Intracellular Correlates of Fast (>200 Hz) Electrical Oscillations in Rat Somatosensory Cortex

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Jones, Michael S., Kurt D. MacDonald, ByungJu Choi, F. Edward Dudek, and Daniel S. Barth. Intracellular correlates of fast (>200 Hz) electrical oscillations in rat somatosensory cortex. J Neurophysiol 84: 1505–1518, 2000. Oscillatory activity in excess of several hundred hertz has been observed in somatosensory evoked potentials (SEP) recorded in both humans and animals and is attracting increasing interest regarding its role in brain function. Currently, however, little is known about the cellular events underlying these oscillations. The present study employed simultaneous in-vivo intracellular and epipial field-potential recording to investigate the cellular correlates of fast oscillations in rat somatosensory cortex evoked by vibrissae stimulation. Two distinct types of fast oscillations were observed, here termed “fast oscillations” (FO) (200–400 Hz) and “very fast oscillations” (VFO) (400–600 Hz). FO coincided with the earliest slow-wave components of the SEP whereas VFO typically were later and of smaller amplitude. Regular spiking (RS) cells exhibited vibrissa-evoked responses associated with one or both types of fast oscillations and consisted of combinations of spike and/or subthreshold events that, when superimposed across trials, clustered at latencies separated by successive cycles of FO or VFO activity, or a combination of both. Fast spiking (FS) cells responded to vibrissae stimulation with bursts of action potentials that closely approximated the periodicity of the surface VFO. No cells were encountered that produced action potential bursts related to FO activity in an analogous fashion. We propose that fast oscillations define preferred latencies for action potential generation in cortical RS cells, with VFO generated by inhibitory interneurons and FO reflecting both sequential and recurrent activity of stations in the cortical lamina.

INTRODUCTION

Transient somatosensory stimuli produce a series of stereotyped cellular events within the cerebral cortex that are reflected in field-potential recordings as the somatosensory evoked potential (SEP) complex. The SEP waveform consists of a surface positive/negative waveform (P1/N1) beginning 10–15 ms post-stimulus followed by a slower biphasic waveform (P2/N2) of 200–300 ms duration. Because this waveform exhibits a similar morphology across a variety of species (Allison and Hume 1981) and may be recorded both intra- and extracranially, the SEP is one of few signals that may be used to relate noninvasive studies of somatosensory function in humans to detailed information of the underlying physiological processes obtained from animal models. In addition, intracranial measurements using multichannel electrode arrays placed directly on the cortex reveal that the SEP is a focal response with a spatial organization that closely reflects the organization of the somatosensory forebrain (Di and Barth 1991; Di et al. 1989, 1994) and thus provides a means of studying rapid spatiotemporal interactions that occur among large cell populations during cortical interaction processing (Jones and Barth 1997).

Although historically considered a slow wave phenomenon, the SEP exhibits high-frequency (>200 Hz) content that is receiving increasing interest as to its role in cortical information processing. This activity is often apparent as a series of small amplitude deflections superimposed on the underlying slow-wave components of the evoked potential complex that, following high-pass filtering, are seen to correspond to a burst of high-frequency field potential oscillations. These fast oscillations have been observed extracranially in humans (Curio et al. 1994a,b, 1997; Eisen et al. 1984; Emori et al. 1991; Gobbelé et al. 1998; Green et al. 1986; Hashimoto et al. 1996; Maccabee et al. 1983; Yamada et al. 1984, 1988) as well as in intracranial recordings in the awake and in the ketamine-anaesthetized rat (Jones and Barth 1999b; Kandel and Buzsaki 1997).

Using high-resolution epipial mapping of the vibrissa-evoked response in rat vibrissa/barrel cortex, our laboratory determined that fast oscillations triggered by transient displacement of individual vibrissae are somatotopically organized, propagate rapidly within somatosensory cortex, and interact in a manner that suggests that they play a role in precisely timing the arrival of sensory afferent information (Jones and Barth 1999b). Laminar recordings demonstrate that vibrissa-evoked fast oscillations exhibit a dipolar pattern of extracellular field potentials oriented perpendicular to the cortical surface and extending throughout the cortical layers (Jones and Barth 1999b; Kandel and Buzsaki 1997). These high-frequency dendritic currents have been shown to influence the timing of action potentials at least in the infragranular layers, where multi-unit activity in layer V has been correlated with the periodicity of the fast oscillatory response (Kandel and Buzsaki 1997). Recent in-vitro results suggest that small but rapid
fluctuations of intracellular potential may serve as a powerful mechanism for controlling the timing of action potential generation in individual cells with a resolution in the sub-millisecond range (Mainen and Sejnowski 1995). Thus, sensory-evoked fast oscillations may mediate the precise timing of interactions between adjacent neural circuits in somatosensory cortex, providing a mechanism for discriminating slight latency differences in the activation of the somatotopic cortical map established by rapid movement of an object across the receptive surface. However, little is known about the neural generators or functional significance of these sensory-evoked fast oscillations at the cellular level.

In the present study, the cellular correlates of fast oscillations in rat vibrissa/barrel cortex were investigated in detail with combined intracellular and epipial field-potential recording in the intact, ketamine-anesthetized animal. Intracellular responses in rat somatosensory cortex evoked by transient vibrissae stimulation were recorded while field potentials at the cortical surface were simultaneously monitored. This allowed comparison of the cellular response with fast oscillatory components of the SEP extracted from the surface record. Our specific objectives were to determine 1) the types and laminar distribution of cortical cells participating in the oscillatory response, 2) whether extracellular fast oscillations evident within apical pyramidal dendrites are associated with high-frequency intracellular currents recordable at the cell soma and if these rapid transients might therefore serve as a mechanism for precisely controlling spike timing, and 3) whether there are distinct subpopulations of cells producing sensory-evoked trains of action potentials closely associated in frequency, phase, and post-stimulus latency to fast oscillations. Preliminary results of this study were reported in abstract form (Jones and Barth 1999a).

METHODS

Surgical preparation

All procedures were performed in accordance with University of Colorado Institutional Animal Care and Use Committee guidelines for the humane use of laboratory animals in biological research. Male Sprague-Dawley rats (250–350 g) were anesthetized to surgical levels using intramuscular injections of ketamine HCl (100 mg/kg) and xylazine (25 mg/kg) and were secured in a stereotaxic frame. A unilateral craniotomy extending from bregma to lambda and lateral to the temporal bone was performed over the right hemisphere, exposing a wide region of parietotemporal cortex where the dura was reflected. The exposed cortical surface was regularly bathed with physiological saline and body temperature was maintained with a regulated heating pad. Additional ketamine and xylazine were administered as required to maintain a level of anesthesia such that the corneal reflex could barely be elicited. Animals were killed by anesthesia overdose, without regaining consciousness, at the conclusion of the experiment.

Stimulation

The 25 large facial vibrissae of the contralateral (left) mystacial pad were tied together and displaced simultaneously at ~1 cm from their base with a laboratory-built stimulator that converted computer-generated square-wave pulses (duration 0.3 ms) into silent dorsolateral displacements (~0.5 mm) of the stimulator arm.

Surface and laminar recording

Epipial maps of the vibrissa-evoked SEP complex were recorded with a flat multi-channel electrode array consisting of 64 silver wires arranged in an $8 \times 8$ grid (tip diameter 100 μm, inter-electrode spacing 500 μm) covering a $3.5 \times 3.5$ mm area of the cortical surface in a single placement (see Fig. 1). Laminar recordings were performed with a linear array of 24 platinum electrodes (20 μm diameter, 125 μm spacing) (EEG KFT, Budapest, Hungary) inserted perpendicular to the cortical surface in the approximate center of the barrel field until the top electrode was barely visible at the cortical surface. Laminar potentials were preamplified with a unity gain buffer (2 fA input bias current). Both laminar and epipial potentials were referenced to a silver ball electrode secured over the contralateral frontal bone and were simultaneously amplified ($\times$10,000), analog filtered (band-pass cutoff $-6$ dB at 0.001–3000 Hz, roll-off 5 dB/octave), and digitized at 10 KHz. Surface and laminar potentials were recorded following 50–100 presentations of vibrissae stimulation, with data from individual trials stored digitally for subsequent analysis. All filtering used in the analysis was performed digitally and employed a zero-phase shift algorithm that processed the input data in both the forward and backward directions to prevent the introduction of systematic phase distortion. Band-pass filtering was implemented using a second-order Butterworth filter exhibiting a maximally flat passband to avoid frequency-dependent amplitude distortion. The signal-to-noise ratio of the field potential data was ~20, estimated by comparing the variance of evoked oscillatory potentials with that of the prestimulus baseline.

Intracellular recording

Intracellular recording was performed using glass micropipettes pulled from thin-walled aluminosilicate glass on a Flaming-Brown micropipette puller (Sutter Instruments, model P-87) and filled with K$^+$-acetate (3 M). In some instances, micropipettes were beveled to improve penetration into smaller cells. In-vivo impedance of electrodes ranged from 80 to 120 mohm. Recording and current injection was performed with an Axoclamp 2-A amplifier (Axon Instruments) equipped with a 0.1 gain headstage (Axon Instruments, model HS-2A). Exposed cortex in the center of the vibrissa/barrel region was stabilized with a small Kevlar plate containing a small (1 mm) access hole, through which the microelectrode was inserted, that was brought into contact with the pia. A silver wire was permanently mounted at the immediate periphery of the access hole for simultaneous monitoring of the surface SEP. Micropipettes were advanced perpendicularly into the cortex in 0.5-μm increments using a piezo translator equipped with a compensating motor drive (Märzhäuser PM-10) and micrometer that indicated the depth of the electrode tip. Criteria for an acceptable cell impalement were a resting membrane potential of at least −60 mV with overshooting action potentials and satisfactory characterization of the cell using depolarizing current pulses as described by McCormick et al. (1985). Intrinsic characterization was also used to infer the cell morphology, with regular spiking (RS) and intrinsic bursting (IB) cells presumed to be pyramidal or stellate in morphology and fast spiking (FS) cells assumed to be aspiny inhibitory interneurons (Connors and Gutnick 1990; McCormick et al. 1985). When a stable cell impalement was obtained, 50–100 200-ms trials were recorded during vibrissa stimulation (100 ms baseline + 100 ms post-stimulus). Intracellular and surface records were low-pass filtered at 3 KHz ($-6$ dB at 3 KHz, roll-off 5 dB/octave), digitized at 10 KHz, and stored for subsequent analysis. Numerical results are presented as mean ± SE.

RESULTS

Surface and laminar extracellular recording

Figure 1 illustrates a typical cortical response recorded using the 64-channel surface array following transient displacement...
of the vibrissae. The averaged evoked-response recorded with the array consisted of a stereotyped biphasic waveform (Fig. 1B; n = 50), the morphology and distribution of which was similar to that obtained in previous studies (Jones and Barth 1997, 1999b). The SEP was characterized by an early positive/negative response beginning 10–15 ms after stimulus onset (Fig. 1C1); the peaks of these events have been designated P1 and N1, respectively, to indicate their polarity and sequence of occurrence. In addition to these slow waves (here designated SW), higher-frequency content was evident as a series of small deflections superimposed on the underlying SW (Fig. 1C1, arrows). Following high-pass filtering (>200 Hz), these deflections appeared as a burst of high-frequency oscillations accompanying the SW (Fig. 1C2; note difference in amplitude scale). Spectral analysis of the high-pass filtered data consistently demonstrated two discrete spectral peaks (Fig. 1C, left). Averaged spectra suggested that the high-frequency activity accompanying the SW be segregated into two frequency bands, the first including activity between 200 and 400 Hz (here designated as fast oscillations or FO) and a second frequency band including activity between 400 and 600 Hz (here designated as very fast oscillations or VFO). The broadness of these frequency bands reflects the variability of the fast oscillatory activity observed across animals and, to a lesser extent, within a given animal over the recording session. Within a given set of trials, the activity in these bands was almost sinusoidal, as demonstrated by filtering the SW for these two frequencies as shown in Fig. 1, C3 and C4. The relative amplitude and latency demonstrated by fast oscillations in this example was typical, with FO usually larger in amplitude and earlier in latency than the accompanying VFO activity. Although this trend repeatedly appeared in the field potential data, oscillatory bursts were somewhat variable in latency, duration, and amplitude across trials and between animals.

The SEP complex exhibited considerable variability in post-stimulus latency across trials, as revealed by superimposing the SW responses (Fig. 2A, left). This variability made the construction of averages of fast oscillatory activity difficult because it too exhibited intertrial latency variability (Fig. 2A, right), which resulted in the attenuation of stimulus-locked averages (Fig. 2B, right). However, fast oscillations exhibited a consistent latency relationship to the SW, as demonstrated by using the latency of the P1 peak to align the oscillatory activity across trials (Fig. 2C; subsequently referred to as “P1-aligned”). This resulted in a marked improvement of time-locked averages of FO and VFO activity compared with that obtained using the stimulus onset (compare Fig. 2, A and B). As such, P1 alignment was subsequently applied to all extracellular and intracellular responses included in the study.

Figure 3 depicts P1-aligned responses in SW, FO, and VFO frequency bands recorded at the 24 electrodes of the laminar array in six animals (Fig. 3, A, B, and C, respectively; left, individual animal averages; right, grand average across all animals). Only the top 18 electrodes of the array were located within cortical lamina. The surface SW was identifiable at the most superficial electrode (Fig. 3A) and exhibited a complex pattern of polarity reversal in the depth, which was in agreement with previous laminar studies of sensory cortex (Abbes et al. 1991; Bode-Greuel et al. 1987; Di et al. 1990; Sukov and Barth 1998). Depth profiles of fast oscillatory activity were obtained by filtering the wideband laminar data on a channel-
per-channel basis. Although there was substantial overlap of the two types of oscillations when results were superimposed across animals (Fig. 3, B and C, left), the grand average confirmed a general trend of earlier onset of FO activity in the response (Fig. 3, B and C, right). Both FO and VFO exhibited the same polarity reversal across all of their respective oscillatory peaks, with FO reversal occurring at ~900 µm and VFO at ~750 µm (R in Fig. 3, B and C). The depth reversal of FO and VFO indicates that these oscillations were not volume-conducted from extracortical structures but rather that the activity was generated locally within cortical cell populations. The laminar results furthermore indicate that fast oscillations primarily reflect activity within infragranular cells because the depth at which polarity reversal occurs is necessarily located superficial to the somata of cells participating in the response.

Intracellular recording

Stable recordings were obtained from 67 cells in 13 animals. Four cells were classified as fast spiking (FS), five as intrinsically bursting (IB), and the remainder as regular spiking (RS). RS cells exhibited long-lasting (>0.5 ms) spikes and strong spike-frequency adaptation in response to injection of depolarizing current pulses (0.5–1.0 nA; 100 ms). IB cells were distinguished by their response to depolarizing current injections with bursts consisting of two or more action potentials, with spike amplitude within a burst usually successively decreased. Action potentials in IB cells were otherwise indistinguishable from those of RS cells and, in some instances, injection of strong levels of depolarizing current often evoked a constant firing pattern similar to that of an RS cell. FS cells exhibited short-duration (<0.5 ms) action potentials caused by a rapid rate of repolarization. The response of these cells to depolarizing current injection exhibited a wide dynamic range and little or no spike frequency adaptation, with strong stimulation evoking a sustained firing rate in excess of 500 Hz. Representative examples of the responses of the three cell classes to depolarizing current injection are shown in Fig. 4, A–C. Only IB cells exhibited a preferred laminar distribution and were found exclusively in layer V, whereas RS and FS cells were uniformly distributed in the cortical lamina (Fig. 4D). The sample size of IB cells (n = 5) may have been a factor in their limited distribution as these cells have been observed in more superficial lamina in other studies (see, e.g., Contreras et al. 1997).

Excitatory postsynaptic potential (EPSP) onset latency varied according to cell location within the cortical lamina (Fig. 5). Earliest post-stimulus latencies were observed in middle cortical layers, followed by cells in superficial layers, and then in deep lamina. Organized into granular, supragranular, and infragranular responses based on the approximate laminar extent of these cytoarchitectural regions (Fig. 5, g, s, and i, respectively), EPSP latency could be ranked as follows: granular cells, 5.1 ± 0.12 ms; supragranular cells, 6.0 ± 0.20 ms; infragranular cells, 7.6 ± 0.28 ms. These latencies are consistent with the established functional anatomy of the SEP, in which the termination of thalamocortical afferents initiates excitation in middle cortical lamina, which then passes to cells in supragranular and then infragranular layers (Abbes et al. 1991; Di et al. 1990). These results are also in agreement with response latencies obtained in previous in-vivo intracortical (Carvell and Simons 1988; Moore and Nelson 1998; Zhu and Connors 1999) and extracortical (Armstrong-James et al. 1992; Simons 1978) unit studies of barrel cortex.
Spike response

RS CELLS. Almost all RS cells responded to vibrissae stimulation in at least some trials with one or more action potentials. In nine cells, the vibrissa-evoked response remained subthreshold regardless of stimulus amplitude; these cells were not included in the analysis. Similar to FO and VFO activity (Fig. 2), the use of P1 alignment greatly reduced the latency variability of the intracellular data and, furthermore, often revealed FO or VFO periodicity in the response of a given cell. An example of the emergence of FO rhythmicity in the intracellular response is shown in Fig. 6. Although in most trials the cell response consisted of a double spike (Fig. 6A, inset), this was not reflected in the post-stimulus time histogram, which was not strongly bimodal. However, when spike latencies were computed relative to the P1 peak of the accompanying surface response in each trial rather than to the stimulus onset, the cell response was more faithfully reproduced in the resulting histogram, which consisted of two large spike clusters (Fig. 6B). When superimposed over the averaged epipial FO from these

FIG. 3. Laminar distribution of the slow and fast components of the SEP complex. Left: average for each animal. Right: grand averages across all animals was constructed using P1-peak alignment. A: wideband field potentials recorded using a 24-channel laminar electrode (20 μm electrode diameter on 125 μm spacing) inserted normal to the cortical surface at the approximate middle of the barrel field. The P1 and N1 peaks of the surface SEP complex are identifiable at the top electrode position. The observed reversal pattern of the slow-wave response is consistent with the asynchronous activation of supragranular and infragranular pyramidal cells as described in detail in Di et al. (1990) and Sukov and Barth (1998). B: laminar potentials in A filtered for FO activity and plotted at expanded vertical scale. FO exhibited a similar laminar distribution across animals, with oscillations of largest amplitude evident at superficial recording sites and a second, smaller site of activity in the infragranular layers. Oscillations in the two laminar foci were of opposite polarity, as demonstrated by comparing subsequent peaks in the grand average (right, vertical lines). The depth of polarity reversal was approximately 900 μm (R). C: laminar potentials in A filtered for VFO. As in FO activity, VFO exhibited a single polarity reversal in the depth that was consistent across all oscillatory cycles. Polarity reversal for VFO was more superficial than that of FO, with grand averages suggesting a value of ~750 μm.

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FIG. 4. Summary of the laminar distribution of intracellular recordings obtained in the study, organized by intrinsic characterization. Cells were classified as regular spiking (RS), intrinsically bursting (IB), or fast spiking (FS) according to their response to injections of brief depolarizing current pulses. A–C: representative examples of the three types of intrinsic response. D: stable impalements were obtained in 58 RS cells, 5 IB cells, and 4 FS cells. RS cells were encountered in all lamina, FS cells in all but the most superficial and deep locations, and IB cells were located exclusively in layer V. This laminar distribution is similar to that obtained in previous in-vivo intracellular studies of barrel cortex (see Agmon and Connors 1992; Moore and Nelson 1998).
trials (Fig. 6B, gray trace), the histogram spike clusters are seen to be separated by one period of the surface FO activity.

The emergence of fast-rhythmic organization in spike histograms such as that demonstrated in Fig. 6 did not require a multi-spike burst response but rather could arise from variations in a cell’s response that collectively exhibited latency features related to one or both classes of surface oscillations. An example of this behavior is shown in Fig. 7, which depicts sub- and suprathreshold events that were coordinated with FO activity. Vibrissa stimulation evoked either a subthreshold response (Fig. 7B) or two distinct single-spike responses, the first with latency similar to that of the rising phase of the EPSP and the second consisting of a single action potential that was delayed 4.7 ms relative to the earlier spike response (Fig. 7C and D, respectively). Within the subthreshold response, small deflections of membrane potential occurred at the latency of the two spike events (Fig. 7B, arrowheads). This latency difference was similar to the period of the FO activity recorded in these trials and a comparison of the average latency of the two types of spike responses to the accompanying FO shows that they were separated by one cycle of oscillatory activity (Fig. 7A). The evoked response demonstrated in this example was typical of RS cells, in which a consistent and rather stereotyped response occurring in a majority of trials (e.g., Fig. 7C, n = 48/78) was mixed with responses in a small subset of trials exhibiting variations in the timing and/or the number of spikes elicited (Fig. 7B, n = 20/78, and Fig. 7D, n = 10/78). Such response variations did not evolve systematically with repeated stimulus presentations but rather appeared to be randomly distributed across trials.

RS cells also exhibited spike and subthreshold events that were aligned with surface VFO activity. Figure 8 shows an RS cell with spike responses that consisted of either a double spike (Fig. 8D) or a single spike at one of two latencies (Fig. 8B and C). In addition, subthreshold events accompanied each of the three spike responses: a pair of subthreshold deflections that followed the early single-spike response (Fig. 8B, arrowheads), deflections in the membrane potential before and after the late single-spike response (Fig. 8C, arrowheads), and a subthreshold deflection interposed between spikes in the double-spike response (Fig. 8D, arrowheads). The latencies of the subthreshold and spike events were similar across all three responses and occurred on a time scale (2-ms period) similar to that of VFO activity recorded from the cortical surface in these trials. A comparison of the intracellular traces and surface VFO shows that the initiation of the three events in any given response aligns with three successive oscillatory cycles of the accompanying surface VFO (Fig. 8A, arrowheads).

Evoked responses associated with a combination of FO and VFO activity were also observed. Figure 9 depicts data recorded from an RS cell that fired bursts of two or three action potentials in response to vibrissa stimulation (Fig. 9, C and D, respectively). In both conditions, the first two action potentials were separated by 3.2 ms and were coincident with two successive peaks of the surface FO (Fig. 9A, open arrowheads). However, the double-spike response was followed by two subthreshold deflections (Fig. 9C, filled arrowheads) that were

![Figure 5](Expected Image Link)  
**Fig. 5.** Laminar distribution of excitatory postsynaptic potential (EPSP) onset resulting from vibrissa stimulation. Onset was defined as latency at which membrane potential preceding a spike response exceeded the prestimulus baseline by 5% (inset) averaged across all trials. Cells in the granular layer tended to exhibit earliest EPSP onset (g), with intermediate latency typical in supragranular layers (s) and longest latency onset occurring in infragranular lamina (i).

![Figure 6](Expected Image Link)  
**Fig. 6.** Spike latency histograms demonstrating the emergence of fast rhythmic activity following P1 alignment. A: post-stimulus time histogram (PSTH) constructed from intracellular data across all trials (n = 100). Bin width = 0.1 ms. Although in most trials the cellular response consisted of a double spike, this is not reflected in the PSTH, which instead displays three somewhat diffuse peaks. B: same data as in A except spike latencies were computed relative to that of the P1 peak of the accompanying SEP on a trial-per-trial basis rather than to the stimulus onset (note change in abscissa). The resulting spike histogram contained two large peaks that were separated by one cycle of the averaged surface FO activity (gray trace).
of the same period (2.1 ms) as the surface VFO (Fig. 9B, arrowheads). The first subthreshold deflection was aligned with the third action potential of the triple-spike response (Fig. 9D); however, in no trials did an action potential occur at the latency of the second subthreshold deflection.

A summary of the extent to which FO and VFO rhythmicity was observed in regular spiking cells as a population is shown in Fig. 10. Power spectral density of the P1-aligned spike histogram envelope was computed for each cell and normalized power in the FO and VFO bands was plotted according to cell depth and response latency. Approximately half of the RS cells exhibited rhythmicity in one or both frequency bands and cell responses with strong FO or VFO content were found throughout the lamina and at various post-stimulus latencies. However, a greater proportion of cells in granular and supra-granular lamina exhibited response rhythmicity compared with the proportion of cells in the infragranular layer. Several cells in the granular layer exhibited disproportionately large-amplitude FO power that, when used to normalize values obtained in the remaining cells, resulted in the apparent low amplitude of the FO response shown in supragranular and infragranular lamina.

FS CELLS. FS cells exhibited a unique response to vibrissae stimulation (Fig. 11) that consisted of a burst of 2–5 action potentials with an average interspike frequency (508 ± 6.7 Hz) similar to the average frequency of surface VFO (515 ± 2.2 Hz) of the same period (2.1 ms) as the surface VFO (Fig. 9B, arrowheads). The first subthreshold deflection was aligned with the third action potential of the triple-spike response (Fig. 9D); however, in no trials did an action potential occur at the latency of the second subthreshold deflection.

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The latency of FS spike-bursts coincided with that of VFO activity, and action potentials within a given burst tended to align with peaks of the surface response (Fig. 11, gray bars). In two of the four cells, phase alignment of the spike and surface responses was remarkably consistent across all spikes in the burst (Fig. 11, A and B). The response in the remaining two FS cells exhibited a similar phase relationship to the surface activity but also included some spikes early or late in the response that were not aligned with VFO peaks (Fig. 11, C and D).

IB CELLS. The evoked response of intrinsically bursting cells was qualitatively similar to that of FS cells in that it consisted of multi-spike bursts of two or more action potentials (Fig. 12). The response in three of the five IB cells was highly variable (Fig. 12, C–E, gray traces) and, in contrast with the FO- or VFO-related variability demonstrated by RS cells (cf. Figs. 7–9), was not coordinated with surface oscillatory activity. The interspike frequency of the evoked bursts in IB cells was $372 \pm 2.0$ Hz, which was approximately midway between that of FO and VFO, and a comparison of intracellular responses with the accompanying surface data demonstrated that spikes in the response were not aligned with a consistent phase of either type of fast oscillation (Fig. 12, gray bars). Thus the IB response appeared to be unrelated to the fast oscillations present in the surface SEP complex.
DISCUSSION

These results demonstrate that short-latency extracellular slow waves of the SEP complex recorded at the cortical surface are accompanied by two distinct oscillatory bursts with center frequencies of approximately 300 Hz (FO) and 500 Hz (VFO), respectively. Laminar extracellular potentials associated with both FO and VFO reverse polarity, indicating that they are generated by coherent currents within the parallel apical dendrites of cortical pyramidal cells. Intracellular recordings reveal that these high-frequency dendritic currents are sufficient to produce fast potentials at the soma, serving as a mechanism to precisely control the timing of action potentials in RS cells.

FS cells produce distinct bursts of action potentials in response to sensory stimulation that are closely associated in frequency, phase, and post-stimulus latency to VFO. No specific cell population could be identified that exhibited an analogous relationship with FO activity, which suggests that this response may be produced by local circuit interactions.

Physiological interpretation of the evoked-potential complex is based on several key assumptions regarding the neural generation of extracellular field potentials in laminar cortex (Creutzfeldt et al. 1966; Mitzdorf 1985). The optimal cellular geometry for the production of field potentials that can be recorded either extracranially, or intracranially on the cortical surface and across the laminae as considered in the present study, is that of a long, vertically oriented dendritic process with active synaptic sites of limited spatial extent. In populations of cells, when such processes are aligned in parallel and are synchronously activated at approximately the same location along their lengths, the resulting summed extracellular potentials approximate an open field geometry that can be recorded at a distance. The radially and symmetrically organized dendritic processes of most interneurons produce a closed potential field that cannot be recorded with large extracellular surface and laminar electrodes (Llinas and Nicholson 1976). However, the activity of interneurons may be recorded indirectly via their postsynaptic contact with the apical dendrites of pyramidal cells. The open field of synchronized potentials in large populations of apical dendrites typically conforms to that expected from an equivalent current dipole.
defined by complementary source/sink pairs that reflect the
locations of underlying transmembrane currents (Mitzdorf
1985). The extracellular slow waves and fast oscillations re-
corded in the present study must therefore reflect highly syn-
chronized synaptic input impinging on the apical dendrites of
populations of cortical pyramidal cells.

FIG. 12. Stimulus-evoked activity in IB cells. A–E: P1-aligned evoked responses recorded from the 5 intrinsically bursting cells included in the study. Bottom traces: superimposed intracellular responses. Top traces: averaged FO and VFO from same trials. IB cells typically responded to vibrissae stimulation with a burst of 2 or 3 action potentials (black traces); however, there was marked response variability in some of the IB cells (gray traces). The interspike frequency of these bursts was 372 ± 2.2 Hz, approximately midway between the FO and VFO bands. A comparison of the spike response with the averaged surface activity suggests that the IB response was not related to either band of fast oscillatory activity (gray bars). Typical response of these cells to a depolarizing current pulse is shown in Fig. 4B.
Laminar recording indicates that the P1 and N1 slow waves of the SEP are produced by the activation of a vertically oriented current dipole in the supragranular layers followed by the activation of a dipole extending from the cortical surface through the infragranular layers (Fig. 3). This finding replicates previous work that demonstrated that the P1/N1 wave of the vibrissa-evoked SEP is produced by sequential activation of supra- and infragranular pyramidial cells in the barrel field (Di et al. 1990), which reflects a vertical cascade of excitatory activity during the P1/N1 that has been described for evoked responses in the somatosensory (Abbes et al. 1991; Di et al. 1990), auditory (Barth and Di 1990; Sukov and Barth 1998), and visual (Bode-Greuel et al. 1987; Mitzdorf 1987) cortex.

In contrast to slow wave activity, the spatiotemporal characteristics of which suggest the sequential activation of supra- and infragranular pyramidial cells, both FO and VFO exhibit a relatively simple laminar profile characterized by a single reversal point common to all oscillatory peaks that is positioned in middle cortical lamina, which implies that cells in deep cortical lamina dominate the response. Thus laminar data suggest that the presynaptic elements generating fast oscillations are distinct from those responsible for slow wave activity. In addition, both laminar results and surface data suggest that the presynaptic elements generating FO are distinct from those responsible for VFO activity. Not only do the two types of fast oscillations reverse polarity at different depths, but the amplitude and latency differ as well, with FO typically of larger amplitude and earlier in latency than accompanying VFO activity (Figs. 1–3). These characteristics would not be expected if the two types of fast oscillations shared a common neural generator.

**Intracellular correlates of slow and fast extracellular field potentials in RS cells**

The predominant cell type yielding intracellular records in the present study was RS cells distributed throughout the supra- and infragranular layers. Vibrissa stimulation evoked a long-lasting EPSP at the soma, with a delayed onset in the infragranular, as compared with the supragranular, cells (Fig. 5). The latency and duration of these depolarizing slow waves appear to follow the P1 and N1 and probably reflect intracellular correlates of dendritic currents giving rise to the slow wave components of the SEP. The post-stimulus latency, phase, frequency, and relative amplitude of the fast deflections superimposed on slow intracellular EPSPs in RS cells resemble simultaneously recorded extracellular FO and VFO superimposed on the P1/N1 slow waves of the SEP. We propose that these deflections reflect intracellular correlates of fast oscillatory dendritic currents conducted to the soma.

The alignment of action potentials and subthreshold events at common latencies across trials (Figs. 7–9) suggests that, although small in amplitude, fast deflections are effective in bringing a cell to threshold for action potential generation. This observation is consistent with recent in-vitro results that suggest that small but rapid fluctuations of intracellular potential may serve as a mechanism for triggering action potentials in individual cells (Mainen and Sejnowski 1995; Nowak et al. 1997). It is also consistent with previous extracellular unit studies that demonstrate an association between increases in multi-unit activity (MUA) in layer V and fast oscillations (400–600 Hz) that occur during high-voltage population spikes and when evoked by thalamic stimulation (Kandel and Buzsáki 1997). MUA was phase-locked to fast oscillations, which indicates that the rapid synaptic currents reflected in field potential recordings control the timing of action potential generation in populations of pyramidial cells with a precision in the millisecond range. An analogous functional arrangement was demonstrated in the hippocampus, where fast oscillations serve to quantize spike latencies of CA1 pyramidial cells (Buzsáki et al. 1992). Action potential bursts of hippocampal inhibitory interneurons are consistently aligned to peaks of 200 Hz oscillatory bursts in local field potentials whereas their pyramidial cell targets tend to fire single action potentials aligned with oscillatory troughs or not at all. Although the phase alignment of RS cells to fast oscillations demonstrated in the present study is more variable than that reported for hippocampal pyramidial cells, such variability is not unexpected given that the fast oscillatory response was extracted from surface rather than local field-potential data. Nonetheless, in a large portion of the RS cells studied, fast oscillations reflect a series of preferred latencies at which a given cell is likely to fire.

**Neurogenerator of VFO**

The close association of stimulus-evoked activity in fast-spiking cells with the surface VFO suggests that FS cells are the generators of these oscillations. This is supported by three features of the data. 1) The evoked response in fast-spiking cells is coincident with VFO activity. As shown in Fig. 11, FS cells responded to vibrissa stimulation with bursts of 2–6 action potentials at latencies that overlap the post-stimulus latency of the accompanying VFO. 2) The interspike interval of FS bursts was almost identical to the periodicity of the VFO (action potential frequency, 508 ± 6.7 Hz; VFO, 515 ± 2.2 Hz). No other cells in the study were found to exhibit stimulus-evoked spike bursts of this frequency. 3) Action potentials in FS cell bursts exhibited a consistent phase alignment with the surface VFO (Fig. 11, gray bars) and thus with each other, suggesting that as a population their activity would summate coherently in field potentials measured at the cortical surface. The proposed emergence of VFO as a consequence of within-burst synchronization of fast-spiking cells implies the coordination of activity across a large cell population at an extraordinarily fine temporal scale. However, such results are consistent with accumulating evidence that FS cells form a functionally continuous network. Synchronous firing of FS cells to within 1 ms was previously reported in barrel cortex (Swadlow et al. 1998) and is strongest in cells in middle cortical layers that receive monosynaptic input from somatosensory thalamus (Swadlow 1995; Swadlow et al. 1998). Synchronization may be sustained as other lamina become active through a combination of electrical and chemical synaptic interactions. It was recently demonstrated using dual intracellular recording that electrotonic coupling acts to promote the synchronous firing of local (~100 μm) networks of FS cells (Galarreta and Hestrin 1999; Gibson et al. 1999). Application of the gap junction blocker halothane eliminated synchronized activity in these preparations; similarly, we found that the administration of halothane suppresses fast oscillations in the intact brain (M. S. Jones and D. S. Barth, unpublished obser-
tions). Over longer distances, synchronization of FS cells may be chemically mediated. Anatomical (Keller and White 1987) and physiological (Connors et al. 1988; Galanerreta and Hestrin 1999; Gibson et al. 1999) studies have demonstrated that FS cells receive projections from other FS cells; computational models suggest that reciprocal inhibitory connections will result in synchronous firing given a sufficiently narrow spike width (van Vreeswijk et al. 1995). Such reciprocal interactions may achieve synchrony only as a steady-state condition; this may explain why some FS cells recorded in the present study exhibited early (Fig. 11C) or late (Fig. 11D) spikes that were not aligned with the remainder of the response.

It is unlikely that extracellular VFO is produced by dendritic currents within FS cells themselves. It is more likely that it reflects the synaptic activity of the postsynaptic targets of FS cells. FS cells in neocortex have been identified as sparsely spiny or smooth GABAergic inhibitory interneurons (Azouz et al. 1997; Kawaguchi 1993; McCormick et al. 1985). As noted above, interneuron activity cannot contribute directly to extracellular field potentials because of their closed field geometry. The model that emerges from this constraint is that burst firing in FS cells is the source of rhythmic activity in the VFO band and appears in field potentials recorded at the cortical surface by virtue of projections of FS cells onto the apical dendrites of pyramidal cells. The induced synaptic currents in pyramidal cell targets act to phase-lock action potentials, perhaps as a rebound spike following a rapid inhibitory postsynaptic potential (IPSP) or, more directly, as a transient hyperpolarization superimposed on concurrent excitation (cf. Mainen and Sejnowski 1995). This description is admittedly incomplete; for example, it does not account for additional complexities of neocortical circuitry such as the reciprocal inhibitory connections described in the preceding paragraph. However, the proposed circuitry is consistent with many features of the present data, such as the similarity of the spike frequency of burst firing in FS cells and VFO frequency, the VFO rhythmicity observed in the spike responses of putative pyramidal cells, and the somewhat late latency of VFO relative to the evoked-potential complex, which suggests that it is at least disynaptic with respect to thalamic afferents. Experimental confirmation of this model might be possible through the selective destruction of GABAergic neurons, which should result in the disappearance of VFO, although this may be difficult to accomplish without introducing pathological epileptiform activity.

Although intracellular results indicate that VFO-aligned responses occur in cells located throughout the cortical lamina (Fig. 10), the depth profile of VFO activity suggests that it is largely generated by cells in infragranular layers (Fig. 3). The polarity reversal of the oscillations, which is necessarily located superficial to the soma of the cells participating in the response, lies in middle cortical depths (750 μm). At minimum, this demonstrates that the contribution of the supragranular cells is occluded by the infragranular response, if it is present at all. The apparent paradox between the intracellular and extracellular distribution of VFO-related activity may be explained by features of cortical anatomy that favor the contribution of infragranular cells to fast oscillatory field potentials measured at the cortical surface. The apical dendrites of infragranular cells are longer than those of pyramidal cells in more superficial lamina, thus providing a more optimal dendritic geometry for generating large synaptically induced current dipoles. Furthermore, there is greater horizontal convergence of GABAergic projections in infragranular layers (Nicoll et al. 1996; Salin and Prince 1996). Therefore, large-amplitude field potentials in these lamina may be a reflection of increased inhibitory synaptic currents. It is interesting to note that IB cells, although located in infragranular lamina, probably do not participate in the VFO response because this cell type receives few projections from GABAergic interneurons (Nicoll et al. 1996).

**Neurogenerator of FO**

Because no specific cell type or subpopulation could be identified that appeared to be responsible for generating FO activity, it is believed that these oscillations may reflect local interactions among populations of cortical cells. The onset latency of FO activity relative to the evoked potential complex is similar to the “initial fast response” observed in some of the earliest recordings of the SEP (Dempsey and Morison 1943; Perl and Whitlock 1955; see also Arezzo et al. 1986). The initial fast response consists of a series of surface-positive deflections superimposed on the first component of the primary evoked response (P1). Whereas the first of these deflections exhibits very short latency and has been attributed to the discharge of thalamocortical projection fibers, the remainder are thought to reflect the sequential activation of cortical elements following arrival of the afferent volley because they can be elicited using single shocks of the internal capsule (Dempsey and Morison 1943; Landau and Clare 1956; Perl and Whitlock 1955) and because they survive extensive lesioning of ventrobasal thalamus (Morin and Steriade 1981).

Recent studies in monkey somatosensory cortex suggest that the initial fast components of the SEP result from the sequential activation of stellate and then supragranular and infragranular pyramidal cells (Nicholson-Peterson et al. 1995). Although this activation sequence is consistent with the laminar progression of EPSP latencies demonstrated in the present study (Fig. 5), several features of the current data suggest that the generation of FO may not be entirely based on sequential activation of stations in the cortical hierarchy. A principal finding of the present study was that, in at least some cells, action potentials in the vibrissae-evoked response may align with more than one peak of FO activity (Figs. 6, 7, and 9). Distributed over a large cell population, these preferred spike latencies would be passed on to postsynaptic targets and, concurrently, projections onto apical dendrites of neighboring pyramidal cells would contribute to different peaks of the oscillatory field potential measured at the cortical surface. In this manner, a given station in the cortical hierarchy may contribute to different peaks of the surface response. Such neural circuit interactions would not only effectively produce FO in a localized region of somatosensory cortex, but would also explain how these oscillations rapidly propagate through the somatotopic cortical map, as noted in previous epipial recordings (Jones and Barth 1999a).

A second feature of the present results that is apparently at odds with the sequential generation of FO is the laminar distribution of this activity. As shown in Fig. 3, FO was found to exhibit a relatively simple reversal pattern dominated by activity in the infragranular layers. Sequential activation would...
be expected to lead to a more complex laminar profile similar to that of slow wave activity (Abbes et al. 1991; Bode-Greuel et al. 1987; Di et al. 1990; Sukov and Barth 1998). Although features of the postsynaptic elements participating in the response may account for this discrepancy, it should be noted that because multi-vibrissae stimulation was used, the evoked response includes both thalamically mediated input from the principal whisker and intracortically propagated afferents from surrounding vibrissae, including both slow wave and fast oscillatory activity (Jones and Barth 1999a). As such, any hierarchical activation resulting from the principal response may be poorly reflected in the laminar profiles of Fig. 3. Whole-field stimulation was employed in the present study because it results in large-amplitude fast oscillations that exhibit a relatively consistent morphology across trials; however it likely elicits a disparate response pattern compared with that evoked by displacement of a single vibrissa. The alterations in extracellular and unit responses that occur when using single-vibrissa stimulation will be the subject of a subsequent study.

Conclusions

Fast oscillations presented in the present study, and the intracellular events associated with them, extend the classical picture of cellular processes underlying the evoked potential complex. An incoming thalamocortical volley may not result merely in a temporally fused slow depolarization in middle laminar pyramidal cells but, at least in some cells, may also establish preferred latencies of action potential generation at the FO frequency. The sequential and recurrent propagation of action potentials at these latencies throughout the pyramidal cell population may explain the persistence of FO activity beyond the PI peak well after thalamic afferents are waning. Such a model would account for the oscillatory variations in MUA at FO frequencies observed in infragranular layers (Kandel and Buzsaki 1997) and in the intracortical propagation of fast oscillations following displacement of individual vibrissae (Jones and Barth 1999b). Furthermore, intracellular correlates of surface VFO activity suggest that the role of inhibitory interneurons may be more complex than is suggested by the accepted roles of receptive field modification or excitability suppression in that their burst activity may provide a mechanism of high-resolution spike timing that can act in isolation (Fig. 8) or in concert with the slower FO activity (Fig. 9). Although these events are an order of magnitude faster than current established models of synchronized cortical information processing (Bullock 1992), such time scales are directly relevant to recent findings that the cortical response arising from paired-vibrissae stimulation is sensitive to interstimulus interval changes at the millisecond level (Jones and Barth 1999b; Shimegi et al. 1999).

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REFERENCES


