

MOTOR CONTROL MECHANISMS OF WHISKING IN RAT

by

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ABSTRACT

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Rhythmic movements underlie a large number of critically important motor behaviors. How different levels of neural organization and control interact to produce these movements, then, is a central question in motor control. Using the whisker system in rat we investigate the role played by the motor cortex, a key structure in the generation of complex movements, in the control of whisking. We begin by measuring the movements of the whiskers and the mastacial pad, controlled by the intrinsic and extrinsic muscles, respectively, to clarify the interaction of these two components in the organization of whisking. We find that though both contribute to movement kinematics, the whisker alone makes a relatively larger contribution. We next investigate the relationship between local field potential (LFP) activity and exploratory whisking and find that brief increases in vMCx activity precede both whisking onset and changes in whisking patterns. Lastly, we investigate the relationship between unit activity in the rhythmic subregion of vMCx and whisking in head-fixed rats engaged in one of two

behavioral tasks. We find that spike rate is most often correlated with whisking amplitude/velocity, and that this relationship is independent of behavioral context. We also find occurrences of significant coherence between whisker movements and unit activity, which vary with behavioral task. These results suggest that, though the motor pattern underlying whisking is generated subcortically, vMCx plays a role in both initiating movement and modulating kinematic properties of whisking.

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TABLE OF CONTENTS

ABSTRACT	IV
ACKNOWLEDGEMENTS	VI
TABLE OF CONTENTS	VIII
LIST OF ABBREVIATIONS	XI
LIST OF FIGURES	XII
1. GENERAL INTRODUCTION	1
<i>1.2. The Whisker Motor Plant</i>	2
<i>1.3. Modulation of Whisking Behavior by Stimulus and Reinforcer: Evidence of Voluntary Control</i>	3
<i>1.4. The Generation of Rhythmic Whisker Movements</i>	5
1.4.1. The Pattern Generator	5
1.4.2. Whisker Motor Cortex	7
<i>1.5. Thesis Outline</i>	11
2. GENERAL METHODS	13
<i>2.1 Head Mount Procedure</i>	13
<i>2.2. Optoelectronic Whisker Tracking</i>	14
2.2.1. Whisker Monitoring	14
2.2.2. Movement Calibration	15
3. WHISKING TOPOGRAPHY: INTERACTION OF WHISKER AND PAD	17
<i>3.1. Introduction</i>	17
<i>3.2. Materials and Methods</i>	18
3.2.1. Subjects	18
3.2.2. Data Collection	18

3.2.3. Behavioral Training	19
3.3. Results	20
3.4. Discussion	26
4. ANTICIPATORY ACTIVITY OF MOTOR CORTEX IN RELATION TO RHYTHMIC WHISKING	32
4.1. Introduction.....	32
4.2. Materials and Methods	34
4.2.1. Surgical Procedures	34
4.2.2. Data Collection	35
4.2.3. Identification of Recording Sites	36
4.2.4. Data Analysis	36
4.3. Results	38
4.3.1. Whisking Epochs	38
4.3.2. Neural Activity	38
4.4. Discussion	42
5. THE RELATIONSHIP BETWEEN WHISKER MOTOR CORTEX AND WHISKING KINEMATICS IN THE BEHAVING RAT	45
5.1. Introduction.....	45
5.2. Materials and Methods	48
5.2.1. Surgical Procedures	48
5.2.2. Electrophysiology	49
5.2.3. Behavioral Recordings	49
5.2.4. Behavioral Training	51
5.2.5. Analyses	53
5.2.6. Identification of recording sites	55

<i>5.3. Results</i>	56
5.3.1. Whisking Frequency	57
5.3.2. Relationship between pre-movement neural activity and whisking	58
5.3.3. Coherence Between Whisker Movements and vMCx	60
5.3.4. Trial-Averaged Coherence Between Whisker Movements and vMCx	63
5.3.5. Relationship between whisking frequency and neural activity	65
5.3.6. Relationship between whisking amplitude and neural activity	67
<i>5.4. Discussion</i>	69
5.4.1. Pre-movement activity	69
5.4.2. Spectral coherence	71
5.4.3. Covariation of cortical activity and whisking frequency	72
5.4.4. Covariation of cortical activity and whisking amplitude	73
5.5.5. The strength of relationships between whisking and cortical activity	74
6. GENERAL DISCUSSION	78
6.1. Motor cortex in the production of rhythmic movements	78
6.2. The Adaptive Modulation of Whisker Movements	79
6.3. Summary of findings	81
6.4. The vMCx and the control of whisking movement parameters	84
REFERENCES	90

LIST OF ABBREVIATIONS

5-HT	–	serotonin
AP	–	Anterior-posterior
CPG	–	central pattern generator
DV	–	Dorsal-ventral
EMG	–	electromyogram
ICMS	–	intracortical microstimulation
IM	–	intramuscular injection
IP	–	intraperitoneal injection
LFP	–	local field potential
PSD	–	power spectral density estimate
SQ	–	sub-cutaneous injection
vMCx	–	vibrissa motor cortex
vMN	–	vibrissa motoneuron

LIST OF FIGURES

Figure 2.1 - Tracking method for vibrissa and pad movements in head-restrained rats	15
Figure 3.1 - Diagram of the rat's face indicating the location of marked whiskers	19
Figure 3.2 - Sample of the AP movements of a pair of whiskers	21
Figure 3.3 - Movements of the mystacial pad at two different locations during 'exploratory' whisking in air	22
Figure 3.4 - Movements of the C-1 whisker and of a pad marker adjacent to whisker B-3, plotted in the AP plane	24
Figure 3.5 - Whisker and pad movements share a common frequency	25
Figure 3.6 - Contribution of pad movement to protraction amplitude	26
Figure 4.1 - Whisker trajectory evoked by microstimulation of vibrissal representation in motor cortex and coronal section depicting recording location	37
Figure 4.2 - Raw trace of EMG activity with corresponding local field potential traces	39
Figure 4.3 - Time course of increases in LFP and EMG activity recorded from 49 epochs in 5 animals	40
Figure 4.4 - Raw trace of EMG activity with corresponding local field potential traces	41
Figure 5.1 - Experimental setup for recording neural activity in head-fixed rats engaged in a whisker contact task	50
Figure 5.2 - Nissle-stained coronal section depicting a lesion made at the recording site in the rhythmic region of vMCx and whisker trajectories evoked by microstimulation	56
Figure 5.3 - Movement traces with corresponding raw neural traces and frequency histogram	57
Figure 5.4 - Raster plot of all epochs from a single recording site and spike histogram	59
Figure 5.5 - Example of coherence between movement and multi-unit activity in vMCx during 1s of movement	60

Figure 5.6 - Comparison of proportion of coherent segments before and after data were shuffled	62
Figure 5.7 - Two examples of recording sessions where coherence between spiking and whisker movements was significant after trial-averaging	63
Figure 5.8 - Comparison of proportion of trial-averaged coherent sessions before and after data were shuffled	65
Figure 5.9 - Comparison of the number of recording sites at which whisking frequency and MUA/Single Unit firing rate were significantly correlated	67
Figure 5.10 - Comparison of the number of recording sites at which whisking amplitude and MUA/Single Unit firing rate were significantly correlated	68

1. GENERAL INTRODUCCION

Organisms move through their environment, actively seeking information that they use to guide their behavior. A number of adaptations for gathering this information, such as walking, sniffing and licking, are based on rhythmic movement patterns that are stereotyped yet adaptively modifiable. Ensembles of premotor neurons that generate patterned drive to motor neurons form the neural basis of rhythmic movement generation. While these “pattern generators” generally continue to produce rhythmic movements in the absence of phasic peripheral influences, they are also known to be susceptible both to modulation by sensory inputs and to voluntary control. The pervasiveness of rhythmic movements in behaviors critical to the survival of organisms, combined with their reliance on both sub-cortical rhythm generators and higher-level inputs, make the study of these behaviors central to understanding how different levels of neural organization and control interact to produce movement.

The rodent whisker system has been extensively studied by neurobiologists for decades, and a number of features make it an ideal model for studying the role of higher motor centers in the control of rhythmic movements. The system has a relatively simple motor plant and its peripheral innervation and central sensory and motor pathways are well described. Removal of the whisker alters the ability of animals to swim (Richter, 1957), copulate (Bialy & Beck, 1993), and act defensively (Shishelova, 2006), suggesting that the system is critically important to the animal’s survival. Rhythmic whisker movements (“whisking”) derive in part from a pattern generator, yet can be modulated by the animal for different purposes and to produce non-stereotypical movements. While sensory inputs to the brainstem are known to form a feedback loop that can alter whisker

movements directly, the relatively large whisker representation in primary motor cortex (vMCx) suggests that cortical inputs play a significant role in normal whisking behavior.

1.2. The Whisker Motor Plant

The mystacial vibrissae are sinus hairs that individually emerge from a follicle embedded in the mystacial (whisker) pad. Movement of the whiskers is achieved through the contraction and relaxation of the intrinsic and extrinsic muscles, which are innervated by whisking motoneurons located in the intermediate and lateral subdivision of the facial motor nucleus (Semba & Egger, 1986). Each of the intrinsic muscles encircles one follicle and inserts into the face of the adjacent, more caudal follicle (Dorfl, 1982). The largest component of a whisker protraction results from the contraction of these muscles (Bermejo, Friedman, & Zeigler, 2005), which causes the backward displacement of the follicle bases. However, the whisker pad also contributes to rhythmic whisking, extending the span of the whiskers beyond what could be achieved solely by pivoting at the whisker base. The whisker pad, for instance, is pulled forward by the most rostral extrinsic muscle, musculus (m.) nasalis, which initiates the protraction phase just prior to intrinsic muscle contraction (Hill, Bermejo, Zeigler, & Kleinfeld, 2008). Whisker retraction is in part the result of the elastic properties of the tissue surrounding the follicles, occurring passively when both the intrinsic muscles and m. nasalis cease contracting. The active contraction of the two caudal extrinsic muscles, m. nasolabialis and m. maxillolabialis, also contribute by pulling the pad and the vibrissae backward (Berg & Kleinfeld, 2002; Hill et al., 2008; Wineski, 1985) thereby furthering the extent of the retraction motion. The intrinsic and extrinsic muscles work in concert, then, in an

anti-phasic manner to produce the periodic motion of the vibrissae and mystacial pad. It has been suggested that the combination of extrinsic and intrinsic muscle activity leads to a more extended range of vibrissa motion than would be available from the intrinsic muscles alone (Hill et al., 2008).

1.3. Modulation of Whisking Behavior by Stimulus and Reinforcer:

Evidence of Voluntary Control

When exploring their environment, rats characteristically generate a stereotyped whisking pattern consisting of a series of rhythmic protractions and retractions. These movements are far from invariant, however, and individuals modulate their movements in different contexts and to achieve specific goals (Carvell & Simons, 1995; Gao, Ploog, & Zeigler, 2003b). Typical “exploratory” whisking involves large amplitude movements in air at between 5 and 15 Hz. When the rat is moving in a straight line towards a destination, as when an obstacle is approached from the front, bilateral whisking is primarily synchronous (Mitchinson, Martin, Grant, & Prescott, 2007) and whisking amplitude increases in anticipation of contact (Carvell & Simons, 1990; Mitchinson et al., 2007). The bilateral whisker movements made during more circuitous exploratory locomotion, however, often differ between the two sides of the face (Towal & Hartmann, 2006). As the animal rotates his head during exploration, for example, the relative positions of whiskers on the two sides change in concert with the speed and direction of head movements. These changes result in an increase in the difference between the angular positions of the whiskers on opposing sides of the face (e.g., an increase in asymmetry) as the animal reaches ahead (protracts) with the whiskers contralateral to the

head rotation and explores more laterally and at smaller amplitudes with the whiskers ipsilateral to the head turn (Towal & Hartmann, 2006).

Contact with objects in the environment significantly alters exploratory whisking patterns, presumably as movements are modulated to maximize processing of sensory information. Rats are able to use their whiskers to discriminate between different textures (Carvell & Simons, 1990; Guic-Robles, Jenkins, & Bravo, 1992; Guic-Robles, Valdivieso, & Guajardo, 1989), shapes (Brecht, Preilowski, & Merzenich, 1997), orientations (Polley, Rickert, & Frostig, 2005) and distances (Krupa, Matell, Brisben, Oliveira, & Nicolelis, 2001; Shuler, Krupa, & Nicolelis, 2002), and discrimination success is correlated with the whisking patterns employed (Carvell & Simons, 1995; Harvey, Bermejo, & Zeigler, 2001a). During discriminations, movement modulations involve changes in amplitude (Carvell & Simons, 1990, 1995; Grant, Mitchinson, Fox, & Prescott, 2009), velocity (Carvell & Simons, 1990, 1995; Grant et al., 2009) and frequency (Carvell & Simons, 1995; Harvey et al., 2001a). As with whisking in air, contact with an object can also lead to differences between movements on the two sides of the face. Unilateral contact with an object results in a decrease in whisk amplitude on the contacted side while contralateral whiskers increase their movement amplitude, possibly to reach the location of the contacted object (Mitchinson et al., 2007).

Changes in whisking patterns in response to environmental interactions often occur within a single whisk cycle (Mitchinson et al., 2007; Towal & Hartmann, 2006). The rapidity of these changes indicates a direct feedback loop from primary afferent neurons (Mitchinson et al., 2007), which are known to project directly to the facial motor nucleus (Dauvergne, Zerari-Mailly, Buisseret, Buisseret-Delmas, & Pinganaud, 2002). However,

context-dependent variations in responses to contact suggest that the animal has the capacity to override this feedback loop, particularly when their attention is focused elsewhere. Mitchinson, Martin et al (2007) observed, for instance, that when a rat appeared to be attending to an object that lay ahead of it, an obstruction on one side resulted neither in the lateral suppression of whisking on the contacted side nor in the increase in whisk amplitude contralateral to contact that was seen when the encountered obstruction was explored.

Further evidence that rats are able to directly control whisking has come from behavioral studies that use conditioning techniques to bring whisking behavior under operant control. Specifically, whisking onset (Bermejo, Harvey, Gao, & Zeigler, 1996) and rate (Gao et al., 2003b) have been manipulated using stimulus cueing and variable reinforcements schedules. The ability of rats to alter whisking kinematics to make fine-grained discriminations under experimental conditions, to dampen their response to an obstacle when focused on a different target and to tailor their rate and timing of whisking to maximize reward delivery all suggest that rats can exert top-down control over whisking behavior.

1.4. The Generation of Rhythmic Whisker Movements

1.4.1. The Pattern Generator

Stimulation of vMCx evokes rhythmic whisker movements and so cortex could, in principle, be the whisking control center. However, neither MCx ablation (Gao, Hattox, Jones, Keller, & Zeigler, 2003a; Semba & Komisaruk, 1984) nor decerebration (Lovick, 1972) abolish whisking so that, while this region may make a critical contribution to

aspects of whisking behavior, it cannot be the primary source of whisker movements. That sensory denervation of the vibrissa pad also fails to suppress whisking (Gao, Bermejo, & Zeigler, 2001) suggests that, as with other important rhythmic movements such as locomotion and chewing, a central pattern generator (CPG) sets the basic rhythmic whisking pattern. The output of this rhythm generator, in turn, may be modulated by direct inputs from higher-level structures and by convergent projections onto the vibrissa motoneurons (vMN) that gate pattern generator inputs.

Retrograde labeling studies have highlighted a few subcortical regions that project to the vMN and may play important roles in generating the whisking rhythm (Cramer, Li, & Keller, 2007; Hattox, Li, & Keller, 2003). In terms of rhythm production and control, regions such as the brainstem reticular formation, the brainstem ambiguous and prepositus nuclei and the superior colliculus (Hattox, Priest, & Keller, 2002) are of particular interest because they both send dense projections to the vMN and are densely innervated by vMCx. Of these, the reticular formation has a number of features that make it a prime candidate. For instance, the reticular formation contains a group serotonergic neurons, the raphe nuclei, that projects to whisker motoneurons (Hattox et al., 2002). The presence of serotonergic neurons is important because infusion of serotonin (5-HT) receptor antagonists into the facial nucleus, *in vivo*, suppresses voluntary whisking, while *in vitro*, the exogenous application of 5-HT induces rhythmic firing, at whisking frequencies, in vMNs (Hattox et al., 2003). Furthermore, stimulation of the raphe nuclei evokes whisker movements similar to exploratory whisking (Hattox et al., 2003). Lastly, the intensity of ICMS in vMCx is positively correlated with the number of evoked spikes in these reticular formation pre-motoneurons, which in turn is correlated with the

frequency of evoked whisker movement (Cramer et al., 2007). Together these findings offer strong evidence that neuron clusters within the reticular formation are part of the whisking pattern generator – a conclusion consistent with studies of other mammalian CPGs (MacKay-Lyons, 2002).

While the available data is compatible with a critical contribution by central pattern generating mechanisms to the control of whisker movements, what remains unclear is the nature of the relationship between cortical and brainstem mechanisms in the generation and control of such movements.

1.4.2. Whisker Motor Cortex

Research efforts over the last few years have brought a greater understanding of the whisking pattern generator. However, whether and how higher-level structures, such as vMCx, interact with pattern generating regions to influence the whisking rhythm remains unclear. Studies of other rhythmic movements implicate the MCx in coordinating the different muscles that contribute to patterned movements (Larson, Byrd, Garthwaite, & Luschei, 1980) and in adapting the basic movement pattern to accommodate environmental fluctuations by integrating incoming sensory information from other systems (e.g., visual) with the current motor state (Drew, Jiang, Kably, & Lavoie, 1996). Whether similar mechanisms mediate cortical–CPG interactions in the whisker system is a focus of current research. Four experimental approaches, lesion, cortical recording, cortical stimulation and anatomical tracing, have provided data regarding the contribution of vMCx to the control of whisking. While stimulation and tracer studies have gone a long way in identifying the pathways through which vMCx may influence whisking,

there is still a paucity of information regarding the movement features modulated by cortical activity.

Lesion studies have found that vMCx is not necessary for the production of exploratory rhythmic whisking patterns (Gao et al., 2003a; Semba & Komisaruk, 1984), suggesting an absence of direct cortical control over movements. Analysis of post-lesion movement patterns, however, have revealed reductions in the dominant whisking frequency (Gao et al., 2003a; Semba & Komisaruk, 1984), as well as changes in whisk amplitude (both decreases (Semba & Komisaruk, 1984) and increases (Gao et al., 2003a)) and velocity profiles (Gao et al., 2003a), suggesting that these parameters may be indirectly influenced by vMCx.

Extracellular recordings from single vMCx neurons have shown that unit activity increases just prior to, and peaks at, whisking onset (Carvell, Miller, & Simons, 1996). Additionally, unit activity has not been found to co-vary with any parameter of movement kinematics, and in fact returns to baseline levels soon after movement onset (Carvell et al., 1996). The temporal relationship between unit activity and whisker movements supports the assertion that MCx exerts its influence indirectly, via subcortical structures. In contrast, phase-locked activity between local field potentials and the intrinsic whisker muscles has also been recorded (Ahrens & Kleinfeld, 2004; Castro-Alamancos, 2006). This finding of coherence opens the possibility that vMCx may directly drive whisker movements in certain contexts.

Intracortical microstimulation (ICMS) studies have shown that low intensity stimulation of different parts vMCx can evoke both whisker retractions (Berg & Kleinfeld, 2003; Donoghue & Wise, 1982) and rhythmic movements similar to

exploratory whisking (Berg & Kleinfeld, 2003; Brecht, Schneider, Sakmann, & Margrie, 2004; Cramer & Keller, 2006; Lang, Sugihara, & Llinas, 2006). Experiments mapping the vibrissa motor cortex of anesthetized rats have identified two functionally distinct subregions, each responsible for one of these two types of movements (Haiss & Schwarz, 2005; Sanderson, Welker, & Shambes, 1984): a larger anterolateral region in which ICMS evokes retractions in the vibrissae coupled with pad movements, and a smaller posteromedial region where ICMS evokes a series of rhythmic protractions.

A number of features of these stimulation studies provide evidence consistent with the hypothesis that vMCx is modulating whisking patterns indirectly through the whisking pattern generator and provide some insights into the kinematic properties that are under cortical influence. First, both single cortical cell stimulation (Brecht et al., 2004) and extracellular stimulation within the rhythmic whisking region (Cramer & Keller, 2006; Haiss & Schwarz, 2005) result in whisking epochs lasting beyond the period of stimulation. Second, an increase in stimulation *intensity* results in an increase in *frequency* of evoked rhythmic movements (Cramer & Keller, 2006; Haiss & Schwarz, 2005). Third, vMCx stimulation evokes firing in the facial motor nucleus at longer latencies than would be expected in a monosynaptic projection (Cramer & Keller, 2006). Lastly, injection of a serotonin receptor antagonist within the pattern generating region abolishes whisker movements evoked by vMCx stimulation (Cramer & Keller, 2006). Like the recoding studies discussed above, these findings suggest that vMCx primarily plays an indirect role in the control of whisker movements. These findings additionally imply that whisking frequency may be modulated by vMCx.

Results from ICMS studies also support the contention that vMCx has the ability to directly control whisking. While it has been reported that whisker movements do not reflect stimulus frequencies (Brecht et al., 2004; Cramer & Keller, 2006; Haiss & Schwarz, 2005), evoked movements have been shown to phase lock with stimulation in the 7-10 Hz range (Berg & Kleinfeld, 2003; Brecht et al., 2004). Importantly, Brecht and colleagues (2004) found that whether or not evoked movements were time locked to the stimulation of single cells depended on the cortical cell layer. Specifically, stimulation of layer 6 cells resulted in a burst of whisker movements that outlasted and was out of phase with the stimulus, while stimulation of layer 5 cells evoked movements that were phase-locked to the stimulus.

Finally, tracer studies have identified both dense projections from vMCx to the brainstem pattern generating region and sparse projections directly to whisker motoneurons (Grinevich, Brecht, & Osten, 2005). To date, no anatomical studies have distinguished between projections from the two physiologically distinct subregions within vMCx, and therefore there is no available data on whether or not the functional division is reflected in anatomical differences. Though there are neural pathways that could enable both the direct and indirect control of movement by vMCx, the conditions under which each of these alternatives may be implemented is not known. Taken together, these observations suggest that, as is the case for other rhythmic behaviors (Larson et al., 1980; MacKay-Lyons, 2002; Nakamura & Katakura, 1995), the rhythmicity of whisking originates in brainstem patterning generating mechanisms whereas its voluntary initiation and pattern modulation are at least in part controlled by cortical inputs.

1.5. Dissertation Outline

The purpose of my dissertation was to explore the relationship between vMCx and whisking behavior, using a combination of behavioral, anatomical and electrophysiological techniques. I specifically investigated the hypothesis that voluntary control of the rhythmic whisking pattern is achieved indirectly, via a pattern generator, and attempted to identify kinematic features subject to cortical modulation.

The first study of this dissertation, described in Chapter 2, aimed at giving a fuller description of whisking behavior than had been given to date, focusing in particular on the relative contributions of the whiskers and mystacial pad to movement. This study found that individual whiskers, controlled by the intrinsic muscles, make the greatest contribution to whisk amplitude. Though the pad contributed proportionally less to overall movement amplitude than the whiskers, the pad was found to move at the same rhythmic frequency and to have a greater dorsoventral range. My finding that the pad moved at whisking frequencies, along with more recent work in our lab showing that activity in the extrinsic muscles is anti-phasic with intrinsic muscle activity, may in fact suggest that whisking involves more than one pattern generator (Hill et al., 2008).

In Chapter 3 I challenged the hypothesis that the vMCx is the source of the whisking motor pattern in that it directly drives whisking on a cycle-by-cycle basis. I test the prediction that neuronal activity in vMCx, as reflected in LFP recordings, is time shifted relative to the onset of intrinsic muscle EMG activity and does not covary with the EMG bursts underlying individual whisks. My results support the proposal that vMCx does not directly generate the whisking motor pattern itself, but rather initiates whisker movements.

The final study, reported in Chapter 4, further investigates the relationship between vMCx and whisking through unit recordings in the awake, behaving animal. This study builds upon the studies described in Chapter 2 and 3 in four important ways. First, head-fixation enabled more accurate tracking of whisker movements than was achieved in Chapter 3 using EMG recordings. Second, extracellular recording facilitated a more detailed analysis of the temporal and frequency relationships between cortical activity and movement than did LFP recordings. Third, in contrast to earlier studies, recording sites were restricted to the rhythmic sub-region of vMCx, which specifically evokes rhythmic whisker movements and therefore is likely to act via the whisker CPG. Lastly, context-dependent differences in these relationships were investigated using two different behavioral reinforcement paradigms. The results indicate that the relationship between vMCx and whisking can differ across movement epochs, and in some cases the likelihood of a relationship occurring varies with the behavioral task engaged in by the animal.

2. GENERAL METHODS

This chapter describes research methods that are common to more than one experiment. Variations in these methods within an experiment and methods unique to a single study are described in the Methods sections of each chapter.

All experimental procedures were conducted in accordance with NIH Guidelines for the Use and Care of Experimental Animals and were approved by an Institutional Animal Care and Use Committee.

2.1 Head Mount Procedure

All experiments involved the surgical application of a head-mount, with some variation as required for the EMG/cortical recording experiments. Under aseptic surgical conditions, animals were anesthetized (Nembutal, Ketamine/Xylazine or Isoflurene/O₂) and placed in a stereotaxic apparatus. Throughout all surgical procedures, body temperature was maintained at 37°C with a servo-controlled heating pad. The eyes were lubricated throughout the procedure, as needed, with a topical ophthalmic ointment. A scalp incision was made, the periosteum retracted, the skull roughened by scraping and several (5-9) jeweler's screws were inserted to provide a support for a dental cement skull platform. A pair of mounting screws, used to fix the head during behavioral testing, was held above the intact portion of the skull, and the skull and mounting screws were embedded in a dental cement platform. The scalp incision was sutured around the mount to close any large gaps, and the entire surgical site was treated with a topical antibiotic ointment.

2.2. Optoelectronic Whisker Tracking

2.2.1. Whisker Monitoring

Whisker and/or pad movements were recorded in head-fixed rats using an optoelectronic monitoring system. The device consists of a linear CCD array opposite an infrared laser emitter (Alpha X07, Metralight, San Mateo, CA), and has a spatial resolution of 7 μm , and a range of 28 mm. A marker (see individual studies for marker details) was attached close to, but not touching, the whisker base (Figure 2.1A). This marker increases the relative size of the whisker of interest, enabling the marked whisker locale to be monitored with all whiskers intact using a size recognition threshold, and has no significant effect on whisker movements (Bermejo, Houben, & Zeigler, 1998). Interruption of the emitted laser beam by the marked whisker produces a voltage shift in a subset of shaded sensors and signals its position along the CCD array. Position information was sampled by a microprocessor at a rate of either 500 Hz or 1 KHz (see individual studies), and was displayed on a screen for on-line monitoring.

A vertically aligned emitter/detector set was used to track single-dimension whisker movements in the anterior-posterior (AP) plane (AP Array in Figure 2.1A). When movements were tracked in both AP and DV (dorsal-ventral) planes, an additional emitter/detector set was positioned perpendicular to the vertical (AP) set such that the laser curtains were overlapping, creating a 2-dimensional square (28 mm x 28 mm) within which the whisker could be tracked.

Each emitter/detector pair was mounted face-to-face using a custom made metal holder, which in turn was attached to a stereotaxic carrier that made it possible to change the monitor's position in both the AP and DV planes. The holder(s) were aligned parallel

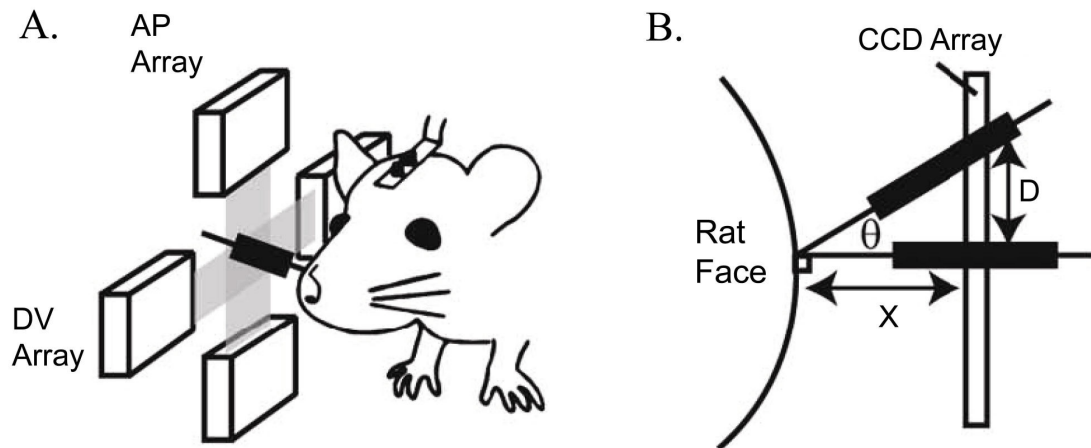


Figure 2.1: Tracking method for vibrissa and pad movements in head-restrained rats. A, Apparatus used for tracking in the A-P and/or D-V planes. One or two whiskers/pad were marked to enlarge its shadow on the linear CCD array(s). B, Schematic of the method used to calculate the angle of the marked whisker(s) or pad. The mid-point of the linear array was aligned with a line normal to the face at the base of the tracked whisker/pad marker, and was defined as 90° (the calibration point). The angle of the whisker/pad marker was calculated using the distance between the array and the face, X, and the distance between the calibration point and the whisker/pad marker, D.

to the rat's face, and were adjusted such that a whisker positioned perpendicular to the face intercepted the center of the laser curtain(s)

2.2.2. Movement Calibration

Because each rat's whisker crosses the monitoring device at a different distance from the face ($\sim 10\text{mm}$), a calibration procedure was carried out to convert micrometer measurements on the sensor to degrees (angle from face) (see Figure 2.1B). Degree measurements are independent of distance between the CCD device and the whisker base and so can be compared across animals, and enable analyses of group data. Each animal was calibrated by positioning the tip of a rod immediately adjacent to the base of the whisker or pad marker being monitored. This rod was held and moved by a micromanipulator, which was fixed relative to the monitor(s) and protruded

perpendicular to the laser curtain(s) and the rat's face. Because the rod crossed the curtain(s) at the center, placement of the rod at the base of the tracked whisker/pad locale situated the monitor(s) such that the marked whisker crossed through the center of the curtain(s) when extended perpendicular to the face (Figure 2.1B). This calibration point was defined as 90° , with 0° representing the most posterior/ventral position and 180° the most anterior/dorsal position. The distance from the face to the laser curtain(s) was measured using the micromanipulator and pointer. The angular displacement of the tracked whisker marker in each plane was then calculated using the formula $\theta = \arctan(D/X)$, where D is the distance moved along the CCD array from the calibration point, and X is the distance of the CCD array from the base of the whisker/pad marker on the face. The procedure is illustrated schematically in Figure 2.1B.

3. WHISKING TOPOGRAPHY: INTERACTION OF WHISKER AND PAD

3.1. Introduction

The whiskers (mystacial vibrissae) of some rodents function as mobile sensors. Whisking provides a continuous stream of temporally organized information about environmental features via the rhythmic movements of the whisker array. Contact with objects may elicit a shift in the whisking pattern involving the repositioning the whisker pad and modulation of whisking frequency, amplitude and location, to provide more detailed information about object features (Carvell & Simons, 1990; Harvey et al., 2001a). The effector system controlling the whiskers thus has three quite different functions—rhythm generation, control of pad position and modulation of whisking parameters—that must be integrated to maximize information flow within the animal's whisking space. The peripheral effector system mediating these functions has two main components, the extrinsic and intrinsic muscles (Dorfl, 1982). The intrinsic (follicular) muscles form a sling surrounding a pair of adjacent follicles of the same rostral-caudal row and their contraction produces vibrissa protraction. The extrinsic muscles produce movements of the entire mystacial pad and may also contribute to whisk amplitude by shifting the base of the whiskers (their pivot point) concurrent with whisking (Berg & Kleinfeld, 2003; Hill et al., 2008). The size, shape and location of the whisking field thus reflect the integrated output of these components interacting with the biomechanical properties of the whisker and pad. Understanding the mechanisms generating the rat's

complex scanning movements would be aided by an analysis of the kinematics and temporal organization of both whisker and pad movements (a) in two dimensions, (b) without confounding head or body movements and (c) in the absence of contact generated (ex-afferent) whisker inputs—i.e. under quasi-‘open-loop’ conditions. The aim of the present study was to monitor both pad and whisker movements, adding to past work characterizing individual whisker movements (Bermejo, Vyas, & Zeigler, 2002), in order to clarify the interaction of these two components in the organization of whisking. This chapter is based on a published paper (Bermejo et al., 2005).

3.2. Materials and Methods

3.2.1. Subjects

Subjects were eight female Long-Evans rats maintained at about 90% of ad lib weight by food deprivation. Under general anesthesia rats were fitted with a dental cement head mount (see General Methods, Chapter 2), which was used to immobilize the head during all behavioral testing.

3.2.2. Data Collection

Movements of the whisker and pad were simultaneously monitored on the right side of the face. By positioning two sets of monitoring devices at right angles to each other we were able to monitor whisker and/or pad movements in both the AP and DV planes (see General Methods, Chapter 2).

A small (<4 mg) foam marker was attached close to, but not touching, the base of the whisker of interest, in order to track 1 or 2 whiskers with all other whiskers remaining

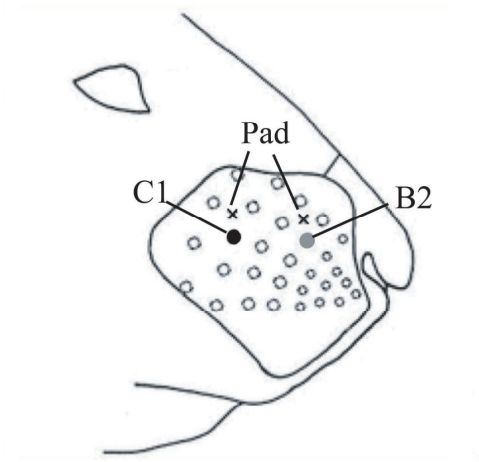


Figure 3.1. Diagram of the rat's face indicating the location of the marked whiskers (circles: C-1 solid; B-3 shaded) and the pad markers (x). For whisker-pad recordings, the caudal-most whisker and rostral-most pad locations were used.

intact. Pad movements were tracked by attaching a similar marker perpendicular to the pad with cyanoacrylate glue. Whisker markers were attached to C-1 and B-3; pad markers were placed just dorsal and posterior to these whiskers, in the space between vibrissae and out of the path of any adjacent whisker trajectories (Figure 3.1). Using whiskers/pad locations that were separated by a few mm in space ensured that the shadows on the tracking device cast by the markers remained spatially segregated (did not overlap), enabling them to be tracked separately but at the same time. We monitored the movement of a single whisker and the pad in all animals. In seven of the eight animals, we also monitored movements of the pad at two locations on the snout and in five of these we monitored movements of a pair of whiskers at two different locations on the face.

3.2.3. Behavioral Training

Animals were trained in an operant 'whisker positioning' task, designed to increase the probability of whisking without reinforcing specific whisking patterns. In this task,

whisker trajectories were continuously monitored and movement of a single marked whisker (C-1) into and out of a ‘window’ within the whisking field was signaled, respectively, by the onset and offset of a tone (CS). Each trial, the size and location of this electronically defined window was randomly shifted, and reinforcement was contingent on the presence of the whisker within the window for a randomly predetermined period (1–3 s). This trial-by-trial shifting of the target location generates periods of active whisking in air, similar to ‘exploratory’ whisking, as the animal searches for the reward window. Test sessions were divided into 30 trials, terminated either by the delivery of a reinforcer (“Yoohoo” chocolate drink) or after 30 s without reinforcement.

3.3. Results

Figures 3.2-3.4 present three samples of typical whisker and/or pad movements under testing conditions, all from a single animal. Movements of the whiskers occur in bursts, separated by periods of little or no activity and whisking frequency may vary over successive bouts. Because whisking was monitored for extended periods, these records also illustrate the manner in which the whisking set point shifts over time, reflecting shifts in the position of the mystacial pad. Most of the records show movement in the AP plane, but two-dimensional reconstructions of movement trajectories are also presented.

3.3.1. Whisker Movements at Different Locations

The top panel of Figure 3.2 presents a 20 s sample of the movements made in the AP plane by two whiskers on the same side of the face. The bottom left panel shows 2 s of

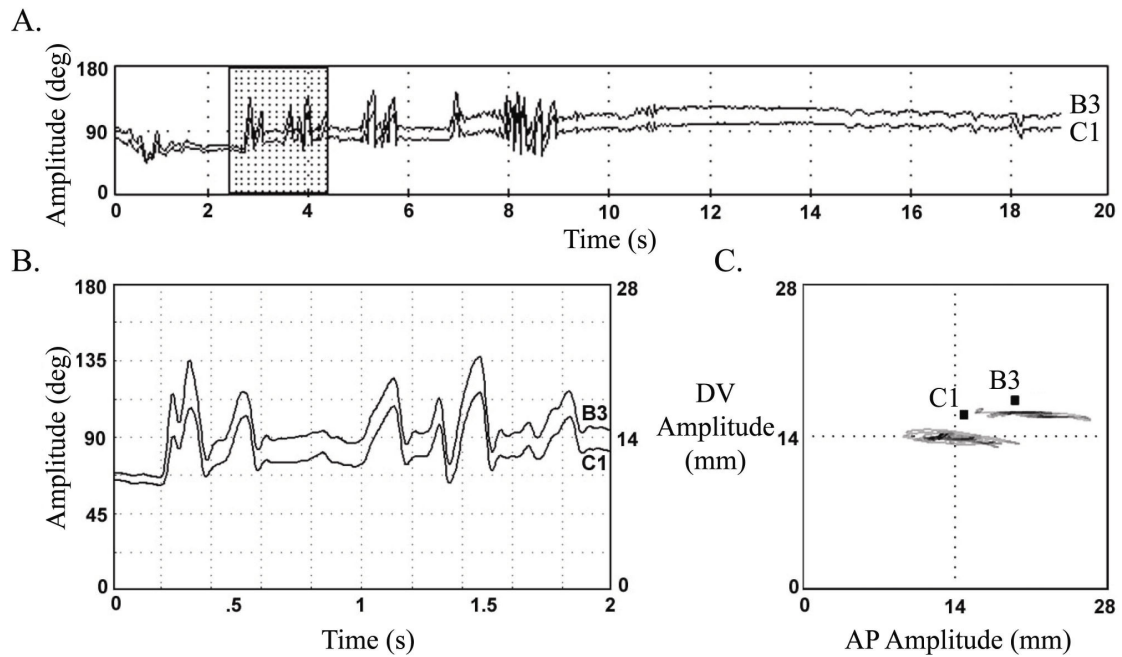


Figure 3.2. Sample of the AP movements of a pair of whiskers (C-1, B-3, locations shown in Figure 3.1) recorded during a whisker-positioning task. A. All movements during a 20 s long trial. B. High-resolution plot of the shaded region in A, above. C. Two-dimensional reconstructions of the whisker movement trajectories during the 2 s data sample in B. The solid squares represent calibration points for each whisker with respect to the intersection of the AP and DV laser curtains (see General Methods, Ch. 2.2.2).

that data (shaded frame in top panel) at a higher resolution, to illustrate the relation between the movement patterns generated by a pair of whiskers during a bout of free-air whisking. Movements of the two whiskers appear synchronous, with similar protraction amplitudes and topographies. Inspection of hundreds of individual whisks revealed no examples of the two whiskers moving in different directions and no cases in which a protraction was seen to occur in only one member of the pair. The bottom right panel of Figure 3.2 represents the same 2 s of movement in two-dimensions, illustrating the movement trajectory in both the AP and DV planes. Here, and in subsequent figures, the two black squares represent calibration points for each of the whiskers with respect to the intersection of the AP and DV laser curtains. As previously reported (Bermejo et al.,

2002), the relative contribution of the DV component to the whisker movement trajectory is minimal.

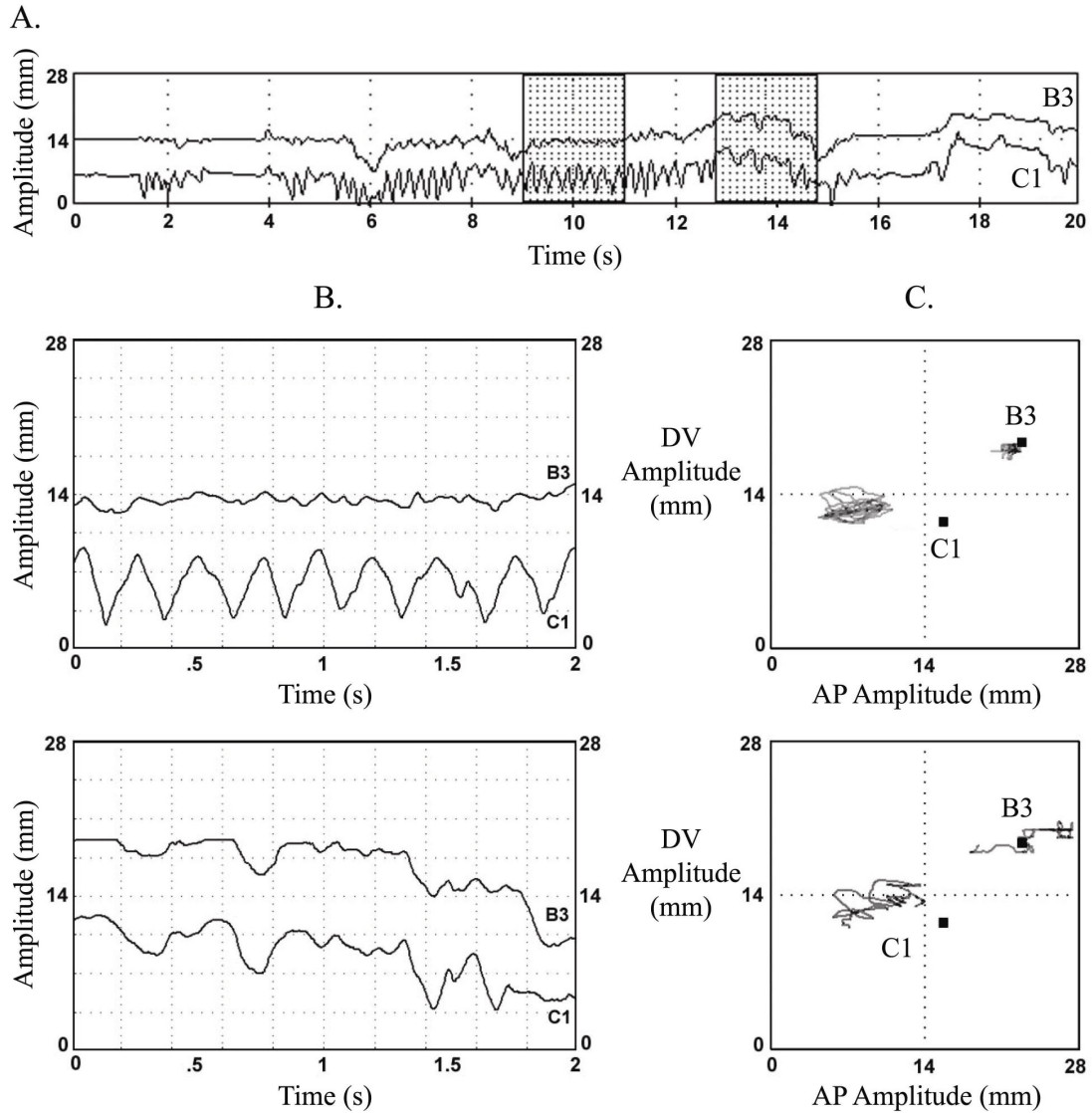


Figure 3.3. Movements of the mystacial pad at two different locations during 'exploratory' whisking in air, as inferred from the displacement of markers attached to the face. A. 20 s of AP movement at each location. B. High-resolution plot of the two, 2 s long epochs in the shaded windows in A. C. Two-dimensional reconstructions for each sample in B.

3.3.2. *Pad Movements at Different Locations*

Figure 3.3 provides records of pad movements monitored simultaneously at two different locations on the face. In the top panel (3.3A), the shaded portions of the record define two different movement samples, selected to illustrate how different locations on the whisker pad can change relative to one another over time. The lower two panels on the left (3.3B) plot the same 2 s samples at a higher resolution. As with whisker movements, movements of the pad exhibit a distinct rhythm whose frequency may vary over successive bouts. Pad movements at the two locations have synchronous onsets and similarly shaped movement trajectories. The illustrations are interesting, however, in that the amplitudes of the pad movements at the two facial locations differ in one sample, but are seemingly similar in the other sample. The panels on right (3.3C) show the corresponding two-dimensional reconstructions of the pad trajectories. Unlike the whisker movements described above, a relatively substantial DV contribution is evident in both samples.

3.3.3. *Interaction of Whisker and Pad Movements*

Figure 3.4A shows a typical 20 s sample of data from a session in which both whisker (C-1) and pad (adjacent to B-3) movements were monitored. The pattern of bursts of movement, separated by periods of relatively little or no activity, illustrated in this sample is representative of how the whiskers and pad moved in the majority of the samples investigated. The 2-D reconstructions plotted in the bottom right panel (3.4C) illustrate the relative contributions of the AP and DV components to movement in both the whisker and the pad. As in the figure, the relative DV component is typically greater

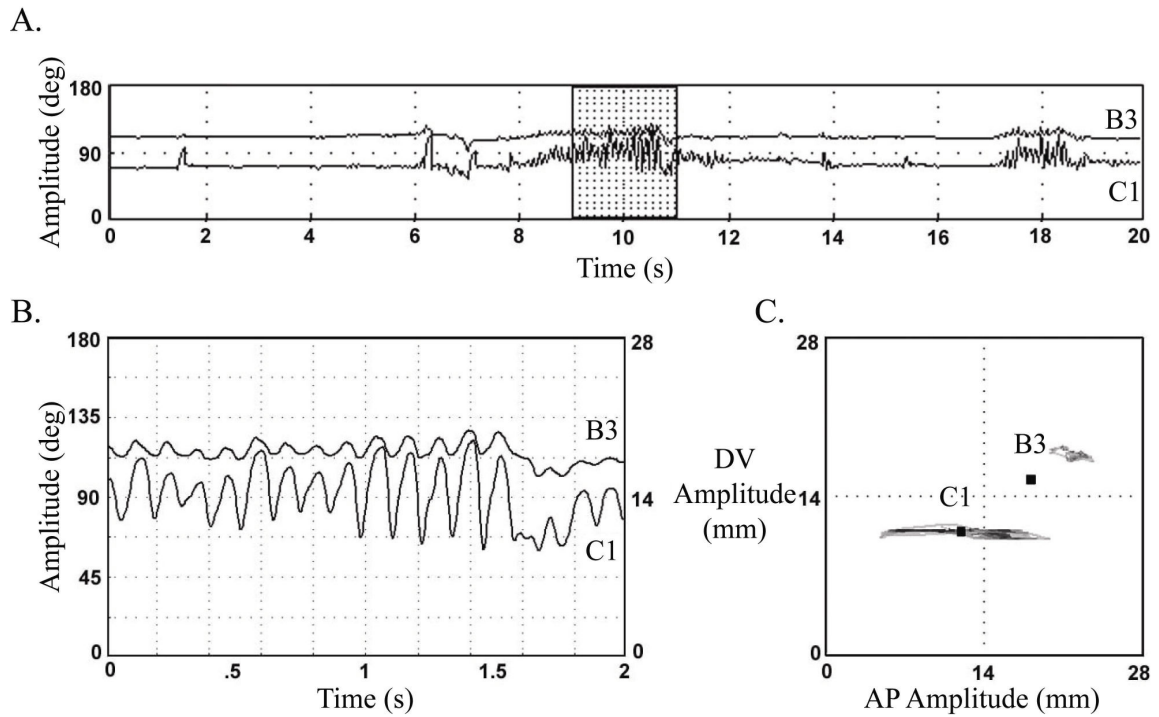


Figure 3.4. A. Movements of the C-1 whisker and of a pad marker adjacent to whisker B-3, plotted in the AP plane. B. High-resolution plot of the shaded region in A, above. Note the near-synchronicity of the whisker and pad movements and the difference in their amplitudes. C. Two-dimensional reconstructions of the whisker (C1) and pad (B3) trajectories during the 2 s of movement from B (left).

in pad movement than in whisker movements. Additionally, the amplitude of whisker movements typically was substantially larger than that of pad movements, as illustrated in the bottom left panel.

The whiskers and pad display the same range of whisking frequencies, and movements within a burst generally appear to be at the same frequency. Movement frequency during a relatively long bout of whisking is illustrated in Figure 3.5. The top panel of this figure (3.5A) depicts the movement traces for both the whisker and pad, while panel 3.5B shows their respective power spectra (PSDs). A comparison of

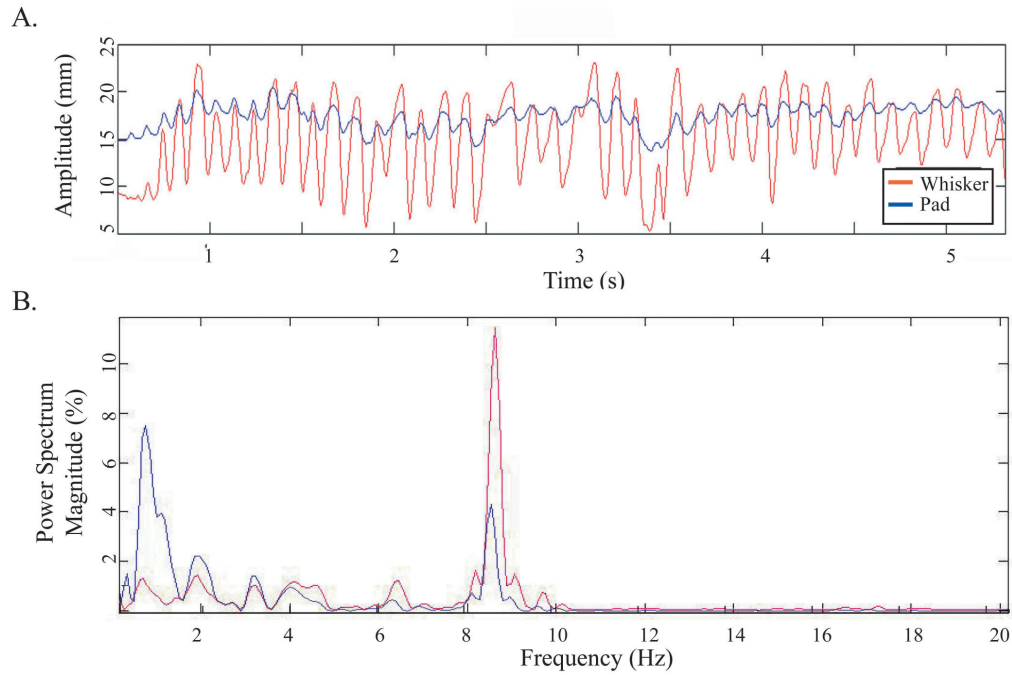


Figure 3.5. Whisker-pad movements share a common frequency. Spectral analysis of simultaneously recorded whisker and pad movements. A. Movement records for the whisker (red) and pad (blue) monitored in the AP plane. Note the near synchrony of the two movements. B. Power spectrum of the whisker (red) and pad (blue) movements. Peaks at the same frequency band are clearly present in both spectra.

the two PSDs reveals that both the whisker and pad are moving at the same frequency, which falls at approximately 9 Hz.

Because the whisker is set in a movable pad, whisker movement records will also reflect a contribution by the pad. A crude method for estimating that contribution is illustrated in Figure 3.6, which plots both whisker (red trace) and pad (blue trace) movements in the AP plane. For the first four movements, we have subtracted the amplitude of the pad movements from the amplitude of the whisker movements. The difference between the two whisker movement traces represents the extent of the pad contribution and is indicated by the green shaded area in the bottom trace. Note that this

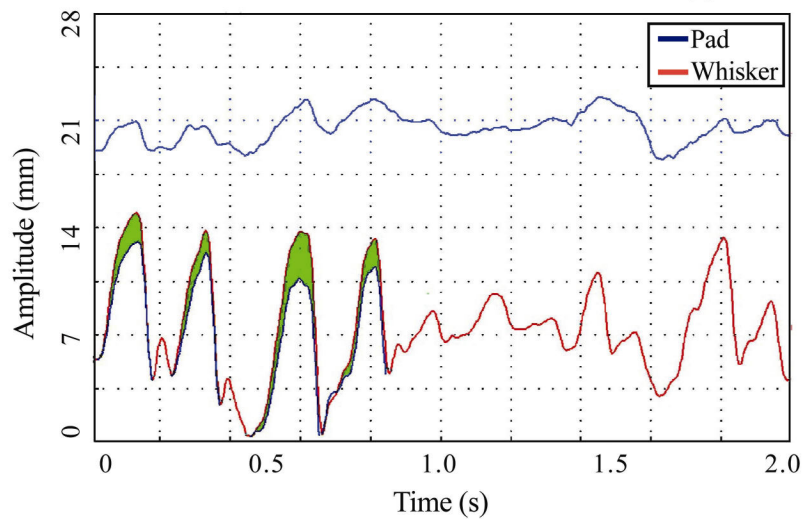


Figure 3.6. Contribution of pad movement to protraction amplitude. The figure illustrates how rhythmic movements of the pad (blue trace) might contribute to the overall protraction amplitude (red trace). The amplitude of the pad movement has been subtracted from the first 4 whisks, as an estimate of its contribution (shaded green region) to whisking amplitude. Note that this contribution is not uniform.

approach assumes that the amount of pad movement is the same at both recording locations.

3.4. Discussion

The rat's whisker system comprises an array of individual sensory hairs capable of varying both their degree of erection and their angle of rotation (Wineski, 1985) and are set into a mobile collagenous base (the mystacial pad). Researchers using whisking behavior as an animal model have made two simplifying assumptions: first, that individual whiskers have only a single degree of freedom in their movements and, second, that all whiskers in the array on one side of the face are activated in synchrony. Observations in a previous study from our lab (Bermejo et al., 2002) have tended to

confirm the first of these assumptions, showing that whisker movements are relatively limited in their degrees of freedom. The present study has confirmed the second assumption. During whisking in air in the head-fixed rat, whisker movements on the same side of the face were always synchronous and similar in amplitude (see also Carvell and Simons (1990)). Other studies that have reported instances of whiskers on the same side moving in different directions (Hartmann, Johnson, Towal, & Assad, 2003; Sachdev, Sato, & Ebner, 2002), or at different amplitudes (Grant et al., 2009), have all involved whisker contacts with object surfaces. This finding suggests that coordinated movement of the entire whisker array is the default condition for this system. Our findings also raise the question of how such coordination is achieved, given that a substantial population of motoneurons in the facial motor nucleus innervates individual whisker muscles. One possibility is that a single whisker motoneuron innervates several whisker follicles. However, a study by Klein and Rhoades (1985) found no double-labeled neurons in facial nucleus after multiple injections into the whisker pad. Another possibility is that the observed synchrony may reflect electrical coupling of facial (whisker) motoneurons, though to date there has been no published work in support of this hypothesis. A third possibility is that the motoneurons receive a common trigger input, such as that from a central pattern generator (Cramer & Keller, 2006; Hattox et al., 2003).

The primary goal of this study was to examine the interaction of whisker and pad movements. Unlike the whiskers, with their relatively limited degrees of freedom, the mystacial pad is capable of substantial translation in the DV and AP planes. When movements of the two structures are integrated, as they are during normal whisking, their combined activity provides the system with multiple degrees of freedom in modulating

the shape, size and location of the whisker sensory field. Given its behavioral flexibility and its two-dimensional operation during active sensing, an analysis of whisker-pad interactions is an important starting point for behavioral and neural modeling of the whisker system.

In a recent EMG study, Berg and Kleinfeld (2002) monitored whisking movements videographically while recording from both intrinsic and extrinsic muscles. These authors distinguished between two types of whisking: ‘exploratory’ and ‘foveal’. The former, characterized by relatively large protraction amplitudes and a frequency range of 5–15 Hz, tends to occur prior to whisker contacts; the latter involves movements of higher frequency (15–25 Hz) and lower amplitude and is seen during rhythmic palpation of an object. They found that during ‘foveal’ whisking, the extrinsic muscle was largely inactive. However, retraction of the whiskers during ‘exploratory’ whisking was accompanied by rhythmic EMG activity in the extrinsic muscles. The present study provides an analysis of whisker and pad movements under conditions in which movements of both structures are monitored simultaneously and with high spatio-temporal resolution. We saw no evidence of ‘foveal whisking’ in our fixed head preparation, rather our observations reflect movement properties associated with ‘exploratory’ whisking in air.

It has been conventionally assumed that the primary contribution of pad translation movements is to control the whisking set point (Carvell & Simons, 1990) over time periods substantially longer than the timeframe of individual whisks. Indeed, variations in the whisking set point are evident in the extended data samples presented in this report. Against this background, the most novel finding of this study, implicit in an earlier study

(Berg & Kleinfeld, 2002), is the observation that rhythmicity is a shared property of both whisker and pad movements. While pad and whisker movements may differ to varying extents in their amplitude and general topography, both structures exhibit the characteristic frequency range of the rodent's 'exploratory' behavior, suggesting that the pad muscles, like the whisker (follicular) muscles receive input from a pattern generating mechanism. In addition, this work demonstrates for the first time a low frequency pad oscillation upon which both whisking rotation and pad translation movements are superimposed.

Whisker and pad movements are mediated by a combination of neuromuscular and hydrostatic mechanisms; some of the muscles act to distort the shape of the pad, while others act to change the tilt of the vibrissae (e.g. Berg and Kleinfeld (2002)). Both operations are occurring simultaneously and the similarity in their temporal organization presumably reflects the fact that both receive input from the same central rhythm-generating mechanisms. Differences in whisker and pad amplitude and topography presumably reflect differences in the biomechanical properties of the structures receiving that input.

Although researchers have tended to view protraction amplitude as reflecting primarily the activity of the intrinsic muscles, it is clear from our findings that translation movements of the pad (via the extrinsic muscles) may also contribute to variations in protraction amplitude. Note that pad displacement is solely translational while the whisker movement reflects both its own pivotal movement and a contribution derived from pad displacement. As Figure 3.6 indicates, this contribution may be substantial. The estimate shown in Figure 3.6 is based on a comparison of protraction amplitude before

and after subtraction of the simultaneous pad movement. The procedure assumes that the amount of pad movement is the same at any two locations on the face, a simplifying assumption not always justified by our data. However, even with this caveat it is clear that pad translation may contribute substantially to measured whisker movement amplitude. An additional caveat is that, because the pad movement is translational its distance cannot be converted into degrees, which can only describe a pendular movement. Degrees are useful when comparing data from multiple animals, as the measure remains the same regardless of the distance of the recording array from the face. Because all comparisons in this study were between two whiskers or pad locations on the same animal, the conversion into degrees was unnecessary and therefore does not impact on the conclusions of this study.

The relatively close proximity and posterior location of our pad markers would tend to minimize differences between the pad movements monitored at different locations. Moreover, we did not collect data during ‘foveal’ whisking, which might have been expected to exaggerate differences between posterior and more anterior pad movements. Nevertheless, as the traces in Figure 3.3 indicate, even under the present testing conditions there may be substantial differences in the amplitude of pad movements recorded from two different locations within a period of a few seconds. Thus, the data generated by the present experimental situation, which was restricted to ‘exploratory’ whisking, may tend to oversimplify the complexity of whisker/pad interaction.

Even under the present simplified conditions the two-dimensional nature of pad movements contributes substantially to the complexity of whisker–pad interactions. Pad movements contribute to the topography of whisker movement trajectories by controlling

the degree of both AP and DV translation of the whisker array. This is evident in the trajectory plots in Figure 3.4 where a contribution by pad movements to the position of the whisker is evident. It is even more clearly seen in the plot of pad movements presented in Figure 3.3. The biomechanical and muscular events generating these movement records is best understood with reference to the lines of force calculated for specific extrinsic muscles (Hill et al., 2008; Wineski, 1985). That is, activation of the extrinsic muscles will translate the pad along a trajectory that reflects the vector sum of the differential activation of different extrinsic muscles. The trajectory will be primarily AP with dorsal and ventral components.

4. ANTICIPATORY ACTIVITY OF MOTOR CORTEX IN RELATION TO RHYTHMIC WHISKING

4.1. Introduction

Experiment 1 provided a qualitative description of movements of the whisker and pad, and their interaction, during exploratory whisking in rats. The results of that study, specifically with regard to the relative timing of whisker and pad movements during epochs of rhythmic whisking, suggest that both types of movement are the result of pattern generating mechanisms. In fact, a long line of research has implicated a pattern generator in the production of rhythmic whisker movements (Berg & Kleinfeld, 2002; Gao et al., 2001; Gao et al., 2003a; Harvey, Sachdev, & Zeigler, 2001b; Semba & Komisaruk, 1984). More recently, serotonergic pre-motoneurons in the brainstem have been shown to be both necessary and sufficient for producing rhythmic whisking (Hattox et al., 2003), and thus are considered to form part of a whisking central pattern generator (CPG). The contribution of other structures in whisker sensory-motor pathways to the production and modulation of whisker movement patterns, however, remain to be determined. The current chapter describes the first of two studies in this dissertation focused on understanding the contribution of motor cortex to the control of whisking behavior.

In rats, the involvement of a large region of motor cortex is suggested by the observation that low intensity stimulation of different parts of this region (vMCx) can evoke both small vibrissal retractions (Donoghue & Wise, 1982) and rhythmic

movements similar to exploratory whisking (Berg & Kleinfeld, 2003; Brecht et al., 2004; Haiss & Schwarz, 2005). Additionally, vMCx is now known to send extensive projections to the serotonergic pre-motoneurons that form part of the whisking pattern generator (Hattox et al., 2002).

Several observations suggest that, as is the case for other rhythmic behaviors (Larson et al., 1980; MacKay-Lyons, 2002; Nakamura & Katakura, 1995; Thompson & Watson, 2005), motor cortex may be responsible for both the initiation and the modulation of the pattern generated and maintained by the whisking CPG. 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). However, demonstration of coherence between cortical field oscillations and vibrissal EMG activity (Ahrens & Kleinfeld, 2004) suggests that vibrissae movements may also be controlled by vMCx on a cycle-by-cycle basis.

If vMCx contributes to the initiation of whisking behavior by activating the CPG then the onset of whisking will be preceded by changes in motor cortical activity. The present study tested this prediction. This chapter is based on a previously published paper (Friedman, Jones, Cramer, Kwegyir-Afful, Zeigler, & Keller, 2006).

4.2. Materials and Methods

4.2.1. 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.

Subjects were gentled daily for at least 2 weeks prior to surgery. Under sodium pentobarbital anesthesia (60 mg/kg, IP; 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 (2 mm ML; 4 mm AP) was made (bilaterally in one, unilaterally in the others) directly above the vMCx (Weiss & Keller, 1994), and an eight-channel array (quartz-insulated platinum electrodes, 500 μ m 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 5th 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 (see General Methods, Chapter 2).

Animals were given Buprenorphine HCl (0.03 mg/kg SQ) to alleviate pain and antibiotic (Baytril 0.05 mg/kg IM) immediately and 12 hours post-surgery.

4.2.2. Data Collection

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 (4x4) silicon-based multi-electrode 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 O₂ 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 (see General Methods, Chapter 2). 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. LFPs were low-pass filtered at 300 Hz.

4.2.3. 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 μ sec long pulses delivered at 50 Hz for 1 s; $\geq 100 \mu$ A). We monitored vibrissae movements with an optoelectronic monitoring system (see General Methods, Chapter 2.3). As described previously by Haiss and Schwarz (2005), these stimulation parameters evoked large vibrissae movements that resembled natural rhythmic whisking (Figure 4.1A), confirming that the electrodes were implanted in vMCx (Haiss & 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 (Figure 4.1B).

4.2.4. 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

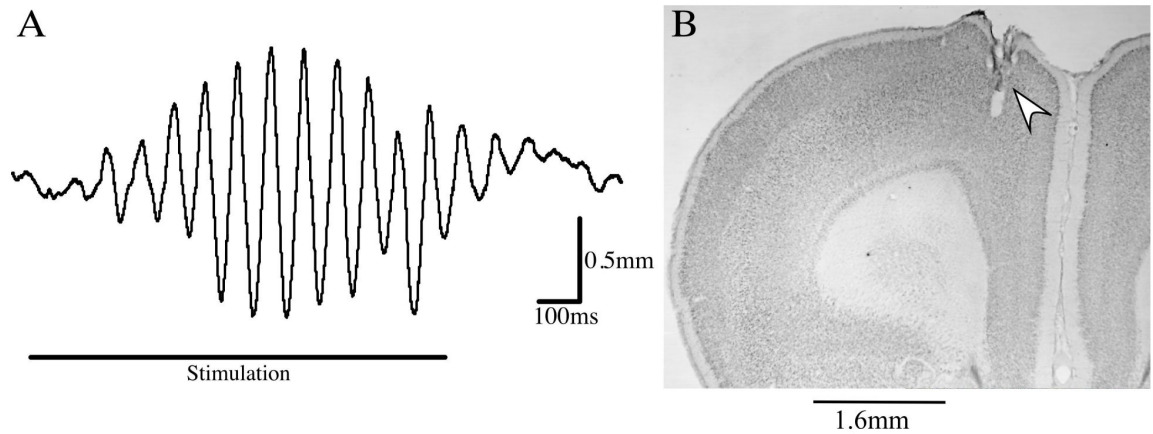


Figure 4.1. A: whisker trajectory (protraction, up) evoked by microstimulation of vibrissal representation in motor cortex (vMCx) and recorded with a charge coupled device (CCD). B: Nissl-stained coronal sections depicting electrode tracks through the agranularmedial (AGm) region of the motor cortex.

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%) for activity at frequencies up to 20 Hz using the jackknife method (Thomson & Chave, 1991) and computed using the Chronux package (www.chronux.org).

4.3. 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.

4.3.1. 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. Figures 4.2 and 4.4 show EMG waveforms recorded during typical whisking epochs (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.

4.3.2. 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 (Figures 4.2 & 4.4). LFP spectrograms enabled us to quantify the

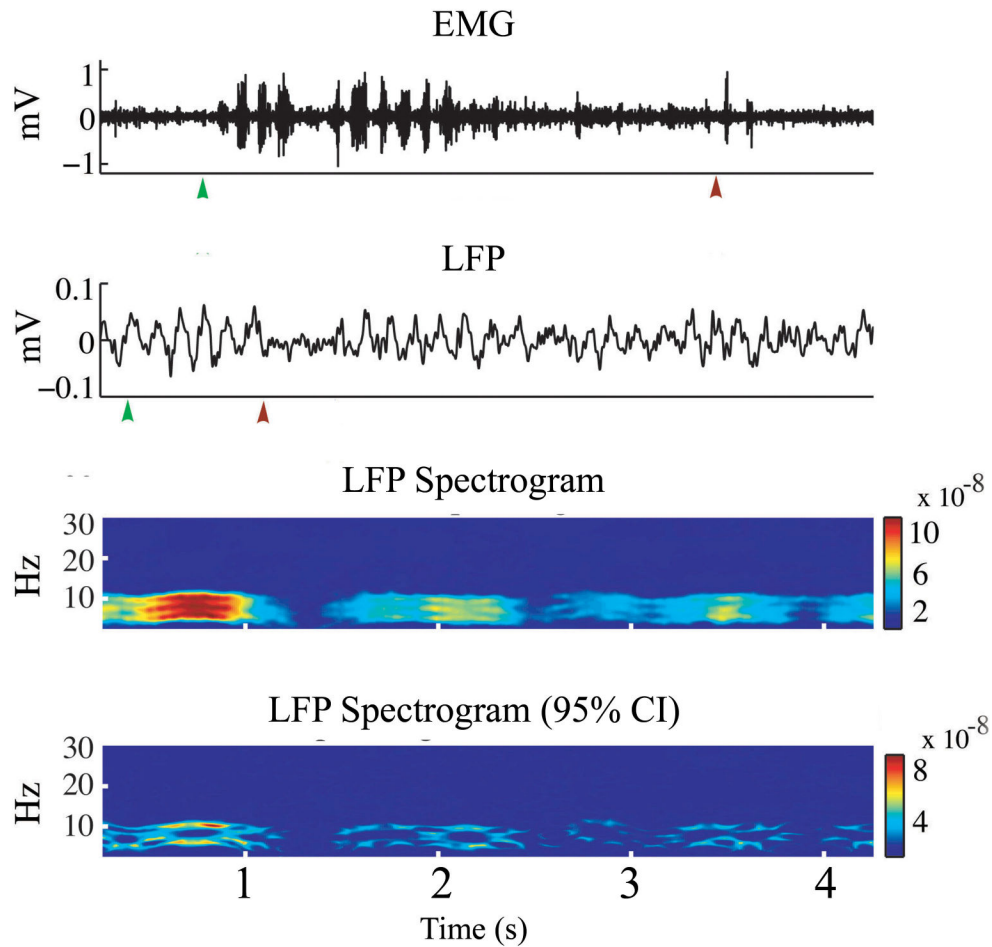


Figure 4.2. Raw trace of EMG activity (top) with corresponding local field potential (LFP) traces below. Onset (green arrowhead) and offset (red arrowhead) in electromyographic (EMG) and LFP activity are depicted on the corresponding traces. Increases in LFP just before whisking onset and again just prior to whisking offset are evidenced as an increase in power in the LFP spectrogram (3rd panel). Changes in power that are statistically significant above the 95% confidence level are plotted in the bottom panel.

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

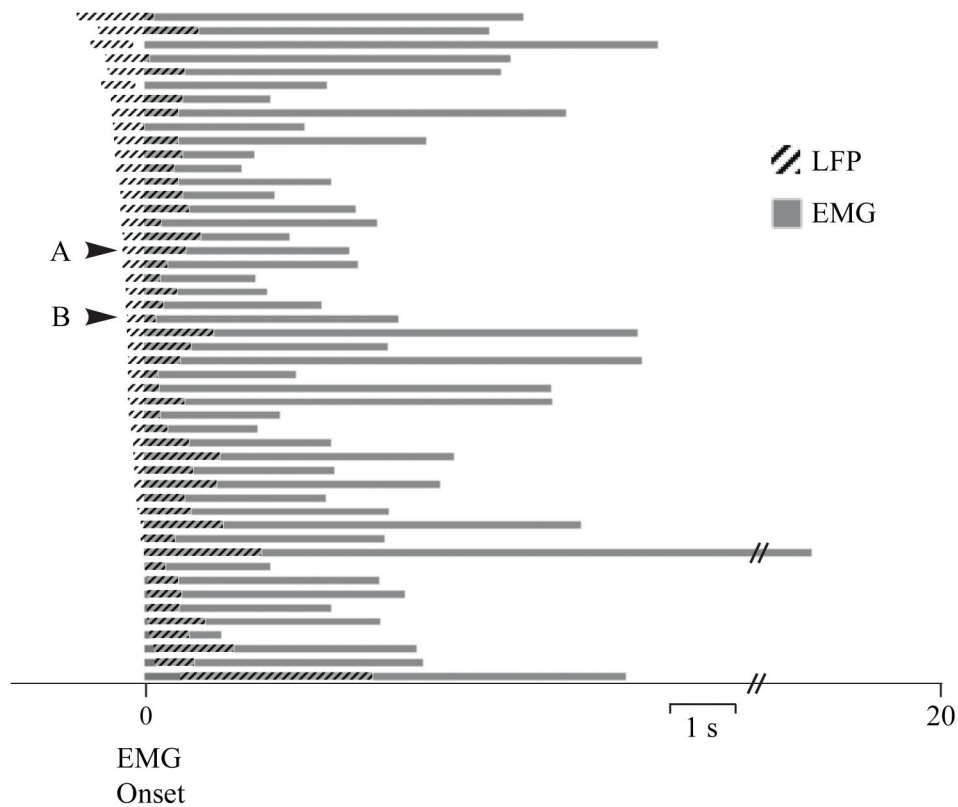


Figure 4.3. Time course of increases in LFP and EMG activity recorded from 49 epochs in 5 animals. Data are aligned to whisking onset. Changes in LFPs preceded whisking onset in 39 of the 49 epochs and ended prior to whisking offset in all samples. Overlaps in activity are illustrated by bar overlaps. Bars marked A and B correspond to data depicted in Figure 4.2 and 4.3, respectively.

determined significant changes in LFP activity by computing activity levels that exceeded the 95% confidence level (Figures 4.2 & 4.4, 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%; Figure 4.3). 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

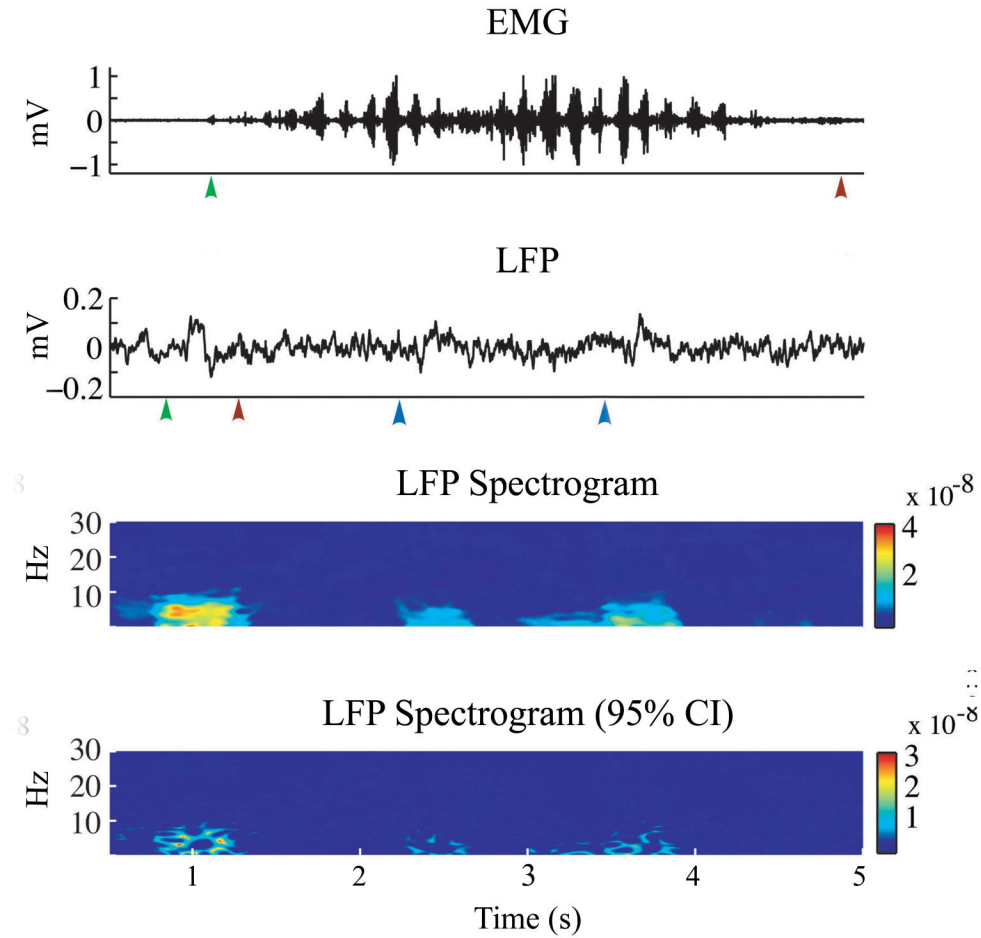


Figure 4.4. Raw trace of EMG activity (top) with corresponding local field potential (LFP) trace below. Onset and offset indicated by green and red arrowheads, respectively (top two panels). All panels are as in Figure 4.2. Instances in which a change in whisking dynamics during an epoch is reflected in LFP activity are indicated with blue arrowheads.

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 (Figure 4.4).

4.4. Discussion

It has long been known that stimulation of the vMCx can result in vibrissae movements (Donoghue & 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 (Cramer & Keller, 2006).

This study was designed to test 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, Nawrot, Pistohl, Aertsen, Schulze-Bonhage, & Mehring, 2004; Mehring, Rickert, Vaadia, Cardosa de Oliveira, Aertsen, & Rotter, 2003; Scherberger, Jarvis, & Andersen, 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. The findings are also consistent with the work of Carvell, Miller *et al.* (1996), who showed that unit activity recorded from vMCx increases immediately prior to whisking onset (51 ± 75 msec). The present study found vMCx activity precedes whisking onset by, on average, 240 msec. The longer pre-movement LFP activity, compared to that reported by Carvell, Miller *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. That is, activity *level* in any given neuron may not necessary increase at the same time that the overall LFP activity increases. Instead, LFP activity increases when there is an increase in *synchrony*, which likely (though not necessarily) is accompanied by an increase in some, but not necessarily all, neurons in a recording area.

Pre-vibrissae movement activity has also been reported to occur in the rat somatosensory cortex (Hamada, Miyashita, & Tanaka, 1999). Indeed, that cortical activity precedes and may “anticipate” voluntary movements was demonstrated 30 years ago (Tanji & Evarts, 1976).

Our findings also demonstrate that vMCx activity does not persist throughout the full time course of whisking epochs (Figure 4.3). These findings are consistent with the hypothesis that vMCx initiates whisking by activating a subcortical CPG, but it does not maintain whisking continuously throughout epochs of movement. 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). The 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 & Marder, 1991; Hooper & 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, Schneider 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 & 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; Hattox et al., 2002), is consistent with the hypothesis that motor cortex can modulate the whisking pattern through its actions on subcortical pattern generators.

5. THE RELATIONSHIP BETWEEN WHISKER MOTOR CORTEX AND WHISKING KINEMATICS IN THE BEHAVING RAT

5.1. Introduction

The role of primary motor cortex (MCx) in the control of movements has been studied for decades. Though a large body of work on this region exists we still do not know how the activity of motor cortical neurons encodes movement parameters. In studies of both tightly controlled movements, such as reaching and grasping, and rhythmic behaviors, such as locomotion and chewing, different cortical cells vary in their relationship to movement. In humans and non-human primates, for instance, MCx has been found to mediate the force, direction, and planning of tightly controlled movements (Grillner, Georgopoulos, & Jordan, 1997; Rickert, Oliveira, Vaadia, Aertsen, Rotter, & Mehring, 2005; Tanji & Evarts, 1976) and individual neurons can encode different parameters of these movements depending on context (Thach, 1978). A similar complexity appears to exist in the control of rhythmic movements. For instance, cortical activity is modulated with changes in the locomotion rhythm during walking in only some classes of MCx neurons (Beloozerova, Sirota, & Swadlow, 2003a), and the role of individual neurons during similar movements can vary with context (Beloozerova, Sirota, Orlovsky, & Deliagina, 2006). Importantly, though motor cortical inputs are not required for the basic production of many rhythmic behaviors, the contribution of MCx appears to

increase with the complexity of the environmental landscape (Beloozerova & Sirota, 1993; Drew et al., 1996; Larson et al., 1980).

As with the previous chapter, the aim of this study was to examine the relationship between vMCx activity and whisker movements during normal whisking behavior. The design of this study, however, sought to expand on the work described above in four important ways.

First, in the previous study whisker movements were inferred from EMG activity of the intrinsic muscles rather than directly tracking the whisker. In the present study movement kinematics were characterized with high spatio-temporal resolution by recording the movements of a single whisker using an optoelectronic monitoring system. As was demonstrated in the first experiment of this dissertation (Chapter 3), monitoring of a single whisker under these conditions provides a valid account of the movement of other whiskers in the same array.

Second, while the local field potentials (LFPs) used in the previous study are an excellent indicator of the general activity in a given region, they do not give information about the precise firing patterns of individual neurons. In fact, though we and others have shown that changes in cortical activity accompany whisking (Carvell et al., 1996; Friedman et al., 2006), the function of these changes still remain unknown. We therefore recorded unit activity using a movable electrode, which enabled us to sample a large population of neurons within the region of interest. By recording single and multi-unit activity we could then analyze the relationship between spike rate and timing and specific kinematic parameters, which is necessary to characterize the encoding properties of specific neurons.

Third, in the previous study we examined neuronal activity in a region of cortex defined as “motor” solely on the grounds of its general location with respect to landmarks and the ability to elicit whisker movement. Since that study, the classical whisker motor region has been reclassified as two functionally distinct subregions, a “rhythmic whisking” area and a “retraction” area (Haiss & Schwarz, 2005). No recording studies that we know of have taken this heterogeneity into account. By restricting our cortical recording sites to the rhythmic sub-region of vMCx, we aimed to increase the likelihood that activity patterns across recorded units would represent similar codes.

Finally, the current study examined unit activity during two different classes of whisking behavior, whisking in air and whisking towards an object, both of which are part of the rat’s normal behavioral repertoire. As was discussed above in Chapter 4.4, the results from intracellular stimulation indicate that the relationship between cellular activity and movement may differ across neurons, and may be context dependent. The use of these two tasks enabled us to investigate if, and how, the role of vMCx during whisking changes with behavioral context.

We focused our analyses on whisking frequency and amplitude, the two cardinal parameters that define whisking behavior. Given the finding that increasing stimulation intensity during intracortical microstimulation in vMCx increases the frequency of evoked whisker movements (Cramer & Keller, 2006) we hypothesized that there would be a correlation between the rate and/or timing of cortical activity and whisking frequency in at least one, if not both, behavioral contexts. However, our results indicate that activity in this cortical region is most frequently associated with the amplitude of

vibrissa movements, and that the coherence between whisking and cortical activity may be task-dependent.

5.2. Materials and Methods

5.2.1. Surgical Procedures

Data for this study were collected from 7 adult female Long Evans rats (200 – 300 g). Animals were handled daily for a minimum of 2 weeks prior to surgical procedures.

Under aseptic conditions animals were anesthetized with isoflurane anesthesia, and fitted with a headmount as described under General Methods, Chapter 2. In order to make a craniotomy after completion of the behavioral training process, a small window (~ 2 x 2 mm) was left in the dental cement at the stereotaxic coordinates (1.3 medial and 1.3 anterior to Bregma) of the rhythmic whisking region of whisker motor cortex.

Following adaptation to head fixation and consistent performance on the operant task (see below) the animals were re-anesthetized with Isoflurane, an injection of Dexamethazone (2mg/kg IM) was given to control brain swelling and an approximately 1.5 x 1.5 mm opening was made in the exposed skull. The opening was filled with saline-soaked sterile cotton and a thin layer of dental cement was placed over the cotton. Each day prior to recording animals were head-fixed and the dental cement and cotton were gently removed with a scalpel and forceps. Saline was added to the opening throughout recording sessions to keep the brain moist and sterile saline-soaked cotton and dental cement were reapplied at the completion of each session.

5.2.2. Electrophysiology

Neuronal activity was recorded while rats generated whisker movements in one of two operant tasks (see below). Electrophysiological activity was amplified and band pass-filtered from 1 - 10 kHz and digitized to disk (Alpha-Map, Alpha Omega, Israel) at a sampling rate of 25 kHz.

Using a manual stereotaxic microdrive, electrodes (FHC, stainless steel, 2-4 M Ω , 250 μ m shank) were inserted through the dura and slowly lowered to the deep layers of whisker motor cortex ($\sim$ 1.5mm). Data collection began after audio-visual isolation of a neuron. If isolation was lost, the electrode was moved until a new neuron was encountered. Care was taken not to exceed a depth of 1.9 mm. Sessions lasted 45-65 minutes.

Activity generated during body movements was eliminated from the analysis, as were periods of licking, which were monitored by connecting the metal sipper tube that delivered the reward to the rat's ground. Contact between the rat's tongue and the tube produced a junction potential that could be identified in the electrophysiological recording.

5.2.3. Behavioral Recordings

Movements of the C2 whisker in the anterior-posterior plane were recorded in head-fixed rats using an optoelectronic monitoring system (See General Methods, Chapter 2, for detailed description). The whisker was enlarged by attaching either a light strip of foam (<4 mg) with glue backing, or by placing it within a concentric set of light polyimide tubes (Small Parts; total weight $\sim$ 0.6 mg), so that it could be differentiated

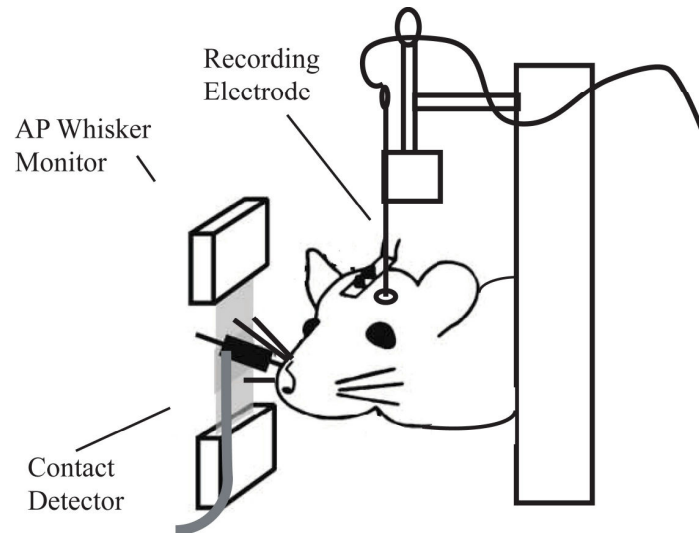


Figure 5.1. Experimental setup for recording neural activity in head-fixed rats engaged in a whisker contact task. A single electrode is inserted into the brain at the start of the recording sessions, and can be moved during the session as necessary. The contact detector is attached to a phonograph needle (not shown). Contact deflects the needle, causing a voltage transient used as the touch indicator.

from the other intact whiskers. The set of tubes consisted of an inner tube (diameter: 0.32mm; length: ~25.5mm) set within a slightly shorter outer tube (diameter: 1.29mm; length: ~20mm) and painted with an opaque black paint. Position information was acquired by a microprocessor at 500 Hz. In order to ensure precise synchrony, the behavioral data was outputted from this microprocessor at 500Hz and re-sampled by the electrophysiology equipment at a rate of 12 KHz.

Whisker contacts were detected using an L-shaped glass rod glued to a phonograph needle (Stylus for Chuo Denshi CZ800, Radio Shack) seated within a magnetic cartridge (Chuo Denshi CZ-800, Radio Shack). The cartridge was connected to a stepper motor, used to mechanically move the device into and out of the whisking field. When the marked whisker touched the vertical arm of the glass rod, the needle transmuted the deflection into steep voltage transients (Hentschke, Haiss, & Schwarz, 2006). Transients

that exceeded a minimum criterion (1.5 or 2.0 V, depending on baseline activity for the session) were taken to indicate contact.

All behavioral recordings were made with the room lights off. There was a low level of illumination, which originated from two computer monitors in the room.

5.2.4. Behavioral Training

Rats were tested under a food deprivation schedule and maintained at 85-90% of ad libitum body weight. Following ~14 days (longer if necessary) of post-surgery recovery and gentling, animals were adapted to head fixation by being kept in a fixation container on a tabletop for 5-15 minutes / day (5 days a week) until they appeared comfortable (usually 1 week) and accepted a treat (NutraCal). Next, animals were adapted to the behavior chamber by delivering the reinforcer (“Yoohoo”, a chocolate drink) on a variable interval (VI10) time schedule for 15-30 minutes / day until they consistently accepted the reinforcer (~2-4 days).

Rats were trained in one of two different behavioral tasks. Because whisking in head-fixed rats will extinguish in the absence of reinforcement (Gao et al., 2003b), the operant training paradigms were designed primarily to maintain exploratory whisking over a large number of testing sessions. They differed in that reinforcement was contingent upon patterns of whisking in air in Task 1 and upon whisks to a contact detector in Task 2.

Task 1 – Exploratory Whisking: The flash of a house light inside the test chamber signaled the start of each trial, but did not remain on during recording. Animals (n=3) were reinforced for epochs of rhythmic whisking in air above a specified frequency and

that contained whisks meeting an amplitude criterion. Initially, a burst of tones (5 Hz) was paired with reinforcer delivery. Once an association between the tones and the presentation of the reward was made, as evidenced by anticipatory licking prior to reinforcer presentation, the occurrence of the tone became contingent on the animal producing a whisk(s) > 5mm in amplitude. Reinforcement, in turn, was contingent on the occurrence of 4 tones (e.g., 4 whisks, each > 5mm) within 1 s (4 Hz whisking). The result of this paradigm was the maintenance of whisking at frequencies within the range of exploratory whisking throughout the recording session. In addition to reinforcement of whisking, the operant contingencies also served to reduce the probability of reinforcing other oromotor behaviors (e.g., tooth grinding and licking). Recording sessions were composed of 45-65, 60s trials.

Task 2 – Contact Whisking: The flash of a house light inside the test chamber signaled the start of each trial, but did not remain on during recording. Animals (n=4) were reinforced for movements of the C2 whisker that made contact with the vertical component of an L-shaped glass rod fixed in the most rostral 2 mms of the behavior-monitoring device (Figure 5.1). All whiskers rostral to C2 were trimmed to a length that precluded them from contacting the rod (~ 7-9mm). The glass rod was mounted on a movable arm, which swung the rod into position at the start of each trial and out of reach at its end. All trials were 60s long, and each daily recording session consisted of 50-65 trials. All contacts $\geq$ 3s from the previous contact were rewarded.

At the start of each animal's training, the contact rod was placed near the resting position of the marked whisker (C2). After the rats had learned the association between contact and reward, as evidenced by the occurrence of licks immediately after contact and

an increase in the number of contacts made, the rod position was moved to progressively more rostral positions in order to increase the amplitude of whisks.

5.2.5. Analyses

Behavioral and neurophysiology data were imported into Neuroexplorer (Nex Technologies, Littleton, MA). Whisking data were selected for analysis by identifying epochs that lasted for ≥ 1 s, were preceded by ≥ 500 ms of non-whisking and did not contain contacts, though they might have led up to a contact. Individual whisking epochs meeting selection criteria were isolated in Neuroexplorer by placing a time stamp at the start and end of each epoch. These time stamps were then used as interval filters on both the raw behavior and neural signals. Once epochs within trials were isolated, all trials from the same electrode location within a recording session were merged together into a single file and exported to the Plexon Offline Sorter (Dallas, Texas) for sorting of multiunit and, when possible, isolation of single-unit activity. Multi- and single-unit spike times and behavioral data were then exported to Matlab (MathWorks, Natick, MA) for further analysis.

The movement records were decimated to 500 Hz prior to all analyses. In order to capture changes in whisking over time and to enable trial averaging, we used 1s sliding window (500 ms overlap) to segment each whisking epoch. For each segment we calculated the spectral power (multi-tapered power spectrum, PSD) of the movement and spike records and the coherence between these two measures (for all analyses, tapers = [2 3]; pad = 2). The dominant frequency (between 2-20 Hz) for whisking and spiking were taken as the location of the peak in the respective PSD.

Coherence between the whisking segments and the corresponding spiking activity was computed using the Chronux software package (www.chronux.org). The confidence limit for coherence was estimated using the equation: $\text{confidence} = \sqrt{1 - p^{(NK-1)-1}}$, where N is the number of segments, NK is the degrees of freedom and $p = 0.05 / 0.01$ represents the 95% and 99% confidence limits, respectively (Jarvis and Mitra, 2001). For $N = 1$ epoch, $K = 3$, and $p = 0.05$, for instance, the coherence between two unrelated signals is 0.88, while coherence at $p = 0.01$ is 0.95.

In order to correct for differences in set point caused by location of the pad (see Chapter 3), the movement record was detrended by subtracting the best-fit line for each whisking segment, and the area under the curve was taken as a measure of whisking amplitude. For all sessions containing ≥ 5 segments both whisking frequency and segment amplitude were correlated with firing rate using Spearman's ρ .

To determine if occurrences of statistical significance were greater than expected by chance, neural and movement data were temporally dissociated by randomly shuffling the position of the spike data relative to the movement data. That is, for each analysis each segment of spike times was analyzed with reference to a segment of the movement record that occurred at a different point in time from the spikes. Comparisons were made between the proportion of significant sessions/segments for the shuffled and unshuffled data using either the Fisher's Exact Probability Test (when $n \leq 50$) or the z-Test for Comparing Two Proportions (when $n > 50$).

Comparisons between proportions of significant sessions/segments for Task 1 and Task 2 animals were also made for all analyses. When no differences by Task were

found, data were combined and analyzed as a group. When differences between groups were found results for each group are reported separately.

5.2.6. Identification of recording sites

The locations of recording sites within vMCx were verified through cortical microstimulation after all recording sessions for each animal were complete. The animals were either anesthetized with Ketamine (100 mg/kg) and Xylazine (8 mg kg/1) (n=4), or head-fixed in the awake state (n=3), and vibrissae movements were evoked by intracortical microstimulation (200 μ s long pulses delivered at 50 Hz for 1 s; $\leq 100 \mu$ A). Six of the 7 animals were stimulated at the same coordinates used during recording. In the 7th animal, the recording locations could not be precisely identified and so stimulation was made at sample sites throughout the craniotomy. Stimulation in the majority of sites across animals resulted in movements characterized by a strong rhythmic component (Figure 5.2B), as described previously by Haiss and Schwarz (2005) and Cramer and Keller (2006). These results confirm that the electrodes were implanted in the “rhythmic whisking” region of vMCx, (Haiss and Schwarz 2005; Sanderson et al. 1984). Stimulation at a few sites evoked a single retraction, with no rhythmic component (Figure 5.2C). Recordings made from this ‘retraction region’ were excluded from analyses.

At the completion of stimulation sessions, rats were deeply anesthetized (pentobarbital 60 mg/kg) and perfused with 4% buffered paraformaldehyde. Brains of 6 of the 7 animals were removed, sectioned in the coronal plane, and Nissl-stained to confirm the location of the electrode tracks in MCx (Figure 5.2A). The 7th rat died before the perfusion process could be completed.

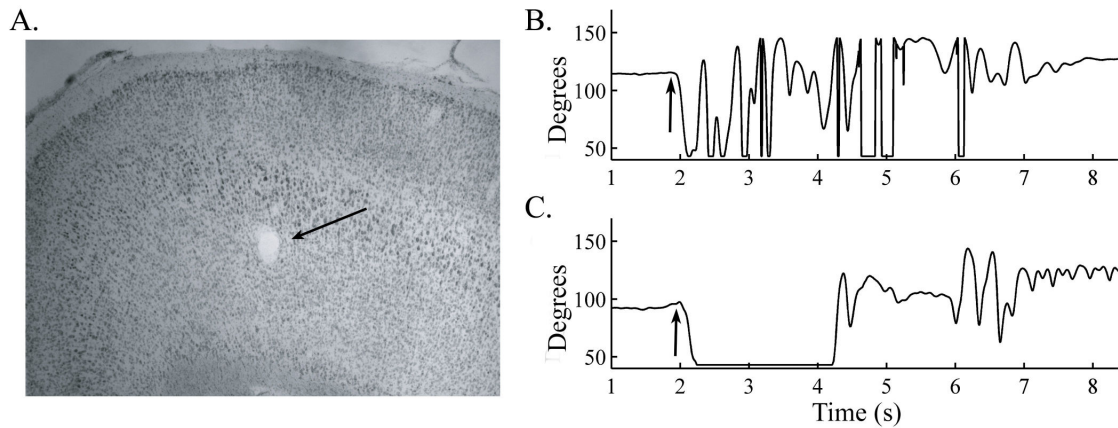


Figure 5.2. A: Nissle-stained coronal section depicting a lesion made at the recording site in the rhythmic region of the whisker motor cortex (vMCx). Stimulation of this region results in rhythmic whisker movements (B), while stimulation of the retraction region evokes a prolonged retraction movement (C). Approximate time of stimulations indicated by vertical arrows in B & C.

5.3. Results

Our goal was to examine the relationship between neuronal activity in vMCx and the kinematics of vibrissae movements, and to determine if that relationship varies with behavioral context. Our analyses included recordings from 32 well-isolated single units (from 4 animals) and MUA recordings from 53 recording locations (7 animals). Neural activity was correlated with simultaneously recorded epochs of voluntary whisking, each lasting at least 1s. Prior to analysis, epochs longer than 1s were parsed into 1s long segments using a sliding window with a 500 ms overlap (see Materials and Methods). In all, we analyzed MUAs from 1249 whisking segments (475 epochs) and single units from 700 segments (263 epochs).

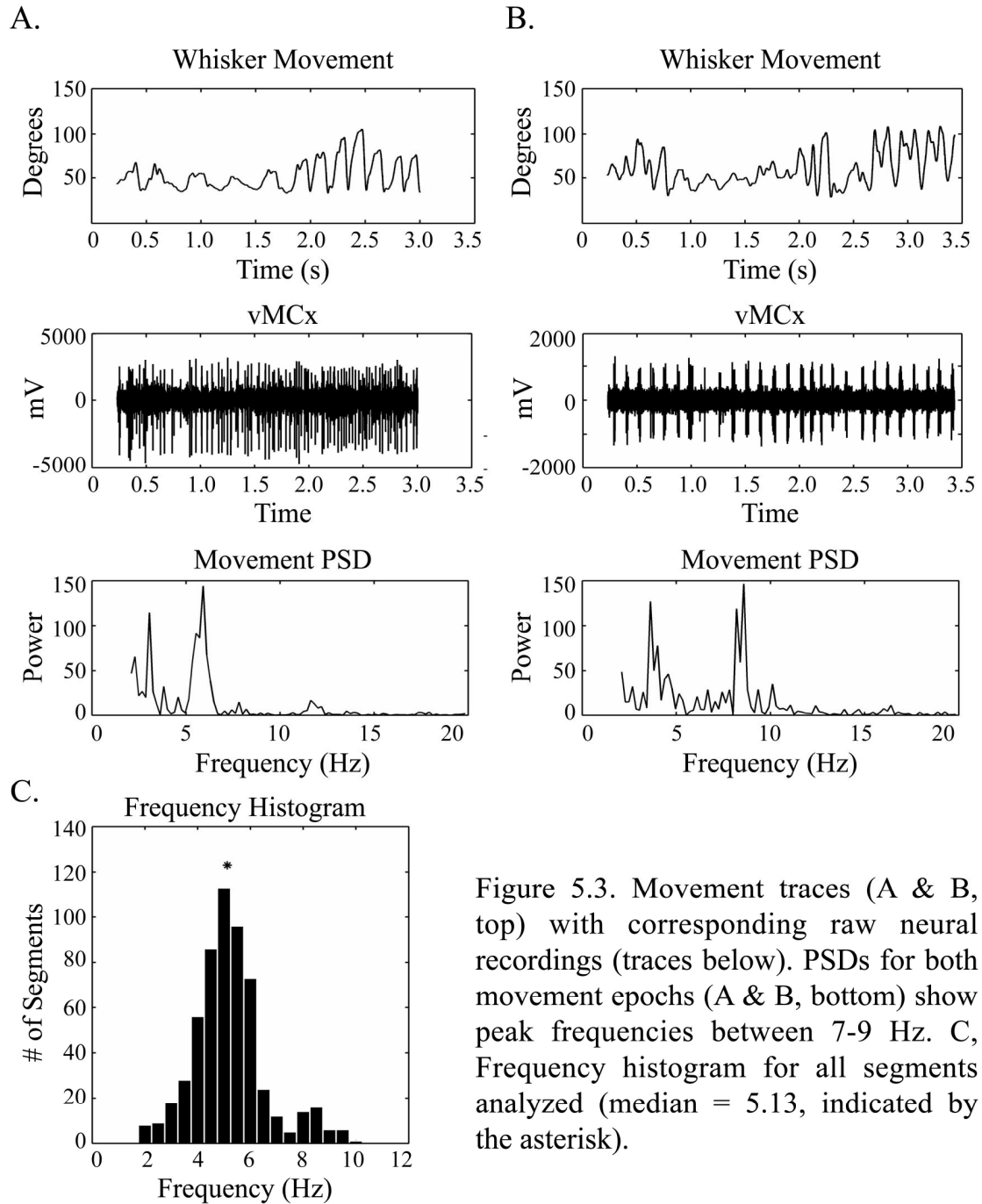


Figure 5.3. Movement traces (A & B, top) with corresponding raw neural recordings (traces below). PSDs for both movement epochs (A & B, bottom) show peak frequencies between 7-9 Hz. C, Frequency histogram for all segments analyzed (median = 5.13, indicated by the asterisk).

5.3.1. Whisking Frequency

We calculated the power spectral density (PSD) of the movement record for each whisking segment, and identified the whisking frequency as the spectrum's peak. Figures

5.3A & B (top panels) illustrate typical epochs of whisking and the PSDs (bottom panels) for those epochs. Also shown are the raw neural records for the same time periods (middle panels). Whisking frequencies within selected segments ranged from 2 to 10 Hz (median = 5.13, Figure 5.3C), which are within the typical range of frequencies for exploratory whisking (Hill et al., 2008; Jin, Witzemann, & Brecht, 2004).

5.3.2. Relationship between pre-movement neural activity and whisking

We, and others, have previously shown that vMCx activity increases before the onset of a whisking epoch (Carvell et al., 1996; Friedman et al., 2006). As an illustration, Figure 5.4 presents raster plots (top) of spike activity and the spike histogram (bottom) from 0.5 s prior to movement onset to 1 s post-movement onset for all epochs from a single recording site. To determine whether the pre-movement activity co-varies with whisking frequency and/or amplitude, we computed correlations (Spearman's rank correlation coefficient, ρ , which makes no assumption of linearity) between these variables (calculated over the first 1 s of movement for each epoch) and neuronal firing rates during the 500 ms preceding each whisking epoch. We selected this time interval because, as reported in the previous chapter, LFP activity increases 200 to 500 ms prior to movement onset ((Friedman et al., 2006). We used the integral of the movement trace as a measure of the amplitude of individual whisks. We performed this analysis on 172 whisking epochs from 14 recording sites and MUAs recorded from animals engaged in Task 1.

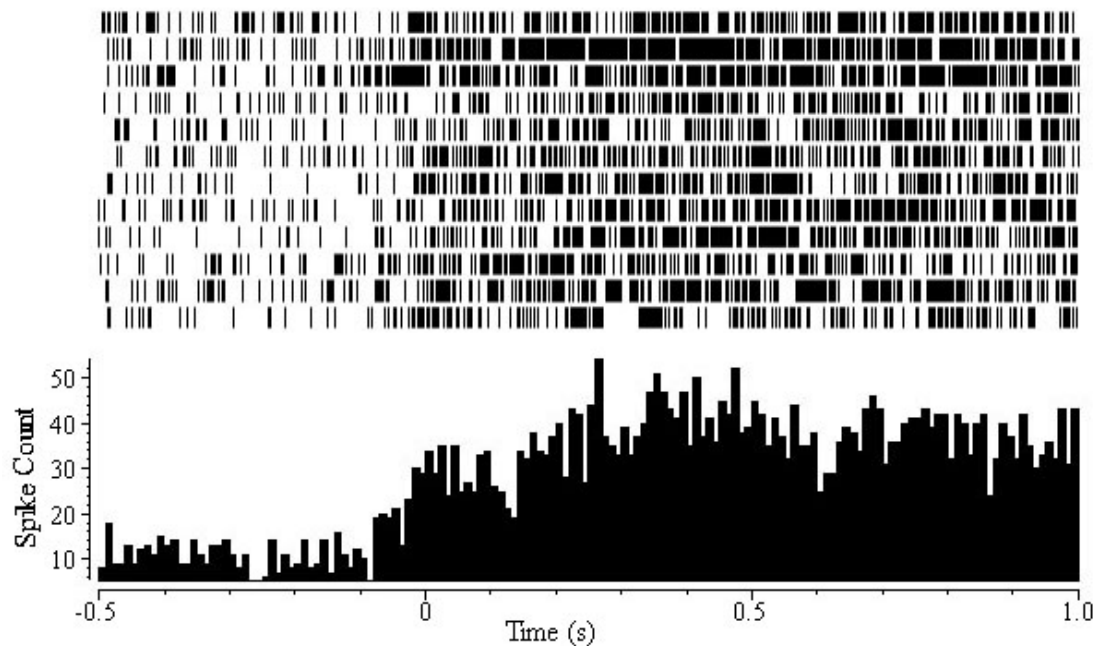


Figure 5.4. Raster plot (top) of all epochs from a single recording site and spike histogram (bottom), from 0.5 s prior to movement onset to 1.0 s post-movement. Movement onset = 0. An increase in activity can be seen just prior to movement onset, which ramps up further at onset.

Frequency: Correlation values ranged from -0.46 to 0.64 (median = 0.13), and their significance values were all >0.05 . This finding indicates that pre-movement cortical activity is not systematically correlated with ensuing whisking frequency.

Amplitude: Correlation values ranged from -0.58 to 1.0 (median = 0.14), and revealed a significant correlation ($p < 0.05$) between pre-movement spike rate and whisking amplitude in 2 of the 14 sessions (14.3%) that met the criteria for analysis (>4 epochs). Because the correlation was negative for one session and positive for another, we did not consider there to be a consistent relationship between prewhisking spike rate and whisking amplitude.

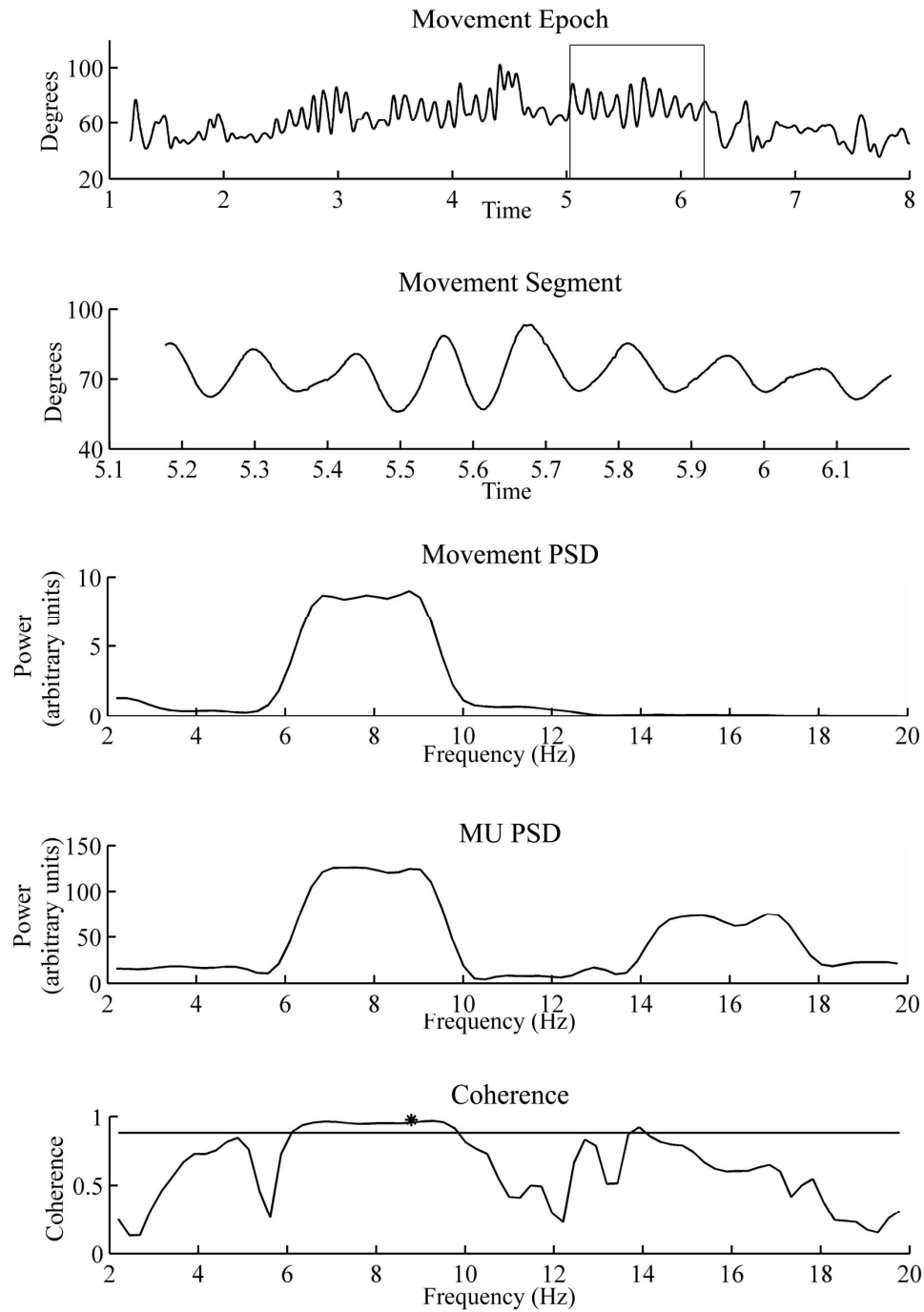


Figure 5.5. Example of coherence between movement and multi-unit activity in vMCx during 1s of movement. Top, the full whisking epoch from which the 1s segment (below) was extracted. 3rd & 4th panels, whisker and MUA frequency power spectra, respectively. Bottom, coherence between movement and MUAs, over the range of 2 to 20 Hz. Horizontal black line = 95% CI; asterisk = central whisking frequency for the segment.

5.3.3. Coherence Between Whisker Movements and vMCx

Having established that pre-movement cortical activity does not consistently correlate with *subsequent* whisking kinematics, we asked whether cortical activity correlates with *ongoing* whisking. We investigated the relationship between cortical activity and whisking in the time-frequency domain by computing, for each segment, the spectral coherence between the movement record and cortical activity. The confidence limits for significant coherence during a single whisking epoch were determined to be 0.88 for $p=0.05$ and 0.95 for $p=0.01$ (see Methods). An example of this analysis is illustrated in Figure 5.5. The movement record is shown in the top panel, the movement and MUA PSDs are in the 3rd and 4th panels, respectively. Coherence values over the range of 2 to 20 Hz are plotted in the bottom panel. The horizontal black line marks the 95% confidence limit for coherence, while the asterisk marks the central whisking frequency for the segment illustrated. In this example, movement and MUA were coherent at the whisking frequency.

MUA: Because the results from our two behavioral tasks were significantly different ($p<0.001$) we report them separately. Movement records and MUA were coherent at the whisking frequency in 86 of 496 (17.3%) segments from Task 1 animals, but in only 42 of the 746 (5.6%) segments from Task 2 animals (Figure 5.6). To determine whether the occurrence of coherent segments is greater than what would be predicted by chance, we re-ran the analysis after shuffling the data, dissociating neural and movement activity. The proportion of coherent segments was significantly different

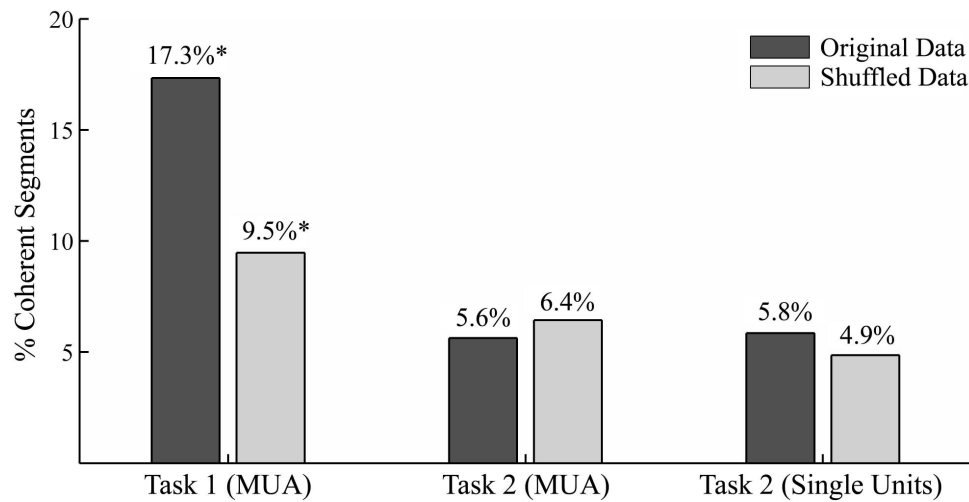


Figure 5.6. Comparison of proportion of coherent segments before and after data were shuffled. The proportion of coherent segments was significantly different from the shuffled data for MUAs in Task 1 only (significance at $p = 0.05$ indicated by asterisk).

from the shuffled data for Task 1 (17.3% versus 9.5%, $p=0.0001$), but not Task 2, animals (Figure 5.6).

Single Units: Movement and single unit activity were coherent at the whisking frequency in 41 of the 700 (5.9%) whisking segments, all of which were from Task 2 animals. Similar to the MUA analysis for these animals, when the data were shuffled and reanalyzed the proportion of coherent segments in the unshuffled data was not significantly different from the proportion in the shuffled data (5.9% versus 4.9%, $p=0.203$, Figure 5.6).

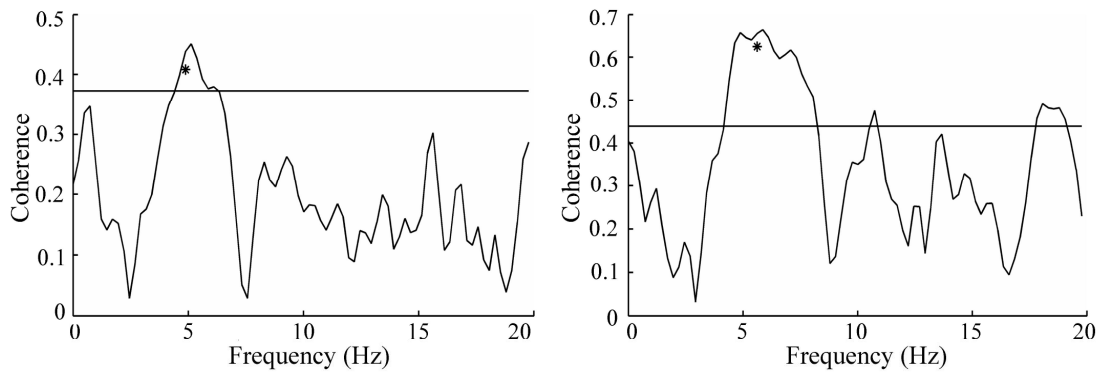


Figure 5.7. Two examples of recording sessions where coherence between spiking and whisker movements was significant after trial-averaging. Trial averaged spectral coherence values are plotted as a function of frequency. Horizontal lines mark the 95% confidence level. The peak movement frequencies after averaging across movement segments are indicated by the asterisks.

5.3.4. Trial-Averaged Coherence Between Whisker Movements and vMCx

We reported above that we found significant coherence between neural activity and movement records in a proportion of the recordings. Such significant coherence indicates that these two signals covary and have a consistent phase relationship. This segment-by-segment analysis, however, does not determine if that phase relationship is consistent over time, across segments. To test if phase-locking was consistent across segments we computed the trial-averaged coherence for each recording site. If the phase relationship between MUA and whisking varied across segments, averaging would diminish the level of coherence between these two variables. Conversely, if the phase relationships were consistent, averaging would enhance the coherence between these variables. Trial-averaged coherence between whisker movements and cortical activity within a session was considered significant if the magnitude of the coherence exceeded the significance limit at the peak whisking frequency, computed from the average movement PSD. Figure

5.7 shows two example of trial averaged coherence. In these cases, the magnitude of the coherence was significant ($p < 0.05$) at the peak average movement frequency.

MUA: As with the trial-by-trial coherence analysis, the results from our two behavioral tasks were significantly different and so we report them separately. Fifteen of 27 (55.6%) MUA recording sites for Task 1 animals and 6 of 26 (23.1%) recording sites for Task 2 animals were coherent (95% confidence interval) after trial averaging. To assess whether the occurrence of coherence is higher than what would be predicted by chance, we shuffled and reanalyzed the data. The proportion of coherent trial-averaged sessions was significantly different from the shuffled data for both Task 1 (55.6% versus 14.8%, $p=0.0018$) and Task 2 (23.1% versus 3.8%, $p=0.0496$) animals (Figure 5.8). The increase in the number of coherent trials following averaging (e.g. to 55.6% from 17.3% from Task 1 trials) suggests that the neuronal signals and movement records have a significant and consistent phase relationship.

Single Units: After trial averaging, movement and single unit activity, all of which is from Task 2 animals, were coherent at the peak average whisking frequency in 12 of 32 (37.5%) sessions. When the data were shuffled and reanalyzed the proportion of coherent trial-averaged sessions in the unshuffled data was not significantly different from the proportion in the shuffled data (37.5% versus 18.8%, $p=0.0818$, Figure 5.8).

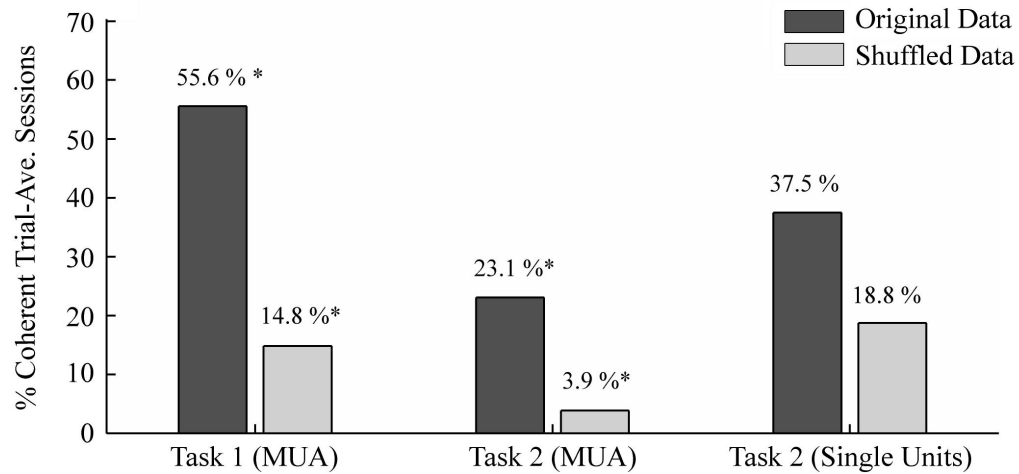


Figure 5.8. Comparison of proportion of coherent trial-averaged recording sessions before and after data were shuffled. The proportion of coherent trial-averaged sessions was significantly different from the shuffled data for MUAs in both Task 1 and Task 2 (significance at $p = 0.05$ indicated by asterisks).

5.3.5. Relationship between whisking frequency and neural activity

To determine if vMCx activity modulates frequency during whisking we correlated whisking frequencies with concurrent neuronal activity. To capture changes in whisking frequency that might be reflected in changing cortical activity, we parsed whisking epochs into 1s segments (as above) and calculated the frequency for each segment. Next we calculated Spearman's ρ to quantify the relationship between firing rates and the center whisking frequencies for all segments from a recording location. Because no difference between the two behavioral contexts was found, data from both Tasks were grouped together for analysis.

MUAs: Six of 46 (13%) recording locations revealed a significant correlation ($p < 0.05$) between whisking frequency and MUA firing rate during the 1 s segments. To determine if this proportion of correlated sessions is significantly different from what can

occur by chance we recalculated the p values after shuffling all segment data for each recording site. The correlation between firing rate and movement frequency, after shuffling, was significant for 2 of the 46 (4.3%) MUA recordings. This proportion is not significantly different from that computed from the unshuffled data, though it approached significance (Fisher's Exact Probability Test, $p = 0.0694$, Figure 5.9). We are therefore unable to reject the null hypothesis that the proportion of MUAs with a significant correlation between firing rate and movement frequency is equal to what might occur by chance.

Single units: We conducted the same analysis described above to determine if there was a significant correlation between movement frequency and firing rate of single units. Twenty-eight units met the criteria for the correlation analysis (i.e., were recorded during at least 5 whisking segments). While there was a significant correlation between firing rate and whisking frequency for 7 of the 28 (25%) units isolated, this number was not significantly different from what occurred after data were shuffled and reanalyzed (25% versus 7.1%, Fisher's Exact Probability Test, $p = 0.071$, Figure 5.9).

Thus, these findings do not support the hypothesis that firing rates of vMCx neurons reliably encode whisking frequency.

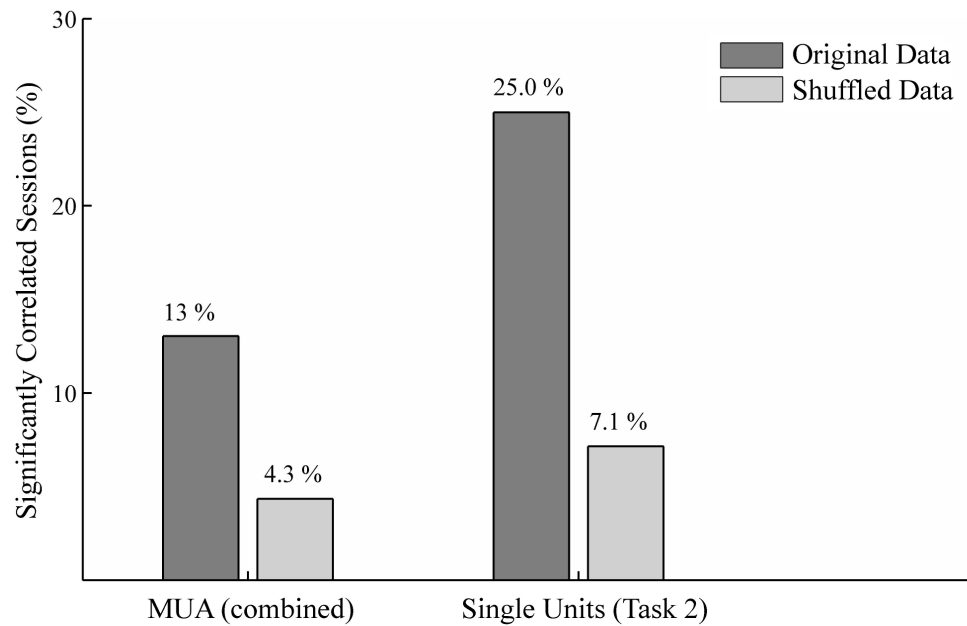


Figure 5.9. Comparison of the number of recording sites at which whisking frequency and MUA / Single Unit firing rate was significantly correlated, before and after data were shuffled. The proportion of correlated sessions was not significantly different from chance for either MUAs or single units.

5.3.6. Relationship between whisking amplitude and neural activity

As with whisking frequency, we determined if there was a correlation between firing rate during whisking and movement amplitude. The amplitude of whisks within epochs varies over time, such that the mean amplitude of an entire epoch might not provide a representative approximation of whisk amplitudes across time. We therefore calculated the correlation (Spearman's ρ) between firing rate and the amplitude of 1s segments of whisking, to capture differences in whisk amplitudes within the larger epochs. As with the frequency analysis, above, no difference was found between contexts and so data from both Task groups were analyzed together.

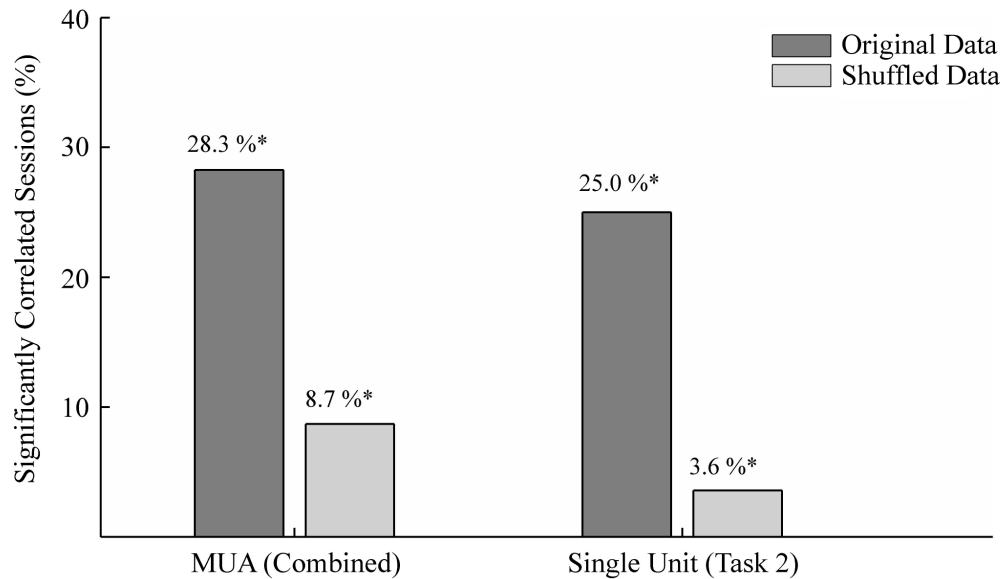


Figure 5.10. Comparison of the number of recording sites at which whisking amplitude and MUA / Single Unit firing rate were significantly correlated, before and after data were shuffled. The proportion of correlated sessions was significantly different from chance for both MUAs and single units (significance at $p < 0.05$ indicated by asterisks).

MUA: Firing rate and segment amplitude were significantly correlated ($p < 0.05$) in 13 of the 46 (28.3%) recording sites having at least 5 whisking segments. To test if this proportion was greater than might occur by chance, the data were shuffled and the correlation analysis was repeated. The proportion of correlated sites in the unshuffled data was significantly greater than the proportion in the shuffled data (28.3% versus 8.7%, $p=0.0078$, Figure 5.10), suggesting that there is a causal relationship between MUA and movement amplitudes.

Single units: Twenty-eight single units met the criteria for the correlation analysis (> 4 movement segments). Spike rates in 7 of the 28 (25%) isolated units were significantly correlated ($p < 0.05$) with movement amplitude within segments. When the

proportion of correlated units was compared with the proportion after movement and units were dissociated through shuffling, the occurrence in the original data was significantly higher than in the shuffled data (25% versus 3.6%, $p=0.026$, Figure 5.10). In conjunction with the analysis of MUA, these results suggest that vMCx activity can be correlated with the amplitude of individual whisker movements.

5.4. Discussion

Our goal was to determine if activity in the vibrissa motor cortex (vMCx) co-varies with parameters of vibrissa movement. We recorded multi- and single-unit activity in the rhythmic region of vMCx, while precisely measuring vibrissa position in awake, head restrained rats. We focused our analyses on whisking frequency and amplitude, the two cardinal parameters that define whisking behavior. Our results indicate that variation in spike rate in this cortical region most frequently is associated with the amplitude of vibrissa movements. Though co-variation between spike rate and whisking frequency was found, the occurrence was not high enough to exceed chance levels.

5.4.1. Pre-movement activity

Chapter 4 reported that activity in vMCx, monitored through recording local field potentials (LFPs), increases 200 to 500 msec before whisking onset (Friedman et al., 2006). Pre-whisking activity was also recorded previously from single units in vMCx, though on a smaller timescale (51 ± 75 msec before whisking) (Carvell et al., 1996). Here we asked whether this pre-movement activity encodes the kinematics of oncoming vibrissae movements. We found no significant correlations between pre-whisking cortical

firing rates and whisking frequency. In a small number of recordings (2, or 12.5%) there were significant correlations between pre-whisking cortical activity and whisking amplitude. However, while one of these recording sessions was highly correlated ($r = 1.0$, $n = 5$ segments) the other revealed a negative correlation ($r = -0.58$), and so the relationship between cortical activity and amplitude was not consistent.

Increases in LFPs are a reflection of the underlying neural activity in a region. More specifically, field potentials in an area increase as firing synchrony across neurons increases. There are three possible explanations for our finding that unit activity prior to whisking was not correlated with the ensuing movement, and therefore does not appear to reflect LFP activity, as would be expected. The first is that firing synchrony amongst units can increase without an increase in activity. If an increase in synchrony without an increase in firing rate did occur, LFPs would increase but not unit activity as measured through extracellular recordings. Strong correlations have been found between LFPs and unit activity, however, and so it is unlikely that the change in LFPs was not accompanied by a change in unit activity. The second explanation is that there was an increase in unit activity prior to movement onset but that this increase did not systematically co-vary with either movement frequency or amplitude, and so was not reflected in the correlation analysis. Had we determined merely if unit firing-rate increased prior to movement onset, without asking if this rate increased monotonically with frequency, we may have found an increase in firing rate. Because this analysis had already been carried out by Carvell et al. (1996), we did not repeat it here. The third possibility is that any correlation in spike rate and whisking frequency or amplitude occurs over a much smaller time-frame, such as the 51 ± 75 msec prior to movement over which unit activity was found to increase

(Carvell et al., 1996), and therefore was washed out by the longer time interval used in our analysis.

5.4.2. Spectral coherence

Studies in other motor systems have shown that cortical oscillations are phase locked to oscillations in muscle activity, and that the magnitude of coherence between these oscillatory signals is modulated by behavioral context (Kilner, Baker, Salenius, Hari, & Lemon, 2000). These findings suggest that the coherence between cortex and muscles is related to some parameter of motor function. By analyzing recordings of cortical local field potentials in sensory and motor cortex, Kleinfeld and collaborators (Ahrens & Kleinfeld, 2004; Ganguly & Kleinfeld, 2004) demonstrated similar, task-dependent modulation of phase locking between cortical and whisking activity. Because this coherence occurred at the same frequency bandwidth characterizing exploratory whisking, they argued that this phase locking illustrates that vMCx can initiate the motion of the vibrissae on a cycle-by-cycle basis.

Our studies are consistent with the results of Ahrens and Kleinfeld (2004) in that they demonstrate significant and common (55.6% of recordings from Task 1) trial-averaged spectral coherence between multi-unit activity (MUA) and whisking. The incidence of trial-averaged coherence recorded from Task 2 animals was lower (37.5%), suggesting that, as with the studies discussed above, phase locking is task dependent. In those studies the investigators argued that the context-dependent nature of the task was reward dependent. That is, coherence was present only when animals were whisking in search of a reward. While both of our tasks involved whisking behaviors reinforced by a

reward, they differed in the types of movements being rewarded. That is, in Task 1 animals were rewarded for producing epochs of whisking that exceeded met amplitude and frequency criteria, whereas Task 2 animals were rewarded when they contacted an object, even if the contact was made with only a single whisker movement. For both tasks our analyses focused on *epochs* of whisking. It may be, however, that we found coherence during Task 1 because we analyzed the rewarded movements, whereas we analyzed whisking epochs in Task 2 that may or may not have resulted in contact. Our analytic approach was based on the assumption that all movements in Task 2 represented an attempt to make contact. This assumption, however, may not be correct. In this case, coherence may occur only in the whisk that leads to contact. Licking often began before contact was made, however, which introduced movement artifacts into the neural record that precluded analyzing these movements.

We also did not find significant coherence between the activity of single vMCx units and whisking. As in any single unit study, this might reflect a selection bias: we might not have recorded from the relevant neurons. It is also possible that control of whisking parameters occurs through a “population code” involving the co-activation of a large number of vMCx neurons. This would be consistent with findings that MUA (present study) and LFPs (Ahrens & Kleinfeld, 2004), both reflecting the activity of large neuronal populations, are phase locked to whisking.

5.4.3. Covariation of cortical activity and whisking frequency

Spectral coherence between vMCx and whisking activity suggests a causal relation between the two, but does not directly point to the movement parameters controlled by

vMCx. We focused on the 2 cardinal parameters of vibrissae movements: frequency and amplitude. We first asked if there was a correlation between spike counts *during* whisking and whisking frequency, as might be expected if vMCx was setting whisking frequency. Activity of single units and MUAs were correlated with whisking frequency only in a small percentage of our recordings. Neither of these levels of occurrence significantly exceeds what could occur by chance. This suggests that activity in vMCx—measured as firing rates—is not setting the frequency of movements in the epochs analyzed, i.e., that frequency is likely not under cortical control.

5.4.4. Covariation of cortical activity and whisking amplitude

The number of active vibrissae motor units, and their firing rate, correlates strongly with the amplitude of whisks (Cramer and Keller 2006). It may be, then, that vMCx directs motor unit recruitment and activity level during movement. In contrast with whisking frequency, we found a relatively high incidence of co-variation between the amplitude of whisking and spike count for both single units (25% of units) and MUAs (28%). These levels of occurrence were significantly greater than what occurred when the data were shuffled and reanalyzed, indicating that amplitude can be set by vMCx during active whisking.

Whisking amplitude and velocity are highly correlated (Cramer & Keller, 2006; Voigts, Sakmann, & Celikel, 2008), and so it may be that the crucial parameter is whisking velocity rather than amplitude. Recent work characterizing the velocity profiles of individual whisks recorded from behaving animals has shown that velocity often increases and decreases in unpredictable ways, leading to irregularly shaped whisks that

at times even reverse direction in mid protraction (Towal & Hartmann, 2008). These authors suggest that the rat may be controlling these changes in instantaneous velocity in real time to influence the processing of sensory information. It may be, then, that vMCx acts on the whisking rhythm generator to set the velocity rather than the amplitude of whisks and perhaps even controls the velocity profile on a whisk-by-whisk basis.

5.5.5. The strength of relationships between whisking and cortical activity

The activity of motor cortex neurons correlates with a large number of movement parameters (Cheney & Fetz, 1980; Grillner et al., 1997; Mehring et al., 2003; Rickert et al., 2005; Scott, 2003; Tanji & Evarts, 1976; Thach, 1978), and these correlations are strongly task dependent (Fetz, 2007; Thach, 1978). We also found a relationship between neural activity and more than one movement parameter (amplitude and frequency), as well as differences in the strength of relationships between tasks. While we found significant correlations between cortical activity and both amplitude/velocity and frequency, neither relationship occurred amongst a majority of cells. Furthermore, when data was shuffled and reanalyzed to determine if the proportion of significant correlations was greater than could occur by chance, only the relationship with amplitude/velocity was significant. There are a number of important methodological issues and unknowns that should be considered regarding the degree to which significant correlations occurred amongst the recording sessions.

There are two primary methodological issues that may have led to an underestimate of the level of coherence and/or correlations amongst cortical activity and movement parameters. The first concerns the use of the absolute peak of the movement PSD as the

measure of frequency used in the coherence analyses. Using a bandwidth containing a range of frequencies, such as the width at half maximum power, may be a more accurate reflection of the behavior, which contains variability, and may likely capture true coherence rather than using just a single frequency. The second, related issue, regards the movement frequency value used in the correlation analysis. A large number of the movement epochs analyzed were bimodal. While we chose to use the peak frequency in the movement PSD in our evaluation of co-variation between frequency and spike rate, it is possible that in some cases spike activity co-varied with movement at the second, slightly lower, peak in the PSD.

Beyond these methodological questions, a likely and important factor determining the occurrence of correlations is the projection target of different vMCx neurons. Work in the rabbit, for instance, has shown that while activity in layer 5 corticofugal neurons is highly modulated with movement, activity in corticocortical and layer 6 corticofugal neurons remain unchanged during movement (Beloozerova et al., 2003a; Beloozerova, Sirota, Swadlow, Orlovsky, Popova, & Deliagina, 2003b). The vMCx projects not only to the putative whisking rhythm generator, but also to a number of other cortical and sub-cortical areas, such as the superior colliculus (Hattox et al., 2002; Miyashita & Mori, 1995), as well as directly to the vibrissa motoneurons in the lateral facial motor nucleus (Grinevich et al., 2005). The current study did not identify the targets of our recording locations, and it is likely that they varied across penetrations. The inconsistency across our recording sites, then, likely reflects the functional differences amongst the various cellular networks encompassing our recording sites.

Another source of inconsistency might relate to the target muscles indirectly controlled by different motor cortical neurons (Jackson, Mavoori, & Fetz, 2007). Two muscle groups, the intrinsic and extrinsic muscles, can participate in the production of vibrissa movements. Anatomical findings (Dorfl, 1982; Wineski, 1985) suggest that the intrinsic muscles control the pendular motion of the whiskers, while the extrinsic muscles move the vibrissa pad to change the set point of the array. Recent work, however, has shown that both groups of muscles have a rhythmic pattern of activation, and that whisking results from the synergy of the muscles working in specific phase combinations (Hill et al., 2008). Based on this work, Hill et al. suggest that multiple CPGs work together to coordinate the outputs of the contributing muscles, which can be combined in different ways to produce a variety of movement patterns. Importantly, stimulation of different areas within vMCx can produce different types of vibrissa movements, such as retractions, rhythmic protractions, and combinations of the two (Berg & Kleinfeld, 2003; Cramer & Keller, 2006; Haiss & Schwarz, 2005). We do not, however, know how sites within vMCx map onto intrinsic and extrinsic muscle activation. This relationship might be critical to deciphering the relationship between specific cells and vibrissa movement.

The current study attempted to minimize some of this complexity by targeting a known cortical sub-region (Haiss & Schwarz, 2005). Stimulation of this area results in the activation of intrinsic muscles, whose EMG signal is predictive of vibrissa movements (Cramer & Keller, 2006). While this increases the likelihood that we recorded from cells associated with the intrinsic muscles, we have not tested this and so cannot rule out the possibility that this area also plays a role in extrinsic muscle activation. If whisking is the product of multiple, interacting rhythm generators we would

expect the relationship between activity in vMCx and whisking to differ depending on the targets of those cells and the role the associated muscle(s) play in a given movement.

That different areas of vMCx may be controlling different rhythm generators, combined with the diversity of possible targets of our recording sites, may explain why this region appears to have a different relationship with movement at different times.

6. GENERAL DISCUSSION

6.1. Motor cortex in the production of rhythmic movements

Whisking is one of many types of movement that are built upon a basic rhythmic pattern that can be altered to meet the demands of the environment and the goals of the animal. Like these other motor behaviors, such as chewing and walking, the production of whisking involves a CPG, and it does not require an intact motor cortex. Given this, what might this region contribute to these behaviors, and what does our knowledge of these other systems suggest about the role vMCx may play in whisking?

The function of locomotion is to move the organism through the environment and having the ability to make fine adjustments to the pattern is necessary to successfully meet this goal. The goal of chewing is to masticate food, and the complexity of food surfaces requires adaptation, via sensory feedback, by appropriately coordinating the chewing muscles. Though animals can produce the basic patterns for both locomotion (Whelan, 1996) and mastication (Larson et al., 1980) without a motor cortex, they lack the ability to adapt these patterns to the complexities of the natural environment (MacKay-Lyons, 2002). The most obvious role of MCx, then, is to fine tune basic motor programs.

Evidence from other motor systems supports the notion that MCx plays a role in fine-tuning the motor pattern. One line of evidence has come from decerebrate preparations. The cat can either walk independently or on a moving treadmill, depending on the level of decerebration (Orlovsky, Deliagina, & Grillner, 1999), but lacks the ability to adjust the basic motor pattern to overcome obstacles. For instance, movements

requiring integration of visual information and accurate foot placement (Drew et al., 1996), such as walking on a ladder or stepping over obstacles, are lost following interruption of inputs from MCx by cortical ablation (Beloozerova & Sirota, 1993) or disruption of the dorsal lateral funiculi (Drew, Jiang, & Widajewicz, 2002). Ablation of the motor cortex has a similar disruptive effect on mastication in cats (Hiraba & Sato, 2005) and monkeys (Larson et al., 1980). The basic masticatory motor pattern survives the loss of the motor cortex, but the accuracy of tongue and jaw movements declines, a likely result of a lack of muscle coordination (Larson et al., 1980).

Recordings from the motor cortex during locomotion further support the role of the motor cortex in fine-tuning motor patterns. During walking in freely behaving cats, the firing rates of a subset of pyramidal tract neurons are modulated in phase with the locomotor cycle (Beloozerova et al., 2006; Drew et al., 2002). The modulation consists of an increase in the baseline firing rate during a particular phase of the locomotor cycle with a magnitude that varies with the need for accurate foot placement. Firing rates of individual pyramidal tract neurons, recorded during walking across a ladder, exceed those recorded during movement across a flat surface (Beloozerova et al., 2003a), suggesting an increased contribution from MCx during skilled movements.

6.2. The Adaptive Modulation of Whisker Movements

Whisking is set apart from most other rhythmic motor behaviors in terms of its function. Unlike locomotion, which involves sensorimotor integration but whose function is primarily motoric, the whisking system is quintessentially one of “active sensing”. Rats whisk almost continuously as they navigate and the role of the motor component of

the system is to position the whiskers in a manner that optimizes sensory processing from moment-to-moment.

As with other rhythmic behaviors, much of whisking takes place subcortically - whisking continues after removal of vMCx and intrinsic and extrinsic muscle activity can be generated directly by sensory inputs (Fee, Mitra, & Kleinfeld, 1997). Moreover, initial changes in whisker movements in response to object contact often occur so quickly as to preclude cortical involvement (Mitchinson et al., 2007). If vMCx contributes to whisking, as it contributes in other systems, by adaptively modulating the whisking motor pattern in response to environmental context, than inputs from vMCx are likely to become critical in the context of directed information gathering, as occurs during object exploration or, in the laboratory context, during a texture discrimination task, and involve the coding of whisking kinematics.

A review of the behavioral data suggests that such adaptive modulation does indeed occur during object exploration and discrimination. Shifts in whisking kinematics (e.g., amplitude, frequency; velocity; set point) have been reported in tasks involving object detection (Harvey et al., 2001a; Knutsen, Pietr, & Ahissar, 2006), object discrimination (Harvey et al., 2001a) and texture discrimination (Carvell & Simons, 1990, 1995). Not only is successful discrimination associated with patterns of whisking, but developmental manipulations, such as whisker trimming in pups, both significantly impairs discrimination and alters the frequency range of whisking (Carvell & Simons, 1996). Interestingly, which parameters shift differs with the difficulty and the details of the task, and strategies vary across animals (Carvell & Simons, 1990, 1995). Additionally, the relationships amongst kinematic parameters (e.g., the correlation between velocity and

amplitude) further complicates the identification of the parameter(s) of importance when more than one feature is altered.

Whether or not these modulations of whisking kinematics are mediated by motor cortical mechanisms remains to be determined. The studies described in this Dissertation were an attempt to take some important first steps in making this determination. That is, using a combination of high-resolution whisker tracking and unit recording from a well-characterized region of motor cortex, these studies aimed at determining 1) whether vMCx was directly or indirectly, via the whisking pattern generator, influencing whisker movements and 2) which, if any, of the kinematic parameters known to be modulated during exploration/discrimination are encoded by motor cortex neurons. We additionally investigated the influence of context on control mechanisms by engaging animals in both a whisking in air and a contact task. The results of discrimination and localization studies have suggested that which whisking parameters are modulated is task dependent (Carvell & Simons, 1995; Harvey et al., 2001a; Knutsen et al., 2006; Towal & Hartmann, 2006). By varying the behavioral demands of the task, we hoped to determine whether the motor cortical contribution also varied with context.

6.3. Summary of findings

Using the rat vibrissa system as a mammalian model for motor control, I began my work by characterizing the movement of the whiskers and the mystacial pad in relation to each other in order to determine 1) how each moves in two-dimensional space, 2) how movement varies with location in the whisker array, and 3) the relative contribution of the pad and whiskers to the final whisker trajectory. I next investigated the relationship

between motor cortex and whisker movements in different recording and behavioral contexts. Specifically, I tested the hypothesis that vibrissa motor cortex (vMCx) exerts an indirect influence on whisking, most likely via a subcortical pattern generator, by analyzing the temporal relation between cortical activity and whisker movements. I then investigated the relationship between spike rate and whisking frequency and amplitude.

In my first study I found that both the whiskers and the mystacial pad move rhythmically and within the same frequency bandwidth. While the implication of this finding is that both pad and whisker could make equally great contributions to whisking amplitude, in fact the largest component of movement in the AP plane was found to come through the movement of the whiskers; the pad's contribution to overall amplitude was relatively small. Movement in the DV plane, however, is almost completely determined by movements of the pad. Additionally, while whiskers at two locations on the face have similar movements, the pad can move quite differently at two locations.

My second study tested the hypothesis that vMCx was indirectly influencing whisking, perhaps by altering the basic rhythm of the pattern generator, and predicted that changes in cortical activity will precede whisking onset. Consistent with my hypothesis, local field potential activity in vMCx of whisking rats increased prior to the onset of whisking and returned towards baseline prior to the end of the whisking epoch, and changes in whisking kinematics were often preceded by brief increases in LFP activity. LFPs and EMGs of the intrinsic muscles were not coherent with whisking during whisking epochs, however, indicating that activity did not co-vary on a cycle-by-cycle basis. These results suggest that vMCx is not directly generating the drive to vibrissa motoneurons (vMNs) during voluntary whisking.

The final study of this dissertation extended the work of the second study by recording unit activity, in a well-characterized region of vMCx, in head-fixed rats under two different operant reward conditions. By using microstimulation to define the rhythmic sub-region (Cramer & Keller, 2006; Haiss & Schwarz, 2005) I minimized the ambiguity as to locus, which has characterized previous recording studies. Use of head-fixation enabled 1) the precise measurement of whisker trajectories and therefore whisking kinematics, 2) reward delivery based on whisking parameters, which could vary with task and 3) the use of a movable electrode to maximize unit yield. Our analysis asked if spike rate *prior* to movement onset *co-varied* with whisking frequency or amplitude during the subsequent first 1 s of movement, or if spike rate co-varied with these parameters *during* movement. We also investigated the level of coherence between cortical activity and movement during whisking. These analyses found that unit activity prior to movement onset did not consistently correlate with either the frequency or amplitude of subsequent whisking. We did, however, find frequent and significant correlations between unit firing rate and whisking amplitude during whisking, and this finding applied in both behavioral contexts. On the other hand, we did not find evidence of co-variation between unit activity and whisking frequency. We did, however, find a significant occurrence of coherence between spike activity and movement at whisking frequencies during the whisking in air, but not the contact, task. That is, the frequency of unit activity matched the frequency of whisking and also maintained a consistent phase relationship, during a significant number of the 1 s segments analyzed in the whisking in air task. While this result does not *necessarily* reflect a mechanism for controlling frequency (such as spike rate, which would have been suggested by positive correlations

in the correlation analysis), it leaves open the possibility that frequency is being directly controlled via spike timing.

6.4. The vMCx and the control of whisking movement parameters.

Whisking frequency, velocity, amplitude, whisk duration, length of whisker contact and set point all show changes during contact-based whisking tasks. In the present study we found a significant relationship between vMCx unit activity and whisking amplitude/velocity, but found no direct correlation between vMCx unit activity and whisking frequency. We did, however, find significant coherence between unit activity and whisking, which could, in principal, represent the direct cortical control, by spike timing, of whisking frequency.

One surprising finding from this Dissertation was that there was no clear, unambiguous relationship between cortical activity and frequency. Increases in whisking frequency have been reported in tasks involving object detection (Knutsen et al., 2006), object discrimination (Harvey et al., 2001a) and texture discrimination (Carvell & Simons, 1990, 1995). These findings, combined with the result of increases in evoked frequency with increased stimulation intensity during ICMS, had led us to predict a significant relationship between cortical activity and frequency. Detailed descriptions from a few behavioral studies, however, may suggest that whisking frequencies are not actually modulated during discriminations in the manner generally assumed. Firstly, changes in whisking frequency during these tasks is described only in terms of frequency spectra, rather than in terms of precise whisking frequency during the time of contact. Specifically, whisking PSDs are dominated by a peak at around 8 Hz during free air

whisking. During discrimination this peak remains, but the distribution becomes bimodal, with a second, but generally weaker peak, at around 12-14 Hz (Carvell & Simons, 1995; Knutsen et al., 2006), resulting in an increase in the overall frequency bandwidth. A recent study characterizing whisk shapes noted that many whisks themselves are bimodal, with two peaks resulting from brief reversals of movement during the protraction phase of the whisk (Towal & Hartmann, 2008). Carvel and Simons (1995) not only described brief reversals in individual whisks during discrimination, which may be represented as a higher frequency component on the movement PSD, but also noted that success on a relatively difficult discrimination task was associated with whisking in the 8 Hz, rather than the higher frequency, range. Lastly, animals that displayed disproportionately more whisking at high (> 15 Hz) frequencies were generally the worst task performers.

Whisking frequency can be described in terms of whisk velocity, amplitude and duration, and so it may be that changes in these parameters lead to changes in whisking frequency without frequency being the relevant movement feature. Discrimination tasks involve clear reductions in both whisk amplitude (Carvell & Simons, 1990, 1995; Harvey et al., 2001a) and whisk velocity (Carvell & Simons, 1990, 1995; Harvey et al., 2001a; Knutsen et al., 2006), and so one or both of these parameters may be under cortical control. Determining which one might be controlled, however, is complicated by the high correlation between the two parameters. Whisking amplitude was highly correlated with performance in discriminating between two rough surfaces, though not with the less difficult task of discriminating between a rough and smooth surface. Additionally, retraction amplitudes during discrimination are smaller than protraction amplitudes

(Carvell & Simons, 1990), suggesting that amplitudes in this context are being directly controlled. Lastly, lightness of touch, which can be controlled by changes in amplitude, appears to be a factor during discrimination, as success is correlated with lower whisker bending (Carvell & Simons, 1990), and cessation of the protraction phase is mediated by the stimulus itself (Grant et al., 2009). That is, contact immediately triggers an end to the whisk protraction, resulting in “minimal impingement” (Grant et al., 2009) of the whiskers by the encountered object.

Characterizations of the velocity profiles of individual whisks suggests that rats may control at what phase in the whisk cycle maximum velocity is reached (Towal & Hartmann, 2008). These authors suggest that irregularities in whisk shape, such as the reversals in direction described above, are a product of the adaptive control of velocity. That these variations in whisk trajectory are directly controlled rather than the consequence of surface contact is supported by the fact that whisk reversals are large relative to surface features and are synchronous in adjacent whiskers irregardless of contact (Carvell & Simons, 1990). Given that rat barrel cortex responds preferentially to high-velocity inputs (Pinto, Brumberg, & Simons, 2000; Temereanca & Simons, 2003), altering the phase of maximum velocity would enable the rat to maximize sensory processing at the moment of contact. The results of study three of this Dissertation further support the hypothesis that whisking amplitude and/or velocity are being controlled by vMCx, though they cannot distinguish between the two parameters.

Rats palpating objects tend to position the whisker arrays at a more protracted set point than whisking in air (Berg & Kleinfeld, 2003; Carvell & Simons, 1990, 1995; Grant et al., 2009), and object contact is often maintained across several whisks (Grant et al.,

2009). Additionally, the small, immovable “microvibrissae” at the front of the snout maintain constant contact with the surface throughout palpation. It is likely that, in addition to modulating the whisking rhythm, vMCx plays a role in adjusting the whisking set point to maintain this protracted position. Preliminary support for this comes from investigations of primary sensory cortex. Activity in this region reflects both the fast rhythmic pattern of individual whisker movements and a slower pattern that appears to reflect changes in set point. When sensory inputs are abolished via transection of the facial nerve, the fast, but not the slow, firing pattern is diminished (Fee et al., 1997). These results suggest that the slower firing pattern in S1 has a central, rather than peripheral, origin and may reflect a corollary discharge from vMCx. Stimulation of the retraction region of vMCx results in a single movement of the whiskers and pad, albeit a retraction, as opposed to an oscillating motion. Given that the extrinsic muscles facilitate the active component of retraction (Hill et al., 2008), it may be that neurons in the retraction subregion of vMCx are modulating the position of the whisker pad via activation of the extrinsic muscles. The observed retraction movements evoked by stimulation of this cortical subregion likely result from activity of m.nasolabialis and m. maxillolabialis, the caudal most extrinsic muscles. Whether or not the rostral-most extrinsic muscle (m. nasalis), which aids protraction during exploratory whisking (Hill et al., 2008), can be activated by ICMS to produce a protraction of the pad is unknown, but could be determined through further musculotopic mapping of vMCx. In fact, the results of study one of this Dissertation, which found that the pad could simultaneously move in different manners at two locations on the face, suggest that the rostral and caudal

extrinsic muscles can be controlled separately to produce different movements, even at the same time.

We utilized two behavioral paradigms in order to investigate context dependent coding in vMCx. Though neither of our behavioral tasks required the animal to implement the whisking strategies utilized during discriminative whisking, we assumed that by rewarding particular movements we would bring whisking under operant, and therefore “voluntary”, control. While a more ideal experimental paradigm would investigate cortical activity during active sensing tasks under more natural conditions, such as making fine-grained discriminations in an open arena, our work was constrained by our whisker tracking procedure. Since this work was begun, great strides have been made in the development of systems that can accurately track the movements of single whiskers in a freely moving rat without a prohibitive amount of manual data processing. Future studies will need to utilize these newer methods to investigate cortical coding mechanisms during tightly controlled, adaptive whisking behaviors. In fact, investigations of whisking dynamics during object localization (Knutsen & Ahissar, 2009; Knutsen et al., 2006; Towal & Hartmann, 2006), identification (Harvey et al., 2001a) and discrimination (Carvell & Simons, 1995) tasks, as well as across difficulty levels within these tasks, suggests that animals may use very different whisking strategies based on the task demands. More specifically, it has been suggested that localization versus discrimination requires direct control over spatial versus temporal components of whisking, respectively (Knutsen & Ahissar, 2009; Towal & Hartmann, 2006). This proposal makes specific predictions about movement modulation in different contexts. Recording cortical activity in both subregions of vMCx during these different behavioral

tasks offers the opportunity to more fully understand context-depending coding strategies in the whisker system.

This dissertation began with a study of whisking topography, and proceeded by investigating the temporal relationship between changes in cortical activity and whisking. The final step of this work was an attempt to identify what kinematic features of whisking are under cortical control. A central hypothesis was that vMCx is exerting its influence over whisking via the whisking pattern generator, as it is thought to do with other rhythmic behaviors. Because this hypothesis focused on the rhythmic component of whisker movements, as opposed to the slower, more variable movement that can change the whisking offset, we specifically sought to measure unit activity in the rhythmic region of vMCx. Stimulation of this area evokes rhythmic movements that are primarily controlled by the intrinsic muscles. Rhythmic whisker movements in the behaving animal, however, are the product of the intrinsic and extrinsic muscles firing rhythmically and with varying phase relationships to one another. If vMCx is influencing each of these muscles, it is likely that cortical activity will exhibit different patterns during movement depending based on its musculotopic organization. Deciphering this organization, then, is an important next step in understanding the cortical control of whisking.

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