Periodicity and Firing Rate As Candidate Neural Codes for the Frequency of Vibrotactile Stimuli

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The flutter sensation is felt when mechanical vibrations between 5 and 50 Hz are applied to the skin. Neurons with rapidly adapting properties in the somatosensory system of primates are driven very effectively by periodic flutter stimuli; their evoked spike trains typically have a periodic structure with highly regular time differences between spikes. A long-standing conjecture is that, such periodic structure may underlie a subject’s capacity to discriminate the frequencies of periodic vibrotactile stimuli and that, in primary somatosensory areas, stimulus frequency is encoded by the regular time intervals between evoked spikes, not by the mean rate at which these are fired. We examined this hypothesis by analyzing extracellular recordings from primary (S1) and secondary (S2) somatosensory cortices of awake monkeys performing a frequency discrimination task. We quantified stimulus-driven modulations in firing rate and in spike train periodicity, seeking to determine their relevance for frequency discrimination. We found that periodicity was extremely high in S1 but almost absent in S2. We also found that periodicity was enhanced when the stimuli were relevant for behavior. However, periodicity did not covary with psychophysical performance in single trials. On the other hand, rate modulations were similar in both areas, and with periodic and aperiodic stimuli, they were enhanced when stimuli were important for behavior, and were significantly correlated with psychophysical performance in single trials. Thus, the exquisitely timed, stimulus-driven spikes of primary somatosensory neurons may or may not contribute to the neural code for flutter frequency, but firing rate seems to be an important component of it.

Key words: awake monkeys; primary somatosensory cortex; secondary somatosensory cortex; neural coding; flutter; discrimination; periodicity; mutual information

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MATERIALS AND METHODS

Neurophysiology and behavior. The behavioral task is schematized in Figure 1a [see also Romo et al. (1998); Hernández et al. (1997)]. In each trial the monkey had to compare the frequencies of two vibratory stimuli presented consecutively. The sequence of events was as follows. The mechanical probe was lowered, indenting the glabrous (hairless) skin of one digit of the restrained hand; the monkey reacted, placing its free hand on a lever within 1 sec after indentation; after a delay period (1.5–3 sec), the probe oscillated vertically, periodically, at a base frequency; after an interstimulus interval (1–3 sec), a second stimulus was delivered at a comparison frequency; the monkey had to release the lever within 600 msec and press one of two push-buttons to indicate whether the comparison frequency was higher or lower than the base. Both stimuli lasted 500 msec and were delivered to the distal segment of digits 2, 3, or 4 of the left hand via a computer-controlled Chubbuck linear motor stimulator (Chubbuck, 1966), which had a 2 mm round tip. Initial indentation was 500 μm.
Stimulus amplitudes were adjusted to equal subjective intensities (Mountcastle et al., 1980; Hernández et al., 1997). For example, 71 μm at 12 Hz and 51 μm at 34 Hz (1.4% per Hertz). In each trial of the task, a pair of base-comparison frequencies was chosen pseudorandomly from a set typically comprising 10 pairs. For one full data collection run, at least five trials per pair had to accumulate with the same stimulus set. Typically, each run included 10 trials per pair. Figure 1, b and c, shows two stimulus sets comprising different combinations of the 50% correct frequencies in B, smaller and larger differences were combined. In general, monkeys had clear difficulties discriminating when base and comparison frequencies differed by 2 Hz or less.

Stimulus stimuli were used initially; 137 S1 neurons were studied in this way. Later we switched to trains of short mechanical pulses like those illustrated in Figure 1a. Each of these pulses consisted of a single-cycle sinusoid lasting 20 msec. For stimulation at 20 Hz, 11 successive pulses were applied, separated by 50 msec. This interval was measured between the beginnings of successive pulses. The data obtained with sinusoidal stimuli were not used in Figure 2 or in comparisons with S2 responses, but they were included in the comparisons between active and passive conditions and between neuronal and psychophysical responses.

Experiments with aperiodic stimuli were also conducted (Romo et al., 1998). In this situation a frequency of 20 Hz still corresponded to 11 mechanical pulses delivered in a 500 msec period, so the mean interval between pulses was 50 msec, but the times between pulses were random. The mechanical stimuli were separated by a random number between 20 and 500 msec, and the monkeys had to compare the average frequencies of the base and comparison stimuli exactly as before. By average frequency we mean the total number of stimulation pulses divided by the corresponding 500 msec period. These experiments were conducted using three different types: with periodic base and aperiodic comparison, or with both aperiodic. Behavioral results from these two variants of the paradigm were pooled. These experiments were performed in blocks interleaved between blocks of regular discrimination with periodic stimuli. These blocks were also performed with the monkey seated in the active posture, and all three amplitudes (at the PSFP, at the stimulus frequency, and all three amplitudes (at the PSFP, at the stimulus frequency, and at twice the stimulus frequency) were much larger than the average power across all bins. Statistical tests applied to any of these four quantities were always performed also with the other three, but sometimes only the results for the most sensitive one are mentioned.

In each trial, we also computed the average interburst interval (AIBI), which measures how often a burst of spikes is produced. A burst was defined as a group of spikes occurring within a 1-msec time window. The number of spikes per burst was variable, with a minimum of 1, and all interspike intervals within a given burst had to be smaller than 1. Large values produced few bursts with few spikes, whereas small values produced many bursts with few or single spikes. Having fixed τ, the AIBI was then computed as the mean value of the time intervals between consecutive burst endings.

Within conditions, such as differences between hits and errors, time-frequency power functions were used, separated by 50 msec, and the monkeys had to compare the average frequencies of the base and comparison stimuli exactly as before. By average frequency we mean the total number of stimulation pulses divided by the corresponding 500 msec period. These experiments were conducted using three different types: with periodic base and aperiodic comparison, or with both aperiodic. Behavioral results from these two variants of the paradigm were pooled. These experiments were performed in blocks interleaved between blocks of regular discrimination with periodic stimuli. These blocks were also performed with the monkey seated in the active posture, and all three amplitudes (at the PSFP, at the stimulus frequency, and at twice the stimulus frequency) were much larger than the average power across all bins. Statistical tests applied to any of these four quantities were always performed also with the other three, but sometimes only the results for the most sensitive one are mentioned.

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Trial-to-trial covariations in the firing rates of simultaneously recorded neurons were measured using Pearson’s linear correlation coefficient $\rho$ (Press et al., 1992). This coefficient can be computed easily from the same standardized rates described in the previous paragraph. If $r_i$ is the standardized rate of neuron $a$ at trial $i$, the correlation coefficient between neurons $a$ and $b$ is simply:

$$p_{ab} = 1 - \frac{1}{N} \sum_{i=1}^{N} r_{ai}^2,$$

where $N$ is the total number of trials in the run, including all stimulus frequencies.

### Information estimates and other statistics.

Having computed the firing rate, the PSFP, and the AIBI in each trial, we quantified how they varied by a computer, we found that this method to obtain the significance of the standardized rate of neuron $a$ at trial $i$, the correlation coefficient between neurons $a$ and $b$ is simply:

$$I_{r,s} = \frac{1}{N} \sum_{i=1}^{N} \frac{p_{r,s}(i) \log_2 \left( \frac{p_{r,s}(i)}{p_{r}(i)} \right)}{H(I)},$$

where $I$ is the information in bits. The maximum amount of information that can be provided by any signal is $\log_2(N)$ bits. A response carrying these many bits of information lets us distinguish between $N$ different stimuli, just as does the firing rate of a neuron. Thus, the firing rate of a neuron provides a measure of the amount of information in the responses. The firing rate and the stimulus are then maximally correlated. Most information results shown below correspond to experiments in which seven or more stimulus frequencies were used (Treves and Panzeri, 1998). (These Gaussians are response probability distributions specific to each stimulus frequency and should not be confused with the Gaussian tuning curves mentioned above.) Then, a correction for finite sampling, based on Monte Carlo methods, was applied (Treves and Panzeri, 1995; E. Salinas, unpublished results, but see below). The underlying idea is practically the same as for the computation of significance described above. Two distributions are thought to differ in some statistic, for instance in their means. To test the significance of the difference, the two distributions are compared pairwise, other statistical comparisons were based on permutation tests (Siegel and Castellan, 1988). Here the underlying idea is practically the same as for the computation of significance described above. Two distributions are thought to differ in some statistic, for instance in their means. To test the significance of the difference, the two distributions are compared pairwise, other statistical comparisons were based on permutation tests (Siegel and Castellan, 1988). (Fig. 1; and see Materials and Methods). After training, single neurons were recorded extracellularly while the task was performed (Mountcastle et al., 1990; Romo et al., 1998). In primates, processing of somatosensory information from S1 to S2 seems to proceed mostly in a serial fashion (Pons et al., 1987, 1992). We recorded in these two areas to assess any differences in the processing or representation of tactile information. In both areas, a neuron was selected for study if, relative to background activity, it responded in any way to the base or comparison stimulus or during the interstimulus interval. For S1 neurons (areas 3b and 1) the stimulating probe was placed at the receptive field centers. S2 neurons had large receptive fields, often bilateral, spanning all digits and sometimes even reaching the forearm (Pons et al., 1987, 1992; Sinclair and Burton, 1993; Fitzgerald et al., 1999). Stimuli were always applied at the fingertips and, as illustrated in Figure 1a, consisted of trains of short mechanical pulses delivered at various frequencies.

For each neuron, two quantities were computed in each trial: the mean firing rate and the PSFP, which is an estimate of stimulus frequency based on the periodicity of evoked action potentials (see Materials and Methods). We used the PSFP because, just like firing rate, it is a scalar quantity from which stimulus frequency can be estimated on a trial-by-trial basis; however, unlike with firing rate,
the accuracy of this estimation depends on the periodicity of the spike trains. Figure 2, a and c, shows examples of S1 spike trains evoked during the base stimulus in individual trials, and Figure 2, b and d, shows the corresponding power spectra. The PSFP is simply the center (x coordinate) of the frequency bin with the most power. As illustrated in these Figures, the PSFP in S1 tends to be the same across trials. This is because the evoked spikes are phase-locked to the individual stimulation pulses. This can be seen more clearly in Figure 2h, which shows average S1 responses triggered at the time of individual pulses, the onset of which occurs at a time lag equal to 0 msec. The evoked activity reflects the periodicity of the sensory input. Curves for mean PSFP versus frequency were also obtained; this was done by averaging the PSFP over trials with equal stimulus frequency. These curves are shown in Figure 2f. Here the points fall close to the x = y line, confirming that the PSFP typically falls near the stimulus frequency. Curves for mean firing rate versus frequency were also obtained; examples are shown in Figure 2e. Notice that these neurons tend to fire more action potentials at higher stimulus frequencies. This was also true for the population: when straight lines were fit (Press et al., 1992) to the rate-versus-frequency data, most neurons had positive slopes, as shown in Figure 2e. Variations in mean rate across the tested range of frequencies were similar to those observed previously in somatosensory cortex using paradigms based on other tactile stimuli, such as textured surfaces or tactile motion (Sinclair and Burton, 1991, 1993; Gardner et al., 1992; Romo et al., 1996).

Curves like those of Figure 2, e and f, give a rough idea of the strength of association between the stimulus and the evoked variations in firing rate and in PSFP, but comparing them against each other is difficult. Instead, a quantitative measure of association was computed: Shannon’s mutual information (Cover and Thomas, 1991; Abbott et al., 1996) (see Materials and Methods). This statistic is useful because it allows a direct comparison between the two kinds of response in the same units, that is, in terms of their capacity to encode stimulus frequency. The maximum amount of information in these experiments was 3 bits.

In S1, the information that the PSFP—i.e., periodic spike timing—provided about stimulus frequency, \( I_{\text{PSFP}} \), was extremely high (1.71 \pm 0.95 bits, mean \pm SD; 107 of 129 values were significant, \( p < 0.01 \), as can be seen in Figure 2i (right plot). In 12 cases, \( I_{\text{PSFP}} \) > 2.8 bits, which means that by computing the PSFFs of any one of these neurons, on average seven frequencies could in principle be distinguished from each other with 100% reliability. The spike rate of these neurons seems to provide a faithful representation of the stimulus as it progresses in time (Fig. 2a, c, h), and the high \( I_{\text{PSFP}} \) values agree with this subjective impression. Notice in Figure 2i, however, that the mean \( I_{\text{PSFP}} \) dropped considerably from area 3b, which receives the heaviest thalamic projection (Jones, 1975, 1983), to area 1. The average numbers were 1.96 \pm 0.97 bits for area 3b (n = 68) and 1.43 \pm 0.86 bits for area 1 (n = 61), and the difference was highly significant (\( p < 0.001 \)). These \( I_{\text{PSFP}} \) values represent upper bounds on the information provided by the PSFP that is available to neurons downstream from S1, because neuronal mechanisms that may actually implement an approximate Fourier decomposition—for example, operations based on spike train autocorrelations (Cariani and Delgutte, 1996) or intrinsic oscillators (Ahissar and Vaadia, 1990; Ahissar, 1998)—cannot match the accuracy of the numerical methods (Press et al., 1992) used to compute the PSFP.

In contrast to these numbers, the information about stimulus frequency provided by the firing rate, \( I_{\text{RATE}} \), was approximately sixfold lower, but certainly not negligible (0.28 \pm 0.23 bits; 74 of 129 values were significant). The distribution of values is shown in Figure 2i (left). What order of magnitude for \( I_{\text{RATE}} \) should we have expected based on rate curves like those in Figure 2e? To get a better idea of the correspondence between the rate-versus-frequency curves and \( I_{\text{RATE}} \), consider the following idealized but representative example. Suppose the applied stimulus frequency \( s \) can take one of eight values, 8, 12, 16, 20, 24, 28, 32 or 36 Hz, and consider a neuron whose mean firing rate increases linearly with \( s \) with a slope of 0.7 spikes, typical of S1 (Fig. 2g), such that the evoked mean firing rate can be described by:

\[
r(s) = 22 + 0.7s + \sigma \epsilon.
\]

Here \( \epsilon \) represents random Gaussian noise with zero mean and unit variance, so \( \sigma \) is the SD of the mean firing rate. This \( \sigma \) is equivalent to the \( \sigma \) computed from the experimental data, except that, for simplicity, it is considered independent of frequency \( s \). On average, the mean rate of this ideal neuron is 28 spikes/sec when \( s = 8 \) Hz and 47 spikes/sec when \( s = 36 \) Hz; these values are also typical for the minimum and maximum mean rates at which S1 neurons fired during our experiments. For this idealized typical neuron, when the amplitude of the noise is \( \sigma = 3.5 \) spikes/sec, \( I_{\text{RATE}} = 1 \) bit; when \( \sigma = 8.7 \) spikes/sec (close to the average measured value, as seen in Fig. 5c), \( I_{\text{RATE}} = 0.3 \) bits; and when \( \sigma = 16 \) spikes/sec \( I_{\text{RATE}} = 0.1 \) bits. In comparison, a Poisson process, which provides a reasonable first order model for neuronal firing (Softky and Koch, 1993; Shadlen and Newsome, 1998), would give \( I_{\text{RATE}} = 0.3 \) bits, assuming that it fired at the same mean rates and that spikes were counted in a 500 msec time window. So, for cortical standards, 1 bit corresponds to an extremely reliable neuron, and 0.3 bits should be more or less typical given the experimental parameters and the measured rates. This is in agreement with the information values computed from the data.

Taken together, these results confirm that S1 spikes are precisely time-locked to the stimulation pulses (Mountcastle et al., 1969, 1990; Recanzone et al., 1992), but they also show that although periodic firing can in principle provide a better code for stimulus frequency, firing rate cannot be dismissed.

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**Figure 1.** Behavioral paradigm and stimulus sets used. a, Schematic diagram of the task. In each trial, the mechanical probe was lowered so that it touched one of the fingertips of the restrained hand (PD, probe down); the monkey reacted, placing its free hand on a lever within 1 sec after indentation (KD, key down); after a delay period (1.5–3 sec) the probe oscillated vertically, delivering a series of pulses at a base frequency; after an interstimulus interval (1–3 sec), a second set of pulses was delivered at a comparison frequency; after the end of the comparison stimulus, the monkey had to release the lever within 600 msec (KU, key up) and press one of two push-buttons (PB). One button indicated that the comparison frequency was higher than the base, and the other indicated that the comparison was lower than the base. b, Two stimulus sets frequently used in the experiments. The numbers inside the grid indicate the percentage of correct responses for each base-comparison combination. Set A had constant differences of 8 Hz between base and comparison. Percentages are based on the performance of three monkeys throughout 350 runs with this set. Set B was designed to vary the difficulty of the task in a more systematic manner. The percentages shown correspond to 42 runs from two monkeys.
Differences between S1 and S2

Figure 3 shows results, displayed in the same format as those in Figure 2, for a population of S2 neurons. The mean strength of rate modulations in S2 was comparable to that measured in S1: the average \( I_{RATE} \) in S2 was lower (0.14 ± 0.18 bits), but the maximum values were still around 1 bit, as shown in Figure 2, and ~20% of all values (139 of 689) were significant (a calculation similar to the one around Equation 5 in this case gave a typical \( I_{RATE} \) of ~0.2 bits, assuming Poisson statistics). Thus, considerable rate modulation was also present in S2. However, comparison between S1 and S2 responses revealed four major differences.

First, a lack of periodicity in S2 was revealed. This can be seen in the spike rasters of Figure 3, a and c, and in the pulse-triggered responses of Figure 3e. For the latter, the responses of neurons with positive and negative slopes were averaged, which is why the mean firing rates are so similar at the three frequencies shown. Note that phase-locking is hardly noticeable, especially at higher frequencies. Consistent with this, the mean PSFP in S2 is practically independent of stimulus frequency, as illustrated in the examples of Figure 3f. In quantitative terms, the mean \( I_{PSFP} \) in S2, computed for the spikes evoked during the base stimulus, was an order of magnitude smaller than in S1 (0.17 ± 0.34 bits), and only 52 of the 689 neurons had values that were significantly different from zero.

Second, S2 contained a larger proportion of neurons that fired most strongly at low stimulation frequencies. The middle column of Figure 3 illustrates such a unit, and e shows that its rate decreases as a function of frequency. As mentioned above, in S1 stronger activity typically occurred at higher frequencies, as shown in Figure 2e. When firing rates were fitted (Press et al., 1992) as linear...
functions of frequency, in S1 only 8% (10/129) of the resulting slopes were negative, whereas in S2 42% (287/689) of the slopes were negative. This difference can be seen by comparing Figures 2g and 3g. Interestingly, a similar transformation between S1 and S2 representations has been reported for textured surfaces (Sinclair and Burton, 1993). Here we should also mention that, in both areas, most response curves were approximately monotonic. First, the linear fits were acceptable \[ \text{in } 53\% (68/129) \text{ and } 87\% (602/689) \text{ of the neurons in S1 and S2, respectively. When Gaussian tuning curves were fitted to the same data, 98\% of the fits were acceptable in both areas (126/129 in S1, 673/689 in S2). This is not surprising, because Gaussian tuning curves had four parameters: baseline, amplitude, center frequency, and width (see Eq. 1). Still, many of the resulting curves were monotonic, because the centers of the best fitting Gaussians were either outside or at the edges of the frequency interval that contained the data. For instance, the area 1 neuron in Figure 2e (right) had a center frequency \( C = 31 \text{ Hz} \), beyond the highest frequency tested, and a width \( \sigma_G = 18 \text{ Hz} \). For other neurons, the Gaussian curves fitted better the saturation effects often seen at lowest or highest firing rates. For example, the firing rate of the area 3b neuron in Figure 2e (left) is lower for 36 than for 28 Hz, although it has a positive slope. The Gaussian fit for this neuron had center frequency \( C = 29 \text{ Hz} \) and width \( \sigma_G = 18 \text{ Hz} \). We considered a neuron as tuned if the limits \( C \pm \sigma_G \) were both inside the interval of tested frequencies and if the neuron also had a significant \( I_{\text{RATE}} \). The first condition assures that small saturation effects, like that of the area 3b neuron in Figure 2e (left), are not counted as actual tuning, and the second one guarantees that the Gaussian curve is significantly different from flat. Few neurons were found that satisfied these criteria: 12% (15/129) in S1 and 1% (8/689) in S2. In conclusion, most S1 and S2 rate-versus-frequency curves were reasonably monotonic, with negative slopes being more common in S2.

The third difference was that “flat” neurons were more abundant in S2 (62%, 428/689) than in S1 (31%, 40/129). Flat neurons had firing rates that did increase or decrease significantly during stimulation, compared with the baseline activity preceding the base stimulus, but were not affected by stimulus frequency, i.e., \( I_{\text{RATE}} \) was not significant (here we used \( p > 0.05 \) as a criterion). This was also reflected in the distribution of slopes: a larger fraction of S2 neurons had slopes that were close to zero, as can be seen by comparing Figures 2g and 3g. This difference in the proportion of flat neurons could be caused partly by suboptimal stimulation of S2; we have observed that S2 receptive fields have essentially no preference for one or another fingertip (Fitzgerald et al., 1999), but sometimes they do extend beyond the hand (Pons et al., 1987, 1992; Sinclair and Burton, 1993).

Figure 3. Neuronal responses in S2. Left and middle columns show data from two neurons: the firing rate of one increases with increasing stimulus frequency (positive slope), and the firing rate of the other decreases with increasing stimulus frequency (negative slope). Slopes were extracted from the linear fits shown in e. Same format is used as in Figure 2, except in d, middle column, frequency was 27 Hz; in e, \( I_{\text{RATE}} = 0.89 \pm 0.09 \) and \( 0.75 \pm 0.13 \) bits for left and middle columns, respectively (both significant); in h, \( I_{\text{PSFP}} = 0.26 \pm 0.18 \) and \( 0.30 \pm 0.30 \) bits, for left and middle columns, respectively (both not significant). In h, histograms are averages of 250–287 neurons (note same scale as in Fig. 2). Population data in g and i are based on 689 S2 neurons. All data are based on neuronal activity evoked by the base stimulus.
activity lacked a significant oscillatory component. Significant rate
modulations after the stimulus were not observed in S1: four
neurons had significant $I_{RATE}$ during the interstimulus interval,
but three were expected just by chance. Therefore, sustained ac-
tivity was absent in the primary sensory area [compare with Zhou
and Fuster (1996, 1997)]. The significance of maintained S2 activity
is hard to pinpoint. To perform the task correctly, the monkeys had
to store the frequency of the base stimulus in short-term memory
(Hernández et al., 1997; Romo et al., 1999). Some prefrontal
neurons are also active in this task, throughout or at different
points of the interstimulus interval, and their mean firing rates also
increase or decrease quasilinearly as functions of stimulus fre-
quency (Romo et al., 1999). Additionally, we found that the sus-
tained modulation in S2 was greatly reduced during passive stim-
ulation, when the stimuli were applied but did not have to be
remembered (data not shown). Hence, it is tempting to think that
such sustained activity may be related to the working memory
requirements of the task, but this is speculative.

A simple compromise between firing rate and timing

The above results show that, on the basis of single-cell compari-
sions, firing rate modulations in S2 were somewhat weaker than
those in S1 in terms of information content; on average, $I_{RATE}$
differed by a factor of 2. However, the difference in terms of
periodicity was a factor of 10. Although in S2 the actual average
values of $I_{RATE}$ and $I_{PSFP}$ were similar, two points should be
stressed: first, that the fraction of neurons with significant $I_{RATE}$
twice as high as the fraction of neurons with significant $I_{PSFP}$,
and second, that the $I_{PSFP}$ values represent upper bounds.

We also checked whether distinctions between frequencies
could be made based on the AIBI in each trial. A burst is simply a group
of spikes close together in time, like those shown in Figure 2a (left).
We defined a burst through a time window $\tau$ such that any two
spikes within $\tau$ milliseconds of each other belonged to the same
burst. Note that the rate of bursts and the rate of spikes are
correlated—indeed, if $\tau$ is very small each spike equals a burst and
the two rates become equal—but grouping by bursts with more
than one spike may produce more accurate results than simply
counting spikes, especially when long interspike intervals corre-
spond to intervals between consecutive stimulation pulses. The
AIBI represents a plausible middle ground between counting the
total number of spikes, ignoring their temporal distribution, and
taking into account all individual interspike intervals.

For each neuron, the AIBI was obtained in each trial, and the
information that the AIBI provided about stimulus frequency,
$I_{AIBI}$, was computed (see Materials and Methods). Parameter $\tau$ was
set to optimize the average $I_{AIBI}$ in S1. It should be borne in mind
that, having optimized $\tau$, $I_{AIBI}$ is expected to be at least equal to
$I_{RATE}$, because one may always choose $\tau$ close to zero and count
each spike as a burst. A positive value of $I_{AIBI} - I_{RATE}$ means that
additional information is extracted from the timing of spikes, in
excess of the information provided by the rate. With an optimal $\tau$
of 20 msec, the average $I_{AIBI}$ in S1 was 0.58 ± 0.49 bits ($n = 129$).
Thus, although the PSFP was more efficient, the AIBI did capture
some of the periodic structure of the spike trains, providing twice
as much information as the firing rate alone (McLurkin et al.,
1991). This was also true for the maximum values, which were
1.16 ± 0.09 bits for $I_{RATE}$ and 2.29 ± 0.07 for $I_{AIBI}$. In contrast, in
S2 $I_{AIBI}$ was indistinguishable from $I_{RATE}$ ($p > 0.49, n = 689$), and
the maximum value was 0.65 ± 0.12 bits, quite below the maximum
$I_{RATE}$, which was 1.04 ± 0.07 bits. Other values of $\tau$ were also
tested for S2, but the results were similar: the mean $I_{AIBI}$ always
decreased with increasing $\tau$. Hence, grouping spikes by bursts,
which effectively doubled the information about stimulus frequency
reported by the firing rate in S1, was entirely ineffective in S2.
This confirms, with a different method, that phase-locking is strong in S1
and extremely weak in S2.

According to these results, neurons immediately downstream
from S1 may read out stimulus frequency in at least two ways:
either from S1 firing rate modulations or from the periodic struc-

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**Figure 4.** Sustained neuronal responses in S2. The base stimulus turned on
at time zero, lasting 500 msec; stimulus onset and offset are indicated by
dotted vertical lines. Interstimulus interval duration was 1–3 sec. a. Spike
density histograms of a neuron that fired most strongly at high frequencies
(positive slope). For the shown traces, stimulus frequencies were 8, 20, and
28 Hz, as indicated. b, Information (±1 SD) carried by the neuron illus-
trated in a as a function of time. $I_{RATE}$ (black bars) and $I_{PSFP}$ (white bars)
were computed every 250 msec using the spikes contained in a 250 msec
window centered at the midpoint (x coordinate) between bars. Large
and small dots indicate significance levels of $p < 0.01$ and $p < 0.05$,
respectively. c, Spike density histograms of a neuron that fired most strongly
at low frequencies (negative slope); same stimulus frequencies as in
respectively. d, Spike density histograms of a neuron that fired most strongly
at time zero, lasting 500 msec; stimulus onset and offset are indicated by
large bars and $I_{PSFP}$, as in b and d. All spike densities were obtained by convolving
the spike trains with a Gaussian kernel of SD equal to 30 msec and
averaging over trials of equal frequency.

Fourth, many neurons in S2 either sustained their frequency-
specific responses beyond the base stimulus or displayed them only
after stimulus offset, during the interstimulus interval. Figure 4a
illustrates this for a neuron with positive slope that maintained
significant rate modulation even 1 sec after stimulus offset. Figure
4c shows the activity of another, more typical neuron that had a
negative slope and prolonged its response for a few hundred
milliseconds. The histograms in Figure 4, b and d, indicate the
amount and significance of $I_{RATE}$ and $I_{PSFP}$ for these neurons as a
function of time, and the plot in Figure 4e presents the numbers of
S2 neurons with significant information ($I_{RATE}$ and $I_{PSFP}$) also as
a function of time. Notice that ~13% (89/689) of the neurons
displayed significant rate modulations in the 250 msec window after
stimulus offset. Figure 4e also shows that during this same period,
10 neurons also had significant $I_{PSFP}$, however, the number
expected just by chance was seven. This means that the sustained

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**A simple compromise between firing rate and timing**

The above results show that, on the basis of single-cell compari-
sons, firing rate modulations in S2 were somewhat weaker than
those in S1 in terms of information content; on average, $I_{RATE}$
differed by a factor of 2. However, the difference in terms of
periodicity was a factor of 10. Although in S2 the actual average
values of $I_{RATE}$ and $I_{PSFP}$ were similar, two points should be
stressed: first, that the fraction of neurons with significant $I_{RATE}$
twice as high as the fraction of neurons with significant $I_{PSFP}$,
and second, that the $I_{PSFP}$ values represent upper bounds.

We also checked whether distinctions between frequencies
could be made based on the AIBI in each trial. A burst is simply a group
of spikes close together in time, like those shown in Figure 2a (left).
We defined a burst through a time window $\tau$ such that any two
spikes within $\tau$ milliseconds of each other belonged to the same
burst. Notice that the rate of bursts and the rate of spikes are
correlated—indeed, if $\tau$ is very small each spike equals a burst and
the two rates become equal—but grouping by bursts with more
than one spike may produce more accurate results than simply
counting spikes, especially when long interspike intervals corre-
spond to intervals between consecutive stimulation pulses. The
AIBI represents a plausible middle ground between counting the
total number of spikes, ignoring their temporal distribution, and
taking into account all individual interspike intervals.

For each neuron, the AIBI was obtained in each trial, and the
information that the AIBI provided about stimulus frequency,
$I_{AIBI}$, was computed (see Materials and Methods). Parameter $\tau$ was
set to optimize the average $I_{AIBI}$ in S1. It should be borne in mind
that, having optimized $\tau$, $I_{AIBI}$ is expected to be at least equal to
$I_{RATE}$, because one may always choose $\tau$ close to zero and count
each spike as a burst. A positive value of $I_{AIBI} - I_{RATE}$ means that
additional information is extracted from the timing of spikes, in
excess of the information provided by the rate. With an optimal $\tau$
of 20 msec, the average $I_{AIBI}$ in S1 was 0.58 ± 0.49 bits ($n = 129$).
Thus, although the PSFP was more efficient, the AIBI did capture
some of the periodic structure of the spike trains, providing twice
as much information as the firing rate alone (McLurkin et al.,
1991). This was also true for the maximum values, which were
1.16 ± 0.09 bits for $I_{RATE}$ and 2.29 ± 0.07 for $I_{AIBI}$. In contrast, in
S2 $I_{AIBI}$ was indistinguishable from $I_{RATE}$ ($p > 0.49, n = 689$), and
the maximum value was 0.65 ± 0.12 bits, quite below the maximum
$I_{RATE}$, which was 1.04 ± 0.07 bits. Other values of $\tau$ were also
tested for S2, but the results were similar: the mean $I_{AIBI}$ always
decreased with increasing $\tau$. Hence, grouping spikes by bursts,
which effectively doubled the information about stimulus frequency
reported by the firing rate in S1, was entirely ineffective in S2. This
confirms, with a different method, that phase-locking is strong in S1
and extremely weak in S2.

According to these results, neurons immediately downstream
from S1 may read out stimulus frequency in at least two ways:
either from S1 firing rate modulations or from the periodic struc-
tecture of S1 spike trains. In contrast, for neurons downstream from S2, the second possibility may not be available. Hence, two areas involved in somatosensory processing could potentially use fundamentally different codes to represent the same quantity. S1 is extremely important for somatosensory processing: lesions in this area cause severe impairments in discrimination and categorization tasks (LaMotte and Mountcastle, 1979; Zainos et al., 1997), and activity driven by direct microinjection of electrical current into S1 may trigger sensory percepts that probably resemble natural sensations quite closely (Romo et al., 1998; Wickersham and Groth, 1998). Therefore, the crucial question is whether neurons downstream from S1 read out its periodicity and are affected by it. We performed other experiments to try to address this issue.

**Context-dependent modulations of activity**

In general, the attentional state of a subject performing a task may have a strong influence on the neurons involved in it; neuronal responses are often enhanced when attention is focused on a sensory feature that the neurons react to (Hsiao et al., 1993; McAdams and Maunsell, 1999; Treue and Martínez-Trujillo, 1999). We wondered whether spike periodicity or firing rate would be subject to similar modulatory effects. The same sets of stimuli used for discrimination—the active condition—were also delivered passively to the monkeys. During passive stimulation the responding arm was restrained, no behavioral reaction was required, and no reward was delivered.

Figure 5 compares S1 activity evoked during the comparison stimulus in active and passive conditions. Figure 5a shows that the mean $I_{\text{RATE}}$ was significantly higher in the active condition ($0.42 \pm 0.35$ bits in active, $0.27 \pm 0.23$ bits in passive; $n = 50$ neurons with significant information in at least one of the conditions; $p < 0.0004$); indeed, most points fall above the equality line. Other measures of neuronal activity also showed significant variations across conditions. Figure 5c shows the average variability in firing rate across trials ($\sigma$) in the two conditions. In this case, most points fall below the diagonal line, indicating that variability in firing rate was significantly smaller during active discrimination ($\langle \sigma \rangle$ was $8.9 \pm 4.2$ spikes/sec in active, vs $10.5 \pm 4.6$ spikes/sec in passive; $n = 77$ neurons tested in the two conditions; $p < 0.0002$). Across conditions, changes in the signal-to-noise ratio (Eq. 2), which is a simple function of the firing rates, were strongly correlated with changes in $I_{\text{RATE}}$ (linear correlation coefficient was $0.98$, $p < 0.0002$). Thus, with all the measures tested we arrived at the same conclusion: the firing rate in S1 is a more reliable signal during discrimination than during passive stimulation.

We were concerned about this result, however, because we had not taken into account the correlations among neurons, i.e., the stimulus-independent co-fluctuations in numbers of spikes fired. For certain changes in the correlations, the information about stimulus frequency transmitted jointly by the rates of multiple neurons might have actually decreased, despite an increase in the information conveyed by individual neurons (Shadlen and Newsome, 1998; Zohary et al., 1994; Abbott and Dayan, 1999). Two additional results indicated that this was not the case. First, we measured $\rho$, the linear correlation coefficient between pairs of simultaneously recorded neurons averaged over all pairs. For each pair, the coefficient was calculated using Equation 3, and a mean over all pairs was computed. We found that $\rho$ was actually smaller in the active condition, although the difference was not significant ($0.10 \pm 0.18$ in active, $0.16 \pm 0.21$ in passive; $n = 84$ pairs tested in S1; $p > 0.037$). Second, we also computed the information provided jointly by the firing rates of pairs of neurons recorded simultaneously, which takes into account their pairwise correlation, and again we observed, on average, a significant increase in information about stimulus frequency in the active condition with respect to the passive ($p < 0.0002$).

Very similar differences between rate modulation in active and passive conditions were obtained in S2. Figure 6a and c, illustrates this for $I_{\text{RATE}}$ and $\langle \sigma \rangle$, but the same was also true for the signal-to-noise ratio and other measures of activity (Fig. 6, see legend).

Interestingly, the sustained responses after the offset of the base stimulus exhibited similar but larger effects (data not shown). Regarding the correlation coefficients in S2, again, no difference was found between active and passive conditions ($\rho$ was $0.07 \pm 0.20$ in active and $0.08 \pm 0.21$ in passive; $n = 126$ pairs tested in S2; $p > 0.7$), and the information carried jointly by the firing rates of pairs of neurons was also significantly higher during active discrimination ($p < 0.0002$). Therefore, the behavioral context of the task definitely had an impact on the evoked firing rates of S1 and S2 neurons: the numbers of spikes produced were significantly more regular across trials during active discrimination.

The periodicity of evoked spikes in S1 was also different in active and passive tests, although the changes seemed more subtle than for rate. This is shown in Figure 5b. Here a disproportionate number of data points seem to fall above the equality line, in agreement with the finding that the mean $I_{\text{PSFP}}$ was larger in the active condition ($1.62 \pm 0.90$ in active, $1.45 \pm 0.85$ in passive; $n = 63$ S1 neurons with significant $I_{\text{PSFP}}$ at least one condition), but the effect did not reach the significance criterion of 0.01 ($p > 0.025$). However, we also compared the mean power at stimulus frequency (mean $P_S$) across conditions. This quantity is just the percentage of power at the frequency bin that includes the stimulus frequency, averaged over all trials (see Materials and Methods). The data are shown in Figure 5d. The mean $P_S$ was also larger.
conditions were not caused by drift artifacts.

showing that the described differences between active and passive runs were not due to a retraining period; they were able to perform the task from the initial runs. Because S1 neurons emit spikes that are reliably phase-locked to individual stimulation pulses, aperiodic stimuli impose a timing between phase-locked spikes or bursts of spikes that, by design, varies randomly within the stimulation period and across trials. Similar random timing can also be imposed directly through intracortical microstimulation (Romo et al., 1998).

The monkeys’ performance in this task only decreased slightly compared with discrimination of periodic stimuli: overall, 88 versus 80% correct (Romo et al., 1998). We investigated whether neuronal responses paralleled this similarity. Figure 7, a and b, shows the responses of an S1 neuron to periodic and aperiodic stimuli at two frequencies. This neuron responded quite faithfully to individual stimulation pulses. Notice the regular interspike intervals in the periodic condition, in Figure 7a, and the much more variable spike train in the aperiodic condition in Figure 7b. Figure 7c shows that for any given neuron, $I_{\text{RATE}}$ could vary somewhat from the periodic to the aperiodic situation, but on average, firing rate modulations in S1 were indistinguishable across conditions ($I_{\text{RATE}} = 0.44 \pm 0.28$ bits for periodic, $0.38 \pm 0.25$ bits for aperiodic; $n = 31$ S1 neurons tested in both conditions and with significant $I_{\text{RATE}}$ in at least one of them; $p > 0.19$). Differences were slightly larger in S2 ($I_{\text{RATE}} = 0.37 \pm 0.22$ bits for periodic, $0.22 \pm 0.17$ bits for aperiodic; $n = 13; p > 0.055$), but fewer samples were available. These results show that in the two areas, firing rate was, on average, similarly modulated by frequency in periodic and aperiodic conditions.

Not surprisingly, in these experiments $I_{\text{PSFP}}$ practically vanished: of 41 S1/S2 neurons with significant $I_{\text{PSFP}}$ in the periodic condition, only one had a significant value in the aperiodic condition. The same thing happened with the mean power at the PSFP, at the mean stimulus frequency and at twice the mean stimulus frequency. This was expected and simply showed that no consistent modulations in periodicity are seen with aperiodic stimulation; the Fourier spectrum shows no regularity from one trial to the next.

What about bursts of spikes; could they provide a reliable measure of mean stimulus frequency for aperiodic stimuli? In the periodic condition, the AIBI of S1 neurons carried more information about the relevant stimulus feature. The periodicity of the evoked spikes did not change with behavioral context in S2, but it did so in S1. This was surprising and indicates that spike timing may be influenced by attention or behavioral context (Steinmetz et al., 2000). However, at the level of S1, these results do not favor one neural code over the other.

Responses to aperiodic stimuli

Two of the monkeys also discriminated the average frequencies of aperiodic stimuli (Romo et al., 1998) (see Materials and Methods). In this situation, the same numbers of pulses corresponding to each stimulus frequency were delivered in the 500 msec stimulation period, but the times between pulses were random and varied from trial to trial. To obtain a reward, the monkeys had to compare correctly the average frequencies of the base and comparison stimuli, just as with periodic vibrations. These animals did not go through a retraining period; they were able to perform the task from the initial runs. Because S1 neurons emit spikes that are reliably phase-locked to individual stimulation pulses, aperiodic stimuli impose a timing between phase-locked spikes or bursts of spikes that, by design, varies randomly within the stimulation period and across trials. Similar random timing can also be imposed directly through intracortical microstimulation (Romo et al., 1998).

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What about bursts of spikes; could they provide a reliable measure of mean stimulus frequency for aperiodic stimuli? In the periodic condition, the AIBI of S1 neurons carried more information than the rate, as has been described. The AIBI of S1 neurons also provided significant information in the aperiodic condition but, as shown in Figure 7d, $I_{\text{AIBI}}$ in this case was significantly smaller than with periodic stimulation ($0.71 \pm 0.47$ in periodic, $0.32 \pm 0.18$ in aperiodic; $n = 31$ S1 neurons tested in both conditions and with significant $I_{\text{AIBI}}$ in at least one of them; $p < 0.0002$); most data points fall below the equality line. The key observation here is that with aperiodic stimuli, $I_{\text{AIBI}}$ was, on average, slightly smaller than $I_{\text{RATE}}$, and this was the case whether all neurons or only those with significant information were compared. This can be appreciated by comparing the y-axis values in Figure 7, c and d. Comparisons using bursts of other sizes were also made—we used $\tau = 20, 15, 10$, and 5 msec—but the results were
Figure 7. Neuronal responses to periodic and aperiodic stimuli. The four raster plots at the top show spike trains from an S1 neuron that was tested with periodic (a) and aperiodic (b) stimuli at frequencies of 12 and 35 Hz, as indicated. Each set of responses includes 10 trials collected during active discrimination. For a given stimulus frequency, the train of stimulation pulses was identical for all periodic trials but was different for all aperiodic trials. However, at a given mean frequency, the total number of pulses delivered was the same in both conditions. Long vertical lines indicate stimulus onset. In the periodic condition the neuron had intervals altogether. For the few S2 neurons tested, there was no spikes into bursts was just as efficient as ignoring the interspike significantly either. Hence, for aperiodic stimuli, clustering the 0.70

Based on the temporal patterns of spikes evoked during aperiodic 0.10 and 0.11 bits. \( I_{\text{RATE}} \) and \( I_{\text{AIBI}} \) were computed for 41 S1 and 30 S2 neurons tested with periodic and aperiodic stimuli. In both panels, circles and triangles correspond to S1 and S2 neurons, respectively, with significant information in at least one of the two conditions (periodic or aperiodic), and small dots indicate S1 and S2 neurons that had nonsignificant values in the two conditions. Diagonal lines indicate equal values in the two axes. Crosses on the bottom right corners indicate 1 average SD in each direction. \( I_{\text{RATE}} \) did not change across conditions, in either area: data points in c are distributed symmetrically around the diagonal line. \( I_{\text{AIBI}} \) was significantly larger with periodic stimulation; data points in d tend to fall below the diagonal. With aperiodic pulses, \( I_{\text{AIBI}} \) was similar to \( I_{\text{RATE}} \); the y-axis values in c and d are similar (see Results).

Figure 7, a and c, illustrates this procedure for a single S1 neuron, and Figure 9, a and c, illustrates it for a single S2 neuron. Here H and E indicate hit and error categories, respectively, and the subscript indicates the type of trial. Each dot corresponds to a single trial, and the horizontal bars indicate the means for hits and errors in the corresponding categories. In both Figures, the difference between panels a and c lies in the quantity considered as the response.

To detect systematic variations in periodic spike timing, we computed standardized versions of the PSF amplitude, of \( P_s \), and of the amplitude of the power spectrum at twice the stimulus frequency. These three quantities tend to increase the closer a spike train is to a perfectly periodic arrangement, so high values should correspond to better likelihood for correct discrimination, if periodicity is related to performance. In most S1 and S2 cells, these quantities were the same in hit and error trials, as illustrated in Figures 8c and 9c. We did find four S1 neurons for which the mean standardized \( P_s \) was significantly different for hit and error trials, but this number of neurons was not significantly different (\( p > 0.21 \)) from that expected by chance under the null hypothesis that hit and error responses come from the same distribution (among 238 S1 neurons, 2.38 significant tests at the 0.01 level were expected by chance). In other words, the result was not significant. The same was true for the three measures of periodicity, in both S1 and S2, and for type 1 and type 2 trials. The periodicity of spikes in single neurons showed no detectable covariations with behavior.

This negative result, however, was obtained by testing the neurons one at a time, but if higher periodicity tends to produce better performance, then across the population, standardized responses might show a tendency to be larger in hit trials than in error trials.
Nevertheless, no such trend was observed. This is illustrated in Figures 8d and 9d. In these plots, each point corresponds to one neuron. Each x coordinate is the difference between the mean of all type 1 error trials and the mean of all type 1 hit trials, using the standardized $P_S$ as a response, and the y coordinate is the same quantity but for type 2 trials. Observe that the clouds of points are symmetric and centered at 0 in both directions. This is because, on average, differences between responses in hits and errors were not significantly different from zero and were not correlated across types of trials either: for the S1 population in Figure 8d, the correlation coefficient was practically zero (0.008, $p > 0.9$), and for the S2 population in Figure 9d, the correlation coefficient was $-0.23$, but it was not significantly different from zero ($p > 0.025$).

Similar results were obtained when these tests were repeated using the standardized power at the PSFP or the standardized power at twice the stimulus frequency. No significant covariations between periodicity and behavior could be detected in either area.

In contrast, the numbers of evoked spikes did show significant covariations with behavioral performance. Figure 8e shows data from an S1 neuron with large differences between the means of the $H$ and $E$ categories. This neuron had a positive slope of $1.17 \pm 0.12$ spikes. The significant difference between $H_1$ and $E_1$ means that at any given comparison frequency lower than the base, on average, the chances of observing an error were higher when the neuron fired more spikes than usual for the given comparison frequency. This association was not a rare event. Among 219 runs that had at least five type 2 errors, we found 11 S1 neurons whose average standardized rates were significantly different in hit and error trials. This number may appear small, but with 231 samples the chances of finding at least 11 significant values when no real difference exists between two conditions is $< 3 \times 10^{-5}$ (binomial distribution with $p = 0.01$). Among the 219 runs with sufficient type 1 errors, only four neurons with significant differences were found, which was within the range expected by chance ($p > 0.18$), but there was additional evidence for the firing rate being related to behavior. In this case, a significant effect was observed across the population: the differences between standardized responses for hits and errors were significantly anticorrelated across trial types. This is shown in Figure 8f. In this plot the cloud of points is not symmetric; its correlation coefficient is $-0.42$ ($n = 191$, $p < 0.0002$). When a neuron fired, for instance, more spikes in incorrect versus correct discriminations in type 1 trials, it typically fired fewer spikes in incorrect versus correct discriminations of the opposite type. This
result and the 11 neurons with significant differences in type 2 trials demonstrate a link between the firing rate of S1 neurons and psychophysical behavior. In S2 the relationship between rate and behavior was even more evident. In type 1 trials, 28 of 321 neurons had significant differences between hits and errors, and in type 2 trials the numbers were 16 out of 206. The probability of finding these many by chance was, in both cases, <10^{-5}. As can be seen in Figure 9b, differences across trial types were negatively correlated in this area too. These anticorrelation patterns would be expected if a comparison between the average rates of two neuronal populations underlay the comparison between frequencies required by the discrimination process (Salinas and Romo, 1998), but this does not exclude other possibilities.

**DISCUSSION**

In these experiments, we examined the responses of S1 and S2 neurons to mechanical vibrations applied to the fingertips. Our aim was to determine, in our laboratory task where the only relevant stimulus feature is temporal frequency, what attributes of the evoked neuronal activity are important for behavior. In our simplified task this amounts to finding out what is the neural code for stimulus frequency. We specifically examined the hypothesis that such a code is constructed by some neural mechanism that reads out the periodic interspike intervals of the spike trains evoked in S1. As discovered in earlier work, the periodicity of spikes evoked by flutter vibrations was extremely prominent and reliable in this area. Unfortunately, however, we could not determine with certainty whether their precise timing plays a significant functional role in frequency discrimination. Previous studies contemplated a code for flutter frequency based exclusively on periodicity (Mountcastle et al., 1967, 1969, 1990; Talbot et al., 1968; Recanzone et al., 1992). What we did find, instead, is evidence that firing rate plays a significant role in encoding flutter frequency.

The evidence is as follows. First, rate modulations were widespread. We found that, contrary to earlier reports (Mountcastle et al., 1990; Recanzone et al., 1992), the firing rate of neurons in primary areas 3b and 1 varied significantly as a function of flutter frequency (Fig. 2e,i). S2 neurons showed roughly similar rate modulations in terms of the information that rate provides about stimulus frequency (Fig. 3e,i), but interestingly, neurons firing most intensely at low frequencies were much more common in S2 than in S1 (compare Figs. 2g and 3g). In some S2 neurons, rate modulations also persisted beyond the end of the stimulus (Fig. 4). The information conveyed by the rate was significant in ~57 and 20% of the neurons in S1 and S2, respectively. The absolute amounts of information that we obtained were comparable to the numbers that have been reported by several groups working with visual cortical neurons (Richmond and Optican, 1990; Gawne and Richmond, 1993; Gochin et al., 1994; Tovee et al., 1995). These studies were based on various kinds of visual stimuli that were effective at driving the tested neural populations, just as flutter was in our case. The agreement between these information values and ours might be attributable to network properties that are common to widely different cortical areas. Second, rate modulations in S1 and S2 conveyed similar amounts of information in periodic and aperiodic conditions, which raises the possibility that the same code for mean stimulus frequency is used in the two tasks. Third, the firing rate in both areas was modulated by the behavioral relevance of the stimuli, and we estimated the net impact of this contextual modulation: the numbers of spikes fired at a given frequency were more reliable when the animals were actively discriminating than when the stimuli were applied passively and, presumably, were ignored (Figs. 4a,c, 5a,c). On average, then, the firing rate provided a clearer, more reliable signal encoding stimulus frequency when this quantity was relevant to behavior. Last, we also found that the fluctuations in firing rate of some neurons were significantly correlated with the animals’ psychophysical performance on a single trial basis, suggesting that a few additional spikes fired by a single cell may have a detectable impact on discrimination performance, even in the case of a primary sensory neuron (Leopold and Logothetis, 1996).

Although not conclusive in terms of the specific question we pursued, the experiments with periodic stimuli revealed a number of interesting facts about the timing of evoked spikes in the somatosensory cortices. First, as found earlier, periodicity was extremely high in S1 (Fig. 2f,h,i), and presumably it is even higher in primary afferents (Talbot et al., 1968; Vallbo, 1995), but periodicity diminished appreciably from area 3b to area 1 (Fig. 2) and almost vanished in S2, suggesting that it is limited to early cortical representations. In view of this and of the presence of significant rate modulations already at the level of S1, the question that arises is whether the strikingly regular temporal structure of S1 spikes is somehow instantiated independently from variations in mean firing rate to compute or encode stimulus frequency. The second finding regarding timing was that the degree of periodicity in S1 was also affected by the behavioral relevance of the stimuli: evoked spikes were more phase-locked to the stimulus during active discrimination than during passive stimulation (Fig. 4b,d). Not surprisingly, this effect was not seen in S2 (Fig. 5b,d), where periodicity was much less prominent, although other timing effects have recently been described in this area (Steinmetz et al., 2000). Last, we found no relationship between variations in periodicity and psychophysical performance in single trials. None of the measures of periodicity that we tested exhibited significant covariations with behavior (Figs. 8c,d, and 9c,d). Clearly, analyses of this sort can only reveal subtle effects, especially when primary sensory neurons are concerned, but significant covariations between firing rate and performance were indeed found in S1 (Fig. 8a,b). This suggests that firing rate may have a larger weight in determining the neural code for stimulus frequency than the periodic alignment of spikes.

Coding mechanisms based exclusively on periodicity appear to be too rigid, as illustrated by the experiments with aperiodic stimulation; periodicity in S1 activity could not encode mean frequency during stimulation with aperiodic patterns, in the sense that mean frequency could not be read out from a Fourier decomposition of the evoked S1 responses, but temporal integration in a wider sense cannot be ruled out by these results. One key question in the discrimination task performed by our monkeys is how neurons postsynaptic to S1 integrate their incoming inputs: are they able to read out some of the temporal features of the evoked activity? Neurons downstream from S1 must respond to certain features in the temporal structure of S1 activity, and they account for the ability of human subjects to distinguish without difficulty between periodic and aperiodic stimuli. From the comparisons between \( I_{\text{RATE}} \) and \( I_{\text{AHB}} \) it appears that such features need to be more sophisticated than plain bursts of spikes from single neurons, but other schemes are possible. We also found that the precise timing of S1 spikes, in relation to the periodic stimulation pulses, was not appreciably correlated with psychophysical performance, but there might be particular time scales or temporally sensitive processes for which variations in S1 spike timing do have an impact in postsynaptic activity and in the code for flutter frequency, and the present experimental design or the analytic tools that we used may have been insensitive to those time scales. In the present task, the monkeys were not required to extract any features from the temporal structure of the stimuli other than the mean frequency, but the results—in particular the evoked activity in S2—might be different in tasks that do require such detailed temporal analysis.

Evidently, single cell recordings also have limitations; possible neural codes based on coordinated spike timing across multiple neurons have been reported (O’Keefe and Recce, 1993; DeCharms and Merzenich, 1995; Riehle et al., 1997; Dan et al., 1998). The following scenario, for instance, could apply to our task: the rate of synchronized spikes from two neurons might vary independently of the individual firing rates, providing additional information about the stimulus that cannot be extracted if synchronous and nonsynchronous spikes are considered equal and are lumped together to compute the firing rates (Dan et al., 1998). This is just one possible coding scheme based on temporal relationships between spikes. In
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