

TECHNICAL NOTE

One whisker whisking: unit recording during conditioned whisking in ratsR. BERMEJO¹, M. SZWED², W. FRIEDMAN¹, E. AHISSAR² and H.P. ZEIGLER¹¹*Department of Psychology, Hunter College, City University of New York, New York City, NY 10021, USA;*²*Department of Neurobiology, The Weizmann Institute, Rehovot 76100, Israel***Abstract**

Understanding of the functional neurobiology of the rodent whisker system would be advanced by neurobehavioral studies in awake, behaving animals that combine unit recording from structures at various levels of the system with quantitative characterization of the kinematics and temporal organization of whisking. Such studies require the solution of a number of methodological problems. These include: chronic recording procedures insuring unit isolation, stability and maximum yield, monitoring and display of unit activity and whisker movements within the same (ms) timeframe and behavioral paradigms which bring whisking movement parameters under the control of the experimenter rather than the rat. Here we describe a head-fixed rodent preparation which makes possible chronic recording of unit activity in the awake, whisking rat, combined with real-time, high resolution monitoring of whisker and pad movements in two dimensions and under behavioral control. While the head-fixed “whisking” preparation has some inherent limitations, it may be used to address a number of important neurobehavioral problems. We suggest that it should contribute significantly to understanding the functional neurobiology of the whisker system.

Key words: *Whisker, whisking, chronic recording, neurons, conditioning, vibrissa*

Introduction

The rodent vibrissa (“whisker”) system is a widely used model of sensory processing, neuronal plasticity, pattern generation and motor control. Whisker movements scan the environment, generating spatio-temporal patterns of neural activity that guide adaptive behaviors (localization, discrimination and spatial mapping) and function, recursively, to control the whisking movements themselves. Studies in anesthetized animals have characterized the response properties of whisker-related neurons along the sensory part of the vibrissal system (reviews in Jones and Diamond, 1995; Sachdev *et al.*, 2001a). However, our understanding of the functional organization of the whisking system is quite modest. This may reflect the fact that the electrophysiological studies have lacked an essential behavioral dimension. The absence of such correlated neural-behavioral data severely constrains our ability to understand the operation of a closed-loop sensorimotor system such as whisking (Kleinfeld *et al.*, 1999; Ahissar and Kleinfeld, 2003). A functional neurobiology of the whisker system will require neurobehavioral studies which combine recording and analysis of activity in whisker-related neurons

with quantitative characterization of the kinematics and temporal organization of exploratory and discriminative whisking movements, in awake, behaving animals. While some progress towards that goal is indicated in a number of recent reports (e.g., Prigg *et al.*, 2002; Berg and Kleinfeld, 2003; Szwed *et al.*, 2003) further advances will require the solution of a number of methodological problems.

Whisking behavior may involve the movements of up to 30 individual whiskers. Each whisker is set in a movable pad that is fixed to a movable head that is attached to a movable body. These movements are the source of numerous sensory inputs that may confound interpretation of unit activity. Furthermore, whisking is not a reflex (i.e., it is not predictably elicitable by a specified stimulus) and it tends to habituate (i.e., become less probable) fairly rapidly. It is thus a significant challenge to bring whisking behavior under experimenter control. Finally, correlating whisking movements with unit activity requires methods for response monitoring with very high spatio-temporal resolution. The videographic methods currently in use have relatively low spatial resolution and, while high-speed (e.g., 1000 fps) videography is available (Szwed *et al.*, 2003), the time-consuming nature

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of videographic data analysis restricts behavioral sample sizes and constrains analysis of long-term changes in whisking patterns.

Previous studies from Zeigler's laboratory have demonstrated the utility of opto-electronic recording techniques for the high resolution monitoring of individual whisker movements in the head-fixed rodent at high spatio-temporal resolution (Bermejo *et al.*, 1998) and in two dimensions (Bermejo *et al.*, 2002b). More recently we have shown that whisker movements may be brought under experimental control using operant conditioning procedures (Gao *et al.*, 2003). The present study combines these monitoring and control procedures with methods for chronic recording from trigeminal ganglion and "on-line" spike sorting techniques in use by the Ahissar lab. To assess the utility of these combined techniques for electrophysiological studies of the rodent whisker system we have recorded unit activity in the trigeminal ganglion of awake, head-fixed rodents during operantly conditioned whisker movements.

Methods

Experimental procedures were conducted in accordance with NIH guidelines for the use and care of experimental animals and were approved by an institutional animal use and care committee.

1. Opto-electronic monitoring of whisker movements and contacts

A laser emitter-detector system (Bermejo *et al.*, 1998; Gao *et al.*, 2001) provides high spatio-temporal resolution (7 μ m; 1 ms) of whisking movements in the head-fixed animal (Fig. 1A). Interruption of the emitted beam (laser curtain) by a whisker produces a voltage shift in a subset of shaded sensors (CCDs). Whisker movement results in successive displacements in the *position* of that voltage shift that are linearly related to whisker position (Fig. 1B). A comparator circuit identifies

the successive positions of voltages above a preset threshold and outputs the data to a microprocessor for computation and display of the whisker movement trajectory. *To monitor an individual whisker trajectory with neighboring whiskers present*, a light (<5 mg) self-adhesive foam marker is attached to the side of a vibrissa, increasing its relative "visibility" with respect to surrounding whiskers, *but does not affect its kinematics* (Bermejo *et al.*, 1998, Table 1). A single whisker may be monitored with or without the presence of the remaining whiskers. By positioning two sets of monitoring devices at right angles to each other, it is possible to monitor whisking in both the anterior-posterior and dorso-ventral planes so as to reconstruct the trajectories of individual whisks (Bermejo *et al.*, 2002b) or to monitor both whisker movements and pad displacements in the same animal (Bermejo *et al.*, 2002a). Because the pairs of emitter/detectors are stereotactically mounted, they may be moved to any position with respect to the face so that the target whisker may be centered with respect to the sensor array. This allows us to monitor the whisker movement over the widest range of trajectories and minimizes noise produced by intrusion of adjacent whiskers. *In this note we plot only movements in the anterior-posterior plane.*

2. Behavioral control of whisker movements

Unless reinforced by novel sensory input or a specific reward, the probability of whisking in the head-fixed preparation decreases fairly rapidly. However, because our recording methods provide us with a real-time output, operant conditioning paradigms may be used to reinforce the occurrence of whisking. This makes it possible to generate stable periods of whisking over many sessions, to bring whisker movements under stimulus control and to manipulate whisker movement parameters (Gao *et al.*, 2003).

For these initial recording experiments, it was desirable to produce periods of continuous whisking over a wide range of movement parameters. We used

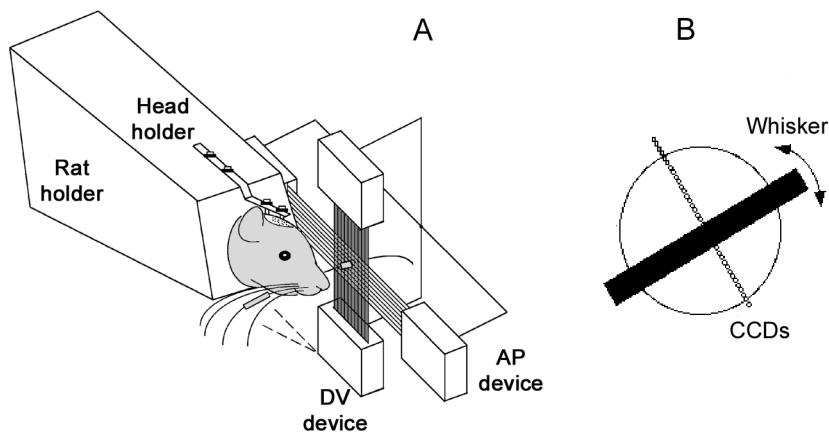


FIGURE 1. Monitoring whisking movements: a single whisker may be monitored in two dimensions with all the whiskers present. (A) Schematic diagram illustrating the placement of the opto-electronic devices in the anterior-posterior and dorso-ventral planes. (B) Schematic diagram illustrating the principle of the monitoring system (see Bermejo *et al.*, 1998, 2002 for details).

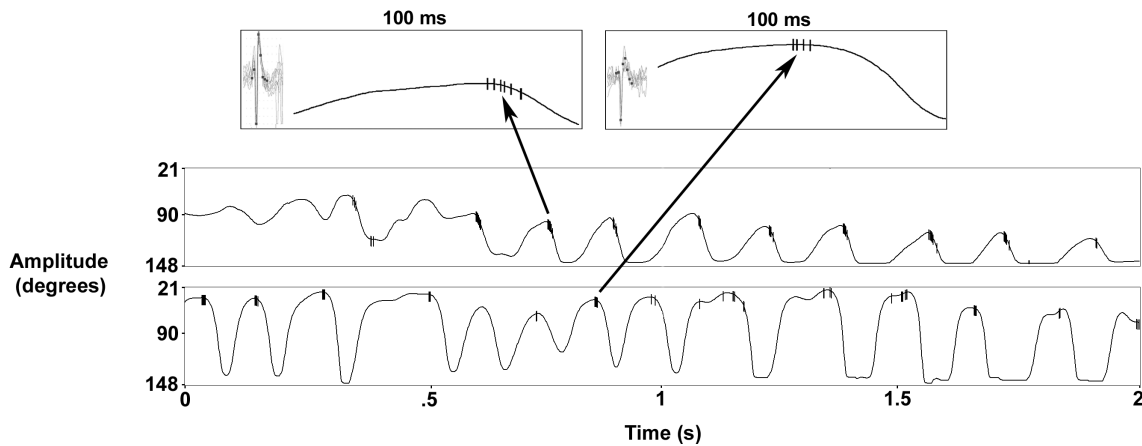


FIGURE 2. Discharge patterns exhibited by two *different* ganglion units in the same animal during bouts of conditioned whisking. The units were recorded from the same electrode in different experiments. For each unit a 2 s sample, taken from a 30 s trial, is shown. Each record is associated with the movement of a single whisker, *plotted only for the anterior–posterior dimension*. For illustrative purposes, the time stamps of each unit are displayed on the movement record. For purposes of calibration 90° (whisker perpendicular to face) is taken as the midpoint of the range of whisker movements. In the behaving animal, of course, the starting point for each whisk may shift over time. (Inset) The records indicated by the two arrows are plotted on an expanded time scale to show that more than one spike is often associated with each whisk. Sorted spike shapes for each of the two units are shown within the insets. The spikes were sorted using a template-matching algorithm. Fifty individual spike traces are shown for each unit. The black boxes represent the template points.

an operant paradigm which requires the head-fixed animal to position and maintain a single marked whisker inside an electronically defined “window”, of variable size and location, within the whisker trajectory (Bermejo *et al.*, 2002b). Whisker trajectories are continuously monitored and movement of a marked whisker into and out of the window is signaled, respectively, by the onset and offset of a conditioning stimulus (a tone/light pair). If the location of the “target” window is randomly shifted on each trial the rat generates defined epochs of active whisking in air under stimulus control (Fig. 2).

3. Surgical, electrode implantation and unit recording procedures

In an initial surgical procedure, animals were fitted with a dental cement headmount (as described in Bermejo *et al.*, 1998). The skull area targeted for subsequent craniotomy/implantation was marked and a 0.003' tungsten wire was attached to one of the posterior head-mounting screws as a ground. Following recovery, the animal was adapted to head fixation and received training on the behavioral paradigm (see 2, above). In a second surgical procedure, a dental drill was used to remove the dental cement over the target area and a craniotomy was performed. Recording electrodes were advanced into the trigeminal ganglion guided by standard stereotaxic coordinates (10–11 mm deep, 2 mm lateral and 2 mm posterior from bregma; Schneider *et al.*, 1981; Lichtenstein *et al.*, 1990) until units drivable by whisker stimulation were encountered. Both surgeries were done under O₂/isoflurane anesthesia.

Because the presence of a thick dura around the trigeminal ganglion precludes the use of wire bundle electrodes commonly employed in chronic recording, we implanted single-ended

epoxy-insulated tungsten electrodes (0.8–1.2 mΩ; F. Haer, Brunswick, ME, Cat. # UE WLECSECN1J). In one pilot experiment, we implanted a rat with four such electrodes, mounted on a Neuralynx microdrive (4-drive; Neuralynx, Tucson, AZ) that permitted us to advance each electrode independently. We modified the microdrive by attaching an 8-mm 18-gauge stainless steel tube to the tip in order to keep the electrodes aligned during penetration. To accommodate both the microdrive and the headmount it was necessary to implant the drive at an 18° angle.

Unit activity (pre-amplified 25× by a pre-amplifier close to the signal source) was amplified (MCP-Plus; Alpha-Omega, Nazareth, Israel) and single units were isolated using on-line template-matching spike sorting techniques (MSD-2; Alpha-Omega). Unit data sorted by the MSD-2 were output as TTL signals (separate channels for each unit), sampled every 50 μs and stored on a PC computer [under a Windows 2000/XP operating system] using custom-written software. The whisker associated with an identified unit was marked (see 1, above) for opto-electronic monitoring. Custom-written software displays both the whisking movements and the associated unit activity (up to six channels) on the same screen, *in real time*, and provides adjustable timeframes for sampling the data at varying degree of temporal resolution.

[Typically a few times per recording session the animal became restless for a few seconds and started fighting inside the body restraint bag. This caused electrophysiological noise that could be observed in the oscilloscope and the spike-sorting software. Moments when the noise occurred could be seen as strong (thousands of “spikes” per second) “bursts” in the non-sorted signal channel of the custom acquisition program. These fragments (that did not usually contain whisking) were then removed from

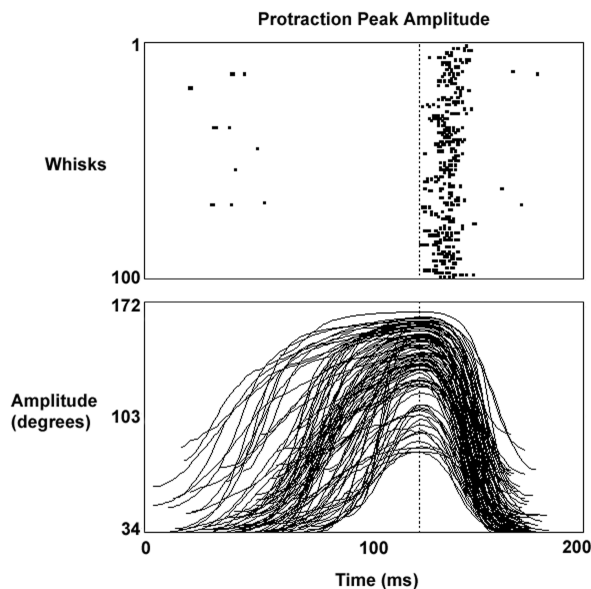


FIGURE 3. Raster plot of ganglion unit spiking activity associated with a population of 100 whisker movements and aligned with peak protraction amplitude.

analysis, as were identified reinforcement periods when the rat was drinking.]

We considered units as single only if they had homogenous spike shapes which did not overlap with other units or noise, and if they exhibited refractory periods of >0.5 ms in their autocorrelation histograms. The methods described above allow one to obtain units that remained stable for periods of 1–2 weeks. In several cases, a change in unit properties was seen after a few sessions suggesting that the electrode spontaneously moved to a new location from which another unit could be recorded. Thus, two or three single units could be recorded from a single electrode. In the pilot microdrive experiment, two of four implanted electrodes reached the ganglion, and each of them yielded two units.

Figure 2 presents records of unit activity recorded during two separate experiments from two neurons in the trigeminal ganglion of a head-fixed rat and associated with conditioned whisking movements. The two insets present portions of the data on an expanded time scale to show that more than one spike is associated with each whisk. Spike shapes of the units generating these responses are shown within the insets.

As an example of the kinds of analysis facilitated by our software, Fig. 3 presents a raster plot of spike activity in a single ganglion unit, associated with a population of 100 whisker movements and aligned with peak protraction amplitude.

Discussion

Advances in the study of other sensorimotor (e.g., oculomotor, somatomotor) systems have involved both the development of chronic recording techniques and refinements in response measurement and behavioral control. The former provides insights

into coding mechanisms; the latter improve control of stimulus and response specification and measurement. They permit the experimenter to manipulate and transduce response parameters (e.g., velocity, amplitude, rate) normally under the control of the animal. They are especially important in studies of behaviors such as whisking which involve continuous interactions between the acquisition of information and the control of adaptive responses.

Our knowledge of encoding mechanisms in the rodent whisker system comes primarily from studies, in *anesthetized* animals, of neural responses to *passive* displacement of the whisker shaft by mechanical stimulation. Both the presence of anesthesia and differences in the biomechanics of vibrissa displacement between the “active” and “passive” modes constrain extrapolations to the natural condition. Understanding the functional organization of the whisker system would therefore be advanced by studies in awake, actively whisking animals.

The development of methods for the study of unit activity in the awake, whisking rodent requires the solution of a number of technical and behavioral problems. These include: (a) the use of recording procedures which facilitate unit isolation and stability; (b) electrode implantation techniques which maximize yield without compromising the animal’s behavior; (c) methods for the monitoring of whisker movements and whisker contacts with a temporal resolution comparable to that of unit recording, so that units and movements share a common timeframe; (d) methods for the rapid acquisition and (computer-assisted) analysis of both unit and whisker activity; and (e) behavioral paradigms which bring whisking movement parameters under the control of the experimenter rather than the rat. Several of these problems (and their solutions) are common to both acute and chronic recording studies. The unique challenges raised by recording in awake, behaving animals relate primarily to response measurement and behavioral control. The data presented in this note suggest that we have taken some important first steps towards their solution.

We describe a head-fixed rodent preparation which facilitates chronic recording of unit activity in central sensory/motor structures associated with whisking behavior. The preparation combines chronic recording and “on-line” spike sorting techniques in use by the Ahissar lab with methods, developed by the Zeigler lab, for head fixation and body restraint, “real-time”, high resolution monitoring of individual whisking movements and behavioral control of whisking movement parameters. Studies involving each of the methods involved have been previously published. The novelty of the present note derives from their combined use in the same study, enabling us to simultaneously monitor unit activity and individual whisker movements in awake, behaving animals within the same timeframe and under tight behavioral control.

While we used on-line spike sorting, unit isolation and identification may also be achieved using off-line techniques. The use of multiple adjustable microdrives in a single preparation should substantially improve data yields, especially since the behavioral methodologies make it possible to gather very large numbers of whisking responses in each behavioral testing trial or session. The data collection software makes it possible to store more than 1 h of combined movement/unit activity, to separate whisking movements, whisker contacts and spikes for independent analysis and to combine them for display. Such displays facilitate recognition and computation of relationships between whisking kinematics and unit activity (see Fig. 2).

Opto-electronic monitoring provides an appropriate level of spatio-temporal resolution and facilitates computer-assisted analysis of very large kinematic and neuronal data-sets. Our procedure for selective monitoring of individual whisker movements while leaving all others intact avoids the complex inhibitory effects known to follow whisker clipping (Kelley *et al.*, 1999). Moreover, because whisking is monitored on-line, habituation of the response may be avoided and pre-selected output values of specific kinematic variables (e.g., rate, amplitude) may be used to trigger selective reinforcement in operant conditioning paradigms. This permits the experimenter to manipulate whisker movement parameters normally under the control of the animal (Gao *et al.*, 2003). These are all obvious advantages of the preparation described in this note. Moreover, while the data illustrated in this note were obtained from the trigeminal ganglion, the system we describe should be usable, with modifications, for recording activity from both central sensory and motor structures in behaving rats. However, some caveats should be noted.

For example, in contrast to the anesthetized preparation, which allows the experimenter to precisely vary whisker displacement parameters over a controlled range of stimulus values, whisker movements in the awake, behaving animal exhibit considerable variability in amplitude, frequency and topography within and between successive epochs of whisking (Gao *et al.*, 2001; Berg and Kleinfeld, 2003). While behavioral manipulations may reduce such variability, they will not eliminate it. Thus, in trying to understand the functional organization of the whisker system, investigators must balance the requirements for experimental control against the need to extrapolate to the natural condition.

This is particularly true for rodent whisking, which is characterized by its extraordinary flexibility and its use in a wide variety of adaptive contexts. It will therefore be important to develop behavioral paradigms for the head-fixed preparation that engage as wide a sample of the natural whisking repertoire as possible. One such paradigm involves the use

of indicator responses (such as lever pressing) in combination with automated presentation of discriminanda. Using such a paradigm (Harvey *et al.*, 2001) we have carried out studies of discriminative whisking in the head-fixed rat that have replicated the essential findings obtained in studies with unrestrained animals (Carvell and Simons, 1990, 1995). Furthermore, by combining operant conditioning paradigms with the use of a piezo-electric sensor it is possible to monitor simultaneously both the trajectory of the whisker movement and the onset and offset of whisker contacts with an object surface (Bermejo and Zeigler, 2000; Sachdev *et al.*, 2001b).

While it will be important to enlarge the range of problems for which it is useful, the fixed-head preparation has a number of inherent limitations. It is certainly incompatible, for example, with the study of head-movement/whisker-movement interactions, a critically important component of whisker system function. Within those limitations, however, we suggest that the preparation described in this note should contribute significantly to understanding the functional neurobiology of the whisker system.

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| 1 | Bermejo <i>et al.</i> 2002b— Okay or 2002a? |
| 2 | 257.8 okay? |
| 3 | Kelley et al. 1999 — author names' okay? |