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Heterogeneity in the Responses of Adjacent Neurons to Natural Stimuli in Cat Striate Cortex

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Yen S-C, Baker J, Gray CM. Heterogeneity in the responses of adjacent neurons to natural stimuli in cat striate cortex. *J Neurophysiol* 97: 1326–1341, 2007. First published November 1, 2006; doi:10.1152/jn.00747.2006. When presented with simple stimuli like bars and gratings, adjacent neurons in striate cortex exhibit shared selectivity for multiple stimulus dimensions, such as orientation, direction, and spatial frequency. This has led to the idea that local averaging of neuronal responses provides a more reliable representation of stimulus properties. However, when stimulated with complex, time-varying natural scenes (i.e., movies), striate neurons exhibit highly sparse responses. This raises the question of how much response heterogeneity the local population exhibits when stimulated with movies, and how it varies with separation distance between cells. We investigated this question by simultaneously recording the responses of groups of neurons in cat striate cortex to the repeated presentation of movies using silicon probes in a multi-tetrode configuration. We found, first, that the responses of striate neurons to movies are brief (tens of milliseconds), decorrelated, and exhibit high population sparseness. Second, we found that adjacent neurons differed significantly in their peak firing rates even when they responded to the same frames of a movie. Third, pairs of adjacent neurons recorded on the same tetrodes exhibited as much heterogeneity in their responses as pairs recorded by different tetrodes. These findings demonstrate that complex natural scenes evoke highly heterogeneous responses within local populations, suggesting that response redundancy in a cortical column is substantially lower than previously thought.

INTRODUCTION

In the striate cortex, populations of adjacent neurons exhibit common preferences to local stimulus features such as orientation, spatial frequency, and direction of motion (Hubel and Wiesel 1977; Hubener et al. 1997; Ohki et al. 2005). This columnar organization is thought to reduce the effects of neuronal variability by enabling the pooling of activity of many nearby neurons that are sensitive to the same features (Mazurek and Shadlen 2002). Adequate coverage of the stimulus space is thought to be achieved by hypercolumnar organization in which the spectrum of featural attributes are represented by continuous variations in the stimulus selectivities of similarly tuned groups of cells (Hubel and Wiesel 1977; Swindale 1991; Swindale et al. 2000).

This model is largely based on the analysis of neuronal responses to simple stimuli, such as bars and sinewave gratings, where parametric variations of individual stimulus features can be easily performed. The widespread use of such stimuli has also revealed that striate neurons are tuned to

multiple stimulus dimensions including orientation, direction, speed, spatial frequency, binocular disparity, and others; the selectivity to one stimulus parameter often depends on the value of other parameters (De Valois et al. 1979; Geisler et al. 2001; Skottun et al. 1994); and influences from beyond the classical receptive field are both nonuniform and nonlinear (e.g., Sillito et al. 1995; Vinje and Gallant 2000; Walker et al. 1999). This multi-dimensional and nonseparable stimulus selectivity, when combined with nonlinear surround influences, suggests that striate neurons should rarely be active during natural vision. Support for this has come from several studies demonstrating that striate neurons in cats, monkeys and ferrets respond with low probabilities when presented with a large sample of natural images (Baddeley et al. 1997; Vinje and Gallant 2000, 2002; Weliky et al. 2003).

The sparsity of neuronal responses to natural scenes also predicts a high degree of response heterogeneity (or population sparseness) among populations of adjacent neurons. Support for this comes from several recent studies where multi-neuron recordings have revealed substantial variations of receptive field properties among adjacent striate neurons (DeAngelis et al. 1999; Maldonado and Gray 1996; Maldonado et al. 1997; Reich et al. 2001). Consistent with this finding, serial recordings of neighboring neurons in striate cortex have revealed that natural images evoke highly decorrelated responses (Vinje and Gallant 2000, 2002). These results suggest that the classical picture of columnar organization, derived from the use of simple stimuli, may be substantially different from the one obtained when using complex, time-varying natural images (Horton and Adams 2005).

We have investigated these issues by simultaneously recording the responses of groups of nearby neurons in cat striate cortex during the presentation of time varying natural scenes (movies). Our aim was to characterize how local populations of neurons jointly represent time-varying natural scenes and to compare the heterogeneity of the responses for cells recorded on the same or different tetrodes.

METHODS

Surgery

All experiments were conducted on anesthetized and paralyzed cats (3–4 kg) of both genders. The surgical methods are described in detail elsewhere (Gray et al. 1995). The protocol used in the experiments was approved by the Institutional Animal Care and Use Committee at Montana State University and conformed to the guidelines recom-

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mended in Preparation and Maintenance of Higher Mammals During Neuroscience Experiments, National Institutes of Health Publication 91-3207 (National Institutes of Health, Bethesda, MD 1991).

Electrophysiology

Recordings were made using either sharp Tungsten electrodes (4 cells) or 16-contact silicon probes (84 cells) provided by the Center for Neural Communication Technology (CNCT) at the University of Michigan. The CNCT probes contained four separate tetrodes and used in two different configurations: one probe type had four shanks with a single tetrode at the end of each shank (4×1), and the second type had two shanks with two tetrodes per shank (2×2). In both configurations, the center-to-center separation between adjacent tetrodes was $150 \mu\text{m}$ (Fig. 1). The impedance of each contact ranged from 0.8 to $1.5 \text{ M}\Omega$ at 1 kHz. All signals were amplified (4000 times), band-pass filtered (0.5–6 kHz), digitized at 30 kHz at a resolution of 12 bits, and stored on a personal computer for off-line analysis. Once stable unit signals were obtained, we determined the borders of the receptive fields, the preferred stimulus orientation, and the dominant eye for each recording site using mouse-controlled light bars and sine-wave gratings. Movies were displayed to the eye that dominated cellular responses at the majority of recording sites (see following text).

Spike Sortings

Spike waveforms were extracted from the raw signals on each channel of the electrode or tetrode using a multi-step procedure. First, the background signal (i.e., the signal in the absence of spikes) was estimated by removing all data points from the raw signal that exceeded its mean ± 4 SD. Local minima in the original signal (i.e., negative peaks in the spike waveforms) were identified if they exceeded 4 SD of the background signal. Spike waveforms were extracted by storing 32 data points (11 points prior to and including the minimum, and 21 points following the minimum), on each channel. An additional constraint was included to prevent local

minima from being within three data points of each other to eliminate repeated extractions of the same waveform that might result from the occurrence of high-frequency noise.

Automated clustering of waveforms was performed using the KlustaKwik software package (Harris et al. 2000) and inspected using the MClust software package (<http://www.cbc.umn.edu/~redish/mclust/>). Clustering was based on the following parameters extracted from each waveform: area, energy, maximum voltage, minimum voltage, maximum to minimum ratio, waveform width, trigger value, fast Fourier transform (FFT) of the waveform, and the first and second principal components of the waveform.

Following the initial cluster analysis, we performed several procedures to edit the data and eliminate artifacts. First, each cluster was carefully inspected, and any waveforms clearly deviating from the average pattern were either deleted or moved to another cluster. The latter were treated as unclassified waveforms and subjected to later analysis. Second, artifactual clusters were deleted. These typically resulted from double triggering of triphasic waveforms or were due to waveforms that just exceeded the spike-extraction threshold. Third, clusters having similar waveforms were merged, but only when the interspike interval histogram (ISI) of the resulting cluster had a clear refractory period. Finally, each candidate cluster had to be nonoverlapping with all other clusters in at least one classification dimension.

Once these steps were completed, the data from a single tetrode consisted of one or more well-resolved clusters, each corresponding to a distinct single unit, and a nonclassified cluster composed of waveforms resulting from superimposed spikes (Goodell and Gray 2003). To identify the single units that contributed to these waveforms, we applied a template-matching algorithm to determine which individual or pair of template waveforms obtained from the initial analysis could best account for each unclassified waveform. The mean waveform of each distinct cluster was used as a template. We computed all possible linear superpositions of templates at all possible phase shifts. This yields $n(n-1)/2$ template pairs, where each pair consists of 125 composite waveforms [$(\pm 31 \text{ phases} * 2) - 1$]. Each unclassified waveform was then compared with each of the superimposed tem-

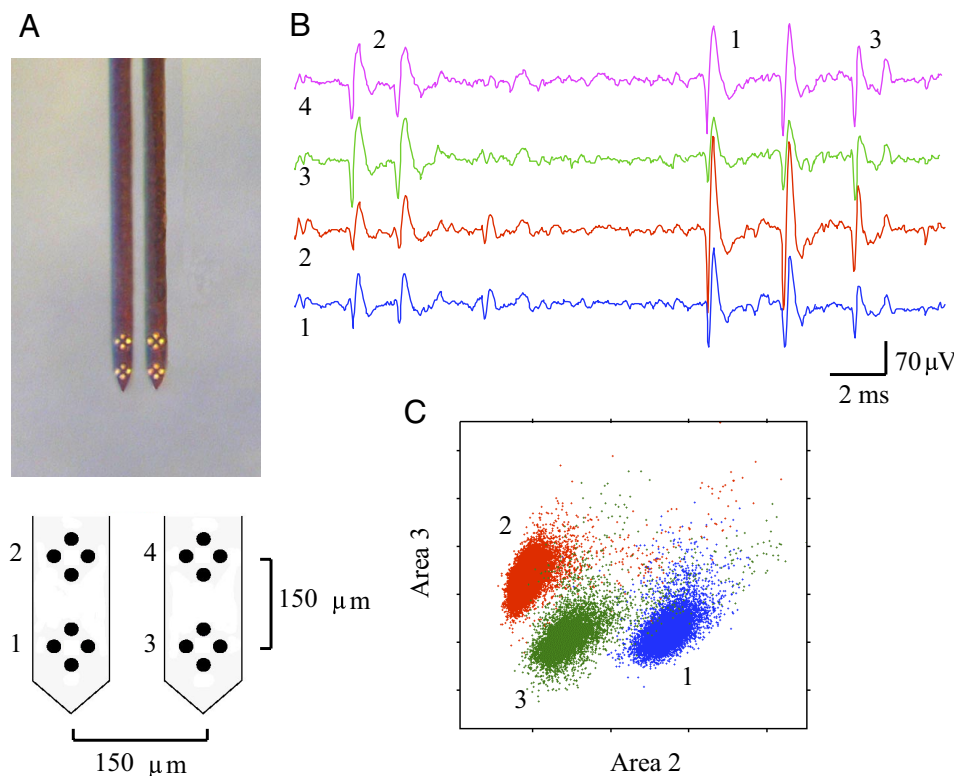


FIG. 1. Tetrode recording of neuronal activity in cat striate cortex. *A*: photograph and schematic of a 4-tetrode silicon probe in the 2×2 configuration. The inter-shank and -tetrode separations are both $150 \mu\text{m}$. Adjacent contacts within a tetrode are separated by $25 \mu\text{m}$. *B*: short segment of raw data showing the spikes of 3 different neurons (1–3) recorded from the 4 channels of tetrode 4 of the probe. The signals are plotted in 4 different colors (blue, red, green, magenta). *C*: scatter plot of waveform area for all spikes of cells 1 (blue), 2 (red), and 3 (green) recorded on channels 2 and 3 of tetrode 4.

plates by computing the sum of the squared differences between each pair of waveforms. The template pair that gave the smallest value was chosen as the best match, and the time difference between the two templates used to compute the best match was stored. If this value was less than or equal to the refractory period used for waveform extraction (± 3 data points), then a copy of the unclassified waveform was assigned to the clusters for both templates. If the phase difference was greater than the refractory period, then the waveform was copied to the cluster whose template had a peak negative value that aligned with that of the triggered waveform. A final editing procedure was performed on each cluster to remove any superimposed waveforms that occurred during the refractory period of the interspike interval distribution.

Visual stimulations

Monochromatic natural movie sequences were presented monocularly, at a distance of 57 cm, using a 20-in color monitor (Viewsonic P810) at a resolution of 800×600 pixels. The luminance distribution of the monitor ($0\text{--}80$ cd/m²) was gamma-corrected prior to the experiments. During the course of this study, we used three different graphics cards with increasing refresh rates of 85.12, 120, and 150 Hz.

Movie frames were digitized from a variety of DVD movies covering a wide range of natural and man-made scenes. These included Hollywood movies like “The Big Lebowski”, “The Matrix”, “Everest”, as well as two documentaries called “The Ultimate Guide—big cats and house cats”, and “Animals in the Wild”. The movie files were de-interlaced, and cropped to remove any visible borders. In some movies, the last few frames of one segment were faded into the first few frames of the next segment so the affected frames had to be removed manually. We did not make any attempts to remove the MPEG2 compression artifacts as they were fairly minor in the video sequences we used.

The individual frames were then converted to grayscale and presented at rates of 3, 4, or 6 refreshes per frame (corresponding to the 85-, 120-, and 150-Hz graphics cards), making the frame rate of the movies 28.3, 30, and 25 Hz, respectively. No temporal up-sampling was attempted, i.e., the identical movie frame was presented for 3, 4, or 6 refreshes. Individual movie frames had dimensions of 640×480 pixels and were positioned at the center of the monitor screen, occupying $32 \times 24^\circ$ of visual angle ($0.05^\circ/\text{pixel}$). For each recording site, we selected a short movie sequence, ranging in duration from 15 to 30 s and presented that clip 60–200 times to the dominant eye.

When presenting movie sequences at a resolution of 640×480 pixels, there are occasional times when the computer cannot keep pace with the requested frame rate. In these instances, short delays occur in the video presentation that result in particular frames of the movie being displayed for more than the specified number of video refreshes. We identified these “extra syncs” by first converting the vertical sync signal from the video card into a 1-ms TTL pulse. We sampled these pulses using a counter/timer card (ACCES CTR-05 ISA) and counted the number of vertical syncs occurring between each frame of the movie. The number of syncs were checked at the end of each frame, and any additional syncs were recorded by specifying the frame number in the movie and the number of extra syncs occurring during that frame. The ability of this setup to track extra syncs was verified by matching the records to the signals produced by a photo-diode placed directly on the monitor.

We were also concerned that scene changes in a movie might cause significant responses due to sudden changes in luminance or contrast in the image. To identify scene changes, we computed the difference in gray level of every pixel between consecutive movie frames, and defined scene transitions as frames where 60% of the pixels changed by ≥ 10 gray levels. We confirmed these scene changes by visual inspection of the movie frames. We found between 2 and 8 scene changes in the movies we presented with an average of 5.3 scene changes per movie. We then performed a simple event-triggered

analysis to see how often events (see *Detection of response events*) were preceded by a scene change. We found only 3.9% (59/1498) of the events occurred within two movie frames of a scene change (a duration of approximately 70 to 80 ms). This led us to conclude that the effect of scene changes in our data were minimal.

Data analysis

The principal goal of our analysis was to characterize the response properties of striate cortical neurons to the presentation of time-varying natural scenes and to determine how similar or different those properties were among neurons recorded simultaneously on either the same or different tetrodes. Based on previous studies (Blanche et al. 2005; Gray et al. 1995; Lemon 1984), we assumed that cells recorded on the same tetrode primarily lie adjacent to one another with the probability of their spatial separation falling off exponentially with a space constant of ~ 30 μm (Gray et al. 1995). Thus by analyzing the responses of cells recorded simultaneously on the same tetrode, it was possible to obtain a sensitive estimate of the heterogeneity of stimulus selectivity among adjacent groups of cells. Because we used two types of probe configurations (2×2 and 4×1), it was possible to compare the estimates of heterogeneity among adjacent cells to those obtained from cells lying in vertically, or horizontally nonadjacent positions. However, because our sample size was small, and the number of possible spatial comparisons was large, we chose to combine all inter-group comparisons.

Compensation for extra syncs

The first step in our analysis was to adjust the spike times for the sessions in which extra syncs were detected on specific frames of the movies. To do this, we removed all spikes that occurred during the extra syncs, and subtracted the duration of the extra syncs (i.e., an integer multiple of the vertical refresh) from all subsequent spike times. We checked the resulting interspike intervals and removed the first adjusted spike if it occurred within 1 ms of the last unadjusted spike time. Across 20 separate movie presentations the median percentage of extra syncs was 0.007%. Thus given their rare occurrence, and our analytical compensation for their effects, the influence of extra syncs on the properties of the neuronal responses is likely to be negligible.

Response reliability

It is well known from physiological experiments in anesthetized animals that there are some neurons in striate cortex that either fail to reliably respond to any stimuli or that change their responsiveness as a function of the state of the animal. Because these effects could have a large impact on our results, we established a measure of response reliability and evaluated our findings with respect to this measure. Because responses to repeated stimulus presentations are likely to be correlated, we devised a simple measure of spike count correlations across trials. On each stimulus repetition, we calculated the number of spikes occurring in each frame of the movie. We then computed the correlation coefficient across all frames of the movie for all possible pair-wise repetitions [$n(n - 1)/2$], and determined the percentage of correlation values that were statistically significant ($P < 0.05$).

Sparsity

To quantify the sparseness of the responses, we utilized the non-parametric statistic employed previously by Vinje and Gallant (2000)

$$S = \left\{ 1 - \left[\left(\frac{\sum_i r_i}{n} \right)^2 / \left(\frac{\sum_i r_i^2}{n} \right) \right] \right\}^{1/2} [1 - 1/n]$$

where r_i is the mean number of spikes observed in a neuron during the presentation of frame i of the movie, and n is the number of movie

frames. S is 0% if a cell responds with the same number of spikes for every frame and is 100% if it responds only to one frame. This measure is often referred to as "lifetime sparseness" to differentiate it from "population sparseness," which is described in the following text (Willmore and Tolhurst 2001). We also computed sparseness using windows of 10, 50, and 100 ms and although S generally decreased with increasing window sizes, the only significant difference in the four distributions was between those computed using 10 and 100 ms ($P = 0.04$ using the Kruskal-Wallis nonparametric one-way ANOVA for all four distributions but was $P = 0.12$ if the distribution computed using 100 ms was excluded and $P = 0.34$ if the 10 ms distribution was excluded).

We also used the same calculation to quantify the population sparseness (PS) for individual frames of the movie. In this case, r_i is the mean number of spikes observed in neuron i during the presentation of a movie frame, and n is the number of simultaneously recorded neurons. S is 0% if all simultaneously recorded cells responded vigorously, and is 100% if only one neuron responded during the presentation of a particular frame. In addition, we also investigated the concept of dispersal (i.e., whether neighboring cells contributed equally to coding a large ensemble of stimuli) for each recording site with more than one cell by creating scree plots (Willmore et al. 2000). This was done by first computing, for each cell, the variance in the mean number of spikes across all movie frames. The variance of all cells at a site were normalized by the highest variance and rank ordered starting with the cell with the highest variance to create the scree plot (see Fig. 6).

Firing rate calculations

To assess the distribution of firing rates in response to the movies, we first calculated the peristimulus time histogram (PSTH) for each cell using bins equal to the frame duration of the movie. In one set of measurements, we computed the mean and the peak firing rate for each cell across all bins in the PSTH to look at the heterogeneity of mean and peak rates for neighboring cells. We also compared the instantaneous differences in firing rates between cell pairs to identify epochs where the cells were responding very differently to the same stimuli. We characterized these differences using the average and the peak values across all bins of the PSTHs.

Comparison of the time course of neuronal responses

To assess variations in the response properties of cells to the movies, we calculated the Pearson's correlation coefficient between the PSTHs of all simultaneously recorded pairs of cells. This measure quantifies the similarity of the time course of the responses (i.e., signal correlation) between each pair of cells, independent of the absolute firing rates (Reich et al. 2001). We also computed the correlation coefficient using windows of 10, 50, and 100 ms, and although the correlation coefficient generally increased with increasing window sizes, the four distributions were not significantly different (Kruskal-Wallis nonparametric 1-way ANOVA $P = 0.17$ for intra-group pairs and $P = 0.09$ for inter-group pairs).

Detection of response events

As in previous studies using time-varying stimuli (Bair and Koch 1996; Berry et al. 1997; Bryant and Segundo 1976; Mainen and Sejnowski 1995), we found that the responses to time-varying natural scenes were often composed of brief events lasting a few tens to hundreds of milliseconds. We were interested in identifying these events at high temporal resolution and characterizing their properties, such as duration, probability and co-occurrence among groups of cells. Significant response events were identified in the PSTH (10-ms bins, shifted in 1-ms steps) by detecting the times when the firing rate exceeded the 75th percentile of the nonzero firing rate distribution.

The bins with a zero firing rate were excluded as they constituted a large fraction of the bins (due to the sparseness of the responses) and led to unsuitably low thresholds. We added the additional criterion that each event had to have ≥ 1 spike on 1/3 of the trials in the session. This had the effect of eliminating events with highly unreliable responses.

Once the events had been detected, we computed their durations and their probability of occurrence (i.e., sum of all event durations divided by the total stimulus duration) for each cell. For cells recorded at the same time, we also determined the periods in which events among any pair of cells overlapped in time. We refer to these as joint-events. We calculated the duration and probability of joint events, and used the latter measure to define a similarity index (SI) expressing how often joint events occurred

$$SI_{ij} = [\text{obs}_{ij}(p) - \text{exp}_{ij}(p)] / [\min_{ij}(p) - \text{exp}_{ij}(p)] \text{ if } \text{obs}_{ij}(p) - \text{exp}_{ij}(p) > 0$$

$$SI_{ij} = [\text{obs}_{ij}(p) - \text{exp}_{ij}(p)] / \text{exp}_{ij}(p) \text{ if } \text{obs}_{ij}(p) - \text{exp}_{ij}(p) < 0$$

where $\text{obs}_{ij}(p)$ is the observed joint probability, $\text{exp}_{ij}(p)$ is the expected probability (i.e., the product of the 2 individual event probabilities), and $\min_{ij}(p)$ is the minimum of the two individual event probabilities for the i th and j th cells. Positive and negative values of SI indicate joint events that occur more or less often than chance (SI = 0), respectively.

RESULTS

We recorded from a total of 88 well-isolated cells in eight experiments. Four of the cells were recorded using sharp Tungsten electrodes, whereas the remaining cells were recorded using silicon multi-tetrode probes provided by the CNCT at the University of Michigan. Once the probes were placed, and a stable recording was obtained, we acquired data during the presentation of time-varying natural scenes (movies). The stimulus sets ranged in duration from 10 to 90 min. Data were collected only as long as the unit isolation remained stable and individual cells could be tracked across sessions.

Table 1 provides a summary of the number of cells recorded at each site. We use the term "site" to refer to recording sessions in which the probe remained at a fixed location in the

TABLE 1. Cells recorded at each site for experiments using either electrodes or tetrodes

Site	No. of Electrodes	No. of Cells	No. of Tetrodes	No. of Cells
1	1	1		
2	1	1		
3	1	1		
4	1	1		
5			1	4
6			2	5
7			1	2
8			2	3
9			1	1
10			2	12
11			4	15
12			3	6
13			1	3
14			1	1
15			3	9
16			3	6
17			3	7
18			1	1
19			2	6
20			1	3

cortical tissue and stable waveforms could be identified from one or more cells. As shown in Table 1, it was rare for us to sample well-isolated single units from all four tetrodes of the silicon probe simultaneously. Across these sites, we typically recorded well-isolated single units on two of the tetrodes, and the average number of cells isolated per tetrode was close to 3. This value is somewhat lower than our previous studies in which we used custom-made tetrodes (Gray et al. 1995; Maldonado and Gray 1996; Maldonado et al. 1997). The difference in yield may be due to the recording properties of the silicon probes. However, in our earlier experiments, we sampled activity from only one tetrode at a time, and this enabled us to adjust the position of the probe to maximize the recording quality. We usually made similar adjustments with the silicon probes to maximize the yield, but the rigid linkage between the tetrodes meant that attempts to improve the yield on one tetrode often led to decreased yield on one or more of the other tetrodes.

One of the goals of our study was to assess the differences in response properties among adjacent neurons. It thus became important to evaluate the spatial extent over which the action potentials of individual neurons could be recorded. This was easily accomplished by tracking each instance in which a cell recorded on one tetrode could also be recorded by one or more of the other tetrodes surrounding it. (Note that the spike waveforms of a cell recorded on 1 tetrode usually appear on >1 of the 4 channels within the tetrode.) Interestingly, we found only one instance where the same cells were sampled by two separate tetrodes. In this case, two cells recorded on one tetrode of a 4×1 probe were also clearly visible in the recording from an adjacent shank of the same probe. Somewhat surprisingly, we found no instance where the same cell was recorded on different tetrodes of the same shank of the probe. We conclude from this that extracellular spikes recorded from these probes are highly localized and that activity from

different cells on the same tetrode most likely comes from adjacent cells.

Response reliability

During the course of this study, it became apparent that cells recorded on the same tetrode often responded in markedly different ways to the same movie. Some cells responded vigorously on every stimulus repetition, whereas others exhibited a much more variable response. Figure 2 shows two examples of the neuronal activity recorded from a tetrode in two different experiments in response to 100 repetitions of a 30-s movie. In each set of plots (A and B), the activities of three well-isolated cells are shown. The *top plots* show the PSTH of each cell, and the *bottom plots* show the corresponding spike rasters for the same three cells. In A, the overall level of activity, as well as the strength and precision of the responses to specific frames of the movie, varied from cell to cell. *Cell 1* fired a total of 26,596 spikes during the 50 min of recording time, whereas *cells 2* and *3* fired 2,059 and 2,508 spikes, respectively. A fourth cell (not shown) fired a total of 47 spikes during the same period. The character of the responses to the stimulus was also different. *Cell 1* displayed both short and prolonged episodes of evoked activity, whereas the activity of *cell 2* was much more sparse and its responses to the stimulus were very brief. The third cell showed only the slightest hint of responding to the movie, and the fourth cell was essentially inactive. In B, the responses to the movie were more uniform. Each cell responded briefly and vigorously to specific frames of the movie, and the responses varied in magnitude and time of occurrence.

As shown in Fig. 2, cells that were visually responsive displayed a consistent pattern of discharge across repetitions of the movie. We therefore devised a simple measure to evaluate the similarity of the spike trains across stimulus repetitions. We

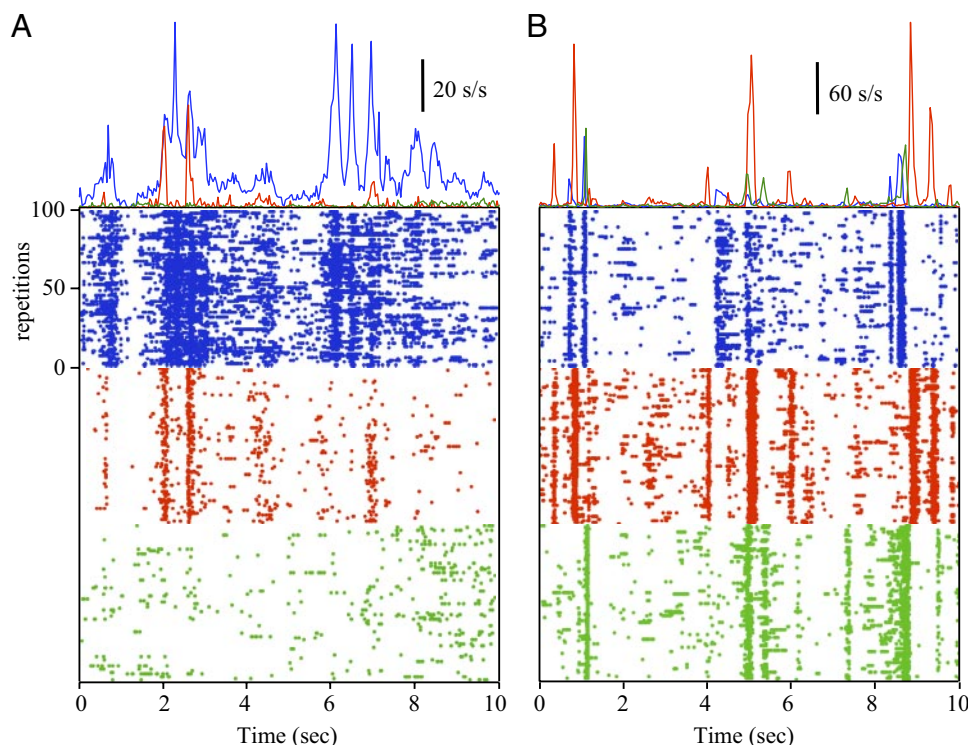


FIG. 2. Peristimulus time histograms (PSTHs) and raster plots of the spike times recorded from a single tetrode in 2 different experiments (A and B) to 100 repetitions of a 30-s movie (10 s of data are shown). In both sets of recordings, the activity of 3 single units are displayed. As in Fig. 1, the activities of cells 1–3 are shown in blue, red, and green, respectively. Responses to specific frames of the movie are apparent from the alignment of spikes across repetitions and the well-defined changes in average firing rate in the PSTHs. These data illustrate that cells recorded on the same tetrode often exhibit clear differences in firing rate, response reliability, and time course.

converted the spike train on each stimulus repetition to a vector of spike counts by calculating the number of spikes in each frame of the movie. We then calculated the correlation coefficient between all pairs of spike count vectors. This yielded $[n(n - 1)/2]$ correlation coefficients, where n is the number of movie repetitions. We used the percentage of significant correlation coefficients ($P < 0.05$) as a measure of response reliability. The result of this analysis, shown in Fig. 3, revealed a broad distribution of values that was skewed to the upper end of the scale. For comparison, the second cell in Fig. 2A (red) displayed relatively clear responses in the PSTH, but response variation across trials, evident in the rasters, led to a relatively low peak firing rate (~ 35 spikes/s) and a low response reliability value (41.7%). In contrast, the second cell in B showed significantly correlated responses on 100% of the comparisons, and its peak firing rate was >300 spikes/s.

We compared the distribution of correlation coefficients obtained for each cell to a distribution of correlation coefficients obtained from 1,000 sets of surrogate spike trains in which the spike counts from the data were randomly shuffled between repetitions and frames. We found the distribution from each of the 88 cells to be significantly different from the surrogates (KS test, $P < 0.001$), suggesting that in spite of the wide range of response variability found in the preceding text, all 88 cells exhibited responses that were significantly different from random spike trains.

There are several possible explanations for the wide variations of response reliability in our sample. One possibility is that variations in the depth of anesthesia and/or changes in the sleep cycle might have led to a global suppression of excitability. In this case, we would expect to find a uniform decrease of response reliability for all the cells at a given recording site. Another possibility is that some cells might have responded unreliably or not at all because the chosen movies did not contain features appropriate to the receptive field properties of the cells. In this case, we might expect to find a mixture of reliably and unreliably responsive cells sampled on the same tetrode. A third possibility is that some cells in visual cortex are simply not responsive to visual stimuli. In this case, the background activity of the cell would yield a measure of low response reliability. This alternative is difficult to test because any chosen stimulus set might not contain the relevant features necessary to activate the cell. Our data set, however, enabled us to distinguish between the first two possible explanations. Figure 4 shows a bar chart of cell response reliability for those tetrodes in which more than one neuron was recorded ($n = 21$).

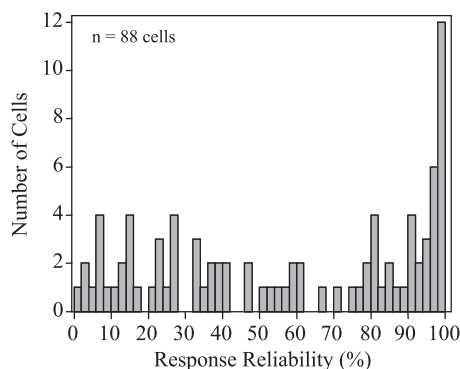


FIG. 3. Histogram of response reliability values calculated for all 88 cells in our sample.

On some recordings (e.g., tetrodes 6, 11, 18, and 20), all the cells have low response reliability values, suggestive of a general decrease of excitability. On other recordings (e.g., tetrodes 2–5, 10, 14–16), all of the cells display high levels of response reliability. More interestingly, however, there are a number of recordings (e.g., tetrodes 1, 7–9, 12, 13, 17, 19, 21) in which the response reliability of adjacent cells shows wide variations. These latter findings suggest that at some sites in cortex adjacent cells may have widely differing receptive field properties, or that regulation of cortical excitability might be cell specific. In either case, they demonstrate that the response reliability of adjacent cortical cells to time-varying natural scenes can be highly heterogeneous.

Response sparsity

A number of theoretical and computational studies have suggested that the efficient coding of natural scenes is best accomplished using a sparse representation (Field 1987; Laughlin et al. 1998; Levy and Baxter 1996; Olshausen and Field 1996, 2004; Simoncelli 2003). This hypothesis predicts that at any given moment the natural visual environment is represented by only a small fraction of active neurons. In turn, this implies that individual cortical neurons will only rarely be active. This prediction was clearly supported by visual inspection of the spike rasters, making it useful to quantify the magnitude of the effect across the population. To accomplish this, we applied a measure of response sparsity (S) that provides a convenient scale from 0 to 1 measuring how selective the responses of a neuron are to a set of stimuli (Vinje and Gallant 2000). A value of 0 indicates that a neuron responds to all the frames of the movie with the same number of spikes. A value of 1 indicates that a neuron responds to only one of the frames of the movie. Figure 5A shows the distribution of S for all 88 cells in our sample. The mean and median values of this distribution are 71 and 81%, respectively, indicating that cellular responses are indeed sparse. The distribution of sparsity values are substantially higher than those reported for cells in the alert monkey (mean = 62%) when stimulated by natural movie sequences having a diameter fourfold larger than the classical receptive field (Vinje and Gallant 2000). This difference in sparsity may reflect a species difference, the possible effects of anesthesia, or the fact that our stimuli spanned a larger portion of the visual field. We also used bin sizes of 10, 50, and 100 ms to compute S and did not find a significant effect in changing bin size (see METHODS).

Unlike with response reliability, we found relatively little heterogeneity in this measure among adjacent cells recorded at the same site (not shown). Sparsity appeared to be a general property of the cells in our database.

A more direct measure of the sparseness of the neural representation can be obtained using the PS measure (this is distinct from the sparseness mentioned in the preceding text, which is usually termed lifetime sparseness). Indeed recent theoretical work has shown that certain codes (e.g., rate codes using cells with Gabor-like receptive fields) may exhibit high lifetime sparseness while simultaneously displaying low population sparseness (Willmore and Tolhurst 2001). In other words, although individual neurons may be rarely active, when they do respond, they respond simultaneously with other neurons. We were able to directly estimate PS using a metric very

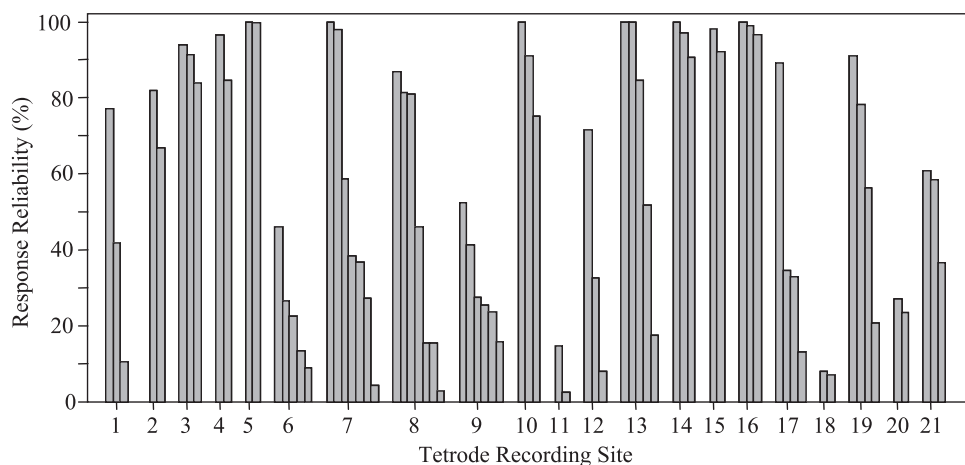


FIG. 4. Bar chart of response reliability values for all tetrode recordings in which >1 cell was recorded on the same tetrode ($n = 21$).

similar to the one used to measure lifetime sparseness in the preceding text (see METHODS). The scale also goes from 0 to 1 with a value of 1 indicating that only one neuron responded during the presentation of one movie frame and a value of 0 indicating that all recorded neurons responded vigorously to the movie frame (frames in which none of the neurons responded were excluded from the analysis). The box plots in Fig. 5B show the distribution of PS values for individual movie frames at 21 recording sites with at least two recorded neurons. The PS values covered a wide range, demonstrating that different movie frames elicited diverse patterns of responses among neighboring neurons. The median PS values at most sites were quite high (except for site 5), with many sites displaying distributions that were highly skewed toward 1 (the extreme being site 20, where the median, upper, and lower quartile were all at a PS value of 1). The mean PS value across all 21 sites was 0.67, with a SD of 0.29, which was higher than the values reported in an earlier study of multi-unit activity in ferret visual cortex (Weliky et al. 2003).

We also looked at the relationship between lifetime sparseness and population sparseness. For this, we took the lifetime sparseness values computed in the preceding text and computed the mean for all the cells at a site. Because the lifetime sparseness values were fairly uniform at each site, this measure was representative of the lifetime sparseness at each site. We then plotted that value against the median population sparseness in Fig. 6A. The data show a strong correlation between the mean lifetime sparseness and the median population sparseness ($r = 0.68$, $P < 0.01$). In comparison to an earlier study (Weliky et al. 2003), both the mean lifetime sparseness and median

population sparseness values in our data were much higher (with the exception of site 5, which had low values for both measures).

It has been suggested that a dispersed code, when evaluated over a large range of stimuli, is one in which all cells contribute equally to coding the stimuli (Willmore et al. 2000). To quantify the individual cellular contributions, we compared individual responses to the groups' representation of the visual stimuli. This is usually illustrated in plots called scree plots in which the variances of all the cells at each site are computed and rank ordered. A flat plot means that the cells contributed equally to coding the stimulus, whereas deviations from a flat plot indicate certain cells are more likely to respond than others. In Fig. 6B, we have normalized the variances by the highest variance at each site, and we have also normalized the cell number at each site. This allows us to compare the data for sites with different numbers of cells. That means for a site with two cells, the data point corresponding to the cell with the higher variance will be plotted at a normalized cell number of 0, whereas the second cell will be plotted at 1. For a site with four cells, the data points will be at 0, 0.33, 0.66, and 1. The data in Fig. 6B have also been grouped according to how many cells were at a site. The six subplots correspond to sites with two ($n = 7$), three ($n = 6$), four ($n = 3$), five ($n = 2$), six ($n = 1$), and seven ($n = 2$) simultaneously recorded neurons. Overall, the plots suggest that the dispersal in our data was fairly low with one or two cells exhibiting much higher variance than the others.

The area under the scree plot is often used as a measure of dispersal, and in our data, the mean area and SD was $0.49 \pm$

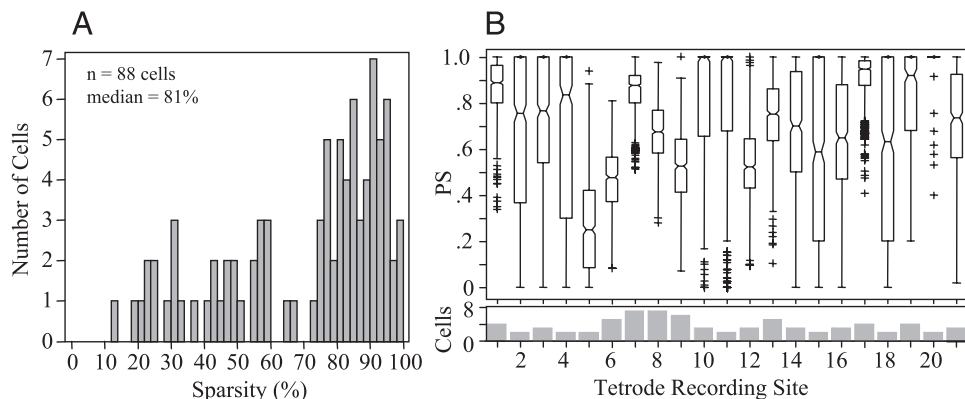


FIG. 5. Lifetime and population sparseness. A: histogram of sparsity values for all cells in our database ($n = 88$). The mean and median values of the distribution are 70.7 and 80.5%, respectively. B: box plots of population sparseness (PS) values at the 21 recording sites with >1 cell present. The distribution of PS values obtained for each movie frame is described by the medians (horizontal lines) and the quartiles (boxes). The notches denote the uncertainty in the estimation of the medians and the whiskers delineate 1.5 times the inter-quartile range. Outliers beyond the extent of the whiskers are indicated by crosses. The bar graphs on the bottom indicate the number of cells recorded at each recording site.

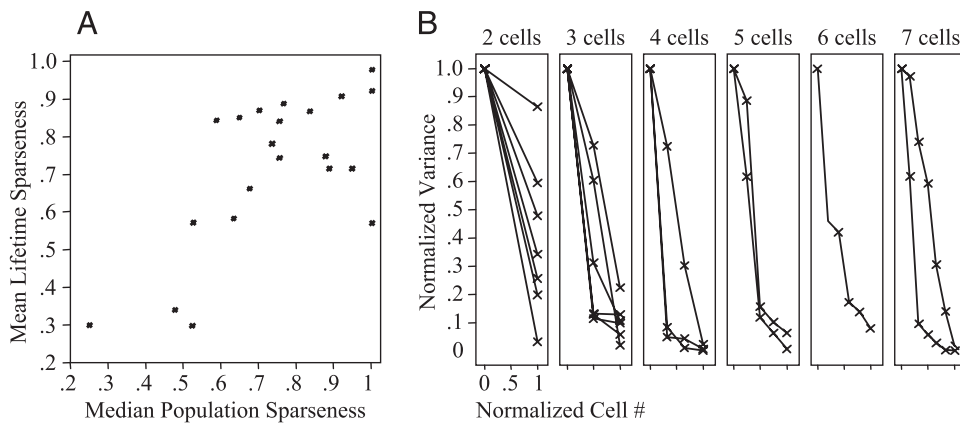


FIG. 6. Population sparseness. *A*: plot of mean lifetime sparseness vs. median population sparseness. *B* plot of normalized variance vs. normalized cell number. See text for details.

0.20. Interestingly, this value is fairly close to the value of 0.56 computed in a theoretical study using sparse coding filters (Willmore et al. 2000). We also attempted to control for differences in variances due to differences in the firing rate distributions (see Fig. 10) by normalizing each firing rate distribution by its mean before computing its variance. This appeared to increase the dispersal, with the mean area and SD of the scree plots increasing to 0.62 ± 0.23 .

These results demonstrate that the neural representation in primary visual cortex exhibits both high lifetime and population sparseness. The contribution of neighboring cells in coding visual stimuli also appears to be as uniform, if not more so, than predictions from a theoretical model of sparse coding.

Response heterogeneity

As is evident from the population sparseness analysis, neurons recorded on the same tetrode often showed striking differences in the time course and magnitude of their responses to the same movie (also reported in Reich et al. 2001 with m-sequence stimuli). An example of these effects is also illustrated in Fig. 2*B*, which reveals three interesting results. First, the responses of each of the cells were vigorous, highly reliable and sparse. The response reliability values were 97, 100, and 99% for the blue, red, and green plots, respectively. The sparsity values for the same three cells were 81, 90, and 85%, respectively. Second, the time course of the responses were very different, even though the cells were presumably adjacent to one another in the cortex. To quantify this relationship, we computed the signal correlation between the PSTHs (Reich et al. 2001). This measure revealed that the responses of

all three cells were quite different from one another. The signal correlations between *cells 1–2* and *2–3* were zero, indicating that their responses to the movie were independent of one another. *Cells 1–3* showed a weak correlation of 0.23 ($P < 0.001$). Third, the firing rates of the cells showed marked variations across the movie, and it was rare for any pair of cells to respond at a high rate at the same time. We quantified this effect by calculating the peak difference in the instantaneous firing rates between each pair of cells across all frames of the movie. These numbers (215, 215, and 87 spikes/s for *cell pairs 1–2*, *2–3*, and *1–3*, respectively) revealed that the peak firing rate in all three cells occurred when the other cells were firing at near-zero rates.

To quantify variations in the time course of responses across the population, we computed the signal correlation for each pair of simultaneously recorded cells. The results were grouped according to whether the cells were recorded on the same tetrode (intra-group) or on different tetrodes (inter-group). This produced 120 intra-group and 170 inter-group pairs of cells recorded from 21 separate tetrode sites.

Figure 7 shows the results of the signal correlation measure as applied to all pairs of cells recorded on the same (intra-group; *A*) and different (inter-group; *B*) tetrodes. The distribution of intra-group signal correlation values is plotted in a box plot in *A* to facilitate comparison with the results from Reich et al. (2001). In general, the signal correlations spanned a broad range of values in both groups. The mean values were relatively low (intra-group = 0.21 ± 0.23 , inter-group = 0.18 ± 0.20 (both distributions were significantly different from a normal distribution with a mean of 0, t -test $P < 0.001$),

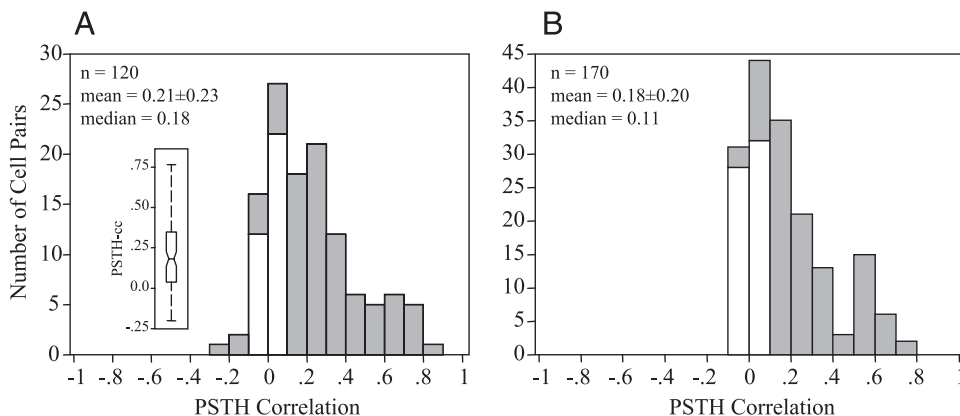


FIG. 7. Histograms of the signal correlations between pairs of cells recorded on the same (*A*) and different (*B*) tetrodes. Correlation coefficients that were not significant (i.e., $P > 0.05$) are shown in white. The medians and quartiles of the distribution of intra-group signal correlations are shown in a box plot in the inset of *A* (the notation is the same as in Fig. 5).

however, and there was a tendency, as might be expected, for the signal correlations to be stronger among cells recorded on the same tetrode. However, we compared the two distributions using a two-sample KS test and found no significant difference ($P = 0.132$). A paired t -test in which the correlation between two neurons recorded on the same tetrode was tested against the correlation between one of the neurons and a third neuron from a different tetrode was also not significant ($P = 0.89$, $n = 103$ pairs). We found a similar result using a separation angle measure (Vinje and Gallant 2000), with mean separation angles of 64 ± 14 for intra-group and $65 \pm 13^\circ$ for inter-group pairs (both distributions were significantly different from a normal distribution with a mean of 0, t -test $P < 0.001$, but the distributions were not significantly different from each other, 2-sample KS test $P = 0.59$). These data indicate, somewhat counterintuitively, that heterogeneity in the time course of responses is as high among adjacent neurons as it is between those located ~ 150 μm apart.

To get a more detailed picture of the response heterogeneity among neighboring neurons, we re-plotted the intra-group PSTH-correlation measure by tetrode recording site (Fig. 8). Pairs that were made up of cells from the same recording site were grouped together (e.g., there were 3 cells at *site 3*, which yielded 3 pairs of cells). This yielded a total of 21 recording sites in which a minimum of two cells were recorded at the same time. At some sites, containing only a single pair of cells, the signal correlations were high (e.g., *site 4*), whereas at other sites, they were low (e.g., *site 20*). More interestingly, the sites containing more than two cells showed a mixture of signal correlations, often spanning a broad range of values (e.g., *sites 6–9*). Cell pairs with nonsignificant correlation coefficients are marked (●).

It could be postulated that neighboring neurons are conveying the same signal and that the low response correlations we observed were simply due to variability in the neurons' responses. For example, we can average the PSTHs from the two cells at *site 4* to create a putative consensus signal and then generate surrogate PSTHs with some variability added to each bin of the consensus signal. With zero variability, the correlation coefficients between the surrogate PSTHs will always be 1. However, as we increase variability, the correlation coefficients start to decrease, until they reach a value that is equal to the experimental data ($r = 0.68$). In the case of the two cells at *site 4*, this corresponded to variability in which the spike-count variance in each bin was five times the mean. Similar variability in spike counts has previously been reported in the cortical neurons of anesthetized animals (Schiller et al. 1976; Tolhurst et al. 1983; Vogels et al. 1989), so it is not entirely implausible that variability could be used to account for the correlation coefficients we observed in the data. However, there is one crucial prediction from this hypothesis: the correlation between repetitions within the same cell should be equivalent to the correlation between repetitions of different cells. We tested this prediction by computing the correlation coefficients for all unique pair-wise repetitions (i.e., if there were 100 repetitions, we would have 2 sets of $99 \times 100/2 = 4,950$ within-cell correlation coefficients, plus $100 \times 100 = 10,000$ cross-cell correlation coefficients). These correlations were computed on the spike counts calculated at the frame level for each repetition. When we compared these three distributions, we found the cross-cell distribution to be significantly lower than at least one of the within-cell distributions for all 120 intra-group pairs (using the Kruskal-Wallis nonparametric 1-way ANOVA, followed by a multiple comparison test of ranks at a significance

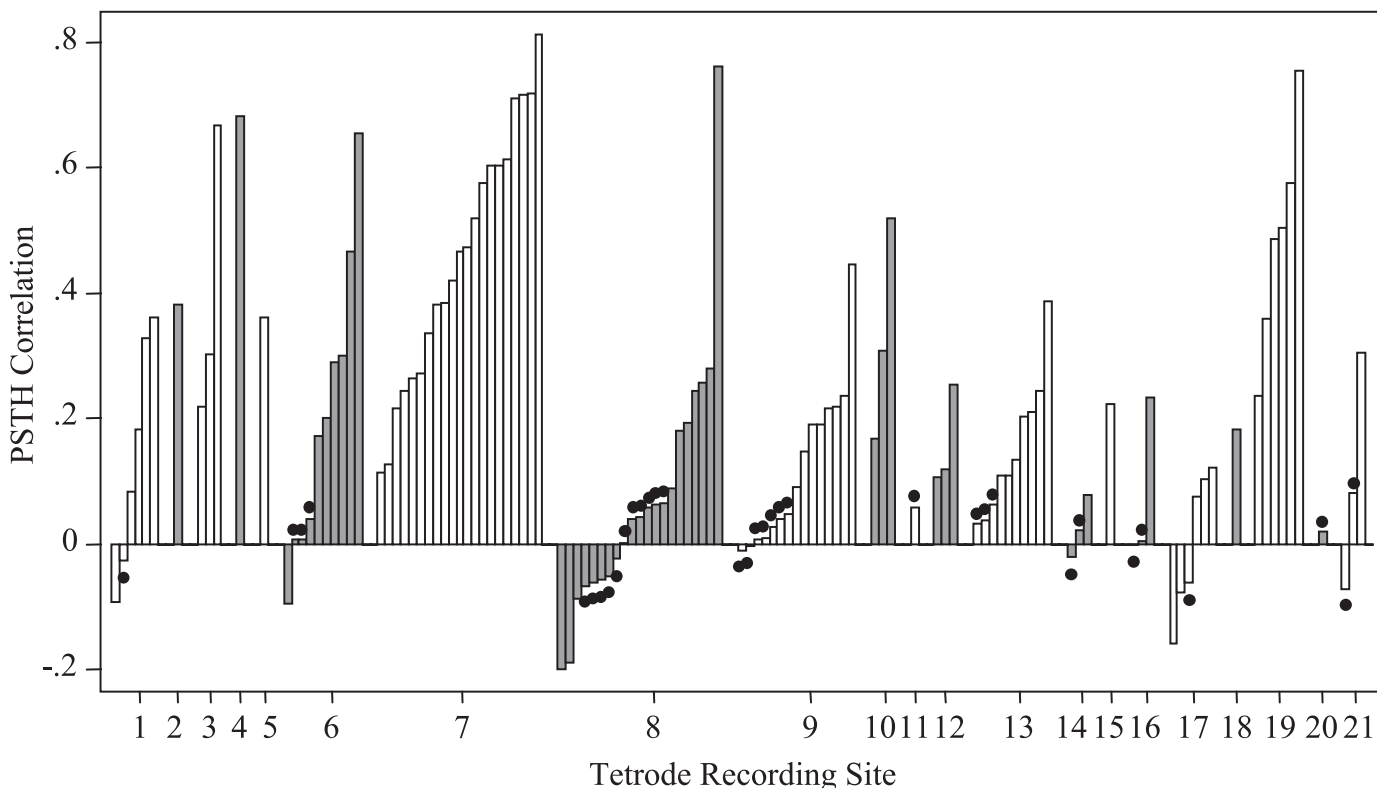


FIG. 8. Bar charts of the signal correlations for all cell pairs recorded at each tetrode site. ● cell pairs that exhibited P values > 0.05 .

level of $P = 0.05$). Moreover, in 82% (98/120) of the intra-group pairs, the cross-cell distributions were lower than both within-cell distributions. This shows that the cells individually exhibit significantly higher response correlations between repetitions than when they are grouped together, leading us to conclude that the response heterogeneity is unlikely to be due to response variability around a common signal.

Another striking property of the heterogeneity of responses to natural scenes was apparent in the widespread differences in firing rates among simultaneously recorded cells. An example of this is shown in Fig. 9 for a pair of cells recorded on the same tetrode. *A* shows the two PSTHs superimposed for a short segment of the movie, whereas part *B* shows the absolute difference in firing rate for each bin of the PSTH. In this example, the cells had a signal correlation of 0.39 but displayed substantially different firing rates during a large number of the movie frames. Even when both cells responded to the same frames of the movie their firing rates tended to be quite different. The mean and the peak firing rate differences were 4.2 and 92.5 spikes/s, respectively. This demonstrates that even when adjacent cells show some correlation in the time course of their responses, there can be large differences in the magnitude of those responses.

To quantify this behavior, we computed the peak firing rate across all bins of the PSTH for each cell (Fig. 10*A*). These values showed broad variation, ranging from 0.6 to 215 spikes/s with a median value of 39 spikes/s. [The mean firing rates were low and tended to show small variations across cells (median = 2.1 spikes/s)]. As with the signal correlations, we found a substantial degree of heterogeneity in peak firing rates among cells recorded on the same tetrode (Fig. 10*B*).

In addition to these differences in peak rate, the relatively low signal correlations indicate that cells tended to respond at different times to the same movie. Moreover, when they did respond to the same frames of a movie, they were likely to fire at substantially different rates. We characterized these rate differences by calculating the peak instantaneous difference in firing rate among all pairs of simultaneously recorded cells. The distributions of rate differences were consistent with the notion that peak firing rates rarely occurred at the same time, or

when they did, they often occurred at substantially different rates. We found no significant difference in the distribution of peak rate differences between intra-group (median difference = 42.7 spikes/s) and inter-group (median difference = 42.4 spikes/s) recordings ($P = 0.49$, KS test), indicating that, similar to the signal correlations, the response heterogeneity among adjacent neurons was equivalent to that between neurons located $\sim 150 \mu\text{m}$ apart.

Because the distribution of peak firing rates was broad, it was difficult to compare the rate differences across recordings. To compensate for this effect, we normalized each cell's firing rate distribution to its own peak value (all bins were divided by the maximum) and then recalculated the peak rate differences. The results, shown in Fig. 11, reveal that the peak rate differences were shifted toward the upper end of the distribution. The median values for the intra-group and inter-group recordings were 92% and 91% of the maximum normalized rate, respectively, indicating that the peak rate differences tended to be very close to their maximum possible values. Again, we found no significant difference between the intra- and inter-group recordings ($P = 0.41$, KS test). These data demonstrate that at any point in time, adjacent cells often convey substantially different signals (in the form of different firing rates) in response to the same stimulus.

Response events

As shown in Figs. 2 and 9, the responses of striate neurons to the movies most often consisted of brief periods of elevated activity that varied in duration and firing rate. One of our goals was to detect these events and characterize their properties. For this, we recomputed the PSTH of each cell using a sliding window of 10 ms advanced in 1-ms steps, and operationally defined response events as those periods in the PSTH when the firing rate exceeded the 75th percentile of the cell's nonzero firing rate distribution. We added the additional arbitrary criterion that each event had to have ≥ 1 spike on 33% of the trials in the session. This excluded unreliable events from the analysis and reduced the number of cells containing events to 77 of 88 cells.

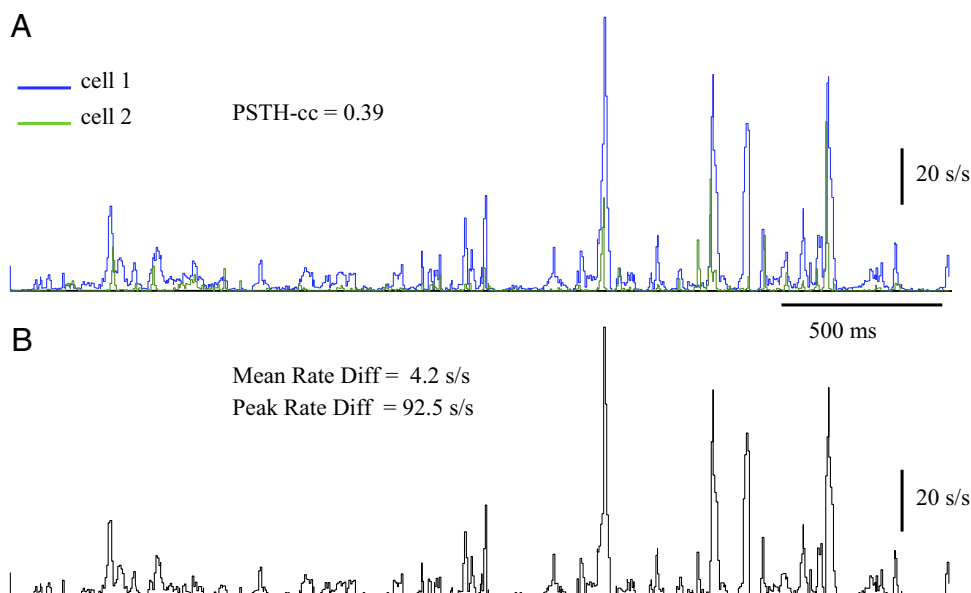


FIG. 9. Neurons recorded on the same tetrode exhibit clear differences in firing rate as a function of time. *A*: plots show a portion (~ 3 s) of the PSTHs, measured in response to a movie, of 2 neurons (blue, green) recorded on the same tetrode. The signal correlation between the 2 cells was 0.39. *B*: plot shows the bin-by-bin difference in average firing rate between the 2 cells. The mean difference in rate across the 30-s movie was 4.2 spikes/s. The peak difference in rate was 92.5 spikes/s, indicating that 1 cell (blue) was firing maximally when the other cell (green) was firing near or below its mean rate.

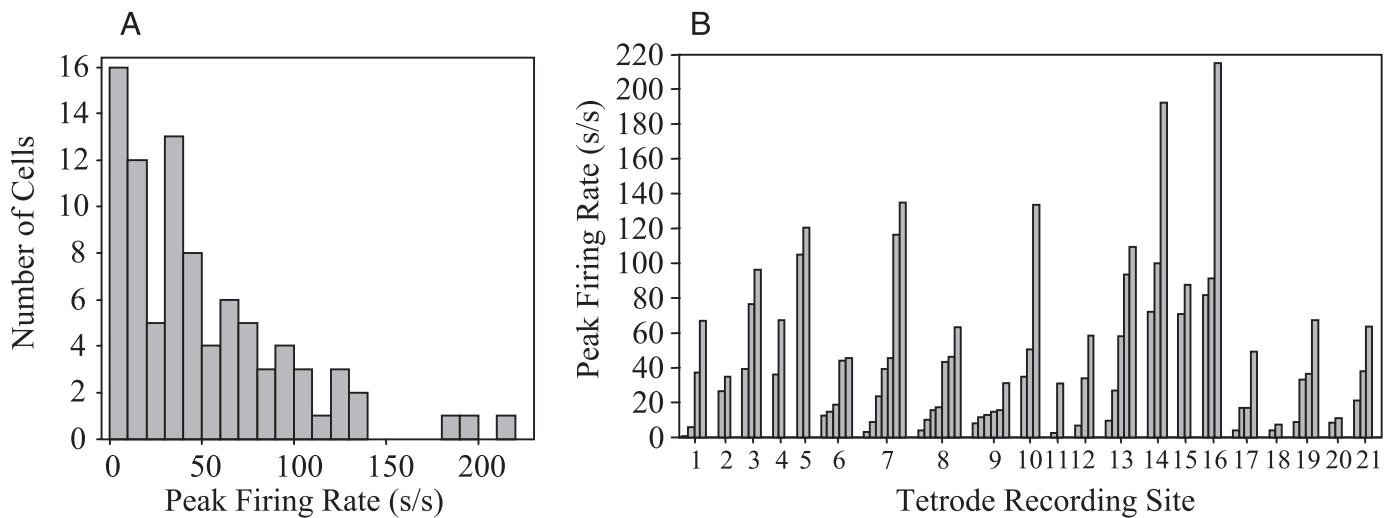


FIG. 10. Distribution of peak firing rates in response to time-varying natural scenes. *A*: histogram of the peak firing rates of all cells ($n = 88$). *B*: bar chart of the peak firing rates for all tetrodes (1–21) in which at least 2 cells were recorded simultaneously.

Figure 12 shows examples of the events detected in three cells during a brief segment of the response to 100 repetitions of a movie. Response events are marked by the colored horizontal bars above the PSTHs. For these three cells, the average event durations and probabilities measured for the entire 30-s movie were 105, 105, and 104 ms and 0.07, 0.087, and 0.066, respectively, demonstrating that the cells rarely engaged in response events. We applied this analysis to all the cells in our database and found the median event duration was 53 ms, whereas the event probability was 0.045 (i.e., the proportion of time a cell was engaged in a response event). Thus a defining characteristic of striate neuronal responses to the time-varying natural scenes used in our experiments, are brief periods of elevated firing which span a broad range of firing rates and occur with very low probability.

Using the same analysis, we identified the periods when response events in pairs of cells overlapped in time (Fig. 12). To characterize these joint events across the population, we computed their probability and duration distributions for all intra- and inter-group cell pairs. The median probability of joint events for the intra-group (0.0026) and inter-group (0.0035) pairs was roughly 5.8 and 7.8%, respectively, of the median probability of individual events. The median duration of joint events was 39 and 49 ms respectively for intra- and inter-group pairs. Joint events were significantly less probable and shorter in duration than the events from single cells (KS test for both probability and duration returned $P < 0.001$ for

both intra- and inter-group cell pairs), indicating that nearby cells tended to respond differently to the same stimulus. Thus coactivity in response to the movies was very rare and very brief. Interestingly, although there was no significant difference in the probabilities of joint events among intra- and inter-group cell-pairs (KS test $P = 0.6$), joint events in intra-group cell-pairs were significantly shorter in duration than inter-group cell-pairs (KS test $P = 0.0056$). These findings further indicate that the heterogeneity in response properties among adjacent cells is similar to, if not higher than, that for cells $\sim 150 \mu\text{m}$ apart.

To compare the joint event probability in a cell pair to the event probabilities of the individual cells, we applied a measure we refer to as the similarity index (SI) (see METHODS). This measure takes the difference between the observed and expected joint event probabilities for each pair of cells and normalizes each value on a scale ranging from -1 to 1 . A value of 0 indicates that joint events occur with a probability expected of two independent responses. Positive and negative values indicate joint events occurring more or less often than expected by chance, respectively. Values of 1 or -1 indicate that joint events occur as often as possible, or not at all. Figure 13 shows the distributions of SI for cell pairs recorded on the same (*A*) and different (*B*) tetrodes. For both distributions, the SI values tended to be positive, indicating some overlap in the responses of cell pairs to the movies. There were, however, a number of cell pairs with negative SI values (27/95 intra-

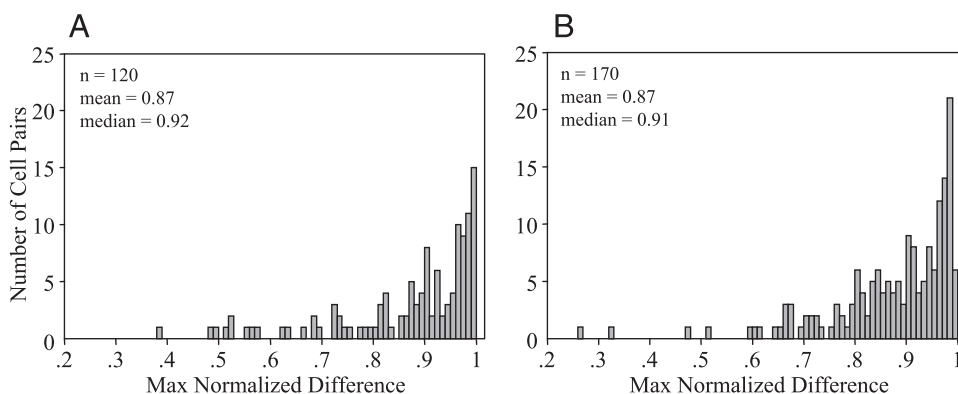


FIG. 11. Histograms of the difference in peak firing rates after each cell was normalized to its respective maximum firing rate. Pairs of cells recorded on the same or different tetrodes are shown in *A* and *B*, respectively.

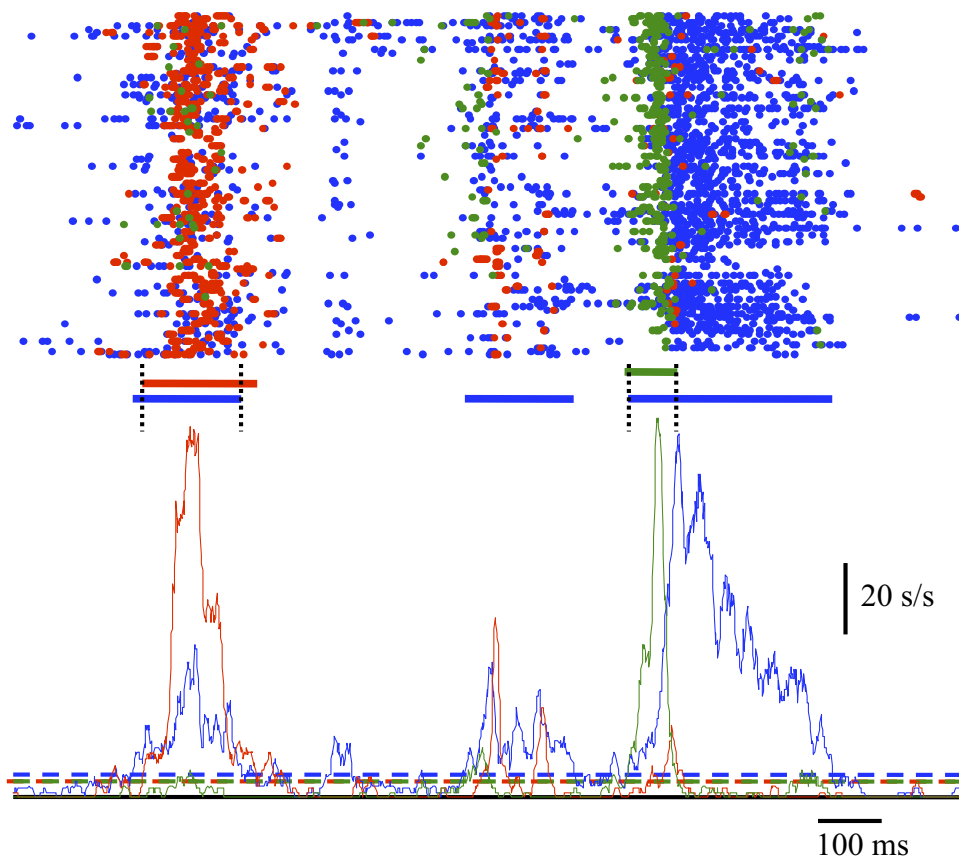


FIG. 12. Detection of response events. A short epoch of data (1.5 s) shows 100 superimposed spike rasters (*top*) and PSTHs (*bottom*) of 3 cells (blue, red, green) recorded from a single tetrode in response to a movie. The dashed horizontal lines indicate the thresholds for event detection (i.e., the 75th percentile of each cell's firing rate distribution). The colored horizontal lines above the PSTHs show the elapsed time of the events in each cell (blue, red, green). Some threshold crossings are not labeled as events, because they did not have a minimum of 1 spike on 33% of the trials. Joint events are marked by the vertical dashed lines. In this short epoch, there were joint events only between *cells 1* (blue) and *2* (red) and *cells 1* and *3* (green) but not between *cells 2* and *3*.

group; 39/140 inter-group), indicating that joint events occurred less often than expected by chance. Most of the cell-pairs with negative intra-group SIs had values of -1 , indicating that their response events to the movies were completely nonoverlapping. Both distributions were non-Gaussian (Lilliefors test) and were not significantly different from one another ($P = 0.66$, KS test).

DISCUSSION

When stimulated with time-varying natural scenes, striate cortical neurons exhibit responses that are brief and that display both high lifetime and population sparseness, consistent with the concept of a dispersed code. Response events, as we have defined them, occurred $\sim 5\%$ of the time and had dura-

tions lasting 50–60 ms on average. The peak firing rates of cells were widely distributed across our sample, ranging from a low of ~ 1 spike/s to a high of >200 spikes/s. When we compared the activity of simultaneously recorded cells, we found a striking degree of response heterogeneity, even among cells recorded on the same tetrode. The signal correlations and joint response probabilities were low, and we found differences in the peak instantaneous firing rates that were close to the maximum expected for independent responses. We commonly observed response epochs in which one neuron was firing at its peak rate, whereas a neighboring neuron was effectively silent. We also found that the response heterogeneity of adjacent cortical neurons (recorded on the same tetrode) was similar to that observed between neurons separated by an average of $150 \mu\text{m}$ (recorded on different tetrodes). These findings demon-

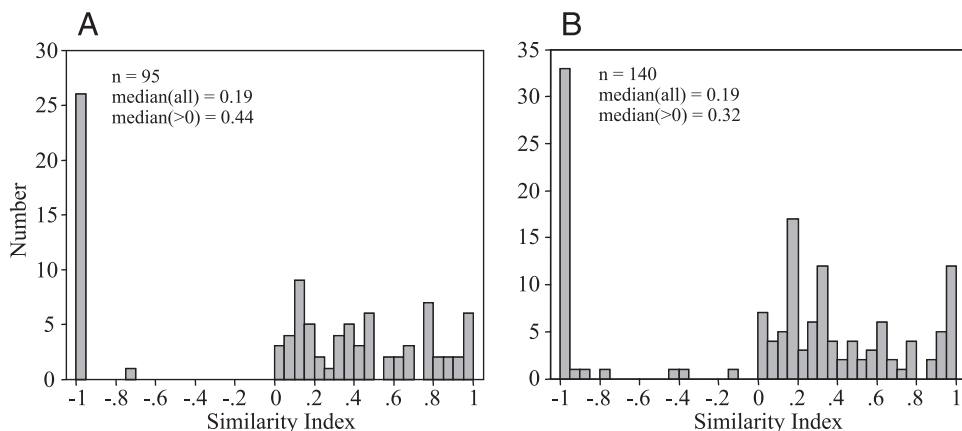


FIG. 13. Histograms of the similarity index for cell pairs recorded on the same (*A*) and different (*B*) tetrodes.

strate a high degree of local response heterogeneity and indicate that adjacent cortical neurons exhibit greater variations in stimulus preferences than previously thought. Before discussing the implications of these findings, it is important to rule out alternative explanations that may have come from methodological problems.

Methodological issues

One possible source of error in our measurements could be due to one or more forms of sampling bias. Like any other neurophysiological study, our study contains sampling biases, some of which we are aware of, and others that are more difficult to quantify. For instance, we recorded only from sites where we were able to isolate single-unit activity. There were a number of instances where vigorous multiunit activity was sampled, but not evaluated, because the individual units could not be isolated. If, for example, the silicon probes were biased toward activity from large cells, then it's possible we may have missed small interneurons and possibly stellate cells in layer 4. This is an issue that could be resolved in future studies. We also selected movie scenes that elicited strong responses from the single units that we did sample. This could have biased our sample toward vigorously responding cells. However, because all of our spike sorting was performed off-line, we avoided the bias that would come from excluding unresponsive or weakly responsive cells during the experiment. Nonetheless, our database may have been skewed toward those recording sites in which at least one neuron responded vigorously to the movie scenes we selected.

A second possible source of error could result from misclassifications in spike sorting. Instabilities in the recording could easily have lead to changes in spike waveforms across the four channels of a tetrode that might have been interpreted as coming from distinct cells. We are acutely aware of this potential problem (Gray et al. 1995) and used several precautions to avoid this type of error (see METHODS). In spite of these precautions, additional problems in sorting might have arisen due to our efforts to recover superimposed waveforms. For example, a pair of cells may have appeared to fire at different times because the periods of joint firing were characterized by the overlap algorithm as belonging to a third cell. We believe this possibility is highly unlikely for several reasons. First, overlapping spikes tend to occur with a distribution of relative phases, making these waveforms very different from one another, thereby avoiding the problem of consistently assigning them to a third cell. Second, we required that the participating waveforms be well isolated and display little or no evidence of instability in shape or amplitude. This made it quite easy to detect overlapping waveforms because we utilized four channels of data to measure each spike and because spike voltages add linearly. Finally, we also carefully inspected the output of the superposition algorithm and manually excluded any overlapping waveforms that clearly did not match the requisite templates. Because of these various precautions, and our extensive experience with the sources of error, we believe that we can largely exclude spike sorting errors as a factor contributing significantly to our results.

Response heterogeneity within cortical columns

How then might we account for the striking heterogeneity of responses among nearby neurons? The most straightforward interpretation is that there are greater variations in the spatio-temporal receptive field (stRF) properties among adjacent clusters of neurons than have been previously identified. This interpretation runs counter to the classical notion that cells with similar response properties are clustered together and is inconsistent with the abundant evidence for columnar organization (Hubel and Wiesel 1962; Hubener et al. 1997; Ohki et al. 2005; Singer 1981). However, at least three lines of evidence lend support to our conclusions. First, there is increasing evidence for heterogeneous response properties among nearby cortical neurons. Multi-neuron recordings have revealed substantial variations of receptive field properties among adjacent striate neurons (DeAngelis et al. 1999; Gray et al. 1995; Hetherington and Swindale 1999; Maldonado and Gray 1996; Maldonado et al. 1997; Mechler et al. 2002; Reich et al. 2001). While part of this effect could be due to the sampling of activity from pinwheel centers in the orientation map (Maldonado et al. 1997), it is unlikely to account for the majority of findings. In our recordings, most sites displayed a broad range of signal correlations, suggesting that the observed heterogeneity was not simply due to recordings from orientation domains and pinwheel centers. In a related manner, heterogeneity in receptive field types has long been known to exist with some cortical layers containing a mixture of simple and complex cell types (Martin and Whitteridge 1984; Martinez et al. 2005). These differences in RF properties could potentially account for a substantial fraction of the response heterogeneity to natural scenes.

A second line of evidence relates to the multidimensional tuning of cortical neurons. It is well known that striate neurons exhibit selectivity to multiple stimulus parameters, with some that are interdependent (De Valois et al. 1979; Geisler et al. 2001; Skottun et al. 1994). With such high dimensional spaces, many neurons with different tuning would be required to provide effective coverage of the entire stimulus space in a local population. However, these differences in high-dimensional space may not be apparent when probed with simple stimuli that vary in only one or two stimulus parameters. Neighboring neurons may appear to exhibit very little response heterogeneity in that case, simply because the full range of stimulus dimensions is not being implemented. Only when probed with rich spatio-temporal visual stimuli, as is the case with natural movies, will the true heterogeneity of the local population be revealed.

A third line of evidence supporting the existence of functional heterogeneity comes from studies of cortical microcircuitry and cellular neurophysiology. Neocortex contains a wide variety of both excitatory and inhibitory cell types that differ in morphology, laminar position, synaptic connectivity, and intrinsic electrophysiological properties (Beierlein et al. 2003; Binzegger et al. 2004; Karube et al. 2004; Markram et al. 2004; Nowak et al. 2003; Thomson and Bannister 2003; Yoshimura et al. 2005). This organization gives rise to a variety of sub-networks with distinct functional and dynamic properties. Thus cells intermingled within the same column might be expected to display a continuum of response properties due to

their selective anatomical connections and intrinsic electrophysiological properties.

Finally, another important factor that might have contributed to the observed response heterogeneity was our use of full-screen movies. These stimuli extended well beyond the boundaries of the classical receptive fields of the cells we recorded and because of their rich spatiotemporal structure would likely have had a variety of nonlinear modulatory influences, both suppressive and facilitatory (Cavanaugh et al. 2002; Jones et al. 2001; Sillito et al. 1995; Vinje and Gallant 2000; Walker et al. 1999; Webb et al. 2003; Yao and Li 2002). Such nonuniform stimulation of the receptive field surround may have had a pronounced influence on the response heterogeneity to natural scenes, particularly given the recent evidence that surround influences are spatially asymmetric. Such effects have been demonstrated indirectly in the study of Vinje and Gallant (2000). These authors found that the signal correlations between pairs of nearby neurons were significantly reduced by increasing the size of movie image patches presented to the cells. These effects were interpreted as evidence for an active, nonlinear process of decorrelation, expected to increase the coding efficiency of cortex by reducing response redundancy (Barlow 1961; Dimitrov and Cowan 1998; Olshausen and Field 1996, 2004).

Implications for pooling models of visual cortex

It is clear from our results that the traditional concept of averaging the responses of neighboring neurons to produce a more reliable signal is problematic. Neighboring neurons in our study were seldom simultaneously active. There was little evidence that the responses of neighboring neurons could be effectively averaged without losing information. [This is consistent with the conclusions of an earlier study that quantified the information present in spike trains responding to m-sequence stimuli (Reich et al. 2001)]. Averaging of neuronal responses could still be useful if knowledge about one particular stimulus parameter is all that is needed. In that case, information from all the other stimulus dimensions could be averaged out and only the relevant stimulus dimension would remain. However, it seems unlikely to us that such reductions in stimulus dimensions would be the primary output of striate cortex.

In our database, we observed rather large differences in the peak firing rates of neighboring neurons. Although it is reasonable to suspect that the neurons with low firing rates might not have been activated by their preferred stimuli, it is also quite possible that neighboring neurons, perhaps those with different intrinsic properties (Connors and Gutnick 1990; Gray and McCormick 1996; Nowak et al. 2003), may have very different peak rates. This possibility, which is well known to physiologists but has seldom been analyzed, especially with respect to responses to natural scenes, raises some interesting problems for downstream neurons. Is the activity of downstream neurons predominantly determined by upstream neurons with high firing rates? Or do the individual neurons contribute equally regardless of their absolute rates, perhaps through some form of synaptic plasticity? These issues raise additional difficulties with the notion of averaging the responses of neighboring neurons to get a consensus signal.

Sparse coding

Several recent studies have looked at the issue of sparse coding in visual cortex (see Olshausen and Field 2004; for a review) but to our knowledge, only one recent abstract has described the population sparseness of multiple, simultaneously recorded single neurons (Blanche et al. 2006). Although our database consisted of recordings with small numbers of cells, our results add an important dimension to the growing consensus that cortical activity is sparse. The advent of newer recording techniques have now made it possible to record simultaneously from much larger numbers of single neurons (Blanche et al. 2005) and it appears that our results scale to larger populations of neurons (Blanche et al. 2006).

It has been argued that a distinction could be made between a compact code (e.g., principal component analysis) and a sparse-dispersed code (e.g., Field 1994) on the basis of dispersal. Although relatively few cells are active at one time in both codes (i.e., both codes are sparse), the same few cells are constantly active when representing different images in a compact code (e.g., cells representing the first few principal components), whereas different cells are active in a sparse-dispersed code (Willmore et al. 2000). Our results suggest that cortical activity is dispersed, but again, due to the relatively small numbers of cells, it remains to be seen if studies with larger numbers of single neurons will show similar dispersal.

Outstanding issues

One outstanding issue in the present work is whether the heterogeneity we have described is due to the use of natural movies in our study, or if artificial stimuli with the appropriate spatio-temporal characteristics (i.e., $1/f$ or white) would be sufficient to elicit similar heterogeneous responses. To properly address this issue, one would have to record the responses to different classes of artificial stimuli, each with different spatial and/or temporal characteristics. Unfortunately, we do not have adequate data to address this issue. Our preliminary analysis suggests that m-sequence stimuli elicit similar heterogeneity, suggesting that spatiotemporal stimulus patterns that are $1/f$ or white have the same effect in eliciting heterogeneous responses.

Another outstanding question is whether sparse coding is a result of network interactions or if it arises from the sharpness of the spatial-temporal tuning of the cells. This issue could be resolved by obtaining the space-time receptive fields of recorded neurons and convolving them with natural movies to compare their outputs with the responses of recorded neurons. Again, we do not have adequate data to address this issue. Our preliminary attempts have highlighted the difficulties in obtaining space-time receptive fields that are of sufficient quality for us to make proper comparisons, and the specific transformations to convert the output of the convolution to a form appropriate for comparisons with the neuronal responses (Olshausen et al. 2004).

Natural scenes

Finally, our findings lend support to the notion that the use of natural scene stimulation is highly informative in the study of vision (Felsen and Dan 2005; Kayser et al. 2004; see Rust and Movshon 2005 for a counter argument). The use of time

varying natural images in our experiments have shed light on the sparseness of the neural representation in primary visual cortex and revealed that local response heterogeneity in striate cortex is substantial. These findings thus raise questions regarding the functional significance of columnar organization (Horton and Adams 2005), and highlight the potential loss of information that would result from the averaging of neighboring responses to obtain a consensus signal (Reich et al. 2001).

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