Physiological Properties of Macaque V1 Neurons are Correlated With Extracellular Spike Amplitude, Duration, and Polarity

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Gur, Moshe, Alexander Beylin, and D. Max Snodderly. Physiological properties of macaque V1 neurons are correlated with extracellular spike amplitude, duration, and polarity. J. Neurophysiol. 82: 1451–1464, 1999. In the lateral geniculate nucleus (LGN) the large neurons of the magnocellular layers are functionally distinct and anatomically segregated from the small neurons of the parvocellular layers. This segregation of large and small cells is not maintained in the primary visual cortex (V1); instead a heterogeneous mixture of cells occurs, particularly in the output layers. Nevertheless, our results indicate that for the middle and upper layers of V1, cell size remains a predictor of physiological properties. We recorded extracellularly from neurons in V1 of alert monkeys and analyzed the amplitude, duration, and polarity of the action potentials of 199 cells. Of 156 cells that could be assigned to specific cortical layers, 137 (88%) were localized to the middle and upper cortical layers, layer 4 and above. We summarize evidence that the large-amplitude spikes are discharged by large cells, whereas small-amplitude spikes are the action potentials of smaller cells. Large spikes were predominantly negative and of longer duration, whereas small spikes were predominantly positive and briefer. The putative large cells had lower ongoing activity, smaller receptive field activating regions and higher selectivity for stimulus geometry and stimulus motion than the small cells. The contrasting properties of the large and the small cells were illustrated dramatically in simultaneous recordings made from adjacent cells. Our results imply that there may be an anatomic pairing or clustering of small and large cells that could be integral to the functional organization of the cortex. We suggest that the small and the large cells of area V1 have different roles, such that the small cells may shape the properties of the large output cells. If some of the small cells are also output cells, then cell size should be a predictor of the type of information being sent to other brain regions. Because of their high activity and relative ease of stimulation, the small cells also may contribute disproportionately to in vivo images based on metabolic responses such as changes in blood flow.

INTRODUCTION

The visual cortex receives multiple channels of information that originate in parallel pathways the identities of which are linked to the sizes of the cells in the pathways. The large cells of the magnocellular (MC) pathway have a distinctively different physiology from the small cells of the parvocellular (PC) pathway (Lee 1996). The differences in response properties help to establish functional specializations, particularly in selectivity for direction of stimulus motion, that endure even through multiple stages of the cortical hierarchy (Andersen 1989; Merigan and Maunsell 1993).

Given the importance of cell size in distinguishing among visual afferent pathways, its influence on information processing within primary visual cortex (V1) has received surprisingly little attention. This relative neglect is probably due to the fact that large and small cells are intermixed in most cortical layers, whereas they are neatly segregated in the afferent pathway by the laminar structure of the lateral geniculate nucleus (LGN). Consequently, extracellular recordings from the LGN have been associated with large or small cells by identifying which layer has been sampled; but for V1, the size of a cell the response of which is recorded extracellularly has usually not been considered.

We were motivated to examine this question because of our observations suggesting that the large cells of V1 appeared to have distinctive physiological properties but not in the manner that would be predicted from the characteristics of the input pathways (Croner and Kaplan 1995). In particular, the cells with the largest extracellular spikes often had small receptive field activating regions (ARs), whereas cells with small spikes had larger ARs and were activated by a broader range of stimuli (Snodderly and Gur 1995).

Many observations in the neuroscience literature support the proposal that differences in spike parameters are associated with functional differences among cell populations (Amitai and Connors 1995). Spike parameters have been related to functional characteristics of neurons in alert animals in the following diverse neural structures: rabbit cortical area V1 (Swadlow 1988), macaque caudate nucleus (Hikosaka et al. 1989), rabbit cortical area S1 (Swadlow 1989), and macaque prefrontal cortex (Wilson et al. 1994). In primate motor cortex, fast-conducting pyramidal cells have larger-amplitude spikes than slow-conducting pyramidal tract neurons (Humphrey and Corrie 1978), and fast-conducting cells are larger than slow-conducting cells (Deschenes et al. 1979; Naito et al. 1969). In somatosensory cortex, small spikes of short-duration are recorded selectively from loci with small cells; the cells with short-duration (thin) spikes have higher spontaneous firing rates, and they follow rapid stimulus changes more faithfully than cells firing longer and slower spikes (Mountcastle et al. 1969; Simons 1978).

In the present paper we relate the amplitude, duration, and polarity of extracellular spikes to the functional properties of neurons in the middle and upper layers of cortical area V1 of alert macaques. We find a consistent set of relationships sug-
suggesting that the large cells are less spontaneously active, have smaller receptive field ARs, and are more selective for stimulus features than the small cells. These contrasting properties are illustrated dramatically when the activity of adjacent cells is simultaneously recorded. Then it becomes clear that large and small cells of area V1 can have different roles, and a balanced view of cortical function requires an understanding of both cell groups.

METHODS

Action potentials were recorded from neurons in area V1 of two adult female monkeys (Macaca mulatta and M. fascicularis) that were trained to hold visual fixation. Details of training (Gur and Snodderly 1987; Snodderly and Kurtz 1985) and recording (Snodderly and Gur 1995) procedures have been published. Briefly, monkeys were trained to fixate on a LED for 5 s while visual stimuli were moved or flashed in the visual field. Eye position was monitored during the fixation trials by a double Purkinje image eye tracker (2–3 minarc resolution).

Visual stimuli were displayed on a Barco 7351 monitor (60-Hz noninterlaced refresh rate) and were red, green, blue, gray, or black bars of optimal orientation, color and spatial configuration 0.9 log units brighter or darker than the background of 1 cd/m2. Chromatic bars of optimal orientation, color and spatial configuration 0.9 log units brighter or darker than the background of 1 cd/m2. Chromatic bars of optimal orientation, color and spatial configuration 0.9 log units brighter or darker than the background of 1 cd/m2.

Electrophysiological recordings

Glass-insulated platinum–iridium electrodes (Snodderly 1973) with a tip diameter of 1–1.5 μm, bare tip length of 5–7 μm, and impedance at 1 kHz of 2.5–4 MΩ were used most frequently to record both single- and multiunit activity. In some experiments, fiber electrodes made from quartz-insulated platinum-tungsten alloy (Eckhorn and Thomas 1993) with bare tip lengths of 5–7 μm were used. The signal was band-pass filtered by our preamplifier (Bak Electronics) between 1 kHz and 20 kHz (24 db/octave). The additional low-frequency filtering enhanced baseline shifts as well as high-frequency noise generated by the awake animal, the amplified signal was filtered further by an adjustable neutral gray background; decremental stimuli were presented on a background of a single color (Snodderly and Gur 1995). The bars were swept across the receptive field in a direction orthogonal to the long axis of the RF at 1.5–4°/s. Luminance was measured with a Gamma Scientific telephotometer. In some experiments, the eye position signal from the eye tracker was added to the stimulus position signal from the computer at the beginning of each video frame to compensate for eye movements during the trial (Gur and Snodderly 1987, 1997a,b; Snodderly and Gur 1995).

Spike separation

Single units and clusters of two or three cells were recorded with a single microelectrode. An example of three simultaneously recorded cells is shown in Fig. 1A. Before processing, the total spike activity for each trial was displayed, (Fig. 1B, top), and the first 30 or so spike waveforms were superimposed (Fig. 1B, bottom). Note that the time of the voltage trigger is not obvious because voltage samples are recorded both before and after the trigger to obtain the full extent of the spike waveform. For trials where more than one cell was recorded or where a single cell was recorded with marginal signal-to-noise ratio, all the spikes were analyzed by the principal components analysis procedure, also known as the Karhunen Loeve expansion (Abeles and Goldstein 1977). In this procedure the spikes are represented by a sum of basic, orthogonal time functions (“templates” or principal components). We found that two such functions were sufficient to account for better than 95% of the spikes’ energy (cf. Abeles and Goldstein 1977). Each spike generated two coefficients by projecting it on the two principal components and could then be represented by a point in a plane whose coordinates are given by the two coefficients (Fig. 1C).

Because using two coefficients usually did not result in complete separation of all spikes into discrete groups, a fuzzy K-mean clustering procedure (Bezdec 1973) then was used. We applied the fuzzy clustering technique by manually defining a center position for each visually observed cluster and then letting the program define the degree to which each spike belonged to one of the groups (Fig. 1C).

In the final stage of clustering, a “hard” assignment to clusters was made based on the values of the datum membership vector. Maximal membership value indicates a maximal likelihood that a given datum belongs to a specific cluster. It is useful, however, to apply a heuristically chosen lower limit for the assignment such that if the largest membership value is less than, say, 0.7, the datum is considered an outlier and is set apart from the valid clusters. This threshold criterion detects corrupted waveforms and recording artifacts and decreases classification errors. Using fuzzy clustering provided us with a first approximate assignment of the spikes to separate groups.

The final assignment was based on visual inspection of superimposed spike waveforms like those in Fig. 1, B and D. If on visual inspection the initially defined group was judged to be composed of more than a single spike class, it could be sorted further and individual spikes could be assigned to other groups, either by repetition of the algorithm or by manual assignment. In this way, practically all spikes were assigned to clusters corresponding to individual cells. When two or more spikes occurred within the sampling window (usually 1.0 ms), they could not easily be separated and were discarded (cf. Abeles and Goldstein 1977). This constraint did not create a serious problem.
1% of all spikes had to be discarded. (Because our analysis enables inspection and marking of superimposed spikes, even such coincident spikes could be assigned manually if necessary).

After separation into individual spike trains, each spike train, for each trial, was displayed along with its associated spike shape (Fig. 1D). Data for each separated spike train then were stored in individual files.

Small spikes—a single cell or a multiunit cluster?

When recording low-amplitude spikes it is sometimes not obvious whether more than one cell is contributing to the record. Several precautions were taken to avoid inclusion of multiple cells in the separated records. During on-line recordings, spike shapes were superimposed continuously and displayed with high time-resolution, which gave a fairly good indication whether more than one spike shape was present. In addition, on-line histograms showed firing rates so that peak rates ~1,000/s signaled the experimenter that two or more units were simultaneously contributing to the record.

For every file, spikes were superimposed off-line (Fig. 2), which enabled us to make a visual judgement whether more than one cell was contributing. Interspike interval (ISI) distributions were calculated for all small spikes (<0.4 mV) to assure that spikes were being correctly attributed to only one cell; again, intervals <1 ms were an indication that more than one cell was contributing spikes to the record (Fig. 2A) while lack of very short ISIs was consistent with
RESULTS

Interpreting and measuring spike waveforms

A total of 199 neurons with receptive fields at 1.3–6.3° eccentricity were studied. Ninety-two of these cells were from multiunit recordings where pairs or triplets of cells were recorded simultaneously and then separated into spike trains representing individual cells. In all, multiunit recordings were made from 37 pairs of cells and 6 triplets.

We have repeatedly observed two spike types, as illustrated in Fig. 3; one type consisted of large, mostly negative spikes, whereas the other consisted of small, mostly positive spikes. The large spikes were characterized by a triphasic shape; small positive, large negative, and midsize positive lobe (Fig. 3A, left). Most small spikes were biphasic with an initial negative and a larger positive lobe, lacking the initial positive phase (Fig. 3A, right).

When interpreting the spike waveforms and quantifying the differences among them, we have been guided by the biophysical modeling of Rall (1962; also see Segev et al. 1995) and the related extracellular study of potential fields by Nelson and Frank (1964). We assume, first of all, that the electrode tip is near the soma of the cell, where we should record the largest potentials and the most stable spike amplitude because small shifts in electrode position should have little effect. For the large, triphasic spikes, the initial, small positive lobe may be due to depolarization in the axon hillock region drawing an outward current from the soma. This lobe is not prominent in published recordings from other brain regions, and we have not included it in our analyses. The large negative lobe is thought to be generated by inward sodium current as the action potential invades the soma. It is followed by a positive lobe that is generated by repolarization of the soma during the outward potassium current, which is accompanied by depolarization of the dendrites.

As our measure of the amplitude of the spike we have taken the potential difference between the peak of the main negative lobe and the peak of the positive lobe that follows it (Fig. 3B). This measure should be dependent on the soma size and also may be influenced by the size and structure of the dendritic tree. Use of the difference between the positive and negative peaks is convenient because the time points defined by these peaks have been used in a prior study (Wilson et al. 1994) as a measure of spike duration. For comparability with their results, we have adopted the time difference between the two peaks as our index of spike duration.

For the small positive, mainly biphasic spikes, we have again selected the negative peak and the following positive peak as our reference points. In this way, the measures of amplitude and duration for small spikes should relate to the same biophysical processes as those considered for the large spikes. The main difference is that the negative lobe of many small spikes is smaller than the positive lobe. It is possible that this difference represents a lesser contribution of the soma and a greater relative contribution of the dendrites to the extracellular potential for small cells, particularly if the dendrites discharge action potentials (e.g., Stuart et al. 1997). Our studies do not provide a way of testing this possibility, but the consistent association between size and polarity of the spikes suggests that it may be a signature for particular cell geometries or specific biophysical relationships.

To interpret and measure spike waveforms, we used the multiunit recordings where pairs or triplets of cells were recorded simultaneously. The large spikes were characterized by a triphasic shape: small positive, large negative, and midsize positive lobe (Fig. 3A, left). Most small spikes were biphasic with an initial negative and a larger positive lobe, lacking the initial positive phase (Fig. 3A, right).

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**FIG. 2.** Interval distribution of a 2-unit cluster and 2 single units. **Left:** superposition of spikes from the cluster (A) and the 2 single units (B and C). **Right:** interspike interval distribution of the units displayed in the left. Insets: histograms of the very short intervals (0–4 ms) with high time resolution.

Discharges of a single cell (Fig. 2, B and C; see also Fig. 6). The ISI distributions of small cells recorded alone and those recorded simultaneously with large cells were analyzed in this way.

Physiological responses of single neurons were analyzed using the separated spike trains. The size of the receptive field AR was measured by moving a bar across the receptive field and determining the boundaries within which 95% of the spikes were elicited (Snodderly and Gur 1995). Data were accepted for analysis only if the stimulus crossed the AR during periods of slow eye drifts and responses were not contaminated by effects of fixational saccades (Snodderly and Gur 1995). Data were accepted for analysis only if the stimulus crossed the AR during periods of slow eye drifts and responses were not contaminated by effects of fixational saccades (Snodderly and Gur 1995). Data were accepted for analysis only if the stimulus crossed the AR during periods of slow eye drifts and responses were not contaminated by effects of fixational saccades (Snodderly and Gur 1995). Data were accepted for analysis only if the stimulus crossed the AR during periods of slow eye drifts and responses were not contaminated by effects of fixational saccades (Snodderly and Gur 1995).
Evidence that action potential amplitude and polarity are related to neuron size

The rationale of this paper is based on the assumption that large spikes and small spikes are recorded from different types of neurons. To justify this assumption, we summarize here the evidence against three other possible origins of the large and the small spikes. The first possibility is that spike amplitude is mainly a function of the electrode distance from the cell body; the closer the electrode, the larger the spike. We reject this interpretation for the following reasons.

First, for most large spikes and for all small ones, either a spike was recorded with a relatively constant amplitude or was quickly lost (cf. Mountcastle et al. 1969). This result was due, in part, to our practice of allowing only forward motion of the electrode and not trying to regain a better recording position by moving the electrode back and forth. We do this to optimize our dye marking and to localize cells in the laminae (Snodderly and Gur 1995). In five unusual cases where we observed a reduction in the amplitude of a large spike as the cell was moved away from a stationary electrode by tissue movement, the decrease in spike amplitude was relatively rapid. Examples are shown in Fig. 4 where during the course of ~4 min the spikes greatly diminished in amplitude but retained the polarity and duration of their components. For these and the three other cells similarly monitored, large spikes (>0.5 mV) were recorded for many minutes; once spike amplitude decreased <0.2 mV, cells were lost within seconds. This sequence is consistent with the quite constrained potential field illustrated by Nelson and Frank (1964) in extracellular recordings from the somata of single neurons...
spinal motoneurons. Our recordings used small electrodes, which would be expected to record spikes preferentially from the high potential field near the cell body.

Second, most large spikes were dominantly negative, whereas most small spikes were dominantly positive. This is consistent with our assumption that large and small spikes are associated with different cell types. Moreover, there was no occasion when advancing the electrode converted a negative large (triphasic) spike into a positive small (biphasic) spike or vice versa. This result is also consistent with the maps of potential fields constructed by Nelson and Frank (1964), who showed that the extracellular spike was predominantly negative at all recording locations and never converted to a predominantly positive spike unless the electrode was virtually penetrating the cell, when the recording soon converted to an intracellular one, or became unstable. Similar observations have been reported by Abeles (1982, p. 7) and by Gustafsson and Jankowska (1976).

Third, also consistent with the results of other authors, our recordings from cells with large spikes were much more stable than those from cells with small spikes (cf. Mountcastle et al. 1969) and were less vulnerable to small movements of the electrode (Humphrey and Corrie 1978; Simons 1978).

Fourth, only small spikes were recorded in layer 4C, which is composed almost exclusively of small cells.

Fifth, most large spikes were of longer duration than small spikes. Spike duration did not change with changes in spike amplitude presumably caused by shifts in electrode position relative to the cell (see Fig. 4).

And finally, if spike amplitude merely reflects the position and distance of the recording electrode relative to the recorded cell, physiological properties should have no relation to amplitude. We show in the following text, however, that there is a very strong correlation between spike amplitude and physiological properties.

A second possibility is that small-amplitude spikes were recorded from LGN axons rather than from V1 cells. We reject this possibility on the grounds that, first, physiological properties of cells discharging small spikes differed from the properties of LGN cells. In most layers, the putative small cells were selective for orientation, most were not color opponent, some were direction-selective, and almost all responded to increment and decrement stimuli at the same spatial location. For 4Cβ cells, AR size was much larger than the AR size of LGN cells at a comparable eccentricity.

Second, even when the electrodes were clearly in the white matter, spikes could usually not be isolated or separated for sufficient times to permit quantitative study, indicating that our electrodes were not effective in recording from axons.

Third, as reported by others (Bishop et al. 1962; Mountcastle et al. 1969), our recordings from cells with large spikes were much more stable than those from cells with small spikes (cf. Mountcastle et al. 1969) and were less vulnerable to small movements of the electrode (Humphrey and Corrie 1978; Simons 1978).

Fourth, only small spikes were recorded in layer 4C, which is composed almost exclusively of small cells.
et al. 1969), the few spikes recorded from axons in the white
matter were held for very short times and had a pure monopha-
sic positive shape. All the small spikes analyzed here were
recorded for some minutes and were all biphasic and thus
differed from spikes recorded from axons.

The third possible alternative interpretation is that the large
spikes are recorded adjacent to cell somata, whereas the small
spikes are recorded adjacent to dendrites such as the apical
dendrites of pyramidal cells that can fire action potentials
(Stuart et al. 1997). We regard this possibility as unlikely
because our small spikes differ from the spikes recorded from
dendrites in the following ways: 1) during a burst of spikes
discharged by a dendrite, the amplitude of the action potentials
decreases markedly (Buzsaki et al. 1996; Kamondi et al. 1998;
Spruston et al. 1995). In contrast, our small positive spikes did
not vary in amplitude during a burst response. Furthermore
many were well isolated so that there was no ambiguity about
the presence of only a single cell. 2) Extracellular spikes
believed to be recorded adjacent to dendrites have a dominant
negative lobe regardless of the spike amplitude (Buzsaki and
Kandel 1998), whereas most of our small spikes (Fig. 3) have
a dominant positive lobe. And 3) the spikes discharged by
dendrites have a longer duration than soma spikes (Buzsaki and
Kandel 1998), but our small spikes have a shorter duration than
large spikes (see following text). Taken together, these facts
suggest that our small spikes are not recorded from neuronal
dendrites.

We conclude that the small spikes we have recorded are
most reasonably assigned to small cortical cells and the large
spikes to large cells. In the next sections, we describe the
functional differences between the cells firing large and small
action potentials.

Results from simultaneously recorded cells

To see the relation between spike size and functional prop-
erties of V1 neurons, it is especially informative to compare
selectivity between large and small cells recorded simulta-
neously. (For brevity, and to avoid acronyms, we will refer to
cells firing large spikes as large cells and cells firing small
spikes as small cells). An example of activity simultaneously
recorded from a large and a small cell is given in Fig. 5. The
two cells differed greatly in their physiological properties. The
large cell had almost no ongoing activity (Fig. 5C), had a small
activating region (AR; Fig. 5E), and was very selective for
orientation and direction of motion of the stimulus (Fig. 5, E
and G). In contrast, the small cell displayed a high level of
ongoing activity (Fig. 5D), had a larger AR (Fig. 5F), and was
less selective for stimulus orientation and direction of motion
(Fig. 5, F and H). Note that this example demonstrates that the
small spike is not generated by a LGN axon because even the
small cortical cell had more selectivity for orientation and
direction than could be attributed to an LGN cell.

More examples of simultaneously recorded cells (1 triplet

![Graph](image-url)
and 4 pairs) are illustrated in Fig. 6. The relation between spike size and physiological properties demonstrated in Fig. 5 is elaborated there. Cells with large spikes have lower ongoing activity, smaller ARs and are more selective for stimulus orientation. Interval distribution histograms of the smallest cells (Fig. 6, right) show no intervals shorter than 1 ms; these indicate that only one cell was contributing.

Because it was clear qualitatively that simultaneously recorded cells firing spikes of very different amplitude differed markedly in stimulus selectivity, we sought an amplitude criterion that would distinguish between small and large spikes and could be applied to many simultaneously recorded clusters of cells. We thus identified 22 large/small pairs or triplets where the large spike was $\geq 0.4$ mV and was larger than the small spike by $\geq 50\%$. In 20/22 sites, the large cells had lower ongoing discharge, smaller AR width, and smaller orientation tuning bandwidth than the small cells. The differences in ongoing discharge, AR width, and orientation bandwidth between the 22 pairs of simultaneously recorded cells firing large and small spikes are shown in Fig. 7. These numbers were generated by subtracting the value of the parameter for the large cell from the value for the simultaneously recorded small cell and constructing histograms of these differences. It can be seen that differences in physiological properties of the large and the small cells were quite substantial. These results suggest that there may be an anatomic pairing or grouping of small and large cells with complementary properties that could be integral to the functional organization of the cortex. Such a grouping has previously been suggested for the pyramidal tract neurons of motor cortex (Humphrey and Corrie 1978).

It is noteworthy that for 18/20 sites where the large cell of the pair was more selective, large spikes were triphasic and dominantly negative, whereas small spikes were biphasic and dominantly positive. There was no site where the more selective cell discharged a biphasic spike and the less selective one a triphasic spike. In the 20 sites where the large cell was more selective, there were 14 sites in which there was a clear difference (>50 $\mu$s) in spike width between the large and small spikes. In 13/14 sites the large spike was of longer duration than the small one. These results confirm our qualitative observations that the more selective cells usually have large, dominantly negative, triphasic spikes of relatively long duration.

**Population characteristics—spike amplitude and polarity**

In the next sections we demonstrate that physiological properties are correlated with spike size and polarity for our entire sample, whether or not the cells were recorded simultaneously. From our total sample of 199 cells, sufficient information was available to assign 156 to individual cortical layers. Of these 156 located cells, 137 were in layer 4 or more superficial layers, and the other 19 were in layer 6. No cells in the current sample were localized to layer 5. Thus our sample is primarily from the middle and upper layers of the cortex—4C and above.

Figure 8 shows the distribution of amplitudes for negative and positive spikes for the entire sample. The two distributions were clearly different ($P < 0.001$). At the extremes of the distributions, there was a clear dichotomy. Almost all the very small spikes ($\leq 0.2$ mV) were dominantly positive while most large spikes ($>0.4$ mV) were dominantly negative. In the amplitude range between 0.2 and 0.4 mV, positive and negative spikes were detected in about equal numbers. Considering the whole distribution, the plot of cumulative percentages of cells discharging positive spikes or negative spikes as a function of spike amplitude (inset) shows that only $\sim 7\%$ of the positive spikes were $>0.4$ mV, whereas $\sim 56\%$ of the negative spikes were $>0.4$ mV.

**Population characteristics**

Next we looked at the relationship of spike amplitude and polarity to physiological properties for our entire population (Table 1). As expected from the data from simultaneous recording, small cells ($\leq 0.4$ mV) had higher ongoing activity, smaller ARs, were less sharply tuned for orientation, and had spikes of shorter duration than large cells ($>0.4$ mV). Similar results were obtained for positive versus negative spikes (Table 1).

**Differences within layers**

Although large and small cells within the whole sample differ considerably in their physiological properties, it is possible that particular layers could disproportionately affect the
results. For example, layer 4Cβ contains mostly small cells with high ongoing activity so that lumping them together with the rest of the sample could obscure whether high ongoing activity is a widely distributed property of small cells or is mainly characteristic of 4Cβ. However, the data from simultaneous recording implies that small cells immediately adjacent to large cells in the same layer have different properties. To extend those results, we analyzed separately the data from layers 2/3 and 4Ca from which we recorded a sufficient number of both large and small cells. Figure 9 shows scatter plots for AR width, ongoing discharge, and orientation bandwidth for all cells in those two layers, whether recorded simultaneously or not. As was the case for the total sample, the large negative cells in these layers were more selective, had less ongoing activity, and had spikes of a longer duration than the small positive ones (Table 2). Spike polarity was significant in that no positive spikes were large ones, with most positive spikes being <0.4 mV. This means that neurons discharging positive spikes will have the properties of small cells. However, there were also many small negative spikes recorded from cells with physiological properties very similar to those of the cells discharging small positive spikes. Consequently, present data do not support a physiological subdivision of the small cells based on spike polarity alone.

The difference between large and small cells is more evident in layer 4Ca than in layer 2/3 where differences in ongoing activity and AR size between small and large cells failed to reach statistical significance. This outcome is partially due to the small sample size but also reflects the smaller range of values in layer 2/3 where cells have low ongoing activity and small ARs (Snodderly and Gur 1995), presumably as a result of a strong tonic inhibition affecting both small and large cells. Thus the picture that emerges is one of regional (including laminar) variations in mean values, but within each region small cells have different properties from large cells.

**Direction selectivity**

We also have considered the relation between spike amplitude and direction selectivity. Although our sample of direction-selective cells (n = 20) is too small for statistical analysis, the preliminary results indicate that large cells tend to be more selective for direction of stimulus motion than small cells. Of 20 direction-selective cells, 14 were very selective (DI > 0.9). The spikes of most of these very selective cells were large (11/14 > 0.4 mV). Of the large cells, 12/14 were dominantly negative, 9/14 were triphasic and 11/14 were of relatively long duration (duration index > 0.65 μs). On the other hand, of the six moderately selective cells with 0.5 < DI < 0.9, all spikes were small and biphasic, 5/6 were dominantly positive, and 4/6 were of relatively short duration (<0.25 μs). There were four

![Plot showing distribution of amplitudes of positive and negative spikes](image-url)

**Table 1. Comparisons of physiological properties for neurons generating spikes of different amplitudes or polarities for our entire cell sample**

<table>
<thead>
<tr>
<th>Amplitude, mV</th>
<th>AR Width</th>
<th>Ongoing Activity</th>
<th>Orientation Bandwidth</th>
<th>Spike Width</th>
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<tbody>
<tr>
<td>≤0.4</td>
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<tr>
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<tr>
<td>Mean</td>
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<tr>
<td>Mean</td>
<td>27.2 ± 15.7</td>
<td>16.9 ± 12.6</td>
<td>13.9 ± 13</td>
<td>6 ± 10.7</td>
</tr>
<tr>
<td>Median</td>
<td>25</td>
<td>14</td>
<td>11.5</td>
<td>1.5</td>
</tr>
<tr>
<td>n</td>
<td>79</td>
<td>81</td>
<td>84</td>
<td>91</td>
</tr>
</tbody>
</table>

Values are means ± SD. All comparisons are significantly different (Mann-Whitney U test, p < 0.0001). Units: spike amplitude, mV; activating region (AR) width, minarc; ongoing activity, imps/s; orientation bandwidth, degrees; spike width index, μs.
pairs of simultaneously recorded cells where both cells were direction selective; at all four sites the larger cell had a larger DL, i.e., was more selective. These results suggest that direction selectivity is related to cell size in a manner similar to orientation selectivity, with the larger cells exhibiting greater stimulus selectivity.

**Laminar distribution of spike parameters**

The distributions of spike amplitudes, durations, and polarities in individual V1 layers are shown in Fig. 10. In the middle input layers 4Cβ and 4Cα, most cells discharged small spikes of short-duration, whereas in the more superficial output layers 2/3 and 4B, broader distributions of spike size and duration are found. The bias toward small spikes is particularly evident in layer 4Cβ where the amplitudes of 11/12 spikes were <0.4 mV and almost all spikes were very brief. The overall pattern is consistent with the domination of layer 4Cβ and 4Cα by small and medium sized cells and a more heterogeneous size distribution in layers 2/3 and 4B (Lund 1988). Note that for the middle and upper layers (4C and above), all but one of the smallest spikes (<0.2 mV) were positive.

In layer 6 there may be greater heterogeneity of spike shapes. Among the small spikes, positive and negative polarities were found in about equal numbers, and the positive spikes had a wide range of durations. While the overall properties of small cells are similar to those found in other layers we do not have enough data from large cells in layer 6 to decide whether the large cell/small cell physiological differences found in the middle and upper layers are valid for layer 6. Because we have not recorded spike shapes from layer 5 cells, our present comparisons of large and small cells apply...
primarily to the granular (4C) and supragranular layers. A large sample will be needed for adequate comparisons within and between all the layers.

**DISCUSSION**

**Neuronal groupings and physiological function**

The complementary relationships between cells recorded simultaneously raises the question whether they are functionally paired or combined. At an abstract level, it has been noted previously that adjacent V1 cells carry dissimilar information (Gawne et al. 1996). In the case of adjacent cells of different sizes, our results indicate some of the reasons for the dissimilarity—namely the small cells have higher ongoing activity, larger ARs, and less selectivity for stimulus geometry. Such a relationship could indicate that the small cells are either directly or indirectly inhibiting the large, silent selective cells (cf. Gasanov 1986), thus shaping the response properties of their immediate neighbors. In fact, they could contribute to the “normalization” process that is posited in models such as those proposed by Heeger (Heeger 1993; Heeger et al. 1996) to explain the nonlinearity of cortical responses. In those models, the normalization influence is assumed to be pooled from many cells because it is broadly tuned. One interesting possibility from our results, however, is that a normalization input derived from the small cells could be based on fewer cells because of their inherently broad tuning.

We previously have shown (Snodderly and Gur 1995) that cells in the cytochrome oxidase (CytOx)-rich layers have higher ongoing activity and are less spatially selective than cells in the CytOx poor layers. In the present work, we show that even within a CytOx-poor layer, such as layer 4B or 2/3, different cells, including adjacent ones, have different degrees of selectivity. It is clear that in primate area V1, spatiotemporal selectivity is generated in several stages. For example, cells in 4C are nonoriented, whereas many cells in layers 2 and 3 are very sharply oriented. Our data raise the possibility that this multistage processing continues within each output layer so that response properties of large output cells are shaped by

**TABLE 2. Comparisons within individual layers 2/3 and 4Cα of physiological properties of neurons generating small and large spikes and neurons generating positive and negative spikes.**

<table>
<thead>
<tr>
<th>Amplitude, mV</th>
<th>AR Width</th>
<th>Ongoing Activity</th>
<th>Orientation Bandwidth</th>
<th>Spike Width</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤0.4</td>
<td>≤0.4</td>
<td>≤0.4</td>
<td>≤0.4</td>
<td>≤0.4</td>
</tr>
<tr>
<td>&gt;0.4</td>
<td>&gt;0.4</td>
<td>&gt;0.4</td>
<td>&gt;0.4</td>
<td>&gt;0.4</td>
</tr>
<tr>
<td>Layer 2/3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>17.10 ± 7.12</td>
<td>12.77 ± 5.83</td>
<td>4.77 ± 7.73</td>
<td>5.55 ± 1.85</td>
</tr>
<tr>
<td>Median</td>
<td>15.00</td>
<td>10.00</td>
<td>1.45</td>
<td>0.00</td>
</tr>
<tr>
<td>n</td>
<td>20</td>
<td>13</td>
<td>14</td>
<td>13</td>
</tr>
<tr>
<td>U test</td>
<td>P &lt; 0.073</td>
<td>P &lt; 0.094</td>
<td>P &lt; 0.0001</td>
<td>P &lt; 0.002</td>
</tr>
<tr>
<td>Layer 4Cα</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>32.50 ± 16.88</td>
<td>14.91 ± 6.88</td>
<td>19.13 ± 13.09</td>
<td>6.33 ± 6.00</td>
</tr>
<tr>
<td>Median</td>
<td>32.00</td>
<td>13.00</td>
<td>15.15</td>
<td>4.05</td>
</tr>
<tr>
<td>n</td>
<td>20</td>
<td>11</td>
<td>18</td>
<td>14</td>
</tr>
<tr>
<td>U test</td>
<td>P &lt; 0.003</td>
<td>P &lt; 0.0008</td>
<td>P &lt; 0.0008</td>
<td>P &lt; 0.006</td>
</tr>
<tr>
<td>Polarity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>42.53 ± 15.99</td>
<td>12.55 ± 9.98</td>
<td>3.13 ± 6.27</td>
<td>2.54 ± 6.04</td>
</tr>
<tr>
<td>Negative</td>
<td>20.00</td>
<td>10.50</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>n</td>
<td>13</td>
<td>20</td>
<td>9</td>
<td>18</td>
</tr>
<tr>
<td>U test</td>
<td>P &lt; 0.004</td>
<td>P &lt; 0.93</td>
<td>P &lt; 0.0005</td>
<td>P &lt; 0.0001</td>
</tr>
<tr>
<td>Layer 2/3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>19.76 ± 5.99</td>
<td>12.55 ± 5.98</td>
<td>3.13 ± 6.27</td>
<td>2.54 ± 6.04</td>
</tr>
<tr>
<td>Median</td>
<td>20.00</td>
<td>10.50</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>n</td>
<td>13</td>
<td>20</td>
<td>9</td>
<td>18</td>
</tr>
<tr>
<td>U test</td>
<td>P &lt; 0.15</td>
<td>P &lt; 0.03</td>
<td>P &lt; 0.05</td>
<td>P &lt; 0.0013</td>
</tr>
</tbody>
</table>

Values are means ± SD. Units as in Table 1.

**FIG. 10. Distributions of spike amplitudes and durations for cells in individual layers of V1.**
excitatory and inhibitory inputs from smaller, less selective neighbors. If some of the smaller, less selective neurons are output cells then later visual areas should receive inputs from cells with distinctively different degrees of selectivity. This diversity of inputs may be useful in later visual areas for generating new response properties.

Connectivity and neuron size

In the upper layers of visual cortex of both cats and monkeys, there is a relationship between the soma size of pyramidal cells and their connectivity. In cat, pyramidal neurons in layers 2 and 3 projecting locally within area V2 have a mean somatic area only 61% as large as neurons sending axons through the corpus callosum to the contralateral hemisphere (Matsubara et al. 1996). Similarly, in macaque monkeys pyramidal neurons in layer 3B of area V1 with only local connections have smaller somata than pyramidal cells that project out of V1 into the white matter (E. M. Callaway, personal communication). Although these observations are very limited, they suggest that acquiring information about cell size through recording of the action potentials may contribute to identifying the functional role of the cell.

Sampling issues

The relationships between spike size and physiological properties of cortical neurons imply that samples of neurons recorded with techniques similar to ours are subject to competing sampling biases. Larger spikes are easier to isolate and to hold for the prolonged periods necessary for quantitative analysis. However, the lower ongoing activity rates and greater stimulus selectivity of the large cells often makes them more difficult to detect and to stimulate effectively. Thus spike size and stimulus selectivity represent competing influences affecting the sampling biases for large and small cells.

It should be evident that other measurement techniques also will be influenced by functional differences among the intermixed populations of cortical neurons. For example, in vivo imaging techniques based on modulations of blood flow or oxygenation are presumably sampling the summed activity of mixed aggregations of cells. Because the small cells are more active, more easily stimulated, and perhaps more numerous, they may dominate the responses. In the case of V1, such a bias could result in an underestimate of the spatiotemporal selectivity of information being transferred from V1 to more central cortical regions.

Ideally, physiological samples should be reconciled with the anatomic populations being probed. The anatomic diversity (Peters 1987) evident in area V1, however, is not usually identifiable in extracellular recording studies, which tend to emphasize unifying organizational principles such as orientation and ocular dominance columns (Hubel and Wiesel 1968) and, more recently, the cytochrome oxidase blobs (Horton and Hubel 1981; Livingstone and Hubel 1982). Although taking such an over-all view of V1 has been very productive, for an understanding of mechanisms, it would be desirable to relate physiological data to the anatomic classes of V1 neurons.

A classical method for identifying the anatomic characteristics of physiologically characterized cells is intracellular recording and staining. However, this approach yields very few cells per animal, requires prompt preservation of the tissue after injection and is impractical for most experiments with valuable trained primates. A possible alternative is to localize extracellular recordings to the cortical laminae and the cytochrome oxidase compartments (Snodderly and Gur 1995) and to use the characteristics of the action potentials to help distinguish among the anatomic types contributing to the recordings.

Action potential duration

Past extracellular studies have focused on the duration of the nerve spike as a possible indicator of the type of neuron being monitored. The rationale for this approach is based on intracellular recordings showing that inhibitory interneurons in several cortical regions fire action potentials of short duration (reviewed by Amitai and Connors 1995). Consequently it has been suggested that neurons with brief extracellular spikes may be inhibitory interneurons (Mountcastle et al. 1969; Wilson et al. 1994), and results from antidromic stimulation experiments to distinguish interneurons from efferent neurons are consistent with this suggestion (Swadlow 1988, 1989). However, short spike duration seems to be associated only with particular subclasses of GABAergic interneurons including, but not limited to, basket cells (McCormick et al. 1983; Naegle and Katz 1990). Other GABAergic neurons known as low-threshold spike (LTS) cells have action potentials with durations similar to pyramidal neurons (Foehring et al. 1991; Kawaguchi and Kubota 1993). Thus short spike duration is not obligatory for GABA neurons.

The anatomic distribution of cells discharging brief spikes is also incompatible with using short spike duration alone to identify inhibitory interneurons. Simons (1978) suggested that the fast (short-duration) spikes in layer 4 of rat somatosensory cortex probably arise from spiny cells with small somata, which presumably exert excitatory effects. His proposal is consistent with our finding that cells in layer 4C of area V1 have small spikes of brief duration (Fig. 10). Anatomic studies have concluded that only ~15% of the neurons in 4C contain GABA (Fitzpatrick et al. 1987; Hendry et al. 1987) so it is unlikely that our recordings are coming solely from GABAergic cells. A more plausible explanation is that small cells discharge spikes of brief duration (Tables 1 and 2), and the spiny stellates that are the major population of 4C (Lund 1988) discharge relatively fast spikes even though they are excitatory. In summary, it is likely that brief spikes are recorded from small excitatory cells as well as some, but not all, GABAergic cells.

Action potential polarity and shape

It is possible that the polarity and shape of the small spikes recorded extracellularly could provide important clues about the biophysics of the cells that discharge them. Unfortunately, recent biophysical experimentation and modeling has emphasized intracellular potentials and does not address this issue. However, if Rall's earlier modeling of spinal cord neurons has heuristic value for understanding the physiology of V1 cells, it could mean that the relatively large positive lobe of the small spikes is evidence of the importance of the dendritic tree in establishing the extracellular currents and potentials. For ex-
ample, the small spiny stellate cells may have a relatively small negative lobe in the extracellular spike because of the small soma but a relatively large positive lobe because of the surrounding dendritic tree. Furthermore the influence of the dendritic tree presumably would be enhanced if the dendrites are not purely passive, as Rall assumed but have voltage-sensitive ion channels.

Recent work on action potentials in dendrites shows that the likelihood of generation of dendritic action potentials and the direction of travel (toward or away from the soma) are both influenced by the patterns of inputs to the cell and hence the state of the network in which the cell is embedded (Buzsaki et al. 1996; Chen et al. 1997; Kamondi et al. 1998; Turner et al. 1999). Thus our use of alert animals, in which the patterns of inputs should be relatively stable over time, is probably favorable for recording spike shapes with stable contributions from dendritic activity. So far, most of the experiments on dendritic action potentials have been done with large pyramidal cells, and we are not aware of any evidence about the ability of the dendrites of nonpyramidal cells of the cortex to conduct action potentials. Such information would be very useful, especially for identifying possible dendritic contributions to the small spikes we have recorded.

Our results point to the desirability of using insights from biophysical experimentation and modeling to increase the information that can be gained from extracellular action potentials recorded from behaving animals. We hope that our observations of the associations between the response properties of the cells and the parameters of their action potentials will help to stimulate this kind of cross-fertilization.

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REFERENCES


