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Anticipatory Activity of Motor Cortex in Relation to Rhythmic Whisking

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Abstract

Rats characteristically generate stereotyped exploratory (5-12 Hz) whisker movements, which can also be adaptively modulated. Here we tested the hypothesis that the vibrissal representation in motor cortex (vMCx) initiates and modulates whisking by acting on a subcortical whisking central pattern generator (CPG). We recorded local field potentials (LFPs) in vMCx of behaving Sprague-Dawley rats while monitoring whisking behavior through mystacial electromyograms (EMGs). Recordings were made during free exploration, under body restraint or in a head-fixed animal. LFP activity increased significantly prior to the onset of a whisking epoch and ended prior to the epoch's termination**.** In addition, shifts in whisking kinematics within a whisk epoch were often reflected in changes in LFP activity. These data support the hypothesis that vMCx may initiate and modulate whisking behavior through its action on a subcortical CPG.

Introduction

Rats use their vibrissae to navigate through their environment. During exploratory behavior they generate stereotyped vibrissal movements; during discriminative behavior these movements may be adaptively modulated. Animals performing texture discrimination tasks, for instance, alter both whisking frequency and amplitude, and performance on these tasks is correlated with the whisking patterns employed (Carvell and Simons 1995; Harvey et al. 2001).

Though the mechanisms underlying such voluntary control of whisking are unknown, they are likely to involve the motor cortex. In primates, motor cortical areas mediate the force, direction and planning of movements (Grillner et al. 1997; Humphrey and Tanji 1991; Tanji and Everts 1976). In rats, a large region of motor cortex is involved in the control of vibrissal movements, and low intensity stimulation of different parts of vibrissal motor cortex (vMCx) can evoke both small vibrissal retractions (Donoghue & Wise, 1982) and rhythmic movements similar to exploratory whisking (Berg and Kleinfeld 2003b; Brecht et al. 2004; Haiss and Schwarz, 2005). vMCx is thought to affect whisking through its extensive projections to multiple pre-motoneurons distributed throughout the mid- and hindbrain (Hattox et al. 2002). One class of pre-motoneurons forms part of a serotonergic central pattern generator (CPG) in the brainstem, and this CPG has been shown to be both necessary and sufficient for producing rhythmic whisking (Hattox et al. 2003).

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Recent demonstration of coherence between cortical field oscillations and vibrissal EMG activity (Ahrens and Kleinfeld 2004) suggest that vibrissae movements may also be controlled by vMCx on a cycle-by-cycle basis. However, other data are inconsistent with this hypothesis. First, stimulation of single cortical cells results in whisking epochs lasting beyond the period of stimulation (Brecht et al. 2004). Second, the periodicity in vibrissae movements is not reflected in the firing patterns of single units in vMCx (Carvell et al. 1996). Third, ablation of vMCx alters the kinematics of whisking, but does not abolish whisking behavior (Gao et al. 2003a).

These observations suggest that, as is the case for other rhythmic behaviors (Larson et al. 1980; MacKay-Lyons 2002; Nakamura and Katakura 1995; Thompson and Watson 2005), rhythmic whisking is maintained by the whisking CPG, while voluntary initiation and modulation of the pattern are mediated by cortical mechanisms. A prediction of this hypothesis is that the onset of whisking will be preceded by changes in motor cortical activity. The present study tested this prediction.

Some of these findings were previously reported in abstract form (Friedman et al. 2004).

Materials and Methods

Surgical Procedures: Five female Sprague-Dawley rats (180-280 g) were used in this study. Four of these animals had chronic electrode implants, while the fifth was recorded from acutely in a head-fixed preparation. All procedures adhered strictly to institutional and federal guidelines.

Subjects were gentled daily for at least 2 weeks prior to surgery. Under sodium pentobarbital anesthesia (60 mg/kg; 10% supplemented as needed) and aseptic surgical conditions, a small incision was made in the face, and a pair of dipolar EMG electrodes (0.003” Teflon-coated stainless steel wire) was tunneled sub-cutaneously into the deep intrinsic musculature. The ends of the wires were run to the top of the head and soldered to pin-connectors. Correct placement of the wires was verified using microstimulation to evoke vibrissae movements.

Subjects were next placed in a stereotaxic apparatus, a scalp incision was made above the midline and the periosteum retracted. After the skull was cleaned and dried, 5-7 skull screws were inserted to provide a support for a dental cement platform, which served to hold the electrode array and EMG connectors in place. Dexamethazone (2 mg/kg IM) was administered to minimize brain swelling. In 4 of the 5 animals, a craniotomy was made (bilaterally in one, unilaterally in the others) directly above the vMCx, and an eight-channel array (quartz-insulated platinum electrodes, 500 μm apart) was lowered into the deep layers of motor cortex (1.5 – 2 mm from surface). A ground wire on each array was soldered to a skull screw. Agarose (1.4% in buffered saline) was placed over the exposed brain to prevent drying and contact with dental cement, and the array and EMG pins were secured to the head with dental acrylic. The surgical incision was held closed with Vetbond.

In the 5^{th} animal, the skull above the right vMCx was thinned and left clear of acrylic in preparation for acute recordings and head-fixation. Two mounting bolts, to which a restraint bar could be attached during recording sessions, were embedded in the acrylic platform (Gao et al. 2003b).

Throughout all surgical procedures, body temperature was maintained at 37°C with a servo-controlled heating pad. Animals were given Buprenorphine HCl (0.03 mg/kg s.q) to alleviate pain and antibiotic (Baytril 0.05 mg/kg IM) immediately and 12 hours post-surgery.

Electrophysiology: In the chronically implanted animals recordings were made both when the rats were unrestrained and when they were restrained in a cloth body sack. The head-fixed animal was recorded from acutely, using a 16-channel (4×4) silicon-based multielectrode probe (Center for Neural Communication Technology, University of Michigan, Ann Arbor, MI). Just prior to recording, this animal was lightly anesthetized with 2% halothane in humidified O_2 and the thinned bone overlying vMCx was carefully peeled away with forceps. Once awake, the rat was put in a cloth sack and placed in a Plexiglas body holder. A restraining bar was attached to both the mounting bolts in the head-mount and to the body holder, thus rendering the head immobile (Bermejo et al. 1998). The electrode probe was slowly lowered to the deep layers of vMCx with a stereotaxic manipulator.

In all 5 animals, LFP and EMG waveforms were acquired simultaneously through different pre-amplifiers and digitized to disk using the Plexon data acquisition system (Dallas,TX) at a sampling rate of 5 kHz.

Identification of Recording Sites: At the completion of data collection, the position of the implanted electrodes in vMCx was verified through cortical microstimulation. The animals were anesthetized with halothane and vibrissae movements were evoked by intracortical microstimulation (200 μsec long pulses delivered at 50 Hz for 1 s; ≥100 μA). We monitored vibrissae movements with a charge-coupled device, as in our previous studies (Bermejo et al. 1998). As described previously by Haiss and Schwarz (2005), these stimulation parameters evoked large vibrissae movements that resembled natural rhythmic whisking (Fig, 1a), confirming that the electrodes were implanted in vMCx, and, specifically, in the posteromedial region of vMCx (Haiss and Schwarz 2005; Sanderson et al. 1984). At the completion of the recording and stimulation sessions, the rats were deeply anesthetized (pentobarbital 60 mg/kg), perfused with 4% buffered paraformaldehyde, and their brains were removed, sectioned in the coronal plane and Nissl-stained to confirm the location of the electrode tracks (Fig. 1b).

Data Analysis: LFP and EMG waveforms were exported to Matlab (MathWorks, Natick, MA) for analysis. EMG data were down-sampled to 500 Hz, low-pass filtered at 50 Hz, and full-wave rectified. Whisking epochs selected for analysis were determined by identifying EMG activity that was >2 standard deviations above baseline, occurred at ≥5 Hz, and lasted for ≥1 s. To disambiguate patterns in the LFPs, we chose whisking epochs that were preceded by ≥500 msec of no significant EMG.

We calculated multitaper spectrograms, which plot the power spectra of the signal over time, using a sliding 0.5 s window (1 msec steps) for both EMG and LFP time series. To unambiguously identify significant changes in LFP activity, we calculated confidence intervals (95%) using the jackknife method (Thomson and Chave 1991) and computed using the Chronux package (Bokil et al. 2005).

Results

Because whisking behavior and local field potential activity were indistinguishable in the freely moving, body restrained and head-fixed conditions, data from all three conditions were combined for the purpose of analysis.

Whisking Epochs: We extracted from the EMGs 49 whisking epochs across the 5 animals (median = 9 epochs from each animal) that met the established criteria (see Materials and Methods). These criteria included the requirement that a whisk epoch be preceded by ≥500 msec of no significant EMG. Because rats in an open field whisk nearly continually, the number of epochs meeting this requirement was relatively small; the 49 epochs chosen were selected from a total of 68.8 minutes of recordings. Figure 2A shows the EMG waveform recorded during a typical whisking epoch (top). The median duration of the selected epochs was 3.03 s;

epochs ranged from 1.07 to 20.04 s (3.65±1.90 s). Whisking frequency within epochs ranged from 5 to 12 Hz. Consistent with previous findings (Berg and Kleinfeld, 2003a), whisking frequency was generally constant within an epoch.

Neural Activity: We extracted LFP time series corresponding to identified whisking epochs. In cases where activity in more than one LFP channel co-varied with EMG, data from only one channel were included in analyses.

In many epochs inspection of the raw data revealed changes in LFP activity that preceded EMG onset (Fig. 2). LFP spectrograms enabled us to quantify the temporal relationship between whisking onset and accompanying increases in LFP power. We identified the start and stop times of the first identifiable increase in LFP power corresponding to the onset of each whisking epoch. We then statistically determined significant changes in LFP activity by computing activity levels that exceeded the 95% confidence level (Fig. 2a-b, bottom; see Materials and Methods).

An increase in LFP power preceding the onset of the EMG activity was clearly observed in 39 of the 49 epochs (79.6%; Fig. 3). The bars in this Figure represent individual epochs aligned to EMG onset. The hatched portion of each bar represents the time over which LFP activity increased; the shaded portion of the bars represents the length of the whisking epoch. LFP frequencies ranged from 4 Hz to 10 Hz. The mean difference between onset of LFP activity and whisking initiation was 241 ± 270 ms, which was significant ($t = -6.23$, $p < 0.001$). The mean overlap time between LFP and EMG activity was 390 ± 320 ms.

Frequently, the rats' behavior consisted of several contiguous epochs of whisking. In most cases, each of these epochs was preceded by significant changes in LFP activity (Fig. 2b).

Discussion

It has long been known that stimulation of the vMCx can result in vibrissae movements (Donoghue and Wise 1982), but the mechanisms mediating that effect are unknown. Two alternative mechanisms have been suggested. One involves direct control of whisking by vMCx on a cycle-by-cycle basis (Ahrens & Kleinfeld 2004), while the other postulates indirect control *via* cortical modulation of a brainstem CPG (see Introduction).

In this report we tested the hypothesis of indirect control by simultaneously recording LFPs and vibrissae EMGs in behaving rats. We focused on LFPs because they reflect activity in populations of neurons, and because an increase in LFP power in a given frequency range is thought to represent an increase in synchrony of the underlying neuronal population (MacKay 2005). Significantly, LFP activity in motor cortex has been shown to be as accurate a predictor of movements as unit activity (Ball et al. 2004; Mehring et al. 2003; Scherberger et al. 2005). We predicted that vMCx activity would increase prior to whisking onset, reflecting vMCx activation of the CPG. The results of our recordings are consistent with this prediction. Our findings are also consistent with the work of Carvell *et al.* (1996), who showed that unit activity recorded from vMCx increases immediately prior to whisking onset (51±75 msec). In the present study we report that vMCx activity precedes whisking onset by, on average, 240 msec. The longer pre-movement LFP activity, compared to that reported by Carvell *et al.* (1996) is likely due to the fact that LFPs represent temporally-integrated synaptic activity from many neurons, whereas unit activity reports discrete spikes from individual neurons.

Pre-vibrissae movement activity has also been reported to occur in the rat somatosensory cortex (Hamada et al. 1999). Indeed, that cortical activity precedes and may “anticipate” voluntary movements was demonstrated 30 years ago (Tanji and Evarts, 1976, who inspired the title of the present manuscript).

Our findings also demonstrate that vMCx activity does not persist throughout the full time course of whisking epochs (Fig. 3). These findings are consistent with the hypothesis that vMCx initiates whisking by activating a subcortical CPG. Finally, we hypothesized that vMCx not only initiates whisking, but also determines whisking kinematics by its actions on the subcortical CPG (Hattox et al. 2003). Our finding that shifts in whisking patterns within an epoch are reflected in changes in LFP activity support this hypothesis. Thus, as in other CPGs (Harris-Warrick and Marder 1991;Hooper and Dicaprio 2004), the output of the whisking CPG may be regulated by phasic inputs from descending command centers, namely vMCx.

The two alternatives presented above are not mutually exclusive, and the mechanism of motor control may be context-dependent. Brecht et al (2004) reported that stimulation of layer V neurons in lightly anesthetized rats evoked movements that were phase locked to stimulation, resulting in similar timing of whisk cycles across trials by resetting the whisking rhythm. Stimulation of layer VI neurons, however, resulted in epochs of whisking that were out of phase across trials. Based on these findings, Brecht (2004) suggested “whisker motor cortex can control whisking in multiple ways – either by a sweep-to-sweep fine control of the movement pattern or more globally by simply turning on and off whisking”. Similarly, a topographical division between cells responsible for movement initiation and guidance has been suggested in primate motor cortex (Humphrey and Tanji 1991). Our finding that activity in vMCx increases *prior to* whisking and usually ends before whisking ceases, together with the fact that vMCx provides inputs to the whisking CPG (Hattox et al. 2003), is consistent with the hypothesis that motor cortex can modulate the whisking pattern through its actions on subcortical pattern generators.

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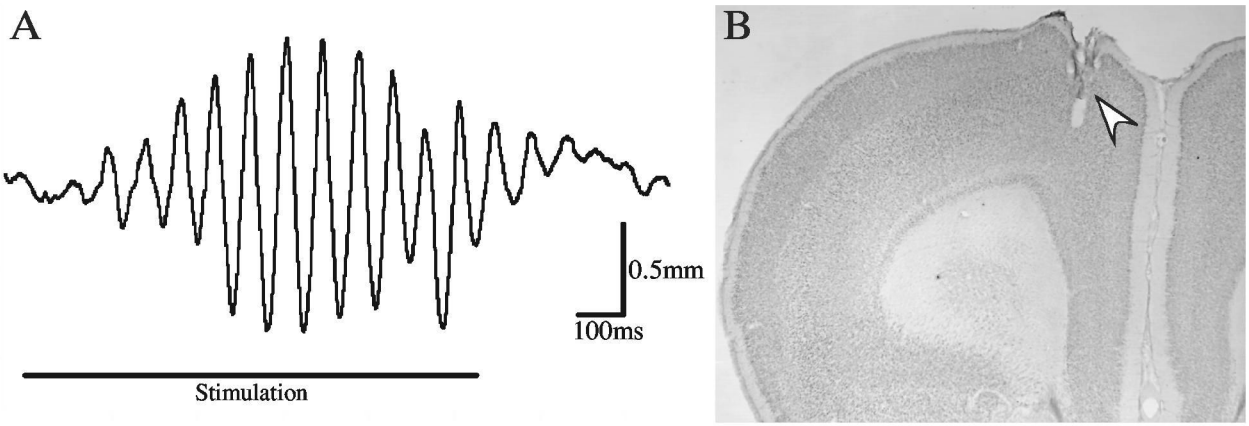

Figure 1.
A: Whisker trajectory (up=protraction) evoked by microstimulation of vMCx and recorded with a CCD. **B:** Nissl-stained coronal sections depicting electrode tracks through the agranular-medial (AGm) region of the motor cortex.

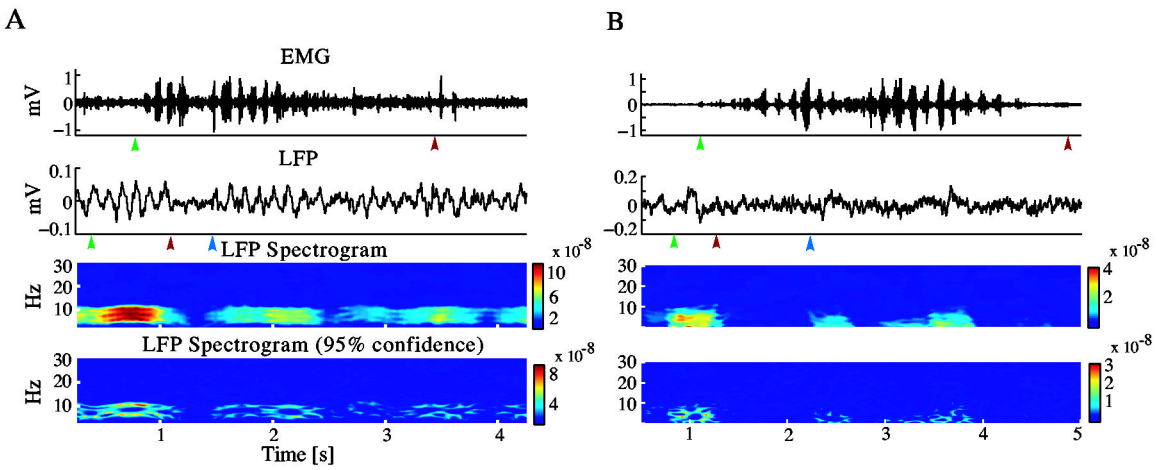

Figure 2.

Raw trace of EMG activity (*A* & *B*, top row) with corresponding LFP traces below. Onset (green arrowhead) and offset (red arrowhead) in EMG and LFP activity, determined using statistical algorithms (see Materials and Methods) are depicted on the corresponding traces. Increases in LFP just before whisking onset and again just prior to whisking offset are evidenced as an increase in power in the LFP spectrogram (third row). Changes in power that are statistically significant above the 95% confidence level are plotted in the bottom panels. Instances in which a change in whisking dynamics during an epoch is reflected in LFP activity are indicated with blue arrowheads.

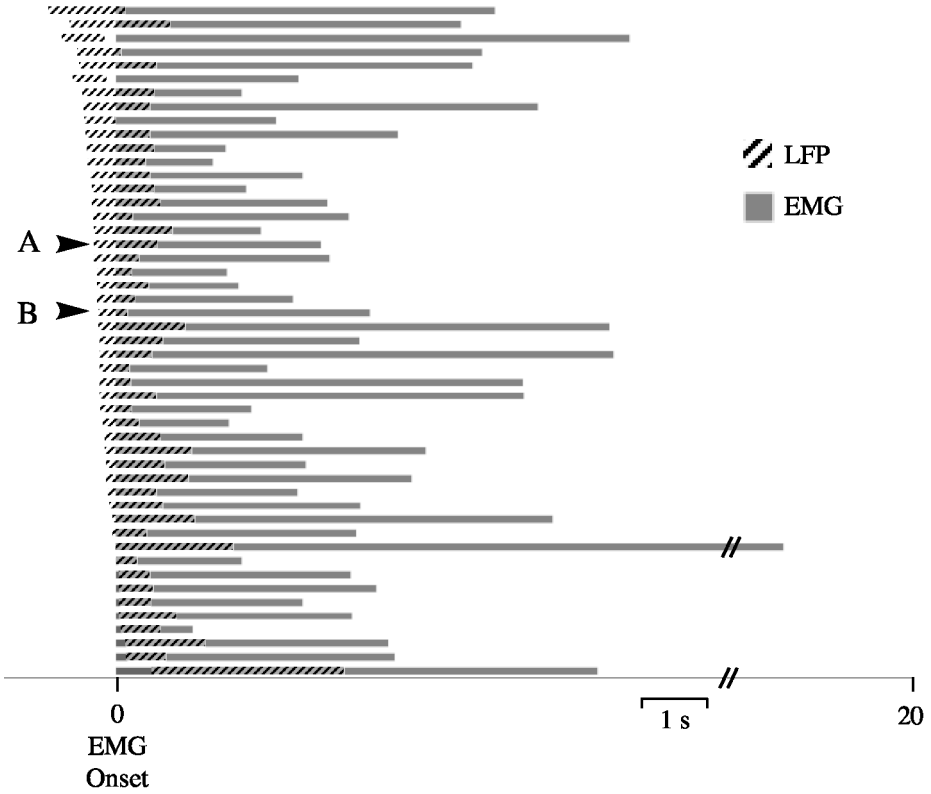

Figure 3.
Time-course of increases in LFP activity (hatched bars) and of EMG activity (filled bars) recorded from 49 epochs in 5 animals. Data are aligned to whisking onset. Changes in LFPs preceded whisking onset in 39 of the 49 epochs, and ended prior to whisking offset in all samples. Overlaps in activity are illustrated by bar overlaps. Bars marked A & B correspond to data depicted in Figs. 2A and 2B.