Neuron
Article

Discrimination of Vibrotactile Stimuli in the Rat Whisker System: Behavior and Neurometrics

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DOI 10.1016/j.neuron.2010.02.007

SUMMARY

Understanding the neural code underlying perception requires the mapping of physical stimulus parameters to both psychophysical decisions and neuronal responses. Here, we employed a novel psychophysical task in head-fixed rats to measure discriminability of vibrotactile whisker deflections. Rats could discriminate 90 Hz from 60 Hz pulsatile stimuli if stimulus intensity covaried with frequency. To pin down the physical parameters used by the rats to discriminate these vibrations, we manipulated stimulus amplitude to arrive at pairs of nondiscriminable stimuli. We found that vibrations matched in intensity (measured as mean absolute velocity), but differing in frequency, were no longer discriminable. Recordings of trigeminal ganglion neurons revealed that the distribution of neurometric sensitivities based on spike counts, but not interspike intervals, matched the rats' inability to discriminate intensity-matched stimuli. In conclusion, we suggest that stimulus mean absolute velocity, encoded in primary afferent spike counts, plays a prominent role for whisker-mediated perception.

INTRODUCTION

Rats use active vibrissa movements to discriminate textures at an amazingly fine level (Carvell and Simons, 1990). However, it is largely unclear which of the physical parameters of vibrissa vibration is encoded by the ascending tactile system and serves as the basis for texture discrimination. There exist several parameters which describe different aspects of vibrotactile signals. First, *kinematic events* can be extracted instantaneously from the vibrotactile signal, e.g., events surpassing a threshold of high amplitude, velocity, or acceleration (Arabzadeh et al., 2005; Jadhav et al., 2009; Wolfe et al., 2008). Second, *physical intensity* (henceforth simply referred to as intensity) is conventionally assessed by variables proportional to temporal integration of powers of velocity (e.g., mean absolute velocity, power, kinetic energy, etc.) (Arabzadeh et al., 2003). Third, *frequency* can be defined as number of cycles per second for repetitive stimuli (present study) or, in the general case, by spectral analysis (e.g., spectral centroid as in Hipp et al. [2006] or best frequency). Like intensity, frequency requires temporal integration of the raw signal. Finally, vibrotactile stimuli may be described by referring to perceptual categories emerging in human descriptions of tactile experience such as *pitch*, *roughness*, and *subjective intensity*. These have been sometimes used in studies of the monkey tactile system (Hernandez et al., 1997; LaMotte and Mountcastle, 1975). However, as perceptual qualities of the whisker-related tactile sense are unknown, we will not use perceptual categories in the present report and focus on the first three physical parameters. To determine the dominant parameter for psychophysical discrimination we used pulsatile stimuli (Salinas et al., 2000) that are helpful for the disentanglement of physical cues because their intensity can be manipulated by changing either interpulse intervals (base frequency) or pulse waveform (kinematic events) independently. With sinusoids, this is not possible because frequency and kinematic events are necessarily interrelated.

Besides the search for relevant stimulus cues, another significant question in the physiology of perception is which coding symbol is used to convey information to subsequent processing stages in the tactile pathway. Recordings from primary afferents in rats (Jones et al., 2004; Shoykhet et al., 2000; Stüttgen et al., 2006), from somatosensory thalamus (Petersen et al., 2008), and from primary somatosensory cortex neurons (''barrel cortex''; Pinto et al., 2000; Stüttgen and Schwarz, 2008) showed that kinematic events of whisker vibrations are represented by spikes with great temporal precision. Furthermore, repetitive whisker deflections up to ~300 Hz evoke one-by-one phase-locked responses in primary afferents, brainstem, thalamocortical, and even barrel cortex units in anesthetized rats (Deschênes et al., 2003; Ewert et al., 2008). This precision suggests that spike intervals may be used to encode vibrotactile signals. However, the issue must be considered to be unresolved, as perceptual

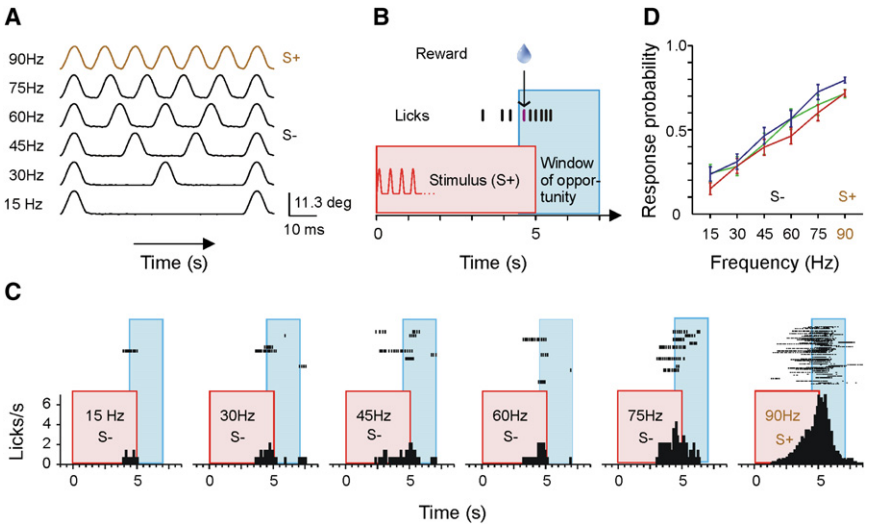

Figure 1. Vibrotactile Discrimination Psychophysics

(A) Cutouts from the pulsatile stimulus waveforms as measured with photodiodes (see Experimental Procedures). One pulse approximated a single period of a sinusoid starting from the curve's minimum. The 90 Hz stimulus was used as rewarded stimulus (S+); the others were unrewarded (S−).

(B) Behavioral paradigm. The presentation of a S+ stimulus is shown schematically. The first lick (marked violet) inside a window of opportunity (blue box) with onset 4.5 s after stimulus onset (red box) produced a drop of water.

(C) Lick histograms and raster plots of responses to S− and S+ stimuli (box colors as in B). Note that the late onset of licking responses after stimulus presentation was deliberately conditioned and does not reflect minimal reaction times.

(D) Psychometric performance of three rats (colors match the ones used in Figure 2C). Error bars are 95% confidence intervals estimated from a binomial model of responses.

measurements in the monkey fingertip system have shown that these animals are able to discriminate mean frequency of pulses given at regular as well as irregular intervals, hinting at the importance of spike counts rather than periodicity as the code for vibrotactile discrimination (Hernandez et al., 2000; Salinas et al., 2000).

The touchstone to identify the relevant encoded parameter and the associated coding symbol for texture discrimination will be the comparison of neuronal sensitivity and the animal's percept as measured by psychophysical performance. As a step toward this end, we established a novel paradigm in head-fixed rats to assess the rats' sensitivity in a vibration discrimination task. We compare the results gained from these experiments to responses of primary afferents obtained in acute experiments. We present evidence that rats use intensity (as opposed to frequency) cues to perform fine discriminations of stimuli in the range of 60 to 90 Hz, a range that carries most of the power of vibrissa vibration elicited by a range of complex fine textures (Hipp et al., 2006). Sensitivity measures of spike counts in primary afferents matched the dependency on intensity for fine discriminations while spike intervals, a possible coding symbol encompassing precise temporal spike timing, failed to do so.

RESULTS

Psychometric Performance

As a first approach, we employed a set of pulsatile stimuli that varied the interpulse interval while keeping the waveform of the pulses constant. In these stimuli, frequency and intensity covary while kinematic events—maximal amplitude, maximal velocity, and maximal acceleration—are constant across the stimulus array (Figure 1A). We trained three animals on a go-no-go task (Figure 1B) to indicate the presence of a pulsatile stimulus at 90 Hz and 11.3° ("go," rewarded, S+) by licking from a water spout in front of their snout during a window of opportunity at the end of the stimulus, while abstaining from responding to frequencies of 15, 30, 45, 60, and 75 Hz having the same amplitude ("no-go," nonrewarded, S−). To achieve good discrimination, it was important to prevent impulsive licking (seen with both S+ and S−) shortly after stimulus onset, a behavior observed with all rats in the early phases of training. To this end, the window of opportunity, which initially started simultaneously with the stimulus, was shifted (across several training sessions) toward the end of the stimulus. Figure 1C demonstrates licking events emitted by a well-trained animal with respect to onset of stimulus (time 0) and the window of opportunity (4.5 s). It can be appreciated that the animal typically only emitted the first licks a few seconds following stimulus onset, which allowed it to concentrate well on the features of the vibration. Only sessions that were recorded after the animals regularly generated this type of responses and that met a criterion of minimal discrimination between the two most different stimuli (15 versus 90 Hz, see Experimental Procedures) entered the present data set. From the trials of these sessions, psychometric curves were calculated (Figure 1D; sessions: n = 38, 36, and 27; trials: n = 4004, 3971, and 2408 for each of the three rats). Response probability increased monotonically with increasing stimulus frequency, indicating increasingly poorer discriminability for S− stimuli with frequencies closer to 90 Hz. Hit rates reached 71.8%, 79.5%, and 71.4% with the rewarded stimulus (90 Hz) and 14.9%, 23.6%, and 24.1% with the nonrewarded stimulus at lowest frequency (15 Hz; each value for one of the three animals).

These findings demonstrated that a difference in kinematic events is not a necessary condition for discrimination—all stimuli used so far featured identical peak amplitude, velocity, and acceleration. Our next goal was, therefore, to shed light on the respective roles of intensity and frequency cues. Before doing this, it had to be clarified which unit of measurement the tactile system actually uses to assess intensity. In general, intensity can be defined by the integral of different powers of velocity. For instance, "mean velocity" is the integral of the first power of velocity while "kinetic energy" and "power" are proportional

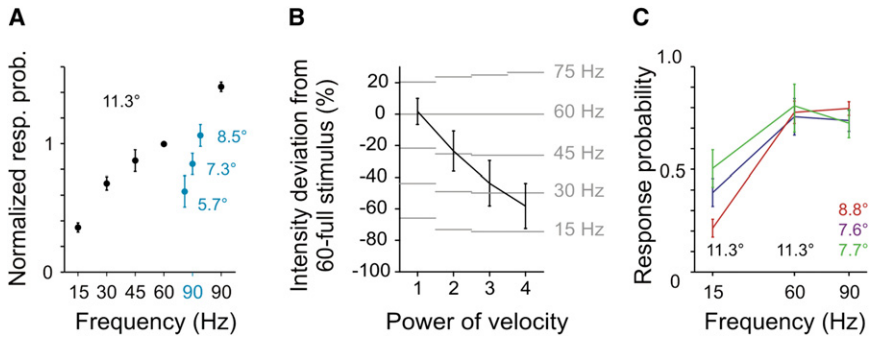

Figure 2. Psychophysical Experiments to Find Intensity-Matched Stimuli

(A) Psychophysical experiment using stimuli as in Figure 1A, except that a 90 Hz stimulus at reduced amplitudes (blue) was substituted for the 75 Hz. Response rates were normalized to the response rate to the 60 Hz stimulus in each session and averaged across rats.

(B) Intensity of full-amplitude stimuli at different frequencies (gray lines and labels) and the mean 90-reduced as measured from three animals (see C, black line, error bars are standard deviation), plotted as deviations from the intensity of the 60-full (ordinate). Different powers of velocity were used to calculate the intensities (abscissa). Trivially, the deviation calculated for the 60-full is 0 for all powers of velocity. The intensity of the 90-reduced stimuli matches the one of 60-full only with velocity taken to the power one, but diverges with higher powers. Using a power of 2, the intensity of the 90-reduced compares to the 45-full and reaches levels below 30-full for the power of 4.

(C) Psychophysical experiment that used 90 Hz stimuli at amplitudes of 7.6°, 7.7°, and 8.8° ("90-reduced") that were found for each rat to match the response probability of 60 Hz at full amplitude (11.3°) ("60-full"). The performance of all three rats is shown in colors corresponding to Figure 1D. All rats readily discriminated 15-full from 90-reduced but failed to discriminate 60-full from 90-reduced. All error bars in (A) and (C) correspond to 95% confidence intervals estimated from a binomial response model.

to integrated squared velocity (throughout this article, we use the term "mean velocity" as a short for "mean absolute velocity"). The use of powers of velocity higher than two seems feasible as well (Arabzadeh et al., 2003). It is important to note that calculating intensity of one and the same vibrotactile stimulus yields different values, depending on which power of velocity is used. On the other hand, two physically different stimuli may feel the same to a subject if they match in intensity. Consequently, determining such a stimulus pair should reveal the relevant physical parameter that is used by the animals. Following this strategy, we employed a behavioral paradigm similar to prior work in the primate tactile system designed to match stimuli for subjective intensity (Goff, 1967; LaMotte and Mountcastle, 1975; Mountcastle et al., 1990; Salinas et al., 2000). We modified our original psychophysical experiment by substituting the 75 Hz stimuli in our stimulus array with several 90 Hz stimuli at different reduced amplitudes (tested amplitudes ranged from 5.7°–8.5° in consecutive blocks of sessions) that would approximate the rats' response probabilities to the well-discriminable pulsatile stimulus of 60 Hz at full amplitude (tagged "60-full"; Figure 2A). As before, the rewarded stimulus was 90 Hz at the full pulse amplitude of 11.3° (tagged "90-full"). The best match of response probabilities to the different 90-reduced and 60-full was determined for each rat individually using regression analysis and were found with stimuli at 8.8°, 7.7°, and 7.6° amplitude (henceforth, the tag "90-reduced" will be used for these best-matching stimuli). To find out if measures of intensity are matched in these stimulus pairs, we analyzed trajectories of all stimuli as tracked by photo diodes (see Experimental Procedures). Comparing the response-matched 90-reduced and 60-full stimuli, as obtained from the three animals, revealed that the mean velocity of both stimuli did in fact match, whereas intensity measures based on higher powers of velocity deviated more and more with higher powers (Figure 2B). This strongly suggested that the whisker-related tactile system measures intensity as mean velocity rather than energy or power.

The fact that intensity as defined by mean velocity was equal in the response-matched stimuli but frequency and kinematic events diverged sharply allowed us to confront the subject with a task to directly discriminate 60-full and 90-reduced (note that in the previous experiment, 60-full and 90-reduced were both presented within the group of nonrewarded stimuli) with the aim to clarify whether the equality of mean velocity of the two stimuli would abolish the ability to discriminate them. In this case, we would conclude that mean velocity is the cue on which discrimination between 60-full and 90-full is based. We retrained the three rats to directly discriminate between the two stimuli using 90-reduced as the new rewarded stimulus. To avoid frustration of the rats when confronted with potentially nondiscriminable stimuli, we first set up an easy task: the discrimination between 90-reduced (rewarded) and 15-full (unrewarded). Once the discrimination performance on this task was good, we introduced the (unrewarded) test stimulus 60-full. In one block of stimuli, 15-full was presented three times, 60-full two times, and 90-reduced five times. The animals were trained to the new task over 14, 9, and 7 additional training sessions over 10, 6, and 6 days to reach criterion performance (computed as difference between the mean of 90-red versus 60-full and 15-full). Two of the animals reached superior discrimination performance as demonstrated by the large difference of response probability to 15-full and 90-reduced (Figure 2C). One rat did not perform as well on this task as on the original one but still reached a highly significant level of discrimination between 15-full and 90-reduced (green curve and confidence intervals in Figure 2C). Despite the high level of general discrimination performance, all rats failed to discriminate between 60-full (unrewarded) and 90-reduced (rewarded; mean differences in response probability < 0.09 for all animals). This suggests that the rats based their discrimination performance on mean velocity, or equivalently, that the distinct frequencies and kinematic events of the stimuli were not used (60-full: 60 Hz, amplitude 11.3°; peak velocity 3600°/s; 90-reduced: 90 Hz, amplitude 7.6° to 8.8°; peak velocity ~2600°/s). We thus suggest that fine

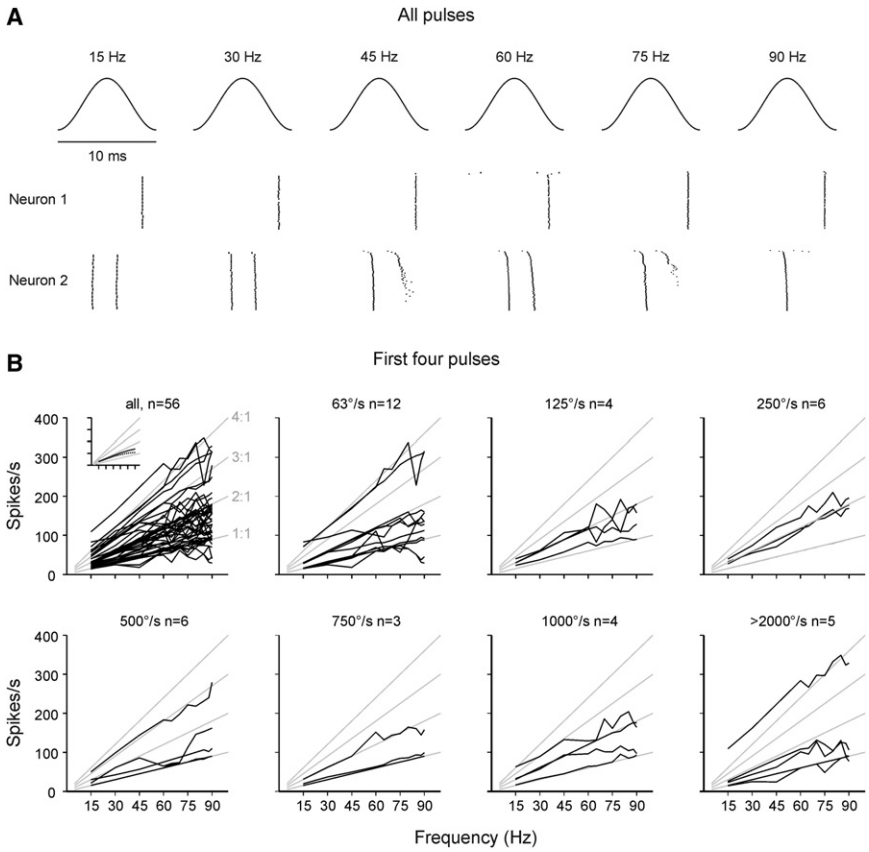

Figure 3. Tuning Curves of Trigeminal Primary Afferents

(A) Two representative neuronal responses to single pulses within the pulsatile stimuli. On top, the waveform of single pulses are shown. The raster plots show spike responses of two neurons to all pulses within each stimulus aligned to the waveforms. The topmost row in the raster plots corresponds to the response to the first pulse in the series, the second row to the second, etc. The first neuron shows spikes at stable latencies, while the second shows systematic shifts in latencies and failure of spiking. For the remainder of the study, we therefore used tuning curves calculated from the first four pulses of a stimulus.

(B) Tuning curves. The panel on the top left shows all tuning curves obtained from the first four stimulus pulses in the present study. The inset shows the mean tuning curves averaged over 56 neurons (continuous line, first 4 pulses; broken line, all pulses) using the same axis scaling as the larger plots. Gray lines indicate the form of hypothetical tuning curves that show exact 1:1 to 4:1 locking ratios as marked in the upper left graph (e.g., 4:1 indicates four spikes per pulse). Tuning curves even within the response to the first four pulses are irregular mostly for frequencies above 60 Hz. The other plots break down a subset (n = 40) of the tuning curves according to their velocity threshold in response to ramps (Stüttgen et al., 2006). The threshold is given in the title of the graphs. Tuning curves wholly or partly deviating from integer locking ratios are observed with cells throughout the ranges of velocity thresholds (across panels) and response strengths (across gray lines within panels).

discrimination of pulsatile frequencies is carried predominantly by intensity cues.

Neurometric Performance of Primary Afferent Neurons

As the trigeminal ganglion shows a coarse somatotopy and the receptive fields of its neurons encompass only one whisker (Leiser and Moxon, 2006), we refrained from trying to sample a sufficient number of unit recordings that respond to whisker C1 in chronically implanted and trained animals. Pooling of neurons with different receptive fields was not an option either, as our experimental design requires highly overtrained animals to obtain asymptotic threshold conditions. Thus, the animals would need to go through complex and lengthy behavioral training in order to make them generalize the discrimination performance across a large fraction of macrovibrissa (some 25–30 of which are present, Brecht et al., 1997) and subsequently work them down to stable thresholds. Therefore, we opted to compare the animals' psychometric performance with neurometric data collected in anesthetized animals, a strategy that has proven fruitful in the past (e.g., Stüttgen et al., 2006). For these experiments, we presented identical stimuli as used in behaving animals plus additional ones between 60 and 90 Hz, while recording from single units in the trigeminal ganglion.

Spike counts as well as interspike intervals that occurred during the entire stimulus presentation were assessed from 56 neurons, all showing strictly single-vibrissa receptive fields. It is important to note that only a minority of primary afferents showed reliable spike response to the pulses contained within all stimuli, characterized by a monotonically ascending tuning curve across frequencies (exemplified by neuron 1 in Figure 3A, 13 out of 56 cases). A majority of cells showed slow latency shifts (36 out of 56) that, in many cases, led to permanent loss of spikes from doublets (triplets) across subsequent stimulus pulses in a highly frequency-dependent way (29 out of 56, exemplified by neuron 2 in Figure 3A). Out of 56, 32 cells showed irregular dips in the tuning curve, and another 10 showed an inverse U-shape of the tuning curve. These slow effects evolving across several pulses within one stimulus presentation may not be relevant given that the presence of complex structures in the whisker-follicle assembly, like blood-filled sinus, etc., may prevent them in awake, actively moving rats. Furthermore, it has been shown that for detection of pulsatile stimuli, only the very first few pulses are evaluated by rats (Stüttgen and Schwarz, 2010). We, therefore, recalculated tuning curves based on spike counts obtained after the first four pulses of a frequency stimulus. As expected, this manipulation removed many instances of spike loss of the sort shown in Figure 3A (neuron 2; 12 out of 29). Accordingly, some nonmonotonic curves were removed by this procedure—a subset of tuning curves straightened and approached a linear course (n = 27).

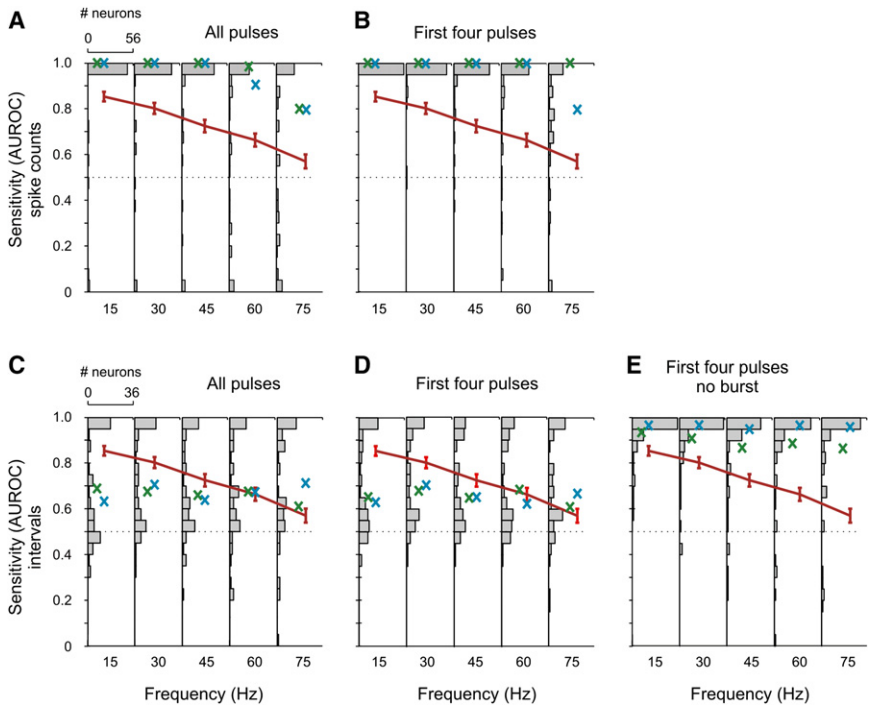

Figure 4. Comparison of Neurometric and Psychometric Sensitivity Expressed as Area Under the ROC Curve

Neurometric sensitivities are presented as histograms for each discrimination pair ([90-full, 15-full], [90-full, 30-full], etc.). The histograms are composed of the sensitivities of the whole sample of primary afferents (n = 56). Median sensitivities are indicated by the blue crosses. Green crosses mark neuronal sensitivities that were achieved in a control experiment, where whisker movement as measured from awake behaving rats and pulsatile stimuli were overlaid. Psychometric sensitivities (cf. Figure 1D) are indicated by red lines. Error bars indicate 95% confidence interval based on a binominal response model.

(A and B) Neurometric sensitivities based on spike counts obtained from responses to all pulses (A), and the first four pulses (B). Neurometric sensitivities exceed the psychometric ones, but the medians reflect the decline in sensitivities for S− closer to 90 Hz.

(C–E) Neurometric sensitivities based interspike intervals obtained in response to all pulses (C), the first four pulses (D), and the first four pulses and additional removal of bursts (i.e., intervals smaller than 6 ms) (E). Neurometric sensitivities partially exceed the psychometric sensitivities if bursts are contained in the data (C and D), but clearly exceed the psychometric ones after burst removal (E). The median sensitivities do not reflect the decline of psychometric sensitivities for S− closer to 90 Hz.

However, another subset of neurons lost their monotonic tuning curve (n = 15): the sample of tuning curves, therefore, still contained a significant number of curves with kinks, most conspicuously for frequencies above 60 Hz. The upper left panel of Figure 3B shows all tuning curves obtained from the first four pulses. Next, we asked if the amplitude of our stimuli drives the neurons into saturation, thus causing the observed nonmonotonic curves. It is clear that varying response properties within the population of trigeminal afferents cover a wide range of naturally occurring vibrotactile stimuli. Thus, for example, cells exist that either show low or high velocity thresholds (Stüttgen et al., 2006). The kinematic events of our stimuli were designed to recruit most of the primary afferents; thus, the kinematic events contained in the pulses were presumably in the upper reaches of the dynamic range covered by the trigeminal ganglion as a whole. If the observed nonmonotonic curves emerge because the neurons with lower thresholds are driven into saturation, we would expect to find a relationship between nonmonotonicity and kinematic threshold (e.g., velocity threshold). We assessed velocity thresholds in 40 cells of our sample using ramp and hold stimuli at an amplitude of 11.3° as described before (Stüttgen et al., 2006). It turned out that, contrary to the expectation, nonmonotonicity was independent of the velocity threshold of the neuron: low (62°/s), as well as high (>2000°/s), threshold cells showed kinks in the tuning curves (Figure 3B).

Next, we applied ROC analysis to convert the distributions of spike counts and spike intervals contained in stimulus-evoked responses to neurometric sensitivity, i.e., the ability of an ideal observer to discriminate the rewarded stimulus (90 Hz) from each of the other stimuli (Green and Swets, 1966; see Figure 5 of Britten et al., 1992 for illustration). The resulting sensitivities for each stimulus pair were then compared to the psychometric sensitivity calculated from the rats' lick responses in the behavioral experiments. Figure 4 plots the psychometric curve (red), averaged across three animals, versus the full distribution of neurometric sensitivities to discriminate each of the nonrewarded stimuli (15, 30, 45, 60, and 75 Hz) from the rewarded stimulus (90 Hz) (gray histograms), as well as the median sensitivity (blue crosses) taken from our sample of 56 primary afferents. It is evident that many neuronal sensitivities exceeded the psychometric ones for all stimulus pairs. Using spike counts sampled across the entire stimulus duration as the coding symbol, neuronal sensitivities were typically found in the best sensitivity bin (>0.95) and far exceeded the psychophysical sensitivity using spike counts (Figure 4A). As rats perform detection of pulsatile stimuli at minimal reaction time, allowing them to evaluate only the very first pulses (Stüttgen and Schwarz, 2010), it is likely that only the very first pulses are used by the rats as well for discrimination. We, therefore, calculated the sensitivity for the first four pulses only. This yielded virtually the same result as when the full stimulus train was used (Figure 4B). The median neuronal sensitivity based on interspike intervals was clearly lower than the one based on spike counts and the psychometric performance (Figure 4C). This was due to a bimodal distribution of neuronal sensitivities that contained many cells at sensitivities close to 0.5 (i.e., chance performance) and another group with

excellent sensitivities close to 1. The bimodal distribution was basically unchanged if only the responses to the first four pulses were analyzed (Figure 4D). However, after elimination of burst responses to individual pulses (cf. the demonstration of doublet, triplet responses to individual pulses; Figures 3) revealed excellent sensitivity of nearly all cells (Figure 4E).

In order to control for the possibility that whisker stimulation in the anesthetized preparation differed from the one in the awake behaving animal due to active whisker movements, we measured residual whisker movements in two rats performing the discrimination task using a photodiode focused on the part of the whisker between the tip of the glass tube used for stimulation and the snout (see Experimental Procedures). Both maximum whisker displacement and mean velocity values showed a strong, positive skew, and in such a low range as to be unlikely to affect trigeminal ganglion, single unit responses significantly. The median of average absolute velocity calculated from each trace was 8.81°/s (interquartile range, 33.62°/s). These kinematic values fall far below the detection threshold of rats assessed with ramp and hold stimuli, which revealed a threshold for the more velocity-sensitive psychophysical channel W1 to be 250°/s (Stüttgen et al., 2006), and would not evoke responses in barrel cortex neurons (Stüttgen and Schwarz, 2008). Still, to completely rule out the possibility that spontaneous whisker movements in this task significantly affect trigeminal ganglion responses, we tested whether unit responses to our stimulus set changed when whisker traces from the behavioral sessions were superimposed on the pure stimuli. To this end, whisker traces with mean velocity higher than the 75th percentile of all mean velocities were randomly chosen with replacement and overlaid on pure stimulus traces (15–90 Hz; 10 repetitions per frequency). The median neurometric sensitivity of these 15 additional neurons is shown as green crosses in Figure 4. It is clear that trigeminal neurometric sensitivity is superior to psychometric sensitivity, even under the unrealistic, worst case assumptions used here, indicating that the difference between neurometric and psychometric curves is largely independent of possible different whisker stimulation in awake versus anesthetized rats.

In summary, the results presented so far do not provide critical arguments to exclude spike counts or intervals as candidates for the coding symbol on which discrimination performance is based. It is well feasible that trigeminal signals are low-pass filtered (removing burst firing) before being decoded, as has been suggested previously (Stüttgen et al., 2006, Stüttgen and Schwarz, 2010). Thus, the superiority of neurometric over psychometric curves (Figures 4B and 4E), indicates that spike counts, as well as interburst intervals, could potentially serve as a carrier of the relevant stimulus information. To further explore this question, we asked whether sensitivities computed from the two coding symbols would bear out the fact that the animals discriminated well between 15-full and 90-reduced but failed to discriminate between intensity-matched 60-full and 90-reduced stimuli. We found that average spike counts to 90 Hz at 7.6° were in the range of the ones obtained with 60-full (94.3 ± 48.1 spikes/s for 60-full versus 90.6 ± 55.5 spikes/s for 90-reduced, mean ± SD), suggesting that the response per individual pulse must have reflected the different

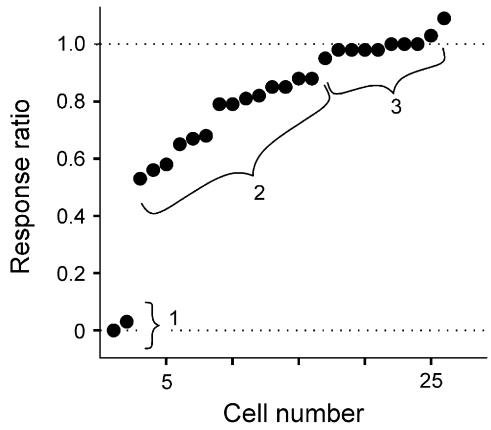

Figure 5. Primary Afferent Responses to Different Intensities
Recruitment of primary afferents (n = 26) by pulse amplitude. The ratio of spike counts with 7.6° and 11.3° amplitude at 90 Hz is plotted for each of the analyzed cells. Two neurons were recruited de novo by the larger pulses (group 1), 14 increased their response (group 2), and 10 showed only minor differences (group 3).

kinematic parameters. Indeed, on average primary afferents generated 50% more spikes to an individual pulse at 11.3° amplitude and ~3600°/s maximal velocity contained in the 60-full compared to the pulses at 7.6° amplitude and ~2600°/s peak velocity composing the 90-reduced stimulus. From 26 primary afferents that were tested with the different 90-reduced stimuli, 2 were recruited de novo by higher amplitudes and peak velocities contained in the 90-full, 14 increased their response to the larger pulse (response ratio: [spikes per pulse 90-reduced]/[spikes per pulse 90-full] < 0.95), and 10 did not change their response (Figure 5). Thus, the 90-reduced and 60-full stimuli induce quite different response profiles but in total yield very similar spike counts.

We then calculated the neuronal sensitivities to discriminate between the two pairs (15-full, 90-reduced) and (60-full, 90-reduced). Based on the distributions of spike counts, the sensitivities for the discrimination between 15-full and 90-reduced was superior to the psychometric performance in most ganglion cells no matter if all spikes during the stimulus were counted (Figure 6A) or just the spikes following the first four pulses (Figure 6B). However, the distribution of sensitivities broadened extensively and covered the entire range of possible sensitivities down to zero for the discrimination between 60-full and 90-reduced. Although a few excellent neuronal sensitivities could still be found, the median sensitivity (blue cross) was close to 0.5 and thus matched the one calculated from the animals' behavior (red line). No such differences for the two discriminations were found using spike intervals as coding symbol. Sensitivities were distributed broadly or in a bimodal fashion with medians close to 0.5 for both discriminations, when using intervals taken from the whole stimulus period (Figure 6C) or from the first four pulses (Figure 6D). After additional removal of intervals within bursts, the distribution of sensitivities was substantially improved and clustered at values close to 1 (Figure 6E). Importantly, in contrast to spike counts, the distribution of interval-based sensitivities did not reveal any covariation with

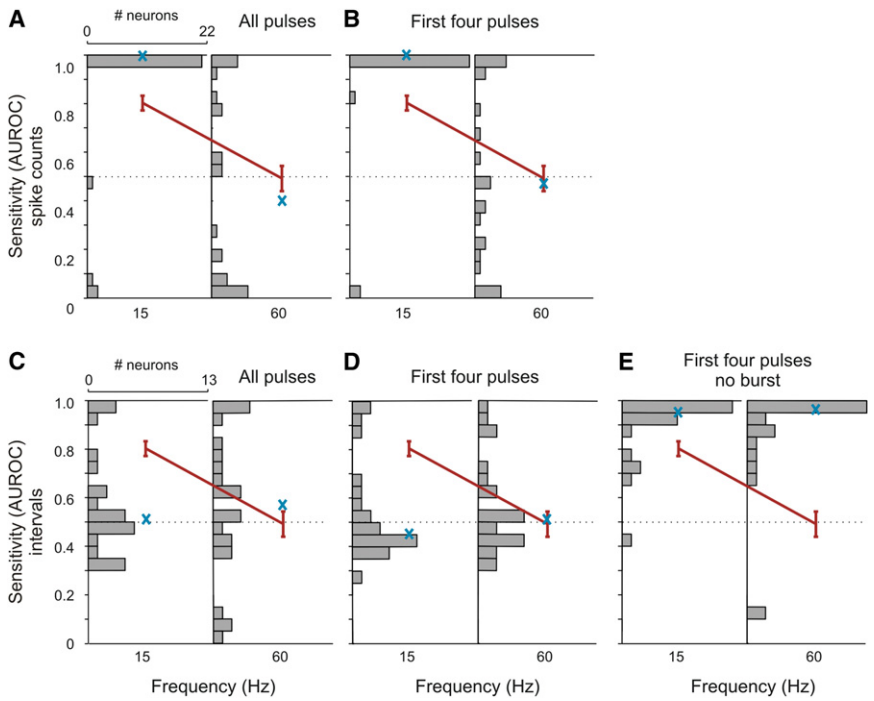

Figure 6. Comparison of Neurometric and Psychometric Sensitivity for Intensity-Matched Stimuli Expressed as Area Under the ROC Curve

Psychometric data are the ones shown in Figure 2C. Conventions are as in Figure 4.

(A and B) Neurometric sensitivities based on spike counts obtained from responses to all pulses (A) and the first four pulses (B). Neurometric sensitivities exceed the psychometric ones, but the medians follow the decline of psychometric sensitivities for the comparison (60-full, 90-reduced) seen in the psychometric data.

(C–E) Neurometric sensitivities based interspike intervals obtained in response to all pulses (C), the first four pulses (D), and additional removal of bursts (i.e., intervals smaller than 6 ms) (E). Neurometric sensitivities partially exceed the psychometric sensitivities if bursts are contained in the data (C and D), but clearly exceed the psychometric ones after burst removal. The psychometric performance is reflected neither in the whole distribution nor in the medians of neurometric sensitivities (blue crosses).

psychophysical performance. They were distributed equally for both discriminations—either broadly or peaked close to a sensitivity value of 1—depending on the inclusion of intervals within bursts.

DISCUSSION

The present study is the first to address the roles played by different physical vibrotactile parameters for whisker-related perception in the rat using behavioral benchmarks. It presents evidence that intensity, measured as mean velocity, plays a prominent role in the rats' perception of whisker vibration in the range between 60 and 90 Hz, a range that carries most of the power of vibrissa vibration elicited by a range of complex fine textures (Hipp et al., 2006). Rats were able to discriminate stimuli matched for kinematic parameters but differing in frequency and intensity. On the other hand, discrimination was abolished with stimuli matched for intensity but differing in frequency and kinematic events. The failure to discriminate intensity-matched stimuli was matched by distributions of primary afferent sensitivity when basing it on spike counts, but not when using spike intervals. These findings argue in favor of spike counts rather than intervals as possible candidate coding symbol used to encode whisker vibrations on the ascending tactile pathway, at least in the parametric range employed in this study.

Physical Parameters Describing Vibration and Their Relevance for Perception

We found a predominance of the intensity cue over both frequency and kinematic feature cues for discrimination of pulsatile stimuli in the vibrissal system (Figure 2). This result is reminiscent of the classic finding in the primate finger/hand system that the Weber fraction for discrimination of subjective intensity is ~0.1, quite smaller than ~0.3, the value estimated for frequency (e.g., Goff, 1967; LaMotte and Mountcastle, 1975). It was further reported that at just detectable intensities frequency discrimination is not possible, leading to the notion of the so-called "atonal" range of vibration close to threshold (LaMotte and Mountcastle, 1975). However, as mentioned in the introduction, these classic studies studied subjective intensity by attenuating sinusoidal stimuli, a manipulation that covaries physical intensity and kinematic events. Theoretically, response-matched stimuli (in the previous as well as the present study) could still carry discriminable cues of *either* frequency *or* kinematic events *or* both. In the present study, we went one step further by demonstrating that rats fail to discriminate when the response-matched stimuli were used as discriminanda. The fact that response-matched stimuli were equal in mean velocity but diverged in other measures of intensity as well as frequency and kinematic events provides strong evidence that mean velocity is a predominant cue for fine discrimination between vibrations at 60 and 90 Hz. In line with this view, a previous study has pointed out that spike counts in the primary cortical somatosensory vibrissa representation (barrel cortex) obtained under urethane anesthesia are monotonically related to intensity measures derived from various powers of velocity (Arabzadeh et al., 2003). Our behavioral results support the explicit coding of intensity in the whisker related system but constrain the conclusions of the previous study by showing that matching intensity, defined by discrimination performance, is reflected by a match of mean velocity rather than of kinematic energy or power—let alone by parameters based on higher powers of velocity (Figure 2B). On the other hand, our finding that mean velocity is the unit of measure for intensity does not necessarily change the conclusions of studies that used measures proportional to higher

powers of velocity (Hipp et al., 2006) because these measures are related in a monotonic fashion.

In previous studies, the observation of precise phase locking on the ascending tactile pathway to sinusoidal or pulsatile stimuli has been put forward to back the notion of a temporally precise code of frequency (Deschênes et al., 2003; Ewert et al., 2008; but see Arabzadeh et al., 2003; Garabedian et al., 2003; Khatri et al., 2004). However, recent work indicates that biomechanical properties of the whisker together with whisker movement transform spatial texture surfaces into a highly irregular vibrotactile signal (Arabzadeh et al., 2005; Ritt et al., 2008; Wolfe et al., 2008). Importantly, Hipp and colleagues (2006) have found that frequency (as measured using the spectral centroid) carries only small amounts of additional information to the one already carried by intensity cues (from 62% achieved by intensity alone to 74%). Furthermore, the spectral centroids evoked by different sandpaper surfaces in the study of Hipp et al. were largely contained in the frequency interval that could not be discriminated by our rats (60 and 90 Hz; inspection of Figure 4E in Hipp et al. (2006) suggests that the statement also holds for the "best frequencies" defined as the ones containing maximum power). With the qualification that naturally occurring surfaces may evoke peaks in power at frequencies different from the ones reported by Hipp et al. using sandpapers, an important role of frequency as a cue for vibration discrimination is thus not supported by the combined results of the present and the previous study.

Neural Coding in the Whisker-Related Primary Afferents

The tuning curves obtained in the present study regularly showed deviations from integer phase-locking ratios (Figure 3B). These deviations occur rapidly (within the duration of the first four pulses) and are likely associated with properties of mechanical or neuronal processes that evolve across small numbers of deflections (Fraser et al., 2006). The pulse waveforms constituting the stimuli feature kinematic values in the higher ranges (7.6°–11.3°; 2600°s^{-1}–3600°/s^{-1}), although they appear to be included well within the dynamic range of primary afferents. Amplitudes of 10° recruit only ~80% of the primary afferents (Gibson and Welker, 1983), and ramps at peak velocity of ~2750°/s barely reach saturation of rapidly adapting cells (Shoykhet et al., 2000). Our present data show that a majority of primary afferents cells could be recruited by increasing kinematic events from 8°/2600°s^{-1} to 11.3°/3600°s^{-1} (cf. Figure 5). Moreover, the deviations from integer phase locking were observed independently of a cell's response magnitude (up to 4 spikes/pulse) and its velocity threshold (63 to larger than 2000°/s), arguing against a saturation phenomenon (cf. Figure 3B). Further arguments for the inclusion of the present pulse parameters within the natural working range of the whisker system are, first, that natural-like surfaces generate kinematic events of similar values (Wolfe et al., 2008) and, second, our present observation, that rats could base reliable discrimination performance on the presented stimuli. In contrast to our present finding that primary afferents often fail to phase-lock at integer ratios with high intensity stimuli, previous studies using stimuli in the lowest intensity range have emphasized precise locking (Deschênes et al., 2003; Jones et al., 2004). In view of this divergence of results, we suggest that the precision of primary afferent spike timing may depend strongly on the kinematic or intensity range studied.

The observed variability in spike responses under the present experimental conditions has important implications for the interpretation of the results: the discrimination performance of the animal in our experimental situation is likely not based on single or very few primary afferents. The reason is that ROC-based neurometric sensitivities of individual trigeminal afferents indicate excellent discriminability of some of the stimuli (values close to 1), while sensitivities drop to around 0.5 (i.e., random performance) at frequencies within kinks in some tuning curves. Therefore, the behavior of the rat can not be explained by one primary afferent but must rely on a sensitivity integrated across the population. Another argument in favor of some form of integration across primary afferents is suggested by the finding that psychometric performance was systematically lower than indicated by sensitivities of individual neurons. One possibility is that mechanical transduction in whisker follicles under anesthesia differs from the awake state due to possible differences in filling the follicle sinus complex (Ebara et al., 2002). Effects of altered blood pressure and/or autonomic nervous system under anesthesia on the sinus are currently unknown. However, in case anesthesia-related effects on the follicle sinus complex play a role, our results would predict that the sensitivity of primary afferents is higher in the anesthetized rat than in the awake one. While this question definitely needs clarification by future experiments, we hold it an unlikely scenario. A second possibility is that sensitivities for the discrimination are reduced on the tactile pathway. In support of this notion, preliminary evidence suggests, that the best sensitivities of barrel cortex neurons are lower compared to trigeminal afferents and are matched closer to the psychometric ones (Gerdjikov et al., 2008). If integration across trigeminal afferent signals governs the readout for perception, it is justified to focus on the distribution of sensitivities, rather than the "best" sensitivities found within the ensemble. Indeed, compared to the psychometric experiments where rats discriminated between 15-full and 90-reduced but fail to do so between 60-full and 90-reduced, the best neuronal sensitivities do not change between the two stimulus pairs: they are found always in the upper bin irrespective of stimulus pair and coding symbol. On the other hand, the *distribution* of sensitivities in principle can change depending on stimulus pairs. In order to be significant for perception, however, such a change must reflect the psychophysical performance. Our observation that the breakdown of discrimination performance is accompanied by a flattening of count-based sensitivities, but not interval-based sensitivities, argues against spike intervals as coding symbol. Spike counts as coding symbols provide a good match to the predominance of intensity over frequency encoding found in our psychophysical experiments, as both require integration across time.

EXPERIMENTAL PROCEDURES

Animals and Surgery

Three male Sprague Dawley rats (Harlan Winkelmann, Borchen, Germany) weighing between 250 and 350 g on arrival were housed together on a 12 hr

reversed light-dark cycle (lights on at 8 p.m.). Food was always freely available. Water was freely available until the start of behavioral testing. Rats were handled for about 5 min every day for two consecutive weeks after arrival. All animals were treated in full compliance with the German Law for the Protection of Animals.

To prevent infection, antibiotic solution (Baytril, Bayer HealthCare AG, Leverkusen, Germany) was added to the drinking water (0.2 mg/ml) for 3 days before and 7 days following surgery. Approximately 2 weeks after arrival at the colony, rats were anesthetized in an induction chamber using a volatile anesthetic (5% isoflurane; Abbott GmbH, Wiesbaden, Germany) mixed with oxygen in a vaporizer system (Drägerwerk AG, Lübeck Germany) and administered at 1.0 l/min. Body temperature was monitored rectally and maintained at 37°C using a homeothermic pad. For fluid replacement, 5% glucose was administered subcutaneously at regular intervals (5 ml total injection volume). Anesthetized animals were fitted to a stereotaxic apparatus and isoflurane was administered at a concentration needed to maintain anesthesia (typically around 1%). After shaving and disinfection, the skin was incised and the pericranium was retracted. Stainless steel screws screwed into predrilled holes in the skull served as anchors for the head mount that was formed by a light-curing dental composite (Heliomolar Flow, Ivoclar Vivadent AG, Schaan, Lichtenstein). A larger screw (5 × 25 mm) was embedded upside down to serve as the head post. Nebacetin antibiotic ointment (Yamanouchi Pharma GmbH, Heidelberg, Germany) was applied before closing the skin with sutures. For analgesia, buprenorphine hydrochloride in solution (0.1 mg/kg; Reckitt Benckiser, Hull, UK) was injected immediately after surgery and twice daily on 3 consecutive days postoperatively. Rats were housed singly after surgery and were given 3 weeks to recover before the start of behavioral testing. Handling resumed 1 week postoperatively and continued throughout the duration of the experiment.

Whisker Stimulation

The whisker stimulator was constructed from a glass capillary (1 mm o.d.) glued to a piezo bender (Physik Instrumente, Karlsruhe, Germany). The tip of the capillary was further thinned through heating until a whisker hair could rest snugly inside the tip opening. Voltage commands were programmed in Matlab (Mathworks, Natick, MA, USA) and delivered using custom-written LabVIEW software (National Instruments, Austin, TX, USA). The stimuli consisted of brief pulsatile deflections (single-period sine wave, 100 Hz, duration 10 ms) presented to the left C1 whisker for 5 s at interpulse intervals of 11 to 66 ms corresponding to frequencies of 15 to 90 Hz (Figure 1A). The deflection amplitude was fixed at 11.3° (i.e., 1 mm at 5 mm distance from the whisker base), except for the second part of the control experiment, where reduced amplitudes were introduced. The stimulator was calibrated with a modified phototransistor with resolution of 20 μs and 1 μm (HLC1395, Honeywell, Morristown, NJ, USA) and an optoelectronic measuring device with a resolution of 1.4 ms and 11 μm (laser emitter and detector; PAS 11 MH; Hama Laboratories, Redwood City, CA, USA) (Stüttgen et al., 2006). Differences in amplitude and peak velocity between frequencies were smaller than three percent. The length of the glass capillary and point of attachment of the piezo element were adjusted such that the ringing of the stimulator was minimal. The capillary tip was positioned 5 mm away from the skin and tilted at an angle of 155° to 175° against the whisker such that the vibrissa rested against the inside wall of the capillary, ensuring that the stimulator immediately engaged the whisker. Stimulation was delivered in the rostral direction. Intensity of the stimuli was calculated from the tracked movements using the phototransistor. The velocity trace was taken as is or taken to the powers of two and three and integrated.

Apparatus

To ensure precise whisker stimulation uncontaminated by body and head movements, rats were carefully habituated to tolerate head immobilization. During head fixation, the rats rested in a box, with the head protruding and fixed by the headpost to a metal bracket extending from the top of the box front. The testing box was placed inside a dark styrofoam-insulated chamber. The chamber was equipped with a water spout for delivering water reward, a metallic arm holding the whisker stimulator, and an infrared camera for monitoring behavior. A piezo element attached to the drinking spout monitored licks at the drinking spout. A data acquisition board and custom-written software were used for experimental control and data acquisition (Labview, National Instruments, Austin, TX, USA). Animals received earplugs (Oropax, Wehrheim, Germany) during behavioral testing and a constant white background noise (70 dB) was produced by an arbitrary waveform generator (W&R Systems, Vienna, Austria) to mask any sound emission of the piezo benders.

Behavioral Procedure

In a first step, the rats underwent a systematic habituation protocol lasting about a month, ensuring that animals were comfortable with head fixation and willing to retrieve rewards. During testing, water intake was restricted to the apparatus where animals were given the opportunity to earn water to satiety. If needed, daily water intake was supplemented after testing to prevent drops in body weight. Rats were initially trained to associate a 90 Hz conditioned stimulus (S+) lasting 1 s with water reward (intertrial interval 15–25 s). To discourage licking during the intertrial interval, a 10 s time-out was introduced if the animal emitted a lick in the 10 s prior to stimulus presentation. The time-out clock was reset with every subsequent lick so that a lick never preceded a stimulus by less than 10 s. Once responding on this task was stable, the stimulus was extended to 5 s and reward was contingent on licking the spout in the period between 500 ms before and 2000 ms after stimulus offset (Figure 1B). Lastly, a range of nonrewarded frequencies was introduced (S−). Responding to the nonrewarded frequencies was discouraged by switching the house light on for 5 s if a lick was emitted during the window of opportunity. Psychophysical testing was conducted using the method of constant stimuli. The behavioral experiment was separated in three different parts, in each of which a different set of stimuli was used. Stimuli were always presented in blocks of ten. Stimulus order was chosen randomly within each block and across blocks. One block consisted of five rewarded stimuli at 90 Hz (at full or reduced amplitudes, respectively) and five nonrewarded stimuli. None of the animals responded consistently in control sessions that were identical to experimental sessions, except that the whisker was detached from the stimulator, assuring that nontactile cues did not play a role in their performance.

In the first part of the experiment, the rewarded stimuli were 90 Hz at 11.3° amplitude and the nonrewarded stimuli consisted of one single presentation of each of the S− stimuli (15, 30, 45, 60, and 75 Hz) at identical amplitude. In a set of rats trained to establish the discrimination task, preliminary results (not presented here) were obtained on the discrimination between the two most extreme stimuli (15 versus 90 Hz) that indicated that the difference between these stimuli was clearly suprathreshold and the discrimination between them was an easy task for the animals. Assuming discriminability, the actual discrimination performance of the rat on this pair, was used to monitor the rats' compliance in the task. To this end, a difference index *di* was calculated using response probabilities *p(r|s)* conditional to a stimulus *s*:

$$di = p(r|90Hz) - p(r|15Hz) \quad (1)$$

Response probabilities are given by $p(r|s) = n_r/n_s$, where n_r is the number of responded stimuli, and n_s is the number of presentations of stimulus *s*. Only sessions entered the data set that contained *di* larger than 0.19, 0.25, and 0.29 corresponding to the upper 80% of the sessions. Response probabilities of the entire data set yielded false alarm rates of 17.7%, 27.4%, and 27.9% with 15 Hz, the lowest unrewarded frequency (14.9%, 23.6%, and 24.1% for the data selection), 42.9%, 57.1%, and 54.7% with 60 Hz (46.3%, 56.6%, and 56.4%) and hit rates of 67.5%, 77.8%, and 70.1% with the rewarded 90 Hz stimulus (71.8%, 79.5%, and 71.4% for the selected data, each value for one of the three animals). Thus, the behavioral results were not changed significantly by the data selection.

In order to measure active whisker movements that could occur when rats performed the discrimination task, we measured active movements using the modified phototransistor (HLC1395, Honeywell, Morristown, NJ, USA) used for calibration. For this purpose, a small polyimide tube was attached to the whisker between the skin and the tip of the glass tube used for stimulation. The photodiode was then set to measure the movement of this tube in rostro-caudal direction. To extract the trajectory due to active movement, a low pass filter (edge frequency 40 Hz) was passed across the measured

trajectory, which would readily remove the fast pulsatile deflections and leave the much slower active movements. Measurements of whisking before the pulsatile deflections showed that active movements do not occur in most of the trials. If they occurred, they typically contained frequencies lower than 5 Hz (in two trials, power up to 15 Hz was detected) and median peak amplitudes of 1.12°. The trajectories stripped of the piezo-induced deflections were used to test the neurometric sensitivity of trigeminal ganglion neurons, assuming a worst case scenario that active whisker movements are present in all trials (Figure 4).

Electrophysiological Recordings and Analysis

Acute experiments were performed under urethane anesthesia (1.5 mg/kg i.p.), and the animals were killed at the end of the experiment with an overdose of pentobarbital. The procedures, except the stimulation protocol, were exactly the same as reported previously (Stüttgen et al., 2006). Briefly, single units were recorded in the trigeminal ganglion using an extracellular amplifier (MultiChannel Systems, Reutlingen, Germany, band-pass filtering 300–5,000 Hz; A/D conversion at 20 kHz). Electrodes consisted of glass-coated platinum tungsten wires pulled and ground to custom shapes in our laboratory (shank diameter 80 μm; diameter of the metal core 23 μm; free tip length 8 μm; impedance, 3–6 MΩ; Thomas Recording, Giessen, Germany). Only clear single unit spikes entered the present data set. For stimulation, the piezo element was attached to the whisker in exactly the same way as done in the awake animals. The stimuli included the ones used for the psychometric investigations of the first part of the behavioral experiment plus five additional frequencies (65, 70, 80, 85, and 88 Hz). In some experiments (26 neurons out of 56), an additional set of three 90 Hz stimuli at reduced intensity (Amplitude: 7.6°, 8.7°, and 9.6°) was presented. Blocks thus consisted of 11 or 14 different stimuli that lasted 1 s and were repeated each ten times in a pseudorandom order with interstimulus intervals of 3 s. In some experiments, the stimuli presented were the sum of the pulsatile deflection and the trajectories of active whisker movement as measured in two awake behaving animals performing the same discrimination task (see above).

Burst Elimination

Multiple spike responses to a single pulse were generated at short time intervals. They were eliminated by leaving the first spike and deleting the consecutive spikes at interspikes intervals smaller than 6 ms from the train.

Comparison of Neurometric and Psychometric Sensitivity

Psychophysical data assessed as response-probabilities was converted into sensitivity d' using the following equation:

$$d' = \Phi^{-1} p_{hit} - \Phi^{-1} p_{FA} \quad (2)$$

where p_{hit} signifies the probability of correct responses, p_{FA} the probability of false alarms, and Φ^{-1} is the probit function (Stüttgen and Schwarz, 2008). In order to compare psychometric with neurometric sensitivities d' values were converted to area under the receiver operating curve (AUROC) (Stanislaw and Todorov, 1999):

$$AUROC = \frac{\Phi(d')}{\sqrt{2}} \quad (3)$$

Neuronal sensitivities were computed for two possible coding symbols: spike count and interspike intervals. In both cases, the probability distributions for the occurrence of spike numbers or spike intervals were computed. Then ROC curves were constructed by shifting a criterion c in steps of one spike or 1 ms intervals to yield hit and false alarm rates. This was done for all pairs of S+ and S− stimuli. Neuronal sensitivity was then calculated as the AUROC which corresponds to the percentage correct responses of an unbiased, ideal observer under the conditions of a two alternative forced choice procedure (Green and Swets, 1966). The discrimination rule was the following: if the neurometric variable (spike count or interval) is larger than c, choose S+; otherwise, choose S−. This rule has been a standard for the analysis of spike counts (Britten et al., 1992; Parker and Newsome, 1998) and was adapted here in addition for the use with spike intervals. It should be noted that it is based on a yes/no task (or go/no go, for that matter) and, thus, demanded the division by $\sqrt{2}$ in Equation 3 to yield comparable psychometric and neurometric variables (cf. Green and Swets, 1966, section 3.2.4). Our procedure for intervals implies that the observer generates a decision after the occurrence of each interval, while in the experimental reality, the rat only makes a decision toward the end of the stimulus—after having observed many intervals. We opted, nevertheless, for this procedure because it reflects the temporal properties of the spike train better than any variable that can be extracted from a set of intervals occurring in response to a single stimulus presentation. Thus, it seems best suited to answer the question whether or not information in precise timing is used by the animal to generate its percept—our prime goal in the present study. Nevertheless, in order to generate an intuition about sensitivities based on variables that distill one number from all intervals generated during a single stimulus presentation, we repeated the ROC analysis using several approaches. First, we computed power-spectra from the spike trains and calculated AUROC values from the median frequencies around the maximum of the spectrum in the same way as has been done before (Hernandez et al., 2000; Luna et al., 2005). Second, we used the median interval (the mean interval is not an appropriate alternative because it is equivalent with the spike rate). These calculations yielded sensitivities that were near optimal (sensitivity of 1) for most neurons across all frequencies tested. Most importantly, this was the case also for the stimulus pair (60-full, 90-reduced) (data not shown). Therefore, calculating neuronal sensitivities on these alternatives, despite being more realistic about the number of intervals on which a decision is based, would not change the major conclusions of the study, that the distribution of neuronal sensitivities based on intervals does not reflect the decline of psychometric sensitivity for intensity-matched stimuli (cf. Figure 6).

Error bars of psychometric data in this study signify 95% confidence intervals calculated from a binomial model setting the animals response probability to the probability of a Bernoulli trial, except for Figure 2A, where error bars display 95% confidence intervals calculated from a t distribution. All calculations were done in Matlab (MathWorks, Natick, MA, USA).

ACKNOWLEDGMENTS

This research was supported by grants from the Deutsche Forschungsgemeinschaft (SFB 550-B11 and SCHW577/10-1). T.V.G. was supported by a postdoctoral fellowship from the Hertie Foundation. C.G.B. was supported by a stipend from IZKF Medical School, University of Tübingen. Further support was provided by the Hertie Foundation and the Hermann and Lilly Schilling Foundation.

Accepted: February 5, 2010
Published: February 24, 2010

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