Dynamic Translation of Surface Coarseness Into Whisker Vibrations

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Lottem E, Azouz R. Dynamic translation of surface coarseness into whisker vibrations. J Neurophysiol 100: 2852–2865, 2008. First published September 17, 2008; doi:10.1152/jn.90302.2008. Rodents in their natural environment use their whiskers to distinguish between surfaces having subtly different textures and shapes. They do so by actively sweeping their whiskers across surfaces in a rhythmic motion. To determine how textures are transformed into vibration signals in whiskers and how these vibrations are expressed in neuronal discharges, we induced active whisking in anesthetized rats, monitored the movement of whiskers across surfaces, and concurrently recorded from trigeminal ganglion (TG) neurons. We show that tactile information is transmitted through high-frequency micromotions superimposed on whisking macro motions. Consistent with this, we find that in most TG neurons, spike activity, and high-frequency micromotions are closely correlated. To determine whether these vibration signals can support texture discrimination, we examined their dependence on surface roughness and found that both vibration signals carry information about surface coarseness. Despite a large variability in this translation process, different textures are translated into distinct vibrations profiles. These profiles depend on whiskers properties, on radial distance to the surface, and on whisking frequency. Using the characteristics of these signals, we employ linear discriminant analysis and found that all whiskers were able to discriminate between different textures. While deteriorating with radial distance, this classification did not depend on whisking frequency. Finally, increasing the number of whiskers and integrating tactile information from multiple whiskers improved texture discrimination. These results indicate that surface roughness is translated into distinct whisker vibration signals that result in neuronal discharges. However, due to the dynamic nature of this translation process, we propose that texture discrimination may require the integration of signals from multiple spatial and temporal sensory channels to disambiguate surface roughness.

INTRODUCTION

Behaviorally relevant tactile stimuli have complex spatial and temporal structures, and animals can readily perceive and discriminate among these stimuli to guide their behavior. Most of our knowledge of somatosensory function has been obtained using stimuli consisting of simple features designed to tease apart neuronal stimulus selectivity. Thus little is known about the behavior of the somatosensory system in response to behaviorally relevant stimuli that are found within the animal’s natural sensory environment.

The system is highly specialized for processing fine tactile information acquired by the array of whiskers on the facial mystacial pad. Rats actively sweep their whiskers across surfaces in a rhythmic forward and backward motion with a frequency ranging from 5 to 25 Hz, called whisking, to locate and distinguish objects in the animal’s immediate sensory environment (Berg and Kleinfeld 2003; Bermejo et al. 2002; Carvel and Simons 1990; Sachdev et al. 2001). Whisker movements excite several hundred primary afferent fibers that innervate mechanoreceptors on each whisker shaft (Ebara et al. 2002). These signals travel along the sensory nerve to the brain stem. The axons of the brain stem neurons then cross the brain midline and travel to the thalamic somatosensory nuclei. Thalamic neurons project to the primary somatosensory cortex, conveying information to layer 4 cell populations called “barrels” (Welker 1971; Woolsey and Van der Loos 1970). Psychophysical studies have demonstrated that rats can reliably detect, using their whiskers, small differences in surfaces textures (Carvel and Simons 1990, 1995; Guic-Robles et al. 1989; Ritt et al. 2008; von Heimendahl et al. 2007). Thus the somatosensory system appears well suited for conveying and processing complex sensory information rapidly and reliably (Diamond et al. 2008).

What mechanisms might underlie this striking capacity for texture discrimination? One attractive model posits that the resonant properties of whiskers, which vary systematically with whisker length along whisker rows, will mediate texture discrimination (Brecht et al. 1997; Hartmann et al. 2003; Kleinfeld et al. 2006; Mehta and Kleinfeld 2004; Moore and Andermann 2005; Neimark et al. 2003). When rats actively sweep their whiskers across a surface, that vibrissa whose resonance frequency most closely matches the texture-induced input frequency will transmit the largest vibrations to the follicle (Andermann et al. 2004). Different whiskers would be tuned to different frequencies and thus split the tactile vibration signals into labeled frequency lines in the cortex (Andermann et al. 2004; Hartmann et al. 2003; Mehta and Kleinfeld 2004; Moore 2004; Moore and Andermann 2005; Neimark et al. 2003). An alternative view suggests that the mechanical properties of the vibrissae determine the translation of surface roughness into different intrinsic frequency or velocity modes in each vibrissa (Andermann et al. 2004; Hipp et al. 2006). The extent to which different modes are favored within each vibrissa reflects the differences between surfaces (Fend et al. 2003; Mehta and Kleinfeld 2004). The neural representation of these modes can either be expressed in discharge probabilities that increase in proportion to stimulus velocity (Arabzadeh et al. 2003–2006; Jones et al. 2004; Shoykhet et al. 2000) or by the preservation of the velocity profile of the vibration in the temporal pattern of spikes (Arabzadeh et al. 2005, 2006; Deschenes et al. 2003; Gibson and Welker 1983a,b; Hipp et al. 2006; Lichtenstein et al. 1990; Shoykhet et al. 2000).

Recent seminal studies, using artificial whisking across textures, have concluded that the velocity profile signal in whis-
kers transmits texture coarseness to the whisker shaft (Albarracin et al. 2006; Arbazadeh et al. 2003–2005; Kleinfeld et al. 2006). Texture discrimination can then be achieved using cortical spike rate and patterns (Arbazadeh et al. 2006). While these studies have advanced our understanding of the representation of “natural” stimuli such as textures in the rat’s whisker somatosensory system, several key issues remain unresolved: what are the characteristics of texture-related whisker vibrations that are transmitted to the whisker shaft? How are these vibrations translated to trigeminal ganglion (TG) neuronal discharges? Are these features able to support texture discrimination? What are the functional consequences of large variability in this translation process? To address these issues, we induced artificial whisking across textures in anesthetized rats, monitored whiskers’ vibrations at the shaft, and concurrently recorded from TG neurons. We first examined how whiskers’ motion along a surface is converted to discharge patterns in TG neurons. Then we characterized the texture-related whisker vibrations that underlie these discharge patterns. Using linear discriminant analysis (LDA), we were able to determine whether whisker vibrations are able to support texture discrimination and the extent to which it depends on intrinsic and extrinsic variables.

METHODS

Surgical procedures

Adult male Sprague Dawley rats (n = 50; 250–350 g) were used. All experiments were conducted in accordance with international and institutional standards for the care and use of animals in research. Surgical anesthesia was induced by urethane (1.5 g/kg ip) and maintained at a constant level by monitoring forepaw withdrawal and corneal reflex and administering extra doses (10% of original dose) as necessary. Atropine methyl nitrate (0.3 mg/kg im) was administered after general anesthesia to prevent respiratory complications. Body temperature was maintained near 37°C using a servo-controlled heating blanket (Harvard, Holliston, MA).

Recording and stimulation

After placing subjects in a stereotaxic apparatus (TSE, Bad Homburg, Germany), an opening was made in the skull overlying the trigeminal ganglion, and tungsten microelectrodes (2 MΩ, NanoBio Sensors) were lowered according to known stereotaxic coordinates of NV (1.5–3 ML, 0.5–2.5 AP) (Leiser and Moxon 2006; Shoykhet et al. 2000) until units drivable by whisker stimulations were encountered. The recorded signals were amplified (×1,000), band-pass filtered (1 Hz to 10 kHz), digitized (25 kHz), and stored for offline spike sorting and analysis. The data were then separated to local field potentials (LFP; 1–150 Hz), and isolated single-unit activity (0.5–10 kHz). Spike extraction and sorting was accomplished with MC1ust (by A.D. Redish available from http://www.cbc.umn.edu/~redish/mc1ust), a Matlab (Mathworks, Natick, MA)-based spike-sorting software. The extracted and sorted spikes were stored at a 0.1-ms resolution and peri-stimulus time histograms (PSTHs) were computed.

Artificial whisking (Fig. 1A) (Brown and Waite 1974; Szwed et al. 2003) was induced by stimulating the buccolabialis motor branch of the facial nerve (Sembra and Egger 1986). The nerve was cut and its proximal end was positioned on bipolar tungsten electrodes and kept moist. Bipolar rectangular electrical pulses (10–15 pulses of 100 μs at 143 Hz for 70–105 ms) were applied through an isolated pulse stimulator (ISO-Flex; A.M.P.I.) to produce whisker protraction, followed by a passive whisker retraction at frequencies in the range of 6–12 Hz. The stimulation magnitude was adjusted at the beginning of each recording session to the minimum value that reliably generated the maximal possible movement amplitude (50–200 μA).

Whisker displacements transmitted to the receptors in the follicle were measured by an infrared photo-sensor (resolution: 1 μm; Panasonic: CNZ1120) placed 2 mm from the pad. The voltage signals were digitized at 10 kHz and amplified (x500; FLA-01, Cygnus Technology, Delaware). (See supplementary material: part 1 and corresponding Fig. S1 for details of sensor operation and a description of sensor calibration.) During “single whisker” experiments, other whiskers were trimmed off. With animals for which multiple whiskers were studied, we chose distant whiskers and trimmed the rest.

Whisker movements were measured under different conditions: whisking with no object contact (“free whisk”), whisking on smooth surface (smooth), and whisking on sandpapers of five different grades [from fine-grained to coarse-grained (the numbers in the parentheses indicate the average grain diameter): P60 (269 μm), P100 (162 μm), P150 (100 μm), P220 (68 μm), and P420 (46 μm)]. These grades were chosen in accordance with previous studies (Arbazadeh et al. 2005; Hipp et al. 2006) and were also based on the findings that rats can discriminate α between sandpapers with grain sizes of 400 and 2,000 μm (Guic-Robles et al. 1989) and b between smooth surfaces and rough surfaces having grooves spaced at 90-μm intervals (Carvell and Simons 1990). The textures were mounted on a flat surface (15 × 15 mm). The surfaces were oriented so that the whisker rested on them and remained in contact during the entire whisk trajectory. The proximal edge of the surface was ~10 or 30 mm from the base of the whisker (see Methodological considerations for a detailed account of this stimulation configuration).

For each texture and full free whisking, we recorded 10 trials once every 60 s. Each trial contained 10 whisks at 6 or 12 Hz, giving a total of 100 whisks per stimulus. During each experiment, the textures were randomly interleaved. To monitor the drift in whisking and sensor performance, we measured whisker vibrations during free whisking between textures. To minimize the number of stimulus combinations and to cover all ranges of whisker types (Moore 2004; Moore and Andermann 2005; Neimark et al. 2003), we monitored the movements of the following whiskers: β, B2, A2, C3, B4 (Fig. 1B).

Data analysis

Whisker movements resulted in high-frequency micromotions (HFs) superimposed on whisking macromotions (WMs; Fig. 1C). To separate HFM and WM signals, we used band-pass Butterworth type II filter of fourth order: cutoff frequencies 1–50 Hz and cutoff frequencies 50–300 Hz, respectively (Fig. 1, C and D, I and 2). Because responses of neurons during various stages of the whisker somatosensory system better reflect whisker deflection velocity rather than amplitude (Arabzadeh et al. 2004; Jones et al. 2004; Shoykhet et al. 2000), we also calculated the velocity profile of HFM (Fig. 1D). Whisker angle was computed at base (the most proximal point of the tracked whisker): angle at base = arctan (measured whisker trajectory/ measuring distance), measuring distance = 2 mm.

To determine whisk protraction and retraction onset and ending, we identified the trajectory inflection points at the beginning and ending of each phase (Fig. 1D1). Protraction onset and retraction ending were determined by locating the time points of the first and last peaks in the second derivative of whisker trajectory. Protraction ending and retraction onset were determined by locating the time points of the first and last troughs in the second derivative of whisker trajectory. We separated the protraction and retraction phases of each whisk (Fig. 1D1, gray background).

We selected a wide range of parameters that came from HFs and WM signals. WMs signals were characterized by: WMs amplitude, defined as the distance between the highest and the lowest points of whisker retraction; WMs velocity, defined as the 10–90% retraction
slope. HFM s were calculated by initially converting the trajectory signal to velocity. An event was defined as the velocity trace comprising three successive local extrema (Fig. 1D, bottom). The scanning for micromotions started from retraction onset. The first extrema during the retraction period was set to be the starting point of the first event (peak – circle), the second – the peak (valley-diamond) of the first event, and the third – the end of the first event (peak – circle). The third point was also the first point of the second event, the fourth – the peak of the second event and so on. Shifting the frames for event detection did not alter the distribution of event parameters significantly (P ≪ 0.001). To reduce the detection of spurious micromotions, we included an amplitude threshold criterion. The threshold was set as the mean micromotion amplitude ±3 SD of a control segment (taken from the time between whisking bouts). HFM s with amplitudes lower than the threshold were not included in the analysis. An example of detected HFMs in the velocity signal (Fig. 1D3) is shown above the trace as a raster plot. Each HFM is indicated by a vertical line.

The following parameters characterizes HFMs: HFM s magnitude defined as the distance between the peak velocity and the average of the two valleys (Fig. 1D, bottom); HFM s latency is the value given to the average latency from retraction onset to all events within a whisk; Inter HFMs interval (ITI) defined as the time interval between two successive HFMs; number of HFMs defined as the number of HFMs occurring in one whisk. HFMs parameters from each whisk were represented by the average. The significance of the differences between the measured parameters was evaluated using one-way ANOVA. When significant differences were indicated in the F ratio test (P < 0.05), the Tukey method for multiple comparisons was employed to determine those pairs of measured parameters that differed significantly within the pair (P < 0.05 or P < 0.01). Averaged data are expressed as means ± SE. Error bars in all the figures indicate the SE.

We characterized the correlation between the spike train and the HFMs using the cross-correlogram (CCG) (Perkel et al. 1967), calculated using the methods of Bair et al. (2001). We represented the spike train of the cell and the HFMs that crossed threshold as binary time series’ with 1-ms resolution.

The CCG was then computed as follows

\[
CCG(\tau) = \frac{1}{M} \sum_{i=1}^{N} \sum_{t=1}^{t} x_i(t)x_i(t + \tau)
\]

where \(M\) is the number of whisks, \(N\) is the number of bins in each whisk, \(x_i\) and \(x_i\) are the spike trains of the neuron and HFMs on trial \(i\), respectively. \(\tau\) is the time lag. A correction was applied to each CCG to compensate for the edge effects that arise when a correlation histogram is computed on windowed data because the spikes at the beginning and ending of the whisk time interval are surrounding by stimulus-elevated firing rates only on one side. The result is often a broad peak in the CCG. Each bin of the CCG was multiplied by the factor \(F\)

\[
F = M/(M - N)
\]

where \(M\) is the whisk length and \(N\) is the absolute value of the time lag.

Our objective in quantifying the synchrony between HFMs and spikes was to establish a measure of the magnitude and statistical
significance of the temporal correlation between HFMs and spikes that occurred within ±10 ms of zero time lag. To determine the magnitude of correlation above and beyond that which might have been introduced by whisking, we used a modification of the method by Maldonado et al. (2000). We utilized the variance in the interval-shuffled control correlograms to establish the confidence limits for significance. Our measure, referred to as the significance ratio (SR), is the ratio of two integral values: a peak value \( P \) (Fig. 3C, black + gray), representing the magnitude of HFMs spikes correlation, computed by taking the sum of the bins in the central 20 ms of the CCG, and a variance value \( R \) (Fig. 3C, gray), representing the expected occurrence of coincident HFMs and spikes, computed from the sum of the central 20 ms in each histogram lying below the 95% confidence limits. This ratio measure is illustrated in Fig. 3 and was computed as follows:

\[ SR = \frac{P}{R} \]

To establish a confidence limit, for each experimental spike train and events, we computed an interval-shuffled spike train obtained by randomly shuffling the time of occurrence of the interspike intervals in the experimental data. This simulations yielded control spike trains identical in spike count, mean firing rate, and duration to the experimental data and preserved the interspike interval distribution of the original data but resulted in a random shuffling of interval times. The interval-shuffled spike trains are used to control for the possibility that the temporal structure of the firing patterns might yield statistically significant correlations of a spurious origin.

The simulation was repeated 200 times for each whisk. The CCG was computed from each simulated spike train and corresponding correlograms were calculated. The resulting correlograms were edge corrected and the mean value for each bin (1 ms) was then computed. This yielded a set of 200 control values for each bin in experimental CCG. We assigned a confidence limit for statistical significance by choosing the \( R \) value for each bin in the control distributions that was >95% of all the values. To provide a measure of correlation strength that was independent of the total spike count in the data, we then calculated the \( SR \) value for each bin and averaged across all \( SR \) values in the central 20 ms of the CCG. Values of this \( SR > 1.0 \) were considered significant.

**Classification**

Linear discriminant analysis (LDA) (Krzanowski 1988; Seber 1984) was used to evaluate whether vibration signals in whisker movements could predict surface roughness. Classification was accomplished using a set of variables that came from WMs and HFMs characteristics (see preceding text). This analysis was employed to statistically determine whether: first, whisker vibration signals can serve to discriminate between textures; second, the influence of whisker identity on texture discrimination; third, the influence of texture distance on texture discrimination; fourth, the influence of whisking frequency on texture discrimination. Here the multivariate data set consisted of WMs and HFMs parameters for each stimulus (see preceding text). For the different conditions, the 100 whisks in each session were divided randomly to “training” and “testing” whisk. For each texture in the training whisk, the analysis determines the location of the point that represents the means for all WMs and HFMs parameters in the multivariate space in the model. These points are called group centroids. An example of two-dimension multivariate space is shown in Supplementary Fig. S2A. For each of the testing whisk, the analysis computes the distances (of the respective whisk) from each of the training texture group centroids. This measure, which is normalized by the within-group variance (similar to z score), provides an indication of whether or not an observation is an outlier with respect to the independent variable values. Finally the analysis classifies the whisk as belonging to the group to which it is closest, that is, where the distance is smallest (Mahalanobian distance). This whisk-by-whisk prediction of texture identity was then compared with the real texture identity of each whisk, and a percentage of correctly predicted whisks was obtained for each group. This resulted in a hit matrix in which the diagonal reflects the percentage of correct predicted whisks (Supplementary Fig. S2B). The sum of all the values in the diagonal gives the overall correct classification. To correct for varying number of textures, we normalized the percentage of correct predicted whisks to the corresponding chance level. \( P_{\text{correct}} = \frac{P_{\text{correct}} - P_{\text{chance}}}{100 - P_{\text{chance}}} \)

Linear discriminant analysis was accomplished with statistical toolbox in Matlab.

**RESULTS**

We induced artificial whisking in anesthetized rats by stimulation of the facial motor nerve, monitored the movement of whiskers across surfaces, and concurrently recorded from TG neurons. Our aim was to characterize how textures are translated into vibration signals in whiskers and how these vibrations are transformed into discharge pattern of TG neurons.

An example of the experimental set is shown in Fig. 1A. Artificial whisking was induced by electrically stimulating cranial nerve 7, generating 6- and 12-Hz whisking movements (Brown and Waite 1974; Szwed et al. 2003) that resemble whisker trajectories in awake rats (Berg and Kleinfeld 2003). An optical sensor measured whisker displacements transmitted to the receptors in the follicle. Whisker movements were measured under different conditions: “free whisk,” smooth surface, and sandpapers of five different grades [Fig. 1C; see METHODS: P60 (\( n = 14 \)), P100 (\( n = 11 \)), P150 (\( n = 23 \)), P220 (\( n = 28 \)), and P320 (\( n = 6 \)). The textures were mounted on flat surface that was oriented so that the whiskers rested on it and remained in contact (see METHODS: \( B (n = 8) \), B2 (\( n = 8 \)), A2 (\( n = 3 \)), C3 (\( n = 3 \)), B4 (\( n = 3 \)) (Fig. 1B)). Whisker movement across surfaces resulted in HFMs riding on WMs signals (Fig. 1C). To separate these, we employed band-pass filters (see METHODS) which resulted in the traces shown in Fig. 1D, 1, and 2.

Before going into a detailed description of our results, it is important to discuss some of our methodological considerations: first, because it was suggested that responses of neurons in various stages of the whisker somatosensory system better reflect whisker deflection velocity than amplitude (Arbabzadeh et al. 2003–2006; Jones et al. 2004; Shoykhet et al. 2000), we used in the current study, the velocity profile of the HFMs (Fig. 1D3). A comparison between the discrimination abilities of trajectory and velocity signals reveals no significant differences between these two signals (supplementary material: part 2 and corresponding Fig. S3). Second, although whisking is not restricted to a single plane of motion (Bermejo et al. 2002), it was claimed elsewhere that movement in the anterior-posterior axis contains most texture-specific signals (Arbabzadeh et al. 2005). To validate this assertion and to simplify our experimental paradigm, we have tested this claim in our experimental setup and found that the majority of texture-related tactile information is carried by the horizontal plane (see supplementary material: part 3 and corresponding Fig. S4 for a study of multi-dimension whisker signal). Therefore we examined the principal direction of vibrissae movements, corresponding to the horizontal forward-backward axis. Finally, during protracion, in some animals, we have seen movement modulation
locked to the electrical stimulus (see also Szwed et al. 2003). Therefore we separated the protraction and retraction phases of each whisk (Fig. 1D1, gray background) and performed the complete analysis for each phase. Results were qualitatively similar for the two phases, although discrimination performance was slightly lower for the protraction phase. Therefore in the current study we restricted the analysis to the retraction phase (see supplementary material: part 4 and corresponding Fig. S5 for a comparison of texture-related signals during protraction and retraction phases).

To examine the relationship between whisker vibrations and neuronal discharges, we recorded extracellularly from 25 TG neurons. All these neurons could be driven by manual stimulation of one of the whiskers, and all had single whisker receptive fields. We first compared whisker vibrations and neuronal discharges during free whisking and during contact with textures. An example of the change in these variables between these two conditions is shown for a single neuron responding to B2 whisker in Fig. 2. The columns on the left and the right depict whisker vibrations and neuronal discharges during free whisking and during contact with textures. An example of the change in these variables between these two conditions is shown for a single neuron responding to B2 whisker in Fig. 2. The columns on the left and the right depict whisker vibrations and neuronal discharges during free whisking and texture contact, respectively. The top and bottom panels in A show all whisker trajectories and HFMs in gray, respectively. The average whisk and HFMs are marked in black. The panels in B show the raster plots of the threshold crossing events and spikes. We represented the spike train of the cell and the HFMs that crossed threshold as binary time series’ with 1 ms resolution. As can be seen in Fig. 2, B and C, during contact with textures, TG neurons increased their discharge rate in response to an increase in HFMs rate and amplitude (more examples are shown in Supplementary Fig. S6). These results suggest that while TG neurons may convey whisking information they increase their firing during contact with textures.

To further examine the temporal relationship between spike train and the HFMs, we used several approaches. We first calculated the CCG between the two signals. Figure 3B shows an example of a B2 neuron that increased its firing rate during a HFMs in response to contact with P150 texture. This association was expressed in the CCG as a tight temporal correlation between its spikes and the HFMs in which the peak near time “0” indicates spikes that were temporally locked to specific HFMs (Fig. 3C). We applied an additional measure to determine the relationship between HFMs and spike activity by calculating the spike-triggered average (STA) of whisker trajectory over a 50-ms time lag for all spikes in a session. This measure revealed the average relationship between spikes and textures.
the underlying whisker trajectory and showed that whisking-evoked action potentials in TG neurons commonly arise from HFMs (Fig. 3D; more examples are shown in Supplementary Fig. S6; during free whisking, only 2 of 10 responsive cells showed a temporal correlation between HFMs and spikes). As a control, we randomly shuffled spike times and recomputed the STA. This led to a complete flattening of the waveform, confirming a correlation between HFMs and the occurrence of action potentials (dashed trace in D).

To quantify the temporal relationship between neuronal discharges during HFMs, we calculated the SR ratio of the sum of bins in the central 20 ms of the experimental CCG that exceed the 99% confidence limits (black and gray area; see METHODS) and the sum of the central 20 ms in the corresponding CCG of the interval-shuffled events and spike trains lying below the 99% confidence limits (gray area). We found that this ratio ranges from 0.45 to 2.9 (Fig. 2E). Any experimental SR ratio value equal to or >1 was considered statistically significant. The degree of correlation as expressed in the SR value did not depend on the firing rate of the neurons (not shown) and was not related to whether the neurons fired during whisking in free air (Fig. 2). Together these results suggest that in addition to discharging throughout the whisk, most TG neurons responses are locked to HFMs.

We next sought to determine whether the characteristics of HFMs and WMs signals are able to support texture discrimination. We quantified the properties of the WMs and HFMs, by computing WMs amplitude, WMs velocity, HFMs magnitude, HFMs latency, ITI and number of HFMs (Fig. 1D; see METHODS). A distribution of these parameters is shown in Fig. 4 for a single B2 whisker presented with texture P220. For each parameter, we calculated the average within a whisk and plotted the distribution across all whisks (n = 100). Several points are noteworthy. First, HFMs parameters across whisks for a single texture are broadly distributed. This illustrates the highly dynamic nature of texture signals transmitted to the follicle. Second, whisking signals are highly variable, possibly reflecting an intrinsic variability in muscular activation.

To determine whether these signals transmit information about surface roughness, we examined the influence of different sandpapers (from fine-grained to coarse-grained) on these characteristics. The results of these calculations are shown in Fig. 5 for B2 whisker [P60 (n = 5), P100 (n = 8), P150 (n = 15), P220 (n = 15), and smooth (n = 6)] presented with different textures. The dashed horizontal line indicates the corresponding values during free whisking. This figure reveals several important findings: first, observed HFMs during active surface palpation show broad distributions of magnitudes and latencies but less variance in their number and frequency. The dependence of HFMs parameters on surface roughness suggests that these signals may be employed to transmit tactile information to whisker follicles. Second, the influence of surface roughness on WMs parameters demonstrates that texture discrimination may be based on information composed of a
mixture of WMs and HFMs. Third, in extreme cases where whiskers either stick permanently to a surface (P60; due to large bumps on the surface) or do not vibrate (smooth surface), the dependence of these characteristics on surface roughness deteriorates. Fourth, the range of WMs amplitudes in the current study corresponds to those that were shown in freely behaving rats (Berg and Kleinfeld 2003; Carvell and Simons 1990; Knutsen et al. 2005). Fifth, the range of HFMs magnitudes measured across several surfaces corresponds to the higher end of the range of micromotions used in passive stimulation (Arabzadeh et al. 2003; Hartings and Simons 1998; Pinto et al. 2000; Shoykhet et al. 2000; Temereanca and Simons 2003; Wilent and Contreras 2004, to name a few) In none of these studies were velocities $>2.5^\circ$/ms employed. However, our results are well within the range of micromotion velocities reported in a recent study, in awake behaving rats (Fig. 8B in Ritt et al. 2008). Together these findings indicate that artificial whisking imitates some aspects of whisking in awake behaving rats and that the properties of whisker vibration signals are tightly linked to surface roughness.

LDA was used to quantify the ability of whiskers to “discriminate,” on a whisk-by-whisk basis, surface roughnesses. By providing a rigorous statistical characterization of the predictive value of tactile sensory information contained within the sample of mystacial whiskers, LDA allowed us to assess the hypothetical ability of whiskers to transmit tactile information to TG neurons. We generated a whisk-by-whisk prediction of texture identity and compared it with the real texture identity. The percentage of correct predicted whiskers was obtained for all textures in each whisker. This resulted in a classification matrix in which the diagonal reflects the percentage of correct predicted whiskers. The sum of all the values in the diagonal gave the overall correct classification. To correct for varying number of groups (number of textures), we normalized the percentage of correct predicted whiskers to the corresponding chance level (see METHODS). An example of this analysis is shown in Fig. 6 for B2 whiskers in response to six textures and free whisking [P60 ($n = 8$), P100 ($n = 3$), P150 ($n = 8$), P220 ($n = 8$), and P320 ($n = 3$), smooth ($n = 7$), and free whisking ($n = 8$)]. We initially computed the classification scores based on WMs, HFMs, and the combination of all variables for each whisker. To correct for varying number of textures for each whisker, we normalized the percentage of correct predicted whiskers to the corresponding chance level (see METHODS). This analysis resulted in three $7 \times 7$ hit matrices (WMs, HFMs, all), in which each element in each matrix was the average across several whiskers. We then divided the diagonals in each of the hit matrices into three categories: coarse (P60, P100, P150), fine...
(P200, P320, smooth), and free whisking. When using all variables, texture identity was correctly classified in 88% of whiskers. Interestingly, WMs variables had a similar discriminative power to HFMs parameters (P < 0.01; Fig. 6A; during free whisking, HFMs were infrequent, thereby reducing their discriminative power). We applied this analysis to all the whiskers in the study (n = 25) and found the same relationship (92 ± 12%). To determine which of the specific parameters is most informative, we employed the Partial Wilks’ lambda. In general, Wilks’ lambda is the standard statistic that is used to denote the statistical significance of the discriminatory power for the current model. Partial Wilks’ lambda is the Wilks’ lambda for the unique contribution of the respective variable to the discrimination between groups. Its value will range from 1.0 (no discriminatory power) to 0 (perfect discriminatory power). We found that all parameters had a Partial Wilks’ lambda of ~0.4. No significant differences were found between variables. These results indicate that whiskers vibrations provide a potential cue that might be used for the accurate discrimination of tactile stimuli.

To determine the temporal constraints on texture discrimination, we varied the number of whiskers on which the prediction of texture identity was made. Figure 6B shows two examples of texture discrimination as expressed by the percent of correct texture classification in two whiskers. Addition of whiskers increased the discriminative power in all whiskers. In fact, increasing the number of whiskers from two to three whiskers led to an increase to the 80–90% of the maximal available classification. Further increases in the number of whiskers had only marginal effects on texture discrimination. Together these results indicate that mystacial whiskers may carry tactile information about surface roughness rapidly and reliably.

The analyses shown in Figs. 4–6 were done mostly on B2 whiskers. To determine the influence of the mechanical properties of the whiskers on the translation of surface roughness into whisker vibration signal, we compared the influence of different sandpapers (from fine-grained to coarse-grained) on the aforementioned characteristics in different whiskers. The results of these calculations are shown in Fig. 7A for β (n = 8) and C3 (n = 3) whiskers presented with different textures. This example shows that while in both whiskers, HFMs magnitude is dependent on surface roughness, their dependency profiles are different. The disparity between the different whiskers is further verified by the comparison between all studied variables in these whiskers in response to the same texture (Fig. 7B; P220). Because whiskers responses were taken from different animals, we normalized them to the highest value. The panel shows that in thin rostral whiskers, whisking velocity decreases while their amplitude increases. Examination of HFMs parameters reveals that their number and magnitude increases in rostral whisker. These results suggest that whis-

**Fig. 5.** Whisker vibrations are dependent on surface roughness. Dependence of HFMs amplitude (A), latency (B), number (C), and interval (D) and WMs velocity (E) and amplitude (F) on surface roughness in B2 whisker. The dashed horizontal lines indicate the value of the corresponding parameter during whisking in free air. Error bars in the figure indicate SE.

**Fig. 6.** Whiskers are able to discriminate texture identity on a whisk-by-whisk basis. A: classification scores of all B2 whiskers. The analysis was divided into 3 categories (see corresponding text): coarse (left), Fine (middle), and free whisking (right). The classification scores came from WMs signals (gray), HFMs (white), and all variables (black). B: the influence of number of whiskers on texture discrimination. Increasing the number of whiskers results in an increase of the classification score. Error bars in the figure indicate SE.
kers’ mechanical properties may influence the transformation of surface roughness into whisker vibrations.

We next evaluated how well different whiskers performed when LDA was used to classify single whiskers into the different textures. We compared classification scores using all parameters in H9252 and C3 whiskers taken from the same animal (n = 3). While the different whiskers presented different response profiles, their ability to discriminate between different sandpapers did not falter. Figure 7C shows the discrimination performance of the two whiskers. Examination of the percent of correct classification for all variables revealed that texture discrimination was not significantly different in the two whiskers and was correctly classified in 87% of whiskers. These findings suggest that tactile information may be available in all whiskers.

It has been suggested that texture or form discrimination may require the integration of signals from multiple vibrissae (Carvell and Simons 1995; Krupa et al. 2001; Neimark et al. 2003) to disambiguate differences in whisker properties and spatial frequency components among textures. Therefore we examined whether integration of tactile information from multiple whiskers would increase the discrimination between different textures. In this analysis, we used all variables from two whiskers resulting in 12 parameters for the LDA. Figure 7D shows the classification scores as expressed in percent correct for each whisker and for both. These calculations reveal that integration of information from multiple whiskers resulted in an enhancement of texture discrimination. Together these results suggest that the availability of tactile information from multiple channels may facilitate texture discrimination.

To determine the influence of surface location on texture discrimination, the proximal edge of the surface was placed at 10 and 30 mm from the base of the whisker. An example of whisker B2 responses to texture P100 at these two distances is shown in Fig. 8A. This example shows that surface distance has a critical influence on WMs and HFMs. The difference between responses at the different distances is further verified by a comparison between all studied variables in these B2 and β whiskers in response to the same texture (n = 4; P100) at two distances (Fig. 7B; because the influence of distance on whisker vibrations was similar in the 2 whiskers, the values in the plot are an average of the 2 whiskers normalized to the maximum response). The panel shows that at distant object location, WMs velocity and amplitude approach their values during free whisking (Fig. 5, E and F), while HFMs number and magnitudes decrease. These results suggest that at long distances, tactile information may be transmitted mostly through HFMs while at short distances, tactile information may be conveyed through WMs and HFMs. To test this prediction, we used LDA and compared classification scores using WMs, HFMs, and all parameters for the two distances. When calculating the classification scores using all variables, we found

![Figure 7](http://jn.physiology.org/)

**FIG. 7.** The influence of whisker identity on transmission of textures signals. A: the influence of surface roughness on HFMs magnitude in β and C3 whiskers. B: comparison of all texture related variables between β (black) and C3 (gray) whiskers. The values were normalized to the maximal between the 2. C: classification scores for β (black) and C3 (gray) whiskers taken from the same animal. D: the influence of combining vibration signals from multiple whiskers on texture discrimination. Increasing the number of whiskers, results in an increase of the classification score (see corresponding text for a detailed explanation). Error bars in the figure indicate the SE.

![Figure 8](http://jn.physiology.org/)

**FIG. 8.** The influence of surface distance on whisker vibrations. A: sample B2 whisker trajectories across P100 texture at 2 distances (top). Bottom: the corresponding HFMs signals. B: comparison of HFMs and WMs variables between close (black) and distant (gray) surface. The values were normalized to the maximal between the 2. C: classification scores for close and distant surfaces using WMs variables (gray), HFMs variables (white), and all variables (Black). Error bars in the figure indicate the SE.
that if a surface is located distantly from the pad, the different whiskers transmit texture information less reliably and accurately. This is the result of a reduction in the contribution of WMs signals (Fig. 8C). Finally, to determine whether distance information may be inferred from vibration signals, we employed the same texture at different distances and used LDA on HFMs and WMs characteristics. We found that relative distance information can be inferred from the vibration signals. Examination of the percentage of correct classifications for all variables revealed that distance discrimination was correctly classified in 95% of whisks (n = 4).

Rats actively sweep their whiskers across surfaces in a rhythmic forward and backward motion with a frequency ranging from 5 to 25 Hz. To determine the influence of whisking frequency on texture discrimination, we induced artificial whisking at frequencies of 6 and 12 Hz. An example of whisker B2 responses to texture P150 at these frequencies is shown in Fig. 9A. Whisking frequency had a considerable influence on vibration signals transmitted by the whisker. The difference between the two frequencies was further confirmed by comparison between all studied variables in response to the same texture (n = 3; Fig. 8B; B2, P150). The panel shows that at higher whisking frequency, most variables were reduced in magnitude due to a smaller whisking trajectory.

We next evaluated the influence of whisking frequency on how well the whiskers performed when LDA was used to classify single whisks into the different textures. While the whiskers presented different response profiles at the different frequencies, their ability to discriminate between different sandpapers did not decline. Figure 9C depicts an example of the discrimination performance of B2 whisker. As before, we calculated classification scores using all variables and found that whisking frequency did not influence the transmission of texture information.

**DISCUSSION**

Active sensation in the rodent vibrissa sensory system is commonly used to discriminate the shape, size, and texture of objects (Ahissar and Kleinfeld 2003; Kleinfeld et al. 2006). Despite broad interest in this system and the consensus that whisker vibrations carry tactile information, most studies that analyzed whisker vibrations used passive whiskers stimulation (but see Arabzadeh et al. 2005). The present report provides a systematic analysis of whisker vibration signals during artificial whisking across textures in anesthetized rats. We discovered that tactile information is transmitted through high-frequency micromotions superimposed on whisking macromotions. Consistent with this, we found that in most TG neurons, spike activity and high-frequency micromotions are closely correlated. Despite a large variability in the translation process, different textures are translated into distinct vibration profiles. This translation can be mediated by all whisks and is influenced by the mechanical properties of the whiskers, the radial distance of the surface, and whisking frequency. Hence in natural conditions, numerous factors influence texture-dependent whisker vibration signal as they arrive at the follicle and may influence texture discrimination. We propose that texture discrimination may require the integration of signals from multiple whisks and whiskers to disambiguate surface roughness. We tested this prediction by combining tactile information from multiple whisks and whiskers. Our results show that increasing the number of whisks and integration of information from multiple whisks improved texture discrimination. The general picture that emerges from the present experiments is that although tactile information can be transmitted through a single whisker, multi-dimensional stimulus space may require spatio-temporal integration of several sensory channels.

**Methodological considerations**

Before discussing the implications of these findings, it is important to consider the set of assumptions on which interpretation is based and the similarities and the differences between our results and those that were reported for awake behaving animals. The first concerns stimulus configuration. In awake whisking animals, the whiskers move rostrocaudally as they scrape against the surface of an object (Brecht et al. 1997; Carvell and Simons 1990; Hartmann 2001; Ritt et al. 2008). In
the current study, whisker stimulation is done by having a flat surface contact a whisker. Textures are located so that plane of the surface is placed in parallel to the whisker axis. This configuration is a modified stimulus arrangement that was employed recently (Arbazedeh et al. 2005) and was chosen for the following reasons: first, under natural conditions, rats may contact a surface at the tip, in the middle, or close to the base of the vibrissa (Brecht et al. 1997; Hartmann 2001; Ritt et al. 2008) and they frequently sample textures with the vibrissae pressed against the sensory surface (Carvell and Simons 1990, 1995 observed typical contact points at \( \approx 15 \) mm from the face, whereas Ritt et al. have seen contact points at 8–12 mm from the face). In several studies that investigated the translation of surface roughness into whisker vibrations, the vibrissae were pinned horizontally against a rotating surface with a contact point at \( \approx 15 \) mm from face (Neimark et al. 2003) or were lying on a 3 \( \times \) 3 mm surface 7 mm from the face (Arbazedeh et al. 2005). In contrast, we included artificial whisking and large surfaces at realistic distances. In some experiments in which only the tip made contact with the surface the vibrations transmitted to the shaft were diminished (see Fig. 8), and the duration of contact were changing from whisk to whisk. In these conditions, the whiskers were more prone to get stuck in grooves of the surface, resulting in a large variability in vibration signals from whisk to whisk (it was technically not feasible to view the tips of the whiskers and to place them in an accurate and precise location to create reliable responses). These problems may be alleviated in the awake animals by including head and body movements (Ritt et al. 2008). Second, although whisking is not restricted to a single plane of motion, it was claimed elsewhere that movement along the anterior-posterior axis contains most texture-specific signals (Arbazedeh et al. 2005). We have confirmed this in the current study, having found that dorso-ventral motion carries only insignificant tactile information (Supplementary Fig. S4). Therefore we examined the principal direction of vibrissae movement, corresponding to the horizontal forward-backward axis. Third, artificial whisking generates whisking-like movement of the vibrissae. However, it should be kept in mind that the actual displacement generated by electrical whisking deviates from naturally occurring whisking. For example, whiskers movement is controlled by the facial motor nerve, which innervates two classes of muscles: intrinsic and extrinsic muscles. In awake animals, whisker retraction probably involves activation of extrinsic facial muscles (Berg and Kleinfeld 2003), whereas in our experimental paradigm, retraction is passive. Furthermore, artificial whisking results in movement of the whole pad and simultaneous activation of all muscle groups that work at odds with one another during protraction. While whisking movements during artificial and natural conditions may differ in detail, the range of HFMs magnitudes (Fig. 5A) (Ritt et al. 2008) and whisking amplitudes (Fig. 5E) (Berg and Kleinfeld 2003; Carvell and Simons 1990; Knutsen et al. 2005), which are well within the range reported in awake behaving animals, imply that the principles by which surface roughness is translated into whisker movement properties should be similar. Finally, in awake animals, whisking patterns vary across cycles (Carvell and Simons 1990; Gao et al. 2001) and between whisking bouts (O’Connor et al. 2002). Furthermore, whisker movements are actively controlled so as to increase the likelihood of environmental contacts (Knutsen et al. 2006; Mitchinson et al. 2007). During artificial whisking the contraction of whisker muscles is completely independent of contact. Hence although the basic pattern of movement trajectory during constant frequency bouts is preserved in our experiments, the details of movements and mechanical interactions probably differ.

Texture coding

Rats use their whiskers to discriminate surface roughness (Carvel and Simons 1990, 1995; Guic-Robles and Jenkins 1992; Guic-Robles et al. 1989). One model posits that different whiskers are tuned to different resonant frequencies, which vary systematically with whisker length along whisker rows and thus split the tactile vibration signals into labeled frequency lines in the cortex (Andermann et al. 2004; Hartmann et al. 2003; Mehta and Kleinfeld 2004; Moore 2004; Moore and Andermann 2005; Neimark et al. 2003). Our results do not rule out the possibility that rats utilize the gradient of resonant frequencies across the whisker array, as proposed by this theory. However, we did not observe an obvious gradient of prominent resonance frequencies across the whisker array as expressed in HFMs interval (Fig. 7B). This may be due to our stimulus configuration in which the whiskers were lying on the surface in contrast to the configuration reported by Neimark and colleagues (2003). The gradient of mechanical properties across the whisker array was evident in other characteristics such as the number and amplitude of HFMs. Moreover, altering these mechanical properties by trimming the whiskers leads to a reduction in texture-related events, amplitude and ITI, suggesting that whisker length plays a critical role in the translation of surface roughness to whisker vibrations (not shown). Finally, placing the surfaces at different distances modifies all aspects of vibration signals reaching the follicles (Fig. 7B) (see also Fig. 7A in Ritt et al. 2008), suggesting that the mechanical properties of whiskers play a critical role in the translation of surface roughness to whisker vibrations. Alternatively, it was shown that the mechanical properties of the vibrissae act to translate surface roughness into different intrinsic frequency or velocity modes for each vibrissa (Andermann et al. 2004; Hipp et al. 2006). The differences between surfaces are expressed by the extent to which the different modes are favored within each vibrissa (Fend et al. 2003; Mehta and Kleinfeld 2004). In line with these earlier findings, our results show that individual whiskers provide information about a broad range of textures and that whisker vibrations provide a potential cue that might be used for the accurate discrimination of tactile stimuli. These results do not rule out the use of resonance-based vibration signals comparisons across whiskers for texture discrimination; however, they suggest that a whisker should not be regarded as a band-pass filter for tactile information. Taken together, the findings of the current study indicate that although additional information might be available due to differences in the mechanical properties among whiskers, even a single whisker can transmit large amounts of texture-specific information to its downstream neural circuits.

While these models remain to be rigorously tested, potentially important properties of active whisking were neglected. First, under natural whisking conditions, the contact point of the whisker with the surface and the forces at the contact point are likely to change continuously, producing a dynamic mod-
ulation of the mechanical properties of the system and variable vibratory tactile information. Here the whiskers were moved forth and back across textures in an attempt to mimic the dynamics of natural whisking. However, changes in the distance of surfaces (Fig. 8) or repositioning of the surfaces at the same distance (not shown) resulted in a critical alteration in vibration signal and the reliability and accuracy in which whiskers transmit this information. Second, as rats whisk across object surfaces they continuously change the geometry of the whisker relative to the texture. They can change the amplitude, speed, and direction of motion (Mitchinson et al. 2007; Sachdev et al. 2001; Towal et al. 2008), producing ever-changing patterns of sensory inputs in different sets of fibers during the scan of regularly textured surfaces. We have tested these, in part, in the current study and found that vibration signals that transmit tactile information are dependent on the radial distance of the textures and on whisking frequency. Third, there is evidence for changes in whisking motor strategy according to the discrimination task. In general, rats vary their whisking amplitude and speed (Berg and Kleinfeld 2003), depending on task type (Carvell and Simons 1995), which, in turn, may modulate the mechanical properties of the whiskers. This fits well with our results which show that whisking frequency modulated all whisker vibration characteristics (Fig. 9). Finally, active whisking movements are always associated with head and body movements. When rats are engaged in a localization task, in which they estimate the location of objects relative to their head, they typically whisk (Knutsen et al. 2005). However, rats often rely solely on passive movement of the vibrissa secondary to body and head movements. Specifically, rats use their vibrissae but do not whisk as they maintain contact with walls and surfaces (Milani et al. 1989; Ritt et al. 2008) while running and while performing an aperture-size discrimination task (Krupa et al. 2001). Thus the vibrations induced in a whisker as it sweeps over a surface are likely to depend, in addition to the structure of the textured surface, on intrinsic and extrinsic variables such as the properties of active whisking (frequency, sweep amplitude, whisker mechanical properties), the distance between whisker shaft and the surface, etc.

Several studies have examined the spatial and temporal constraints on texture discrimination and object localization. These studies have argued that tactile information may be available through a single whisker (Carvel and Simons 1995; Hipp et al. 2006; Knutsen et al. 2006; Krupa et al. 2001) on the time scale of a couple of whiskses (Mehta et al. 2007). It should be noted, however, that these studies have examined whisking behaviors during tactile discrimination tasks that necessarily require intense training of the animals and constrain the way in which the animal contacts the discriminandum. For example, single whisker tasks occur after the animals were heavily trained with all whiskers intact. Animals in single whisker tasks succeeded only in the easier discrimination tasks (Fig. 7 in Carvel and Simons 1995; Krupa et al. 2001). In contrast, other studies have shown that rodents employ several whisks for texture discrimination (von Heimendahl et al. 2007) and object localization (Knutsen et al. 2006) and in some experimental setups, animals used several whiskers for texture discrimination (Ritt et al. 2008).

We propose, based on our results and the literature (as discussed above), that due to a large variability in the translation of surface roughness to vibration signals, and the influence of numerous variables (see preceding text) on this translation, texture discrimination may require the integration of signals from multiple spatial and temporal sensory channels (whiskers, whiskers, neurons). In this framework, the relay of sensory inputs may not actually rely on the frequency-following ability of individual TG neurons (Andermann et al. 2004; Jones et al. 2004) but rather on coordinated discharges among an ensemble of neurons having different response preferences. This conjecture is consistent with recent studies showing that individual TG neurons encode different aspects of tactile signals that may underlie object localization (Szwed et al. 2003, 2006), provide information about the movement of whiskers in the air and encode information regarding contact with an object (Leiser and Moxon 2007), and mediate the detectability of a single whisker deflection as a function of amplitude and peak velocity (Stütten et al. 2006). Thus the discrimination of behaviorally relevant tactile stimuli can be explained, in part, by dynamic mechanisms that integrate sensory information from multiple channels. For example, disambiguation of texture signals at different distances can be achieved when the animal can access information about its distance from the surface and its self-generated whisking motion. This information can be inferred from whisker vibration signal, and from distance signal (Afissar and Kleinfeld 2003; Szwed et al. 2006) and whisking signal (Crochet and Petersen 2006; Fee et al. 1997; Gauguly and Kleinfeld 2004; Kleinfeld et al. 2006; O’Connor et al. 2002). The distance signal is relayed by the sensory afferents. The whisking signal, in contrast, could originate either as an efferent copy relayed from the motor system to the sensory system (Ahrens and Kleinfeld 2004) or as a sensory signal from the whisker follicle (Szwed et al. 2003). In summary, the rat’s knowledge of spatial coordinates and of its own motor output could make the sensory input more reliably decodable.

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