HVRS discharges markedly differed, particularly in middle-/long-latency (>30 ms) evoked responses (Fig. 8C). Evoked potentials under HVRS discharges were very similar to those of SWS with the exception that their magnitudes were smaller than those of SWS (Fig. 8C). Evoked responses under awake and SWS conditions are corroborated by previous studies (Cauller and Kulics 1988; Shaw and Chew 2003; Shaw et al. 1999a).

Pharmacological manipulation of HVRS discharges

To evaluate the functional role of spontaneous HVRS discharges, two convulsants, PEN and PTZ, were intraperitoneally injected into Long Evans rats with \((n = 3)\) or without \((n = 2)\) spontaneous HVRS discharges. Rats with spontaneous HVRSs showed head jerks, then forelimb myoclonus after initial administration of the convulsant. A clear drop of the head and rhythmic nodding behavior were accompanied by whisker twitching 20 min later. By contrast, rats without spontaneous HVRSs only showed head jerks initially after drug injection. An obvious head-down posture and rhythmic nodding behavior as well as whisker twitching, in turn, were also observed 10 min later. In general, spontaneous HVRS discharges often occurred when rats showed behavioral arrest (immobility) 40 min and later after drug administration. Paroxysmal HVRS discharges 20–30 min after injection of both convulsants were selected for comparison of temporal morphology. A barrage of HVRS discharges induced by PEN and PTZ in rats lacking spontaneous HVRSs resembled spontaneous HVRSs, and there was also no phasic neck muscle activity (Fig. 9B). However, a group of phasic muscle activities, which was associated with nodding behavior, was almost synchronous with HVRS discharges in rats with spontaneous HVRSs (Fig. 9A). The discrepancy of behavioral responses exhibited in rats with or without spontaneous HVRSs might be due to different thresholds or reaction times to convulsants. Although the behavior showed a small difference at the same moment after drug administration, HVRS discharges consistently oscillated in the range of 7–10 Hz.

Ethosuximide was injected into rats with inherited spontaneous HVRS discharges \((n = 6)\). HVRS discharges were significantly decreased by ethosuximide administration (Fig. 9C; \( P < 0.00001 \) by 1-way repeated-measures ANOVA).

DISCUSSION

In the present study, spontaneous HVRS discharges were present in most Long Evans rats (90%). These HVRS activities occurred in several specific vigilance states, being particularly abundant in the short-lasting period before vigilance changes (Fig. 4). Although numerous aberrant HVRS discharges have been observed in Long Evans rats, durations of wake-sleep states in a 6-h recording period were close to those reported in normal rats (Maloney et al. 1997; Trachsel et al. 1991). Several characteristics, such as durations, oscillatory frequencies, and
ISIs of HVRS segments, are altered during wake-sleep states (Figs. 5 and 6). HVRS discharges were widely distributed in bilateral cortical areas (Fig. 7). Innocuous somatic cortical evoked potentials were altered under HVRS discharges in comparison with those of waking state (Fig. 8). Moreover, spontaneous HVRS activities were very similar to those induced by PEN and PTZ (Fig. 9. A and B). The incidence of spontaneous HVRS discharges was significantly decreased by ethosuximide administration (Fig. 9C).

Possible functional role of spontaneous HVRS discharges in Long Evans rats

At least three possible functional hypotheses of HVRS discharges have been proposed for Long Evans rats: alpha tremor/mu rhythm (Semba and Komisaruk 1984; Semba et al. 1980), attention/mu rhythm (Nicolelis and Fanselow 2002; Nicolelis et al. 1995), and absence seizure (Kaplan 1985). Several lines of evidence shown in the present study indicate that spontaneous HVRS discharges may be an absence-like seizure activity rather than a mu rhythm. First, HVRS discharges appeared under AW, SWS, IS, and PS (Figs. 2, 3, 5, and 6). Semba and Komisaruk (1984) also observed spontaneous HVRS discharges under AW and PS. In addition, most HVRS discharges were usually clustered together and occurred at the transition of vigilance states (Fig. 4). In humans, mu rhythm is usually observed under a quiescent resting condition and is also shown in rapid-eye-movement sleep (Duntley et al. 2001; Niedermeyer 1999a; Yamada and Kooi 1975). Whether the mu wave exists in light sleep (stages 1 and 2) remains inconclusive.

**FIG. 5.** Changes in oscillatory frequencies of HVRS episodes under different vigilance states. A: comparison of HVRS oscillatory frequencies under different vigilance states. Oscillatory frequencies of the AW state were significantly higher than those of other states. *, $P < 0.05$ by Dunn's test. B: relationship between oscillatory frequencies and durations of HVRSs. A negative correlation was found between oscillatory frequency and durations of HVRSs, particularly in AW and SWS with statistical significance.
Accordingly, the relation between the mu rhythm and vigilance states needs to be validated with further evidence (Niedermeyer 1999a). By contrast, a lot of evidence in humans indicates that the occurrence of absence seizure activity depends on vigilance states, particularly in the transition of vigilance states (Kellaway 1985; Niedermeyer 1999b; Sato et al. 1973; Terzano et al. 1989).

Second, most HVRSs occurred during passive wakefulness (immobility; Fig. 2). This is corroborated by previous studies (Fanselow et al. 2001; Kaplan 1985; Nicolelis et al. 1995; Semba et al. 1980). These HVRS discharges are considered an endogenous component and are attributed to the thalamocortical system (Nicolelis et al. 1995; Semba and Komisaruk 1984). Previous studies primarily focused on the rhythmic whisker movement (whisker twitching) under AW state. However, only 66% of HVRS discharges under AW state were found with whisker twitching in the present study. Moreover, HVRS discharges were also observed during sleep (Figs. 3 and 4; Table 1). Accordingly, HVRS discharges are not simple motor-dependent products. Nicolelis and colleagues (Nicolelis and Fanselow 2002; Nicolelis et al. 1995) proposed that the 9-Hz thalamocortical alpha wave might be related to attentive behavior or texture analysis. However, the gamma wave (20–50 Hz) is considered an index of attention and feature binding (Bressler 1990; Pulvermüller et al. 1997; Steriade 2001; Steriade et al. 1993). An attentive gamma wave (~30 Hz) is often followed by exploring whisking behavior (Hamada et al. 1999), which plays an important functional role in texture discrimination in rodents (Carvell and Simons 1990; Nicolelis and Fanselow 2002). Similar gamma-oscillation neuronal activity, which is occasionally accompanied by theta rhythmic neuronal activity, is found during texture discrimination in primates (Ahissar and Vaadia 1990). In addition, previous studies have also indicated that the whisking behavior with a cortical theta rhythm strikingly differs from the whisker twitch-
FIG. 7. Spatiotemporal characteristic of a representative HVRS discharge. A spontaneous HVRS discharge is illustrated in bilateral cortical areas. According to the location of 12 electrodes (inset), a relatively higher amplitude during the HVRS episode was found in the frontal and lateral parietal cortical areas. Time lags between CH2 and CH12 and between CH1 and CH11 were ~0.5 s (7 cycles). Coherent oscillations with variable latencies are shown in inter- and intra-hemisphere cross-correlograms (CROSSs) with reference to CH1. The oscillatory frequency of the HVRS episode was ~9 Hz.
ing with a cortical alpha rhythm in several aspects, including behavior and spontaneous/evoked brain activities (Carvell and Simons 1990; Nicolelis and Fanselow 2002). In the present study, I could find no gamma wave followed by HVRS discharges. In addition, the occurrence of HVRS discharges varied during a 6-h recording period in Long Evans rats. That is, HVRS discharges appear not to be a common behavior in Long Evans rats. Moreover, 66% of HVRS discharges under AW state were found with whisker twitching. All HVRS activities were observed before the onset of whisker twitching, but the reverse was never seen. In this manner, whisker twitching might be an automatic behavior concomitant with HVRS discharges. Based on these observations, spontaneous HVRS activity might not be involved in attentive function or texture analysis.

Third, the Rolandic mu rhythm is functionally associated with the resting sensorimotor alpha wave, and it is easily terminated by sensory/motor stimulation (Duntley et al. 2001; Niedermeyer 1999a). In the present study, mild electrical stimuli elicited obvious cortical responses under AW state (Fig. 8C). However, over 90% of ongoing HVRS discharges could not be stopped by mild electrical stimulation (Fig. 8B). Similar phenomenon obtained by deflecting the vibrissae was also described in previous studies (Nicolelis et al. 1995; Fanselow et al. 2001). In addition, the evoked cortical field potential under HVRS discharges was analogous to that of SWS (Fig. 8C). These evoked responses under SWS and HVRS discharges resembled those recorded under light anesthesia [2–3 h of Fig. 2A in Shaw et al. (2001)]. Under situations of SWS and light anesthesia, sensory information processing of the cerebral cortex in response to extrinsic stimuli would be slightly impaired in comparison with that under the awake behavioral state (Cauller and Kulics 1988; Shaw et al. 2001; Steriade et al. 1997). Accordingly, the functional state of the cerebral cortex under HVRS discharges should be depressed. The results would be analogous to a loss of consciousness and/or unre-
sponsiveness to external stimuli during absence seizures (Nie-

dermeyer 1999b).

Finally, spontaneous HVRS activities resembled those in-
duced by PEN and PTZ (Fig. 9, A and B). In addition, the
incidence of HVRS discharges was significantly decreased by
ethosuximide, which is an anti-absence medicine (Fig. 9
C). According to these results, spontaneous HVRS

discharges in Long Evans rats should be an absence-like sei-
zure activity.

Comparison of HVRS discharges during wake-sleep states

Several aspects of HVRS discharges in Long Evans rats
were similar to those reported in WAG/Rij and GAERS rats
(Coenen et al. 1991; Danober et al. 1998; Marescaux et al.
1992; Snead et al. 1999), including the temporal morphology,
spatial distribution, bilateral synchronization, vigilance depen-
dence, and pharmacological interventions. Although the oc-
currence of HVRS discharges was dependent on vigilance levels,
the distribution of HVRS discharges during wake-sleep states
strikingly differed in the three strains of rats (Table 1). In
comparison with WAG/Rij and GAERS rats, the distribution of
occurrence under wake-sleep states in Long Evans rats was
relatively close to that of GAERS rats except with a higher
probability for HVRS discharges under a PS state (Table 1).

In comparison of absence-seizure discharges in humans and
rats, durations of HVRSs or SWDs under AW state are longer
than those of sleep in both species. However, several charac-
teristics of rat’s HVRSs remarkably differed from those of
human’s SWDs under wake-sleep states. First, the patterns of
HVRS discharges remains relatively constant during wake-
sleep states either in Long Evans rats (Figs. 2 and 3) or in other
strains (Coenen et al. 1991; Danober et al. 1998; Jando et al.
1995; Kandel and Buzsáki 1997; Marescaux et al. 1992). By
contrast, morphologies of 3-Hz SWDs are altered during wake-
sleep states in humans (Kellaway 1985; Niedermeyer 1999b;
Sato et al. 1973). Polyspikes and/or irregular slow-wave pat-
tern usually dominant during quiet sleep (Sato et al. 1973).
Second, the frequency of human’s SWDs under stage II sleep
is higher than those of AW state (Niedermeyer 1999b; Sato et
al. 1973). The elevated frequency under light sleep might be
due to the combination of polyspikes into SWDs. By contrast,
oscillatory frequencies of HVRSs under AW state were higher
than those of other vigilance states (Fig. 5A) because ISIs at the
beginning of HVRS discharges under AW state were signifi-
cantly shorter than those of other states, but ISIs at the end
showed little obvious difference during wake-sleep states ex-
cept SWS state (Fig. 6B). Indeed, a shorter ISI will lead to a
higher frequency peak. Accordingly, different distributions of
ISIs at the beginning and the end of HVRS discharges can

FIG. 9. Comparison of spontaneous HVRSs and epileptic drug-induced activities. Analogous paroxysmal aberrant HVRS

activities induced by penicillin (PEN) and pentylenetetrazol (PTZ) were observed in rats with spontaneous HVRSs (SPONT; A)

and without spontaneous HVRSs (NO SPONT; B). C: comparison of the incidence of spontaneous HVRS discharges before and after

ethosuximide administration (n = 6). The frequency of spontaneous HVRS occurrences significantly decreased for ≤6 h after

ethosuximide administration. * P < 0.05 by Dunn’s test.
explain oscillatory frequency shifts during wake-sleep states. A decrease in noradrenergic and serotonergic drives as well as dynamic changes in cholinergic activities have been observed in SWS-PS but not AW states (Steriade et al. 1997). Alteration of neuromodulation may be responsible for the frequency shift of HVRS discharges during wake-sleep states.

In humans, responsiveness to mild stimuli is impaired and reaction time is prolonged during paroxysmal SWDs (Brown et al. 1974; Porter et al. 1973; Tassinari 1968). By contrast, a strong stimulation often terminates seizure discharges (Tassinari 1968). A compatible phenomenon was also observed here (Fig. 8). In the present study, a mild electrical stimulation could not terminate most HVRS discharges (Fig. 8, A and B). It is congruent with previous results to mild somatic (Fanselow et al. 2001; Nicolelis et al. 1995), auditory (Meeren et al. 2001), and visual (Meeren et al. 1998) stimuli. In a sharp contrast, a high-intensity current (>0.8 mA) immediately stopped most ongoing HVRS discharges in company with behavior indicative of discomfort, such as tail flick (Fig. 8B). The nociceptive stimulation will activate the medial thalamus, which widespread projects to the cerebral cortex, then lead to desynchronous cortical activities (Steriade et al. 1997). It may be the reason why a high-intensity current can immediately terminate most HVRS discharges.

Possible mechanisms for generation and termination of paroxysmal HVRS activities

Several studies have indicated that absence-seizure activities are associated with spindle oscillations (Gloor and Fariello 1988; Kellaway 1985; Kostopoulos et al. 1981; Steriade 1974), thus the thalamic reticular nucleus (TRN) is closely linked to the generation of SWD or HVRS activities (Avanzini et al. 1999; McCormick and Contreras 2001; Steriade et al. 1993, 1997). Recently, several pieces of evidence suggest that the cortex plays an important role in the generation of absence-seizure activities. First, the cerebral cortex of athalamic cats could give rise to SWDs by an intracortical administration of bicuculline, but only variably recurring spindles could be induced by intrathalamic injection of bicuculline in complete decorticated cats (Steriade and Contreras 1998). In addition, layer 5 cortical neurons in isolated neocortical blocks could initiate the paroxysmal synchronous bursts, which may be correlated to epileptic activities, by administration of PEN and bicuculline (Connors and Telfeian 2000). Accordingly, the cortex should be a minimal substrate to produce seizure activities. Second, the activities of cortical neurons preceded several cycles of thalamic neuronal activities during SWD/HVRS activities in Long Evans rats (Fanselow et al. 2001; Nicolelis et al. 1995) and other strains of rats (Seidenbecher et al. 1998). At the beginning of HVRS discharges, the neuronal activities in the lateral parietal cortical region precede those from both other cortical areas and the thalamus (Meeren et al. 2002). In addition, cortical neuronal activities precede or follow TRN neuronal activities during SWD/HVRS discharges (Avanzini et al. 1999; Slaght et al. 2002; Yen et al. 1999), but the cortex is 5–10 ms ahead of the ventroposterior medial nucleus and TRN by peri-event histograms (Yen et al. 1999). Moreover, the SWD appears in the cortex before the thalamus (Fishcr and Prince 1977), but the reverse has never been seen (Gloor and Fariello 1988). Thus the cortex may drive paroxysmal seizure activities. In the present study, ∼75% of 4327 HVRS activities occurred under AW state and a few during sleep (Table 1). In addition, a barrage of HVRS discharges often occurred before the transition of vigilance states, i.e., the state consisting of abundant sleep spindles (Fig. 4). Thus the HVRS may not start from a spindle. This was also corroborated in GAERS rats (Pinault et al. 2001). Moreover, posterior parietal site usually lagged behind frontoparietal areas (Figs. 3, 7, and 9), and subtle phase shifts between inter- and intra-hemisphere channels also were measured during HVRS discharges. The results imply that intracortical propagation through mono- and multisynaptic projections may take place during paroxysmal HVRS discharges. Based on these results, the cortex may play a crucial role in the generation and propagation of HVRS discharges.

Spontaneous HVRS discharges usually exhibited a wax-and-wane pattern with variable cycles (Figs. 2, 3, 7, and 9A). In addition, a single or oligo high-amplitude spike with high magnitude was followed by a small-amplitude spike, whereas HVRS activities were usually terminated after a large number of high-amplitude spikes (Figs. 2, 3, and 6A). The present results indicate that persistent activation may terminate ongoing paroxysmal HVRS activities. Similar phenomenon was also described by an in vitro study (Bal and McCormick 1996). It has been proposed that paroxysmal activities might give rise to the generation of afterdepolarization and the relative refractory period in thalamocortical cells after persistent HVRS discharges and afterward terminate the spontaneous synchronized oscillations (Bal and McCormick 1996). However, the duration of persistent activation that blocks paroxysmal HVRS discharges still remains to be determined. Moreover, persistent HVRS discharges might lead to progressive hyperpolarization of thalamocortical neurons via the interaction of GABergic neurons, thereafter shifting brain activities into other synchronized oscillations. For instance, slight hyperpolarization of thalamocortical neurons will lead to alterations in brain activities from high-frequency waves to sleep spindles or from sleep spindles to delta waves (Steriade 2001; Steriade et al. 1997). A series of HVRS discharges under AW state was occasionally followed by HVRS with abundant sleep spindles (Fig. 4). That might result in hyperpolarization of thalamocortical cells. Consequently, dynamic changes in membrane potentials of corticothalamic/thalamocortical cells might account for the termination of spontaneous HVRS discharges and alterations in vigilance levels.

In summary, the results about HVRS discharges shown in the present study, coinciding with immobile behavior, high incidence during the transition of vigilance states, bilateral synchronization and diffusive distribution in frontoparietal cortical areas, similar temporal patterns of innocuous evoked potentials under SWS and HVRS discharges, analogous temporal profiles between spontaneous and proconvulsant-induced discharges, and reduction of frequencies of HVRS discharges by ethosuximide, suggest that spontaneous paroxysmal HVRS discharges in Long Evans rats might be an absence-like seizure activity. Several basic properties of human’s SWDs and rat’s HVRSs during wake-sleep states were compared. Possible functional operation of cortico-thalamocortical networks for generation and termination of HVRS discharges was also discussed.
recording and analysis of cortical field potentials in freely moving rats. 


