Synchronization of Local Neural Networks in the Somatosensory Cortex: A Comparison of Stationary and Moving Stimuli

STEPHANE ROY AND KEVIN D. ALLOWAY

Department of Neuroscience and Anatomy, Milton S. Hershey Medical Center, Penn State University College of Medicine, Hershey, Pennsylvania 17033-2255

INTRODUCTION

Recent work in the visual system suggests that perceptual objects are represented by synchronous activity among populations of neurons that respond to the individual components of the object (for reviews, see Singer and Gray 1995; Singer et al. 1997). Cross-correlation analysis of neuronal activity in striate cortex, for example, has shown that neurons representing adjacent parts of the visual field become synchronized if they have similar orientation preferences and are activated by a single bar of light that stretches across their receptive fields (Gray et al. 1989). This result has prompted the hypothesis that cortical synchronization represents a dynamic mechanism for increasing the salience of activity among those cortical neurons that respond to different segments of the same linear stimulus. According to this view, synchronization in striate cortex is mediated by intracortical connections that provide a substrate for linking separate neural populations into functional assemblies for the perception of contours and other stimulus features that have spatial continuity (Singer and Gray 1995; Ts’o et al. 1986).

If neuronal synchronization is a universal principle of cortical physiology that underlies aspects of perception in all sensory modalities, then stimulus-induced synchronization should occur in other sensory regions, including the somatosensory cortex. In support of this hypothesis, neurons in layer III of somatosensory cortex have extensive intracortical projections that allow them to communicate with neighboring neurons that have similar receptive fields (Bernardo et al. 1990; Burton and Fabri 1995; Jones et al. 1978; Lund et al. 1993; Schwark and Jones 1989). Such connections allow SI neurons to receive sensory information from outside their receptive field, but these subthreshold inputs are not apparent unless the local inhibitory circuits are antagonized (Alloway and Burton 1991; Alloway et al. 1989; Dykes et al. 1984; Kyriazi et al. 1996). Although intracortical connections probably are involved in reorganizing SI cortex after digit amputation, nerve transection, or other forms of sensory deprivation (Diamond et al. 1994; Fox 1994; Merzenich et al. 1984; Pons et al. 1991), their functional role during normal somatosensory processing remains unclear.

One possible function for intracortical connections within SI cortex is to synchronize the activity of adjacent populations of neurons during certain stimulus conditions. It is conceivable, for example, that intracortical connections might prime neighboring cortical populations to respond more effectively to a cutaneous stimulus that moves across the skin. To determine whether neuronal synchronization might have a role in coding somatosensory information, we compared the amount of synchronization present in spontaneous activity with that produced by cutaneous stimulation. Furthermore we also tested the possibility that a moving stimulus enhances synchronization in SI cortex more than a stationary stimulus.

METHODS

Four adult cats were used in this study and were treated according to National Institutes of Health guidelines for the use and care of laboratory animals. Most experimental procedures were described...
previously and are only briefly reported here (Johnson and Alloway 1994).

Sterile operating techniques were used to expose SI cortex and to implant a stainless steel recording chamber onto the surrounding cranium. During this operation, a stainless steel bolt was attached to the occipital ridge to immobilize the animal’s head during subsequent recording experiments. After implantation of the recording chamber, SI activity was recorded from each cat twice per week for 4–6 wk. During each recording session, the animal was intubated through the oral cavity and ventilated with a 2:1 gaseous mixture of nitrous oxide and oxygen containing 0.5–1.0% halothane. Heart rate and end-tidal CO₂ were monitored continuously, and body temperature was maintained at 37°C by a thermostatically controlled heating pad.

Cortical electrophysiology

During each electrophysiological session, an array of 3–6 tungsten electrodes (2–5 MΩ; Frederick Haer, New Brunswick, ME) was used to record neuronal discharges in SI cortex. In virtually all experiments, the electrode arrays were configured to sample neurons separated by no more than 600 μm to ensure that all of the recorded neurons had overlapping receptive fields (Dykes and Gabor 1981). Only three or four electrodes, arranged in a linear configuration (1 × 3 or 1 × 4; 300-μm separation), were used in the initial experiments. In later experiments, a matrix of six electrodes (2 × 3; 250-μm separation) was used to record a larger number of neuron pairs simultaneously. The electrode array entered the forearm representation of SI cortex located in the rostromedial bank of the coronal sulcus (Felleman et al. 1989). Electrodes penetrated the cortex at a 25° angle to the parasagittal plane and were advanced by a hydraulic microdrive until single neurons could be isolated on at least two or more electrodes. Recordings were made only from layers III or IV because the neurons in those layers are the most responsive to cutaneous stimulation (Johnson and Alloway 1996). Extracellular neuronal waveforms were displayed on an oscilloscope and converted into digital signals for off-line data analysis (DataWave Technologies, Broomfield, CO).

Cutaneous stimulation

Once neurons were isolated on multiple electrodes, their receptive fields (RFs) were mapped by manually stroking the hairy skin while listening to their neuronal discharges over an acoustic speaker. Most neurons recorded in this study were sensitive to hair movements and could be activated by jets of air that stimulated their RFs.

Computer-controlled air jets were presented in blocks of 100 or 200 trials. Each trial was subdivided into three periods: a prestimulus period for recording spontaneous activity, a stimulation period that contained a series of stationary and/or moving air jets, and a poststimulus period. Neuronal activity was recorded during all three periods but was not recorded during intertrial intervals, which lasted 2 s. Prestimulus and poststimulus periods lasted 3 and 2 s, respectively. The duration of the stimulation period ranged from 2 to 6 s and depended on the number of air jets that were delivered. In the initial experiments, the stimulus period contained only stationary air jets or a moving air jet; in later experiments both types of air jets were presented within each trial.

Stationary air jets were delivered by three or four hollow tubes (1 mm ID) that were aligned in a micromanipulator. The tubes were spaced at equal intervals, ranging from 5 to 20 mm, and were oriented orthogonal to the hairy skin surface. Each tube was connected to a four-channel manifold in which each channel’s air flow was controlled by an electronic valve (Clippard ET-2 M). The electronic valve for each channel was controlled by a digital timer that was triggered by the data acquisition system (DataWave Technologies). Air pressure (20 psi) to the manifold was regulated by a needle valve in series with a pressure gauge.

Previous work has shown that moving air jets activate discrete regions of the hairy skin without producing the lateral distortions caused by dragging a probe across the skin (Ray et al. 1985). Therefore we modified a Grass polygraph pen module to deliver moving air jets in a curvilinear trajectory. The ink pen of the polygraph module was replaced by a tube identical to those used for the stationary air jets except that the end of the tube was curved to direct a jet of air orthogonal to the sweeping motion of the tube. Air flow through the tube was controlled by an electronic valve as described for the stationary air jets. A waveform generator in series with a DC-coupled amplifier was used to produce constant velocity sawtooth movements of the air-jet tube. The waveform generator cycled at 0.5 or 1.0 Hz so that a moving jet of air traversed forward and backward across the skin for 1 or 2 s. The amplitude of the waveform generator was adjusted to produce a trajectory of movement that corresponded to the length of the RFs combined from all recording sites. Given the variability in RF sizes, the stimulus velocities for moving air jets ranged between 4 and 14 cm/s and averaged nearly 10 cm/s. For each experiment, the moving air jet was positioned to pass over the same sites stimulated by the stationary air jets.

In the initial experiments, stationary and moving air jets were presented in separate blocks of trials. In later experiments, a single block of trials was administered in which moving and stationary air jets were presented sequentially during each trial.

Analysis of neuronal responses

Neuronal discharges were sorted on the basis of several parameters including spike width, spike amplitude, and time of maximum spike peak. The time of each neuronal discharge was recorded to within 0.1 ms, and time stamps from each group of sorted waveforms were used to generate summed peristimulus histograms (PSTHs) and cross-correlograms (CCGs). Binwidths for the PSTHs and CCGs were 25 and 50 ms, respectively.

Cross-correlation analysis was used to characterize neuronal activity at one electrode as a function of neuronal activity recorded at a second electrode (Perkel et al. 1967). In a stimulus-based paradigm, an increase in correlated neuronal activity can be produced by stimulus coordination or may occur by chance due to the increased rate of neuronal discharges during peripheral stimulation. To estimate the magnitude of these effects, a linear shift predictor was subtracted from the raw CCG to produce a shift-corrected CCG (Alloway et al. 1993; Gerstein and Perkel 1972; Johnson and Alloway 1996). For our analyses, the shift predictor was the mean of three CCGs calculated from pairing the first 97 of 100 reference responses (or 197 of 200 responses when 200 trials were administered) with subsequent target responses shifted by one, two, or three stimulus trials. Using only 97 trial responses in a linear shift, rather than all 100 trial responses in a circular shift, avoided the pairing of responses having large time separations. Because stimulus-induced responses are not identical from one trial to the next, subtraction of the shift predictor may not remove all instances of stimulus coordination. Nonetheless, the shift predictor can detect many instances of stimulus coordination, especially at stimulus onset when neurons are most responsive and their response latencies are similar across trials. The shift predictor also was used because it represents a convenient tool for determining if correlated events are statistically significant. Because the shift predictor was based on independent spike trains (recorded in response to separate stimuli), the counts in each bin of the shift predictor were assumed to reflect a Poisson process and were used to calculate a Z score to evaluate the significance of values obtained in the shift-corrected CCG. The square root of each value in the shift predictor was multiplied by 1.96 to yield a 95% confidence limit (Aertsen et al. 1989). Peaks within the shift-corrected CCG that exceeded the 95% confidence limits on two or more contiguous bins were considered statistically significant (Aertsen et al. 1989; Gochin et al. 1989).

CORRELATION COEFFICIENT. The correlation coefficient, ρ(r), was calculated to indicate the proportion of discharges in the spike trains...
that were correlated (Abeles 1982). The formula for calculating the cross-correlation coefficient was adapted from Eggermont (1992)

\[ \rho(t_\tau) = \frac{[\text{CE}] / \left[ (N_A - (N_A)^2/T)(N_B - (N_B)^2/T) \right]^{1/2} }{ } \]

where CE is the number of correlated events in the two tallest adjacent bins of a significant peak in the raw or shift-corrected CCG, T is time interval over which the CCG was calculated, and \( N_A \) and \( N_B \) represent the number of neuronal discharges recorded from the A and B electrodes during time T.

SYNCHRONIZATION RATE. Because the correlation coefficient is independent of firing rate and does not indicate how often neuron pairs discharge simultaneously, we also calculated the rate of synchronized discharges from the raw and shift-corrected CCGs. For this parameter, the number of coincident events in the highest 2-ms peak was divided by the total recording time. Thus synchronization rate expresses the number of coincident events occurring per second. An interval of 2 ms was chosen for measuring synchronization rate because this duration encompasses most sharply synchronized events in local regions of SI cortex (Swadlow et al. 1998).

PEAK HALF-WIDTH. We measured the peak half-widths of the shift-corrected CCGs to determine the amount of temporal variability among correlated discharges. Peak half-width was obtained by measuring the width of the CCG peak at half the height of its tallest bin. For spike trains having low rates of synchronized activity, peak half-width was difficult to measure because the bin heights were highly variable. For this reason, we ignored single 0.5-ms bins that dipped into a broader CCG peak. We also measured peak half-widths from both smoothed and unsmoothed CCGs. Smoothed CCGs were generated by averaging each bin in the unsmoothed CCG with its two adjacent bins. Although smoothing removes much of the variability in CCG peak, it may cause an increase in the width of CCG peaks, which consist of only one or two tall bins. Thus smoothing was useful for measuring peak half-widths in CCGs based on low rates of spontaneous activity but was less accurate for measuring highly synchronized responses evoked by peripheral stimulation. Therefore we used smoothed CCGs to compare spontaneous and stimulus-induced synchronized activity but used unsmoothed CCGs to compare the degree of synchronization produced by stationary and moving air jets.

Autocorrelation analysis

Autocorrelograms (ACGs) were constructed from spontaneous and stimulus-induced activity to detect oscillations among neurons showing coordinated responses in the shift-corrected CCGs. For this analysis, ACG peaks exceeding the expectancy value by at least 2 SDs (95% confidence limits) were considered statistically significant, and the ACG had to contain three or more significant peaks at regular temporal intervals to be classified as oscillating (Eggermont 1992). Each ACG was displayed over a time frame of at least ±125 ms.

Histology

The final recording session for each animal was terminated by deeply anesthetizing the animal with an intravenous injection of pentobarbital sodium followed by an injection of 20 mg lidocaine and 1,000 USP units of heparin. The animal was transcardially perfused with 0.9% saline, neutral formalin, and 10% sucrose in formalin. The brain was removed and placed into 30% sucrose in formalin until it sank. The SI cortex was blocked, frozen, and cut into 50 μm coronal sections that were mounted onto chrom-alum-coated slides and stained with thionin.

RESULTS

We recorded extracellular discharges from ~700 SI neurons but excluded from analysis those neurons that did not respond to air-jet stimulation or that failed to discharge at least five times per stimulus trial. On the basis of these criteria, we acquired stimulus-induced responses from 544 SI neurons. In this sample, 263 neurons were stimulated by moving air jets in one block of trials and by stationary air jets in a separate blocks of trials; the remaining 281 neurons were stimulated by both types of air jets in a single block of trials. For this latter group, Table 1 displays the mean discharge rates recorded during spontaneous activity and in response to moving and stationary air jet stimulation. As Table 1 indicates, the SI neurons in this study exhibited low rates of spontaneous activity and were activated by stationary and moving air jets. Statistical comparison of the responses evoked from the most effective stationary site and those evoked by the preferred direction of movement indicates that these neurons were more responsive to moving stimulation (paired \( t = 6.25, P < 0.0001 \)).

We assessed the coordination of stimulus-induced neuronal activity in 880 pairs of SI neurons. This group includes neuron pairs in which stationary and moving air jets were both administered within the same block of trials (\( n = 577 \)) as well as those in which moving and stationary air jets were administered in separate blocks of trials (\( n = 303 \)). Inspection of the shift-corrected CCGs indicated that 10% (\( n = 88 \)) of these neuron pairs were synchronized during both spontaneous and stimulus-induced activity.

Modulation of SI synchronization by stationary air jets

Stationary air-jet stimulation produced synchronization of SI activity in 87 neuron pairs. These data were obtained from experiments in which stationary air jets were delivered alone (\( n = 27 \)) or were interleaved with moving air jets on each trial (\( n = 60 \)).

An example of SI synchronization during spontaneous activity and in responses to a series of stationary air jets is shown in Fig. 1. In this case, neuron CC52a was excited by each of the three air jets aimed at the ventral forelimb, while neuron CC52b preferred air-jet stimulation at the more distal sites. The smoothed shift-corrected CCG compiled from spontaneous activity revealed weak synchronization in which the peak half-width lasted 15 ms and the correlation coefficient was only 0.0236. Cortical synchronization was enhanced noticeably dur-
ing air-jet stimulation as indicated by the fact that the peak half-widths were narrower (2–6.5 ms) and the correlation coefficients were larger (0.0259–0.0283) than those obtained during spontaneous activity.

The effect of cutaneous stimulation on SI synchronization is illustrated by a pair of scatter plots, which show the distribution of correlation coefficients and peak half-widths for 87 neuron pairs tested with stationary air jets (Fig. 2). Because more than one RF site was stimulated for each neuron pair, each point in Fig. 2 represents the air-jet response having the largest correlation coefficient. Compared with spontaneous activity, air-jet stimulation caused a larger proportion of SI cortical activity to become synchronized. Thus the mean correlation coefficient increased from 0.01569 ± 0.0009 (mean ±

**FIG. 1.** Responses to stationary air jets by a pair of primary (SI) somatosensory cortex neurons that had overlapping receptive fields (RFs) and were separated by a distance of 300 μm. A: outline drawing of the forepaw showing the RF boundaries for neurons CC52a and CC52b and the sites (●) that were stimulated by a series of stationary air jets. B: peristimulus histograms (PSTHs) of the responses evoked by stimulating the sites indicated in A; binwidth, 25 ms. Mean extracellular waveforms of neurons CC52a and CC52b are shown (inset); bars, 1.0 ms and 50 μV. C: smoothed, shift-corrected cross-correlograms (CCGs) show the pattern of correlated discharges occurring spontaneously and in response to stationary air-jet stimulation. Magnitude of the correlation coefficients and peak half-widths are indicated on each CCG. - - -, 95% confidence limits; binwidth, 0.5 ms.

**FIG. 2.** Scatter plots comparing the parameters of cortical synchronization for 87 neuron pairs that had correlated discharges during spontaneous activity and stationary air-jet stimulation. Left and right: illustrations of correlation coefficients and peak half-widths, respectively, which were obtained from smoothed, shift-corrected CCGs. Because multiple sites in the RFs were stimulated, each data point is based on the response obtained from the stimulus site with the largest correlation coefficient. - - -, where correlation coefficients or peak half-widths were identical during spontaneous and stimulus-induced activity. →, data obtained from the pair of neurons illustrated in Fig. 1.
Modulation of SI synchronization by moving air jets

Cutaneous stimulation with a moving air jet caused synchronization in 88 neuron pairs. These data represent the combined results from experiments in which moving air jets were administered alone \((n = 28)\) as well as experiments in which moving and stationary air jets were interleaved on each trial \((n = 60)\). Figure 3 illustrates the effects of a moving air jet on the same pair of neurons the responses of which to stationary air jets were shown in Fig. 1. The mean extracellular waveforms in Figs. 1 and 3 show that both neurons remained well isolated, although the waveform for neuron CC52a had increased in amplitude since the time the data in Fig. 1 were recorded. The spontaneous activity of neuron CC52a also had declined slightly, but both neurons continued to respond vigorously to air jets moving distally or proximally across their RFs. Although cross-correlation analysis indicated little change in the half-widths of the CCG peaks obtained during spontaneous activity \((4 \text{ ms})\) or in response to a moving air jet \((2 \text{ ms})\), the change in the proportion of correlated discharges was quite striking. The smoothed shift-corrected CCG for spontaneous-activity contained a small peak near \(t = 0\), which had a correlation coefficient of 0.0157. During stimulation with a moving air jet, the correlation coefficient increased to 0.029 during movement in the preferred (forward) direction but declined to 0.0151 when the air jet moved in the nonpreferred (reverse) direction even though both neurons showed similar rates of activity \((8.85 \text{ vs. } 5.57 \text{ spikes/s for CC52-a1 and } 60.0 \text{ vs. } 50.6 \text{ spikes/s for CC52-b1})\) in both directions. The lack of a relationship between firing rate and correlation coefficient was underscored by the fact that the correlation coefficient during movement in the nonpreferred direction \((0.0151)\) was lower than during spontaneous activity \((0.0157)\) even though the discharge rates of both neurons was several times higher during cutaneous stimulation. This was not an isolated case as 17\% \((n = 15)\) of the neuron pairs showed directional prefer-
enses in synchronization without showing corresponding changes in their underlying rate of activity. Furthermore smoothing the shift-corrected CCGs was not a factor in these cases because the unsmoothed CCGs displayed the same degree of directional preferences in their correlation coefficients.

The effects of moving air jets on cortical synchronization are summarized for all 88 neuron pairs in Fig. 4. The scatter plots in this figure show the distribution of correlation coefficients and peak half-widths obtained during spontaneous activity and moving air-jet stimulation. Because the pattern of coordination often differed when the air jet moved in opposite directions, each data point represents the air-jet response having the largest correlation coefficient. Compared with spontaneous activity, moving air jets produced a substantial increase in cortical synchronization. Thus the correlation coefficients increased from a mean of 0.01692 ± 0.0012 during spontaneous activity to 0.02284 ± 0.0011 during air-jet movements in the preferred direction. A matched-sample t-test indicated that these differences were highly significant (paired t = 4.63; P < 0.0001). A similar comparison of mean peak half-widths obtained during spontaneous activity (7.03 ± 1.31 ms) and during air-jet movement (3.03 ± 0.30 ms) indicated that moving air jets caused a decrease in the temporal variability of correlated activity (paired t = 3.11; P < 0.002).

Comparison of synchronization induced by stationary and moving air jets

We compared the effects of stationary and moving air-jet stimulation on 60 neuron pairs that showed significant levels of cortical synchronization during stationary and moving air-jet stimulation. This analysis was conducted only on neuron pairs in which stationary and moving air jets were both administered within the same block of trials. This restriction was necessary because many spike trains showed clear signs of nonstationarity when different blocks of trials were compared (see Figs. 1 and 3).

In addition to analyzing single neurons, we also analyzed multiunit responses recorded across pairs of electrodes. Multiunit activity was analyzed because many electrodes recorded two or three distinguishable waveforms, but cross-correlation analysis usually failed to detect coordination among any of the single neuron pairs even though their PSTHs were highly similar. Cross-correlation analysis of multiunit responses revealed significant levels of correlated activity across 79 pairs of electrodes.

A representative example comparing the effects of stationary and moving air-jet stimulation on SI synchronization is presented in Fig. 5. In this case, three distinct waveforms were recorded from one electrode (CC69a) and two neuronal waveforms were recorded simultaneously by an electrode located 250 μm away (CC69b) to yield a total of six neuron pairs. Cross-correlation analysis revealed substantial amounts of synchronization in the multiunit responses and in four of the six single neuron pairs. The shift-corrected CCGs obtained from the multiunit responses contained tall peaks in which correlation coefficients were largest during moving air-jet stimulation (0.120) and smallest during spontaneous activity (0.104). The half-widths of these peaks showed that the relative timing of correlated activity was less variable during moving air-jet stimulation (0.5 ms) than during spontaneous activity (1.5 ms) or during stationary air-jet stimulation (2.5 ms). Comparison of the multiunit CCGs with those obtained from single neurons revealed noticeable variability in the coordination of specific pairs of neurons. In one pair of neurons (a3 and b2), for example, each of the CCGs obtained from spontaneous and stimulus-induced activity contained a prominent peak centered around time 0. In another pair of neurons (a1 and b1), correlated activity was barely detected during the moving air-jet response, whereas the stationary air jets produced a broad peak of correlated activity that was located 1–2 ms to the right of time 0.

Although the correlation coefficients for CC69 appeared similar during for both moving and stationary air-jet responses, mean firing rates were higher during air-jet movement. This difference was not apparent from CCGs generated from the complete stimulus period because the stationary air jets lasted 1,000 ms whereas the moving air jets lasted only 500 ms in each direction. Furthermore although each stationary air jet was aimed at overlapping regions of the neuron’s RFs, the moving air jets traversed the entire length of each RF, and this meant that the beginning and end of each sweep of the moving air jet was ineffective for activating both neurons. Therefore to fully appreciate any differences in the rate of synchronized activity produced by moving and stationary air jets, it is necessary to examine the rate of correlated activity produced during equivalent time periods when both types of air jets stimulate overlapping portions of the RFs. Because moving air-jet responses were largest in the midst of the sweep, we
FIG. 5. Comparison of moving and stationary air jets on neuronal synchronization in SI cortex. A: outline drawing of the forepaw showing the overlapping RF boundaries for the multiunit activity recorded by electrodes CC69a and CC69b. Moving air jet pursued a curvilinear trajectory between points 1 and 2 (curved line) and was followed by a series of stationary air jets directed at sites A and B (filled circles). B: PSTHs showing the responses of multiple neurons recorded by a pair of electrodes (CC69a and CC69b) separated by 250 μm. Binwidths, 25 ms. C: shift-corrected CCGs showing the patterns of correlated discharges that occurred during spontaneous activity and in response to moving and stationary air jets. Top: unsmoothed CCGs that were obtained from an analysis of multiunit activity. Bottom: CCGs obtained from an analysis of 2 pairs of single neurons (a3 and b2, a1 and b1) during spontaneous activity (smoothed) or in response to stationary and moving air jets (unsmoothed). Each CCG portrays changes in the activity of CC69a as a function of CC69b activity at time 0. Binwidths, 0.5 ms.
conducted cross-correlation analysis on the activity occurring in the middle portion (100–400 ms) of an air jet moving in the preferred direction. Furthermore, we also conducted cross-correlation analysis on equivalent 300-ms periods at the beginning, middle, and end of the best stationary air jets. Figure 6 illustrates the differences produced by moving and stationary air jets by presenting the raw and shift-corrected CCGs calculated from 300 ms periods for the same multiunit responses shown in Fig. 5. The differential effects of moving and stationary air jets on the rate of synchronized activity is clear from comparing the amplitude of the CCG peaks in Fig. 6. Whereas stationary air jets produced relatively short and broad CCG peaks for each 300-ms period, the moving air jet produced a much taller peak of correlated activity. In fact, most of the correlated activity produced by the moving air jet occurred precisely at time 0 in the raw and shift-corrected CCGs, and the rate of synchronization during the moving air jet (44.6 coincident events/s) was substantially higher than that produced by the stationary air jet (ranging from 26.4–16.8 coincident events/s). The same pattern also was present when the shift-corrected CCGs were analyzed. Binwidths, 0.5 ms.

To compare the proportion of activity synchronized by stationary and moving air jets, we calculated the correlation coefficients for the single- and multiunit responses that had the highest synchronization rates. As Fig. 8 indicates, mean correlation coefficients for multiunit responses were substantially larger than those obtained from pairs of single neurons. This result is consistent with other evidence that correlation coefficients are larger when multiunit responses are analyzed because there are more opportunities for detecting correlated discharges that occur among subsets of different neuron pairs (Bedenbaugh and Gerstein 1997).

As shown by Fig. 8, the raw CCGs for both single- and multiunit activity had higher correlation coefficients during the moving air jets than during the stationary air jets (paired t = 3.47, P < 0.001 for single neurons). Analysis of the shift-corrected CCGs, however, failed to reveal any significant difference in the proportion of correlated activity produced by moving or stationary air jets among pairs of single or multiple neurons. Thus subtraction of the shift-predictor caused considerable...
reduction in the correlation coefficients, but the resulting levels remained above those obtained during spontaneous activity.

A comparison of the mean peak half-widths obtained from the shift-corrected CCGs for single and multiple neurons is shown in Fig. 9. We did not measure peak half-widths for the raw CCGs because we frequently found that half the height of the tallest peak was within the background level of correlated activity and rendered this parameter meaningless. Consistent with the results shown previously in Figs. 2 and 4, both moving and stationary air jets produced substantial decreases in CCG peak half-widths when compared with spontaneous activity. Peak half-widths for the stationary air-jet responses were larger when the entire 1,000-ms period was analyzed, and this result parallels a trend seen in 90% of the single- and multiunit responses in which the rate and proportion of synchronized activity gradually declines during successive 300-ms periods of the stationary air jet (see Figs. 6–8). Analysis of the multiunit responses showed that moving air jets produced slightly less variability in the timing of correlated activity than stationary air jets (paired \( t = 2.04, P < 0.05 \) for comparison with the best 300-ms period). A similar comparison of single neuron pairs, however, failed to detect significant differences in the peak half-widths produced by moving and stationary air jets (paired \( t = 2.005, P < 0.052 \) for comparison with the entire 1,000-ms period).

Effects of electrode separation on stimulus-induced cortical synchronization

There was considerable RF overlap when neurons were separated by only 250–300 \( \mu m \), but the amount of overlap declined with increasing distance and was much less than 50% of the combined RFs for neurons separated by 600 \( \mu m \). This was consistent with other reports showing that RF overlap varies systematically with cortical separation (Alloway and Burton 1985; Dykes and Gabor 1981; Sur et al. 1980). Unless objective criteria are used, however, RF boundaries are diffi-

FIG. 7. Comparison of the stationary and moving air jets on synchronization rate. Each bar indicates the mean rate of coincident events during experiments in which stationary and moving air jets were both presented on each trial. These data were obtained from raw CCGs (top) and shift-corrected CCGs (bottom) constructed from discharges in the 3,000-ms prestimulus period (spontaneous), the entire stationary air-jet period (stationary 1,000 ms), the best 300-ms period during the stationary air jet (stationary 300 ms), and the middle 300-ms period of the moving air jet (moving). All CCGs were unsmoothed except for those constructed from the spontaneous activity of single units. Brackets indicate SE. Asterisks indicate responses to the stationary air jets that were significantly different from the moving air-jet response (matched-sample \( t \)-tests; ***\( P < 0.001 \)).

FIG. 8. Comparison of the proportion of activity that was correlated during stationary and moving air jets. Each bar indicates the mean correlation coefficient obtained from the same CCGs represented in Fig. 7. Brackets indicate SE. Asterisks indicate responses to the stationary air jets that were significantly different from the moving air-jet responses (matched-sample \( t \)-tests; ***\( P < 0.001 \)).

FIG. 9. Comparison of stationary and moving air jets on the timing of synchronized activity. Each bar indicates the mean peak half-width obtained from the shift-corrected CCGs represented in Figs. 7 and 8. Except for the CCGs representing the spontaneous coordination of single units, all other CCGs were unsmoothed. Brackets indicate SE. Asterisks indicate stationary air-jet responses that were significantly different from the moving air-jet response (matched-sample \( t \)-tests; *\( P < 0.05 \)).
synchronization rates were recorded at separations of 300 μm. Air jets at each of the electrode separations, and the highest synchronization rates were higher for moving air jets than for stationary ones. Pairs, but there was little difference in this parameter at separations of 250 or 354 μm. At separations of 600 μm, however, peak half-widths increased to a mean value of 2.77 ± 1.13 ms for the moving air jets and 4.33 ± 0.26 ms for the stationary air jets (paired t = 2.35, P < 0.055).

Table 2 indicates that the probability of detecting cortical synchronization tended to decline with increasing distance between recording sites. Because this trend was less evident among single neuron pairs, we analyzed only the CCGs of multiunit responses to determine how cortical synchronization varied with electrode separation. For both the raw and shift-corrected CCGs, electrode separation had a significant effect on synchronization rate (F = 72.4, P < 0.0001 for raw CCGs; F = 66.5, P < 0.0001 for shift-corrected CCGs). Synchronization rates were higher for moving air jets than for stationary air jets at each of the electrode separations, and the highest synchronization rates were recorded at separations of 300 μm (Fig. 10). Unexpectedly, synchronization rates were higher for neurons separated by 600 μm than for neurons separated by 250 or 354 μm. Electrode separation also had a significant effect on correlation coefficients, but this effect was more evident in the raw CCGs than in the shift-corrected CCGs (F = 32.7, P < 0.0001 for raw CCGs; F = 5.5, P < 0.01 for shift-corrected CCGs). Analysis of correlation coefficients from the raw CCGs also revealed a propensity for greater amounts of synchronization at 300-μm increments (Fig. 11). When the shift-corrected CCGs were analyzed, however, the proportion of correlated activity was highest at 300-μm intervals, but there was little difference in this parameter at separations of 250, 354, or 600 μm. Electrode separation also had a significant effect on the temporal variability of synchronized discharges (F = 23.8, P < 0.001), but this effect was more apparent for spontaneous than for stimulus-induced synchronization (Fig. 12). Thus the mean half-width of CCG peaks constructed from spontaneous activity increased from ≤6 ms at the short intervals (250–354 μm) to nearly 15 ms at intervals of 600 μm. By contrast, cortical synchronization produced by moving or stationary air jets had little temporal variability and mean peak half-widths remained <2.40 ms at the shorter intervals (250–354 μm). At separations of 600 μm, however, peak half-widths increased to a mean value of 2.77 ± 1.13 ms for the moving air jets and 4.33 ± 0.26 ms for the stationary air jets (paired t = 2.35, P < 0.055).

Lack of oscillations during stimulus-induced synchronization

Many reports indicate that neuronal oscillations may play an essential role for synchronizing activity in segregated cortical areas (Bressler et al. 1993; Eckhorn et al. 1988; Gray and McCormick 1996; Gray et al. 1989, 1992; Konig et al. 1995; Murthy and Fetz 1996a,b; Steriade et al. 1994). Therefore we applied autocorrelation analysis to the multiunit responses to determine if stimulus-induced synchronization was dependent

\[ \text{Probability of synchronization across SI recording sites separated by varying distances} \]

\[
\begin{array}{cccccccc}
\text{Distance Separating Electrodes, μm} & 250 & 300 & 354 & 500 & 560 & 600 & 900 \\
\hline
\text{Total} & 123 & 43 & 19 & 31 & 7 & 9 & 11 & 3 \\
\text{Pairs recorded} & 79 & 30 & 19 & 19 & 14 & 11 & 9 & 0 \\
\text{Significant pairs} & 64 & 70 & 100 & 61 & 14 & 11 & 82 & 0 \\
\text{Percentage, %} & 64 & 70 & 100 & 61 & 14 & 11 & 82 & 0 \\
\end{array}
\]
on oscillatory activity. In this analysis, oscillatory activity was considered to be present if the ACG contained three or more peaks which exceeded the expectancy level by 2 SDs at regular temporal intervals (Eggermont 1992). Only five recording sites showed clear cases of oscillatory activity during air-jet stimulation, but two of these responses were identical to oscillatory patterns that appeared spontaneously. None of the remaining multiunit responses contained any clear patterns of oscillation, and this is consistent with other reports indicating that oscillations are not necessary to synchronize cortical neurons separated by ≤2 mm (Konig et al. 1995; Swadlow et al. 1998). Figure 13 illustrates some examples of multiunit responses that were synchronized during moving and stationary air-jet stimulation yet failed to display any oscillations.

**DISCUSSION**

The results of this study demonstrate that neuronal synchronization is an important part of the cortical response to tactile stimulation. Consistent with the view that neuronal synchronization is a potential mechanism for coding certain aspects of sensory stimuli, our results indicate that correlated activity in somatosensory cortex may supplement the changes in firing rate that code intensity and other attributes of a cutaneous stimulus. Although many studies have shown that neuronal synchronization might play a role in visual perception, this is one of the first studies to show the potential utility of stimulus-induced synchrony in somatosensory cortex.

**Anatomic factors affecting synchrony in SI cortex**

The probability of detecting synchronized responses to a discrete air jet was highest for SI neurons located across an interval of 300 μm and was substantially lower for neurons separated by 354–600 μm (see Table 2). One factor that appears to be related to this trend is the degree of RF overlap. Neurons separated by 250–300 μm share the majority of their RFs, whereas neurons separated by 354–600 μm share only a small portion of their RFs. This observation confirms reports showing that RF overlap in somatosensory cortex is related inversely to the distance intervening between neurons (Alloway and Burton 1985; Dykes and Gabor 1981; Sur et al. 1980). Those studies also found that neurons separated by 600–800 μm had nonoverlapping RFs that represented adjacent skin regions. Because we rarely recorded responses from sites separated by >600 μm, we do not know if a discrete moving stimulus can synchronize SI populations that are spaced more widely.

The incidence and strength of synchronization between neighboring parts of SI cortex also appears to be related to anatomic factors. Like other cortical areas, pyramidal neurons in SI cortex, especially those in layer III, give rise to axonal collaterals that have extensive contacts with other neurons in the vicinity of the soma as well as neurons located more distantly (Bernardo et al. 1990; Burton and Fabri 1995; DeFelipe et al. 1986; Juliano et al. 1990; Lund et al. 1993; Schwark and Jones 1989). Although the spatial distribution of intracortical connections probably is related to RF overlap, any discontinuities in this distribution might explain why the incidence and rate of synchronization were higher for neurons separated by 600 μm than for neurons separated by 250, 354, 500, or 560 μm. Intracortical connections in striate cortex, for example, cluster at regular intervals (Gilbert 1992), and some evidence indicates that focal collateralizations also may occur at regular intervals in SI cortex (DeFelipe and Jones 1986).

Common inputs from thalamocortical projections are also likely to play a major role in synchronizing adjacent groups of neurons.
SI neurons. Thalamocortical relay neurons have axon collaterals that terminate in multiple patches of SI cortex and may span a distance of $600\,\mu$m (Garraghty and Sur 1990; Landry and Deschenes 1981; Snow et al. 1988). Consistent with this finding, our CCG peaks usually straddled time 0, a coordination pattern that suggests the presence of common inputs from a third source (Fetz et al. 1991; Perkel et al. 1967).

**Synchronization in SI cortex and sensory coding**

Compared with spontaneous activity, both stationary and moving air jets caused substantial increases in the rate, proportion, and temporal precision of synchronized activity in local regions of SI cortex. Although both types of stimuli produced large increases in the rate of synchronized activity, moving air jets were significantly more effective than stationary air jets in boosting this parameter. Furthermore the increased rate of synchronized activity produced during moving air-jet stimulation was not just the result of an increase in neuronal firing rate but was accompanied by significant increases in the proportion of correlated activity as measured by the correlation coefficients for the raw CCGs. Finally, differences in the rate of synchronization produced by stationary and moving air jets were most prominent among neural populations that were separated by $600\,\mu$m and thus had minimal RF overlap. This finding is important for sensory coding because the stationary and moving air jets were identical with respect to the skin area that was stimulated at any moment in time.

Whereas a previous study found that a discrete tactile stimulus can synchronize SI cortical neurons separated by $\leq 500\,\mu$m (Metherate and Dykes 1985), our study extends those results by suggesting that synchronization may occur over wider regions of SI cortex if the stimulus sequentially activates groups of neurons representing contiguous skin regions.

Some evidence suggests that sensory stimulation evokes recurrent excitation among neighboring cortical populations that may, under certain conditions, interact with incoming thalamocortical activity to enhance cortical synchronization (Douglas et al. 1995). On the basis of the differences in firing rate and proportion of correlated discharges produced by moving and stationary stimuli, we believe that moving stimuli are more effective than stationary stimuli in promoting cooperativity among related thalamocortical and corticocortical networks. In our view, moving stimuli sequentially recruit neighboring populations of thalamocortical neurons that project to the subliminal fringe of excitation surrounding the cortical area activated in the preceding time frame. Compared with a stationary stimulus, a moving stimulus evokes more cortical excitation and continuously activates neighboring populations of thalamocortical and corticocortical networks that are strongly interconnected. One consequence of this appears to be a tremendous increase in the rate of highly synchronized activity in neighboring regions of cortex and suggests that local regions of cortex are wired to become synchronized when the same stimulus activates neighboring parts of this network. We speculate that the cortical area over which a single moving stimulus may...
cause neuronal synchronization probably is related to the speed of stimulus motion and the time period over which recurrent excitation persists. In any case, our findings are consistent with the view that synchronization is a plausible mechanism for linking adjacent cortical populations and suggest that shifts in highly synchronized activity from one cortical region to the next is an important neural correlate of the sensation of movement produced by a single moving stimulus.

We also obtained preliminary evidence suggesting that neuronal synchronization in SI cortex can signal more specific attributes of a cutaneous stimulus. Thus 15 of our neuron pairs were strongly synchronized by air jets moving in one direction but not the other even though the underlying rate of activity was similar for both directions of movement. Although earlier studies indicate that some SI neurons are directionally sensitive (Ruiz et al. 1995; Warren et al. 1986; Whitsel et al. 1972), those studies did not analyze whether groups of such neurons become synchronized or whether synchronization might code stimulus direction independent of changes in firing rate. Although our results suggest that synchronization might play a role in coding direction of movement, we only tested stimuli that moved back and forth for one stimulus cycle. Hence in these cases, we do not know whether synchronization might vary with the level of adaptation, the direction of the initial stimulus, or other factors. To examine these effects, we are currently studying SI responses to repetitive back-and-forth movements.

Parallels with other sensory systems

The presence of synchronized activity within distributed populations of cortical neurons has been investigated in many sensory systems because of its theoretical importance as a potential coding mechanism (Konig and Engel 1995), and many of the findings in those studies bear a resemblance to our results in SI cortex. In auditory cortex, for example, synchronization among local groups of neurons is substantially greater during sound stimulation than during periods of spontaneous activity (de Charms and Merzenich 1996; Dickson and Gerstein 1974; Eggermont 1994; Frostig et al. 1983). In addition, neighboring neurons in auditory cortex display stimulus-induced interactions that have narrower CCG peak widths and larger correlation coefficients than neuron pairs that are more widely separated (Eggermont 1997). Finally, for a significant fraction of neurons in auditory cortex, synchronization is sensitive to the direction of sound movement (Ahissar et al. 1992).

Just as we have observed that synchronization in SI cortex is more likely for neurons sharing similar RF properties, a large body of data indicate that synchronization in visual cortex is governed by similar principles (Singer and Gray 1995). Thus local populations of striate neurons are more likely to become synchronized when they have similar response properties and overlapping RFs (Gray and Singer 1989; Toyama et al. 1981a,b). Consist with the Gestalt criteria for visual perception, adjacent populations of neurons in striate cortex are more likely to be synchronized if they have similar orientation and directional preferences (Ts’o et al. 1986) and represent colinear portions of the visual field (Gray et al. 1989). These findings indicate that the spatial continuity of a visual stimulus is important for organizing striate cortical neurons into functional assemblies (Singer and Gray 1995).

Comparisons of multiple and single neuron responses

Many laboratories have analyzed multiunit activity to reveal cortical synchronization during sensory stimulation. Cross-correlation analysis of multiunit activity has been used widely to demonstrate that neuronal synchronization is a potential coding mechanism in visual and auditory cortex (de Charms and Merzenich 1996; Eckhorn et al. 1988; Engel et al. 1990, 1991; Gray and Singer 1989, Gray and Engel 1992). In addition, temporal analysis of local field potentials also has been used to determine whether segregated populations of neurons become synchronized during different sensory or behavioral conditions (Bressler et al. 1993; Engel et al. 1990; Gray and Singer 1989; MacKay and Mendonca 1995; Murthy and Fetz 1996b; Sanes and Donoghue 1993).

Some investigators have concluded that cross-correlation analysis of multiunit activity is more sensitive for detecting cortical synchronization than an analysis of single neuron pairs (Bedenbaugh and Gerstein 1997; de Charms and Merzenich 1996). We agree with this view because synchronized activity was apparent in only 10% of our single neuron pairs, yet appeared among 64% of our electrode pairs when multiunit responses were analyzed. Although the proportion of synchronized neuron pairs varied as a function of electrode separation, the ability to detect cortical synchronization over longer distances was improved greatly when multiunit responses were analyzed. Finally, small differences in the effects of stationary and moving air jets on mean peak half-widths were detected by our analysis of multiunit responses but not by a similar analysis of single neuron pairs.

Although cross-correlation analysis of multiunit activity appears to be more sensitive for detecting neuronal coordination, the data acquired with this technique must be interpreted carefully (Bedenbaugh and Gerstein 1997). A potential problem with comparing multiunit responses with particular stimuli concerns the recruitment of different sets of neurons. If stationary and moving air jets activate different groups of neurons, any change in correlated activity produced by these stimuli might reflect a sampling difference rather than a change in functional connectivity. Moving air jets evoked higher rates of activity than stationary air jets (see Table 1), and it could be argued that a moving stimulus recruits more SI neurons than a stationary air jet. There are two reasons why this possibility does not explain the differences that we observed. First, we noted the characteristics of the extracellular waveforms recorded during each stimulus and did not observe differences in the shape, amplitude, or width of discharges evoked by moving and stationary stimuli. Second, a comparison of the responses to stationary and moving air jets showed that the CCG peak half-widths were slightly narrower during stimulus movement (see Fig. 9). If moving air jets activated a larger population of cortical neurons, then this should have produced an increase, not a decrease, in the temporal variability of their coincident discharges.

Interpretation of raw and shift-corrected CCGs

In this study we presented data from both the raw and shift-corrected CCGs for a variety of reasons. First, raw CCGs represent the actual patterns of synchronized activity that are available to the organism for sensory perception and discrimination. Second, our stimuli are relatively long in duration (500–1000 ms), and responses to moving and stationary air jets can be highly
variable from one trial to another. Thus it could be argued that subtraction of the shift predictor in our paradigm does not really remove enough stimulus-coordinated events to portray accurately the amount of correlated activity mediated by neural connections. Finally, we wished to determine whether the raw and shift-corrected CCGs revealed similar patterns in the relative amounts of synchronization produced by moving and stationary air jets. We found that moving air jets produced higher synchronization rates than stationary air jets and that this difference was present in both the raw and shift-corrected CCGs. By contrast, correlation coefficients were significantly higher for the moving air-jet responses when the raw CCGs were analyzed, but this difference disappeared when the shift predictor was subtracted.

It is not immediately obvious why the correlation coefficients produced by moving and stationary air jets should be different in the raw but not in the shift-corrected CCGs. Any plausible explanation must consider what the shift predictor represents in these experiments. Consistent with the flat appearance of the 95% confidence limits, which are derived from the shift predictor, we did not observe any prominent peaks in the shift predictor around time 0. Instead, the shift predictor was either completely flat or, in a few instances, contained relatively broad elevations that gradually tapered away from time 0. The lack of a prominent peak suggests that events in the shift predictor do not reflect temporal characteristics of the stimulus but could reflect correlations due to chance. In cases where neurons are not interconnected and do not share any common inputs, the shift predictor accurately indicates the probability of chance correlations, and this value is determined largely by the rate of activity in the recorded neurons. Because discharge rates were significantly greater during moving air jets than during stationary air jets, it could be argued that correlations due to chance are disproportionately greater for the responses to moving air jets. In cases where neurons are likely to be interconnected or to share common inputs, however, the coincident events subtracted from the raw CCG are likely to represent a combination of chance correlations and correlations produced by direct neuronal interactions or common inputs. In experiments such as ours, in which the neurons share RFs and are likely to share thalamocortical inputs, the differential effects of stationary and moving stimuli on synchronization rate may reflect true differences in the proportion of correlated activity produced by neuronal connections. Hence the larger correlation coefficients observed in the raw CCGs during moving air-jet stimulation suggest that this stimulus enhances the cooperativity of thalamocortical projections to common postspike targets.

The authors thank Dr. Barry Dworkin for providing the polygraph pen module that was used for moving air-jet stimulus.

This work was supported by National Institute of Neurological Disorders and Stroke Grant NS-29363 to K. D. Alloway.

Address for reprint requests to K. D. Alloway.

Received 15 May 1998; accepted in final form 3 November 1998.

REFERENCES


NEURONAL SYNCHRONIZATION IN SOMATOSENSORY CORTEX


