Anticipatory Activity of Motor Cortex in Relation to Rhythmic Whisking

Wendy A. Friedman, Lauren M. Jones, Nathan P. Cramer, Ernest E. Kwegyir-Afful, H. Philip Zeigler, and Asaf Keller

Department of Psychology, Hunter College, City University of New York, New York, New York; and Department of Anatomy and Neurobiology and the Program in Neuroscience, University of Maryland School of Medicine, Baltimore, Maryland

Submitted 7 September 2005; accepted in final form 24 October 2005


Anticipatory activity of motor cortex in relation to rhythmic whisking. J Neurophysiol 95: 1274–1277, 2006. First published October 26, 2005; doi:10.1152/jn.00945.2005. 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). Although 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 retraction (Donoghue and Wise 1982) and rhythmic movements similar to exploratory whisking (Berg and Kleinfeld 2004; Brecht et al. 2004; Haiss and Schwarz 2005). vMCx is thought to affect whisking through its extensive projections to multiple premotorneurons distributed throughout the mid- and hindbrain (Hattox et al. 2002). One class of premotorneurons forms part of a serotonergic central pattern generator (CPG) in the brain stem, and this CPG has been shown to be both necessary and sufficient for producing rhythmic whisking (Hattox et al. 2003). Recent demonstration of coherence between cortical field oscillations and vibrissal electromyographic (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).

Although 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, whereas 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).

METHODS

Surgical procedures

Five female Sprague-Dawley rats (180–280 g) were used in this study. Four of these animals had chronic electrode implants, whereas 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 ≥2 wk prior to surgery. Under pentobarbital sodium anesthesia (60 mg/kg; 10% supplemented as needed) and aseptic surgical conditions, a small incision was made in the face, and a pair of bipolar EMG electrodes (0.003-in Teflon-coated stainless steel wire) was tunneled subcutaneously 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 scalpel incision was made above the midline, and the periosteum was retracted. After the skull was cleaned and dried, five to seven 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 four of the five animals, a craniotomy was made (bilaterally in 1, 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.
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 fifth animal, the skull above the right VMX 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 h 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 halothane, and vibrissae movements were evoked by intracortical microstimulation (200–500 μA peak-to-peak) using a 16-channel (4 × 4) silicon-based multielectrode probe (Center for Neural Communication Technology, University of Michigan, Ann Arbor, MI) coupled to a charge-coupled device as in our previous studies. We monitored vibrissae movements with a charge-coupled device as in our previous studies (Bermejo et al. 1998). The electrode probe was slowly lowered to the deep layers of VMX with a stereotaxic manipulator.

In all five animals, LFP and EMG waveforms were acquired simultaneously through different preamplifiers 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 VMX was verified through cortical microstimulation. The animals were anesthetized with halothane, and vibrissae movements were evoked by intracortical microstimulation (200-μs-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 VMX, and, specifically, in the posteromedial region of VMX (Haiss and Schwarz 2005; Sanderson et al. 1984). At the completion of the recording and stimulation sessions, the rats were killed (pentobarbital 60 mg/kg) and 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 SD 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 ms of no significant EMG activity.

We calculated multitaper spectrograms, which plot the power spectra of the signal over time, using a sliding 0.5-s window (1-ms 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 software package (www.chronux.org).

RESULTS

Because whisking behavior and LFP 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 five animals (median = 9 epochs from each animal) that met the established criteria (see METHODS). These criteria included the requirement that a whisk epoch be preceded by ≥500 ms of no significant EMG. Because rats in a new environment 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 min 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

![FIG. 1. A: whisker trajectory (up = protraction) evoked by microstimulation of vibrissal representation in motor cortex (vMCX) and recorded with a charge-coupled device (CCD). B: Nissl-stained coronal sections depicting electrode tracks through the agranular-medial (AGm) region of the motor cortex.](http://jn.physiology.org/doi/abs/10.1152/jn.00582.2005)
of each whisking epoch. We then statistically determined significant changes in LFP activity by computing activity levels that exceeded the 95% confidence level (Fig. 2, A and B, bottom; see 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 of each bar represents the time over which LFP activity was increased; the ■ of the bars represents the length of the whisking epoch. LFP frequencies ranged from 4 to 10 Hz. The mean difference between onset of LFP activity and whisking initiation was 241 ± 110 ms, which was significant (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 and Kleinfeld 2004), whereas the other postulates indirect control via cortical modulation of a brain stem 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 ms). In the present study, we report that vMCx activity precedes whisking onset by, on average, 240 ms. The longer pre-movement LFP activity, compared with 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.

Previbrissae 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 yr 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.

ACKNOWLEDGMENTS

This work was supported by National Institute of Neurological Disorders and Stroke Grants NS-35360 to A. Keller and NS-39107 to H. P. Zeigler and Individual Kirschstein-National Research Service Awards NS-050042 to W. Friedman and NS-46100 L. M. Jones.

REFERENCES


