Relation Between Activities of the Cortex and Vibrissae Muscles During High-Voltage Rhythmic Spike Discharges in Rats

Fu-Zen Shaw¹ and Yi-Fang Liao²

¹Institute of Neuroscience and ²Institute of Medical Sciences, Tzu Chi University, Hualien, Taiwan

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Shaw, Fu-Zen and Yi-Fang Liao. Relation between activities of the cortex and vibrissae muscles during high-voltage rhythmic spike discharges in rats. J Neurophysiol 93: 2435–2448, 2005. First published December 29, 2004; doi:10.1152/jn.00999.2004. Paroxysmal 5- to 12-Hz high-voltage rhythmic spike (HVRS) activities, which are accompanied by whisker twitching (WT), are found in Long Evans rats, but the function of these HVRS activities is still debated. In four major functional hypotheses of HVRS discharges, i.e., alpha tremor, attention/mu rhythm, idling/mu rhythm, and absence seizure, the first two hypotheses emphasize WT behavior in HVRS bouts. Whisker movement is primarily determined by activation of intrinsic and extrinsic muscles. To clarify the role of WT in HVRS activities, simultaneous recording of the activities from the cortex and intrinsic/extrinsic neck muscles were performed. Most HVRS bouts (68.8%) revealed no time-locked WT behavior in a 2-h recording session. In addition, WT primarily arose from active protraction due to activation of intrinsic muscles followed by passive retraction. A small portion of WT resulted from activation of both vibrissae muscles with dynamic frequency-dependent phase shifts. Onset of the rhythmic vibrissae EMG significantly lagged behind HVRS onset, and the mean duration of vibrissae muscle activity was one-third to a one-half of a HVRS bout. Moreover, a greater number of HVRS bouts were associated with a longer HVRS duration and higher oscillation frequency. Oscillation frequencies of HVRS activities without WT behavior were significantly lower than those with WT. Under peripheral sensory/motor blockade by xylocaine injection, oscillation frequencies of HVRS bouts significantly decreased, but no remarkable changes in the number or duration of HVRS bouts were observed. Compared with vibrissa muscle activity during WT and exploratory whisking, the duration of muscular activity in each cycle was apparently longer during whisking bouts. Based on these results, overemphasis of the role of WT on HVRS activities might not be appropriate. Instead, HVRS discharges may be associated with absence seizure or idling state. In addition, peripheral inputs, including WT, may elevate the oscillation frequency of HVRS bouts. Moreover, different muscular controls may exist between WT and whisking.

INTRODUCTION

Paroxysmal 5- to 12-Hz high-voltage rhythmic spike (HVRS) activities or compatible rhythmic neuronal activities have been found in Long Evans rats by several laboratories (Kaplan 1985; King 1979; Nicolelis et al. 1995; O’Connor et al. 2002; Semba et al. 1980; Shaw 2004; Vanderwolf 2000). However, the function of these HVRS activities in Long Evans rats is still the subject of some debate. At least four possible functional hypotheses have been proposed: alpha tremor/mu rhythm (Semba and Komisaruk 1984; Semba et al. 1980), attention/mu rhythm (Fanselow et al. 2001; Nicolelis and Fanselow 2002; Nicolelis et al. 1995), idling/mu rhythm (Fontanini and Katz 2005), and absence seizure (Buzsáki et al. 1990; Kaplan 1985; Shaw 2004). Previously, we showed that paroxysmal HVRS discharges may be associated with absence seizure based on several lines of evidence (Shaw 2004); bilateral cortical synchronous pattern, the appearance of HVRSs under sudden arrest behavior (immobility), mostly occurring at the transition of vigilance states, rare immediate responsiveness to innocuous stimulation, the identical pattern between spontaneous HVRS and pentylenetetrazol- and penicillin-induced rhythmic activities, and a reduction in the HVRS number by ethosuximide. In addition, intermittent rhythmic whisker twitching (WT), which phase-locks to cortical spikes or neural bursts, is observed during HVRS bouts ( Nicolelis et al. 1995; Semba et al. 1980; Shaw 2004). The phenomena observed in Long Evans rats are similar to observations from genetic epileptic rats, such as WAG/Rij and GAERS strains (Coenen et al. 1991; Danober et al. 1998; Snead et al. 1999), and are similar to those found in humans with absence epilepsy (Niedermeyer 1999b). Because rhythmic WT activity falls in the alpha frequency range and is controlled by the thalamocortical network (Semba and Komisaruk 1984; Semba et al. 1980), WT behavior is suggested to be associated with a movement disorder, i.e., alpha tremor. Moreover, WT vigorously affects the thalamocortical response from the vibrissae (Fanselow and Nicolelis 1999; Fanselow et al. 2001), and afterward, a dynamic modulatory role in sensory information coming from vibrissae was proposed (Nicolelis and Fanselow 2002). These two functional hypotheses of HVRS activities emphasize the motor outcomes of the vibrissae. In this scenario, WT should be observed in most HVRS bouts and most rats, and it also should display a considerable duration within a HVRS bout. To elucidate the contribution of WT to spontaneous HVRS activities, the portion and duration of WT during HVRS discharges must be quantified.

Rats use the vibrissae to acquire tactile sensory information by a series of coordinated, rhythmic sweeps (Carvell and Simons 1990; Kleinfeld et al. 1999; Nicolelis and Fanselow 2002). Movement of the follicle is controlled by the facial motor nerve, which innervates two classes of muscles, i.e., intrinsic and extrinsic muscles. Contraction of intrinsic muscles has been shown to correlate with retraction of the vibrissae (Carvell et al. 1991). In contrast, contraction of extrinsic muscles leads to retraction of the vibrissae (Berg and Kleinfeld 2003a). A great number of studies (Carvell et al. 1990; Fanselow and Nicolelis 1999; Krupa et al. 2001;
O’Connor et al. 2002) have been carried out to investigate the functional correlations between brain activity and whisking behaviors, which is associated with textural discrimination in primates (Ahissar and Vaadia 1990; Nicolelis and Fanselow 2002). In particular, exploratory whisking with a large-degree whisker deflection was recently shown to result from activation of both intrinsic and extrinsic muscles with an antiphase relation (Berg and Kleinfeld 2003a). In contrast to extensive
investigations of whisking behavior, muscular control of tremor-like WT remains largely unknown (Semb et al. 1980).

In this study, we asked the following questions. 1) Does time-locked WT behavior appear with most HVRS discharges within a 2-h recording session? What is the distribution of intrinsic/extrinsic muscles in the generation of WT and the phase relation between intrinsic and extrinsic muscles during WT? 2) What is the role of peripheral sensory/motor activities in HVRS discharges? In particular, we quantified the numbers, durations, and oscillation frequencies of HVRS discharges within a 2-h recording before and after injection of xylacaine.

METHODS

Adult Long Evans rats (300–350 g; n = 19) were used in the study. Animals were kept in a sound-attenuated room under a 12:12-h light-dark cycle (7:00 a.m. to 7:00 p.m. lights on) with food and water provided ad libitum. The care and all aspects of experimental manipulation of our animals were in strict accord with guidelines from the National Institutes of Health, and they also were reviewed and approved by the Institute Animal Care and Use Committee of Tzu Chi University. The detailed procedures of animal preparation were described in a previous study (Shaw et al. 2002). Briefly, the recording electrodes were implanted under pentobarbital sodium 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. A midline scalp incision was made. The dorsal surface of the skull was exposed and cleaned. Six stainless steel microwires (7955, A-M systems) were used to record cortical activities. The parietal lead was located in the barrel cortical region (Chapin and Lin 1990). 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 (7935, A-M systems) were first passed through a second-order high-pass Bessel filter (cut-off frequency of 0.5 Hz) to reduce low-frequency drift. Then, constant-current stimulation. The extrinsic muscle was accessed through an incision along the midline of the skull. These muscle groups attach on the frontal bone behind the nasofrontal suture, close to the incision. A better location of wires was identified by obvious whisker retraction after electrical stimulation of the extrinsic muscles. Subsequently, two wires were gently inserted into the fibers and sutured to the connective tissue. 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 (chlorotetracycline) and housed individually in cages for recovery.

Two weeks after surgery, animals were placed in individual clear acrylic chambers, so that their behaviors could easily be observed. To allow rats to habituate to the experimental apparatus, each rat was placed in the recording environment at least two times (1 h/day) before 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 2-h recording period per day in conjunction with video tracking of behavioral changes. To evaluate the peripheral contribution of HVRS activities, xylacaine (8 mg in 0.4 ml), a local anesthetic, was contralaterally injected (n = 9) into the mystacial pad to block contralateral sensory/motor information. Subsequently, a 2-h recording was carried out 30 min after xylacaine injection. All recordings were performed from 10:00 a.m. to 2:00 p.m. to minimize circadian variation. Exploratory whisking behavior was induced by a novel open-top environment as well as placement of another animal near the recording chamber. The experimental rats often stood up when in the presence of another rat and performed abundant spontaneous exploratory whisking behaviors in the air.

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, and the tips of the vibrissae microwire electrodes were visually examined.

Monopolar EEGs (0.3–1,000 Hz) recorded from skull electrodes and bipolar EMGs of the intrinsic/extrinsic vibrissae and neck muscles (100–500 Hz) were buffered with field effect transistors and amplified (Shaw et al. 2002). A grounded plate was placed under the recording chamber to reduce electromagnetic interference (Shaw et al. 2003). All electrical signals were digitized at 2 kHz with a 12-bit A/D card (PC16023E, National Instruments). The differential EMG was full-wave rectified and smoothed by 40-point moving average (∼50 Hz) (Shaw et al. 2002).

The HVRSs were characterized by a barrage of large sharp spike discharges (>0.4 mV) with negative polarity (Fig. 1). HVRSs were found in both frontal and parietal leads, but seldom occurred in the occipital area. In addition, the power spectra of HVRS discharges displayed a dominant frequency peak of around 7–10 Hz in company with several harmonics (Fig. 1). These characteristics of HVRS activities have been well documented in previous studies (Buzsáki et al. 1990; Coenen et al. 1991; Danber et al. 1998; Shaw 2004).

Accordingly, the number of HVRSs in a 2-h recording period could be measured. In an attempt to extract characteristics of HVRS episodes, several analyses were performed. To determine the phase relationship between cortical spikes and smooth EMGs of intrinsic/extrinsic and neck muscles, a spike-triggered average was used herein. The frontal cortical activity was selected as a reference signal because of the neighboring of whisker motor area and the presentation of prominent cortical spike amplitude. Two steps were essential for detecting each individual spike within an HVRS episode. Selected HVRS segments were first passed through a second-order high-pass Bessel filter (cut-off 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 peaks of selected spikes were subsequently identified by searching for minimal/maximal limitations. Therefore the timing of each individual spike could be determined. According to these time points of cortical spikes, smooth EMGs (of the intrinsic/
extrinsic and neck muscles) could be extracted and averaged. The time lags between cortical spikes and smooth EMGs could be measured from time difference between the two peaks. In addition, Fourier transformation with a Hamming window was used to calculate the power spectra of selected HVRS episodes and coincident smooth EMG. Consequently, the prominent frequency peak of a selected HVRS episode in the range of 5–14 Hz could be identified. Furthermore, time lag (\( \tau \)) was transferred into phase lag (\( \phi \)) according to the equation of \( \phi = 2\pi \times \tau \) to further evaluate the relationship between time/phase lag and oscillation frequency (\( f \)). In addition, a cross-correlogram was used to determine the phase relationship between intrinsic and extrinsic muscles during exploratory whisking. The amplifier, data acquisition system, and entire analysis programs have been described in detail elsewhere (Shaw et al. 2002). Data are expressed as the means ± SE in this study.

RESULTS

Seventeen rats (89.5%, \( n = 19 \)) displayed obvious spontaneous HVRS discharges with variable numbers within a 2-h recording session (Table 1). Durations of HVRS bouts varied from 0.88 to 88.14 s (5.71 ± 0.16 s, \( n = 1908 \)). Previous studies (Fanselow et al. 2001; Nicolelis and Fanselow 2002; Nicolelis et al. 1995; Semma and Komisaruk 1984; Semma et al. 1980) emphasized the time-locked phenomenon between rhythmic whisker behavior and cortical spikes during paroxysmal HVRS discharges. Accordingly, we simply classified HVRS episodes into two categories: the first accompanied by phase-locked whisker EMG activities, which were associated with WT, and the other without time-locked whisker behavior or in the absence of a phasic EMG.

### Table 1. Distribution of rhythmic phase-locked intrinsic/extrinsic and neck muscle activities during HVRS discharges within a 2-h recording

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Sum 1,908 1,312 527 6 63 243

NO, no rhythmic intrinsic or extrinsic muscle activity; INT, rhythmic intrinsic muscle activity only; EXT, rhythmic extrinsic muscle activity only; INT + EXT, both rhythmic intrinsic and extrinsic muscle activity; NECK, rhythmic nuchal muscle behavior (nodding behavior); NA, not available because of a bad neck EMG recording.

### Distribution of rhythmic activities of intrinsic/extrinsic and neck muscles during HVRS discharges

Paroxysmal HVRS discharges were often accompanied by a lack of rhythmic phasic activities in both intrinsic and extrinsic muscles (Fig. 1A). In another case, spontaneous rhythmic activity of intrinsic/extrinsic muscle or tooth chatting was seen during an HVRS discharge, but it displayed no clear time-locked relation to cortical spikes (Fig. 1B). This unlocked muscular activity started before HVRS discharge and usually persistent throughout HVRS episode. Thus these unlocked rhythmical muscular activities might not be related to HVRS discharges. A number of EMG recordings revealed the absence of phasic activity (\( n = 1184 \) bouts) or unlocked phasic EMGs (\( n = 128 \)) during HVRS discharges.

In the phase-locked group, at least three patterns were identified. First, rhythmic muscular activity was found in intrinsic muscle and revealed a time-locked pattern with cortical spikes (Fig. 2). In particular, the rhythmic muscular activity occurred at the beginning of an HVRS bout. Second, time-locked activities to cortical spikes were simultaneously found in both intrinsic and extrinsic muscles (Fig. 3). Third, the time-locked behavior was seen only in extrinsic muscle. The distribution of rhythmic time-locked intrinsic, extrinsic, or both muscle activities is summarized in Table 1. Time-locked WT behavior appeared in one-half of the rats, and the distribution of HVRS discharges classified by vibrissae muscle activity showed a particular pattern (\( P < 0.0001 \) by \( \chi^2 \) test). Most HVRSs (68.8%, \( n = 1908 \)) were not associated with time-locked WT behavior. In a total of 1,908 HVRS instances, only 596 HVRSs (31.2%) displayed rhythmic phase-locked activities to cortical spikes of HVRS discharges. WT being locked to a cortical spike primarily arose from activation of intrinsic muscles (27.6%). A small portion of WT (3.3%) seemed to result from activation of both muscles. A very small percentage of WT bouts (0.3%) arose from extrinsic muscle. In addition, we also found a considerable portion of phase-locked activities in neck EMG recordings during HVRS discharges (Table 1). Nuchal muscle activity was recorded in concordance with rhythmic WT behavior (Figs. 2 and 3B) or was independent of whisker movement (Fig. 3A). During 243 instances of rhythmic neck muscle activities, 184 instances (75.7%) were concomitant with WT. In particular, rhythmic nuchal activity appeared in rats with rhythmic WT behavior (Table 1).

### Comparison of basic characteristics of HVRS discharges with/without WT

The number, duration, and oscillation frequency of HVRS discharges within a 2-h recording period were compared. The rats were divided into two groups based on whether or not there was WT behavior. Although rat 16 displayed no obvious WT (Table 1), it revealed a few phase-locked nodding behaviors. Because the pattern of rhythmic neck muscle activity was very similar to that recorded from the vibrissa (Fig. 3B), rat 16 was assigned to the WT group for further analysis. HVRS numbers of rats with WT (174 ± 28) were higher than those without WT (57 ± 23; \( P < 0.01 \) by Student’s t-test). Thus the rhythmic muscular activities appeared in animals with more HVRS bouts. Moreover, mean durations of HVRS bouts in rats with
WT (6.44 ± 1.03) were significantly higher than those without WT (2.66 ± 0.20; P < 0.0001 by Mann Whitney rank-sum test). The oscillation frequency of HVRS discharges also markedly differed in both groups (WT, 8.44 ± 0.07 Hz; no WT, 8.07 ± 0.14; P < 0.05 by Student’s t-test). Nonetheless, increased HVRS numbers were markedly related to a longer HVRS duration (r = 0.52, P < 0.05) and also led to a higher oscillation frequency (r = 0.61, P < 0.01).

To determine the role of WT on HVRS discharges, oscillation frequencies and durations of HVRS episodes were individually compared in rats with WT. Oscillation frequencies of HVRS bouts with WT were consistently higher than those
without WT, and seven of nine rats showed statistical significance (Fig. 4). In contrast, no consistent trend was found between changes in HVRS durations and WT behaviors, although four rats displayed significantly shorter durations under HVRS discharges with WT (Fig. 4).

With regard to cortical spike during HVRS bouts, the time lag of rhythmic intrinsic muscle activity was $-33.5 \pm 0.4$ ms and displayed no remarkable relation with oscillation frequencies of HVRSs (Fig. 5A). Most time lags (77.8%) in intrinsic muscle fell in the range of from $-30$ to $-50$ ms. Alternatively,
a remarkable frequency-dependent relationship was found between phase lags and WT oscillation frequency. Phase lag of intrinsic muscle with regard to cortical spike decreased as WT oscillation frequency increased. In contrast, a frequency-dependent relationship was found in the time lag of the extrinsic muscle group (−26.9 ± 0.8 ms). A large portion (88.2%) of time lags in extrinsic muscle occurred from −20 to −40 ms. Similar positive frequency-dependent trend was also shown in phase lags. Moreover, fluctuations of lag differences between intrinsic and extrinsic muscle pairs revealed a significant relation with the oscillation frequency of HVRS bouts (Fig. 5C). A similar pattern was also seen in phase lags. That is, during WT, phase lags between intrinsic and extrinsic muscle activities were related to oscillation frequency of HVRS discharge. All phase lags between intrinsic and extrinsic muscles were smaller than π/2. Moreover, several WT instances display an in-phase pattern, i.e., lag difference = 0. Thus during WT intrinsic and extrinsic muscles could be activated simultaneously. That is, two antagonist muscles worked against each other.

In consideration of onsets of HVRS bouts and vibrissae muscle activity, only six instances (n = 590 bouts) displayed phase leading or a simultaneous pattern with HVRS onset in intrinsic muscle. No phase leading instance (n = 69) was found in extrinsic muscle. The onsets of rhythmic intrinsic and extrinsic EMGs significantly lagged behind HVRS onsets (intrinsic, 479 ± 22 ms, P < 0.0001 by Wilcoxon signed-rank test; extrinsic, 510 ± 33 ms, P < 0.0001). About 66.8% of all rhythmic intrinsic muscle activities began within 500 ms. In addition, mean durations of rhythmic vibrissae muscle activities were remarkably shorter than those of HVRS bouts (intrinsic, 44.5 ± 1.0%, P < 0.0001; extrinsic, 32.3 ± 3.3%, P < 0.0001). Thus most of the rhythmic vibrissae muscle activity appeared at the beginning of HVRS bouts and on average lasted for one-third to one-half of the HVRS bout. Considering HVRS bouts with rhythmic activities in both intrinsic and extrinsic muscle pairs (n = 63), the onset latency of intrinsic muscle (455 ± 29 ms) was significantly smaller than that of extrinsic muscle (499 ± 31 ms, P < 0.0001 by Wilcoxon signed-rank test), and the proportional duration of intrinsic muscle (51.4 ± 3.5%) was larger than that of extrinsic muscle (32.4 ± 3.4%, P < 0.0001). According to the distribution and duration of intrinsic and extrinsic muscles during HVRS bouts, intrinsic muscle may play a profound role in the generation and maintenance of WT behavior.

**Effect of xylocaine on HVRS discharges**

In an attempt to determine the contribution of peripheral signals to HVRS discharges, a local anesthetic, xylocaine, was contralaterally (n = 9) or bilaterally (n = 3) injected into the mystacial pad. Paroxysmal HVRS discharges displayed in all testing rats after administration of xylocaine either unilaterally or bilaterally. A flattened recording was observed in the intrinsic muscle trace after xylocaine injection, and unperturbed muscular activities were recorded in extrinsic as well as nuchal muscles (Fig. 6). In particular, rhythmic muscular activities were persistently recorded in extrinsic and neck muscles during HVRS bouts. The number, duration, and oscillation frequency of HVRS discharges within a 2-h recording session were compared with/without xylocaine injection. The number (120 ± 34 vs. 110 ± 27, P = 0.64 by paired t-test) and duration (5.26 ± 1.12 ms, 5.34 ± 0.77 ms, P = 0.91) of HVRS discharges within a 2-h recording session showed no remarkable difference before and after xylocaine injection. Most interestingly, oscillation frequencies of HVRS activities without xylocaine administration (8.30 ± 0.12 Hz) were significantly higher than those with xylocaine injection (7.77 ± 0.09 Hz, P < 0.0001).

**Comparison of exploratory whisking and WT**

From a visual inspection of both whisker behaviors, the whisker deflection during exploratory whisking was obviously larger than that of the tremor-like WT. This is consistent with previous observations (Semba and Komisaruk 1984; Semba et al. 1980). However, the detailed muscular operation of intrinsic and extrinsic muscles during WT and whisking remains largely unknown. Exploratory whisking is a voluntary movement. Therefore clear theta activity was recorded in the occipital region (Fig. 7). In addition, intrinsic and extrinsic muscle activity displayed an antiphase pattern during exploratory whisking. These results are consistent with previous studies (Berg and Kleinfeld 2003a; Kleinfeld et al. 1999). Based on cross-correlogram of rectified intrinsic and extrinsic muscle activities, an almost out-of-phase relation was found in explora-
atory whisking (Fig. 8A). In addition, phase lags between intrinsic and extrinsic EMGs were not significant to be related with whisking frequency (Fig. 8B), which is consistent with previous observations (Berg and Kleinfeld 2003a). Most phase lags of whisking (~90%) between intrinsic and extrinsic muscles were >3/4π. In contrast, a small phase shift with temporal overlapping pattern was seen in WT instance at time 0 (Fig. 8A). In addition, durations of intrinsic and extrinsic muscle
activities during whisking were significantly longer than those during WT (Fig. 8C).

**DISCUSSION**

In this study, we observed that spontaneous HVRS discharges were present in a number of Long Evans rats. The number of HVRS discharges was variable. Most HVRS bouts (68.8%) did not show time-locked WT behavior. In addition, WT primarily arose from active protraction followed by passive retraction. A small portion of WT resulted from activation of both vibrissae muscles with considerable overlap in their muscular activities. Onsets of rhythmic intrinsic/extrinsic EMGs significantly lagged behind that of HVRS bout, and the mean duration of vibrissa muscle activity lasted for one-third to one-half of an HVRS bout. In addition, a higher HVRS appearance was accompanied by a longer HVRS duration and higher oscillation frequency. Oscillation frequencies of HVRS activities without WT behavior were significantly lower than those with WT. In the case of vibrissae sensory/motor blockade by a xylocaine injection, oscillation frequencies of HVRS bouts were significantly lower, but without a remarkable change in the number or duration of HVRS discharge. In comparison of intrinsic and extrinsic muscle activities during WT and exploratory whisking, the duration of muscular activity in each cycle was apparently longer during whisking bouts.

**Technique consideration**

In this study, the skull EEG was recorded and used to evaluate phase relationship between cortical activity and vibrissae muscles. The spike amplitude of HVRS in frontal area was higher than that of parietal, and HVRS was less extent to occipital region (Fig. 1A). It is corroborated by previous studies (Meeren et al. 2002; Shaw 2004). With regard to frontal cortical spike, we found subtle relationship between HVRS activities and vibrissae muscle activity, particularly for WT. The skull EEG signal recorded from screw is contributed by brain activity from both local brain region and neighboring areas. It might be too coarse to delineate fine control of muscular activity from brain. It is known that local field potential (LFP) mostly reflects the summation of postsynaptic potentials within a local region, and the amplitude of LFP signals is correlated to the degree of coherent activity in a population of neurons (Buzsáki et al. 1988; Lopes da Silva 1991). Accordingly, LFP may be more beneficial in elucidating the detailed relationship between brain activity and behavioral state rather than skull EEG (Buzsáki et al. 1988; Meeren et al. 2002).

**Possible functional roles of spontaneous HVRS discharges in Long Evans rats**

In this study, we found that 31.2% of HVRS discharges in 17 rats revealed WT. It is lower than those observed in previous studies [e.g., ~48.7% from 15 rats (Shaw 2004) and 79.1% from 5 rats (Nicollelis et al. 1995)]. In both previous studies, they seemed to analyze the animals with abundant HVRS occurrence. In this study, ~90% of rats displayed variable number of HVRS activity, and WT particularly occurred at the rats with higher HVRS number (Table 1). In our samples, one-half of the rats only showed a few HVRS bouts during a 2-h recording session. Accordingly, the lower proportion of WT during HVRS discharges observed here might arise from a considerable portion of animals with lower HVRS number. Based on these results, WT may not appear in most rats and most HVRS discharges.

At least four 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 (Fanselow et al. 2001; Nicolelis and Fanselow 2002; Nicolelis et al. 1995), idling/mu rhythm (Fontanini and Katz 2005), and absence seizure (Buzsáki et al. 1990; Kaplan 1985; Shaw 2004). The first two hypotheses emphasize WT behavior during HVRS bouts. However, we
found that WT was not commonly observed in all Long Evans rats. The data are corroborated by observations of humans with absence epilepsy, i.e., facial twitching or eye blinking is not exhibited in every patient (Holmes et al. 1987; Niedermeyer 1999b). In addition, several lines of evidence, including bilateral cortical synchronous pattern, the appearance of HVRSs under sudden arrest behavior (immobility), mostly occurring at the transition of vigilance states, rare immediate responsiveness to innocuous stimulation, identical patterns between spontaneous HVRSs and pentylenetetrazol- and penicillin-induced rhythmic activities, and a reduction in the HVRS appearance by ethosuximide, indicate that spontaneous HVRS discharges may be associated with absence seizure (Shaw 2004). According to these results, overemphasis of the role of tremor-like WT in HVRS bouts might not be appropriate. In addition, during WT activation of intrinsic/extrinsic muscles were temporally

**FIG. 7.** Typical polygraphic recordings of exploratory whisking. Whisking is a voluntary movement, which was shown by the presence of theta activity in the occipital cortex (inset). Exploratory whisking is composed of an interplay of rhythmic EMGs of intrinsic and extrinsic muscles with an antiphase relation (expanded at the bottom).

**FIG. 8.** Comparison of the duration and relation of activities of the intrinsic and extrinsic muscles under whisking and WT. A: cross-correlogram of intrinsic and extrinsic EMGs during exploratory whisking and WT. An antiphase relation was found in whisking. A temporally overlapping pattern was identified from the time 0 activity of the correlogram of intrinsic and extrinsic EMGs during WT. B: phase lags between intrinsic and extrinsic EMGs displayed no remarkable trend with whisking frequency ($n = 248$ bouts). A large portion of phase lags ($\sim 90\%$) were larger than $3/4\pi$. C: comparison of durations of intrinsic and extrinsic EMGs during individual cycles of whisking and WT bouts. Durations in both intrinsic and extrinsic muscles under whisking were significantly longer than those of WT. $^*P < 0.01; ^{**}P < 0.0001$ by Student’s $t$-test.
Mu rhythm in humans or sensorimotor rhythm in cats appears in a quiet, motionless state, and its frequency falls in the alpha frequency range (Niedermeyer 1999a). Rolandic mu activity is dampened by limb movements and tactile stimulation (Hari and Salmelin 1997; Pfurtscheller 1999). Furthermore, sensorimotor mu rhythm may be associated with an idling/ inertia state (Pfurtscheller et al. 1996). Although paroxysmal HVRS discharges exhibited during abrupt immobility and their oscillation frequencies primarily fell in the alpha frequency range, several remarkable differences from mu rhythm have been observed. First, HVRS activity appears simultaneously in both hemispheres (Shaw 2004). The maximal amplitude of HVRS activity was found at the frontal and/or lateral parietal region (Fig. 1A) (Meeren et al. 2002; Shaw 2004). This differs from mu rhythm, which is dominant in the rolandic central region but lower in the frontal region (Hari and Salmelin 1997; Kuhlman 1978). Mu rhythm is primarily localized in the hand representative area of the postcentral gyrus (Hari and Salmelin 1997; Kuhlman 1978), and it can begin in one hemisphere and then develop quas simultaneity pattern in both hemispheres (Bouyer et al. 1983; Pfurtscheller 1999). Second, tremor-like WT behavior during HVRS bouts was emphasized by Semba’s and Nicolelis’s groups (Fanselow et al. 2001; Nicolelis and Fanselow 2002; Nicolelis et al. 1995; Semb and Komisaruk 1984; Semb et al. 1980). A considerable appearance of WT was also shown in several rats (Table 1). In contrast, no comparable tremor activity has been reported during presentation of mu rhythm (Hari and Salmelin 1997; Niedermeyer 1999a). On the other hand, limb movement stops hand-dominant mu rhythm (Hari and Salmelin 1997; Niedermeyer 1999a), but tremor-like WT behavior is coincident with HVRS activity (Table 1) (Nicolelis et al. 1995; Semb and Komisaruk 1984; Semb et al. 1980). Third, gentle tactile stimulation is able to terminate mu rhythm (Hari and Salmelin 1997). In contrast, innocuous stimulation applied to either the whisker or tail is not enough to stop HVRS activities (Nicolelis et al. 1995; Shaw 2004). Instead, nociceptive stimulation ceases most HVRS discharges (Shaw 2004). Last, effects of proconvulsants and anticonvulsant on HVRS activities in Long Evans rats (Shaw 2004) are similar to those observed from genetic absence epileptic rats (Coenen et al. 1991; Danober et al. 1998; Snead et al. 1999). The anticonvulsant result is also consistent with that of absence epileptic patient (Niedermeyer 1999b). Based on these data, paroxysmal HVRS discharges may be related to absence seizure rather than sensorimotor mu rhythm.

### Contribution of peripheral inputs to HVRS discharges

Nicolelis and colleagues (Fanselow and Nicolelis 1999; Fanselow et al. 2001; Nicolelis and Fanselow 2002) emphasized a cyclic modulatory role in incoming information from the whiskers during HVRS bouts, particularly for the time-locked WT behavior. In this study, during HVRS bouts, a considerable portion of nodding behavior (75.7%, n = 243) was found to be associated with WT (Semb et al. 1980), which has not been discussed in previous studies (Nicolelis and Fanselow 2002; Nicolelis et al. 1995; Semb and Komisaruk 1984; Shaw 2004). That is, time-locked rhythmic muscular activity is not only found in the vibrissae/facial region, but may also possibly be present in other muscle groups. In addition, a number of previous studies have indicated that a near-synchronous HVRS activity displays in bilateral frontoparietal areas (Coenen et al. 1991; Danober et al. 1998; Meeren et al. 2002; Shaw 2004). Thus HVRS activity is not limited to whisker representative areas. Moreover, a comparable cycle-dependent change in the early component of somatosensory evoked potentials elicited by electrical stimulation of a rat’s tail was found during HVRS bouts (FZ Shaw and SY Lee, unpublished observations). Based on these observations, the modulatory role of sensory inputs during an HVRS bout may be a global effect and might not be limited to whisker-related brain regions.

In addition to sensory modulatory function, we found that oscillation frequencies of HVRS bouts with WT were consistently higher than those without WT (Fig. 4). It is consistent with our previous result (Shaw 2004) that oscillation frequencies of HVRS activities under the waking state are higher than those under sleep, which displayed no WT. In particular, increase of oscillation frequency of HVRS discharge might be associated with shortening interspike intervals of HVRS activ-


Different muscular controls of whisker movement during HVRS discharges and whisking

The whisker deflection of WT was smaller than that of exploratory whisking from behavioral observations. Displacement depends on both the magnitude and duration of the rectified EMGs. The amplitude of intrinsic EMG of tremor-like WT is lower than that of whisking (Kleinfeld et al. 1999; Sembra and Komisaruk 1984). We observed similar patterns in most rats (Fig. 3A). In addition, we also found that the durations of individual cycles of both intrinsic and extrinsic EMGs during WT were significantly shorter than those of whisking (Fig. 8C). We found that most WTs resulted from activation of the intrinsic muscles (Table 1). According to these observations, whisker deflection of WT will be smaller than that of whisking. Considering the case of activation of both vibrissae muscles, an antiphase interaction between intrinsic and extrinsic muscles was shown in exploratory whisking (Figs. 7 and 8A). Most phase lags (~90%) between intrinsic and extrinsic muscles were >3/4 \( \pi \). Thus during whisking, full protraction of the vibrissae (activation of intrinsic muscles) is followed by the beginning of retraction of the vibrissae (activation of extrinsic muscles) (Berg and Kleinfeld 2003a). In contrast, a temporal overlap of muscular activities was observed between intrinsic and extrinsic muscles (Figs. 3 and 8A). The time difference between extrinsic and intrinsic EMGs was within the activating duration of extrinsic and intrinsic EMGs (Figs. 5C and 8C). All phase lags between intrinsic and extrinsic muscles were smaller than \( \pi /2 \). That is, the vibrissae retract before finishing the protraction. This counteracts whisker movement. Based on these results, the whisker deflection of tremor-like WT should be smaller than that of whisking.

The control of whisking is believed to primarily be generated by a central pattern generator located in the medulla in cooperation with several hierarchical sensorimotor loops (for review, see Kleinfeld et al. 1999). Based on simultaneous recordings of unit activities from multiple vibrissae-related regions (Nicolelis et al. 1995) and a lesion study (Sembra and Komisaruk 1984), the tremor-like WT is controlled by the corticothalamic network, particularly for the cortex. A general concept of the generation of WT results from activation of intrinsic muscles only. We also confirmed this viewpoint (Table 1). An almost constant time lag was found between cortical spikes and intrinsic EMGs (Fig. 5A) (Nicolelis and Fanselow 2002; Nicolelis et al. 1995). This may imply the existence of direct cortical influence on the intrinsic muscles. In addition to simple activation of intrinsic muscles in WT, two more situations of activations of intrinsic and/or extrinsic muscles were found (Table 1). In particular, a frequency-dependent phase shift was found in a small portion of WT (Fig. 5C; Table 1). This strikingly differs from the constant phase during whisking (Fig. 8B) (Berg and Kleinfeld 2003a). Determining what mechanism accounts for the frequency-dependent presentation in both vibrissae muscles remains to be further studied. A model of simple sensory feedback from the vibrissae and a push-pull circuitry model, which have been described in detail elsewhere (Berg and Kleinfeld 2003a), might be rationally ruled out because time differences of several cases were approximate to zero and WT can be caused by activation of extrinsic muscles only (Fig. 5C; Table 1). Instead, direct cortical control accompanied by selective activation of neuromodulators, such as serotonin on facial motor neurons (McCall and Aghajanian 1979), may be a good candidate. This concept is partially supported by a recent study, which showed that direct rhythmic microstimulation of the motor cortex can drive frequency-compatible whisking-like behavior (Berg and Kleinfeld 2003b).

In summary, we found that most HVRS bouts contained no time-locked WT. Thus overemphasis of the role of tremor-like WT in HVRS activity might not be appropriate. WT primarily arises from active protraction followed by passive retraction. A small portion of WT resulted from activation of both vibrissae muscles with dynamic frequency-dependent phase shifts, which remarkably differs from the motor control of whisking. WT onset lagged behind HVRS onset, and WT appeared at the beginning of HVRS bouts and lasted for one-half the duration of the HVRS bout. After peripheral sensory/motor blockade by xylocaine, no obvious changes were seen in the number or duration of HVRS bouts. This may imply that WT is an inherent component of HVRS bouts. According to the intrinsic properties of WT and electrophysiological and pharmacological evidence (Shaw 2004), HVRS discharges may be associated with absence seizure rather than mu rhythm. Furthermore, peripheral signals, including WT, may drive HVRS bouts to a higher oscillation frequency.

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