PROBING THE CORTICAL NEURONAL CORRELATES
OF A SENSORY DISCRIMINATION PROCESS

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INTRODUCTION

Humans and monkeys have the same ability to discriminate the difference in frequency between two mechanical vibrations delivered sequentially to the fingertips (5, 7). What are the neuronal correlates of this sensory discrimination task? Cutaneous afferents of the quickly adapting (QA) submodality linked to Meissner's corpuscles respond with impulses which are phase-locked to the mechanical sinusoids delivered to the center of their receptive fields (14). Neurons of the dorsal column nuclei (DC) of the spinal cord respond as a function of the incoming afferent input and neurons of the ventrobasal complex (VBC) of the thalamus maintain the representation provided by the DC (1, 3). The same type of processing is observed in QA neurons of areas 3b and 1 of the somatosensory cortex (S1) (7).

Here, we tested whether the S1 representation of the vibrotactile stimulus is sufficient for sensory discrimination. This was done by recording from QA neurons in S1 while trained monkeys discriminated between two mechanical vibrations delivered to the fingertips (5, 6, 12). We sought to determine which signal in the evoked activity best matches the psychophysical performance (6, 12). This was made by computing receiver operating characteristic (ROC) curves both from the periodic spike intervals and from the mean firing rate (4, 6). The results indicate that neurometric thresholds from the mean firing rate are almost similar to the animal's discrimination thresholds, whereas those from the spike intervals are much better than the animal's discrimination thresholds (6). We then sought to determine whether these representations are causally related to the psychophysical performance. This was probed by substituting the mechanical vibrations delivered to the fingertips by electrical current pulses delivered directly to the S1 neurons which mapped the mechanical vibrations during the vibrotactile discrimination task (10, 11). The discrimination performance was almost similar to that obtained with the natural stimuli. These results demonstrate unequivocally that the S1 representation of the vibrotactile stimuli is sufficient for the psychophysical performance of this sensory discrimination task.

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METHODS

Discrimination task.

Two vibrotactile stimuli were delivered sequentially to the skin of the distal segments of one digit of the right, restrained hand, via a computer-controlled motor stimulator (BME Systems, Inc; 2-mm round tip). The initial indentation was 500 µm. Vibrotactile stimuli were trains of short mechanical pulses. Stimulus amplitudes were adjusted to equal subjective intensities (5, 7). For example, 71 µm at 12 Hz and 51 µm at 34 Hz (~ 1.4% per Hz). During trials, two vibrotactile stimuli were delivered consecutively to the glabrous (hairless) skin, separated by an interstimulus delay of 1-3 s, and the monkey was rewarded for correct discrimination with a drop of liquid. Discrimination was indicated by pressing one of two push-buttons. Performance was measured through psychometric techniques (5, 7). Animals were handled in accordance with the institutional standards of the NIH and the Society for Neuroscience.

Recording sessions and sites.

Neuronal recordings were obtained with an array of seven independent, moveable microelectrodes (2-3 MΩ) inserted into S1 (6, 10-12) (areas 3b and 1; four monkeys). Recording sites changed from session to session and standard histological procedures were used to construct surface maps of all the penetrations in S1. This was done first by marking the edges of the small chambers (7 mm diameter) placed above S1. Additionally, in the last recording sessions, we made small lesions at different depths in the recording area. We considered neurons recorded in area 1, from the top of the cortex to 2500 µm, and in area 3b, from 2500 µm down. All these neurons had small cutaneous receptive fields confined to the distal segments of fingertips 2, 3, or 4, and had QA properties.

Data analysis.

For each neuron studied during the discrimination task, off-line analysis and statistical tests were done using custom and Matlab software. The analysis was restricted to the stimulus periods, according to two criteria. First, we devised a measure that quantified the capacity of the neurons to represent the periodicity of the stimulus. For each trial, the power spectrum of the spike train evoked during the stimulus period was computed (FFT, $N = 2$ (16), sampling frequency = 10 kHz; resolution, 0.15 Hz; range, 6-100 Hz) (8). As an estimate of the periodicity, we calculated the median frequency around the peak power spectrum frequency, weighted according to the power at each frequency. The frequencies used for this measure were limited to those within a factor of 1.8 of the peak frequency (to avoid contamination by harmonics) and to frequencies with a power greater than 15% of the peak power (to avoid noise). The median frequency calculated in this way was considered a quantitative measure of periodicity evoked in S1 neurons by the periodic mechanical stimuli. Second, for each trial we calculated the mean firing rate over the stimulus periods. For each stimulus frequency, we computed the mean ± S.D. of both periodicity and firing rate over all trials with that stimulus frequency. For further analysis, we selected those neurons that had the best linear fit (χ^2, Q > 0.05) of the periodicity and/or firing rate values as a function of the stimulus frequency (8). We also required the slope of this linear fit to be significantly different from zero (permutation test, n = 1000, P (0.05) (13), and that the slopes calculated separately for each the two stimulus periods (base and comparison) were not significantly different from each other (α < 0.05 as computed using the S.D. of the linear fits) (2).

The discrimination task requires the comparison of the second stimulus frequency against the first. We observed that QA neurons of S1 provide a reliable representation of the two stimulus frequencies. We then determined the probability that an observer (a cortical region central to S1) could distinguish the difference between the two stimuli. This could be based on a comparison of the neuronal response distributions of the second stimulus frequency (f2) made against the neuronal response distributions of the first stimulus frequency (f1). According to this, the observer could use a simple rule: if the number of spikes during the second stimulus is higher than during the first stimulus, then f2 is higher than f1. The same rule can be used when considering the periodicity values: if the periodicity values during the second stimulus period (f2) are higher than during the first stimulus (f1), then f2 is higher than f1. This rule can be tested by determining the area

under the curve ROC generated by the neuronal response distributions for each pair of stimulus frequencies, using both periodicity and firing rate values (4). In pairs of stimulus frequencies where the neuronal response distributions of f2 are much higher than the neuronal response distributions of f1, ROC values are close to 1; if the neuronal response distributions of f2 are much lower than the neuronal response distributions of f1, ROC values are close to 0; for overlapping distributions, intermediate ROC values are found. The ROC values were then used to compute neurometric functions. Psychophysical and neuronal discrimination thresholds were calculated as half the difference between the stimulus frequency identified as higher than the standard in 75% of the trials and that frequency identified as higher in 25% of the trials (5, 7). These were read directly from the logistic functions (Boltzman's equation) expressed in terms of Hz.

Microstimulation.

A computer-controlled pulse generator (Coulbourn), in series with an optical stimulus isolation unit, produced biphasic current pulses with the cathodal phase leading. Each phase lasted 0.2 ms, with 0.05 ms between phases. Two-pulse bursts, with 0.5 ms between pulses, were delivered at the base or both base and comparison frequencies. Current amplitude varied between 65 µA and 100 µA; this range has been proven to be very effective to produce behavioral responses than are indistinguishable from those elicited by the mechanical stimuli delivered to the fingertips (10, 11). Within each session, current amplitude was maintained fixed across all stimulus frequencies.

RESULTS

General.

Six monkeys (*Macaca mulatta*) were trained to discriminate the difference in frequency between two mechanical vibrations delivered sequentially to their fingertips (6, 10, 11) and they learned to indicate whether the second frequency was higher or lower than the first. Neurophysiological recordings were made in S1 (areas 3b and 1) contralateral to the mechanical stimulation while four monkeys performed the discrimination task (6, 10, 11). The neurons selected for study in S1 had small, cutaneous receptive fields confined to the smooth, hairless skin of one fingertip of digits 2, 3 or 4. All neurons had QA properties. The neuronal responses from S1 were collected while monkeys discriminated frequencies at psychophysical thresholds. We microstimulated clusters of QA neurons in area 3b in four monkeys while they performed the discrimination task. In two monkeys we substituted the comparison, mechanical stimulus frequency with current pulses which mimicked the natural stimuli. In the two other monkeys we substituted the base stimulus frequency with current pulses which mimicked the natural stimuli. The neuronal correlates and the microstimulation experiments were designed to probe whether the evoked responses in the QA neurons of S1 cortex are sufficient for this somatosensory discrimination task.

Periodicity and firing rate as candidate code for the frequency of vibrotactile stimuli.

We first determined the responses of 223 QA neurons of S1 (135 in area 3b and 88 in area 1) as a function of the stimulus frequency while monkeys performed the vibrotactile discrimination task (panels a and d of Figure 1 show examples of two types of responses) (6). This was done by measuring for each neuron the periodicity and the mean firing rate during the stimulus periods in both single trials and in
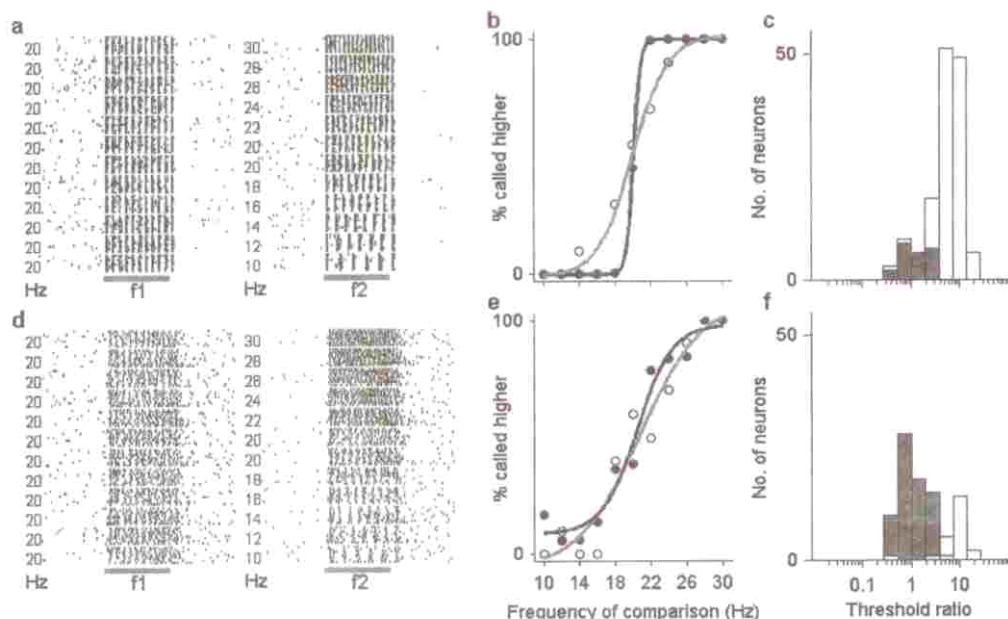
Fig. 1. - *Neuronal correlates of vibrotactile discrimination (6) (see Methods' section for details of the task, recording and analysis).*
a, Phase locked responses to the mechanical sinusoids of the vibrotactile stimuli of an S1 neuron. *d,* Modulation of the firing rate as a function of the vibrotactile stimuli of an S1 neuron. *b* and *e,* Relationship between the neurometric functions from the example neurons of *a* and *d* (black dots and lines) and the psychometric performances (open circles and gray lines). *c* and *f,* Psychometric/neurometric threshold ratios based on periodicity (open bars) and mean firing rate (gray bars). Labels f1 and f2 indicate base and comparison stimulus periods (500 ms each), respectively. Numbers on the left are stimulus frequencies for f1 and f2.

blocks of the same trials. The results of these two measures indicate that the stimulus frequency can be represented both in the periodicity and in the mean firing rate responses to varying degrees across the QA neuronal population (not shown). Based on this analysis, 188 neurons (113 in area 3b and 75 in area 1) gave information about the stimulus frequency. One hundred and thirty-nine neurons responded with periodic spike intervals at a frequency that reliably represented the input stimulus frequency (94 in area 3b and 45 in area 1), and 72 other neurons increased their firing rate as a function of the stimulus frequency (49 in area 3b and 23 in area 1). Only 23 neurons (14 in area 3b and 9 in area 1) provided information about the stimulus frequency in terms of the periodicity and mean firing rate.

Neuronal correlates of vibrotactile discrimination in S1.

Having quantified the responses of S1 as a function of the stimulus frequency, we proceeded to determine whether these neural signals carry information that might be associated with psychophysical behavior. For each neuron, we computed neurometric functions by using the periodic or the firing rate values. We first focused our attention on those neurons that responded with periodic spike inter-

vals at a frequency of the input stimulus. Figure 1a shows the responses of a S1 neuron during the two stimulus periods while the monkey discriminated between pairs of frequencies. The responses of this neuron matched the input stimulus frequency. The question is then whether in the periodic spike intervals a neural signal is to be found that matches the animal's psychophysical performance. Figure 1b shows the relationship between the psychometric and neurometric functions for neuron of Figure 1a. Notice that the psychometric threshold (2.08 Hz) is higher than the neurometric threshold (0.20 Hz); the psychometric threshold ratio = 10.4. Figure 1c shows the psychometric/neurometric threshold ratio [6.53 ± 3.87 (mean ± s.d.); open bars] over the population of periodic neurons, and it is clear that, based on response periodicity, these neurons discriminate vibrotactile stimuli (neurometric threshold = 0.79 ± 1.22 Hz) much better than the animals do (psychometric threshold = 2.95 ± 1.87 Hz).

As indicated above, some QA neurons of S1 modulate their firing rate as a function of the increasing stimulus frequency (Fig. 1d). Are these neural signals associated with the animal's psychophysical performance? We computed neurometric functions for each of these neurons by using the firing rate values. Figure 1e shows the relationship between the psychometric and neurometric functions for the example neuron (Fig. 1d). The neurometric threshold (2.48 Hz) computed from this neuron is slightly lower than the animal's psychometric threshold (3.22 Hz); the psychometric/neurometric threshold ratio = 1.29. Figure 1f (gray bars) shows the relationship between the psychometric (3.07 ± 0.34 Hz) and neurometric (3.37 ± 1.82 Hz) thresholds for the population of modulated firing rate neurons; the psychometric/neurometric threshold ratio = 1.31 ±0.94.

It is clear from these two measures that neurometric thresholds based on periodicity are far lower than the psychometric thresholds, whereas neurometric thresholds based on mean firing rate are close to the psychometric thresholds.

A minority of neurons (23 of 188) provided information about the stimulus in both of their periodic spike intervals (the neurometric threshold = 1.31 ± 0.94 Hz: the psychometric/neurometric threshold ratio = 8.99 ± 3.68; open bars of Figure 1f), and in their mean firing rate (the neurometric threshold = 2.95 ± 1.87 Hz; the psychometric/neurometric threshold ratio 1.56 ± 1.0; gray bars of Figure 1c). Once again, for this subpopulation of neurons, psychometric thresholds are far higher than neurometric thresholds based on periodicity, but are similar to neurometric thresholds based on firing rate.

Frequency discrimination based on S1 microstimulation.

Neurophysiological studies often reveal close associations between neuronal activity and sensory events, as shown above, but does such activity have an impact on perception and subsequent behavior? We typically assume so, but this is hard to verify. To verify whether the evoked neuronal activity in the QA circuit of S1 is sufficient for vibrotactile discrimination, we manipulated the S1 representation of the vibrotactile stimulus by injecting current bursts of electrical stimuli at the same mechanical frequencies (10, 11).

A first approach was to manipulate the comparison stimulus frequency during the discrimination task (11). In each trial of the task, the monkeys discriminated between the frequency of the two successively presented mechanical vibrations delivered to the fingertips, termed the base stimulus and the comparison stimulus. After the animals, mastered the discrimination of the mechanical stimuli, microstimulation of area 3b substituted the mechanical, comparison stimulus frequency in half of the trials. Artificial stimuli consisted of periodic current bursts delivered at the same comparison frequencies as the mechanical comparison stimuli (Fig. 2a). Microstimulation sites in area 3b were selected to have QA neurons with receptive fields on the fingertip at the location of the mechanical stimulating probe. Remarkably, the monkeys could discriminate between the mechanical (base) and the electrical (comparison) signals with performance profiles indistinguishable from those obtained with natural stimuli only (Fig. 2b).

We wondered whether, in addition to using artificial stimuli during the compari-
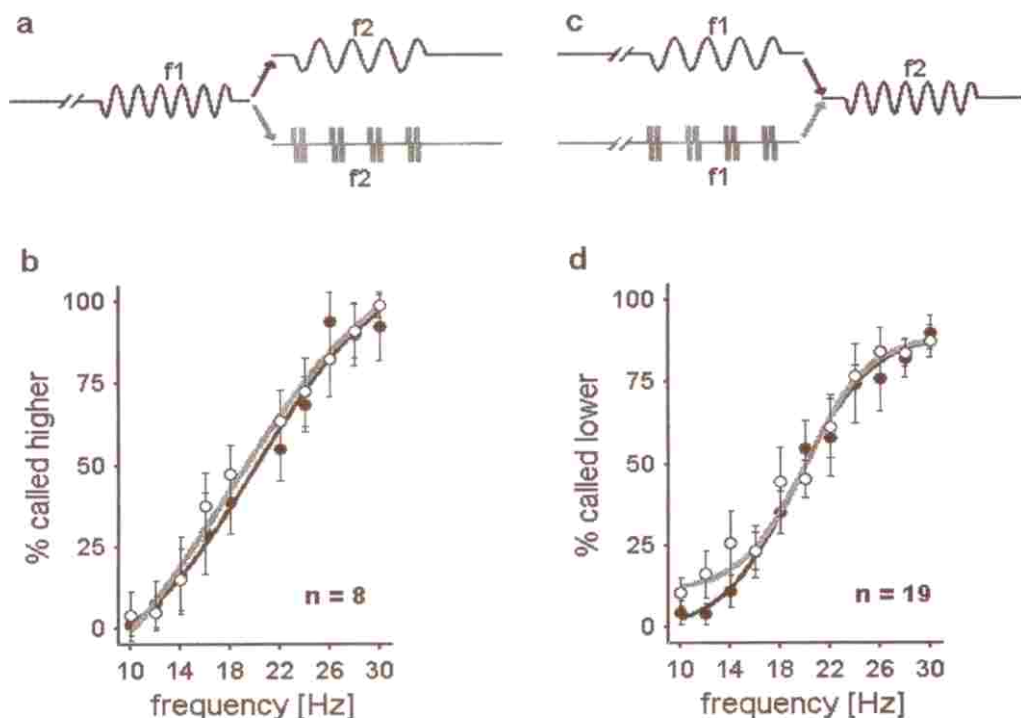

Fig. 2. - *Discrimination performance with natural and artificial stimulus frequencies delivered to the fingertips and to S1 (10, 11) (see Methods).*
b, Psychometric functions with natural stimuli delivered to the fingertips (black dots and line) and when the second, comparison stimulus (f2, gray line) was substituted with current pulses (a) injected in clusters of QA neurons of S1 (open dots and gray line). d, Psychometric functions with natural stimuli delivered to the fingertips (black dots and line) and when the first, base stimulus (f1, gray line) was substituted with current pulses (c) injected in clusters of QA neurons of S1 (open dots and gray line). Dots and bars are mean ± s.d.

son period of the task, monkeys could store and use a quantitative trace of an electrical stimulus delivered to clusters of QA neurons in S1 cortex in place of the first mechanical stimulus (10). We also wondered whether monkeys could perform the entire task on the basis of purely artificial stimuli (10). This would demonstrate that activation of the QA circuit of S1 was sufficient to initiate the entire cognitive process involved in the task.

Again, the mixed mechanical/microstimulation protocol was used, in which microstimulation trials were randomly intermixed with standard, purely mechanical trials. The frequency pairs and event sequence were the same in both mechanical and microstimulation trials, except that in microstimulation trials the first (Fig. 2c) or both mechanical stimuli were substituted by trains of current pulses injected in S1 and delivered at the frequency of the mechanical stimulus they were replacing. Design of the stimulus set assured to explore the working memory component of the task and determine discrimination thresholds.

Psychophysical performance with electrical microstimulation patterns in S1 cortex at the mechanical base stimulus frequencies they were replacing was almost similar to that measured with the mechanical stimulus (Fig. 2d). These results show that monkeys were able to memorize the base artificial stimulus frequency and make comparisons of the second stimulus against the memory trace left by the artificial stimulus. As for substituting the comparison stimulus with electrical patterns, monkeys could not reach the usual level of performance when clusters of slowly adapting (SA) neurons were microstimulated. Nor they could discriminate when microstimulation patterns were made at the border between QA and SA clusters. These control experiments tell us about the specificity of the QA circuit in flutter discrimination. Finally, in most sessions in which the two mechanical stimuli were replaced by microstimulated patterns, monkeys were able to reach discrimination levels close to those measured with mechanical stimuli delivered to the fingertips. This indicates that microstimulation elicits quantitative memorizable and discriminable percepts, and shows that activation of the QA circuit of S1 is sufficient to initiate the entire subsequent neural process associated with vibrotactile discrimination.

DISCUSSION

The results obtained in these two sets of experiments suggest that QA neurons from S1 cortex represent the stimulus frequencies both in the periodic spike intervals and in the mean firing rate (6, 12), and that these forms of representations are sufficient to initiate the entire chain of discrimination processes of this task (10, 11). The conclusion previously found in the literature, that frequency discrimination is based on periodicity, came from the observation that a small number of studied QA neurons from S1 reproduce in their activity the periodicity of the mechanical stimulus frequency, and also from the fact that these neurons did not have average firing rates that were modulated by the stimulus frequency (7). However, the study that reached this conclusion only determined the relationship between the neuronal

responses to the mechanical stimulus frequencies; no attempt was made to quantify the neurometric thresholds based on periodicity and to compare these to the psychophysical thresholds. Our analysis shows that neurometric thresholds using the periodicity values are far lower than the psychometric thresholds. What is then the functional meaning of this neural signal? One possible role is that this simply represents the temporal structure of the stimulus and that monkeys do not use this exquisite representation for frequency discrimination. Consistent with this interpretation, we found QA neurons in S1 whose firing rates are modulated by the stimulus frequencies, and their neurometric thresholds based on this measure are similar to the monkey's psychophysical thresholds.

These results also suggest that QA neurons of S1, which are classified according to their capacity to react to a slight mechanical indentation applied to the center of their receptive fields, may in fact be composed of two subpopulations, each of which behaves differently in response to a periodic mechanical stimulus. These two subpopulations might be organized in hierarchical fashion: QA neurons that respond periodically might be closer to the input stimulus, and those that modulate their firing rate might integrate the responses of the periodic neurons and transform them into a rate code. Such last order neurons of the QA circuit could distribute the neural representation of the stimulus to those structures anatomically linked to S1, to solve the sensory discrimination task. Further studies will be needed to test whether this is so.

Observation of a neuronal correlate does not prove that the neuronal response is sufficient for frequency discrimination. However, the results obtained in the microstimulation experiments show that the relationship between these neuronal responses and the animal's behavior are not simple coincidences (10, 11). Monkeys are able to discriminate the stimulus frequencies either delivered to the fingertips or artificially injected into a cluster of QA neurons. The specificity of QA stimulation for frequency discrimination is suggested by the fact SA stimulation cannot produce discrimination. Interestingly, it has been shown that activity in a single cutaneous afferent fiber could produce localized somatic sensations, and frequency microstimulation of QA afferents linked to Meissner's corpuscles produced the sensation of flutter (15). These observations strongly support the notion that the activity initiated in specific mechanoreceptors is read out by S1; this reading is then widely distributed to those anatomical structures that are linked to S1. The whole sequence of events associated with this sensory discrimination task must depend on this distributed neural signal. We predict that recording of neuronal activity (6, 9, 12) and artificial activation of these structures linked to S1 will reveal the components of the discrimination task processed by each structure (Romo et al., unpublished results). This study, therefore, has directly established a strong link between neuronal activity and perception.

SUMMARY

Key to understanding perception is the form of how sensory stimuli are represented in the evoked activity of the brain. Here, we addressed the question of which components of the evoked neuronal activity in the somatosensory cortex represent the stimulus features while trained monkeys discriminated the difference in frequency between two vibrotactile stimuli. We probed whether these cortical neuronal representations are essential to perception. The results show a strong link between the cortical representation of the stimulus and perception.

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