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PATTERN GENERATION IN THE RAT VIBRISSA SYSTEM: BEHAVIORAL EVIDENCE FOR A CENTRAL PATTERN GENERATOR

by

PUHONG GAO

A dissertation submitted to the Graduate Faculty in Psychology in partial fulfillment of the requirements for the degree of Doctor of Philosophy, The City University of New York

2001

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This manuscript has been read and accepted for the Graduate Faculty in Psychology in satisfaction of the requirement for the degree of Doctor of Philosophy.

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Abstract

PATTERN GENERATION IN THE RAT VIBRISSA SYSTEM: BEHAVIORAL EVIDENCE FOR A CENTRAL PATTERN GENERATOR

by

Puhong Gao

Advisor: Professor H. Philip Zeigler

Even in the absence of explicit stimulation, rats emit patterns of rhythmic whisking movements. Because of their stereotyped nature and their persistence after sensory denervation and cortical ablation, whisking movements have been assumed to reflect the output of a central pattern generator (CPG). However, identification of a movement pattern as the product of a CPG requires evidence that its generation, patterning and coordination are independent of sensory input. To provide such evidence we used optoelectronic instrumentation to obtain high resolution records of the movement trajectories of individual whiskers, in rats whose heads were fixed to isolate their exploratory whisking from ex-afferent inputs. Unconditioned whisking patterns were quantitatively characterized by a biometric analysis of the kinematics, rhythmicity and coordination of bilaterally homologous vibrissa movements. Unilateral and bilateral section of the infraorbital nerve, which innervates the whiskers, was then carried out to block *re*afferent inputs generated by the animals own whisking movements. Unilateral section of the nerve has no effect on whisking kinematics but is followed by a significant, but relatively transient bilateral increase in whisking frequency.

However, bilateral deafferentation, when carried out in a single-stage procedure, does not disrupt either the generation, patterning or bilateral coordination of whisking patterns in the rat. The findings provide strong behavioral evidence for a whisking CPG, and are discussed in relation to its possible location and properties.

Acknowledgements

I would like to thank the following people for their help towards the completion of this dissertation.

First, I would like to thank my dissertation advisor, Dr. H. Philip Zeigler, for his intellectual and financial support during the process of this study. I am very grateful for his patience and understanding when things were moving slow along the way of my completing this dissertation.

Many thanks to my supervisory committee, Dr. Sheila Chase and Dr. Peter Moller. To Dr. Sheila Chase, thanks for her many years' emotional support and friendship. Thank Dr. Peter Moller for his careful and valuable comments towards the completion of this manuscript.

I would also like to thank my outside readers, Dr. PoKay Ma and Dr. Bertram Ploog, for their positive comments and encouragement. I am very grateful for their brother-like friendship.

Special thanks to Dr. Roberto Bermejo and Dirk Houben for their ingenious incorporation of hardware with software that made this study possible. I am grateful to Dr. Bermejo for his friendship and valuable help in computer programming. I would like to express my sincere appreciation to Dr. James Gordon for his generous help. Though he is not on my committee, he has been always available for my questions.

Finally, I would like to dedicate this piece of work to my two lovely children, Yang and Alice.

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Introduction

Sensory vs central control of behavior

A major goal of neuroscience is to understand how the brain processes information about the external world and uses it to control movements. The extent to which motor output is controlled by sensory input vs central mechanisms has been a major concern of behavioral biology. Historically there have been two main attempts to answer these questions. One is the peripheral control theory. Derived from Sherringtonian reflexology, it hypothesizes that coordinated motor output is built up from smaller, discrete phases of movement, linked together by chain reflexes, with sensory feedback from each phase of the movement reflexly eliciting each subsequent phase. Behavior is controlled from moment to moment by environmental stimuli (Burke, 1971). The other is central control theory. It suggests that feedback from the environment is unnecessary for the elaboration of the motor output, because the central nervous system contains all the information necessary for the patterning of behavior (DeLong, 1971). The only role for stimuli is to serve as triggers to unlock or release the behavior, such as the fixed action pattern (FAP) and innate releasing mechanism (IRM) suggested by ethologists (e. g., Tinbergen, 1951).

Both control theories seemed to be supported by some neurophysiological experiments. The classic studies of the feeding activity in the blowfly by Dethier and Bodenstein (1969) provide a good example of a self-regulated feedback control system. The study illustrated that the whole feeding processes was under tight peripheral (sensory) control. On the other hand,

numerous types of motor activities seem to operate without obvious sensory regulation (feedback--reafference, exafference, or both). Examples can be found both in vertebrate and invertebrate animals, stepping in a cat (Grillner and Wallen, 1991), snapping in a toad (Ewert, 1985), flight in Locust (Wilson, 1961), singing in a cricket (Kutsch and Huber, 1989), and copulation in praying mantis (Roeder, 1935, 1967), as examples, in which a basic pattern of motor output is mainly determined by a certain neural circuitry, a central pattern generator (CPG). Most of such examples are connected to a sterotyped, species typical behavior or a repetitive, or rhythmic movements.

It has been shown that neither peripheral control nor central control theory alone can explain the variety of movements exhibited by animals. The interaction between central control mechanisms and peripheral factors is more often the case than either "pure" central or "pure" peripheral control. Even though the feeding activities of a hungry fly is mainly driven by peripheral factors (input from oral sugar receptors), a normal fly will cease feeding eventually whether the peripheral factors are present or not. On the other hand, a centrally generated motor pattern needs to interact with sensory input to become an adaptive behavior. For example, animals are capable of rhythmic stepping even after transection of spinal cord and elimination of inputs from sensory receptors, but the speed of walking is slowed and the locomotor synergy is less well-coordinated. These animals cannot compensate for external disturbances (Grillner and Wallen, 1991).

Sensation for Action: Action for sensation--A Comparative perspective.

Historically, we tend to describe a behavior as being elicited by a particular stimulus or stimuli. The issue of central vs peripheral control becomes even more complex when we consider a group of behaviors whose initiation appears to be correlated with the *absence*, rather than the presence of eliciting stimuli. In recent years there has been increasing interest in a class of behaviors whose common function appears to be to acquire information about the environment. For examples, a reptile often flicks its tongue in a searching pattern, either to sample a chemical gradient or to recatch lost stimulus. In some species of crickets, the females locate males for mating by orienting and locomoting toward them in response to their species' specific auditory call. They move back and forth in a zigzagging track that would take them directly to the sound source. The zigzagging movement appears to lead the females out of an "ambiguity zone" directly in front of the sound source (Willis and Edmund, 1997). For a behavior to be adaptive, a subject must actively search and use the related information in its immediate environment to regulate the behavior. In essence, acting to sense is a fundamental characteristic of sensorimotor systems (Willis and Arbas, 1997).

The class of receptors related to "active touch" provides excellent examples of the adaptation of a sensory system for the acquisition of proximal sensory input. The primate hand provides the most obvious example. For many other mammals, however, "the mouth is the hand of the face" (Goethe). Facial tactile hairs (vibrissae) are present in the vast majority of mammals. Differing

from regular body hair, these enlongated tactile hair organs have larger follicles that are surrounded by encapsulated blood sinuses and receive much rich nerve innervation. Vibrissae may be divided into two classes with respect to their potential for mobility ("active" vs "passive" sense organs). A further classification into four categories—with respect to motility- has been suggested by Wineski (1983). (1) *Stationary* (immobile), which is seen canids, chiropterans, higher primates, cetaceans, etc. Those vibrissae typically are small, few, and less organized. (2) *Sporadic,* seen in most pinnipeds, larger rodents, lower primates, etc. Those vibrissae are large and are typically well-organized in some sort of grid-like pattern. Those animals occasionally or periodically move their vibrissae, but not in a rapid, repetitive, whisking fashion. (3) *Rapid* (whisking mammals), normally seen in small, nocturnal creatures—many small rodents, for examples. They have large vibrissae that are highly organized on the snout. Movement of those vibrissae is quick, repetitive, and long-lasting. (4) *Uncertain.* There is not enough information about whether some mammals move their vibrissae independently or the movement is just a by-product of movements of face -- shrews for example. Available information suggests that all vibrissae, either mobile or not, have a similar sensory capability and morphology, but functionality of vibrissal mobility remains unclear (Wineski, 1983). It is suggested that whisking may be most important in providing one species with environmental information which may be acquired in other species using other (e.g. visual) modalities. The rat's (mystacial) vibrissa provides an excellent example of a sensorimotor system with a highly developed "active touch" function which

serves to provide information about the "business end" (orofacial region) of the animal.

The rodent vibrissal system: Morphology and central representation

The rat's mystacial vibrissal (whisker) system is morphologically and anatomically well defined. The vibrissal system comprises an array of 25-30 whiskers arranged as a grid on the animal's snout in an invariant species/strain-specific pattern (five horizontal rows, A, B, C, D, E, from dorsal to ventral respectively). Each row of whiskers bears the same orientation dorsoventrally (Brecht et al., 1997). Each whisker hair is associated with a follicle-sinus complex (F-SC) (Rice et al., 1986). Individual vibrissae are both sensory and effector organs. Their sensory innervation is provided by the infraorbital nerve of the trigeminal maxillary division, which conveys afference from a variety of mechanoreceptors (*both fast and slow adapting)* (Dorfl, 1982; Rice et al., 1986). Their sensory capacities are comparable to that of the primate hands (Carvell and Simon, 1990). Vibrissa movements are controlled by facial nerve efferents to two distinct groups of muscles, extrinsic (orofacial) and intrinsic (follicular) muscles. The extrinsic muscles mediate the movements (rostro-caudal/dorso-ventral) of the entire mystacial pad. The intrinsic muscles are sling-like muscles that surround two adjacent follicles in the same row. Based upon the morphological data, it is hypothesized that protraction is produced *actively* by the contraction of intrinsic muscles while retraction is a *passive* event, mediated by the elastic rebound of the follicular connective tissue (Dorfl, 1982, 1985; Wineski, 1983, 1985). No muscle spindles or any afferent innervation to either extrinsic or

intrinsic muscles has been found (Bowden and Mahran, 1956; Semba and Egger, 1986). These observations suggest that whisker position is inferred from activity in trigeminal sensory afferents (reafference) or from centrally-generated reference signals (corollary discharge or efferent copy). Figure 1A illustrates the morphology and innervation of the rodent vibrissa system.

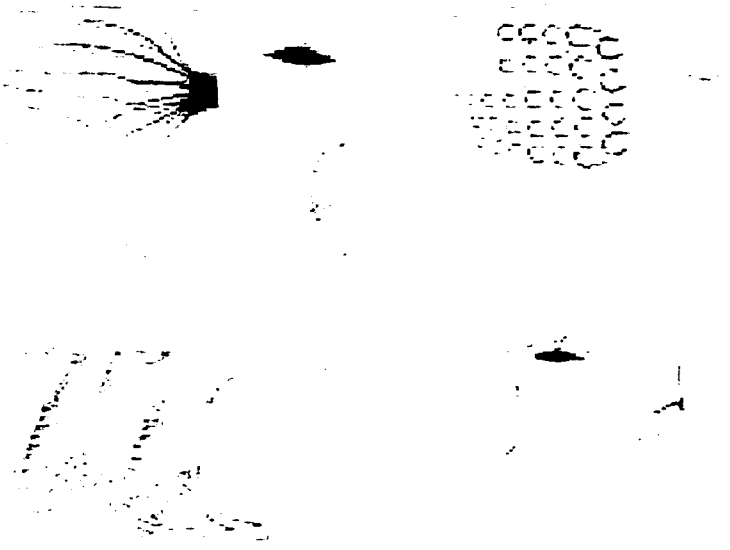
The vibrissae are represented at successive levels of the trigeminal neuraxis—(brainstem, thalamus, cortex)-- by discrete neuronal modules ("barrelettes, barreloids, barrels") (Figure 1B). The spatial organization of the vibrissae representation at various brain levels replicates, both structurally and functionally--as indicated by 2-deoxyglucose (2-DG) studies -- the peripheral organization of the whisker array. Furthermore, the development of that modular representation pattern is dependent upon sensory inputs from the whiskers during critical neonatal periods (Woolsey, 1990) The "barrel" field is the source of a massive topographically organized projection upon both the ventral posterior nucleus of the dorsal thalamus (VPM) and upon trigeminal brainstem structures, with dense terminations in the vibrissae regions of the trigeminal brainstem complex (TBC-- principalaris, spinal oralis, interpolaris, and caudalis) (Jacquin, et al., 1990). These projections may regulate the receptive field properties of central trigeminal neurons. The somatosensory cortex (SI) is also the origin of an intracortical projection upon vibrissal motor cortex. Cortical control of vibrissal movements involves descending projections from vibrissal motor cortex terminating upon, *inter alia,* basal ganglia, thalamus, superior colliculus and brainstem (spinal trigeminal nuclei, lateral reticular formation: Miyashita, et al.

1994).

Figure 1. (Top) Innervation of the mystacial pad of the mouse. (upper left) Schematic drawing of sensory innervation by the infraorbital nerve. mouse. (lower right) Motor innervation by branches of the facial nerve. (upper right). Schematic drawing of the intrinsic musculature of the left mystacial region. (lower left, medial view). Two neighboring mystacial follicles. Adopted from Dorfl (1982, 1985).

Bottom: Central representation of the vibrissa. Each peripheral axon in the trigeminal nerve (N.V.) innervates one whisker follicle. Centrally the axons are aggregated in whisker-related bundles that terminate in several nuclei in the brainstem. From top to bottom these are: principal sensory nucleus and spinal trigeminal complex subnuclei oralis, interpolaris and caudalis. Whisker-related aggregations of afferent fibers and cell bodies are present in all but subnucleus oralis. Such whisker-related aggregations are also observed in the contralateral thalamus and cerebral cortex.

A



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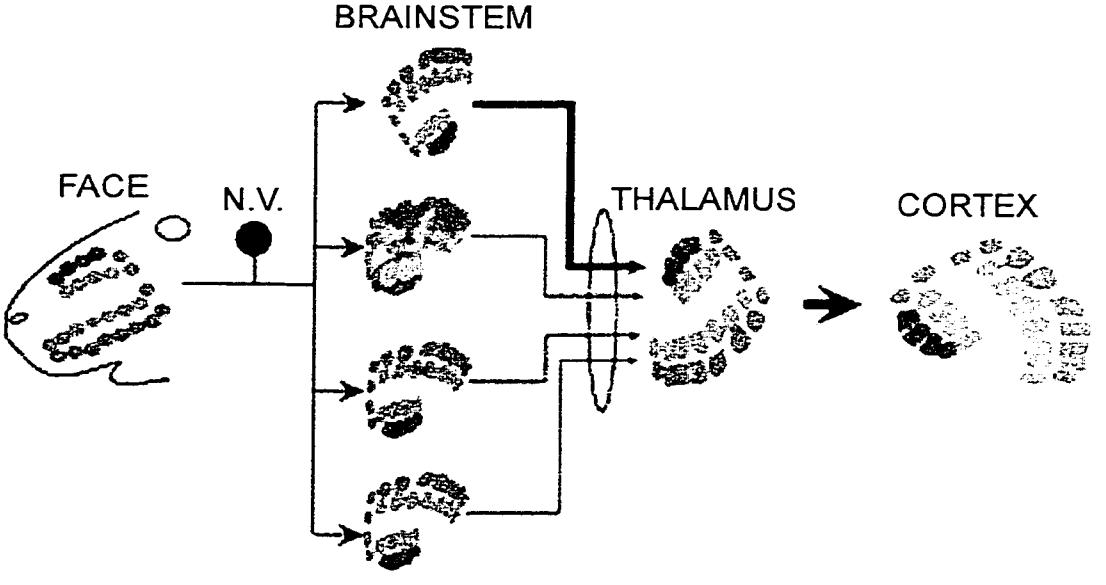


Whisking Behavior: Functional considerations.

The best way to characterize whisker function is to view the mystacial vibrissae as "mobile sensors". When the rat is placed in a novel environment he emits bursts of rhythmic whisking, which are synchronized with sniffing movements (Welker, 1964). Moreover, the whisking movements of the two sides of the face are clearly coordinated (Kleinfeld, 1999). Under resting conditions, they may be highly synchronous; under conditions of exploration they may be quite asynchronous. We know from some very early experiments that sensory inputs from the vibrissa are used to orient the rat in the maze and to localize objects in the animal's "personal space" (Vincent, 1912). More recent experiments have shown that the animal can use the whiskers to discriminate between textured surfaces, in a jumping stand paradigm, at a resolution comparable of the human fingertips (Guic-Robles, et al., 1989; Carvell and Simons, 1990, 1995). Videographic analysis showed that the decision to jump is preceded by bouts of intensive whisking activity (Carvell and Simons, 1990, 1995). It is clear from such studies that, the rat's mystacial whiskers, like the human hand, function as an active touch system. During tactile discriminations, the whiskers are swept across the surface of objects such that different parts of the array are stimulated to different degrees. As in the hand (Morley et al., 1983; Connor et al., 1990; Connor et al., 1992), these scanning movements generate trains of impulses in mechanoreceptors; which serves to code the spatial properties of objects as spatio-temporal patterns of neural activity.

There is another way in which the whiskers resemble the human hand. Success in different types of discrimination tasks is correlated with the adoption of task-dependent whisking strategies, which involve the rat's modulation of its own whisking parameters, like amplitude or frequency. Similarly, humans, when using their hands to discriminate among hardness, volume, or shape, typically use hand movements specific for each task (Morley et al., 1983; Connor et al., 1990; Connor et al., 1992). Such movement strategies correspond, to what cognitive psychologists call "observing responses" --behaviors, which, while not directly reinforced during discrimination, (a) provide exposure to the relevant stimuli and (b) mediate encoding of critical stimulus properties. In the rat, such observing responses appear to be acquired during development. Animals whose whiskers are clipped during the first 45 days of life are impaired on some tactile discriminations as adults (Carvell and Simons, 1996). It is not clear whether the deficit is related to sensory processing, to the control of movement parameters, or in the in the linkage of sensory and motor processes.

Finally, whisking and active touch both involve the generation of scanning movements which are constantly being modulated by the very inputs which they generate: That is, whisking involves a continuous and dynamic interaction of motor patterns whose generation is modulated by recurrent sensory inputs from the vibrissae.

Rat's mystacial system as a model system for studying control of behavior

All of these characteristics—simple and well-defined system and richness in behavior--make whisking an extraordinarily rich neurobehavioral model

system. Whisking involves only a single active movement, protraction, by a simple effector organ (Dorfl, 1982; Wineski, 1985). Studies suggest that the entire set of whiskers appears to move in synchrony during exploration (Brecht et al., 1997; Wineski, 1983). Therefore, movement of the entire whisker array can be assessed by monitoring the kinematics of a single whisker. Yet, this relatively simply motor system is involved in pattern generation, exploration, object localization and recognition, texture, size and shape discrimination, and spatial mapping of the environment. The system generates a wide range of movements, both stereotyped and highly plastic. It functions as an interface between sensory inputs and exploratory/discriminative behaviors and the sensory and motor processes are quite direct. "Whisking" provides a unique behavioral "window" onto a set of processes that are often lumped together under the rubric of "sensorimotor integration".

Contributions of trigeminal afference to the generation and coordination of whisking patterns: A Behavioral preparation for the study of "Central" Pattern Generation.

Even in the absence of explicit whisker stimulation, rats emit patterns of rhythmic whisking. The existence of a central pattern generator(s) for whisking in the rat is suggested by the persistence of whisker movements after sensory denervation, decerebration, and cortical ablation (Sharp and Evans, 1982; Carvell et al., 1996; Semba and Komisaruk, 1984; Welker, 1964) and the overlap between the frequencies of whisking rhythms and those of other orofacial behaviors (chewing, sucking, licking) known to involve CPGs located in

brainstem reticular regions (Nakamura & Katakura, 1995).

However, identification of a movement pattern as centrally generated requires that we show that its generation, patterning and coordination are independent of sensory input. The rodent whisking system lends itself to such an analysis, because rat moves its whiskers with a rhythmic pattern even in the absence of explicit whisker stimulation and its peripheral sensory innervation is well understood and surgically accessible for manipulations. Moreover, the behavior can be elicited under conditions in which the intrusion of other sources of sensory input, such as from head movements can be eliminated. Finally, our laboratory has developed instrumentation which makes it possible to monitor whisking movement trajectories over a wide range of frequencies and amplitudes with very high spatio-temporal resolution. Kinematic analysis of deafferentation effects upon unconditioned vibrissal movements in air will allow me to evaluate the role of sensory input in initiation, maintenance, bilateral coordination, and/or modulation of these movements.

Rationale and Objectives

Previous physiological studies have provided no data on whisking kinematics, and the available behavioral studies have focused on the complex, tactually modulated, discriminative whisking pattern. The more complex patterns may reflect the modulation of simpler patterns by tactile stimuli. Studying sensorimotor integration will be facilitated by developing a neurobehavioral preparation that permits experimental isolation, monitoring and behavioral control of specific types of vibrissal movements. It can then be used to correlate types of

manipulations with behavioral changes (deficits). A currently employed neurobehavioral strategy, such as the freely-moving paradigm (animal's engaging in a discriminative task), involves simultaneously activation of neck, forepaws, nose, and whiskers (Welker, 1964; Jones and Diamond, 1995). Afference is generated from both whisking contact (exafference) and vibrissal movements (reafference). Such a paradigm, therefore, can not be used to evaluate the role of afference in the animal's fine motor control of vibrissal movements.

The proposed research examines whisking patterns in the head-fixed animal, isolated from tactile contact, in order to characterize the kinematic parameters involved in pattern generation. Kinematic analysis of vibrissal movements in air will allow me to evaluate the role of reafference in initiation, maintenance, bilateral coordination, and/or modulation of these movements by comparing normal and deafferented animals. Kinematic analysis of exploratory vibrissal movements will provide a baseline for the subsequent studies of motor control mechanisms (feedback or feedforward).

Specific aims of the present research

1. To characterize the kinematics and coordination of bilaterally homologous whiskers,
2. To evaluate the effects of deafferentation of the vibrissa pads upon the kinematic variables and bilateral coordination,
3. To relate these data to the hypothesis that there exists a central pattern generator(s) for whisking.

General Methods

Subjects

Eleven female rats (Long-Evans) age six to 12 months, were housed individually under a 12:12 hour reversed light-dark cycle (lights on at 8:00 PM) with food and water available *ad libitum*. During training or testing, subjects were maintained on a 23 hr water deprivation schedule, with food continuously available in the home cage. The availability of water was controlled to maintain body weight at 85-90% of an age-adjusted free-feeding weight. Two animals did not complete the entire experiment and their data is not included.

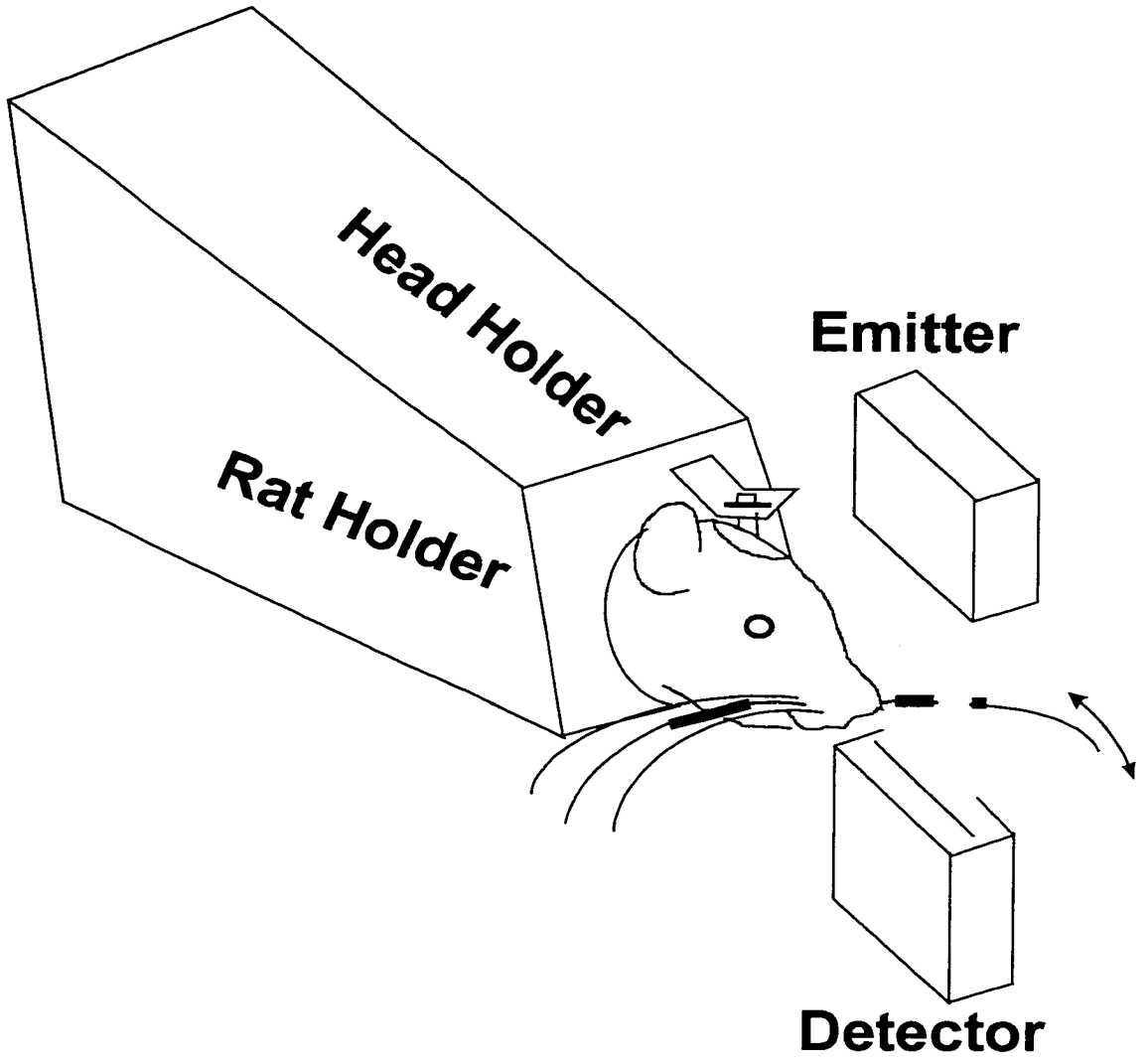
Experimental design

Five subjects (701,704, 707, L5, and L10) were assigned to the experimental, and four (703, 705, L8, and L9) to the sham surgical control group. The experimental group sustained a sequential, two-stage deafferentation of the vibrissae. Following an initial period of adaptation to the testing situation, whisking was monitored in three preoperative test sessions. This was followed by section of the right infraorbital nerve to denervate the whiskers on the right side of the face (Stage I). Following a recovery period and three sessions of behavioral testing, the left infraorbital nerve was sectioned (Stage II). Following recovery, the bilaterally deafferented animals were retested for another three sessions—for a total of nine test sessions. The same testing procedure was used for the animals in the sham surgical control group.

Apparatus

All observations were carried out in a sound-shielded test chamber, (interior dimensions 80 x 60 x 60 cm). The chamber was equipped with a house light, a gravity-driven water delivery system, a tone generator (Radio Shack #273-074A, 2.5 kHz), and a Charge Coupled Device (CCD) camera (Panasonic OEM Board Camera, GP-CX 151) connected to a closed circuit video monitoring system. The rat's body was restrained in a V-shaped acrylic enclosure, bolted to the floor of the chamber (Bermejo, et al.1996). The rat's head protruded through an opening in the enclosure and was fixed to a metal bracket attached to the box by a bolt embedded in a dental cement crown. Water was delivered to the rat's mouth by a gravity driven water delivery system. The system generated pulses of water, whose duration was controlled by a solenoid valve in the system and adjusted to deliver 20-40 μm aliquots of water. Movements of an individual vibrissa were monitored using an optoelectronic device. All these devices were interfaced with a personal computer (486 PC/Alpha interface cards) and driven by customized software written in QuickBasic. The computer was used to control stimulus presentation, reinforcer delivery, and data collection and storage. Figure 2 illustrates the experimental setup.

Figure 2. Schematic diagram of the monitoring system, indicating the position of the laser emitter/detector with respect to the head of the animal. For clarity only a single set of emitter/detectors, and only a single whisker is shown on one side of the face. In all our experiments, all whiskers were intact on both sides of the face.



Optoelectronic monitoring of individual vibrissa movements

The response monitoring system (Bermejo, et al. 1998) consisted of a laser emitter/CCD detector system, configured with respect to the head-fixed rat so as to avoid contact with the whiskers. The 2496 sensors in the detector are arranged in a linear array spanning a distance of 25 mm. Interruption of the emitted beam (laser curtain) by the shadow of a whisker produces a potential shift in a subset of shaded sensors. Whisker movement results in successive displacements in the position of that voltage shift which are linearly related to whisker position. A comparator circuit identifies the successive positions of potential above a preset threshold and outputs the data to a microprocessor for computation and display of the whisker movement trajectory. The monitoring system has a spatio-temporal resolution of 11 μm and 1.4 ms, respectively.

To monitor an individual whisker trajectory with neighboring whiskers present, a light (ca. 3-4 mg) self-adhesive foam marker (17 mm long, 1 mm in diameter) was attached to the side of a vibrissa. This increases its relative "visibility" with respect to surrounding whiskers without affecting either its kinematics or bilateral coordination (Bermejo, et al., 1998).

Calibration

At the start of each animal's test session the position of the monitoring devices was adjusted so that the emitter-detector pairs were aligned in parallel with the mystacial pad, at the same angle, and at a fixed distance from the base of the whisker. Because the laser signal is not visible, we used a pair of custom-made acrylic "curtains" one for each emitter-detector pair. The height of each

curtain is equal to the distance between the laser emitter and the CCD detector and contains an opening at the height of the rat's vibrissa pad when the head is fixed. When the curtain is in place, the opening is positioned in parallel with the CCD array with its center approximating the midpoint (sensor #1248) and it serves to aid in positioning the whisker. With the whisker held at a 90° angle to the pad within the curtain opening, the distance between the mystacial pad and the midpoint of the CCD detectors was set at 10 mm (See below).

Surgical Procedures

Preparation of head mounts. Subjects were anesthetized with Ketamine (100 mg/kg of body weight, ip) and Xylazine (5.5 mg/kg of body weight, im). The head was shaved and lubricating ointment was applied on the eyeballs to protect them from irritation and drying. During the operation, the rat's head was fixed in place using a stereotaxic head holder, with the body in a prone position on a heating pad. A midline incision (about 2.5 cm long) was made in the scalp, beginning at about 1 cm anterior to bregma. Periosteum was retracted with cotton swabs soaked with saline and the skull was exposed. Bleeding was stopped and the skull was scraped clean. Periosteum was then further pushed down to the two sides of the skull. Care was taken not to damage the eyeballs and the nerves or glands attached to them. Six self-tapping stainless steel screws (Small Parts #Q-TX0-2) inserted to a depth of 1 ½ turns were used as anchors for a dental cement platform, using the location of bregma as a reference point. Four of them were set into the top of skull at approximately 3 mm lateral to the midline, with one pair 5 mm rostral and the other 5 mm caudal

to bregma. The remaining two screws were inserted into the two sides of the skull. A mounting screw (Small Parts #Q-TSB-632-12) was embedded in the central portion of the dental cement platform above bregma. The incision was closed with a single suture at its caudal portion to minimize irritation of the incision edges.

Infraorbital nerve section (IOx): Animals were anesthetized with Ketamine/ Xylazine. The fur caudal to the mystacial pad was shaved. The shaved skin was cleaned and an approximately 3-mm incision was made through the skin about 2 mm caudal to the γ-straddler. Access to the infraorbital nerve from this location can minimize the damage to adjacent muscles. The remainder of the operation was carried out with the aid of a Zeiss dissecting microscope. Using blunt dissection through the *levator labii superioris* and around the anterior superficial masseter muscle, the infraorbital nerve (ION) was exposed as it emerges from the infraorbital fissure. Care was taken not to damage the more superficially running facial nerve branches and mystacial pad structures. The infraorbital nerve was lifted and transected with micro scissors proximal to the infraorbital fissure. The incision was closed with one or two sutures and antibiotic ointment was applied. Because postoperative testing was normally completed within 2 weeks of nerve section, no attempt was made to interfere with nerve regeneration.

Surgical Controls. Animals in the control group underwent all the surgical procedures, except for section of the infraorbital nerve. For all subjects, a minimum recovery period of three days intervened between surgery and

adaptation or testing. Several additional animals were denervated and sacrificed after two to three weeks to assess the completeness of the ION section and the regeneration of the ION.

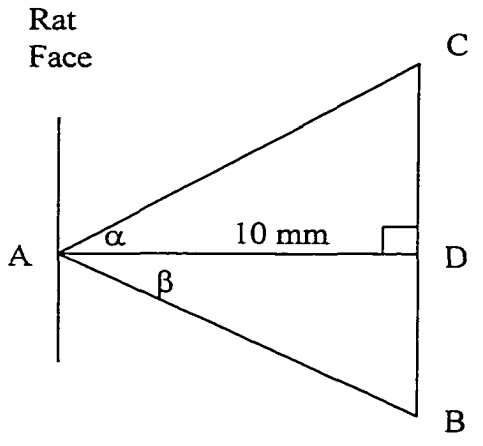
Note: All procedures adopted in the present study were in compliance with the federal and State laws and local regulations and were approved by the Hunter College IACUC.

Behavioral Procedures

Data on the whisking patterns of normal and deafferented animals were obtained by monitoring the movement trajectories of a pair of bilaterally homologous whiskers (right and left C-1) in each of the head-fixed subjects. Subjects were handled daily for at least two weeks prior to and after placement of the headmount. To reduce the stress of immobilization, water was delivered at random intervals during the session (Variable Time schedule) with an average inter-reinforcement time of about 26 seconds. Water delivery was non-contingent; i.e., independent of the whisking response. All observations were made during test sessions, about 30 minutes in duration, on successive days. During testing, the house light was kept on all the time except at the moments of water delivery and during brief period (2 s) during which data were saved to disc. Each water delivery occurred at the midpoint of two consecutive data-saving periods except the first and last deliveries. Each session involved 30 water deliveries.

Data analysis:

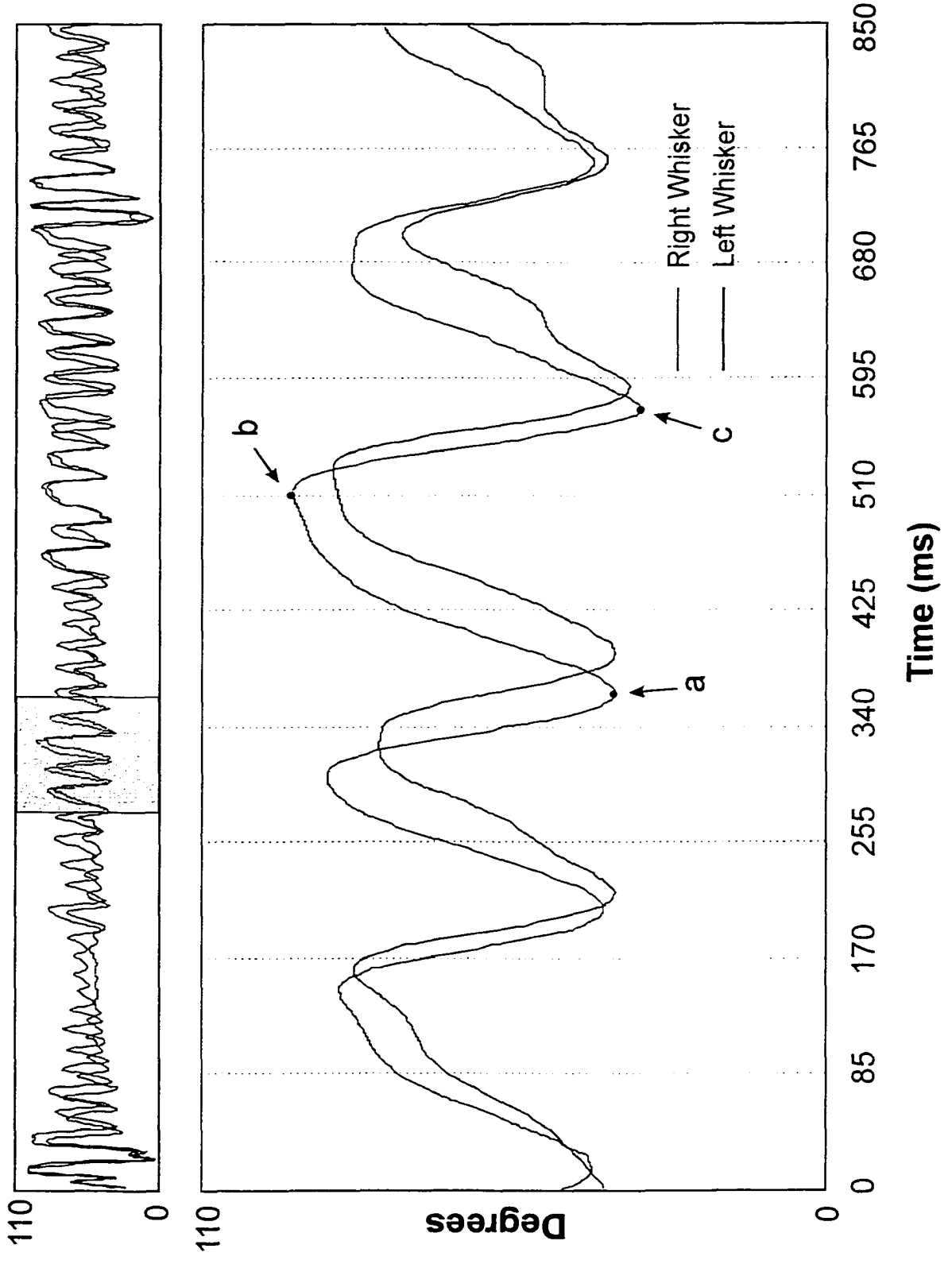
During testing, the data on whisker movements was saved in CCD units; i.e. as a series of numbers between 1 and 2496, indicating the successive positions of the shadow of the marked whisker as it moved across the array of CCD detectors. These data were transformed into angular units to express movement in degrees for subsequent analysis. The principle of that transform is illustrated in the accompanying schematic diagram.



Systematic calibration of the monitoring system at the start of each session provided information about the distance from the base of the whisker to the midpoint (Point D) of the CCD array (Line AD, 10 mm) and the angle of that intersection (90°). Each data point (one per 1.4 ms), such as Point B or Point C in above drawing, has its relative distance to Point D (Line CD or BD). For any data point for example Point B or C, a rectangular triangle (ADC or ADB) can be defined. Given the appropriate information about one side and one angle of an equilateral triangle, the angular distance traversed by the whisker (α or β) can be calculated using the arc tangential relationship between Line DC and Line AD or between Line BD and Line AD. These computations were carried out using a specially written computer program.

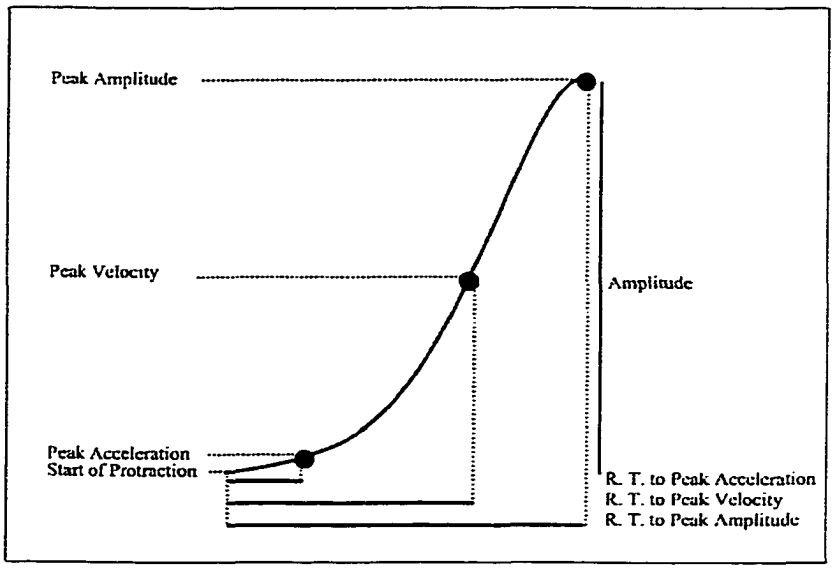
The transformed data for each test session were plotted in angular coordinates and displayed on the computer monitor as a plot of whisker position against time. A specially written, cursor-driven graphics program was used to scan this plot under varying conditions of temporal resolution and select episodes of whisking for subsequent analysis. Figure 3 illustrates the data display and plots the trajectories of the right (darker trace) and left (light trace) C-1 whiskers during a period of whisking. The top portion of the figure represents 8.5 sec. The shaded portion of the record highlights an 850 ms sample that is displayed at higher temporal resolution (all data points used) at the bottom of the figure.

Figure 3. Whisker movement trajectories (angular position/time) for the Right (darker lines) and Left (lighter lines) C-1 whisker in a head-fixed animal during the first test session (resolution 1.4 ms). The upper panel presents an 8.5 s sample plotted at a lower resolution (every 10^{th} data point). The shaded portion of the record highlights an 850 ms sample which is displayed at higher temporal resolution at the bottom of the figure. Upwards and downwards movements represent whisker protractions and retractions, respectively.

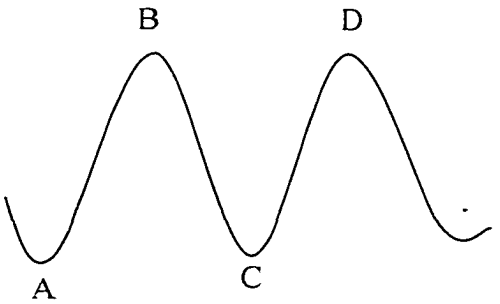


Individual whisks selected for kinematic analysis had protraction amplitudes falling in between 1° degree and 110° (the limit of the detector array). Only those whisks with a single peak, with clearly defined protraction (rising) and retraction (falling) phases and obvious starting and ending points were chosen. These criteria served to exclude some waveforms obscured by noise or highly irregular in form. Whisks were picked up from the beginning of each session, whisk by whisk, until the finish of the last whisk of the session. Bilaterally homologous whisks were selected in pair. Any individual whisks that didn't fit in above criteria were skipped, without concerning whether they were consecutive whisks in a bout of whisking or isolated ones as long as they met the defined criteria. The information extracted with above criteria included kinematic variables (amplitude, velocity, acceleration, and their associated time courses), whisk duration, and synchrony in temporal domain. This information was used for kinematic analysis, frequency and synchrony estimation. For burst duration, which is defined as the time of a continuing bout of whisking, it was extracted with another customized QuickBasic program. This program was used to identify the start and end of each bout of whisking activities and to extract the length of the bout. Both protraction and retraction amplitude of an individual whisk was defined in such a way that it was calculated according to the distance the monitored whisker traveled on the CCD detector without regarding to its absolute starting point.

To characterize the kinematics of whisker movements, we measured a set of kinematic variables including amplitude of the movement (peak amplitude), its



derivative variables (peak velocity and peak acceleration), and the time course associated with each of them. These variables are illustrated in the accompanying schematic diagram. A customized QuickBASIC program was developed to perform this task. With a mouse click on an individual wave form (see diagram), the program finds the three critical points (A, B, and C) using a general algorithm. (In a few cases, the location of one or two of these points was manually adjusted.) If an individual whisk has a well-defined peak and trough, the Basic program can locate the three critical points without any error. Even in the cases where one or two of these points need to be



adjusted manually with my visual guide, the error is still minimal because of (1) the high resolution data and magnified plotting during analysis (precision to each pixel), and (2) only a few occurrences. In the example shown, Point A is the start of an individual whisker protraction, Point B is the peak of the protraction, and the Point C is the end of the retraction. Based upon these values, the program automatically computes a variety of kinematic parameters, including protraction amplitude (vertical distance between A to B), peak velocity, and peak acceleration and the time course associated with each of them. The relation between peak amplitudes and other kinematic variables provided the information about the amplitude-scaling characteristic of the whisker movements. The rise time to peak amplitude was defined as the time elapsed from A to B). The whisking cycle duration (time from point A to C) and inter-whisk time (inter-peak time, time from point B to D) were also extracted. The cycle duration was used to provide a first approximation to whisking frequency. The bilateral synchrony (in the temporal domain) of whisker movements on the two sides of the rat face was computed as the peak time difference between the peaks of two homogenous whisks. At the end of the scoring session, the number of whisker movements analyzed was computed and saved to disk. Kinematic values were transferred to a spreadsheet for additional analysis.

Calculation of whisking frequencies (rhythmicity) was based upon two different measures. (1) It could be inferred from measurement of individual whisk durations-- or (2) It could be calculated for episodes of whisking by applying Finite Fourier Transforms (FFT) to the CCD data after it had been transformed to

angular degrees. Phase relationships among movements of the R and L whiskers were calculated using a cross correlation procedure. Both Fourier analysis and cross correlation procedures were carried out using Software from the SAS Institute, Inc.

All statistical analyses were performed with software from the SAS Institute, Inc. Repeated-measures *t* test (two-tailed) and repeated-measures ANOVA were used to test group data where it applied. Multiple regression method was used for individual subject's data to analyze the relation between/among kinematic variables.

Results

Part I. Topography and kinematics of whisking movements in normal rats.

The present results are based upon an analysis of more than 125,000 whisking movements, recorded from 9 rats over a series of nine test sessions, before and after trigeminal deafferentation (5 rats) or sham operations (4 rats). To illustrate the data set from which the response population was drawn, Figure 4 presents a sample of whisking movement data recorded from a pair of bilaterally homologous whiskers (Right and Left C-1) in an intact rat during a single trial in the first test session. Water was delivered at the beginning of the fourth pane (indicated by arrow). The data are plotted in the low-resolution mode to allow presentation of an extended sample. The rat is whisking in air and successive protractions and retractions of the monitored whiskers appear as a series of upward and downward deflections. Reference to the time scale indicates that the whisking responses occur mainly at a rate of between 5-6 Hz. While movements of the two whiskers are sometimes out of phase, and of differing amplitude, the record conveys an impression of generally synchronized movements of the bilaterally homologous whiskers over a wide range of amplitudes. The results of our kinematic analysis of individual animals are consistent with that general impression.

Results of statistical analyses presented in Part I of this Results section are based on data from all 9 subjects collected in the first testing session.

Figure 4. A sample record of whisking movements, recorded from a pair of bilaterally homologous whiskers (the Right: darker lines; Left: lighter lines) in a normal rat during the first testing session. The data are plotted in a low-resolution mode to allow presentation of an extended data sample. Conventions as in Figure 3. The analyses presented in Figures 4-13 are based on the data from this session. The arrow indicates the point water was delivered.

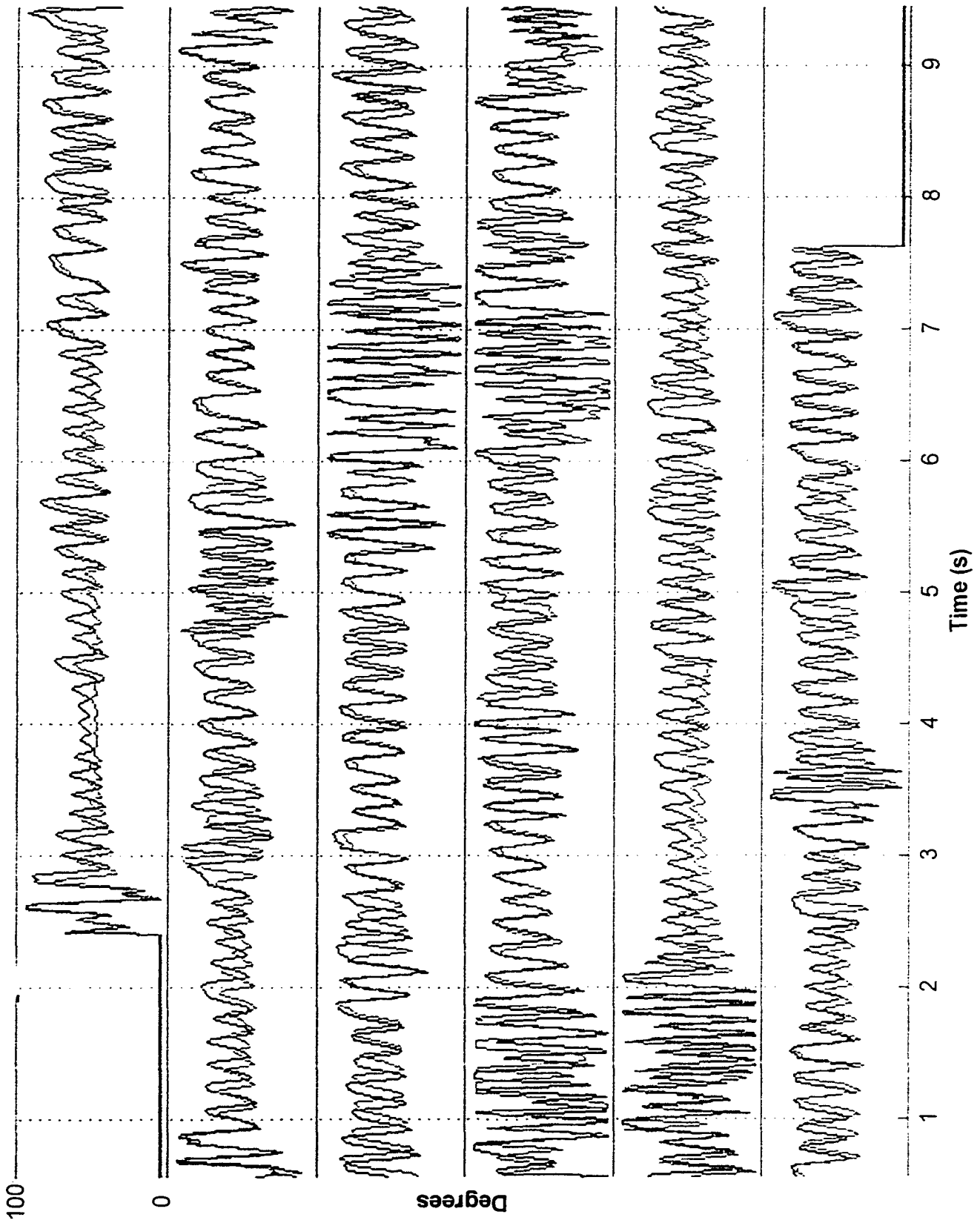


Figure 5 (top) plots the mean amplitudes of protraction and retraction movements for the right (R) and left (L) C-1 whisker in each of four normal subjects. The bottom portion of Figure 5 presents frequency distributions of protraction amplitude for each of the subjects. The data indicate (a) that protraction and retraction amplitudes (Right C-1 whisker: 39.6 ± 4.9° vs 39.9 ± 5.3 °; Left C-1 whisker: 40 ± 9.4 ° vs 40.3 ± 8.6 °) are not significantly different (repeated-measures *t* test, Right C-1 whisker: $t(8) = -2.23$, $p > 0.05$; Left C-1 whisker: $t(8) = -2.13$, $p > 0.05$); (b) that while there are individual differences in the modal protraction amplitude of individual subjects, protraction movements occur over a similar amplitude range, and (c) that there are no significant bilateral differences in protraction and retraction amplitudes (repeated-measures *t* test, Protraction: $t(8) = 1.75$, $p > 0.05$; Retraction: $t(8) = 1.14$, $p > 0.05$). The latter observation was so characteristic of the kinematic analysis that in many subsequent figures only data for the right whisker is presented. Figure 6 (Top) compares protraction and retraction movements with respect to their mean velocity in two representative subjects and the statistical analysis indicates that retraction velocities are significantly higher (repeated-measures *t* test, Right C-1 whisker: $t(8) = -11.38$, $p < 0.001$; Left C-1 whisker: $t(8) = -0.75$, $p < 0.001$). The mean peak protraction and retraction velocity for the right and left C-1 whiskers are given in the Table 1. The bottom portion of Figure 6 indicates an almost perfect correlation between amplitude of the retraction component and its peak velocity. Mean movement times for the two components are compared in the top

portion of Figure 7 and the differences are significant and consistent with the velocity differences seen in Figure 6 (Right C-1 whisker: $t(8) = 18.81$, $p < 0.001$; Left C-1 whisker: $t(8) = 14.94$, $p < 0.001$). In the bottom portion of Figure 7, protraction and retraction times for two subjects are plotted as a function of the duration of the whisk in order to assess the relative contribution of the two components to the total duration of the whisk cycle. It is clear that, for durations over 100 ms, protraction time increases almost linearly with increments in whisk duration while retraction time remains essentially constant. Note, however, that for whisk durations at or below 70 ms. (i.e., whisking frequencies around 14 Hz or faster if the duration is converted into cycles per second.), the protraction phase could be shorter than the retraction phase (see Figure 7, lower left plot). Results of regression analysis which show that the variation in whisk durations is mainly accounted for by the variation of protraction time (Table 2). Again, there is no difference bilaterally ($t(8) = 0.31$, $p > 0.05$).

Figure 5. (A) Mean protraction and retraction amplitudes for the R and L C-1 whiskers in four normal rats (Session 1). Data are based upon number of whisking cycles (n's) for each animal shown in the graph. Error bars = Standard Error of the means. (B). Frequency distribution of protraction amplitudes for each of the subjects whose data is presented in A.

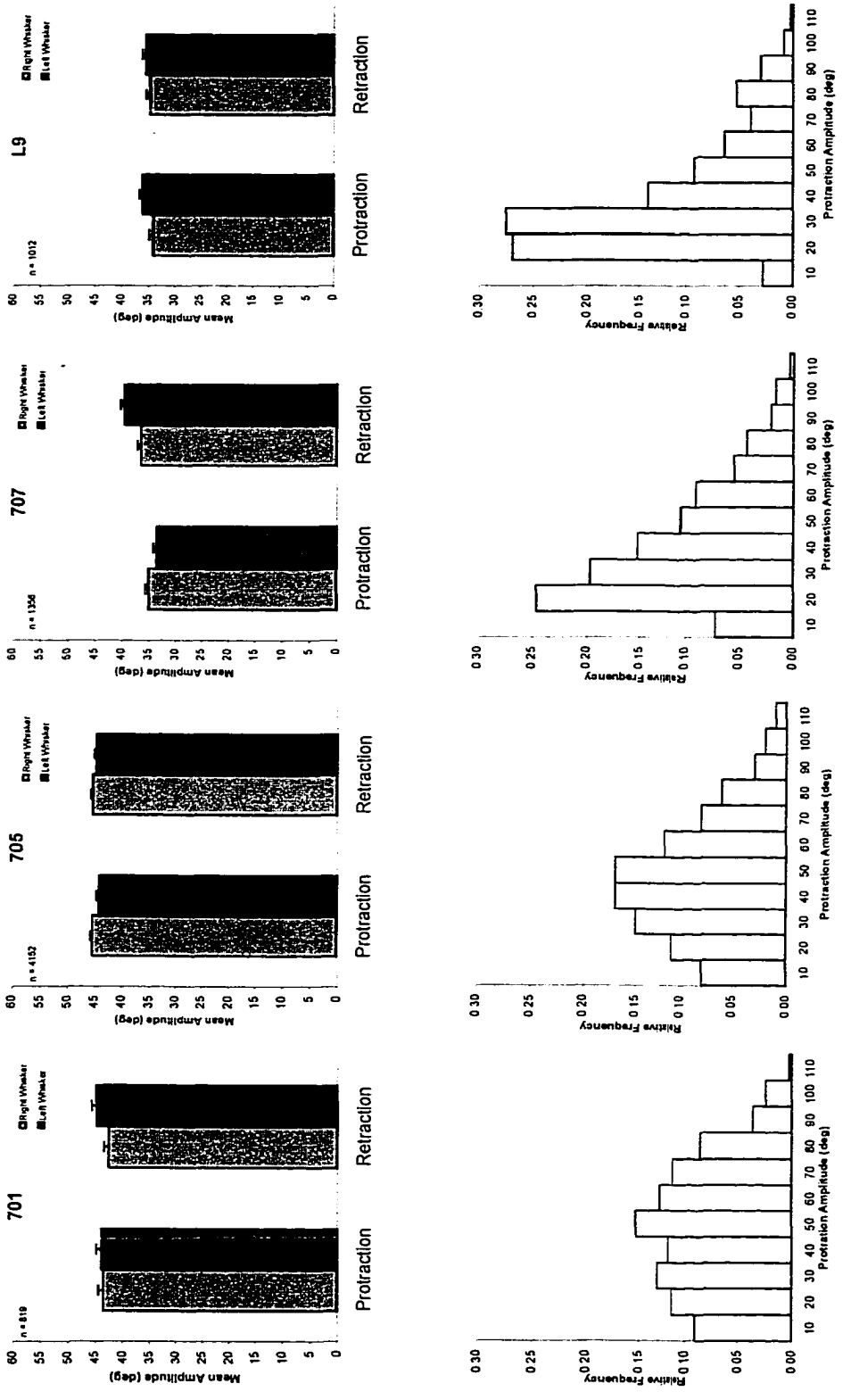


Figure 6. (Top) Comparison of protraction and retraction velocities in two representative subjects. (Bottom) Correlations between retraction amplitude and peak retraction velocity for the same subjects.

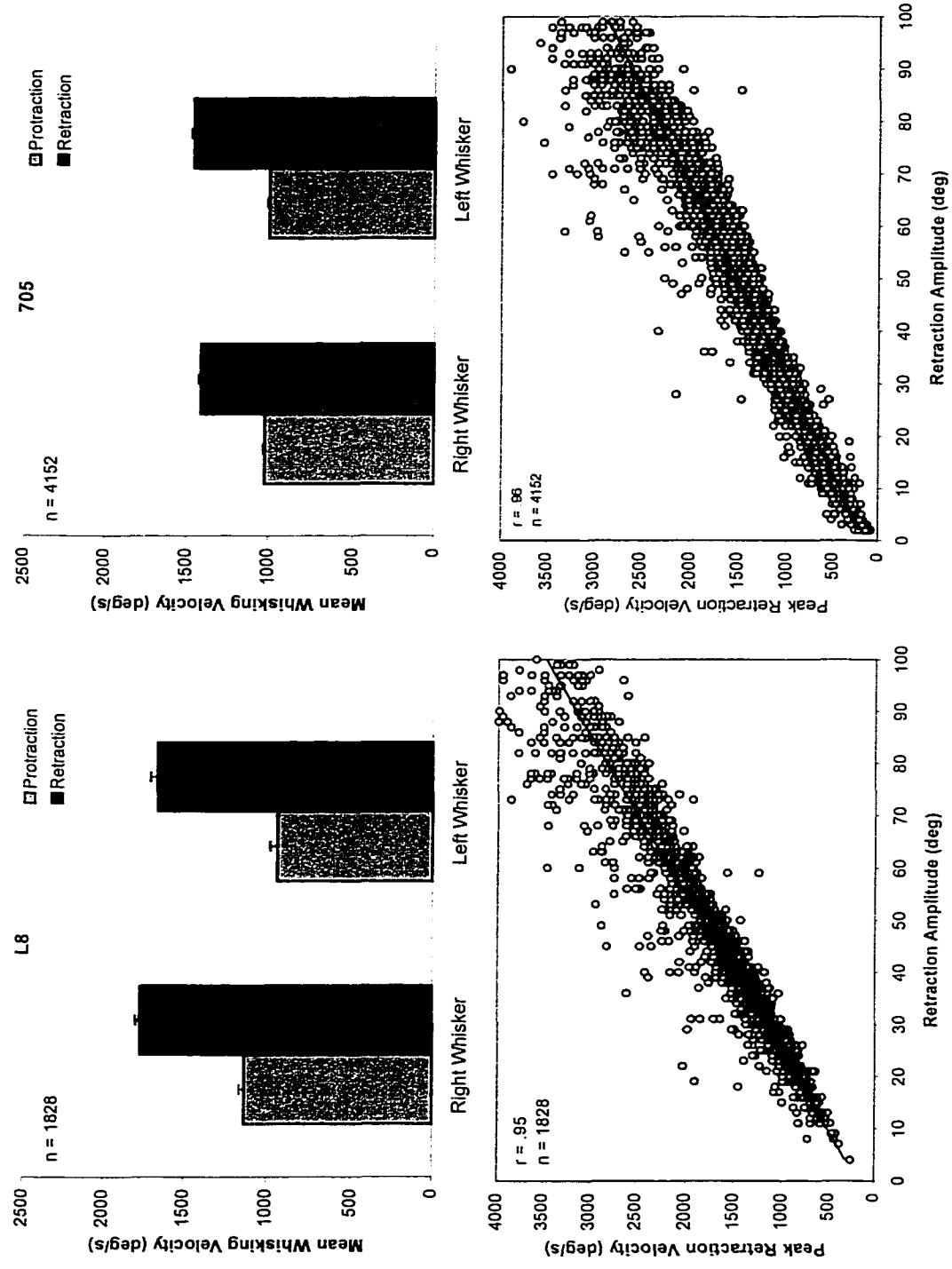
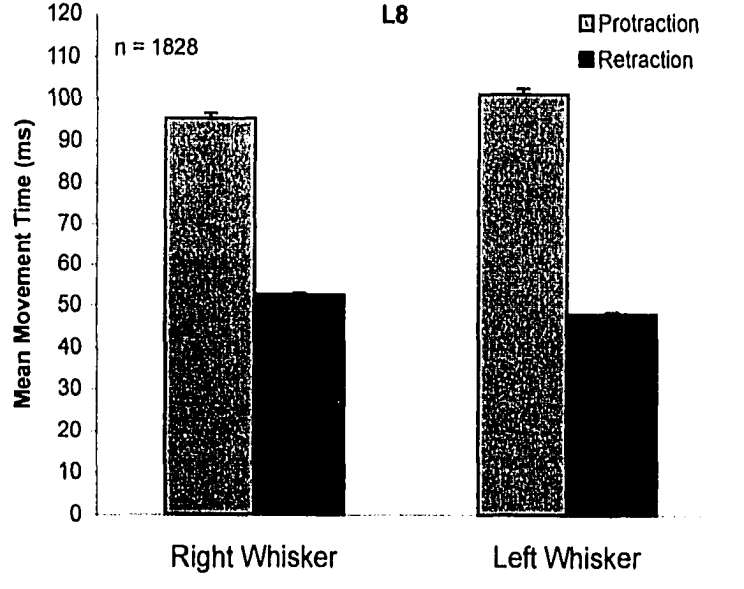
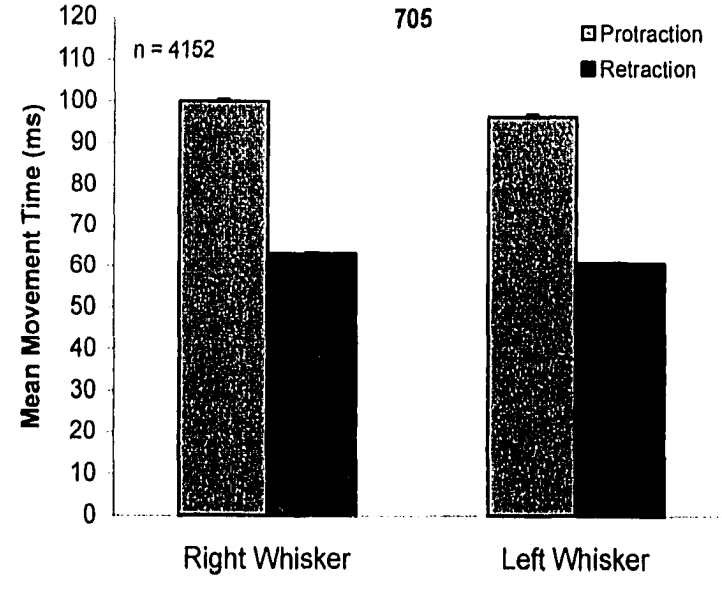
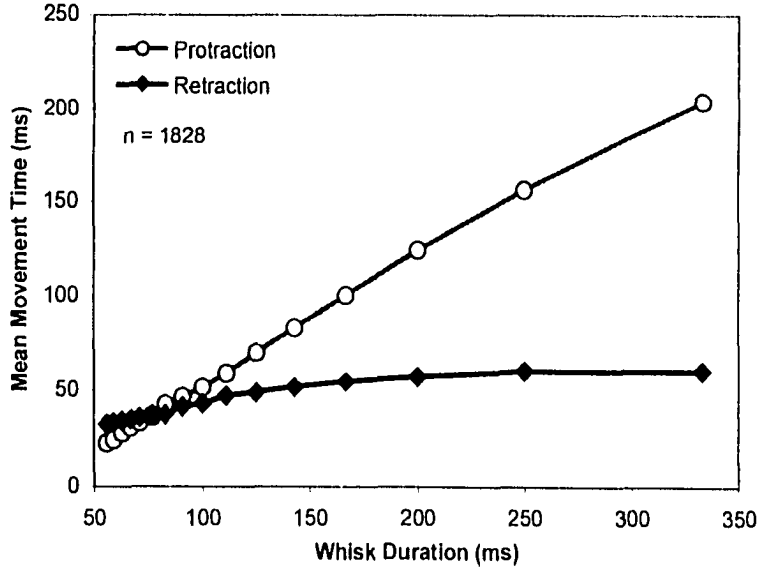


Figure 7. (Top) Movement times (duration) of the protraction and retraction components of whisking cycle for two normal rats. (Bottom) Relation between duration of an individual whisk cycle and the duration of its protraction and retraction components.







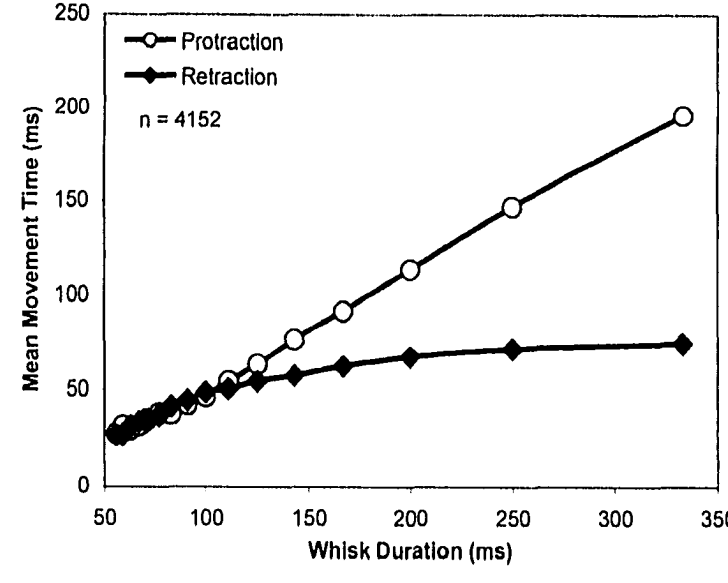


Table 1.

Peak whisking velocity (°/s)

	Protraction Velocity		Retraction Velocity	
Subjects (n=9)	Right	Left	Right	Left
Mean ± SD	1020 ± 212 °/s	1044 ± 257 °/s	1589 ± 286 °/s	1587 ± 372 °/s

Table 2.

Proportion of variance in whisk duration, during whisking, accounted for by Protraction movement time ($r^2_{D.\ PMT}$). The upper numbers in the cells are the results for the right C-1 whisker and lower ones (bold face) are for the left C-1 whisker. For all the results presented in the table, their corresponding F values are statistically significant ($p < .0001$).

Regression	Rat701	Rat703	Rat704	Rat705	Rat707	RatL5	RatL8	RatL9	RatL10
$r^2_{D.\ PMT}$	.913	.972	.909	.929	.902	.80	.957	.96	.967
	.903	**.966**	**867**	**.925**	**.901**	**.907**	**.923**	**.953**	**.922**

Table 3.

Proportion of variance in peak protraction amplitude, during whisking, accounted for by peak protraction velocity ($r^2_{A.\ V}$), rise time to peak protraction amplitude ($r^2_{A.\ RT}$), and the combination of both velocity and rise time ($R^2_{A.\ V,\ RT}$). The upper numbers in the cells are the results for the right C-1 whisker and lower ones (bold face) are for the left C-1 whisker. For all the results presented in the table, their corresponding F values are statistically significant ($p < .0001$) except otherwise labeled (n. s: not significant, **: $p < .001$).

Regression	Rat701	Rat703	Rat704	Rat705	Rat707	RatL5	RatL8	RatL9	RatL10
$r^2_{A.\ V}$	.649 **.671**	.707 **.735**	.631 **.75**	.744 **.705**	.783 **.589**	.51 **.66**	.713 **.694**	.777 **.729**	.62 **.56**
$r^2_{A.\ RT}$	.139 **.214**	.086 **.054**	.035 **.101**	.039 **.091**	.058 **.113**	.025 **.00 (n. s)**	.018 **.024**	.017 **.008****	.026 **.141**
$R^2_{A.\ V,\ RT}$	.859 **.874**	.857 **.847**	.684 **.862**	.837 **.846**	.882 **.671**	.663 **.798**	.83 **.804**	.865 **.844**	.833 **.793**

Amplitude scaling. As Figure 5 (bottom portion) indicates, head-fixed animals tested during periods of initial exposure to the apparatus emit whisks which are unimodally distributed over an amplitude range from about 10° to 100°, with the median of the distribution at about 25° to 45°. Figure 8 examines the relationship between whisking amplitude and two kinematic variables (velocity and rise time). A population of 100 whisks selected at random from the data set of four rats is shown. Multiple regression analysis shows that (with one exception) each of the variables accounts for a significant percentage of the variance in peak protraction amplitude (Table 3 and Figure 9). However, the proportion of variance explained by peak protraction velocity is substantially higher, suggesting that the scaling of whisking amplitude involves primarily control of whisking velocity (see Figure 8 also). This conclusion is consistent with an analysis of the topography of individual whisking responses in Figure 10. In Figure 10A a series of whisks of different amplitudes are aligned with respect to the occurrence of their initial points to demonstrate that increasing peak amplitudes are associated with increases in the slope (velocity) of the protraction component; In Figure 10B, individual whisks of similar amplitude may have velocity quite different rise times. Note also that for those whisks with longer duration (low frequency), the initial rapid protraction is followed by a relatively constant movement "plateau" prior to the start of retraction. Further analysis of the data (Figure 11, Top, Bottom) suggests that a rat may use two different strategies to scale its protraction amplitude. At lower protraction amplitudes, both peak protraction velocity and time increase with increases in amplitude. As the

protraction amplitude increases, the slope of the velocity function increases, while that of the rise time function turns more negative. These shifts are seen in three of the four subjects.

Figure 8. Relative contributions of protraction velocity (Top) and rise time (Bottom) to the amplitude scaling of protraction movements for four normal animals. The analysis was based upon 100 randomly selected whisks.

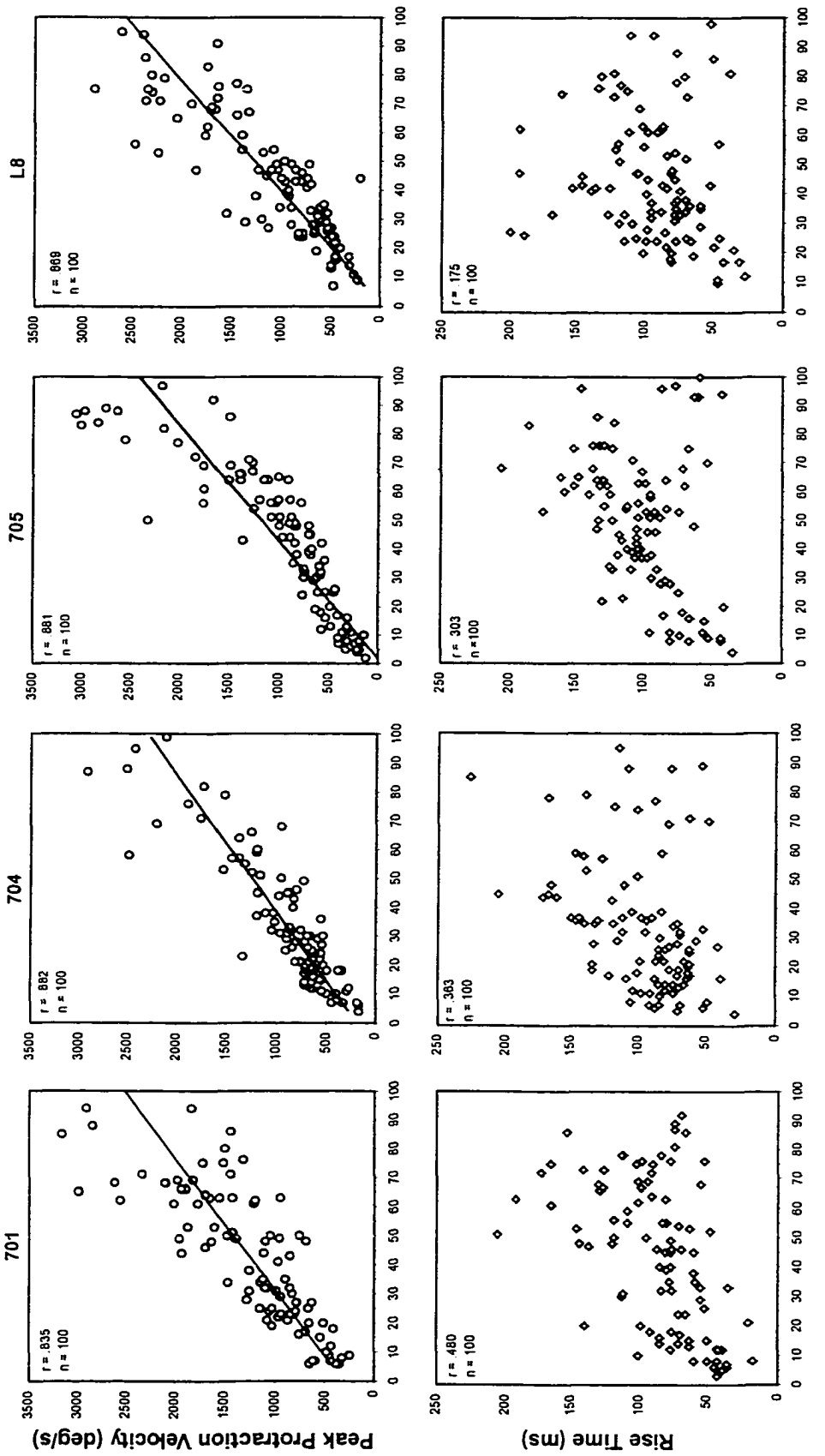


Figure 9. Proportion of variance in protraction amplitude accounted for by peak protraction velocity (white bars), by rise time (gray bars), and by the combined effect of the two kinematic variables. (black bars). Based on the data shown in Table 2.

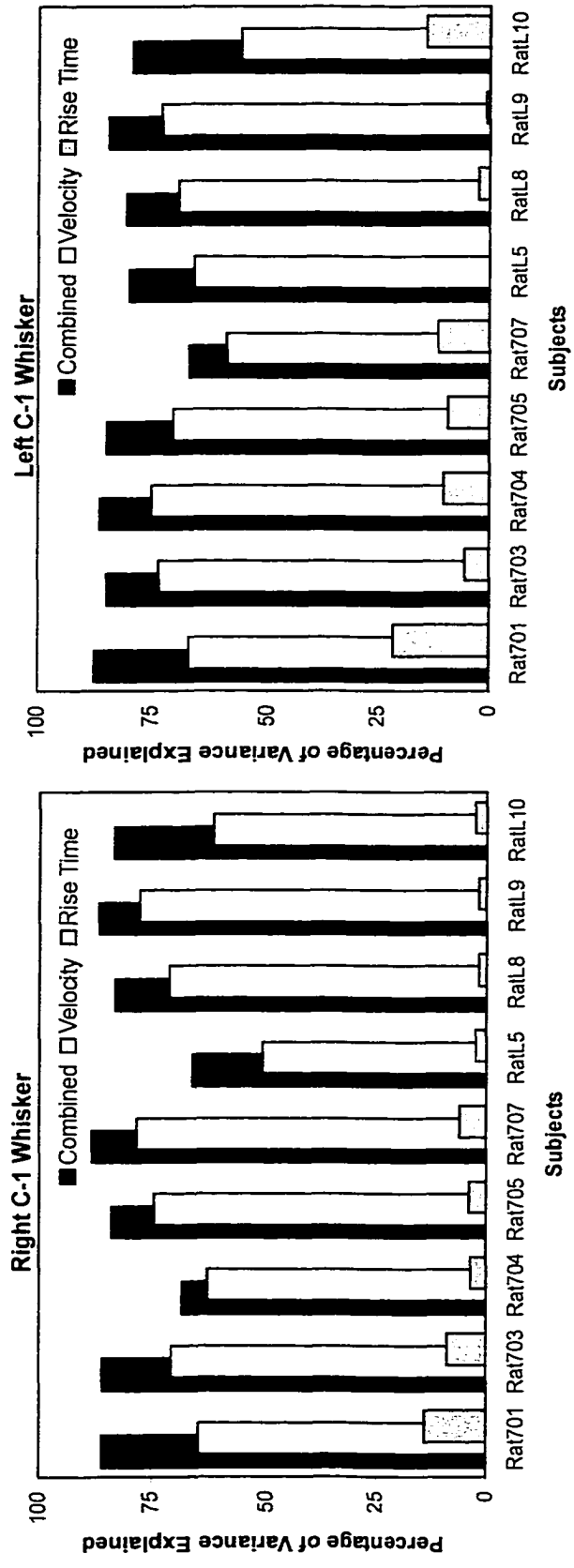


Figure 10. Relation between velocity and rise time during the scaling of protraction amplitude. Panel A plots whisks with the same duration but different peak amplitudes. Panel B plots whisks with the same amplitude but different durations. In both panels, individual whisks are aligned with respect to their starting points.

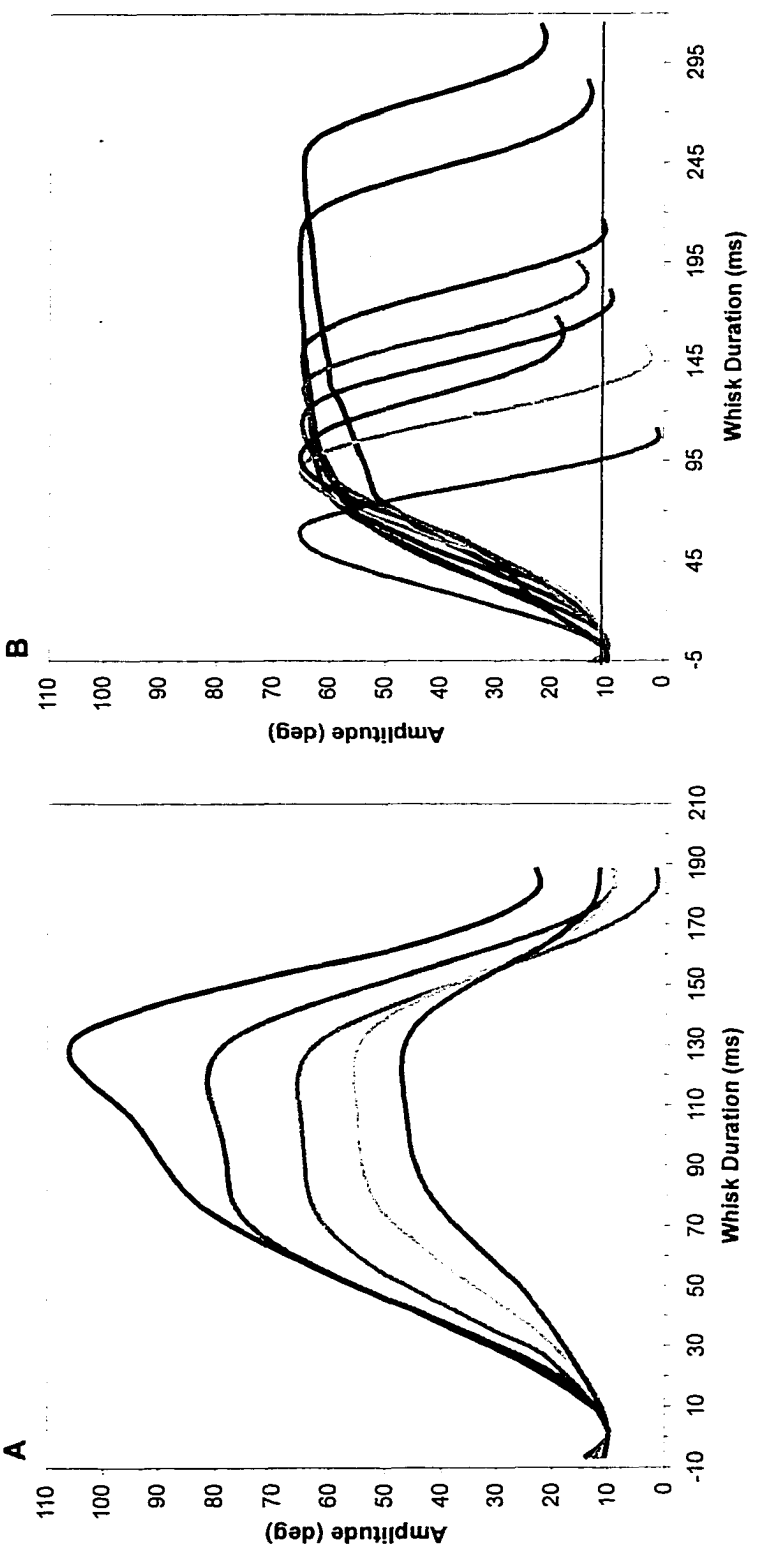
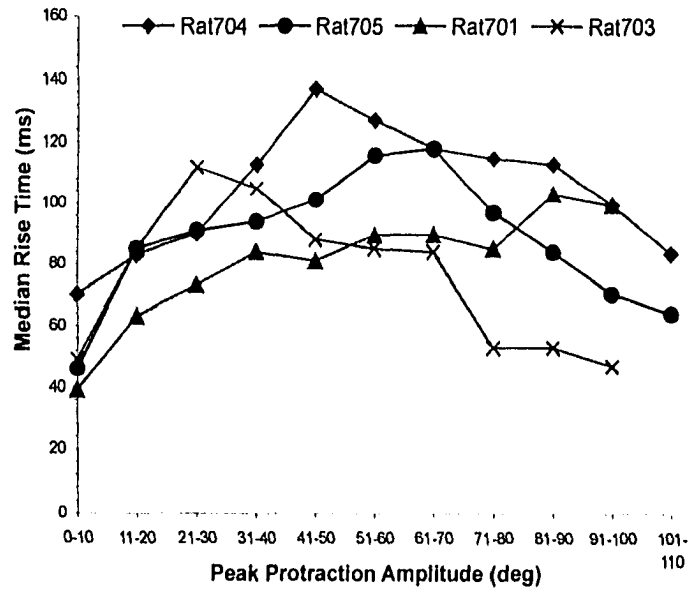
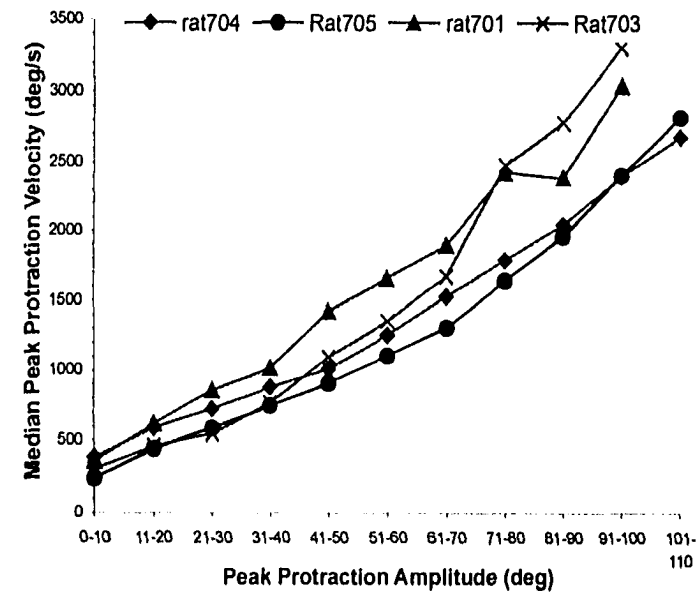
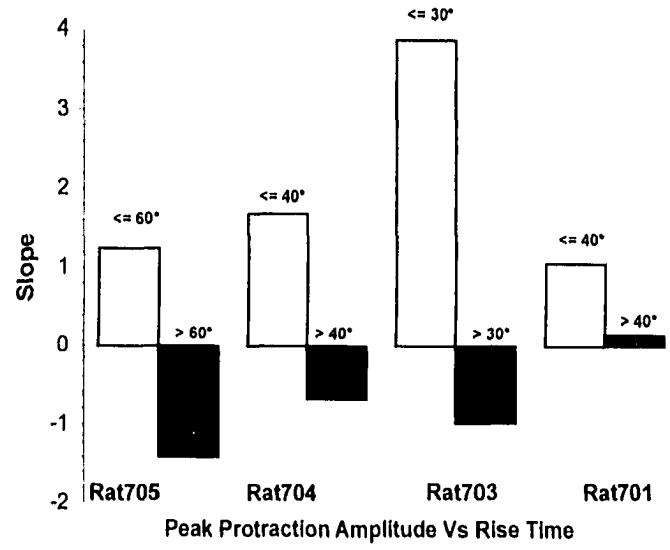
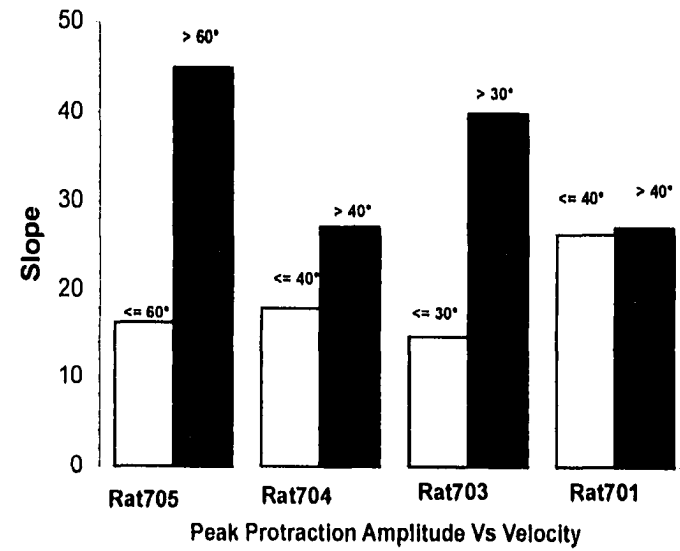


Figure 11. Relation between amplitude scaling strategy and peak protraction amplitude. (Top): The right and left hand panels illustrate the effect of increasing protraction amplitude upon rise time and velocity scaling functions. (Bottom). Effects of increasing protraction amplitude upon the regression slope values for the rise time and velocity scaling functions. For each subject, velocities and rise times were grouped with respect to protraction amplitude and the slope value was calculated for each group.





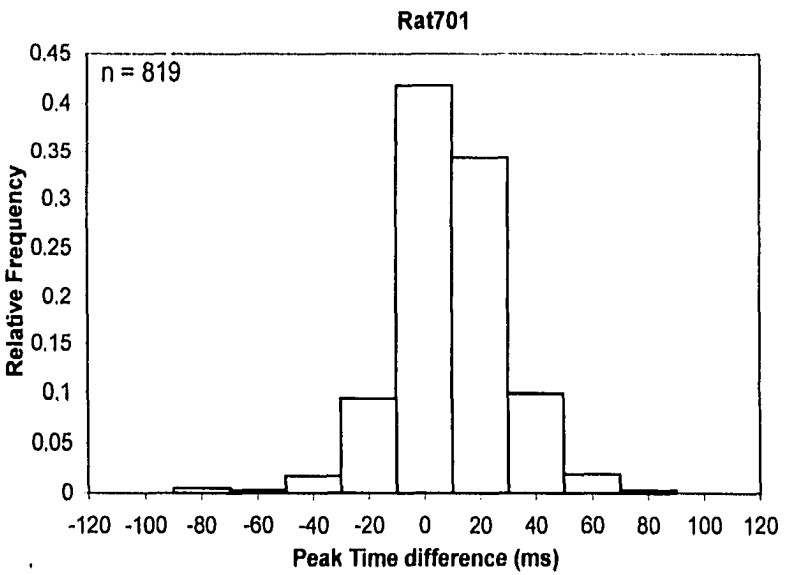


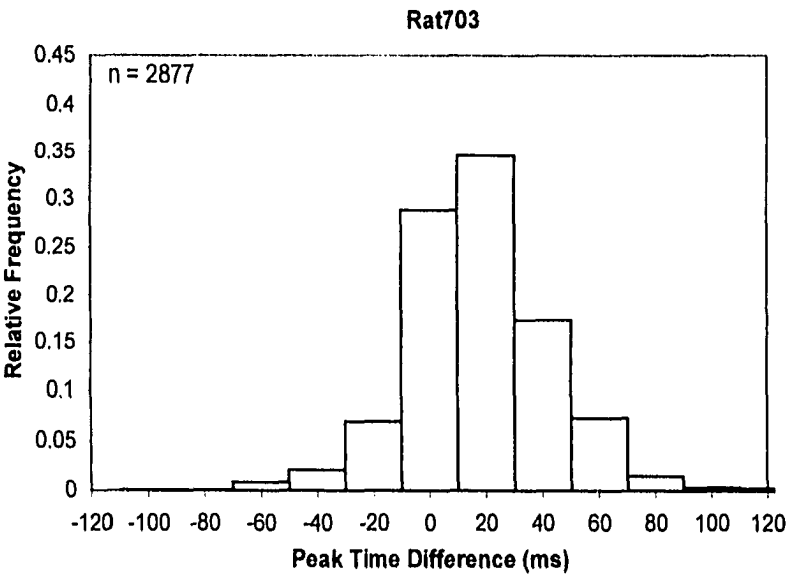


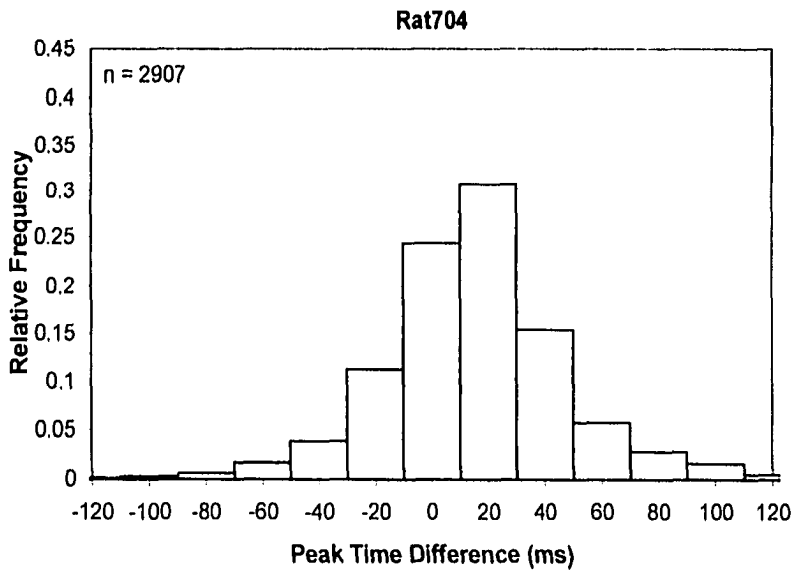
Bilateral coordination of whisking. In Figure 12 coordination is assessed by calculating the time differences between the occurrence of peak protraction in the right and left whiskers. For all four subjects shown, the distributions are centered about zero with small and similar variability. Figure 13 examines phase relationships among whisking rhythms on the two sides of the face in four subjects using cross-correlational methods. For all subjects, crosscorrelograms have a main peak centered about zero with a highly significant correlation value (from +0.5 to +0.65, $p < 0.001$) though there is a slight lag in some subjects (< 10 ms, Lag—showing in the Figure 13 as the main peak does not locate exactly at zero). Crosscorrelograms also show regular repetitions that are quite symmetrical towards both lag directions, and with peaks at about 150 ms—which corresponds to the dominant whisking frequency (5-7 Hz).

Rhythmicity. This is an important property of the whisking system and we have used two different methods of analysis. The first, based upon an analysis of individual whisk durations (Figure 14), indicates that whisking occurred over a range from 3 to 20 Hz, with a mode between 5-7 Hz. Comparable results were obtained using the Finite Fourier transformation (FFT). Power spectra for four subjects are given in Figure 15.

Figure 12. Bilateral coordination of whisking in homologous (R and L C-1) whiskers as measured by the time difference in the occurrence of peak protraction. Frequency distributions of time differences for four normal subjects shown.







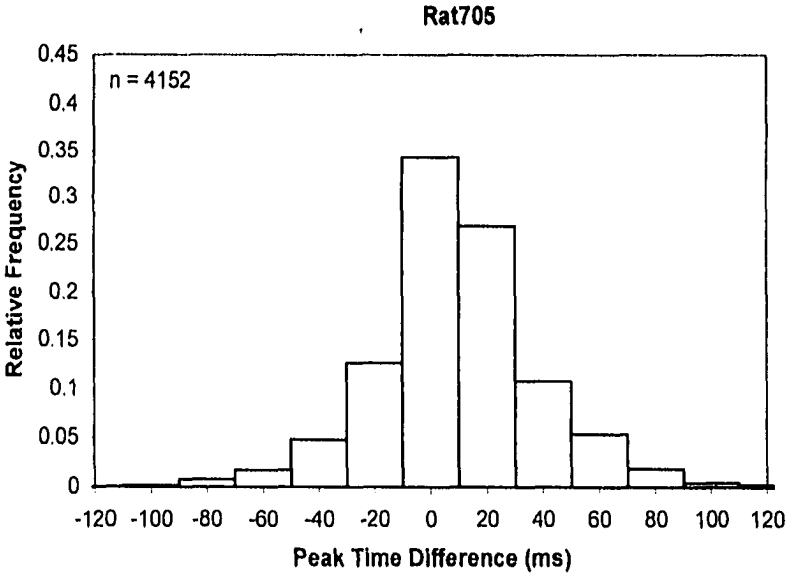


Figure 13. Bilateral coordination of homologous whiskers measured using a cross correlation procedure. The correlograms are based upon data on the phase relationships between the trajectories of R and L whisker movements,. The position of the central peak on the vertical axis indicates the magnitude of the correlation. The position of the secondary peaks along the horizontal axis reflects the dominant whisking frequency (5-6 Hz).

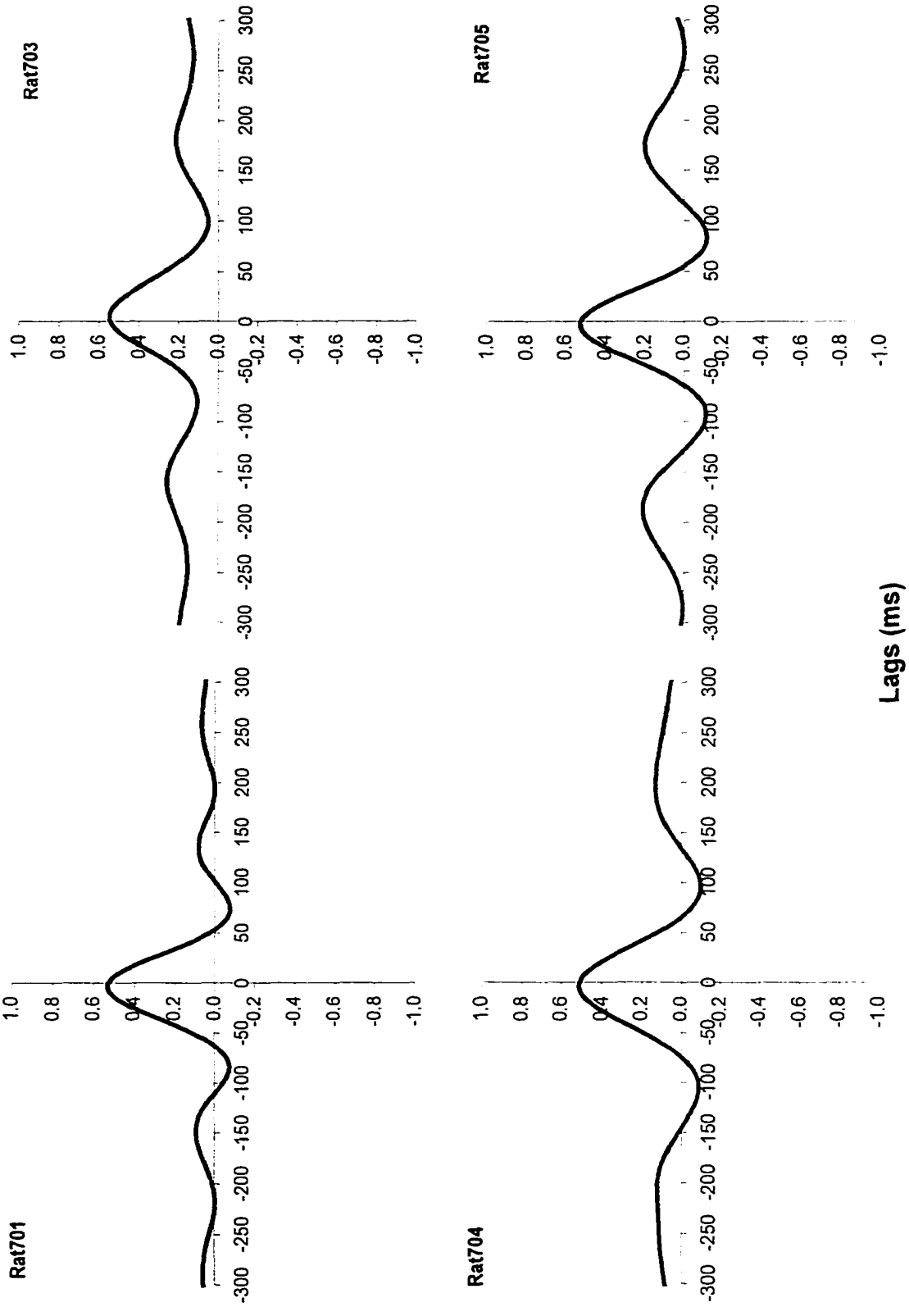


Figure 14. Calculation of whisking frequency based upon direct measurement of individual whisking cycle durations. Data for four normal subjects shown.

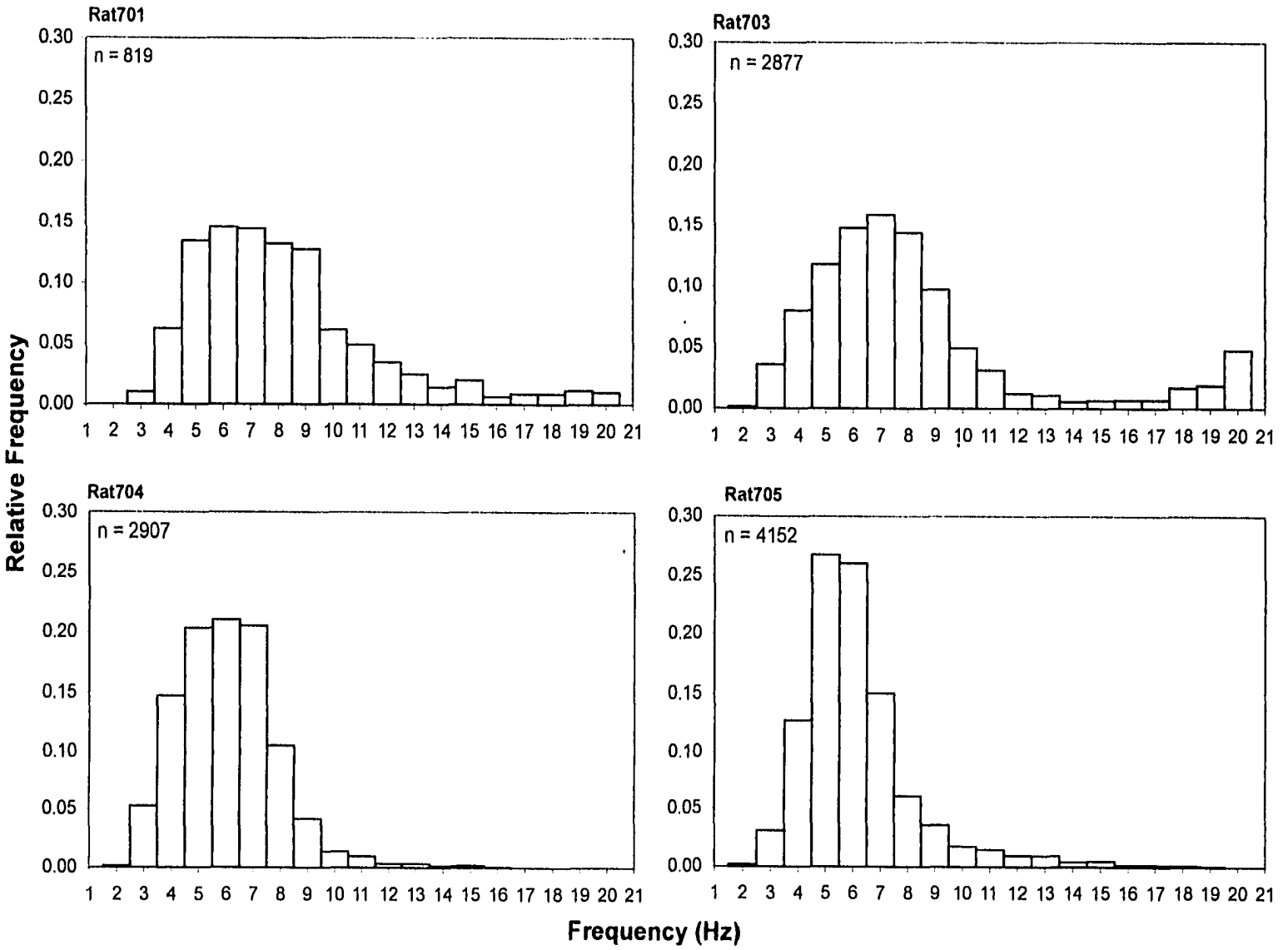
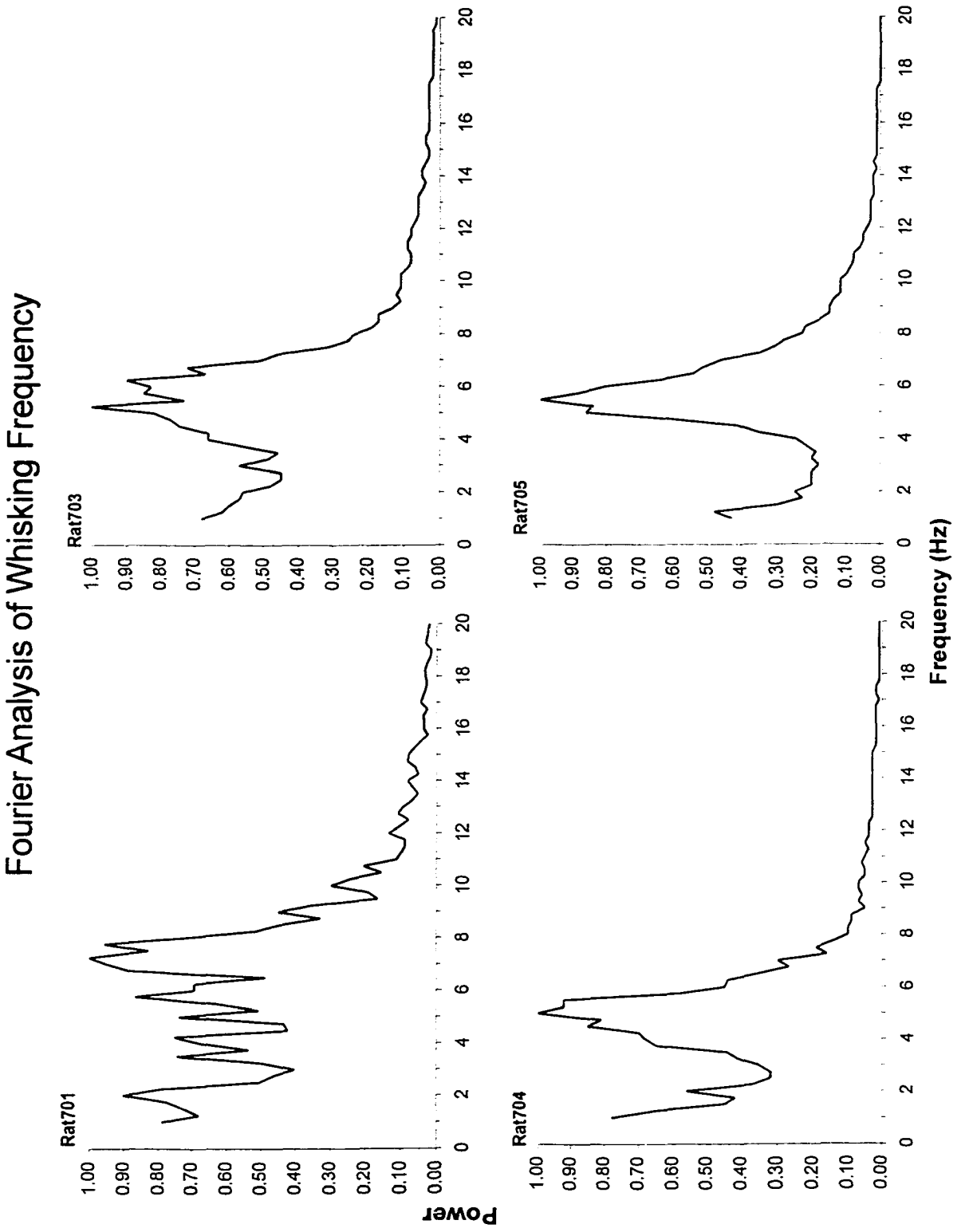


Figure 15. Calculation of whisking frequency based upon Fourier analysis. Power spectra of whisking frequencies for the four subjects of Figure 12.



Effects of repeated testing. The analysis presented in Figure 16 and 17 examines the effects upon several whisking parameters (number of whisks, protraction amplitude, duration of whisking bursts), of repeated exposure to the test situation within and between test sessions. The results presented are based upon the data from the four sham-operated rats. Repeated-measures *t* test was used for statistical analysis. Although the group data do not achieve statistical significance on all three measures in the intersession comparison (Number of whisks per session: $t(3) = 1.58$, $p > 0.05$; Amplitude: $t(3) = 0.26$, $p > 0.05$; Burst duration: $t(3) = 3.12$, $p = 0.052$), all four subjects show reduction in the mean burst duration over the repeated testing and the probability of differences tested is very close to significance ($p = 0.052$). On the other hand, significant reductions in the amount of whisking (number of whisks) are evident in the intra-session data (Figure 17) ($t(3) = 6.61$, $p < 0.01$). This reflects primarily a reduction in the duration of whisking bursts, and an increase in periods of inactivity ($t(3) = 5.80$, $p < 0.02$). No effects on protraction amplitude were found ($t(3) = 0.10$, $p > 0.05$).

Figure 16. Effects of repeated testing upon whisking activity (Intersession effects). The figure plots changes in number of whisks, mean whisking amplitude and burst duration for four sham-operated subjects.

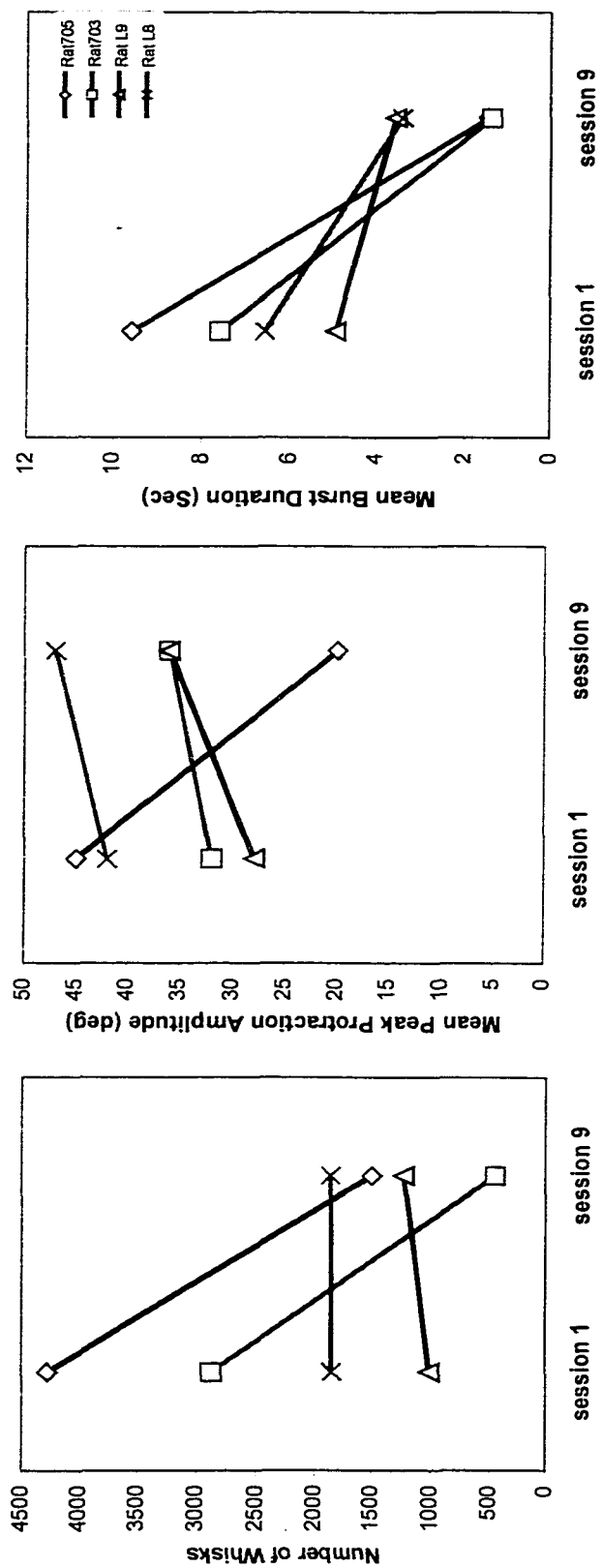
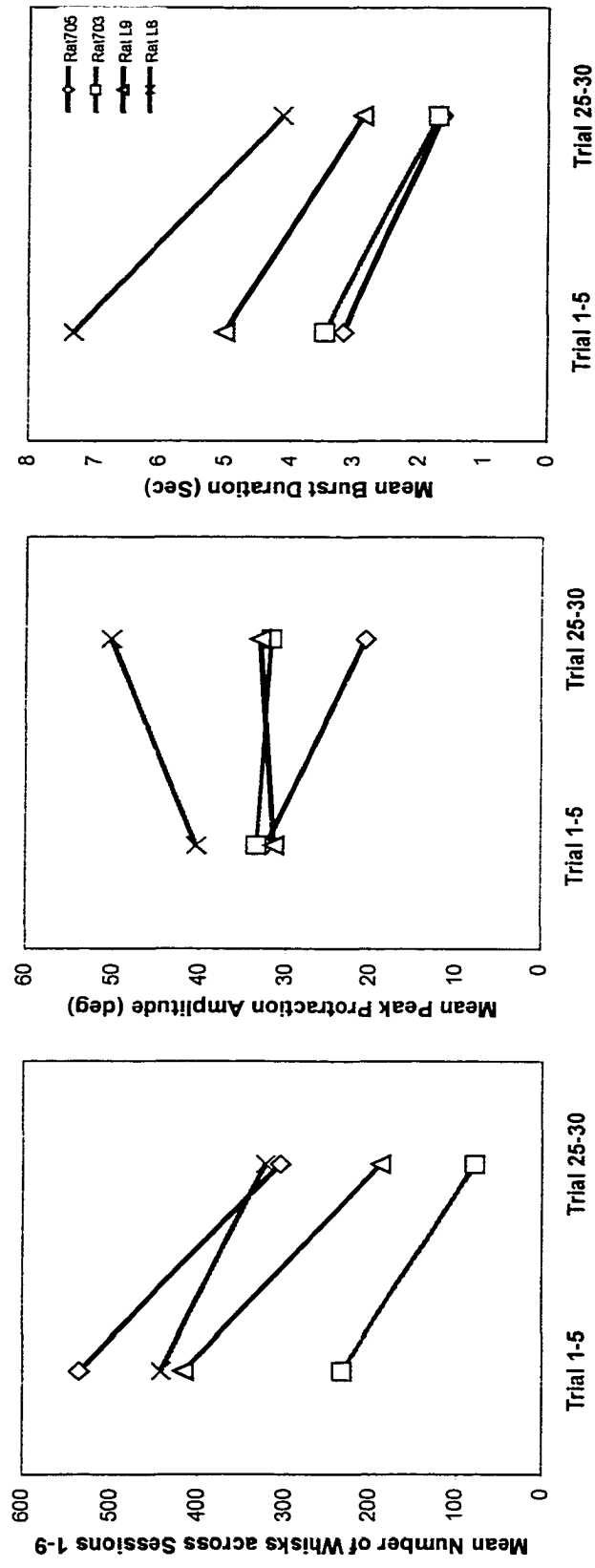


Figure 17. Effects of repeated testing upon whisking activity (Intrasession effects). Subjects and measures as in Figure 14. Data were grouped into 5-trial bins and the medians from each bin were averaged across the 9 test sessions.



Part II. Effects of vibrissal deafferentation upon whisking patterns.

Figure 18 presents, for a single representative subject, a sample of whisking activity during the first preoperative test session and during the first test sessions following sequential deafferentation of the vibrissa. This figure indicates (a) that whisking behavior is not abolished, either after unilateral or bilateral deafferentation, (b) that its topography and kinematics are essentially unchanged and that its rhythmicity is preserved. However, unilateral (but not bilateral) deafferentation appears to produce a substantial increase in whisking frequency. A more detailed analysis of whisking kinematics and coordination provided support for this conclusion.

Whisking Kinematics and amplitude scaling. Figure 19 compares mean protraction amplitudes in deafferented and sham-operated subjects. No systematic trends are seen in either group before and after sequential surgeries (deafferentation or sham) (Session 1—intact; Session 4—unilaterally deafferented or sham; Session 7—bilaterally deafferented or sham). Statistical analyses show no significant differences in either group before and after surgeries, and between the two groups (Repeated-measures ANOVA; Deafferented group: $F(2, 8) = 2.08$, $p > 0.05$; Sham group: $F(2, 6) = 0.02$, $p > 0.05$; between groups: $F(1, 12) = 0.87$, $p > 0.05$). Essential similar results were found for the velocity measures. (Figure 20; Statistics are not shown.).

Figure 18. Effects of trigeminal deafferentation upon whisking behavior. A representative sample of whisking movements of the Right C-1 whisker recorded preoperatively (Top), and after sequential section of the ipsilateral (Middle) and contralateral (Bottom) infraorbital nerve. Conventions as in Figure 4.

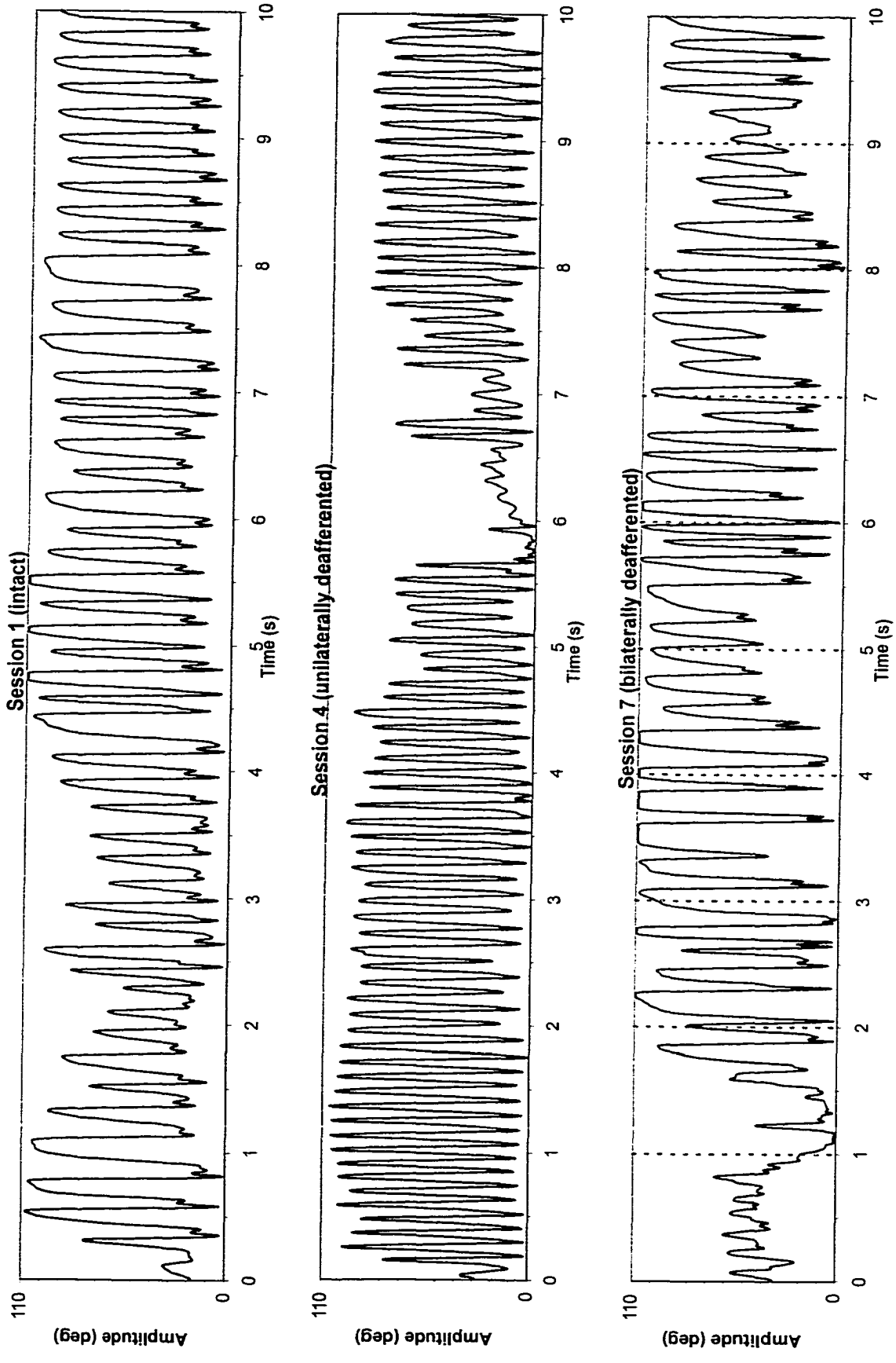


Figure 19. Effects of sequential deafferentation upon whisking amplitudes. The right panel graph shows the mean protraction amplitudes for four subjects preoperatively (Session1) and after the first (Session 4) and second (Session 7) deafferentations. Data from four sham subjects are presented in the left panel for comparison. Error bars denote the standard error of the means.

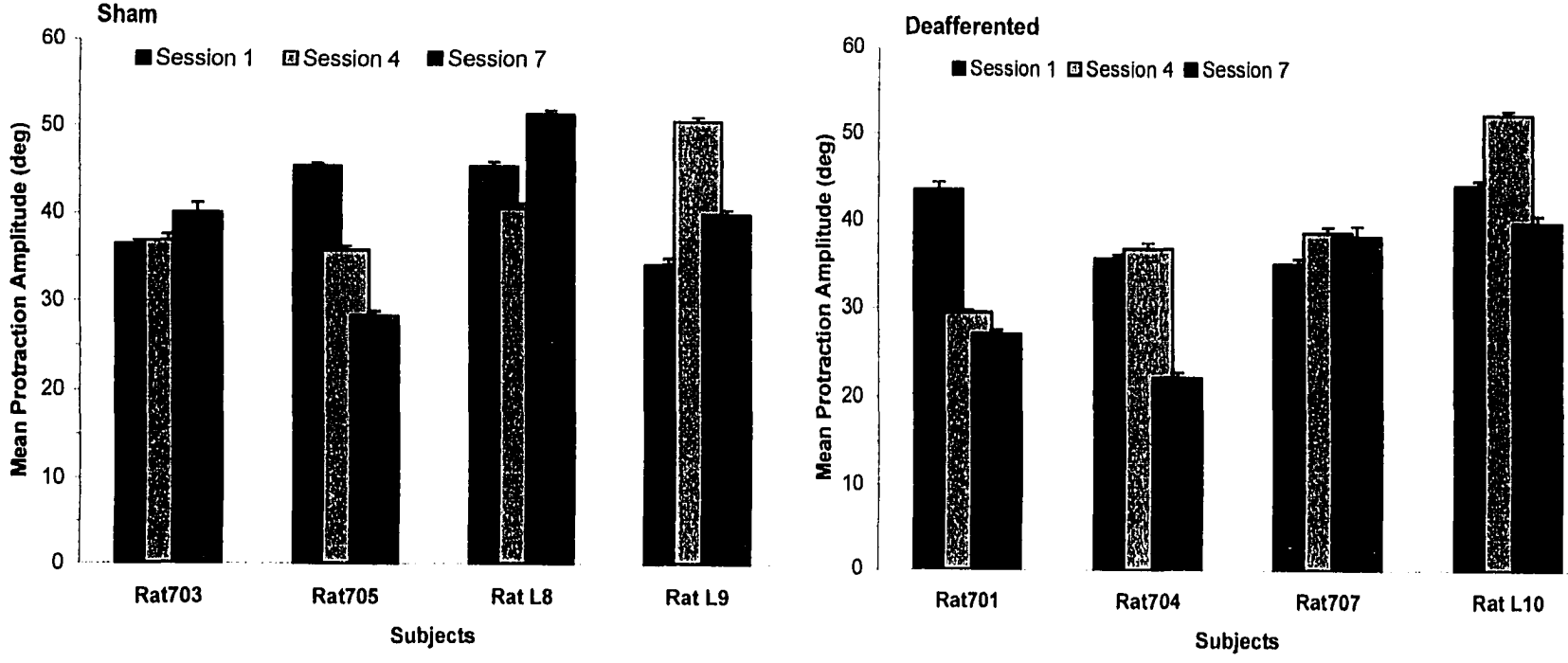
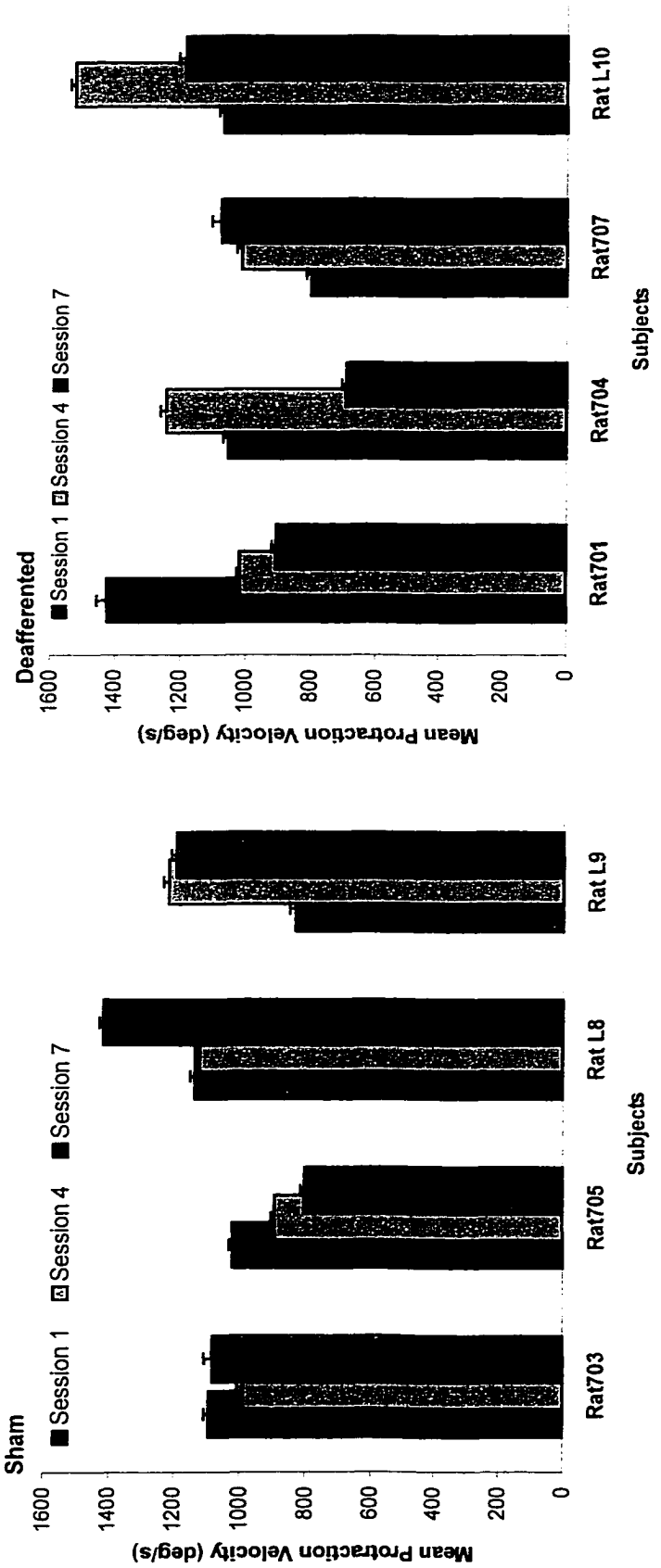


Figure 20. Effects of sequential deafferentation upon whisking velocity. Presentation and conventions as in Figure 19.



Analysis of amplitude scaling mechanisms indicates that deafferented subjects continue to scale amplitude by varying protraction velocity (Figure 21). Multiple regression analyses were done to examine the contributions of peak protraction velocity and rise time to the amplitude scaling in deafferented and sham subjects before and after surgeries. The results are given in Tables 3 and 4. The results are also illustrated in Figure 22 and Figure 23, correspondingly. Statistical analyses based on the derivative data given the tables (percentages of variance accounted by velocity and rise time in each animal) show that no significant change in the contribution of peak protraction velocity to the protraction amplitude scaling is found in either group before and after surgeries (Repeated-measures ANOVA; Deafferented group: $F(2, 8) = 0.44$, $p > 0.05$; Sham group: $F(2, 6) = 1.98$, $p > 0.05$). Both groups show the increasing involvement of rise time in the scaling of amplitude (Deafferented group: $F(2, 8) = 5.78$, $p < 0.05$; Sham group: $F(2, 6) = 6.25$, $p < 0.05$). The overall amplitude scaling by the combination of protraction velocity and rise time after the sequential deafferentation is not significantly different from that of intact ($F(2, 8) = 3.18$, $p > 0.05$). Also, no change is found after sequential sham operations ($F(2, 6) = 0.14$, $p > 0.05$).

Figure 21. Effects of deafferentation upon the scaling of protraction amplitude. Data for a single sham-operated subject (L-8) and three experimental animals are presented. Upper panels are preoperative data, middle and bottom panels reflect data for the unilateral (Session 4) and bilateral (Session 7) conditions, respectively.

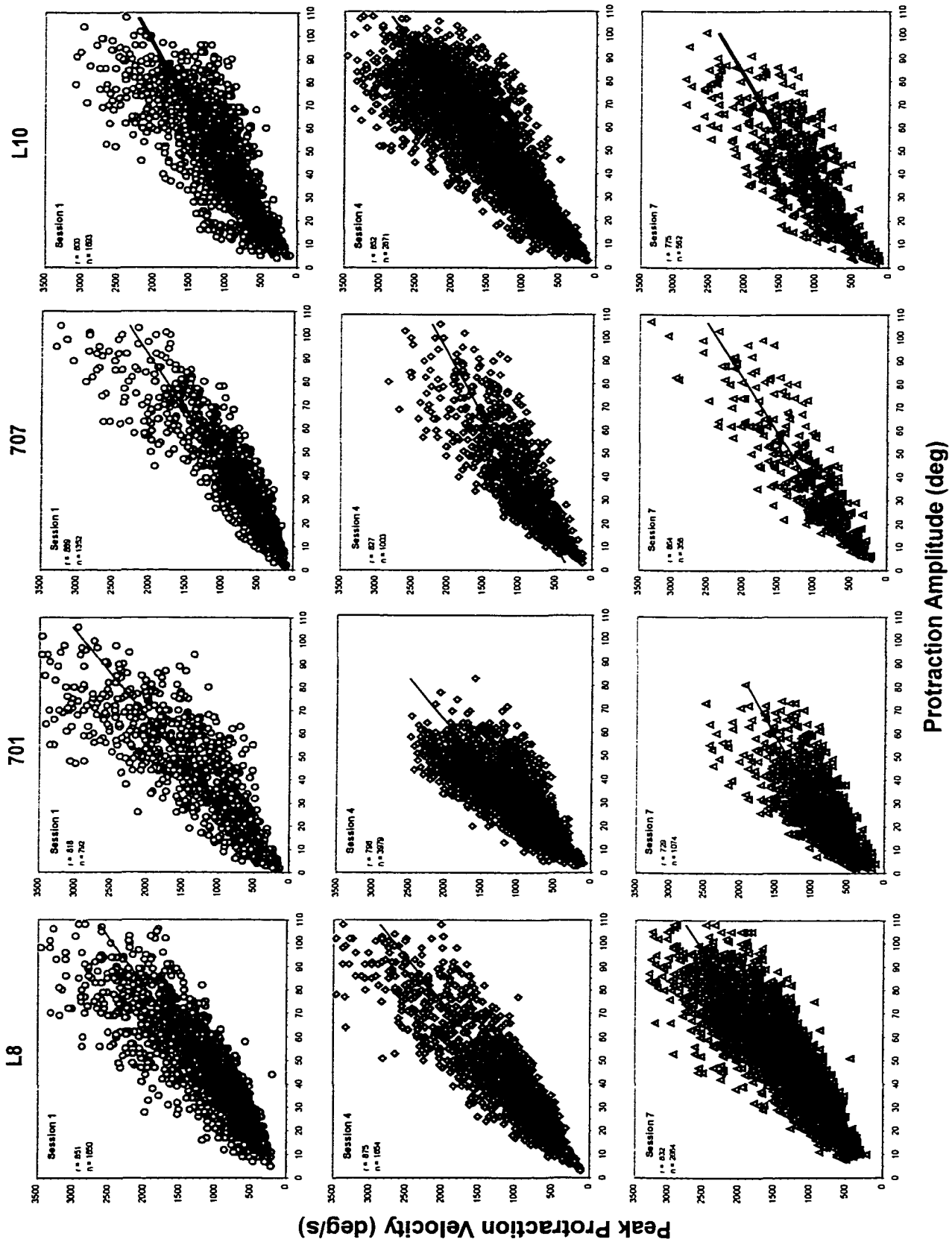
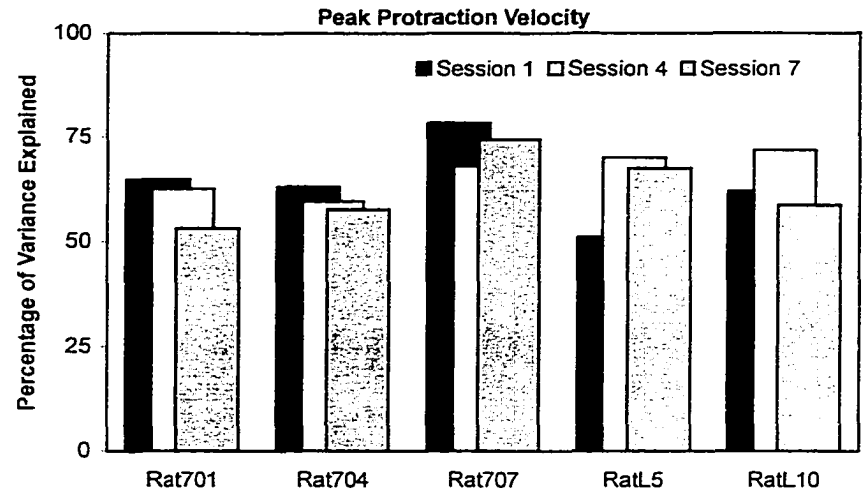
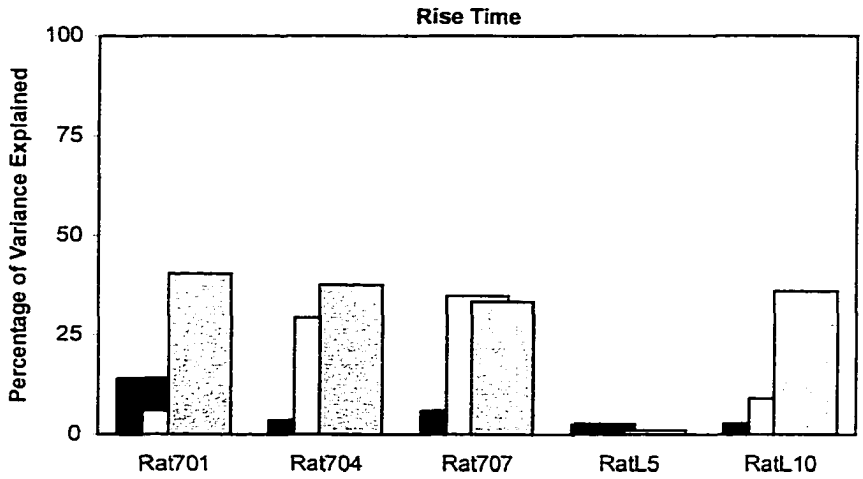


Figure 22. Effect of deafferentation upon amplitude scaling. Proportion of variance in peak protraction accounted for by peak protraction velocity (upper graph), by rise time (middle graph), and by combined effect from both peak protraction velocity and rise time (lower graph) before and after sequential infraorbital nerve section. Data for this Figure are given in Table 3.





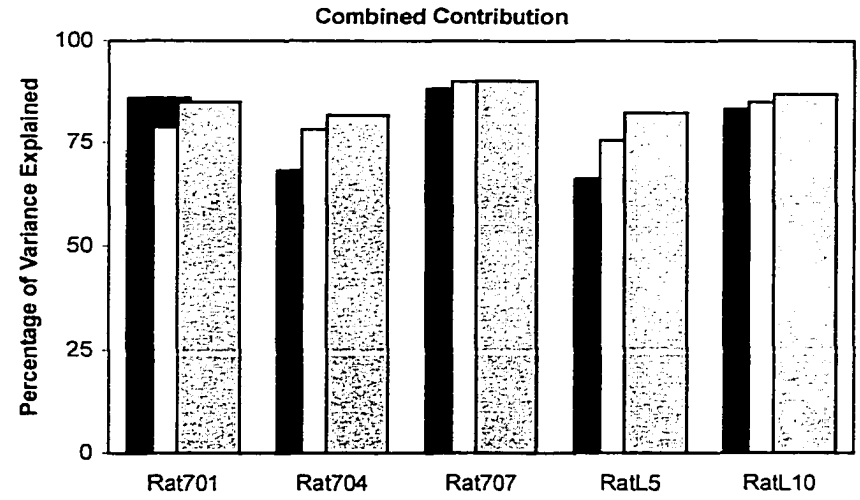
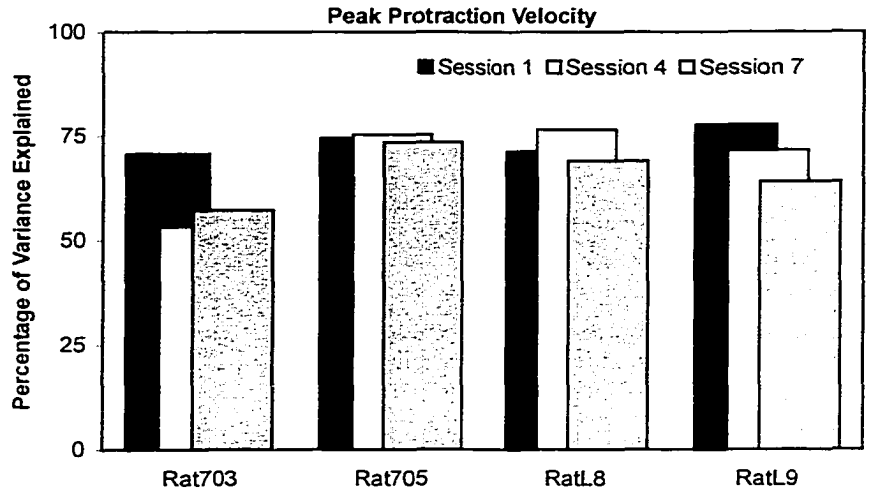
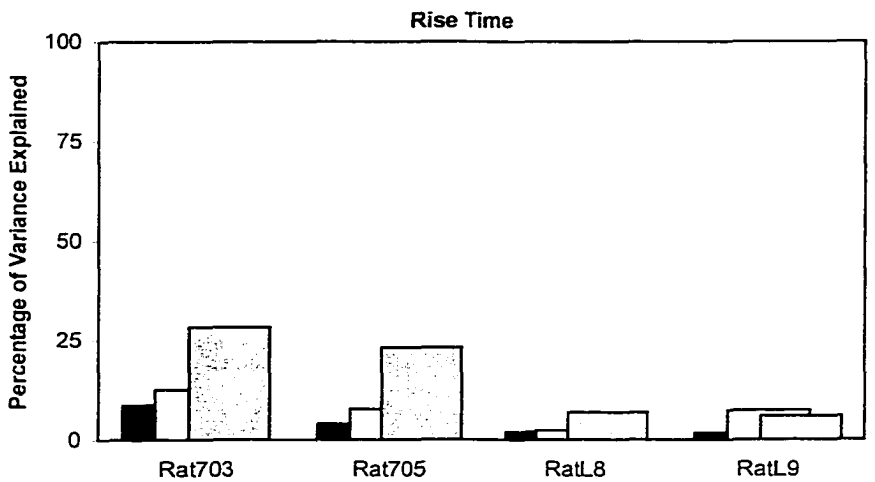


Figure 23. Effect of sham operation upon amplitude scaling. Proportion of variance in peak protraction accounted for by peak protraction velocity (upper graph), by rise time (middle graph), and by combined effect from both peak protraction velocity and rise time (lower graph) before and after sequential sham operations. Data for this Figure are given in Table 4.





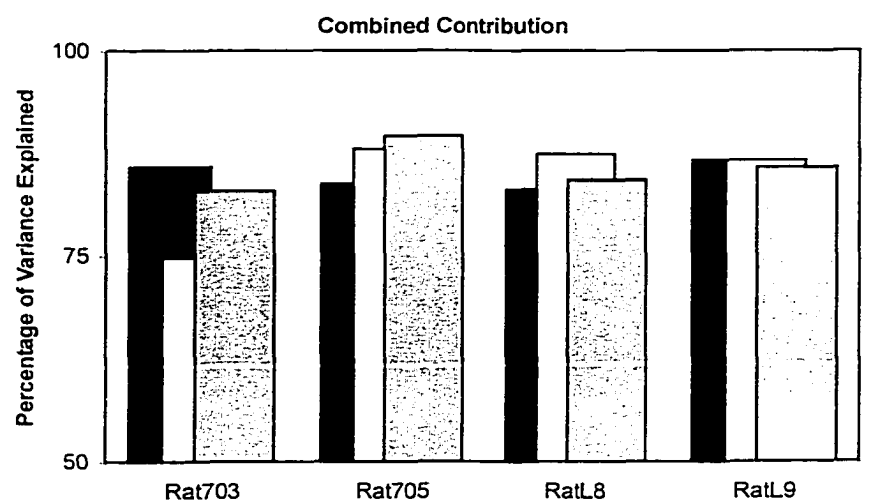


Table 4.

Effects of sequential deafferentation on the proportion of variance in peak protraction amplitude accounted for by peak protraction velocity ($r^2_{A.\ V}$), rise time to peak protraction amplitude ($r^2_{A.\ RT}$), and the combination of both velocity and rise time ($R^2_{A.\ V,\ RT}$). Only results for the right C-1 whisker are shown. The upper numbers in the cells are the results from Session 1 (intact), middle ones are from Session 4 (bold face, unilaterally deafferented—right side), and lower ones (italics) are for Session 7 (bilaterally deafferented. For all the results presented in the table, their corresponding F values are statistically significant ($p < .0001$) except otherwise labeled (n. s: not significant, **: $p < .001$).

Regression	Rat701	Rat704	Rat707	RatL5	RatL10
$R^2_{A.\ V}$	.649 **.626** *.531*	.631 **.596** *.576*	.783 **.682** *.745*	.51 **.701** *.676*	.62 **.719** *.586*
$R^2_{A.\ RT}$	.139 **.059** *.404*	.035 **.293** *.376*	.058 **.348** *.333*	.025 **.008 (n. s)** *.009**	.026 **.089** *.36*
$R^2_{A.\ V,\ RT}$	.859 **.789** *.85*	.684 **.782** *.818*	.882 **.90** *.902*	.663 **.756** *.824*	.833 **.85** *.869*

Table 5.

Effects of sequential sham surgeries on the proportion of variance in peak protraction amplitude accounted for by peak protraction velocity ($r^2_{A.\ V}$), rise time to peak protraction amplitude ($r^2_{A.\ RT}$), and the combination of both velocity and rise time ($R^2_{A.\ V,\ RT}$). Only results for the right C-1 whisker are shown. The upper numbers in the cells are the results from Session 1 (intact), middle ones are from Session 4 (bold face, unilaterally sham operated—right side), and lower ones (italics) are for Session 7 (bilateral sham). For all the results presented in the table, their corresponding F values are statistically significant ($p < .0001$) except otherwise labeled (n. s: not significant, **: $p < .001$).

Regression	Rat703	Rat705	RatL8	RatL9
$r^2_{A.\ V}$	.707 **.533** *.571*	.744 **.753** *.735*	.713 **.766** *.69*	.777 **.716** *.641*
$r^2_{A.\ RT}$	.086 **.124** *.283*	.039 **.077** *.231*	.018 **.024** *.069*	.017 **.073** *.006*
$R^2_{A.\ V,\ RT}$	.857 **.748** *.829*	.837 **.879** *.895*	.83 **.873** *.842*	.865 **.865** ***.857***

Rhythmicity. Figure 24 and 25 presents spectral plots of whisking frequency for sham-operated and deafferented animals, respectively. The plots present data for the first preoperative session and the sessions following unilateral (Session 4) and Bilateral (Session 7) deafferentation. Peak power spectrum frequencies (PPSF) were extracted for each subject before and after surgeries and used for repeated-measure ANOVA. There are no significant differences in whisking frequency after sham surgery, and all four animals showing peaks in the 5-7 Hz range ($F(2, 6) = 0.22$, $p > 0.05$). After unilateral deafferentation, four (shown here) of the five deafferented subjects show a shift in their modal whisking frequency towards the 8-10 Hz range (middle row), which is statistically significant ($F(2, 8) = 8.98$, $p < 0.01$). In three of the four subjects, no such shift is evident after bilateral deafferentation. *Post hoc* test shows that whisking frequency is significantly higher in unilaterally deafferented animals, than in either intact or bilaterally deafferented animals. No such difference was found between intact and bilaterally deafferented subjects.

Bilateral Coordination of Whisking. Figure 26 plots whisking coordination calculated as the time differences between the occurrence of peak protraction in the right and left whiskers. It is clear that the neither unilateral nor bilateral deafferentation disrupt the synchrony of bilaterally homologous whiskers. The cross-correlograms presented in Figure 27 indicate that the phase relations among whisking movements on the two sides of the face are not substantially disrupted. Correlation values remain significant and high ($p < 0.001$), but in three of the experimental animals they are lower in the unilateral than in the intact

condition. However, crosscorrelograms exhibit increased regularity after unilateral deafferentation, which suggests a reduction in the range of whisking frequencies.

Figure 24. Effects of sham surgical procedures upon whisking frequency. Fourier power spectra of whisking behavior before and after sequential sham operations.

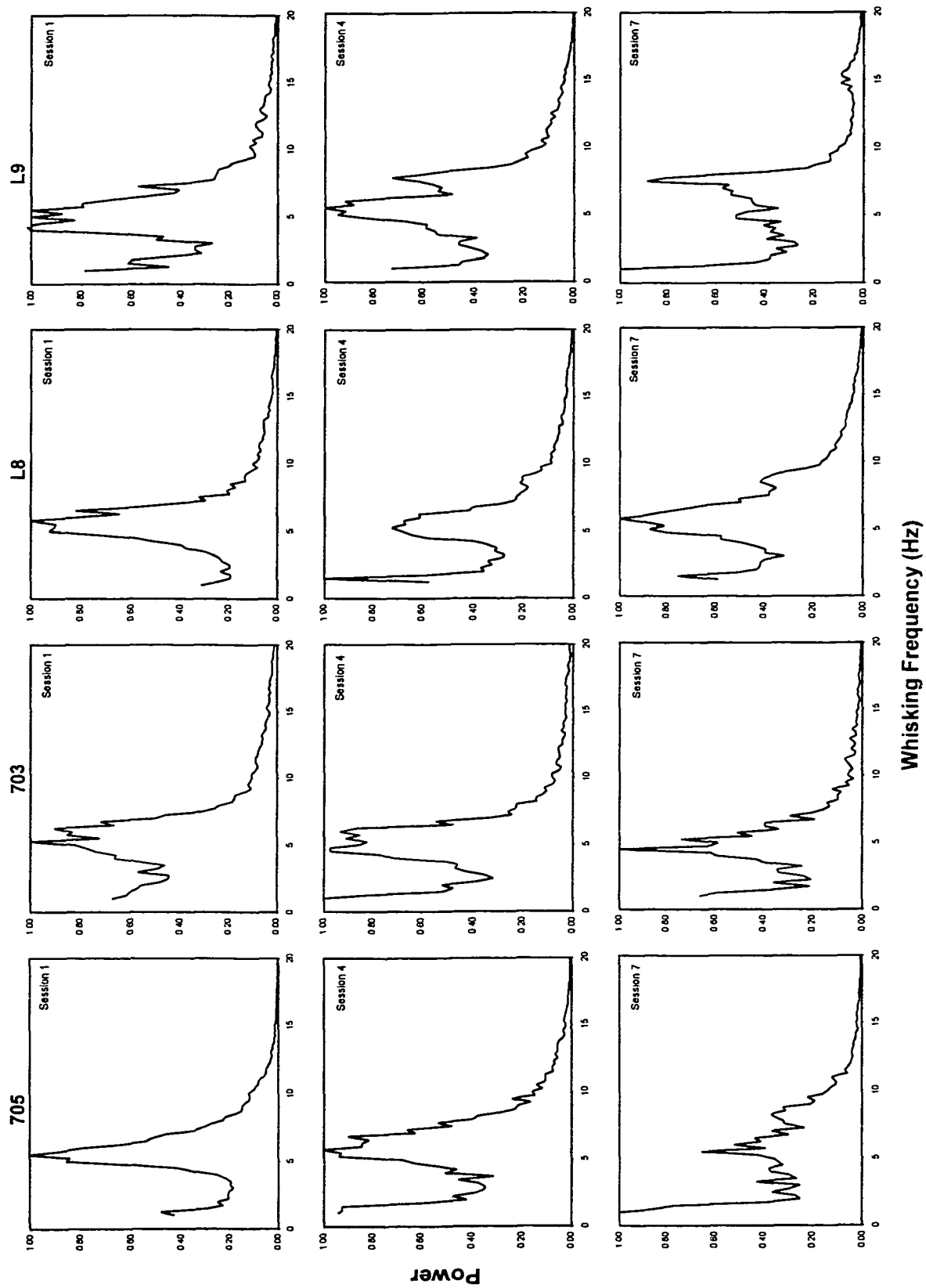


Figure 25. Effects of sequential whisker deafferentation upon whisking frequency. Fourier power spectra of whisking behavior preoperatively and in the unilateral and bilateral conditions.

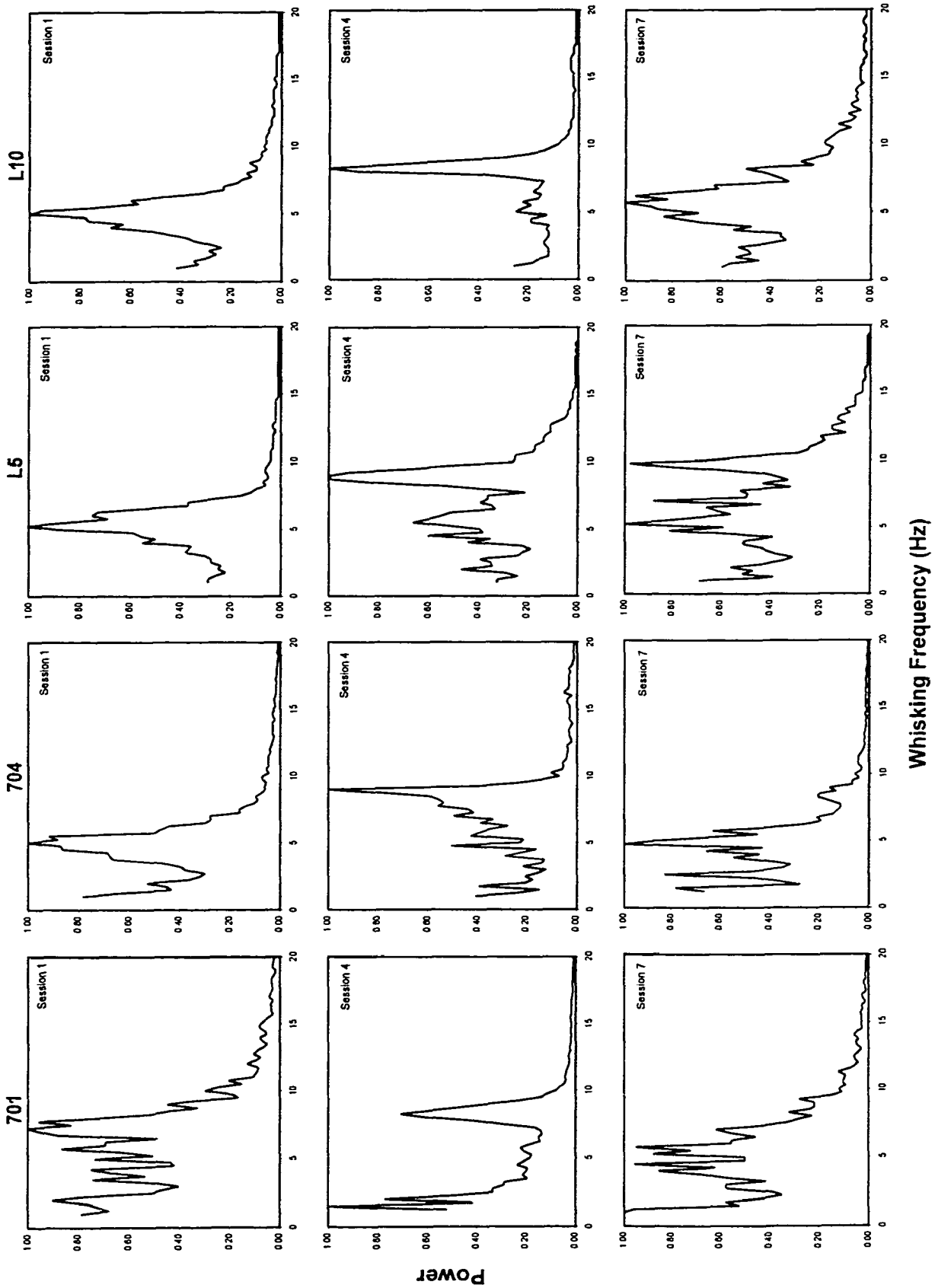


Figure 26. Deafferentation effects upon bilateral coordination of whisking as measured by the time difference in the occurrence of peak protraction in homologous (R and L C-1) whiskers. Frequency distribution of time differences for four deafferented subjects (Session 1, preoperative; session 4, unilateral condition; session 7, bilateral condition).

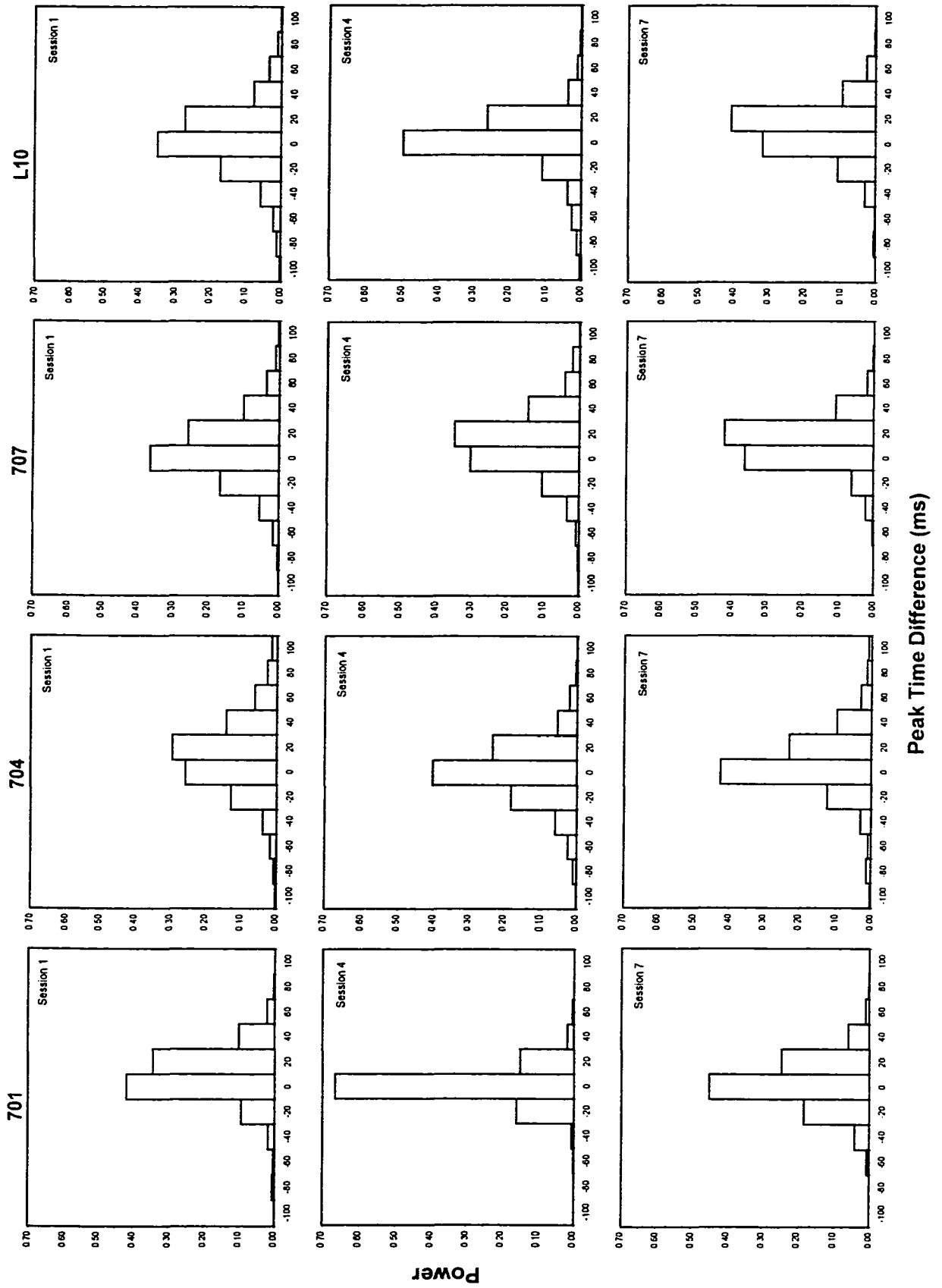
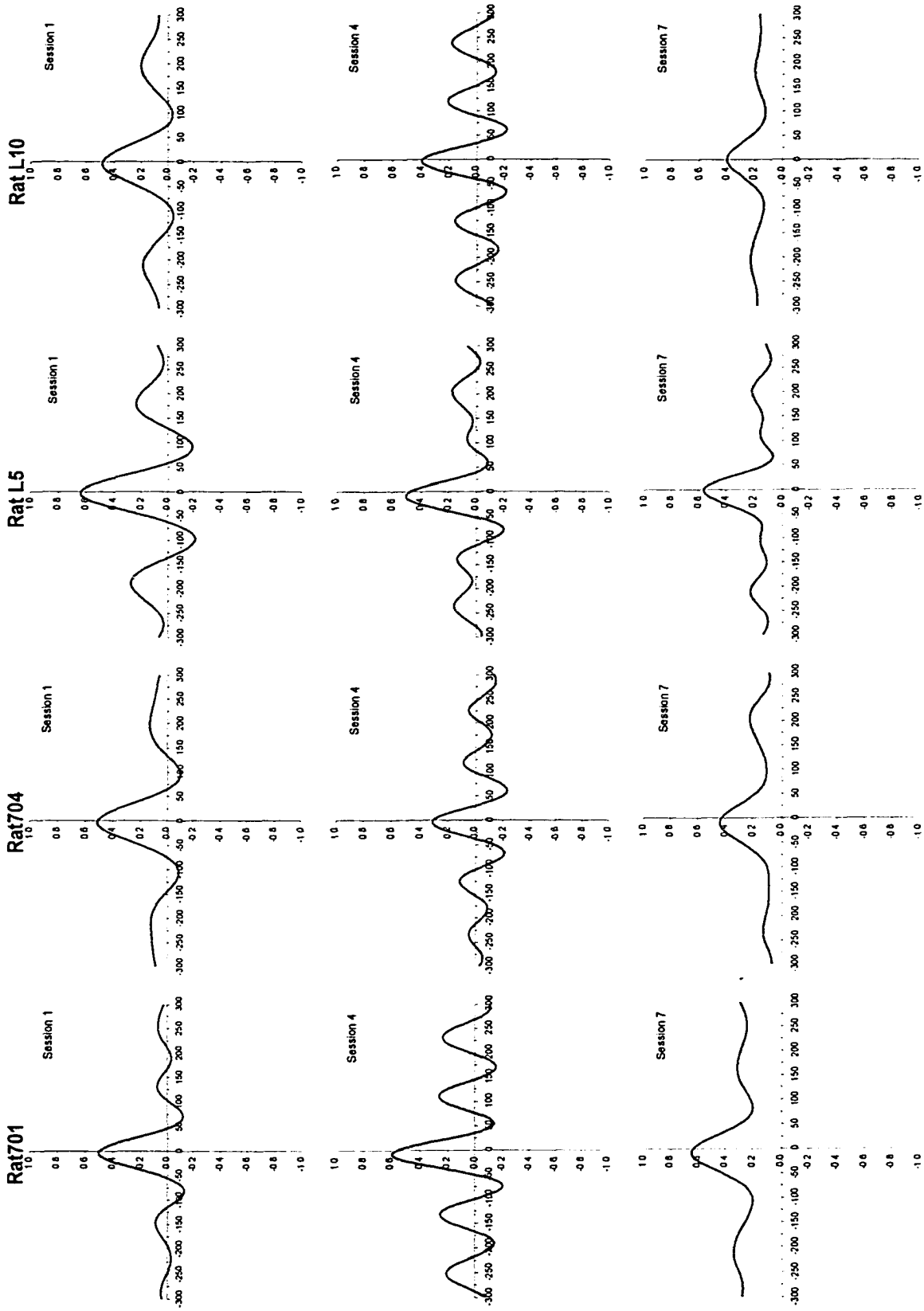


Figure 27. Effects of sequential deafferentation upon bilateral coordination of whisking as measured by phase relationships using a cross correlation procedure (Session 1, preoperative; session 4, unilateral condition; session 7, bilateral condition).



Discussion

The rodent whiskers function as "mobile sensors". Even in the absence of explicit stimuli to the whiskers, the rat emits bursts of rhythmic whisking (protraction/retraction) at a characteristic frequency. My dissertation had two aims: First, to provide data on the kinematics, rhythmicity and bilateral coordination of whisking in normal rats, and second, to assess the contribution of afferent input from the whiskers themselves on the generation and spatio-temporal organization of whisking patterns. To accomplish the first aim I monitored whisking in head-fixed animals, using optoelectronic methods to acquire a very large population of whisking movements, and characterize whisking patterns with high spatio-temporal resolution. To accomplish the second aim, we compared the effects of sequential trigeminal deafferentation of the whiskers mystacial pad with those of sham surgical controls. The employment of head-fixation not only eliminates intrusion of other inputs produced by head movements, it also allows one to monitor bilateral whisker movements simultaneously and to eliminate tactile inputs by monitoring whisking in air. The infraorbital branch of the trigeminal nerve provides the only sensory input to the whisker region, and no afferents travel in the motor nerve.

Part I: Kinematic analysis.

The two previous studies of whisking biometrics (Welker, 1964; and Carvell and Simons, 1990) employed either video-or cinematography. In the first study, rats were freely exploring objects in their home cage; in the second, rats

were engaged in tactile (texture) discrimination on an elevated jump stand. Thus the studies had no control of head movements and the motion analysis had a relatively low spatio-temporal resolution relative to the frequency and amplitude of whisking movement. Moreover, in neither was the whisking pattern recorded under conditions where no tactile inputs were present.

Despite these differences in method and behavioral context, much of our data is consistent with the findings of earlier workers. We have found that protractions made by homologous whiskers on the right and left sides of the snout have highly similar amplitudes and velocities. The mean whisking velocities presented in the current study (Table 1) are higher than those given in the Carvell and Simons' (1990, Table 1) (1020 °/s vs 748 °/s). These differences may reflect differences in the peak amplitudes recorded in the two studies (31.6° vs 39.6°), as well as the fact that I used a peak velocity measure while in Carvell and Simons' study velocity was estimated between two neighboring video-fields and then averaged across fields for protraction and retraction movements. Note, again, that Carvell and Simon's animals were often in contact with discriminanda, resulting in smaller whisk amplitudes. As in previous studies, we found that whisking movements on the two sides of the face show a high degree of coordination, although fine grain analysis of movement records indicates that individual whisks are frequently not in synchrony.

Our data is also consistent with previous morphological (Dorfl, 1982) and electrophysiological studies suggesting that suggested that protraction is the only active process during whisking. That is, protraction of whisking is actively driven

by the follicular muscles and retraction is just the rebound of the deep connecting tissue of vibrissal pad. An electromyographic study of whisking (Carvell and Simons, 1991) found (1) that the magnitude of EMG activity of mystacial pad musculature increased during protraction, (2) that little activity was present during retraction, and (3) that EMG level is highly correlated with the whisker position. In the current study, detailed analysis of the protraction and retraction phases of whisks indicates that mean retraction time is quite constant over a wide range of whisk durations (the indicator of the whisking frequencies). Further examination of the relationship between the retraction velocity and amplitude shows that the retraction velocity increases almost perfectly with the retraction amplitude. This finding is consistent with the notion described above that the retraction is mainly a passive, elastic rebound of the deep connecting tissues of the vibrissae. On the other hand, the protraction movement time increases almost linearly as the whisk duration increases. It suggests that the regulation of whisking frequency is an active process, which is mainly determined by the regulation of protraction time. On average, retraction is faster than protraction. Consistent with the observation by Carvell and Simons (1990), I found, on average, that protractions account for 60% and retractions 40% of the complete cycle (Table 1). However, at higher frequencies (above 13 Hz), protraction time is shorter than retraction time. Given the evidence provided above, it suggests that protraction during whisking is an actively produced by the contraction of follicular muscles and whisking frequency is actively regulated by the protraction process. The speed of elastic rebound of vibrissal connection tissue may determine the upper limit of whisking frequency.

Accurate scaling of the magnitude of a movement (amplitude) to a required task is an important feature of motor control mechanisms. The initial phase of such scaling of many movements is thought to involve the generation of an output pulse in the motoneurons that in turn causes the contractions in the relevant muscles. Rapid, targeted movements are presumably independent of peripheral feedback and their control pulses are determined prior to the initiation of the movements. The model of amplitude scaling in humans was formulated by Gordon and Ghez (1987). In their model, they contrasted "pulse-height" (velocity) vs "pulse-width" (rise time) motor control strategies. They compared the scaling of isometric force trajectories generated by human elbow flexor muscles under "accurate" and "fast" conditions. Under "fast" condition, both velocity and rise time involved, while under "accurate" condition, rise time was relatively constant. These observations suggest that motor control strategies used may vary with the functional requirements of a given task.

In the present study, animals were whisking in air, with no requirement of task accuracy. Kinematic analysis of whisker movements shows that protraction amplitude is highly and consistently correlated with peak protraction velocity. In contrast, evidence for the amplitude scaling due to rise time neither as clear nor as consistent. Though the correlation coefficients are statistically significant across most subjects due to the extreme large sample sizes, scatter plots of protraction amplitude and rise time indicate no obvious trend in increments or decrements of rise time as protraction amplitude increases. There may not be a linear relationship between protraction amplitude and rise time. It is clear from

the regression analyses that the peak protraction velocity accounts for most variance in amplitude scaling. This notation is further supported when individual whisk trajectories are examined (See Figure 10). Individual whisks with the same duration (same frequency), but different peak amplitudes show different peak protraction velocity, while rise time keeps about the same among all the individual whisks. Also, the increments of peak protraction velocity are quite regular as amplitude increases. In contrast, the individual whisks with the same protraction amplitude but with different durations (frequencies) show highly similar peak protraction velocity and slow down when reaching the peak. This "slow-down" phase is more noticeable as the whisk duration gets longer. It is not clear at this moment how the "slow-down" phase is produced in some whisks since all the whisks seem to having a similar initiation. On the other hand, the retraction phases are strikingly similar in all whisks, simple and with almost the same time duration. In the case of whisks with the same duration but different amplitudes, the retractions terminate with different peak velocity. In the case of whisks with the same amplitude but different durations, the retractions terminate with almost identical velocity (also see the scatterplots in Figure 6). This, again, confirms to the anatomical and electrophysiological evidence (Dorfl, 1982; Carvell and Simons, 1991) that retraction of whiskers is the passive, elastic process.

As noted above, there may not be a simple linear relationship between amplitude and rise time. More detailed analysis, which regroups the data according to the different protraction amplitudes, suggests that there may be two

(or more?) processes in the scaling of protraction amplitude (see Figure 11). In most of the subjects, the relationship between protraction amplitude and rise time is positive when protraction amplitude is small. This positive relationship turns to negative one as the amplitude increases, up to some point. It seems that there is a turning point and it differs among subjects. This change in relationship is observed correspondingly in the relationship between amplitude and velocity. When the relation between amplitude and rise time becomes negative, there seems a compensatory process in protraction velocity; that is, the sudden upshift in protraction velocity as protraction amplitude increases. Regression analysis suggested that there was corresponding increase in the contribution of peak protraction velocity in the overall amplitude scaling when protraction amplitude was getting larger. In contrast, the contribution of rise time in the amplitude scaling decreased while protraction amplitude became larger. In general, however, there were more variations in both peak protraction velocity and rise time as protraction amplitude became larger (the correlations between protraction amplitude and peak protraction velocity and between protraction amplitude and rise time decreased.). Therefore, it is not sure from the current study whether there is an absolute increment in the peak protraction velocity in amplitude scaling, though the relative contribution of velocity to amplitude scaling increases comparing to that of rise time. Differences in follicular muscle tones when a protraction is initiated may be one plausible explanation for the higher variation in peak protraction velocity and rise time as amplitude increases. A larger protraction movement is mostly associated with a more retracted position

of whiskers at its initiation of the protraction and the corresponding follicular muscles are more stretched. Medium or small movement is associated with less stretched muscles. Further information is needed to assess the effect of initial muscle tone in the amplitude scaling.

Previous findings in the rat (Welker, 1964; Carvell and Simons, 1990) and in the hamster (Wineski, 1983) suggested that bilaterally homologous whiskers (right and left C-1 whiskers) show highly synchronous movements in amplitude, velocity and rise time, with or without contact. Bilateral coordination is suggested by the findings in the present study. All subjects show high bilateral synchrony with a small and similar variability in the bilaterally homologous whiskers in both temporal and spatial analysis of synchrony. It is interesting to note that in some subjects the movement of one whisker seems to lag behind the whisker on the opposite side of the face (The peak of frequency distribution shifts away from the zero). There is no information available to answer why the lag is present. It may be due to preferential use of whiskers on one side of the face during the development and eventually one side of face responds more readily to the command from the higher center in the central nervous system.

Previous observations have suggested that a rat is capable of whisking at frequencies between 1 to 30 Hz, but mainly at 6-9 Hz during exploratory whisking with or without contact with an object (Welker, 1964) and with a dominant frequency of 8 Hz performing discriminative tasks (Carvell and Simons, 1990, 1995). Though a rat is capable of whisking at a larger range of frequencies, results in both measures (direct measurement and Fourier transforms) from the

current study are quite similar and indicate that all but one subject, (Rat 701), whisked at frequencies at 20 Hz or slower, with a peak whisking frequency at 5-6 Hz. The dominant frequency found in the present study covers the lower end of frequency range suggested in the Welker's study, but slower than the one suggested in the Carvell and Simons' study (1990). The possible reasons that cause the discrepancy are as follow. (1). In the present study, rats were whisking in air, without any contact with an object. Welker's study (1964) employed a free exploratory paradigm, in which a rat explored its home cage that is equipped with some testing concrete blocks. A rat's exploratory whisking analyzed contained both whisks with or without contact with an object. In Carvell and Simons' study (1990), the data were extracted during a rat's performing a tactile (texture) discriminative task. It was suggested from their analysis that contact with an object produces increased frequency and reduced amplitude. (2). The results presented here were based on the analyses of data from head-fixed rats during their first test session. Compared to both Welker's and Carvell and Simons' rats, rats in the current study whisked at a higher stress. Testing situation in the Welker's study (1964) presented a maximal novelty because of presence of different kinds of odors, while Carvell and Simons' rats were performing a learning task. This point is supported by the fact that the rat whisked at a more variable frequency range in the later testing sessions, showing more whisks clocked at 7-9 Hz. The rats were presumably less stressful and more adapted to the testing situation in the later testing sessions. (3). The results from the present study were based on large amount (up to 20 minutes of whisking activity in

individual animals), high-resolution data (sampling space—714 Hz). And data were collected from the rats with head-fixation, which eliminated the intrusion of head and neck movement. Frequency analysis performed in Carvell and Simons (1990, 1995) was based one or two seconds of whisking activity collected in individual trials. Their data were collected from freely moving rats whisking at a discriminadum and had very low resolution (60 data points per second). Also the data were confounded by constant movements from the head region. Therefore, data from the present study can be used to estimate whisking frequency more accurately. (4). Differences may also reflect the fact that both previous studies used (Sprague-Dawley) rather than Long-Evans rats.

It is worth to note that power spectrum shows the low frequency of whisking (< 2 Hz) that is found in all subjects, which is not found with direct measures. By examining whisker movement by trial as shown in Figure 4, it is suggested that this slow phase may include component of the spindling in whisking. Further analytic technique is needed to quantify the spindling of whisking, which is not available in the present study. Records of data from individual trials shows that movement of the whole mystacial pad during whisking is not frequent observed. It is consistent with the fact that the two independent groups of muscles control the movement of either individual follicles or whole mystacial pad. Under present experimental test condition, whisking without contact, the subjects mainly move the follicles (vibrissae) while maintaining the pad muscle at certain tone. There is no regular pad movement pattern observed. Pad movement is also not bilaterally synchronized.

During the test sessions, all animals emit bursts of whisks at amplitudes that are unimodally distributed over a range from about 10° to 90°. No distinction can be made between previously defined "twitches" and whisks suggested by others (Semba and Komisaruk, 1984; Welker, 1964). "Twitches" are usually observed in a still rat without showing gross bodily movement, while rats in the present were head-fixed and water-reinforced (with highest arousal).

After repeated testing, all sham animals (control) show reduction in the number of whisks emitted and in the mean burst durations over the test sessions (about 3 weeks span), though the reduction didn't reach statistically significant because of small number of animals in the sample. Animals showed more and longer paused after repeated tests. These effects of repeated testing upon whisking activity are clearer when looking at intra-session changes of whisking. There is a significant reduction in number of whisking and in mean burst durations towards the end of the testing session. Examination of raw data (plotted as in Figure 4) shows that whisking activity in most subjects' changes from the continuous, long bursts to the much shorter or impulse-like single whisks after repeated testing or towards the end of each session. All subjects in the present study were tested under a variable time schedule (VT). Though reinforcement (water) was used during the testing, there was no contingency between water delivery and any particular behavior for the subjects. Therefore, reduction in rat's whisking activity is not due to the habituation to a particular stimulus (or stimuli). It is an activity-dependent reduction. This activity-dependent reduction in whisking is important in distinguishing between spontaneous

whisking and operant conditioning of whisking. Since a rat stops whisking under non-stimulus contingent situation, maintained whisking under stimulus contingent situation must be operant whisking.

Part II: Effect of deafferentation

Though no action was taken to prevent nerve regeneration of innervation to the vibrissal pad after the infraorbital nerve transection, I am confident that deafferent data presented are free of influence from sensory input from the vibrissal pad. First, all sections were performed under direct visual control. Careful examination was done to make sure that all branches of the infraorbital nerve were sectioned at their exit of bone. Second, post-surgical check-up (visual) was performed on two additional rats (not the subjects used for data collection), two and three weeks after surgery. There was still no sign of any reconnection of the infraorbital nerve where it was transected (a clear gap) even three weeks after surgery. This finding is consistent with the previous evidence that regenerating axons were first seen entering the vibrissae one month following the infraorbital nerve transection (Renehan and Munger, 1986). Data collection on the deafferented rats was done within 10 days after first infraorbital nerve section (unilateral deafferentation). Third, all but one animal showed behavioral change after unilateral deafferentation, which is not seen in any control animals.

After infraorbital nerve section (IOx), there are some morphological and behavioral changes in the animals. In general, after deafferentation, mystacial pad was somewhat pulled dorsally from the normal position once in a while. It

seems that animals could not maintain the pad muscles at certain tone for an extended period of time; therefore, the pad needs to be "voluntarily" restored to its normal position frequently. It indirectly suggests the fact that the facial nerve innervation of the rat mystacial pad lacks sensory feedback by itself (Bowden and Mahran, 1956; Semba and Egger, 1986). Constant output to the pad via the facial nerve branches may need the peripheral input from the trigeminal branches. The other change in the pad morphology is that the whiskers become more irregular and easy to break. This change may be due to the behavioral change observed in the deafferented animals. Those Deafferented animals spent excessively more time on grooming the deafferented pad.

General observation suggested that a deafferented animal could still whisk at a rhythmic fashion with a similar frequency as found before deafferentation. Similar to the sham operated rats, no constant trend of change in mean protraction amplitude and velocity was found in subjects after the sequential deafferentation of bilateral mystacial pads. All deafferented animals still whisked at a similar amplitude and velocity as the sham animals did. Bilateral coordination is well preserved after the sequential deafferentation. Actually, temporal synchrony is somewhat enhanced in all deafferented animals. The same is true for the spatial coordination. Though the regularity is somewhat enhanced, crosscorrelations are at values comparable to those in the intact rats.

Deafferentation does not affect animals' amplitude scaling. In all deafferented subjects, protraction velocity is highly correlated with protraction amplitude after uni- and bilateral deafferentation, which is comparable to those of

sham animals or those before deafferentation. Amplitude scaling by rise time is much more variable, but there is no clear trend of any changes because of deafferentation.

The most striking change found in the present study is the sudden increase in whisking frequency after unilateral deafferentation, which is seen in four out of five deafferented subjects, but not in any sham animals. It has been described above that rats whisked at dominant frequency of 5-6 Hz during initial test session. After unilateral deafferentation, four rats emitted bursts of whisks clocked at about 8.5 Hz. These bursts show strikingly high regularity in frequency with very little variation, which is not seen before deafferentation or in sham animals. There is also very high similarity in amplitude among individual whisks. In general, these bursts last longer than those seen before surgery and the spindling regulation is less frequent. The effect of unilateral deafferentation is bilateral. The increased whisking frequency is also seen in the intact side, which is highly synchronized with the deafferented side. These changes in whisking frequency seem to be transient. Such change lasted several sessions in some rats while it only lasted one session in some others. Though they happen less frequent, the high frequency bursts of whisking can still be seen in the following testing sessions. Little further effect was observed with section of the remaining (contralateral) nerve (bilateral deafferentation). In general, animals became much less active and their dominant whisking frequency returned to that seen in the intact session in all but one animal. One possible cause for this change may be that unilateral deafferentation causes sudden imbalance centrally in receiving

peripheral input/feedback. The consequence is that the central system will try to restore the balance; therefore, it will send more commands to the vibrissae, which causes the sudden increase in whisking activity. Another Possible cause may be the inhibiting role of reafference—removal of input produces an increase. From previous evidence (Carvell and Simons, 1990, 1995), which suggested that the contact with an object increases a rat's whisking frequency but reduces its whisking amplitude, one may speculate that exafference from vibrissae may have an opposite effect on whisking, compared to the effect of reafference.

Part III: Whisking and the concept of a Central Pattern Generator

Following the classical work on the locust flight system (Wilson, 1961) a series of studies has confirmed a major contribution of central patterning mechanisms in motor control. Many behaviors in mammal, for examples chewing, sucking, and licking, appear to involve putative CPGs which have been tentatively located in the parvocellular reticular formation (Nakamura and Katakura, 1995; Lund, et al., 1998; Berhoudt and Zeigler, 1982). The rat's "exploratory" whisking pattern may provide another example.

Rats emit patterns of rhythmic whisking even in the absence of explicit whisker stimulation. The rat's vibrissa system is an integrated sensorimotor system. Its sensory innervation is provided by the infraorbital nerve. Vibrissa movements are controlled by facial nerve efferents to two distinct groups of muscles, extrinsic (orofacial) and intrinsic (follicular) muscles. No muscle spindles or any afferent innervation to either extrinsic or intrinsic muscles has been found (Bowden and Mahran, 1956; Semba and Egger, 1986). It is believed,

therefore, that whisker position must be inferred from reafference from vibrissa system. For both trigeminal ganglion and somatosensory thalamus whisker movements evoked by motor nerve stimulation have been shown to elicit activity in sensory neurons (Zucker and Welker, 1961; Brown and Waite, 1974). Although these data were obtained in anesthetized animals, it is reasonable to assume that trigeminal reafference is continuously generated during whisking. Trigeminal deafferentation may be expected to block this source of input. Thus, strong support for the existence of a CPG would be provided by evidence that the initiation, patterning and bilateral coordination of whisking are unaffected by removal of sensory input to the vibrissa. The present study has provided such evidence. I have shown that deafferentation of the vibrissae (either unilaterally or bilaterally) does not disrupt kinematics of rhythmic whisker movements or coordination of bilaterally homologous whiskers. My results thus provide behavioral evidence for a contribution of central patterning mechanisms to the control of whisking.

These data and others from our lab may also help to define some of the functional properties of a whisking CPG. First, the high degree of bilateral whisking coherence suggests a tight linkage between pattern generating mechanisms on the right and left sides of the animal, in *adults*. Additional evidence for such a linkage comes from the fact that the effects of unilateral deafferentation upon whisking frequency are seen on both sides. On the other hand, though coherence is high, it is far from complete, and periods of asynchrony are seen in head-fixed animals, suggesting the existence of

independent central patterning mechanisms on the two sides. Moreover, a CPG for whisking may include both rhythm-generating and burst-generating components.

Landers (personal communication) has recently shown that, in rat pups, deafferentation during the first few days after the onset of whisking can significantly abolish or disrupt whisking for periods of several days. The effect is seen only on the denervated side: the other side appears normal. This observation suggests (a) that afference is required for normal development of a putative CPG; (b) that the CPGs for each side are initially independent; and (c) that afference generated by whisking may play a role in the entrainment of right-left synchrony during development. In adults, it is possible that re-afference is primarily inhibitory in its effects, since it produces an increase in whisking frequency. Alternately, the increased whisking frequency seen in deafferented adults implies (a) that the system is sensitive to the decrease in *re-afference* and that the increase in frequency may reflect a compensatory process which functions to produce an increased amount of afference. In contrast, studies from several laboratories (Carvell & Simons, 1995: Harvey, Bermejo, and Zeigler, in prep.) have shown that whisking *exafference* (e.g., whisker contact with novel objects or during discriminative whisking) leads to an increase in whisking frequency. As with other putative pattern generators, afferent input from whatever source, seem to serve a modulators of CPG activity. Interestingly, our laboratory (Harvey, Sachdev, and Zeigler, in prep.) has recently found that ablation of the cortical sensory whisker representation produces an increase in

the amplitude of whisking movements in head-fixed rats, without altering the basic firing frequency. These researchers have suggested that the increased amplitude of whisks reflects a disinhibition of a normally inhibitory descending input upon a putative CPG.

There is some evidence that the putative whisking CPG, like those for other rhythmic oromotor behavior, involves neuronal ensembles at brainstem levels. Previous studies (Welker, 1964; Semba and Komisaruk, 1984; Lovick, 1972) showed that whisker movements persisted after deafferentation, decerebration, cerebellectomy, and cortical. Second, the "whisking" rhythm shares basic (6-9 Hz) frequencies with those for licking, sucking and mastication. The parvocellular reticular formation (RPC) has long been a candidate for the location of oromotor CPGs because it receives inputs from a number of brainstem orosensory nuclei and projects upon oromotor nuclei of the Vth, VIIth and XIIth nerves (jaw, whiskers, tongue, respectively). Keller (personal communication) has recently reported that "whisking (VIIm) motoneurons in an *in vitro* slice preparation exhibit spontaneous rhythmic firing at 5-10 Hz." Retrograde labeling of Vm neurons has confirmed that they receive an input from RPC neurons, and electrical stimulation of RPC pre-motoneurons elicits monosynaptic responses in whisking motoneurons. Keller hypothesizes that "rhythmic activity in whisking-motoneurons is generated by interactions between synaptic inputs from pacemaker-like excitatory and inhibitory pre-motoneurons in the parvocellular reticular formation"

The analysis of pattern generation mechanisms requires a combination of

physiological recording procedures and precise measurements of behavioral and kinematic variables under highly controlled conditions. The behavioral preparation developed in my Dissertation should make an important contribution to such analyses.

Bibliography

Bermejo, R., Harvey, M., Gao, P., & Zeigler, H. P. (1996). Conditioned "whisking" in the rat. Somatosensory and Motor Research, 13: 225-233.

Bermejo, R., Houben, D., & Zeigler, H. P. (1998). Optoelectronic monitoring of individual whisker movements in rats. Journal of Neuroscience Methods, 83: 89-96.

Bowden, R.E.M, & Mahran, Z. Y. (1956). The functional significance of the pattern of innervation of the muscle quadratus labii superioris of the rabbit, cat and rat. J. Anat., 90: 217-227.

Brecht, M., Preilowski, B., and Merzenich, M. M. (1997). Functional architecture of the mystacial vibrissae. Behavioural Brain Res, 84: 81-97.

Brown, A. S., & Waite, P. M. E. (1974). Responses to the rat thalamus to whisker movements produced by motor nerve stimulation. Journal of Physiology, 238: 387-401.

Burke, R. (1971). Control systems operating on spinal reflex mechanisms. Neuroscience Research Program Bulletin, 9: 60-85.

Carvell, G. E., & Simons, D. (1990). Biometric analyses of vibrissal tactile discrimination in the rat. Journal of Neuroscience, 10: 2638-2648.

Carvell, G. E., & Simons, D. (1995). Task and subject related differences in sensorimotor behavior during active touch. Somatosensory Motor Research, 12: 1-9.

Carvell, G. E., & Simons, D. (1996). Abnormal tactile experience early in

life disrupts active touch. Journal of Neuroscience, 16: 2750-2757.

Carvell, G. E., Simons, D, Lichtenstein, S. H., & Bryant, P. (1991). Electromyogrphic activity of mystacial pad musculature during whisking behavior in the. Somatosensory Motor Research, 8: 159-164.

Connor, C.E., Hsiao, S. S., Phillips, J. R., & Johnson, K.O. (1990). Tactile roughness: Neural codes that account for psychophysical magnitude estimates. J. Neurosci., 10:3823-3836.

Connor, C.E., & Johnson, K.O. (1992). Neural coding of tactile texture: comparison of spatial and temporal mechanisms for roughness perception. J. Neurosci., 12:3414-3426.

DeLong, M. (1971). Central patterning of movement. Neuroscience Research Program Bulletin, 9, 10-30.

Dethier, V. G., & Bodenstein, D. (1969). Hunger in the Blowfly. In C. G. Gross and H. P. Zeigler (Ed.), Readings in physiological psychology: Motivation (pp. 8-21). Harper and Row.

Dorfl, J. (1982). The musculature of the mystacial vibrissae of the white rat. Journal of Anatomy, 135: 147-154.

Dorfl, J. (1985). The innervation of the mystacial region of the white mouse: a topographical study. Journal of Anatomy, 142: 173-184.

Ewert, J. –P. (1985). Concepts in vertebrate neuroethology. Animal behavior, 33: 1-29.

Gordon, J., & Ghez, C. (1987). Trajectory control in targeted force impulses II. Pulse height control. Exp Brain Res, 67: 241-252

Grillner, S., & Wallen, P. (1991). Central pattern generators for locomotion, with special reference to vertebrates. In E. R. Kandel, J. H. Schwartz, and T. M. Jessell, Principles of Neural Science (pp. 591-594). Elsevier.

Guic-Robles, E., Valdivieso, C., & Guajardo, G. (1989). Rats can learn a roughness discrimination using only their vibrissal system. Behavioral Brain Research, 31: 285-289.

Jacquin, M. F., Wiegand, M. and Renehan, W. E. (1990). Structure-function relationships in rat brainstem interpolaris.VIII. Cortical inputs. J. Neurophysiol., 64: 3-27.

Jones, E. G., & Diamond, I. T. (1995) Cerebral Cortex, Vol. II: The Barrel Cortex of Rodents, Plenum Press, New York.

Kleinfeld, D., Berg, R. W., and O'Conner, S. M. (1999) Anatomical loops and their electrical dynamics in relation to whisking by rat. Somatosens. Motor Res. 16: 69-88.

Kutsch, W., & Huber, F. (1989). Neural basis of song production. In F. Huber, T. E. Moore, & W. Loher (eds.), Cricket behavior and neurobiology (262-309). Cornell University Press, New York.

Lovick, T. A. (1972) The behavioural repertoire of precollicular decerebrate rats. J Phisiol, Lond, 226:4-6.

Lund, J. P., Kolta, A., Westberg, K., & Scott, G. (1998) Brainstem mechanisms underlying feeding behaviors. Current Opinion in Neurobiology, 8: 718-724.

Miyashita, E., Keller, A., & Asanuma, H. Input-output organization of the

rat vibrissal motor cortex. Exp. Brain. Res. 99: 223-232.

Morley, J. W., Goodwih, A.W., & Darian-Smith, (1983). Tactile discrimination of gratings. Exp. Brain Res., 49: 291-299.

Roeder, K. D. (1935). An experimental analysis of the sexual behavior of the praying mantis. Biological Bulletin, 69, 203-220.

Nakamura, Y., & Katakura, N. (1995). Generation of masticatory rhythm in the brainstem. Neurosci Res, 23: 1-19.

Renehan, W. E., & Munger, B. L. (1986). Degeneration and regeneration of peripheral nerve in the rat trigeminal system. II. Response to nerve lesions. J Comp Neurol, 249: 429-459.

Rice, F. L., Mance, A. & Munger, B. (1986). A comparative light microscopic analysis of the sensory innervation of the mystacial pad. I. Innervation of the vibrissal follicle-sinus complexes. J. Comp. Neurol, 252: 154-174.

Roeder, K. D. (1967). Nerve cells and insect behavior (pp. 157-169). Harvard University Press, Cambridge, Massachusetts.

Semba, K. and Egger, M.D. (1986) The facial "motor" nerve of the rat: Control of vibrissal movement and examination of motor and sensory components. J. Com. Neurol., 247: 144-158.

Semba, K. & Komisaruk, B. (1984). Neural substrates of two different rhythmical vibrissal movements in the rat. Neuroscience, 12: 761-774.

Sharp, F. R., & Evans, K. (1982). Regional 14C-2DG uptake during vibrissal movements evoked by rat motor cortex stimulation. Journal of

Comparative Neurology, 208: 255-287.

Tinbergen, N. (1951). The study of instinct. Clarendon Press, Oxford.

Vincent, S. B. (1912). The function of the vibrissae in the behavior of the white rat. Behavior Monograph, 1: 1-81.

Welker, W. I. (1964). Analysis of sniffing of the albino rat. Behaviour, 22: 223-244.

Willis, M. A. & Arbas, E. A. (1997) Centrally patterned behavior generates sensory input for adaptive control. In P. S. G. Stein, S. Grillner, A. I. Selverston, and D. G. Stuart (Ed.), Neurons, Networks, and Motor Behavior (pp. 269-275). MIT.

Wilson, D. M. (1961). The central nervous control of flight in a locust. J. Exp Biol, 38: 471-490.

Wineski, L.E. (1983). Movements of the cranial vibrissae in the golden hamster. J. Zool: Lond., 200: 261-280.

Wineski, L.E. (1985). Facial morphology and vibrissal movement in the golden hamster. J. Morphol., 183: 199-127.

Woolsey, T. A. (1990). Peripheral alteration and somatosensory development. In J. Coleman (Ed.), Development of sensory systems in mammals. Wiley, New York, pp. 461-516.