Vibrissa Myoclonus (Rhythmic Retractions) Driven by Resonance of Excitatory Networks in Motor Cortex

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Submitted 30 April 2006; accepted in final form 21 June 2006

Castro-Alamancos, Manuel A. Vibrissa myoclonus (rhythmic retractions) driven by resonance of excitatory networks in motor cortex. J Neurophysiol 96: 1691–1698, 2006. First published June 28, 2006; doi:10.1152/jn.00454.2006. Rodents use rhythmic vibrissae movements to sense the environment. It is currently unclear whether intrinsic activity in the vibrissa motor cortex (vMI) is capable of driving vibrissa movements on a cycle-by-cycle basis. Disinhibition of vMI results in the occurrence of spontaneous 5- to 15-Hz synchronized oscillations. In behaving rats, this synchronous resonance of vMI is shown here to drive contralateral vibrissa movements that are phase-locked to each cycle of the oscillation. In contrast to active whisking during sensing, which consists of active protractions, the vibrissa movements produced by vMI oscillations consisted of rhythmic retractions. The results demonstrate that rhythmic motor cortex output is capable of driving vibrissa movements on a cycle-by-cycle basis. Such motor output may be primarily expressed during abnormal states such as those related to cortical myoclonous, tremors, and cortical seizures.

INTRODUCTION

Rodents use their vibrissae to navigate the environment by performing fast rhythmic vibrissae movements (Welker 1964). During active exploration, whisking consists in ellipsoid movements through the air and over objects at between 4 and 15 Hz, which are characterized by vibrissa protractions (Berg and Kleinfeld 2003a; Carvell and Simons 1990; Carvell et al. 1991, 1996; Harvey et al. 2001). Low-intensity stimulation within medial areas of motor cortex can evoke both small vibrissa retractions and rhythmic movements similar to exploratory whisking (Berg and Kleinfeld 2003b; Brecht et al. 2004; Donoghue and Wise 1982; Haiss and Schwarz 2005). This region of cortex, hereafter called vibrissae motor cortex (vMI), affects whisking through its extensive projections to premotorneuron networks that form a central pattern generator (CPG) distributed throughout the mid- and hindbrain (Hatton et al. 2002, 2003). In addition, vMI projects directly to facial motoneurons providing a route for direct control of vibrissa movements (Grinevich et al. 2005).

Despite the connectivity, it remains unclear whether spontaneous motor cortex activity can drive vibrissa movements on a cycle-by-cycle basis. Previous work showed that increased spike activity in vMI precedes the onset of whisking but does not correlate with each whisking cycle (Carvell et al. 1996). More recently, field potential activity in vMI was shown to covary with whisking in the absence of sensory feedback indicating that, in principle, vMI can initiate motion of the vibrissae on a cycle-by-cycle basis (Ahrens and Kleinfeld 2004). An additional approach would be to test whether intrinsically generated and spontaneous rhythmic activity in vMI can drive rhythmic vibrissa movements on a cycle-by-cycle basis.

Using slices and anesthetized animals, we previously showed that during disinhibition, a pure excitatory network of motor cortex generates spontaneous 5- to 15-Hz (termed 10 Hz, for simplicity) rhythmic oscillations (Castro-Alamancos 2000; Castro-Alamancos and Rigas 2002). This provides a means to test whether intrinsic rhythmic activity of the vMI, within the frequency range of normal whisking, can drive vibrissa movements on a cycle-by-cycle basis, such as a CPG does. The results demonstrate that the vMI can act as a CPG of rhythmic vibrissa movements.

METHODS

Five male Sprague–Dawley rats (300–350 g) were used in the present study. Briefly, a craniotomy is made over the vMI bilaterally based on previous microstimulation mapping (Castro-Alamancos and Borrell 1993; Donoghue and Wise 1982). Note that placement of electrodes in vMI was later confirmed by the fact that disinhibition produced vibrissa movements (see following text). Several (two to three) microelectrodes are implanted in each hemisphere within vMI between layer V and layer III (depth: 1,100–500 μm). Recordings are obtained with 100-μm-shank-diameter tungsten microelectrodes edged to a fine tip (1–5 MΩ; FHC, Bowdoin, ME) or 75-μm stainless steel wires. In addition, in one of the hemispheres, a guide cannula (Plastics One, Roanoke, VA) fitted with a dummy cannula is implanted at a depth of about 300 μm. The guide cannula serves to allow later insertion of an infusion cannula to apply drugs in the vMI. In addition, pairs of wires are placed in each whisker pad to record EMG signals. The insulated stainless steel wires (with uninsulated tips) are inserted subcutaneously and attach to the miniature pin connector on the skull. The uninsulated tips of the EMG wires terminate close to the skin in the middle of the whisker pad. The electrodes, connector, and the guide cannula are secured with dental cement and small stainless steel screws. Animals are allowed to recover from surgery for about 1 wk.

After recovering from surgery, the animals are placed in a recording chamber where they are free to move. The animal’s head connector is attached to a headstage connector containing unity gain JFET-operational amplifiers. The behavior of the animal is recorded using video (30 fps) synchronized with the electrophysiology. Electrophysiologically recorded movements consisted of bilateral field potential (FP) and multunit activity (MUA) from vMI and EMG signals from each whisker pad. Disinhibition of vMI was produced by infusing γ-aminobutyric acid types A and B (GABA<sub>A</sub> and GABA<sub>B</sub>, respectively) receptor antagonists, bicuculline (BMI; 100–300 μM), and CGP35348 (CGP; 2–10 mM) dissolved in saline. During the recording session, a cannula that is connected to a Hamilton syringe by a long flexible tube is inserted through the dummy cannula on the animal’s head and 0.2–1 μl of
volume is slowly infused over 5–10 min. Thereafter the cannula is withdrawn. This procedure interferes minimally with the behavior of the animal, which can move about during the infusion. Every animal underwent repeated applications of the same drugs on different days. This allowed testing different doses and the reproducibility of the effects per animal. For data analysis, one or two sessions of the same drug-dose application are used per animal. At the end of the experiments the animals were given an overdose of sodium pentobarbital; the brain was extracted and placed in fixative. Coronal slices were cut in a vibratome and Nissl stained. Figure 1A shows the track marking the location of the infusion cannula in the right vMI of one animal.

FP and MUA signals are band-pass filtered (1–300 and 600–6,000 Hz, respectively) and differentially recorded using one of the implanted electrodes as the reference electrode. Multiunits were detected by setting a threshold detector at twice the noise and quantified using a rate meter. The EMG is differentially recorded between the pair of implanted wires on each pad and high-pass filtered (300 Hz). The EMG signal is either plotted raw or rectified by obtaining the absolute values and low-pass filtering (100 Hz). Data analyses were performed using OriginLab (Northampton, MA) and Neuroexplorer (Littleton, MA) software.

RESULTS

Disinhibition of motor cortex in freely moving rats leads to spontaneous synchronous discharges that are followed by rhythmic oscillations of about 10 Hz. Figure 1B shows record-

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**FIG. 1.** Electrophysiological correlates of vibrissa motor cortex (vMI) disinhibition in a behaving rat. A: cresyl-violet–stained coronal section of the right hemisphere showing a track that corresponds to the implanted guide cannula used for infusion of the drugs into the vMI. B: bilateral field potential (FP) and multiunit activity (MUA) recorded from the vMI during disinhibition caused by application of bicuculline and CGP35348 (BMI + CGP, 0.1 + 2 mM, 0.2 μl volume) into the right vMI. Corresponding raw electromyographic (EMG) activity measured from the contralateral whisker pad is also displayed below the FP and MUA. 1: the infused hemisphere and the related (contralateral) whisker pad; 2: the noninfused hemisphere and the related whisker pad. C: close-up of a portion of the activity shown in A.
ings from a freely behaving rat during disinhibition (BMI + CGP; 0.1 + 2 mM, 0.2 μl volume) of the right vMI. The recordings shown consist of simultaneous FP and MUA activity from the vMI of the right and left hemispheres and EMG recordings from the corresponding whisker pads (1 refers to the infused hemisphere and the related whisker pad, 2 refers to the opposite hemisphere and whisker pad). During disinhibition, nearly 10-Hz oscillations occurred spontaneously in the infused (right hemisphere) vMI (FP1 and MUA1) every 5–15 s. The cortical activity in the infused vMI produced vibrissa movements on the contralateral (left) whisker pad (EMG1). The EMG activity evoked contralateral to the infused vMI (EMG1) is phase-locked with the initial cortical discharge and with each cycle of the cortical oscillation (Fig. 1C). In addition, the cortical activity in the vMI spread to the contralateral (left; noninfused) vMI (FP2 and MUA2) where it evoked both FP and MUA at the same frequency. The activity caused by BMI + CGP in the infused vMI (~10-Hz oscillations) and the associated vibrissal movements were completely abolished by infusing tetrodotoxin (TTX, 10 μM; 0.2 μl) by the same cannula in the presence of BMI + CGP. During TTX, the activity in the infused vMI was abolished and the activity in the

![Graphical representation of the data](image-url)
contralateral, noninfused vMI returned to normal (an example of the effect of TTX is shown in Fig. 2). Moreover, cross-correlation analyses show that during BMI + CGP, nearly 10-Hz activity originated in the infused vMI and was followed by longer-latency activity in the noninfused contralateral vMI (not shown). In the cross-correlograms, the MUA peak measured at half-maximal amplitude of the contralateral hemisphere was delayed by $3.7 \pm 0.9$ ms with respect to the infused hemisphere ($n = 5$ experiments). In contrast, during normal oscillatory activity (such as slow-wave sleep oscillations) such dependency on the infused hemisphere did not exist; slow waves occurred nearly simultaneously in both hemispheres. Thus the $\sim 10$-Hz oscillations in the contralateral hemisphere are driven by the $\sim 10$-Hz oscillations in the infused hemisphere. That the roughly 10-Hz oscillations caused by BMI + CGP are generated locally in vMI, and not by subcortical interactions, is demonstrated by the fact that the $\sim 10$-Hz oscillations occur in isolated slices of vMI in vitro (Castro-Alamancos and Rigas 2002) and are not blocked by thalamic inactivation in vivo (Castro-Alamancos 2000).

Figure 3 shows additional examples of simultaneous FP and EMG activities that were observed during application of BMI + CGP in the vMI of freely behaving animals. In some cases, the synchronous discharges in vMI did not produce significant $\sim 10$-Hz oscillations. This was common when the effect of the drugs (BMI + CGP) started to wear out or if BMI was infused alone. Single discharges of vMI activity (without the oscillatory phase) lead to long bursts of EMG activity in the contralateral whisker pad (see Fig. 3A). In some cases, the single discharge of vMI activity could produce a bout of bilateral vibrissa movements in the frequency range of whisking (Fig. 3B). This later behavior indicates that the cortical activity was triggering a brain stem whisking CPG because there was no cortical activity driving each whisking cycle. On most occasions, the activity observed during BMI + CGP resembled that shown in Figs. 1 and 3, C and D. That is, the $\sim 10$-Hz oscillations in vMI produced clearly phase-locked

![Fig. 3](http://jn.physiology.org/)

**Fig. 3.** Different types of EMG activities associated with cortical discharges produced by disinhibition (A–D) or normal active whisking without cortical discharges (E). Shown are FP recordings from the infused vMI (FP1) and the corresponding EMG (Raw EMG1) and the FP recordings from the contralateral (noninfused) vMI (FP2) and the corresponding EMG (Raw EMG2). A and B: cortical discharges that lack an oscillatory phase. C and D: more typical discharges caused by disinhibition containing an $\sim 10$-Hz oscillation. E: normal active whisking on air over a gap crossing in the absence of drugs in cortex. This is shown for comparison with the drug-induced movements. Note that in B, D, and E the movements are bilateral.
FIG. 4. 10-Hz oscillations of vMI caused by disinhibition produce cycles of vibrissa retractions. A: representative example of frame-by-frame tracking of a vibrissa movement caused by vMI disinhibition. White dot tracks the vibrissa movement in each frame (30 fps). B: rectified EMG for the sequence shown in A. C: typical example of frame-by-frame (30 fps) tracking of a vibrissa movement in a different rat during a spontaneous oscillation caused by disinhibition (left) and during normal active whisking in the absence of drugs in cortex (right). Note the vibrissa retraction and return to the point of origin, without protractions, in the whisking caused by cortical disinhibition. Units in the y-axis are relative pixels.
movements in the contralateral whisker pad with no movements in the ipsilateral whisker pad (Figs. 3C and 1) or with ipsilateral movements (Fig. 3D) that were not necessarily phase-locked to the cortical oscillations. In general, movements ipsilateral to the infused cortex were quite variable trial to trial. In some cases, it appears that the cortical activity drives simultaneously phase-locked contralateral movements and a brain stem whisking CPG giving rise to complex EMG patterns as shown in Fig. 3D. For comparison, Fig. 3E shows EMG and FP traces corresponding to normal (no drugs in cortex) active whisking. In this case, the rat was whisking on air over a gap. Because video was recorded synchronized with the electrophysiological activity, it allowed correlating the EMG activity with the type of movement produced. Vibrissae move in a single dimension producing retractions or protractions. Figure 4A shows a sequence of frames taken from the video at 30 fps during a spontaneous rhythmic vibrissa movement sequence caused by infusion of BMI + CGP (0.1 + 2 mM; 0.6 μl) in the right vMI. The first frame is the top left and the last frame is the bottom right. A white dot is placed over one of the vibrissa to track its movement. Note that the first movement is a sustained retraction (frames 4–9) followed by a sequence consisting of the relief of the initial retraction (i.e., the vibrissa returns close to the point of origin) followed by a new retraction. This sequence is repeated for several cycles at about 10 Hz. Figure 4B shows the raw and rectified EMG corresponding to the sequence shown in Fig. 4A. Figure 4C (left) shows the frame by frame tracking of a different movement caused by disinhibition from another rat. Thus the movements produced by the cortical resonance were very characteristic. The initial movement caused by the first discharge is a strong vibrissa retraction. In no case did we see a protraction of the vibrissae caused by the initial discharge (this conclusion is derived after monitoring 150 spontaneous events in 4 animals during BMI + CGP on a frame-by-frame basis). After the initial retraction, the vibrissae would return to roughly the point of origin and retract again in association with each cycle of the ~10-Hz cortical oscillations.

A video containing the sample sequence shown in Fig. 4A and other sample sequences is provided in the supplemental material (Movie1.avi; see Supplemental Information1). Thus the movements evoked by cortical oscillations caused by disinhibition were different from those produced during active whisking on air. Active whisking in the absence of drugs in cortex contained vibrissa protractions and retractions. Figure 4C (right) shows the tracking of a vibrissa movement during normal (no drugs in cortex) active whisking on air over a gap. The results presented indicate that cortical activity during the ~10-Hz oscillations caused by disinhibition can drive vibrissa movements at the same frequency. To test this, cross-correlations were calculated between the EMG activity (i.e., onset of vibrissa movement cycle) and the contralateral MUA and FP activity during the ~10-Hz oscillations. To selectively study the ability of the oscillatory phase of the ~10-Hz oscillations to drive EMG activity, the first large-amplitude discharge that triggers the oscillations was left out of these analyses. Thus these analyses measure the cross-correlation between the rhythmic phase of the discharges caused by disinhibition and the EMG. To calculate the cross-correlations, the EMG signal was rectified and a threshold detector was triggered by each cycle of the EMG signal. In addition, a cross-correlation was calculated between the continuous FP and the triggered EMG signals (EMG-triggered FP average). Figure 5A shows a cross-correlogram between the onset of the EMG activity and the contralateral MUA and FP activity during the ~10-Hz oscillations caused by BMI + CGP. Note the large peak of

1 The online version of this article contains supplemental data.
activity preceding the EMG onset by <20 ms. Moreover, the peak of MUA is well correlated with the corresponding FP activity; a large negative wave precedes EMG onset. This indicates that the cortical activity is driving the EMG activity. A similar analysis computed for this same animal between the EMG activity during normal whisking (in the absence of ~10-Hz oscillations) and the contralateral MUA and FP activities produced flat cross-correlograms for both the MUA and FP (not shown), indicating that the recorded cortical activity under those control conditions had little relation with the vibrissa movements. Population analyses of cross-correlograms computed during the ~10-Hz oscillations caused by BMI + CGP revealed that MUA between 1 and 15 ms before the EMG activity was significantly higher than MUA measured between 35 and 50 ms before EMG activity (t-test, \( P < 0.001; n = 5 \) experiments). In contrast, for the same experiments, there was no significant difference in MUA firing between these periods for cross-correlograms derived during normal vibrissa movements in the absence of the ~10-Hz oscillations (t-test n.s.; \( n = 5 \)).

To further test whether the cortical activity during the ~10-Hz oscillations was driving (entraining) the EMG activities, a coherence analysis was performed between the FP activity in cortex and the contralateral rectified EMG activity (Ahrens and Kleinfeld 2004). Figure 5B shows a coherogram between the cortical FP in the infused hemisphere and the contralateral EMG (FP-EMG coherence) and a power spectrum of the FP activity (FP FFT) for the same activity shown in Fig. 5A. The FP FFT revealed a large peak at about 7 Hz and also did the FP-EMG coherence analysis. Thus the two signals were highly coherent, indicating that the cortical activity is entraining the EMG activity. Population analyses revealed that coherence between the FP and EMG signals during the ~10-Hz oscillations caused by BMI + CGP (at the frequencies of the FP oscillations determined with FP FFT) was significantly stronger than the coherence for these same signals during normal vibrissa movements in the absence of the ~10-Hz oscillations (Fig. 5C; t-test \( P < 0.001; n = 5 \)).

**DISCUSSION**

The present results show that disinhibition of the vMI produces spontaneous 10-Hz oscillations that drive vibrissa movements at the same frequency. In essence, the results demonstrate that the vMI can act as a CPG of vibrissa movements on a cycle-by-cycle basis.

Interestingly, the movements produced by synchronized cortical output were always vibrissa retractions, followed by relief from the retraction and a new retraction. This correlates well with previous studies that used electrical stimulation of MI to drive vibrissa movements (Berg and Kleinfeld 2003b; Brecht et al. 2004; Donoghue and Wise 1982; Haiss and Schwarz 2005). Recent work has also shown that motor cortex stimulation can produce rhythmic vibrissa movements that resemble normal active whisking, including protractions (Berg and Kleinfeld 2003b; Haiss and Schwarz 2005). However, the electrical stimulus frequency is not phase-locked with the whisking cycle, which suggests that the cortex is driving the brain stem CPG. It is also possible that trains of electrical stimulation of vMI drive the ~10-Hz excitatory network of vMI (Castro-Alamancos 2000; Castro-Alamancos and Rigas 2002), which would act as a cortical CPG to produce rhythmic movements. Interestingly, the movements observed in the present study after cortical disinhibition are phase-locked with the cortical activity but lack protractions per se, such as those typically seen during gap crossings in which the animal extends its vibrissae to palpate the environment (Carvell and Simons 1990; Carvell et al. 1991).

The present results do not address whether the vMI output is relayed directly to the muscles by facial nucleus motorneurons (Grinevich et al. 2005) or indirectly through a brain stem CPG (Hattox et al. 2002, 2003). Both possibilities are compatible with the present results. However, the present results indicate that the motor cortex can act as a CPG for rhythmic vibrissa movements.

It is important to note that the intrinsic activity generated in the present study by disinhibition is abnormal. This activity may be related to pathological conditions such as cortical myoclonus or tremor. For instance, in humans, the ~10-Hz rhythmic and synchronized activities, similar to the ~10-Hz oscillations shown here, are associated with frontal lobe epilepsies (Chauvel et al. 1992). In *Epilepsia Partialis Continua* the myoclonus consists of body jerks that occur continuously at regular short intervals for days or weeks (Chauvel et al. 1992), which is quite similar to the motor effects produced by spontaneous oscillations of about 10 Hz caused by disinhibition of the MI in rats. These similarities raise the possibility that some forms of myoclonus may be generated within the neocortex by isolated intrinsic excitatory networks that act as motor CPGs after inhibition has been compromised.

In conclusion, rhythmic and synchronized intrinsic activity of the motor cortex drives phase-locked vibrissa movements that resemble a tremor or cortical myoclonus.

**ACKNOWLEDGMENTS**

The author thanks Y. Tawara-Hirata for excellent technical assistance.

**REFERENCES**


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