Stimulus-Induced Intercolumnar Synchronization of Neuronal Activity in Rat Barrel Cortex: A Laminar Analysis

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Zhang, Mengliang and Kevin D. Alloway. Stimulus-induced intercolumnar synchronization of neuronal activity in rat barrel cortex: a laminar analysis. J Neurophysiol 92: 1464–1478, 2004. First published March 31, 2004; 10.1152/jn.01272.2003. We used cross-correlation analysis to characterize the coordination of stimulus-induced neuronal activity in the primary somatosensory barrel cortex of isoflurane-anesthetized rats. On each trial, multiple whiskers were simultaneously deflected at frequencies that corresponded to 2, 5, 8, or 11 Hz. Among 476 neuron pairs that we examined, 342 (71.8%) displayed significant peaks of synchronized activity that exceeded the 99.9% confidence limits. The incidence and strength of these functional associations varied across different cortical layers. Only 52.9% of neuron pairs in layer IV displayed synchronized responses, whereas 84.1% of the infragranular neuron pairs were synchronized during whisker stimulation. Neuronal synchronization was strongest in the infragranular layers, weakest in layer IV, and varied according to the columnar configuration of the neuron pairs. Thus correlation coefficients were largest for neuron pairs in the same whisker barrel row but were smallest for neurons in different rows and arcs. Spontaneous activity in the infragranular layers was also synchronized to a greater degree than in the other layers. Although infragranular neuron pairs displayed similar amounts of synchronization in response to each stimulus frequency, granular and supragranular neurons were synchronized mainly during stimulation at 2 or 5 Hz. These results are consistent with previous studies indicating that infragranular neurons have intrinsic properties that facilitate synchronized activity, and they suggest that neuronal synchronization plays an important role in transmitting sensory information to other cortical or subcortical brain regions.

INTRODUCTION

In rodents, layer IV of primary somatosensory (SI) cortex contains an isomorphic representation of the mystacial whiskers (Chapin and Lin 1984; Land and Simons 1985; Simons and Woolsey 1979; Welker 1971, 1976; Woolsey and van der Loos 1970). Each whisker is represented by a population of layer IV neurons known as a barrel, and the SI barrel field has a grid-like organization that corresponds to the peripheral arrangement of the whiskers in rows and arcs. Even though neurons in all layers of barrel cortex respond preferentially to stimulation of a single principal whisker (Simons 1978; Welker 1976), their receptive fields subdivide several whiskers, and receptive-field size varies with stimulation parameters, anesthesia, and other factors (Armstrong-James and Fox 1987; Diamond et al. 1992, 1994; Moore and Nelson 1998; Simons 1985; Simons et al. 1992). These findings are consistent with data suggesting that multiple whisker receptive fields may depend on connections between adjacent barrel columns. These horizontal connections are not uniform in all directions, however, but are densest among whisker representations in the same whisker barrel row (Bernardo et al. 1990a,b; Hoelflinger et al. 1995; Kim and Ebner 1999; Petersen et al. 2003).

This asymmetry among intracortical connections in barrel cortex bears similarity to the asymmetry of whisker movements during exploratory behavior. Although whisker motion is not confined within a single spatial plane, behavioral observations indicate that the whiskers move predominately in the rostrocaudal directions (Carvell and Simons 1990; Sachdev et al. 2001; Welker 1964). Furthermore, although individual whiskers may move independently of their neighbors (Sachdev et al. 2002), most exploratory whisking behavior is characterized by synchronous, back-and-forth movements of multiple whiskers at frequencies varying from 1 to 20 Hz but predominately in the 5- to 12-Hz range (Berg and Kleinfeld 2003; Carvell and Simons 1990; Carvell et al. 1991).

Controlled whisker deflections evoke a series of neuronal responses in the corresponding and neighboring barrel columns (Armstrong-James et al. 1992; Petersen and Diamond 2000; Petersen et al. 2003). Very few studies, however, have characterized the temporal coordination of these responses across multiple whisker representations of SI barrel cortex (Erchova et al. 2002; Lebedev et al. 2000; Nicolelis et al. 1995). This is an important issue in systems neuroscience because substantial evidence suggests that neuronal synchronization may play a critical role in coding the distributed features of a sensory stimulus and in transmitting information to postsynaptic targets within a sensory processing stream (Alonso et al. 1996; Gray 1999; Roy and Alloway 2001; Singer and Gray 1995; Usrey 2002).

To characterize the coordination of neighboring columns in SI barrel cortex during whisker stimulation, we recorded extracellular discharges from neurons in neighboring barrel columns while multiple whiskers were activated by a computer-controlled stimulus. In addition to establishing whether neurons in neighboring barrel columns become synchronized during multiple whisker stimulation, we wanted to determine if neuronal coordination varied with the relative location of neurons in the same or different rows of barrel cortex. For this purpose, we used a square array of electrodes to sample multiple pairs of neurons simultaneously, some of which were located in the same rows or arcs, whereas others resided in different rows and arcs. Finally, to determine if coordination varies as a function of laminar position, all electrodes pene-
trated the cortex in tandem, and we compared the incidence and strength of neuronal synchronization at multiple cortical depths within each animal.

METHODS

Data were obtained from 15 male Sprague-Dawley rats ranging from 285 to 425 g. All procedures complied with National Institutes of Health guidelines, and our institutional animal welfare committee approved a detailed description of the experimental protocol.

Each rat was initially anesthetized with an intramuscular injection of ketamine (20 mg/kg) and xylazine (6 mg/kg), and then atropine sulfate (0.05 mg/kg) was administered to reduce bronchial secretions. Each animal was intubated through the oral cavity, placed in a stereotoxic frame, and artificially ventilated with a 2:1 mixture of nitrous oxide and oxygen. As the effects of ketamine subsided, isoflurane was administered at concentrations (0.75–1.25%) just sufficient to prevent reflexive movements. Ophthalmic ointment prevented corneal drying, and the wound margins were infiltrated with 2% lidocaine. Body temperature was maintained at 37°C with a homeothermic heating pad. Continuous monitoring of expired CO2 indicated that the peak-to-peak end-tidal pressure of this gas varied from 15–20 mmHg. Heart rate was also monitored continuously and varied from 275 to 325 beats/min.

Electrophysiology

A craniotomy was made over the right hemisphere ~1–3 mm posterior to bregma and 4–6 mm lateral to the midline. These coordinates correspond with published maps of SI cortex and with anatomical studies of the SI barrel field (Alloway et al. 1999, 2000; Chapin and Lin 1984; Hoover et al. 2003). Immediately after exposing SI barrel cortex, the dura was resected, and a low-impedance carbon fiber electrode (0.5 MΩ) was used to map a small part of the barrel field. Stimulus-induced neuronal discharges were monitored over an acoustic speaker while the whiskers and surrounding face regions were stimulated with a hand-held dowel stick.

Subsequently, an array of four carbon electrodes was advanced into SI barrel cortex. Each carbon fiber electrode had a small tip (12 μm) to facilitate penetration of the cortex. To improve signal-to-noise recording properties, the tip of each electrode was dipped into cyanoacrylic to seal the glass pipette around the etched carbon fiber; this raised impedance to ~1 MΩ. Four electrodes were cemented together in a square configuration so that the distance between electrode tips on each side ranged from 490 to 840 μm. Because of the curvature of the cortical surface, the electrode holder was set to 25 or 34° angle from the sagittal plane so that all electrodes contacted the pial surface simultaneously as they entered SI barrel cortex. The microdrive reading was recorded as each electrode contacted the pial surface, and we analyzed data only from those electrode pairs in which the discrepancy in surface contact was ~200 μm. This requirement ensured that we analyzed the coordinated activities of neurons residing in the same cortical layer. On entering cortex, receptive fields were mapped by hand-held stimuli; whiskers were stimulated once at a consistent time-locked discharges across trials (Pinto et al. 2000); it is necessary, however, to have some trial-by-trial variation in spike timing to detect functional connections with cross-correlation analysis. The 50-ms sawtooth pulse was produced by a LeCroy waveform generator (model LW 420) the output of which was amplified by a DC-coupled amplifier (Techtron LVC608). Similar methods have been used by other investigators to stimulate the mystacial whiskers and activate neurons in SI barrel cortex (Kleinfield and Delaney 1996; Simons 1978).

Each stimulus trial contained four frequency-specific blocks of eight stimulus pulses for a total of 32 stimulus deflections. After a prestimulus period (1,000 ms) for recording spontaneous discharges, whisker deflections were administered in four blocks of 50-ms pulses that corresponded to frequencies of 2, 5, 8, or 11 Hz. A period of 2,000 ms separated each frequency-specific block, and neuronal activity was recorded continuously during these blocks and the interblock intervals. Neuronal discharges were not recorded, however, during the intertrial intervals, which lasted ~2 s. For each set of neurons, stimulus-induced activity was recorded during 300 or 400 trials.

Analysis of neuronal responses

Pairs of simultaneously recorded spike trains were used to construct CCGs that portrayed changes in the probability of one neuronal discharge given that the other neuron discharged at time 0. In a stimulus-based paradigm, correlated neuronal activity can be produced by stimulus-induced coordination or may occur independently as a function of increased neuronal firing rate. We minimized these effects by subtracting a shift predictor from the raw CCG (Alloway et al. 1993; Gerstein and Perkel 1972; Johnson and Alloway 1996; Roy and Alloway 1999). The shift predictor was constructed by shifting one spike train with respect to another by a single adjacent trial. We chose this method because neurons are more likely to be in similar states on adjacent trials, and this should maximize detection of potential instances of stimulus coordination. The shift predictor was also used to calculate the 99.9% confidence limits, which correspond to 3.3 SDs in a normalized distribution. Small peaks that barely exceeded the confidence limits were often observed and, therefore, shift-corrected CCGs (and their confidence limits) were subjected to a smoothing procedure in which the average of each set of three consecutive bins in the unsmoothed CCG was used to determine the
height of the central bin in that set. Peaks were considered statistically significant if they exceeded the 99.9% confidence limits in both the smooth and unsmoothed CCGs (Alloway et al. 2002). For all measurements of correlation coefficients, peak half-widths, and peak times, a total of 100 bins (1 ms each) were used to display interactions occurring 50 ms before and after time 0. Smaller bins of 0.5 ms were used only for subsequent construction of population CCGs shown in Fig. 10. For figures in the results section, the tails of the CCGs were removed so that only the central portion is depicted.

To characterize the strength of neuronal coordination, significant peaks in the shift-corrected CCGs were used to measure correlation coefficients, peak half-widths, and peak times. The correlation coefficient, \( p(\tau) \), indicates the proportion of discharges in a pair of neurons that are correlated. As in previous studies (Eggermont 1992; Roy and Alloway 1999), the correlation coefficient was calculated as: 

\[
p(\tau) = \frac{|CE|}{|N_A - \langle N_A \rangle^2/T||N_B - \langle N_B \rangle^2/T|} \]  

where \( CE \) is the number of correlated events in the tallest 2-ms period of a significant peak, \( T \) is the time interval over which the CCG was calculated, and \( N_A \) and \( N_B \) represent the number of neuronal discharges recorded from cortical neurons A and B during time \( T \). Neurons A and B were always recorded by separate electrodes, and one electrode was arbitrarily selected as the reference neuron, discharges of which are represented at time 0 in the CCG. The correlation coefficient was always measured from CCGs in which each bin was 1 ms in duration.

The temporal precision of neuronal coordination was determined by measuring both the half-width of the largest peak in the shift-corrected CCG as well as its peak time (or lag time). Peak half-width, which is the width of the CCG peak at half the height of its tallest bin, indicates variability in the relative timing of correlated discharges recorded from a pair of neurons. When measuring the half-width of relatively broad peaks, we ignored those instances in which a single bin (1.0 ms) dipped sharply into the peak. The peak time indicates the time of the tallest peak in the shift-corrected CCG, and this represents the most frequent time lag that occurs among the correlated discharges.

**Histology**

At the end of the recording session, each rat received a lethal dose of pentobarbital and was transcardially perfused with physiological saline followed by neutral formalin. The brain was removed, and the cortex was dissected from the underlying hemisphere and flattened between glass slides stored overnight in fixative with 30% sucrose. The pial surface of the flattened cortex was placed directly onto a horizontal plane of frozen substrate on the microtome stage, and then the cortical slab was cut tangentially into 50- or 75-μm-thick sections. This produced sections that were always parallel to the pial surface and ensured that the entire barrel field would be observed in three or four consecutive sections through granular layer IV. These tangential sections through layer IV were processed for the presence of cytochrome oxidase (Land and Simons 1985). The tangential sections located above and below layer IV were mounted onto separate glass slides and were stained with thionin before being dehydrated, defatted, and coverslipped.

**Results**

We recorded extracellular discharges from 316 neurons in SI barrel cortex. Responses to whisker deflections varied with different types of neurons, their laminar location, and the stimulus frequency of the whisker deflections. As shown by Fig. 1, substantial adaptation was apparent during 8- or 11-Hz stimulation as reflected by a decline in response amplitude throughout these frequency-specific blocks. Consistent with previous reports (Ahissar et al. 2001; Sosnik et al. 2001), response latencies also displayed gradual increases as stimuli were administered within each block of eight stimuli. During 5-Hz stimulation, for example, a latency of 12 ms was observed in response to the first stimulus but increased to 20 ms for subsequent stimuli within that block.

The duration of stimulus-induced responses often extended beyond the duration of the whisker deflections. As shown by Fig. 1B, the average neuronal response to all 32 stimuli began ~16 ms after stimulus onset and did not return to spontaneous levels until 80 ms after stimulus onset. Hence the average stimulus-induced response lasted longer than the 50-ms stimulus pulse.

The primary aim of this study was to characterize stimulus-induced neuronal coordination in neighboring parts of SI barrel cortex, and therefore we focused our analysis on discharges that occurred in response to whisker deflections. To accommodate response latencies of ≥10 ms and the fact that responses continued beyond the end of each stimulus pulse, analysis of neuronal coordination was conducted on discharges that occurred within a 50-ms interval beginning 10 ms after each stimulus pulse. Furthermore, among the 316 neurons that we recorded, analysis was limited to 289 neurons in which the mean stimulus-induced response (averaged over 32 stimulus

![Fig. 1](attachment:image.png)

Fig. 1. Temporal relationship between mechanical stimulation and neuronal responsiveness. A: peristimulus-timed histograms (PSTHs) illustrate primary somatosensory (SI) responses to 300 trials of multiple whisker stimulation. Top: responses of a regular spiking neuron to whisker deflections at 2, 5, 8, and 11 Hz. Bottom: the responses to 5-Hz stimulation are shown on an expanded time scale. Vertices below the abscissa indicate the temporal profiles of stimuli 9–16. Each stimulus lasted 50 ms and consisted of 725-μm deflections in the caudal direction during the 1st half of the pulse; see Methods for further details. Bin widths, 10 ms for upper PSTH, 2 ms for lower PSTH. Waveform scale indicates 1 ms and 500 μV. B: PSTH showing the average stimulus-induced response of the same neuron for all 32 stimuli. Bin widths, 2 ms.
periods) exceeded the spontaneous discharge rate by a factor of two or more. From these 289 neurons, a total of 476 neuron pairs was available for cross-correlation analysis. After constructing the shifted-corrected CCGs for these 476 neuron pairs, we found that 71.8% (342 of 476) of them contained stimulus-induced correlated activity that exceeded the 99.9% confidence limits.

**Synchronization of fast- and regular-spiking neurons**

Based on the temporal duration of the isolated extracellular waveforms, neurons were classified as fast or regular spikes (Bruno and Simons 2002; Simons 1978). As shown by Fig. 2, we measured the length of time that the waveform departed from the resting potential and found that the waveform durations had a bimodal distribution. Based on this distribution, 154 neurons with durations <0.75 ms were classified as fast spikes, and 135 neurons with durations >0.80 ms were classified as regular spikes. These values are consistent with previous studies that measured the width of discharges in SI barrel cortex (Armstrong-James et al. 1993; Bruno and Simons 2002; McCormick et al. 1985; Simons 1978). Previous reports indicate that fast-spike neurons, especially in the layer IV barrel field, are local inhibitory neurons (Amitai and Connors 1995; McCormick et al. 1999; Kawaguchi and Kubota 1993; McCormick et al. 1985; Porter et al. 2001). Fast spikes were encountered in all cortical layers but mostly at a depth of 400–900 μm, which contains the barrel field. Although inhibitory neurons are distributed more uniformly throughout the cortical layers (McCasland and Hibbard 1997), a bias in our electrodes may have favored the detection of discharges produced by larger neurons. If this occurred, then small inhibitory neurons may not have been detected except in sites where large neurons were in relatively low densities. Consistent with this view, we encountered regular-spiking neurons in all layers but mainly in the infragranular layers. Other investigators have reported similar laminar distributions of regular-spiking neurons (Agmon and Connors 1992; Amitai and Connors 1995; Simons 1978). However, because we classified neurons only by their extracellular waveforms, the regular spike discharges are likely to represent multiple classes of neurons including intrinsically bursting pyramidal neurons (Agmon and Connors 1992; Amitai and Connors 1995; McCormick et al. 1985).

We examined whether whisker stimulation might differentially synchronize pairs of neurons that displayed fast or regular spike discharges. As indicated by Table 1, when a fast-spike neuron was recorded simultaneously with a regular-spike neuron, only 59.1% of these heterogeneous combinations displayed significant levels of synchronization during multiple whisker stimulation (see Fig. 3). By comparison, when homogeneous neuron pairs were examined, the probability of synchronization increased. Thus nearly 70% of the neuron pairs comprised of fast-spiking neurons displayed significant levels of stimulus-induced synchronization (see Fig. 4), whereas 86.3% of regular-spike neuron pairs became synchronized during whisker stimulation (see Fig. 5). Regardless of which combinations of neuronal subtypes were analyzed, significant peaks in the shift-corrected CCGs did not depend on oscillations in the stimulus-induced spike trains. Thus autocorrelation analysis usually failed to reveal any periodicity in the temporal structure of the stimulus-induced spike trains. On occasions where small peaks appeared in the ACG of one of the neurons, the ACG of the other neuron did not display a corresponding peak (see Figs. 3–5). Hence synchronization did not require the presence of phase-locked oscillations in the discharges of both neurons.

Consistent with the lower incidence of synchronization among pairs of fast- and regular-spike neurons, the strength of correlated events was also weakest for these heterogeneous neuron pairs. As shown in Fig. 6, left, the cumulative frequency distribution of correlation coefficients for fast-regular neuronal combinations showed little overlap with the distributions of fast-fast or regular-regular neuron pairs. A one-way ANOVA

**TABLE 1. Incidence of neuronal synchronization in SI barrel cortex**

<table>
<thead>
<tr>
<th>Neuronal combinations</th>
<th>Recorded Pairs</th>
<th>Synchronized Pairs</th>
<th>Percent of Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fast-fast</td>
<td>154</td>
<td>106</td>
<td>68.8</td>
</tr>
<tr>
<td>Fast-regular</td>
<td>154</td>
<td>91</td>
<td>59.1</td>
</tr>
<tr>
<td>Regular-regular</td>
<td>168</td>
<td>145</td>
<td>86.3</td>
</tr>
<tr>
<td>Laminar location</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>II/III</td>
<td>93</td>
<td>69</td>
<td>74.2</td>
</tr>
<tr>
<td>IV</td>
<td>104</td>
<td>55</td>
<td>52.9</td>
</tr>
<tr>
<td>V/VI</td>
<td>157</td>
<td>132</td>
<td>84.1</td>
</tr>
<tr>
<td>Uncertain</td>
<td>122</td>
<td>86</td>
<td>70.4</td>
</tr>
<tr>
<td>Column configuration</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Same row</td>
<td>147</td>
<td>105</td>
<td>71.4</td>
</tr>
<tr>
<td>Same arc</td>
<td>107</td>
<td>75</td>
<td>70.1</td>
</tr>
<tr>
<td>Disparate</td>
<td>222</td>
<td>162</td>
<td>73.0</td>
</tr>
</tbody>
</table>

SI, primary somatosensory.
indicated that different neuronal combinations had a significant effect on the strength of the synchronization as measured by the correlation coefficient ($F = 3.50; P < 0.05$).

**Neuronal synchronization and laminar location**

Another objective of this study was to compare the incidence and strength of neuronal synchronization across different layers of SI barrel cortex. Therefore after characterizing neuronal responses at one cortical depth, the electrode array was advanced until new neurons were isolated on two or more electrodes. The same whisker stimulation protocol was repeated, thereby allowing us to characterize neuronal coordination at three or four cortical depths within each animal. This laminar analysis is based only on electrode penetrations in which microlesions were histologically recovered. The laminar positions of the unlesioned recording sites were interpolated with respect to the pial surface, the CO-labeled sections through layer IV, and the laminar position of the microlesions.

Data from animals in which microlesions were not recovered were discarded from this laminar analysis. Furthermore, data from recording sites that appeared to be on the border of CO labeling (either between layers III and IV or between layers IV

![FIG. 3](image1.png)

**FIG. 3.** Representative example of coordination among a pair of fast- and regular-spike neurons recorded at a depth of 1,250 µm in columns D2 and E1 (experiment ME56). A: PSTHs illustrate responses to 300 trials of multiple whisker deflections. Bin widths, 10 ms. B: autocorrelograms (ACGs) illustrate the temporal structure of spike discharges for each neuron; analysis based only on discharges occurring in 50-ms periods beginning 10 ms after stimulus onset; bin widths, 0.1 ms. Insets: mean waveforms calculated from 150 to 200 discharges of each neuron; scales indicate 1.0 ms and 500 µV. C: diagram of the mystacial whiskers; black encloses the deflected whiskers. × correspond to the barrel columns for the 2 electrode penetrations. Histologic verification of the electrode penetrations appears in Fig. 8. D: shift-corrected cross-correlation histogram (CCG) illustrating the synchronized responses for this neuron pair during whisker deflections. CCG analysis based only on stimulus-evoked discharges during 50-ms blocks beginning 10 ms after stimulus onset. Strength of correlated activity is indicated by the correlation coefficient (CC). 

![FIG. 4](image2.png)

**FIG. 4.** Representative example of synchronized responses among 2 fast-spike neurons recorded at a depth of 270 µm in columns C3 and C4 (experiment ME64). The stimulator activated 16 whiskers located in arcs 1–4 of rows B–E. C: a tangential section through the layer IV barrel field; electrolytic lesions made by the electrodes are indicated by arrowheads. Waveform scales indicate 1.0 ms and 500 µV. All details of the PSTHs, ACGs, and shift-corrected CCG are identical to Fig. 3.
and V) were also excluded to minimize the possibility of analyzing responses whose laminar position was uncertain. Finally, we did not subdivide the supragranular or infragranular layers into their laminar components because of the difficulty in using cytoarchitectonic criteria in tangential sections. Among 289 neurons showing stimulus-induced responses, we were able to establish the laminar position of 207 neurons.

As indicated by Table 1, the incidence of neuronal synchronization was lowest in layer IV (52.9%), highest in the infragranular layers (84.1%), and intermediate in the supragranular layers (74.2%). Among layer IV neuron pairs that displayed significant amounts of stimulus-induced synchronization, analysis of the correlation coefficients indicated that these correlations were weak. In Fig. 7, in which four neurons were recorded simultaneously in layer IV, only half of the six neuron pairs displayed significant peaks near time 0 of the shift-corrected CCGs, and the correlation coefficients for these cases were low, ranging from 0.013 to 0.016.

By comparison, functional associations in the infragranular layers were characterized by taller peaks near time 0 of the shift-corrected CCGs. To illustrate this result, Fig. 8 shows results from a representative experiment in which four neurons were simultaneously recorded in layer V. As this figure indicates, all six neuron pairs displayed significant amounts of correlated activity near time 0 of the shift-corrected CCGs, and two of these neuron pairs displayed broad CCG peaks with half-widths ranging from 6 ms (D2 × D3) to 20 ms (D2 × E1). The correlation coefficients for half of these neuron pairs exceeded 0.020 (ranging from 0.023 to 0.031). Note that correlation coefficients do not depend on the width of the CCG peaks, the numerator of this ratio is based only on correlated events in the tallest two adjacent bins (see METHODS).

Quantitative analysis of the responses in SI barrel cortex confirmed the view that stimulus-induced synchronization was significantly different across cortical layers. As illustrated by Fig. 6 (middle), the cumulative distribution of correlation...
coefficients for the infragranular layers showed virtually no overlap with corresponding distributions for the granular or supragranular layers. Consistent with this, the mean correlation coefficient was higher for infragranular neuron pairs (0.0224 ± 0.0009; means ± SE) than for neuron pairs in either the granular (0.0186 ± 0.0008) or supragranular (0.0197 ± 0.0009) layers. Furthermore, a one-way ANOVA revealed that laminar location had a significant effect on the correlation coefficients of those neuron pairs that had significant peaks in their shift-corrected CCGs ($F = 6.05; P < 0.01$).

We also detected differences in the temporal precision of correlated activity across the different layers of SI barrel cortex. As shown by Fig. 9, CCG peak half-widths were largest for neuron pairs in the infragranular layers. A one-way ANOVA confirmed that laminar location had a significant effect on this measure of temporal variability ($F = 4.83; P < 0.01$). Because most infragranular neurons displayed regular spike discharges, peak half-widths were also largest for pairs in which both neurons displayed regular spikes (see Fig. 9). Consistent with this, a one-way ANOVA confirmed that different neuronal combinations produced a significant effect on peak half-widths ($F = 6.46; P < 0.01$).

A similar temporal trend appeared among the time lags of neuron pairs recorded in different layers. As shown in Fig. 9, the mean peak time for significant interactions in the supragranular layers was only 1.20 ± 0.22 ms, but this increased to a mean peak time of 2.45 ± 0.28 ms for neuron pairs in the infragranular layers. A one-way ANOVA confirmed that laminar location had a significant effect on peak time ($F = 4.70; P < 0.01$).

**Neuronal synchronization and columnar configuration**

Another goal of this study was to determine whether stimulus-induced synchronization varied according to the columnar configuration of the constituent neurons. We analyzed this factor because there are more interconnections among barrel columns in the same row than among barrel columns in the same arc (Bernardo et al. 1990a,b; Petersen et al. 2003). For this analysis, we inspected the CO-labeled tangential sections through layer IV and noted the location of each electrode penetration within a specific barrel column. Each pair of simultaneously recorded neurons was assigned to one of three groups depending on the respective location of the electrode penetrations: both neurons in the same barrel row (e.g., C1 and C2), both neurons in the same barrel arc (e.g., D2 and C2), or both neurons in disparate rows and arcs (e.g., D1 and C2). For some neuron pairs recorded in layer IV, either one neuron in the pair ($n = 8$) or both neurons ($n = 1$) were located in the septal regions. These neuron pairs were also assigned to one of the three groups according to their axis of alignment. For example, a neuron in barrel C1 and a septal neuron between C2 and C3 would be classified as having a row orientation.

The representative examples of neuronal responses in Figs. 7 and 8 indicate that columnar configuration had an influence on the magnitude of synchronization that we observed in SI...
barrel cortex. In Fig. 7, in which all recorded neurons were located in layer IV, three of the six neuron pairs failed to become synchronized, and these were configured in barrels that resided in different rows (C2/D2, C2/H11003/D3, C3/H11003/D2). For the three neuron pairs that showed significant amounts of synchronization, two were located in barrels that resided in the same row (C2/H11003/C3, D2/H11003/D3), or disparate rows and arcs (D2 × E1, D3 × E1, D3 × E2). Nonetheless, the neuron pairs with the strongest correlation coefficients were the two pairs that resided in the same row (D2 × D3, E1 × E2).

These observations were consistent with the entire data set. As indicated by Table 1, the incidence of significant synchronization in the shift-corrected CCGs did not vary according to the columnar configuration of the constituent neurons. Whether neurons resided in the same row, same arc, or disparate rows and arcs, the incidence of synchronization only varied from 70 to 73%. Quantitative analysis of the correlation coefficients, however, revealed important distinctions between neurons residing in the same row and those that resided in different rows.

FIG. 8. Neuronal responses recorded from neighboring barrel columns in the upper part of layer V (experiment ME55). A: PSTHs illustrate responses to 300 trials of controlled stimulation of 12 whiskers, arcs 1–4 of rows C–E. Columnar location for each neuron indicated in the top left of each PSTH. Bin widths, 10 ms. Amplitude scales represent 500 μV for all neuronal waveforms. B: tangential section through the deepest part of the SI barrel field (depth of 800–850 μm) was labeled for CO to indicate the location of each electrode penetration with respect to specific whisker barrels. The PSTH responses in A were recorded ~60 μm below this CO-labeled section. C: shift-corrected CCGs illustrate the amount of synchronized activity for the neuron pairs located in the same row (D2 × D3, E1 × E2), same arc (D2 × E2), or disparate rows and arcs (D2 × E1, D3 × E1, D3 × E2). All CCGs are shown at the same scale; binwidths, 1.0 ms.

FIG. 9. Changes in the duration and lag times of CCG peaks as a function of different neuronal combinations, laminar location, and columnar configuration. Each bar indicates the mean duration (peak half-width) or mean lag time (peak time) obtained from neuron pairs displaying significant amounts of synchronization; brackets represent SE.
As illustrated by Fig. 6, the cumulative distribution of correlation coefficients for neurons in the same row showed minimal overlap with the distributions for neurons located in the same arc or in disparate rows and arcs. Consistent with this, the mean correlation coefficient was highest for neuron pairs in the same row (0.0219 ± 0.0008) and was lower for neuron pairs in the same arc (0.0193 ± 0.0008) or in disparate rows and arcs (0.0192 ± 0.0005). A one-way ANOVA revealed that columnar configuration had a significant effect on the correlation coefficients of these distributions (F = 5.10; P < 0.01).

We also found that correlated discharges among neurons in the same row had shorter time lags than neurons in different rows. Thus as shown by Fig. 9, the mean time lag (or peak time) for synchronized neurons in the same row was only 1.33 ± 0.19 ms, whereas synchronized activity across disparate rows and arcs displayed a mean peak time of 2.40 ± 0.23 ms. A one-way ANOVA confirmed that columnar configuration had a significant effect on mean peak time (F = 5.45; P < 0.01). Peak half-widths generally varied from 7 to 9 ms (see Fig. 9), and columnar configuration had no effect on this measure of temporal variation (F = 1.30; P > 0.25).

Neuronal synchronization and stimulus frequency

We stimulated whiskers at frequencies (i.e., 2, 5, 8, and 11 Hz) that are within the range of whisking frequencies observed during exploratory behavior (Berg and Kleinfeld 2003; Carvell and Simons 1990; Carvell et al. 1991). Most whisking behavior occurs between 5 and 12 Hz, and we were interested in determining whether certain stimulus frequencies were more effective than others for synchronizing neuronal responses.

To analyze the relative contribution of each frequency in producing synchronized activity, we limited our analysis to neuron pairs having correlation coefficients in the top half of the cumulative distributions shown in Fig. 6, middle. By focusing on neuron pairs with the strongest statistical associations, this increased the likelihood of analyzing correlations that were based on functional connections within the neural network. We constructed shift-corrected CCGs that were based on neuronal responses to the last seven stimuli in each frequency-specific block. Responses to the first stimulus pulse in a block were not included because that stimulus occurred 2 s after the preceding stimulus and, consequently, reflects a stimulation frequency of only 0.5 Hz.

In contrast to CCGs based on 32 stimuli, it was difficult to interpret shift-corrected CCGs that were constructed from frequency-specific responses. This difficulty arose because fewer spikes were available for cross-correlation analysis, and many frequency-specific CCGs did not contain a well-defined peak even though such peaks appeared in CCGs that were based on the cumulative responses to all stimulus frequencies.

To overcome this interpretive difficulty, we constructed normalized CCGs for each neuron pair by dividing the shift-corrected CCG by the number of stimuli that were administered (7 stimuli × 400 trials or 7 stimuli × 300 trials). Then we summed the normalized CCGs for each stimulus frequency and cortical layer combination, and to compensate for laminar differences in recordings, we divided the summed CCGs by the number of neuron pairs obtained from the supragranular, granular, or infragranular layers (i.e., 35, 28, or 66). This procedure generated a normalized population CCG for each stimulus frequency and cortical layer combination. We also applied the same procedure to spontaneous activity that was recorded over a 350-ms period prior to stimulation at 2 Hz. This time period, 350 ms, is equivalent to the amount of time analyzed for each stimulus frequency (i.e., 50-ms responses to 7 stimulus pulses).

The population CCGs for different combinations of stimulus frequency and cortical layer are presented in Fig. 10. As judged by the tallest peaks at time 0 for these CCGs, the greatest amount of synchronization was observed in the infragranular layers during 5-Hz stimulation. In all layers, 5-Hz stimulation produced more synchronization than any other stimulus frequency. Furthermore, for all stimulus frequencies, neuronal synchronization was strongest in the infragranular layers. In fact, spontaneous activity was also more strongly synchronized in the infragranular layers than in other layers. This suggests that similar mechanisms may mediate both spontaneous and stimulus-induced synchronization in the infragranular layers.

Inspection of Fig. 10 also revealed systematic variations in the confidence limits of the population CCGs. This is informative because the confidence limits are derived from the shift predictor and therefore must reflect the number of discharges that are time-locked to whisker deflection, especially at stimulus onset. As Fig. 10 indicates, the confidence limits were wider for the granular layers than for the supragranular layers. Presumably, this reflects the higher fidelity that layer IV neurons exhibit in response to activation of thalamocortical inputs. Furthermore, in each cortical layer, the confidence limits declined in peak height as stimulus frequency increased, and this indicates a parallel decline in the number of time-locked discharges as stimulation frequency increased.

To view the changes in time-locked responses more directly, we constructed normalized population PSTHs for each stimulus frequency and cortical layer combination. The PSTHs in Fig. 11 are based on the same set of neuronal responses used to construct the population CCGs but are expressed as the mean response per stimulus. As this figure indicates, an increase in stimulus frequency produced a clear decrease in response amplitude, regardless of laminar location. The drop in response magnitude was most pronounced as stimulus frequency increased from 5 to 8 Hz. This corresponds to a decrease in the interstimulus interval from 150 ms (5 Hz) to 75 ms (8 Hz) and suggests that local cortical inhibition, which requires 100 ms to dissipate (Laskin and Spencer 1979), might be responsible for the adaptation that we observed during stimulation at 8 or 11 Hz. Alternatively, this frequency-based decline in responsiveness might be mediated by an increase in synaptic depression (Chung et al. 2002) or by a combination of these two mechanisms.

Stimulus-induced responses to 8 or 11 Hz were evident in the supragranular or granular layers but were barely perceptible in the infragranular layers (see Fig. 11). The decline in infragranular responsiveness during 8- or 11-Hz stimulation was also accompanied by an increase in discharge rate that occurred between successive stimuli (i.e., during the interstimulus intervals) in these frequency-specific blocks. This effect is emphasized by comparing the population PSTHs with the mean rate of spontaneous activity that occurred prior to each frequency-specific block (see Fig. 11, right). The interstimulus intervals for 8 or 11 Hz were only 75 or 40.9 ms, respectively, and it is apparent from Fig. 11 that infragranular neuronal activity...
during these periods remained elevated and did not return to spontaneous levels when each stimulus pulse had ended.

Neuronal synchronization and spontaneous activity

To examine the potential influence of spontaneous activity on neuronal coordination, we compared the relative timing and magnitude of correlated events during spontaneous and stimulus-induced activity. For this comparison, cross-correlation analysis of spontaneous activity was conducted on neuron pairs in which each neuron discharged ≥1,000 or more times during the four 1-s periods that occurred prior to the frequency-specific blocks of whisker deflections. The shift predictor and confidence limits for spontaneous interactions were based on shifting the spike trains by adjacent trials; hence, all calculations were identical to those used for the stimulus-induced responses. Among 342 neuron pairs that contained significant amounts of stimulus-induced synchronization (see Table 1), we identified 292 neuron pairs in which spontaneous activity displayed significant amounts of synchronization as indicated by CCG peaks that exceeded the 99.9% confidence limits.

As shown by the scatter plots in Fig. 12, we detected a weak relationship in the temporal coordination of spontaneous and stimulus-induced activity. In ~90% of all cases ($n = 260$), the CCG peaks obtained from spontaneous activity were wider than the CCG peaks obtained from stimulus-induced activity. This indicates that spontaneous activity in neighboring barrel columns was synchronized much more loosely than when activity was evoked by peripheral stimulation. Analysis of the peak half-widths obtained from spontaneous and stimulus-induced activity revealed a weak correlation in this parameter ($r = 0.3614; P < 0.0001$). This relationship was due primarily to neuron pairs recorded in the middle and deeper layers; there was little correlation between the peak half-widths for spontaneous and stimulus-induced activity recorded from the supragranular layers ($r = 0.0437; P > 0.50$).

Correlation coefficients calculated from spontaneous and stimulus-induced responses were moderately correlated ($r = 0.5673; P < 0.0001$). This relationship was similar for neurons pairs recorded in each of the three laminar groups (data not shown). As shown by Fig. 12B, correlation coefficients were larger for spontaneous activity than for the stimulus-induced responses. This difference, of course, is due to the large amount of correlated activity that was subtracted in the shift predictor to compensate for potential instances of stimulus-induced coordination. Hence, the correlation coefficient for a shift-corrected CCG does not indicate the actual proportion of all activity that is correlated in a pair of spike trains, but it provides an unbiased indication of which neurons are likely to be coordinated because they have functional connections. The moderately strong relationship in correlation coefficients obtained during spontaneous and stimulus-induced activity suggests that the same functional connections were operative during both of these periods.

DISCUSSION

Substantial evidence indicates that granular layer IV is the primary input layer for sensory cortex and that the infragranular layers are the main output layers (Jones 1986; Keller 1995). Although this view is oversimplified with respect to many details of cortical synaptic organization, it is consistent with...
results characterizing the serial flow of afferent information within a cortical column (Agmon and Connors 1992; Armstrong-James et al. 1992). It is also consistent with cross-correlation studies showing that thalamic neurons are strongly connected with the middle cortical layers but have weak connections with infragranular neurons (Alloway et al. 1993; Johnson and Alloway 1996).

The present study extends these findings by demonstrating that intercolumnar synchronization is strongest among neuron pairs in the infragranular layers and weakest among neuron pairs in layer IV. Neurons in the infragranular layers displayed similar amounts of synchronization in response to each stimulus frequency, whereas neurons in the other layers were differentially synchronized by low and high stimulus frequencies. This is noteworthy because infragranular neurons display smaller PSTH responses to the higher stimulus frequencies (i.e., 8 or 11 Hz). In addition, spontaneous activity in the infragranular layers was synchronized to a greater degree than in the other cortical layers. Collectively, these results suggest that one function of the infragranular layers is to facilitate stimulus-induced synchronization across neighboring cortical columns. Along with other data, this suggests that infragranular synchronization is a mechanism for transmitting information to other cortical or subcortical brain regions.

FIG. 11. Normalized comparisons of stimulus-induced responses for different combinations of laminar location and stimulus frequency. Each panel depicts a normalized PSTH that shows the average response to a single trial of whisker stimulation. The panels are based on the same set of neurons used to generate the population CCGs in Fig. 10. Only responses to the last 7 stimuli in each frequency-specific block were used to construct the PSTHs. →, the mean rate of spontaneous activity recorded immediately prior to each frequency-specific block of stimuli. The profiles of the stimulus pulses are shown under the abscissa (top row). Bin widths, 2 ms.

FIG. 12. Comparison of spontaneous and stimulus-induced synchronization. A: scatter plot illustrating peak half-widths obtained from shift-corrected CCGs for 292 neuron pairs that displayed significant amounts of spontaneous and stimulus-induced synchronization. The diagonal line indicates where points should appear for neuron pairs in which peak half-widths were identical for spontaneous and stimulus-induced activity. Logarithmic scales were used to expand the region representing the peak half-widths obtained from stimulus-induced responses. B: scatter plot illustrating the correlation coefficients obtained from the same neuron pairs illustrated in A.
Weak synchronization in granular layer IV

Results from most physiology studies suggest that barrels are functionally independent and that very little information is directly transmitted from one barrel to another within layer IV (Goldreich et al. 1999; Laaris and Keller 2002; Petersen and Sakmann 2001; Swadlow et al. 1998). These findings are supported by anatomical studies showing few intercolumnar connections within layer IV (Harris and Woolsey 1983), most of which are in the septal regions (Chapin et al. 1987; Kim and Ebner 1999). Hence, it was surprising to find that whisker stimulation produced synchronized responses among half of the layer IV neuron pairs that we recorded. There are some mechanisms, however, that could synchronize neurons in neighboring barrels without involving direct interconnections. While thalamic projections to cortical layer IV have a precise topographic organization (Chmielewskas et al. 1989; Killackey and Leshin 1975), each cortical barrel receives some inputs from noncorresponding barreloids, and these divergent thalamocortical projections could synchronize target neurons (Arnold et al. 2001; Land et al. 1995). Because we deflected multiple vibrissae simultaneously, concurrent activation of multiple thalamic barreloids should reinforce the synchronizing influence of these parallel, divergent pathways.

Other neuronal substrates may also coordinate neurons in neighboring barrels. Recent data indicate that discrete excitation of one barrel can produce synaptic events in a neighboring barrel (Schubert et al. 2003). These barrel-to-barrel interactions are much weaker than interactions within a barrel, and they have long latencies that suggest multisynaptic transmission. In the absence of direct connections between neighboring barrels, the most likely route for interbarrel communication involves projections from layer IV pyramidal neurons to the supragranular layers of neighboring columns (Lubke et al. 2000), where apical dendrites of layer IV pyramidal neurons may respond to these extracolumnar inputs (Schubert et al. 2001).

Anatomical basis for extragranular synchronization

Compared with granular layer IV, the incidence and strength of synchronization were greater for neuron pairs located in the extragranular layers. Furthermore, synchronization was stronger if both neurons were located in neighboring columns of the same barrel row. The anatomical connections most likely to mediate intercolumnar synchronization are the dense horizontal projections that course through the supragranular and infragranular layers (Bernardo et al. 1990a,b; Chagnac-Amitai et al. 1990; Hoefflinger et al. 1995; Zhang and Deschenes 1997). As described in a landmark paper, the pattern of horizontal labeling after a tracer is injected into barrel cortex “is hour-glass shaped with the waist of the hour glass in layer IV” (Bernardo et al. 1990b). Furthermore, these horizontal connections are predominantly aligned along the barrel rows (Bernardo et al. 1990a,b; Fabri and Burton 1991; Hoefflinger et al. 1995; Petersen et al. 2003).

A functional role for these horizontal connections is suggested by a growing body of physiologic data. For example, supra- and infragranular neurons in barrel cortex have larger receptive fields than neurons in layer IV (Simons 1978; Simons et al. 1992) presumably because sensory information is transmitted from one cortical column to its neighbors after being processed in layer IV (Armstrong-James et al. 1992). Consistent with this view, focal stimulation of supra- or infragranular sites within barrel cortex evokes excitatory responses that propagate horizontally into neighboring barrel columns (Laaris and Keller 2002; Schubert et al. 2001). Voltage-sensitive dye recordings have also shown that whisker deflection produces excitatory responses in the supragranular layers that spread preferentially to neighboring columns in the same SI barrel row (Petersen et al. 2003). This horizontal spread of excitation corresponds to the spatial extent of the axonal arbors of supragranular pyramidal neurons, and it supports the view that horizontal projections may mediate intercolumnar interactions.

Intercolumnar synchronization is probably augmented by divergent interlaminar projections. Although most axonal projections from layer IV neurons terminate within the column of the parent barrel, some collaterals project across these vertical boundaries to innervate neighboring cortical columns. For example, star pyramidal neurons in layer IV send dense projections to layers II/III above the barrel, and collaterals from these “fan out” to adjacent cortical columns (Lubke et al. 2000). In addition, spiny stellate neurons in layer IV project to infragranular layers where they give off numerous collaterals, some of which terminate in adjacent cortical columns (Lubke et al. 2000). Finally, vertical projections between the supra- and infragranular layers often have collaterals that terminate in adjacent barrel columns (Bernardo et al. 1990b; Chagnac-Amitai et al. 1990). Hence, collateral projections within barrel cortex represent a potential mechanism for synchronizing neurons in neighboring columns.

Infragranular facilitation of synchronized responses

The major finding in this study concerns the incidence and strength of neuronal synchronization in the infragranular layers. Nearly 85% of all infragranular neuron pairs displayed stimulus-induced synchronization, and correlation coefficients were highest for neuron pairs in the infragranular layers. Infragranular neuron pairs also displayed similar amounts of synchronization at four different stimulus frequencies despite increased adaptation at the higher frequencies (compare Figs. 10 and 11). This suggests that infragranular neurons have unique biophysical and morphologic properties that facilitate their mutual synchronization at the expense of relaying the temporal attributes of the thalamocortical inputs.

Many results suggest that intrinsically bursting (IB) pyramidal neurons contributed to the synchronization that we observed in the infragranular layers. Based on intracellular recordings, IB neurons exhibit bursts of spikes in response to depolarizing current injections, whereas other pyramidal neurons exhibit regular trains of action potentials to identical current injections (Chagnac-Amitai and Connors 1989; McCormick et al. 1985). We did not characterize intracellular responses to current injection and therefore could not distinguish between these two functional groups of pyramidal neurons. Nonetheless, IB neurons probably contributed substantially to the synchronization that we observed in the infragranular layers. First, IB neurons reside primarily in layer V and are not found in most of the other cortical layers (Agmon and Connors 1989; Chagnac-Amitai et al. 1990). Second, IB neurons are large pyramidal neurons, and their response properties suggest that they receive less inhibition than other pyramidal
neurons (Chagnac-Amitai and Connors 1989; Chagnac-Amitai et al. 1990). These characteristics increase the likelihood of recording IB neurons, especially if our electrodes had a bias for recording large, responsive neurons. Consistent with this, spontaneous activity was highest in the infragranular layers, and both individual and population PSTHs indicate that infragranular discharge rates did not return to spontaneous levels when higher stimulation frequencies (8 or 11 Hz) were administered (see Fig. 11). This supports the view that infragranular neurons received less inhibition than neurons in other layers.

In addition, IB neurons have structural properties that should enhance intercolumnar synchronization (Chagnac-Amitai et al. 1990). These neurons have extensive apical and basal dendritic arborizations, and this allows them to sample a large pool of afferent inputs. Furthermore, IB neurons have horizontal projections that extend 1.5–2.0 mm within layers V and VI. These input-output patterns have prompted the suggestion that IB neurons may “amplify and synchronize cortical outputs” (Wilson and McNaughton 1994). In view of the higher levels of spontaneous activity in the infragranular layers, this suggests that IB neurons should act primarily as coincidence detectors. If groups of interconnected IB neurons discharge primarily when they receive synchronous inputs, then synchronous bursts should appear across extensive parts of the infragranular cortex. Consistent with this prediction, synchronized bursts have been reported in the infragranular layers of rodent SI cortex (Erchova et al. 2002) especially when inhibitory influences are reduced (Chagnac-Amitai and Connors 1989).

**Functional significance of infragranular synchronization**

Many single-tracer anatomy studies indicate that infragranular layers in SI project to the neostriatum,pons, spinal cord, and other brain regions that regulate motor activity (McGeorge and Faull 1989; Welker et al. 1988; White and De Amicis 1977; Wise and Jones 1977). In addition, SI cortex projects to primary motor cortex, and most of these projections originate from the infragranular layers (Isaiahi and Porter 1995). Furthermore, our dual anterograde tracing studies indicate that neighboring barrel columns in SI cortex send overlapping projections to the neostriatum and motor cortex, and tracer overlap is highest for projections originating from the same whisker barrel row (Alloway et al. 1999; Hoffer et al. 2003; Leergaard et al. 2000).

The present study indicates that synchronization is greatest for SI neuron pairs residing in the same barrel row. Given that corticostriatal, -pontine, and -cortical projections from the same row of SI barrel cortex overlap more than projections from different rows, synchronized infragranular neurons in the same barrel row should cooperate with each other to increase activation of common postsynaptic targets. Similar effects of synchronization have previously been shown in the thalamocortical projection system (Alonso et al. 1996; Roy and Alloway 2001; Usrey 2002). Therefore our results suggest that infragranular synchronization in SI barrel cortex might represent a mechanism for facilitating information transmission to brain regions involved in somesthesia-guided motor behaviors. Consistent with the row-like movements of the whiskers and the row-like organization of the corticocortical and -fugal projections, increased infragranular synchronization in SI barrel cortex may inform target brain regions when neighboring barrel columns in the same row are activated simultaneously or in a close temporal sequence.

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**References**


