Dynamics of Striate Cortical Activity in the Alert Macaque: I. Incidence and Stimulus-dependence of Gamma-band Neuronal Oscillations

Using single and multiunit recordings in the striate cortex of alert macaque monkeys, we find that gamma-band (20–70 Hz) oscillations in neuronal firing are a prominent feature of V1 neuronal activity. The properties of this rhythmic activity are very similar to those previously observed in the cat. Gamma-band activity is strongly dependent on visual stimulation, largely absent during spontaneous activity and, under the conditions of our experiment, not time-locked to the vertical refresh of the computer monitor (80 Hz) used to present the stimuli. In our sample, 61% of multiunit activity (MUA) and 46% of single-unit activity (SUA) was significantly oscillatory, with mean frequencies of 48±9 and 42±13 Hz, respectively. Gamma-band activity was most likely to occur when cells were activated by their optimal stimuli, but still occurred, although less often and with lower amplitude, in response to nonoptimal stimuli. The frequency of gamma-band activity also reflected stimulus properties, with drifting gratings evoking higher-frequency oscillations than stationary gratings. In the cat, the spike trains of single cells showing gamma-band oscillations often displayed a pattern of repetitive burst firing, with intraburst firing rates of 300–800 Hz. The overall similarity of rhythmic neuronal activity in the primary visual cortex of cats and monkeys suggests that the phenomenon is not species-specific. The stimulus-dependence of the rhythmic activity is consistent with a functional role in visual perception.

Introduction
Pattern recognition by the mammalian visual system is thought to be preceded by a rapid, pretentive process that groups related features in an image. Because most visual images evoke activity that is distributed over multiple cortical locations, it has been recognized that some mechanism is needed to identify members of a representation as belonging together. One hypothesized mechanism for this grouping process posits that cortical neurons responding to common features exhibit temporally correlated firing on a millisecond time scale (Milner, 1974; von der Malsburg, 1981, 1985; Singer, 1993; Singer and Gray, 1995). Support for this hypothesis has been obtained from experiments demonstrating that synchronous firing in the visual cortex is stimulus-dependent (Gray and Singer, 1987, 1989; Eckhorn et al., 1988. Gray et al., 1989; Engel et al., 1990, 1991a,b; Freiwald et al., 1995; Kreiter and Singer, 1996; Livingstone, 1996; Fries et al., 1997; Gray and Viana Di Prisco, 1997). These studies have led to vigorous debates concerning the origin, properties and functional significance of synchronous activity (Gray, 1999; Shadlen and Movshon, 1999). However, much of the controversy surrounding this debate stems from a confusion of two separate, but related findings. First, the studies cited above have revealed that neurons, located within the same or different areas of the visual cortex, are capable of synchronizing their responses with millisecond precision when activated by appropriate visual stimuli. Second, the same studies show that synchronous activity is often associated with pronounced oscillations of firing probability in the gamma frequency range (20–70 Hz). These findings have led to the proposal that gamma-band activity may contribute to the generation of neuronal synchrony (Gray et al., 1989; König et al., 1995; Gray and McCormick, 1996; Livingstone, 1996). But they have also led to the erroneous notion that the properties of synchronous activity can be inferred from the measurement of gamma-band oscillations at single recording sites (Ghose and Freeman, 1992; Tovee and Rolls, 1992; Young et al., 1992; Bair et al., 1994). The ensuing debate has led to the emergence of two further questions: is gamma-band activity a general feature of neuronal firing in mammalian visual cortex, and if so, what are its properties? And what is the relationship between neuronal synchronization on a millisecond time scale and gamma-band activity? The current paper addresses the first of these questions, while the companion paper (Maldonado et al., 2000) focuses on the relationship between gamma-band oscillations and synchronous activity.

While a number of studies have reported common features of gamma-band activity in cat visual cortex (Gray and Singer, 1987, 1989; Eckhorn et al., 1988; Gray et al., 1990; Fries et al., 1997; Gray and Viana Di Prisco, 1997; Herculano-Houzel et al., 1999) [but see (Ghose and Freeman, 1992)], there is no general agreement on the nature of this activity in the visual cortex of nonhuman primates. Some researchers have explicitly reported a failure to find gamma-band activity in monkey visual cortex (Tovee and Rolls, 1992; Young et al., 1992; Bair et al., 1994; de Oliveira et al., 1997; Nowak et al., 1999). Other groups have found oscillatory activity, but of a higher frequency (60–90 Hz) than in cat (30–60 Hz) (Eckhorn et al., 1993; Fries et al., 1994). Still others have reported the presence of oscillatory firing in monkey visual cortical areas, with frequency ranges comparable to those documented in cat (Kreiter and Singer, 1992, 1996; Livingstone, 1996).

It is difficult to reconcile these divergent findings because the studies vary in (i) the species of monkey used; (ii) the experimental paradigm employed; (iii) the methods of data analysis; and (iv) the cortical area investigated. For example, while both Young et al. and Livingstone (Young et al., 1992; Livingstone, 1996) recorded from the striate cortex of anesthetized monkeys, the first study used macaque monkeys and analyzed cumulative autocorrelograms whereas the second study utilized squirrel monkeys and analyzed single-trial autocorrelograms. Eckhorn et al. and Fries et al. (Eckhorn et al., 1993; Fries et al., 1994) have been the only reports of gamma-band activity in the striate cortex of the alert monkey; however, these investigations employed only one animal, and the neural activity was sampled from large groups of multiple units or from the local field.
potential. In an effort to resolve these differences, we have analyzed single and multunit activities recorded from the striate cortex of two alert macaque monkeys. Our aim was to determine the incidence and properties of gamma-band activity, and thereby clarify whether this form of activity is species-specific or is a more general phenomenon. We focused our investigations on the primary visual cortex because the original reports were based on data collected from areas 17 and 18 of the cat. And we utilized alert macaque monkeys because these animals are most commonly used in behavioral paradigms. In this study, we demonstrate that oscillatory firing in the gamma frequency band (20–70 Hz) is a robust property of visually evoked neuronal activity in the striate cortex of monkeys performing a visual fixation task.

The results of this study and the companion paper (Maldonado et al., 2000) have been reported previously in abstract form (Friedman-Hill et al., 1995; Gray et al., 1995a).

Materials and Methods

Subjects and Surgical Procedures
Two adult, female rhesus monkeys (Macaca mulatta) served as subjects for this study. The monkeys underwent two sterile surgical procedures to prepare them for training and recording. In the first procedure, we implanted a pair of scleral search coils for monitoring eye position (Judge et al., 1980) and a stainless steel post for head restraint (Gray and Viana Di Prisco, 1997). Following the behavioral training described below, the monkeys underwent a second surgical procedure, in which a hard plastic recording chamber was mounted over the opercular surface of striate cortex and secured to the skull with orthopedic screws and dental acrylic. A craniotomy (5 × 10 mm) was made in the bone overlying one hemisphere. The animals were given ~20 days to recover from each surgical procedure before behavioral training or recording was initiated. All surgical procedures were performed at the California Regional Primate Research Center (CRPRC) and were supervised by the attending veterinary staff. Surgical and experimental techniques were in accordance with institutional and NIH guidelines.

Behavioral Training
The monkeys were trained to maintain their gaze within 1° of a fixation spot, in the presence of moving or stationary visual stimuli, for a period of up to 3 s. Successful trials were rewarded with a drop of dilute apple juice. During behavioral training and recording, the monkeys’ access to water was restricted. On days the monkeys were working, they were given a minimum of 30 ml/kg/day of water or juice in the form of fixation rewards or supplemental water. In practice, the monkeys earned an average of 50–80 ml/kg/day. On Fridays and Saturdays, the monkeys received 80 ml/kg/day of water. Throughout the entire experimental period the animals were monitored by the veterinary staff of the CRPRC.

Recording Techniques
Neuronal signals were recorded with multiple (2–6), tungsten microelectrodes (1–2 MΩ resistance) or custom-fabricated nichrome tetrodes (Gray et al., 1995b), separated by either 250 μm or 3–4 mm. The signals were amplified (10K), bandpass filtered (0.6 kHz–6kHz), digitized (30 kHz/channel) and stored for offline spike sorting and analysis. We recorded both multunit activity (MUA) and isolated, single-unit activity (SUA) from both tetrodes and electrodes. On individual penetrations, units were recorded whenever the signal-to-noise ratio exceeded 3:1. After a recording was completed at a given site, the electrodes or tetrodes were always moved at least 200 μm before another recording was obtained. On successive penetrations occurring in the same guide tube, activity was always sampled at least 200 μm deeper than the previous recording session. This sampling procedure was continued until activity could no longer be measured at a given guide tube location, at which time the guide tubes were repositioned. Sometimes the sampling procedure across sessions involved recording from a second layer of V1 in the head of the calcarine sulcus. This was confirmed by changes in receptive field position.

After recording, individual units were resolved from the MUA through principle components analysis of waveforms (Abeles and Goldstein, 1977) or by using the stereo properties of tetrodes to cluster spikes by peak-to-peak amplitude, peak time, spike width, etc. (Gray et al., 1995b). The extracted spike trains were stored at 1.0 ms resolution. We carried out a separate analysis of the incidence of oscillatory activity for MUA and SUA. Because some of the single units were recorded simultaneously from a single electrode or tetrode and separated offline, we also pooled these units and included the pooled data in our MUA analysis. In our discussion below, a ‘site’ refers to an electrode or tetrode position. Cells simultaneously recorded from different electrodes or tetrodes are considered to be different sites; cells simultaneously recorded from the same electrode or tetrode are considered to be the same site.

Visual Stimuli
Visual stimuli, consisting of single or multiple, drifting square-wave or sine-wave gratings (0.5–3.0 cycles/°, 8–12 cd/m² luminance) presented on a dark background, were generated by a personal computer and displayed on a 19-inch color monitor (80 Hz noninterlaced, 1024 × 768 resolution). The vertical refresh of the video monitor was synchronized with the data acquisition at a resolution of 300 μs. The stimuli were presented 5–50 times during each recording session while the animals maintained ocular fixation on a central target. At the start of each trial, the monkey was given 0.5–1 s to look at the fixation point. Data collection began once the target was acquired and continued throughout the period of fixation. If the monkey failed to move its eyes to the fixation point within the required time or broke fixation early, the trial was aborted and replaced with a new trial following a delay. On each successful trial, the stimulus was presented at a frequency of 0.25–0.75 Hz for a duration of 2.0–2.5 s. The interval between the end of one trial and the beginning of the next varied from 1.0 to 4.0 s in duration. The monkey was rewarded with apple juice for each complete trial, but was not penalized for incomplete trials.

Prior to the automated presentation of stimuli, receptive fields were hand-mapped with computer-generated light or dark bars or gratings, while the monkey maintained fixation. The minimum response field was determined following the methods of Barlow et al. (Barlow et al., 1967). We were usually able to make rough estimates of orientation, direction, velocity and spatial frequency preferences during the mapping routine; this information was used to choose a range of stimuli that was subsequently presented in automated tuning curve sequences.

Anatomical Localization
Cells in V1 and V2 were distinguished on the basis of electrode location and depth, as well as published reports of receptive field size versus eccentricity (Hubel and Wiesel, 1977;Gattass et al., 1981). Because of the angle and location of our penetrations, all cells initially encountered within a penetration were from the opercular surface of V1. As the electrodes were advanced, the amount of change in receptive field location and size was used to determine whether a second folding of V1 had been penetrated, or whether the electrode had moved into V2. All cells in our database were from the lower visual field and had receptive fields within 13° of the center of gaze.

Data Analysis
For each recording and stimulus, we computed the peri-stimulus time histogram (PSTH, bin width 50 ms). Using this measure, two epochs were defined to calculate spontaneous (win1) and stimulus-evoked (win2) activity (Gray and Viana Di Prisco, 1997). Mean firing rates were computed within these epochs for each stimulus and were used to construct tuning curves. Direction tuning curves were fit by a Gaussian function and deemed to be ‘tuned’ or ‘untuned’ using the methods employed by Maldonado and Gray (Maldonado and Gray, 1996). Interspike interval histograms (ISIH) were calculated with a bin width of 1 ms and a maximum time lag of 200 ms.

To study rhythmic neuronal firing, we computed autocorrelation histograms (ACH) for individual trials and for cumulative data (summed across trials) (Peirce et al., 1967) with a time resolution of 128 ms. These
calculations were performed separately for both spontaneous (win1) and stimulus-evoked (win2) activity. Correlated firing time-locked to the stimulus was estimated by computing the shift-predictor control correlogram. Each of these histograms (single trial, session and shift-predictor) was then corrected to enable a comparison of the data across cells and stimuli: (i) each bin in the ACH was normalized by dividing by the central bin; (ii) the central bin in the ACH was then replaced by the mean of the two adjacent bins, in order to eliminate a large, sharp peak at zero time lag; and (iii) a correction was applied to each ACH to compensate for the edge effects that arise when a correlation histogram is computed on windowed data. Each bin of the ACH was multiplied by the factor [window length/window length – absolute value of the time lag]. In general, this correction had very little effect on the ACHs computed from the stimulus-evoked data, because these data were collected with long window durations (i.e. 1.0–2.0 s) compared with the time-lag of the ACH. All histograms depicted in the figures accompanying this paper and the companion paper are uncorrected.

To quantify neuronal rhythmicity, we calculated the power spectrum of each ACH (±128 ms) and extracted the frequency and amplitude of the peak value in the frequency range of 20–80 Hz (Gray and Viana Di Prisco, 1997). The statistical significance of the spectral peaks was estimated using a Monte Carlo simulation. For this procedure, we generated an equivalent pseudorandom spike train for each window of data collected on each trial using a random sample from a uniform distribution and a 1 ms refractory period. The ACH and corresponding power spectrum were computed for each simulated data set and the peak value in the spectrum between 20 and 80 Hz was extracted. The simulation was repeated 500 times and any experimental value was considered significant if it was greater than the largest simulated value (the ‘cutoff’). The ratio of the experimental peak to the simulated cutoff served as a measure of oscillation strength or amplitude. We utilized this ratio (peak/cutoff) because it provided a scale that was easy to understand: any ratio >1.00 was statistically significant. A ratio of 3.00 exceeds the cutoff for significance by 300%; a ratio of 1.10 corresponds to a correlogram with very weak oscillations, just visible upon inspection. Spectral amplitudes are expressed as this ratio throughout the study and all statistics regarding oscillation strength were performed with these ratios. This enabled us to compare oscillation strength across different cells or stimuli independent of the total spike count in each correlogram. The increased stringency of the method also allowed us to include ACHs with very low spike counts and avoided the need for assigning an arbitrary spike count cutoff that excludes valuable data from the analysis (Gray and Viana Di Prisco, 1997).

**Results**

**Incidence of Gamma-band Activity**

The data set included in this study consisted of 357 MUA and 169 SUA recordings taken from striate cortex, and a further 33 MUA and 12 SUA recordings sampled from V2. Rhythmic firing of single units or groups of cells was often obvious in the raw data. Figure 1A illustrates the oscillatory activity of a single neuron when stimulated by a drifting square wave grating of optimal orientation and spatial frequency. This cell fired repetitive bursts of spikes during visual stimulation, as can be seen in the expanded trace on the bottom of Figure 1A. The ISIH in Figure 1C has a peak at 2 ms, representing the intraburst firing rate of the cell, and a second peak at 14 ms, corresponding to intervals between the last spike in one burst and the first spike in a subsequent burst. Because the number of spikes within a burst and the length of the burst may vary, the second peak of the ISIH is only a rough estimate of the interburst firing rate. We refer to cells with bursting properties similar to the cell shown in Figure 1A as ‘chattering cells’. We have documented the existence of these cells in extracellular (Gray et al., 1990; Gray and Viana Di Prisco, 1997) and intracellular (Gray and McCormick, 1996) recordings from cats and in extracellular recordings from awake monkeys (Friedman-Hill et al., 1996). The autocorrelation histogram of this cell exhibits robust oscillations at a frequency of 52 Hz (Figure 1D,E).

In most recordings displaying oscillations, we found that the rhythmicity was easily detected and quantified from the data sampled on single trials. Figure 2 shows 10 trials of activity recorded from the same cell as shown in Figure 1. The cumulative ACH and its shift-predictor control are shown in A and B, respectively. The absence of any peaks in the shift-predictor demonstrates that the oscillations are not time-locked to the stimulus. This excludes the possibility that the rhythmic firing is driven by the video refresh (80 Hz) of the computer monitor. Panels C and D show the ACHs and their associated power spectra computed from 10 repetitions of the same stimulus. Strong oscillatory activity near 50 Hz is present on each trial with some fluctuation of frequency and amplitude from trial to trial.
trial. Compared with the episodic and nonstationary neuronal oscillations previously seen in alert cats (Gray and Viana Di Prisco, 1997), we found much more robust and stable oscillatory activity in the alert monkey. The greater incidence and stability of oscillatory activity in the alert macaque may reflect genuine differences between cats and monkeys or may have resulted from differences in stimuli [drifting bars (Gray and Viana Di Prisco, 1997) versus drifting gratings (present study)].

We visually inspected the ACH and power spectra of all the MUA and SUA sites in order to evaluate the descriptive power of the significance measure (i.e. peak/cutoff) derived from the Monte Carlo analysis. The ACHs and power spectra from five recordings are plotted in Figure 3, along with the oscillation amplitude, measured by the peak/cutoff, and the frequency. The correlograms range in strength from very weak oscillations (Figure 3A) to very robust rhythmic activity (Figure 3E). The corresponding peak/cutoff provides an accurate description of the rhythmic structure of the ACH. The weakly oscillatory ACH (Figure 3A) just barely passes the Monte Carlo cutoff, with a significance ratio of 1.09, while the cell with very strong oscillations (Figure 3E) exceeds the cutoff by almost 800%.

Examples of moderate-to-strong oscillations (Fig 3B–D) also exhibit a good correspondence between the ACH and the significance measure. For comparison, we have included the peak/DC measure used in our earlier study in the alert cat (Gray and Viana Di Prisco, 1997). This parameter expresses the peak value in the power spectrum in the 20–70 Hz frequency range relative to the magnitude of the DC component.

Of the total sample of 357 MUA records in V1, 218 (61%) showed significant oscillations in their cumulative ACHs. The median peak/cutoff was 2.88 and the mean oscillation frequency was 48 ± 9 Hz (Figure 4A,B and Table 1). Oscillations were less likely to occur in SUA autocorrelograms (46%; 78 of 169 cells) than in the MUA sample, with a median peak/cutoff of 1.68 and a mean frequency of 42 ± 13 Hz (Figure 4C,D). Thus, the data shown in Figure 3C are representative of the overall sample. It is also evident that the range of oscillation amplitudes and frequencies for MUA and SUA are similar (Figure 4A–D), although there are more MUA sites with oscillation amplitudes >5.0 and a greater percentage of SUA sites with oscillation frequencies <40 Hz.

We found comparable results in our small sample of V2 recordings. Ten out of 33 (30%) MUA ACHs had significant oscillatory peaks. The mean frequency and median peak/cutoff were 43 ± 9 Hz and 2.10, respectively. Four of the 12 (33%) V2 units were significantly oscillatory, with a median peak/cutoff of 1.34 ± 0.16 and a mean frequency of 40 ± 5 Hz.

There were 571 shift-predictor correlograms, but only 27 (5%) of these had significant spectral peaks, and they came primarily from sessions with small numbers of trials and strongly oscillating cells. For some sessions with many stimuli (e.g. orientation tuning curves), the monkey completed only five trials per stimulus or a cell was lost abruptly because of movement of the animal. With small numbers of trials, the data are not adequately shuffled. This is particularly problematic for strongly oscillatory neurons. The significant shift-predictors had spectral

Figure 2. Oscillatory activity is clearly present in individual trial data and is not due to screen refresh. (A) The cumulative ACH obtained from the same cell as shown in Figure 1D. (B) The trial-shuffled ACH from this cell is flat, indicating that oscillations are not time-locked to the stimulus, as would be expected if they reflected monitor flicker. (C) ACHs from 10 individual trials. (D) Power spectra of each of the single-trial ACHs shown in C. Spectral amplitudes >1.0 are considered statistically significant.

Figure 3. ACHs and associated power spectra for 5 single or multiunit recordings illustrating gradations in the strength of oscillatory activity. The amplitude of oscillation (A = peak/cutoff) ranges from just greater than detection threshold (A) to very strong (E).
peaks ranging in frequency from 39 to 54 Hz, well below the 80 Hz refresh rate of the monitor. These data strongly suggest that the oscillations seen in the experimental correlograms (Figure 4A–D) were not driven, in a time-locked fashion, by the visual stimulus.

It has been suggested that neuronal oscillations reflect spontaneous rhythmicity that is unrelated to visual stimulation (Ghose and Freeman, 1992, 1997). We addressed this question in two ways. First, we computed ACHs for 200–500 ms windows prior to the onset of the visual stimulus. Of 571 spontaneous ACHs, only 29 (5%) were significantly oscillatory. These spontaneous oscillations had a mean frequency of 36 ± 10 Hz (range: 23–66 Hz) and a median peak/cutoff of 1.36 (range: 1.02–4.85). Second, in a subset of our recordings, we included fixation trials in which no stimulus was presented. This enabled us to compare spontaneous and visually evoked activity recorded under comparable conditions. From this sample, 14 of the sites displayed vigorous gamma-band activity in response to an optimal drifting grating. None of these recordings, however, showed significant oscillations during an equivalent period of spontaneous firing. The mean peak/cutoff values for spontaneous and stimulus-evoked activity were 0.61 ± 0.41 and 4.57 ± 2.28, respectively (P < 0.0001, paired t-test). Together these data indicate that striate cortical neurons rarely exhibit gamma-band activity in the absence of a stimulus, and when they do it is usually of low amplitude.

In addition to the cumulative autocorrelograms, we also computed the ACHs for individual trials (Table 2). From the total sample obtained in V1, 25% of the MUA trials and 9% of the SUA trials displayed significant oscillations. For V2, 9% of the MUA trial correlograms and 1% of SUA trial correlograms were significant. Figure 5 plots the amplitudes and frequencies for individual trials for striate MUA (A,B) and SUA (C,D). The strength of oscillations for single trial ACHs was somewhat weaker than the cumulative ACHs (compare Figure 5 with Figure 4), but significant rhythmic activity was still present for single trials. Moreover, the distribution of oscillation frequencies for trial and session ACHs were also similar.

Throughout the recording phase of these experiments, we developed the subjective impression that oscillatory activity
evoked by drifting stimuli was most prominent in the 40–50 Hz frequency range. This impression was confirmed by our finding that the mean oscillation frequency was near 48 Hz. Low-frequency (20–30 Hz) oscillations were not usually significant for single trial correlograms, but when accumulated across many trials, weak oscillations sometimes reached statistical significance (Figure 4D). However, as illustrated in Figure 6, the strongest oscillatory activity in our sample was in the 40–50 Hz frequency range. These results reveal that the most robust oscillations, those with spectral amplitudes >5.0, correspond to gamma activity in the 30–60 Hz range. Thus, the rhythmic activity occurring at the limits of the frequency distribution in our sample (i.e. 20–30 and 60–80 Hz) tended to be lower in amplitude.

On the basis of earlier studies (Engel et al., 1990; Gray et al., 1990), and evidence presented below, it could be argued that oscillatory firing reflects activity in the cortical network when the cells are driven to fire at high rates. Using this line of reasoning, we should expect to see a greater incidence of significant oscillatory activity in those cells that fire at high rates. We examined this question by comparing the firing rates of the oscillatory and nonoscillatory single units in our sample of 169 V1 cells. The results are shown in Figure 7. Both oscillatory (Figure 7A) and nonoscillatory (Figure 7B) cells displayed a wide range of firing rates. Although oscillatory activity tended to occur in neurons firing at higher mean rates, significant oscillations were not limited solely to cells firing at high rates; there were many units that fired rhythmically at rates of <20 spikes/s. The distributions of firing rates for oscillatory and nonoscillatory units differed significantly (P < 0.0001, Mann–Whitney U-test), perhaps reflecting the fact that many of the oscillatory units are likely to be chattering cells with high intraburst firing rates (Friedman-Hill et al., 1996). Figure 7C plots oscillation strength (peak/cutoff) as a function of firing rate for significantly oscillatory single units. From this figure, it is clear that absolute firing rate and oscillation strength were largely independent: There were units with high response rates but very weak oscillatory activity, and there were units which fired very rhythmically but at low rates.

Because other researchers have observed a difference in oscillation frequency between subjects (M.S. Livingstone, personal communication), we compared the data from the two monkeys that participated in this study. We found small, but significant differences in the incidence, strength and frequency of rhythmic activity in the two animals. In one animal, 66% of the recordings were significantly oscillatory, while in the other animal 45% of the recordings were rhythmic. The amplitude and frequency values were also different. In the monkey with the higher percentage of oscillatory cells, the ACHs had a mean peak/cutoff of 3.99 and a mean frequency of 45.4 Hz. In the second monkey, the mean peak/cutoff was 2.87 and the mean frequency was 46.5 Hz. Both of these differences were statistically significant.
These data indicate that the properties of gamma-band activity can vary from animal to animal, but it is difficult to estimate the extent to which sampling biases may have contributed to the differences.

Oscillations Reflect Orientation and Direction Preference

Aside from documenting the existence of rhythmic neuronal activity in the visual cortex, another major aim of this study was to determine how the presence or absence of oscillations was related to orientation and direction preference. Although this issue has been previously investigated in the anesthetized cat (Gray and Singer, 1987, 1989; Eckhorn et al., 1988; Gray et al., 1990), it has received much less attention in the awake monkey. Only one study has examined the influence of stimulus direction or orientation on oscillatory activity in the alert monkey and this report included only 11 MUA sites recorded in a single monkey (Eckhorn et al., 1993). Our sample consisted of 188 direction-tuning curves. For the majority of sessions, 16 directions of a drifting square-wave grating were presented in randomly interleaved trials. For one session, only eight directions of motion were tested.

The effect of changing stimulus orientation on oscillatory activity is illustrated in Figure 8 for a direction-selective V1 single unit. The ACHs of the six stimuli that encompassed the preferred direction are shown on the left side of the figure (B); the corresponding power spectra appear on the right (C). Stimuli that caused a vigorous response (e.g. 3 and 4) evoked strong oscillations, while nonoptimal stimuli elicited fewer spikes (e.g. 1 and 6) and yielded weakly oscillatory or nonoscillatory correlograms. Interestingly, the strongest oscillations (as quantified by the peak/cutoff) occurred in response to the stimulus which was 22° away (stimulus 4) from the ‘optimal’ stimulus (i.e. the peak of the tuning curve). Thus, the stimulus that elicited the most spikes was not always the stimulus that evoked the strongest oscillations.

In order to understand the effect of orientation preference on the probability and strength of oscillatory firing for our entire sample, we compared correlograms for preferred and nonpreferred stimuli, for sessions displaying significant oscillations in response to the optimal stimulus. If rhythmic firing is completely independent of the stimulus, then one might expect to find significant correlograms for all stimuli capable of evoking a visual response. On the other hand, if rhythmic neuronal activity reflects stimulus preferences, then one might expect that optimal stimuli would be associated with a higher incidence of oscillatory activity than nonoptimal stimuli. We identified sites that were orientation tuned and which were oscillatory for at least the orientation that elicited the maximal firing rate. Of the 180 tuning curves, 123 were tuned for orientation; of these, 76 (62%) demonstrated oscillations for the optimal orientation. Figure 9A illustrates that the probability of significant oscillatory firing in this latter population decreased with deviation from the preferred orientation. Stimuli at the preferred orientation were nearly seven times more likely to evoke oscillatory firing than at the orthogonal orientation.

We also examined whether the amplitude and/or frequency of oscillation was modulated by stimulus orientation. Figure 9B plots the strength of oscillation (mean peak/cutoff) as a function of normalized firing rate. It is evident that nonoptimal stimuli elicit weaker oscillations than do optimal or near-optimal stimuli. However, it is also worth noting that even when cells are firing at less than half their maximal firing rates, they may still exhibit...
significant oscillations. For our entire sample of direction tuning curves, we examined the oscillation frequency for all stimuli that yielded significant oscillations. Oscillation frequency did not vary in any systematic manner as a function of stimulus direction. This finding is consistent with similar results from the anesthetized cat (Gray et al., 1990).

Finally, we were interested in how well the tuning based on firing rate matches the tuning based on oscillation strength. As noted above, the amplitude of oscillation for some cells was greater for near-optimal stimuli than for stimuli at the peak of the tuning curve. Figure 9C shows a histogram of the orientation difference between the best stimulus in terms of firing rate versus optimal stimulus for oscillation magnitude. The majority of cells show a match between the two measures. However, there are quite a few cells that oscillate more strongly for a stimulus that is 22 or 180° away from optimal. Thus, while oscillatory activity tends to reflect orientation preference in general, the relationship between firing rate and the incidence and strength of oscillation is not simple.

Differences in Oscillation Frequency between Stationary and Moving Stimuli

In our preliminary recordings, we discovered an interesting difference in oscillation frequency between responses to stationary and moving gratings. Because we and others have previously reported that oscillations in area 17 of the anesthetized cat rarely occur in response to stationary stimuli (Eckhorn et al., 1988; Engel et al., 1990; Gray et al., 1990), we chose to pursue this initial finding in more depth. We recorded 84 sessions where presentations of a stationary bar or grating were interleaved with presentations of a bar or grating drifting at the preferred velocity. Figure 10A.B shows an example of MUA which exhibited significant oscillations in response to a stationary bar (A), but not a moving bar (B). It is worth noting that the firing rate in response to the moving stimulus was actually greater than that evoked by the stationary stimulus. An

![Figure 9](image9.png)

**Figure 9.** Percentage and strength of oscillatory activity, as a function of orientation. (A) Incidence of oscillatory activity reflects orientation tuning preferences, with non-optimal stimuli yielding fewer significant correlograms than optimal or near-optimal stimuli. The analysis was limited to sites that were: (1) orientation tuned and (2) significantly oscillatory for the stimulus that elicited the maximal firing rate (n = 76). (B) Mean oscillation amplitude (peak/cutoff) as a function of normalized firing rate for stimuli yielding significant oscillations. Error bars indicate 2 SD. (C) Histogram comparing the orientation evoking the strongest oscillations with the orientation yielding the maximal firing rate.

![Figure 10](image10.png)

**Figure 10.** Autocorrelograms (columns 1 and 3) and corresponding power spectra (columns 2 and 4) computed from responses to stationary and drifting stimuli. (A,B) MUA displaying significant oscillations for static, but not moving stimuli. (C,D) A single V1 unit showing rhythmic firing in response to a drifting grating, but very few spikes in response to a stationary grating. (E,F) Oscillatory MUA recorded in response to both stationary and moving stimuli.
example of a single unit that fired rhythmically in response to a moving grating (C) but not to a stationary grating (D) is shown in the second row of Figure 10. Although this cell did not fire at a high rate, oscillations are clearly visible in the ACH for the drifting stimulus. Finally, some neurons exhibited oscillatory firing for both stationary (E) and drifting stimuli (F), as illustrated in the third row of Figure 10. Even though both correlograms are significant, they have spectral peaks at very different frequencies (i.e. 35 versus 47 Hz).

Of the entire sample, there were eight sessions where stationary stimuli elicited significant oscillations, but moving stimuli did not; six sessions where moving stimuli led to significant oscillations, but static stimuli did not; and 39 sessions where both types of stimuli evoked significant rhythmicity. For the latter 39 sessions, we found that oscillations evoked by stationary stimuli were lower in frequency (mean = 36 ± 6 Hz) than those evoked by stimuli moving at the preferred velocity (mean = 47 ± 8 Hz) (Wilcoxon Signed Rank, P < 0.001). This trend is illustrated in the upper plot of Figure 11, where nearly all the data points fall below the unity slope line. Although stimulus motion consistently led to higher oscillation frequencies, the two types of stimuli evoked equally strong rhythmic responses (P = 0.70). The mean peak/cutoff value for stationary stimuli was 4.33 ± 7.2; for moving stimuli the mean was 4.64 ± 10.4. In the bottom plot of Figure 11 the data points fall equally above and below the unity slope line. These findings differ from those reported in anesthetized cat (Gray et al., 1990), where stationary stimuli often failed to elicit rhythmic activity or evoked oscillations of a smaller amplitude than moving stimuli. Whether this is a genuine difference between cat and monkey striate cells, whether it is due to the effects of anesthesia, or whether it reflects a difference in sampling or the effects of residual eye movements in the alert state remains to be explored.

Possible Confounding Effects of Flicker Due to Monitor Refresh Rate

It has been suggested that oscillatory neuronal responses may be confounded by the rhythmic activity induced by the flicker of the video monitor employed for visual stimulation (Kiper et al., 1996; Mechler et al., 1996). Thus, it became important to assess the effect of video monitor refresh on the properties of oscillatory responses. Other studies have documented the existence of oscillatory activity using stimuli generated by a projector with a DC powered light source (Eckhorn et al., 1988, 1993; Gray and Singer, 1989) or by a tungsten light source (Livingstone, 1996). We have frequently observed rhythmic firing when using gratings or bars employing a DC light source to roughly assess receptive field position. Figure 12 compares the response of a single unit stimulated by 20 presentations of a stationary square-wave grating generated by the computer (left) or by 40 presentations of a hand-held DC-generated grating (right). The hand-held stimulus consisted of a black and white slide, taped to a flashlight and placed in the receptive field by the experimenter. A fixation point was illuminated on the computer monitor, but aside from this small dot, the rest of the monitor screen was covered with opaque black paper. Shortly after the fixation point was turned on, the stimulus was presented until the fixation point was turned off. It is evident in Figure 12 that significant oscillations can result when cells are stimulated by DC-generated stimuli. The frequency of oscillation (i.e. 35 Hz) is identical to the frequency observed when the cell was stimulated by a computer-generated square-wave grating.
by the computer-generated stimulus. We have found results similar to those in Figure 12 at several other sites.

Discussion

Incidence and Frequency of Oscillatory Activity

Our results demonstrate that the properties of gamma-band activity in the striate cortex of the alert macaque monkey are very similar to those observed in the cat (Eckhorn et al., 1988; Gray et al., 1990; Fries et al., 1997; Gray and Viana Di Prisco, 1997). Thus, contrary to the view espoused by Young et al. (Young et al., 1992) [see also the discussion of Shastri and Ajanagadde (Shastri and Ajanagadde, 1993)], these data show that gamma-band oscillations are not a physiological curiosity of the cat, but rather are a robust and general feature of neuronal activity in the striate cortex of monkeys.

It is not clear, however, why some research groups have found little or no evidence for gamma-band activity (Krüger and Aiple, 1988; Young et al., 1992; Nowak et al., 1999) or have observed oscillatory activity of a different frequency range (Eckhorn et al., 1993; Frien et al., 1994; Livingstone, 1996). One possible confounding factor is the use of anesthesia. Gray and Viana Di Prisco (1996) reported that gamma-band activity in cat striate cortex occurs with greater probability and larger amplitude when the animals are alert, suggesting that the state of arousal could affect the strength of oscillatory firing (Gray and Viana Di Prisco, 1997). Support for this conjecture comes from the finding that the probability and magnitude of stimulus-induced oscillations are closely correlated with the state of electroencephalogram (EEG) arousal and activation of the mesencephalic reticular formation (Munk et al., 1996; Herculano-Houzel et al., 1999). Thus, in experiments where the reported incidence of oscillations is low, it is possible that the bulk of the data were collected during states of EEG synchronization [see the discussion of Maldonado et al. (Maldonado et al., 2000)].

Variations in neuronal sampling may provide another explanation for the reported differences among studies. Sampling biases can arise in a variety of ways that depend on electrode properties, cellular responsiveness, laminar position and the bias of the experimenter. We attempted to reduce sampling bias by collecting data whenever the signal-to-noise ratio of our recordings was at least 3:1, regardless of the degree of visual responsiveness of the neurons. We also minimized the re-sampling of previously recorded neurons by restricting our recordings to sites that were at least 200 µm apart. Throughout our study, we found that the occurrence of oscillatory activity frequently varied with the recording depth, and we often encountered regions of strong oscillatory activity extending several hundred microns along a penetration. This apparent laminar specificity is supported by Livingstone’s (Livingstone, 1996) finding that rhythmic firing in the monkey is most prevalent in superficial layers 2/3 and 4B, and by the study of Gray and McCormick (Gray and McCormick, 1996), who reported that rhythmically bursting neurons are localized in layer 2/3 of the cat. These findings suggest that oscillatory activity is more prevalent in the superficial layers of cortex. If this is the case, variations in the incidence of rhythmic activity might stem from disproportionate sampling from the superficial layers. Another form of bias may also have crept into our measurements, however. Because our aim was to assess the properties as well as the prevalence of gamma-band activity, we often spent additional time collecting data when this activity was encountered. Consequently, less recording time was available for sampling neuronal activity at other cortical depths and locations. We cannot exclude the possibility that each of these types of bias may have contributed to our estimates of the incidence and properties of gamma-band activity.

A further reason for variations in the reported incidence of rhythmic activity may stem from fluctuations in the frequency and probability of oscillations across trials. Intertrial frequency variability can dampen the cumulative ACH, even when strong oscillations are present on single trials (Livingstone, 1996; Gray and Viana Di Prisco, 1997). Livingstone (Livingstone, 1996) discussed this problem and chose to classify sessions as oscillatory if 25% of the single trial correlograms were significantly oscillatory. Intertrial variability of oscillatory firing appears to be less of a factor in our data, however. In many instances, we observed strong oscillations that were sustained throughout each trial and varied little in their frequency content across trials (see Figure 2). A possible explanation for this apparent difference between our findings and those of Livingstone may again relate to anesthesia or to differences in the stimuli or species of monkey used.

Finally, another potential source of variation between studies may stem from the differences in analysis methods used by different investigators. For example, some studies have relied on simple visual inspection of raw auto- and cross-correlation histograms (Krüger and Aiple, 1988). Others have employed curve-fitting procedures to identify rhythmic components in correlation histograms (Engel et al., 1990; Young et al., 1992; Livingstone, 1996), and still others have applied spectral analysis to auto- and cross-correlation histograms (Ghose and Freeman, 1992; Gray and Viana Di Prisco, 1997), to unit activity directly (Bair et al., 1994) or to local field potential recordings (Eckhorn et al., 1988, 1993; Gray and Singer, 1989; Engel et al., 1990; Frien et al., 1994). These differences in analysis methods are complicated by the fact that the measures used to ascribe statistical significance also vary widely across studies. Given that each method is associated with its own set of pitfalls, it is reasonable to surmise that these factors contribute to the disagreement between studies. In this study, we attempted to maintain consistency with our previous method (Gray and Viana Di Prisco, 1997), but at the same time introduced a new measure of statistical significance based on a Monte Carlo simulation of pseudorandom spike trains. While this method does not provide an adequate model to deal with such factors as nonstationarity and burst firing, it does provide a rigorous test of the null hypothesis that neuronal spike trains are Poisson-like in their behavior. Agreement among investigators, however, will probably not be obtained until a uniform set of experimental and analysis methods can be applied to all studies.

Another question raised by our findings is why MUA is more often rhythmic than SUA. One possible explanation is that MUA recordings may contain a mix of oscillatory and nonoscillatory units. If one or more simultaneously recorded units is firing rhythmically and at a high rate, the ACH of the entire group is even more robust for local field potentials than for MUA (Engel et al., 1992; Gray and Viana Di Prisco, 1997), to unit activity directly (Bair et al., 1994) or to auto- and cross-correlation histograms (Ghose and Freeman, 1992, 1993; Gray and Singer, 1989; Engel et al., 1990; Frien et al., 1994). These differences in analysis methods are complicated by the fact that the measures used to ascribe statistical significance also vary widely across studies. Given that each method is associated with its own set of pitfalls, it is reasonable to surmise that these factors contribute to the disagreement between studies. In this study, we attempted to maintain consistency with our previous method (Gray and Viana Di Prisco, 1997), but at the same time introduced a new measure of statistical significance based on a Monte Carlo simulation of pseudorandom spike trains. While this method does not provide an adequate model to deal with such factors as nonstationarity and burst firing, it does provide a rigorous test of the null hypothesis that neuronal spike trains are Poisson-like in their behavior. Agreement among investigators, however, will probably not be obtained until a uniform set of experimental and analysis methods can be applied to all studies.

Another question raised by our findings is why MUA is more often rhythmic than SUA. One possible explanation is that MUA recordings may contain a mix of oscillatory and nonoscillatory units. If one or more simultaneously recorded units is firing rhythmically and at a high rate, the ACH of the entire group is likely to show significant oscillations, even though some of the units in the group are not oscillatory. This masking of nonoscillatory firing can also explain why rhythmic activity is even more robust for local field potentials than for MUA (Eckhorn et al., 1988, 1993; Gray and Singer, 1989; Frien et al., 1994). Another possible explanation for the greater incidence of oscillatory MUA could be that rhythmic activity is a population phenomenon. In this scenario, individual cells may display little or no evidence of oscillatory firing in their ACHs, but are correlated with a population of cells that collectively display
rhythmic activity. This type of behavior, which is well documented in the olfactory system, hippocampus, motor cortex and cat visual cortex (Gray, 1994; Gray and Viana Di Prisco, 1997), can occur when individual cells fire at low rates and the population behavior is variable in frequency. In such instances, it is easy to underestimate the incidence of rhythmic activity when recording solely from single units.

In addition to variations in the incidence of oscillations, some studies have reported higher-frequency oscillations in the monkey compared with the cat or with the data reported here (Eckhorn et al., 1993; Frien et al., 1994; Livingstone, 1996). At present, we are unable to account for these differences, although it is possible that the relation between stimulus velocity and oscillation frequency could provide a partial explanation (Eckhorn et al., 1988; Gray et al., 1989; Gray and Viana Di Prisco, 1997). The results reported here were obtained with drift velocities in the range of 2–6°/s. In a separate study, we have used drift velocities as high as 9°/s and in no instance have we observed significant oscillatory responses that exceeded a frequency of 70 Hz (Maldonado and Gray, 1997). Although Livingstone (Livingstone, 1996) did not report the stimulus velocities used in her study, Eckhorn et al. (Eckhorn et al., 1994) used drift velocities in the range of 1–2°/s. Thus, it appears unlikely that significantly higher stimulus velocities can account for the higher oscillation frequencies observed by these authors compared with the results presented here.

**Stimulus Dependence of Oscillatory Activity**

The stimulus-dependence of oscillatory activity in V1 has been a further point of contention in the literature. Ghose and Freeman (Ghose and Freeman, 1992, 1997) have argued that cortical oscillations are not stimulus-dependent and may be generated by oscillatory input occurring spontaneously in the lateral geniculate nucleus (LGN). Several lines of evidence are inconsistent with this hypothesis. First, oscillatory firing in the LGN of the anesthetized cat occurs in a frequency range that only partially overlaps with that observed in the cortex (Lauer and Verzeano, 1967; Ghose and Freeman, 1992; Ito et al., 1994; Neunenschwander and Singer, 1996; Castelo-Branco et al., 1998). Second, under conditions identical to those employed here, we have recently recorded unit activity in the LGN of one of the monkeys used in this study. While we found numerous instances of rhythmic firing time-locked to the vertical refresh of our video monitor, only 9% (8/86) of our recordings showed evidence of weak stimulus-induced oscillations in the frequency range of 30–100 Hz (Yen and Gray, 1999). Third, this study, as well as numerous others, have consistently found that gamma-band activity is not only induced by visual stimulation, but its properties, such as frequency and probability of occurrence, are influenced by the properties of the stimuli [for reviews see (Singer and Gray, 1995; Gray, 1999; Singer, 1999)]. These findings clearly demonstrate that the occurrence and properties of gamma-band activity in V1 are dependent on visual stimulation.

In this context, we found on average that oscillatory firing occurred most often and with greatest magnitude with those stimuli that evoked the highest firing rates in each cell. However, we found no correlation between the occurrence of gamma-band oscillations and the absolute firing rate across the population of single units. We found many oscillatory cells that fired at rates below 30 Hz in response to their preferred stimuli. In contrast, neither the orientation nor the direction of motion of the stimulus bore a consistent relationship to the frequency of oscillation. Stimulus motion was the only parameter we tested that showed a systematic influence on oscillation frequency. Drifting stimuli consistently evoked higher-frequency oscillations than stationary stimuli, even though oscillation amplitude was the same for drifting and stationary gratings. Together these findings demonstrate that the magnitude and probability of oscillatory activity is directly correlated with the tuning preferences of the cells, and hence their relative firing rates. Oscillation frequency, on the other hand, appears to be dissociated from firing rate.

The finding that stationary stimuli evoke lower-frequency oscillations is interesting, on the one hand, because previous reports have suggested that static stimuli elicit nonrhythmic firing in the cat visual cortex (Eckhorn et al., 1988; Gray et al., 1990). However, the data in both these reports were taken from a small sample and could represent the subgroup of cells in our study that fire rhythmically only in response to moving gratings. On the other hand, velocity-sensitive oscillations have been documented in anesthetized (Eckhorn et al., 1988; Gray et al., 1990) and awake cats (Gray and Viana Di Prisco, 1997), and in awake monkeys (Eckhorn et al., 1993; Maldonado and Gray, 1997). In these studies, oscillation frequency was directly proportional to stimulus velocity. Thus, the low-frequency oscillations we observed in response to stationary stimuli may simply represent the lower end-point of the speed spectrum.

Finally, although oscillatory activity is stimulus-dependent, several lines of evidence indicate that, under the conditions of our experiment (i.e. low luminance intensity), it is not time-locked to the stimulus or the refresh rate (80 Hz) of our monitor (but see Meckler et al., 1996). Vigorous oscillatory responses could be evoked by a handheld DC-generated stimulus, and the distribution of oscillation frequencies in response to computer-generated stimuli was well below the 80 Hz refresh rate of the monitor. Only a small percentage (~5%) of the trial-shuffled correlograms displayed significant spectral peaks. None of these had frequencies at or close to 80 Hz and their magnitudes were smaller than those measured from the unshuffled data. We also found that oscillatory responses displayed variations in frequency and amplitude across repeated trials to the same stimulus. Each of these findings closely parallel similar observations in the cat (Eckhorn et al., 1988; Gray et al., 1990, 1992) and provide strong evidence for a neural induction of oscillatory responses rather than a strictly stimulus-locked process.

**Notes**

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