Mechanisms Underlying Somatosensory Cortical Dynamics: II. *In vitro* Studies

Chang-Joong Lee, Barry L. Whitsel, and Mark Tommerdahl

Department of Physiology, The University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599-7545

The response of the sensorimotor cortical slice to repetitive, single-site afferent drive is mapped using both evoked potential and metabolic mapping [2-deoxyglucose (2DG)] methods. Systematic changes (increases or decreases) in the evoked potential occur during repetitive 3–5 Hz stimulation. These resemble the changes in SI neuron response observed in the *in vivo* studies of the preceding companion article; they occur rapidly, recover within 1 min and are reproducible if stimulus parameters remain unchanged. Place, timing, and intensity of repetitive stimulation influence the amplitude and form of the response alterations observed at a given cortical locus. The neuron populations that exhibit different response modifications to the same repetitive stimulus are distributed nonrandomly in the slice: neurons occupying column-shaped aggregates undergo a common response alteration (either an increase or decrease) during repetitive stimulation, with sharp boundaries separating neighboring aggregates distinguishable on the basis of their dynamic behaviors. The distribution of stimulus-evoked 2DG uptake in the slice is "columnar," the dimensions of the 2DG columns corresponding to those mapped with neurophysiological methods.

Taken together, the findings support the concept that repetitive stimulation causes the intrinsic network of somatosensory cortex to modify dynamically the network's response to extrinsic excitatory drive so that the local differences in the pattern of extrinsic excitatory drive to neighboring cortical columns are enhanced.

The *in vivo* experiments summarized in the preceding companion article (Lee and Whitsel, 1992) used a preparation that retains the natural state of the cortical network, thus permitting study of the effects of moving tactile stimuli resembling those the network must process during everyday life. This advantage is considerable, but the limitations of *in vivo* experimentation are also significant. In particular, studies of the SI cortex in intact subjects do not allow direct experimental evaluation of the principal idea advanced in the preceding article (Lee and Whitsel, 1992), that stimulus-evoked dynamic pericolumnar interactions underlie the cortical changes that occur during repetitive afferent drive.

Significant practical advantages make the cortical slice preparation useful for the study of a number of important issues. These include easy accessibility for the placement of stimulating or recording electrodes, and precise control of the spatiotemporal parameters of stimulation (Dingledine, 1984; Ried et al., 1988). In addition, specific neuropharmacological manipulations with the slice preparation are not only feasible, but can be done with relative ease. These advantages have allowed the slice to be used effectively to obtain observations about local mechanisms operative in networks located at a wide variety of levels of the CNS. While studies of the slice have contributed significantly to current views of cortical neuron membrane properties, local circuits, and the characteristics of cortical neurotransmission (Connors et al., 1982; Harrison and Simmonds, 1985; McCormick, 1989; Konnerth, 1990), few workers have used the slice to investigate the mechanisms of cortical information processing, neuronal feature extraction, or the dynamics of the sensory neocortical response to input drive. This situation appears to be attributable to the widely held assumption that investigation of the underlying mechanisms requires the use of "natural" stimuli. The process of slice preparation, of course, makes this requirement difficult if not impossible to meet.

Three sets of experiments utilizing the cortical slice are described in this article. The first set sought to ascertain if the cortical slice retains those mechanisms that in the intact somatosensory cortex enable repetitive stimulus-evoked input drive to sculpture (via dynamic pericolumnar interactions) a global activity pattern made up of multiple, column-shaped neuronal aggregates. This issue was approached by map-

Cerebral Cortex Mar/Apr 1992;2:107–133; 1047-3211/92/$4.00

ping (in both the radial and tangential dimensions) the response of the slice to precisely controlled input drive provided at a single site in the cortical white matter. In the second type of experiments, the effects of the prior history of input drive on the slice's response to single-site input drive were evaluated. Finally, in a third experimental series, it was determined if, and to what extent, preexposure to brief periods of repetitive input drive can modify the global spatial activity pattern evoked in the slice by single site stimulation. To this end, the spatiotemporal patterns of response of sensorimotor cortical slices to precisely controlled trains of afferent drive were mapped using several different, complementary, experimental strategies that employed the methods of evoked potential recording and 2-deoxyglucose (2DG) metabolic mapping.

The results reported have been described previously in preliminary communications (Lee et al., 1990; Tommerdahl et al., 1991).

Materials and Methods

Cortical Slice Preparation

Sensorimotor cortical slices were prepared using young, fully developed rats (Sprague–Dawley, 150–250 gm) of either sex. Subsequent to decapitation, the dura overlying the sensorimotor cortex was reflected, and a large block of cortex and underlying white matter was rapidly removed. After washing the block in ice-cold artificial cerebrospinal fluid (ACSF; in mM: 118 NaCl, 4.8 KCl, 2.5 $CaCl_2$, 1.2 $MgSO_4$, 25 $NaHCO_3$, 1.2 KH_2PO_4, 10 glucose, pH 7.4), a smaller block of cortex and underlying white matter (corresponding to sensorimotor cortex) was dissected and transferred to a bath of cold, oxygenated ACSF. From this smaller block, 500 μm coronal slices (usually four to six slices from each hemisphere) were cut using a tissue slicer and placed in a temperature-controlled equilibration chamber. The entire procedure from decapitation to placement of the slices in the equilibration chamber took no more than 15 min (Fig. 1*A–C*). Slices prepared in this way and maintained in 35°C oxygenated ACSF yielded stable neural responses to input drive and demonstrated levels of glucose uptake comparable to those obtainable from healthy, intact animals. An interface-type chamber (Kelso et al., 1983; Fig. 1*D*) was used to obtain neurophysiological recordings and metabolic mapping data. This arrangement allowed fresh, oxygenated, and pH-controlled ACSF to flow continuously over the inferior surface of the slice. The perfusion rate of the chamber was approximately 1 ml/min; its temperature was maintained constant at 35°C by means of a servo-controlled temperature controller. The chamber consisted of (1) a central inner tissue bath with a nylon net to support the slice, a thermocouple, and an Ag-AgCl ground wire; and (2) an outer bath in which ACSF was bubbled with a 5% CO_2, 95% O_2 gas mixture and maintained at constant temperature and pH. Flow of oxygenated and pH-controlled ACSF between a remote reservoir and the inner bath occurred via tubing that first passed through the outer chamber. In this way, the ACSF was warmed immediately prior to accessing the inner bath. The fluid level in the inner bath was held constant by an overflow port.

Electrophysiological Recording

Slices were submerged and allowed to equilibrate in a constant-temperature, ACSF-filled chamber for at least 1 hr prior to recordings. In all but the earliest studies, constant-current, square-wave stimuli (AMPI Master-8 constant current stimulator and isolation transformer) were used. Stimuli were applied using a concentric (diameter, 200 μm) bipolar stimulating electrode; stimulus duration was 200 μsec; frequencies ranged between 0.1 and 5 Hz. Stimulus strength was one to two times the threshold for the appearance of an evoked postsynaptic response in the part of layer IV immediately overlying the stimulating electrode. Cortical evoked potential recordings were made using glass micropipettes filled with 1 M NaCl; impedance was 1–2 MΩ at 20 KHz. Evoked potentials generally were uncontaminated with unitary discharges; recording system band pass was 0.6–600 Hz. Evoked responses and times of occurrence of stimulus events were stored on magnetic tape using a Vetter model 4000A PLM recording adaptor and a standard VHS VCR. Acquisition, display, and analysis of the evoked potential recordings were carried out using a PDP 11/73 computer system. Analog waveforms were digitized at 12-bit resolution at a rate of 20 KHz for 60 msec following stimulus onset and stored on disk. Software allowed the data for individual stimulus presentations to be displayed and analyzed, and permitted averaging of the data collected over series of stimulus presentations. A variety of measurements could be extracted from the data for any time epoch after stimulus onset; these measurements included peak response amplitude (maximum voltage obtained during an identified time epoch) for a single trial or series of trials, response latency (delay between stimulus onset and peak response amplitude), and average integrated response amplitude (absolute value of the area between baseline and the half- or full-wave-rectified evoked response within a defined time epoch) for the responses to selected stimulus presentations. For the purposes of this report, plots of the amplitude of particular evoked response components against stimulus repetition number ("trend" plots) proved particularly useful. Twenty-five slices provided the neurophysiological data.

2-Deoxyglucose Metabolic Mapping

For 2DG metabolic mapping, a tracer amount of ^{14}C-2DG ($1–3 \times 10^{-6}$ M) was introduced into the chamber through the reservoir. At the end of the time period allowed for stimulus-evoked 2DG uptake (typically 15 min), stimulation was terminated and the slice was rapidly (1 min) cleared of the remaining extracellular 2DG by perfusing with 2DG-free ACSF. Following exposure to ^{14}C-2DG, the slice was removed from the chamber with a fine brush and placed on a clean glass slide. M1 freezing medium was applied to the ex-

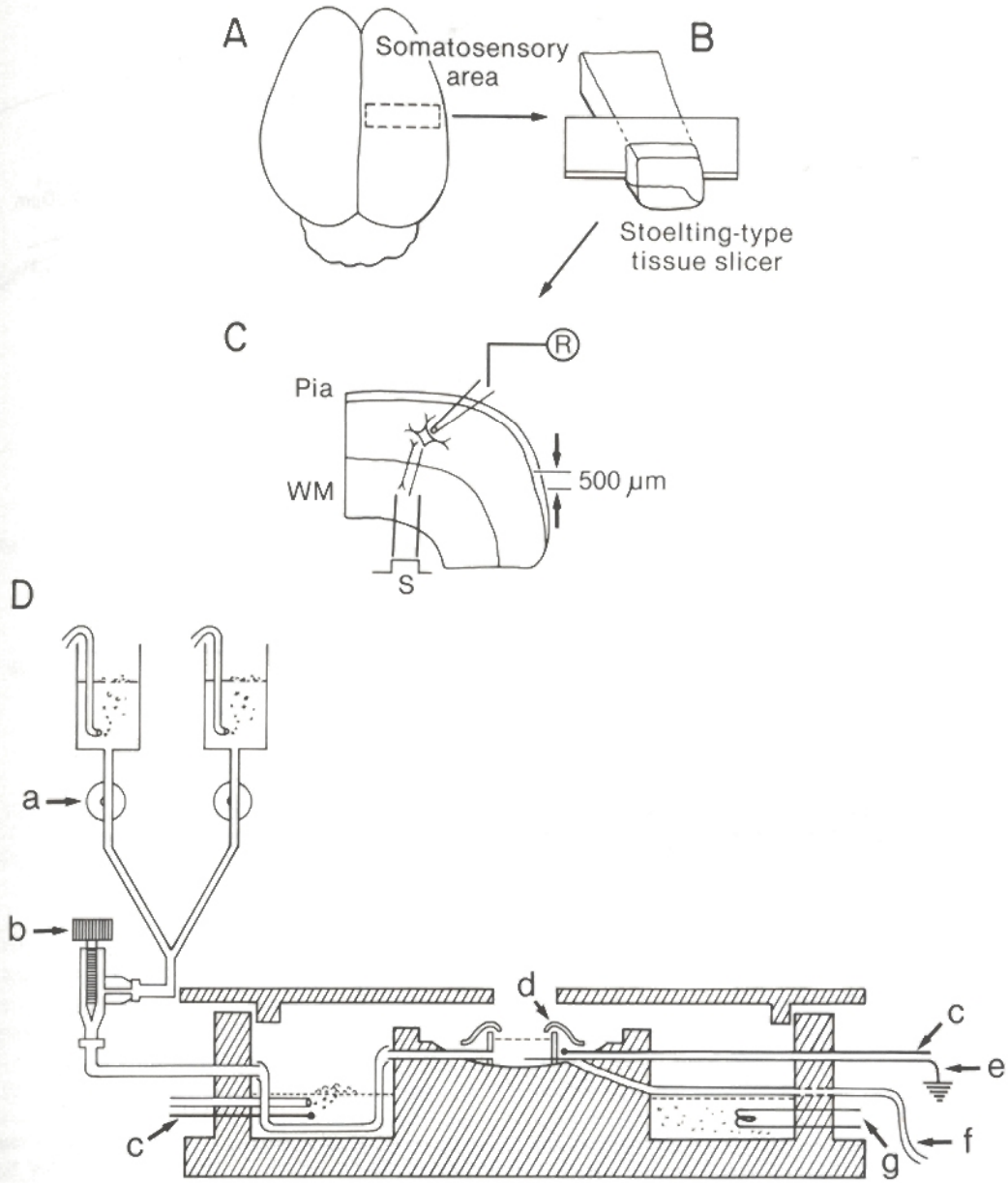

Figure 1. Preparation and utilization of sensorimotor cortical slice. *A,* Top view of rat brain. The *broken rectangle* indicates region of sensorimotor cortex selected for preparation of slices. *B,* Coronal sectioning of cortex using Stoelting-type slicer. *C,* Cortical slice and experimental setup. Horizontal length, 1 cm; vertical height (from white matter to pia), 1.5 mm; thickness, 0.5 mm. *S,* stimulus applied to white matter (*WM*); *R,* recording system connected to electrolyte-filled pipette. *D,* Recording chamber and perfusion system. Reservoir bottles (50 ml) containing oxygenated perfusion solution and solutions containing drugs or tracer used in metabolic labeling experiments are at *upper left*; *a,* on/off valve; *b,* adjustable flow valve; *c,* thermocouple; *d,* paper wick; *e,* ground wire; *f,* overflow port; *g,* heating coil.

posed surface of the slice; a rubber gasket (thickness, 0.5 cm) placed around the slice confined the M1 medium to the immediate vicinity of the slice. The embedded slice, gasket, and slide were then slowly immersed in Freon 22 (temperature, −45°C). The frozen cylindrical pellet of M1 medium (containing the slice) created by this procedure permitted accurate alignment of the surface of the slice for the cutting of serial 20-μm sections using a cryostat with a rotatable chuck.

Autoradiographic film images of the distribution of ^{14}C-2DG labeling in the sections were prepared following standard protocols (Sokoloff et al., 1977). The film images were digitized and analyzed, and the spatial pattern of ^{14}C labeling reconstructed and quantified using a custom-designed image analysis system (Tommerdahl et al., 1985; Tommerdahl, 1989). Individual autoradiographs were transilluminated using a homogeneous light source, and viewed with a macroscope. A Fairchild CCD camera mounted on the macroscope generated a standard RS-170 video image of the autoradiograph, and a flash A/D converter (on a Datacube imaging board interfaced to a PDP 11/73) digitized the image at 8-bit resolution at a rate of 30 frames/sec. Custom software allowed the images to be stored, displayed (in pseudocolor), enhanced, and analyzed. Reconstructions of the spatial distri-

slices, response amplitude fell off more rapidly on one side of the stimulating electrode than on the other (Fig. 3), and more rarely, the maximum response was detected at a location to one side or the other of the stimulating electrode. Variations in the spatial pattern and locus of the maximal cortical response to single site stimulation were anticipated given the likelihood of regional variations in slice integrity, and of regional differences in the status of the intrinsic GABAergic inhibitory neurotransmitter system known to shape and delimit the responding neuronal population in the slice (Shaw and Teyler, 1982; Chagnac-Amati and Connors, 1988; see also S. M. Lee et al., 1991). Apart from such experiment-to-experiment variations in the shape and spatial distribution (relative to the stimulating electrode) of the responding cortical neuron population, the observations obtained in these studies replicate those of others (Lee, 1982; Shaw and Teyler, 1982), and are presented to illustrate that in our hands the sensorimotor cortical slice retains essential features of the organization determined in *in vivo* studies. Most importantly, it retains the capacity to respond to afferent drive in a manner that (1) is constrained by the cortical intrinsic inhibitory network and (2) resembles the "columnar" response patterns observed in studies of the intact cortex.

The Behavior of the Evoked Potential during Repetitive Drive

In the next experiments, we sought to determine whether systematic, stimulus-evoked changes in neuronal response could be detected in the sensorimotor slice preparation. Such changes were anticipated on the basis of the finding (Lee and Whitsel, 1992) that repetitive brushing stimuli frequently lead individual SI neurons and neuron groups to modify their response to repetitive afferent drive. In our *in vivo* studies, almost one-third (32%) of the SI neurons sampled showed increased activity in response to repetitive stimulation of their receptive fields, 10% showed no significant change, and the majority (58%) showed decreased responsivity.

The results revealed systematic changes in the cortical response to repetitive 3–5 Hz stimulation; moreover, these changes were comparable in many respects to those we had observed in our *in vivo* studies. Figure 4, *A–C* illustrates three representative patterns of response change observed in the slice with repetitive (3–5 Hz), single site stimulation. In each example provided in Figure 4, postsynaptic response amplitude is plotted as a function of stimulus number (in the manner of the "trend" plots of the preceding article). In addition, the plots are normalized to facilitate direct comparison. For the examples shown in Figure 4, *A* and *B*, response magnitude decreases with stimulation, but with a different time course. In Figure 4*C*, response magnitude increases with stimulus repetition.

In the experiments summarized in Figure 4, as in all the experiments described in this article, a specific component of the evoked response was preferentially affected by repeated stimulation. This becomes evident upon comparison of the averaged responses to the early and late stimuli of each run, shown in Figure 4*A*, right; for these responses, postsynaptic responses clearly are evident in the period 4–14 msec after stimulus onset for the first two stimuli, but with repetitive stimulation they become greatly attenuated or disappear completely. This time course is apparent in the trend plot in Figure 4*A*, left: the change in postsynaptic response magnitude occurred very rapidly, reaching a steady state after only three stimuli. In addition, the response recovered completely within 30 sec of the cessation of repetitive stimulation (not shown). In contrast, for the recording data summarized in Figure 4*B* there was a much slower decrease in response—a steady state was not reached even after the delivery of 150 stimuli. Again, however, it was the long-latency response components that were selectively and reversibly altered by repetitive stimulation. Of some interest is the fact that the late postsynaptic evoked potential components increased in latency as well as decreased in amplitude with repetitive stimulation, while the early postsynaptic components of the response underwent only slight modifications in amplitude or latency. For the recording data summarized in Figure 4*C*, there occurred a gradual increase in postsynaptic response magnitude over the first 50 stimuli, and thereafter a steady state was attained. As in Figure 4, *A* and *B*, it was the late component of the postsynaptic response that underwent modification. Across all experiments of the type illustrated in Figure 4, 59% of recording sites (35 sites) showed decreases in response magnitude, 22% (13 sites) showed increases, and 19% (11 sites) showed no change to 5 Hz stimuli. All changes were short-lasting, all recovered within 3 min after the cessation of repetitive stimulation, and in no case was there any change in the size of the presynaptic response components.

Effects of Stimulus Frequency and Intensity

The results described above indicate that in the cortical slice 5 Hz repetitive input drive frequently is accompanied by diverse changes in the magnitude of the postsynaptic neuronal population response, and shows that these changes disappear rapidly following the termination of stimulation. These results led us to attempt in subsequent experiments to identify the parameter(s) of input drive that influence the changes in postsynaptic response magnitude. Two previous observations suggested that stimulus frequency is of primary importance: first, in the *in vivo* studies of the preceding article, Lee and Whitsel (1992) showed that changes in single neuron response magnitude were more prominent when interstimulus interval was short, and second, similar results were reported in postsynaptic evoked potential studies of human subjects (e.g., Angel et al., 1985; Delberghe et al., 1990). With these observations in mind, a study of the effects of stimulus frequency on response magnitude was carried out in the cortical slice.

Figure 5 shows change in the postsynaptic re-

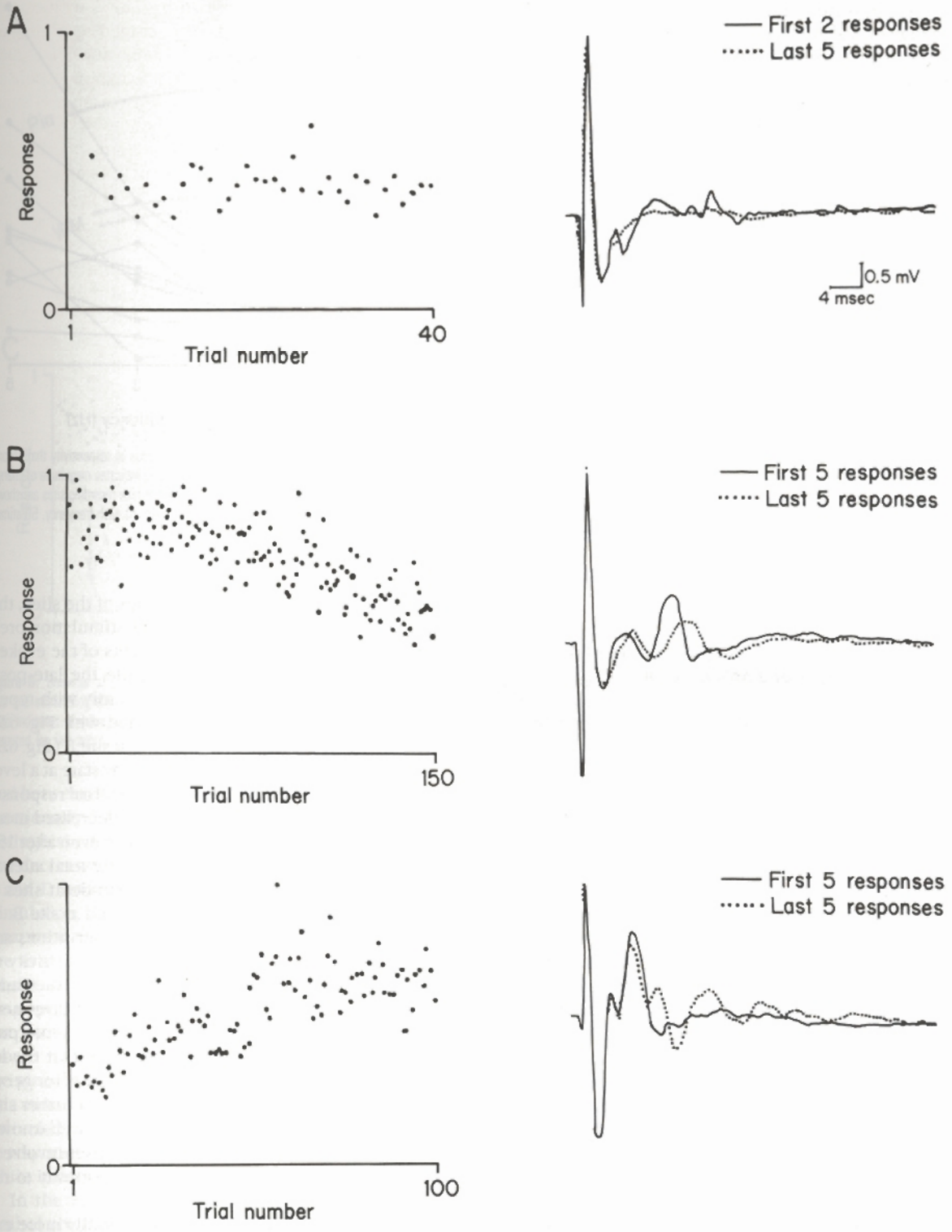

Figure 4. Changes in postsynaptic response during repetitive stimulation. Data from three recording sites (*A–C*) from different sites are presented. *Left column,* Magnitude of response to each stimulus plotted as a function of stimulus number. Response amplitude within a given poststimulus time window was obtained by measuring the area between the baseline and the response after half-wave rectification. *A,* response amplitude for poststimulus window between 5 and 10 msec; *B,* between 7 and 12 msec; and *C,* between 8 and 15 msec. *Right column,* Averaged evoked responses obtained from slice providing data for trend plot shown at *left* in same row. *A,* Comparison of average response to the first two (*solid line*) and last five stimuli (*broken line*). *B* and *C,* Comparison of average evoked response to the first five and last five stimuli.

sponse (normalized) as a function of stimulus frequency for nine different recording sites studied in three different slices. Although not shown in this figure, the early presynaptic components of the evoked potential were insensitive to the range of frequencies of input drive used (see Fig. 4). Percentage change in the evoked postsynaptic response with stimulus repetition was measured using a two-step procedure:

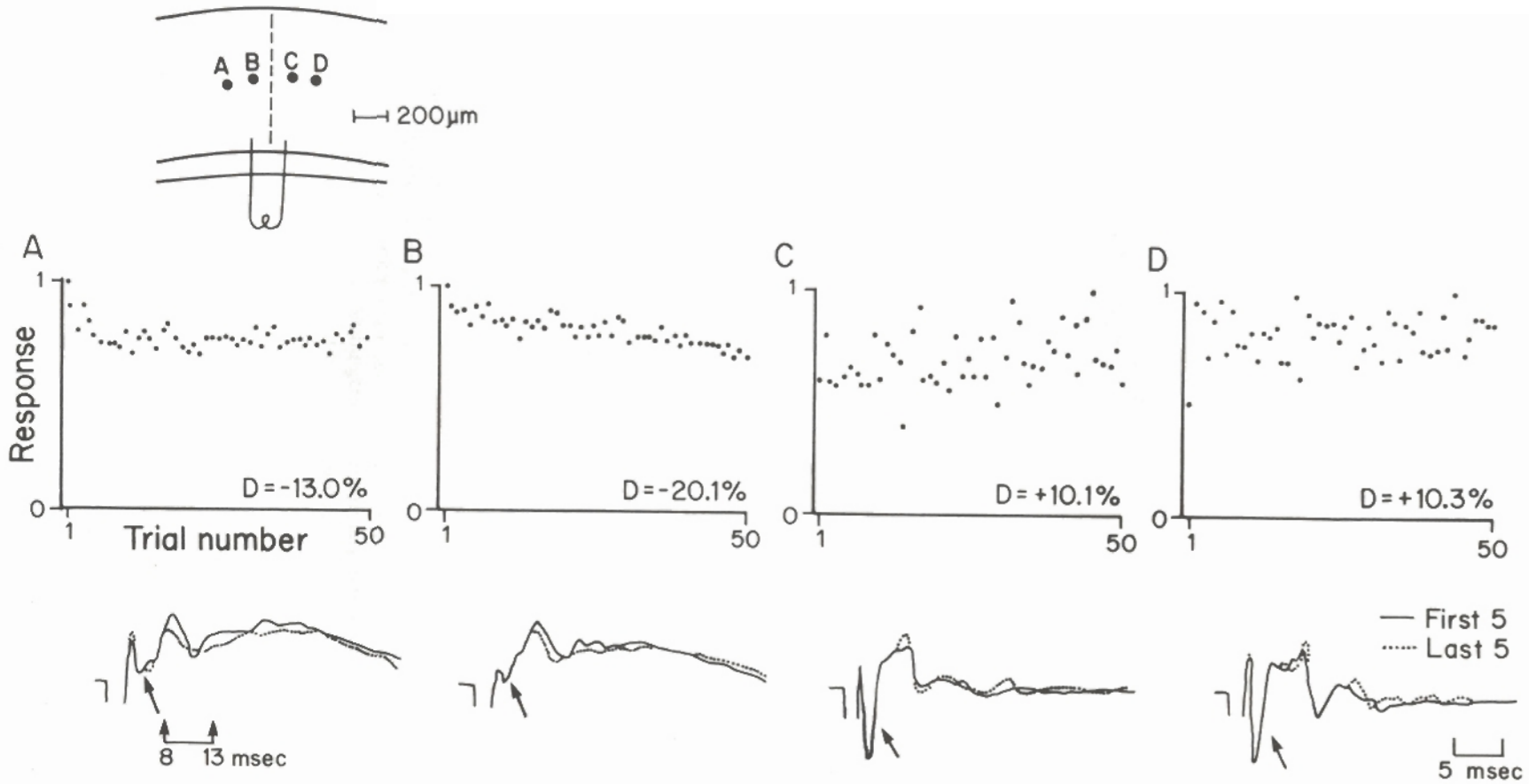

Figure 7. Behavior of evoked response at four different sites in "different columns" during 5 Hz stimulation. Locations of recording sites and stimulating electrode are shown at the *top*. *A–D, top row,* show changes in response are plotted as function of stimulus number. *X-axis* indicates stimulus number; *y-axis* indicates average magnitude (normalized) of postsynaptic response observed 8–13 msec after stimulus onset. *Bottom row,* For each site, averaged evoked response computed for two sets of five successive stimuli (average of first five trials, *solid line*; last five trials, *broken line*). The *arrows* indicate afferent volley. The *broken line* in the slice diagram at the *top* indicates the existence of a functional boundary separating neural populations exhibiting different dynamic behaviors to the same repetitive stimulus.

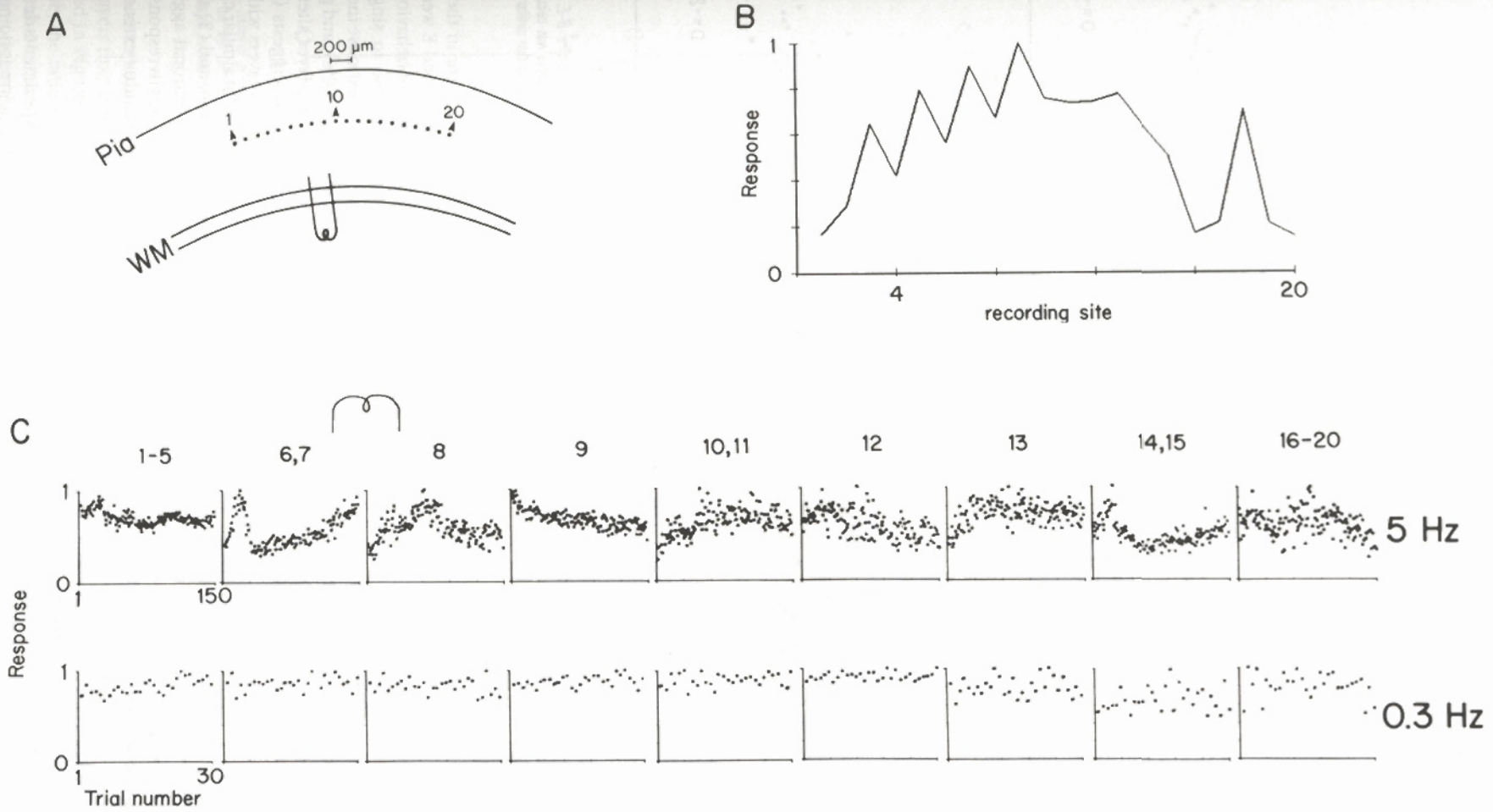

Figure 8. Behavior of evoked response in same slice at an extensive tangential array of sites. *A*, Locations of recording sites and stimulating electrode. Distance between neighboring recording sites was 100 μm. *B*, Relative strength of the unadapted postsynaptic response at each site; postsynaptic response between 6 and 11 msec was measured. *C*. *Bottom*, repetitive stimulation at 0.3 Hz did not lead to changes in response at any site. Format of plots is identical to that used in the previous figures. *Top*, At 5 Hz, response magnitude changes at most sites, and exhibits different time courses and patterns of changes at the different sites.

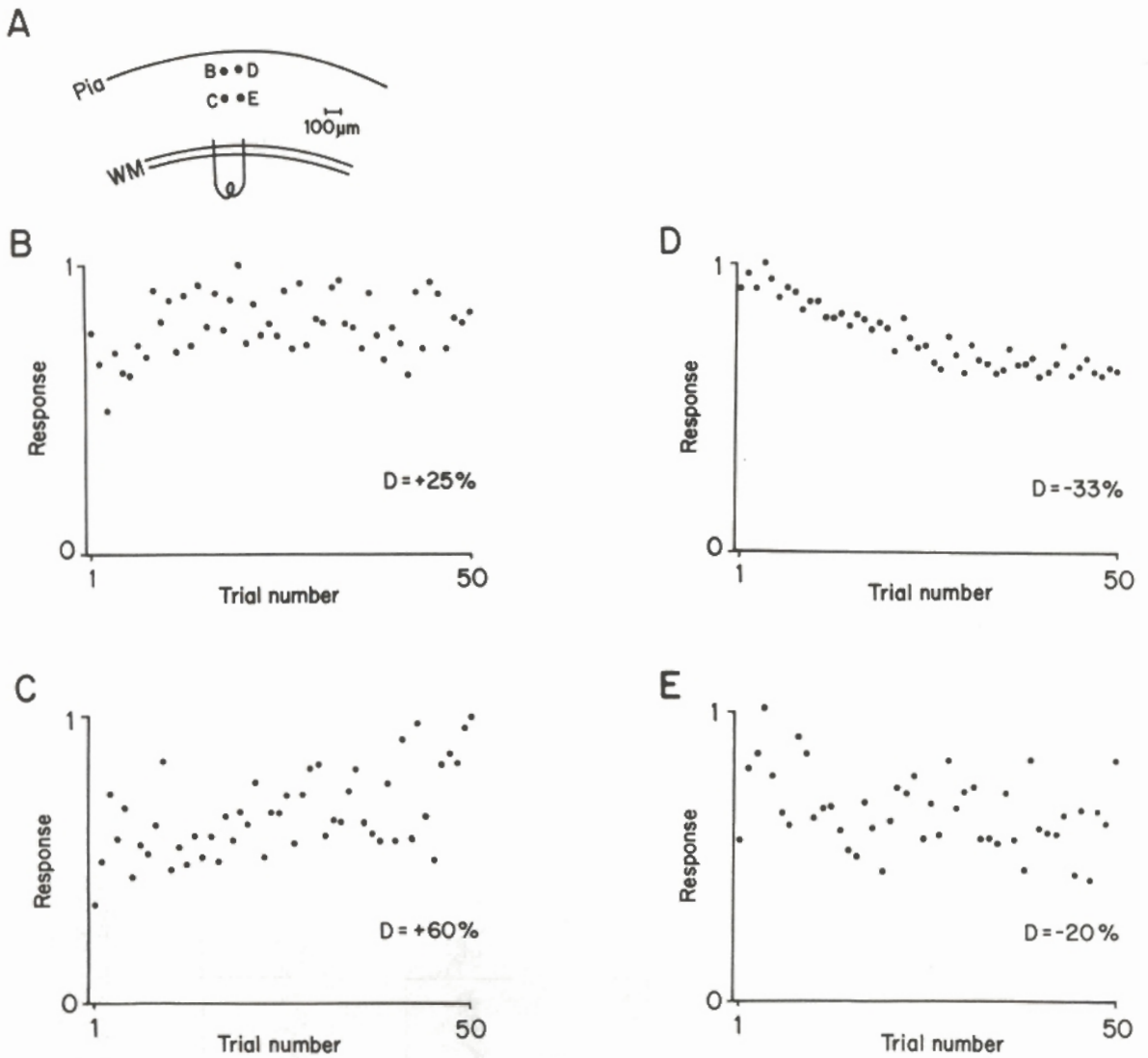

Figure 9. Behavior of evoked response at different vertical and horizontal locations in the same slice. *A*, Arrangement of recording sites and stimulating site. *B–E*, Changes in response as a function of stimulus number for the four recording sites indicated in *A*. Postsynaptic activity was measured between 5 and 10 msec. D-value was calculated as in Materials and Methods. Note that opposite tendencies for responsivity to change with repetitive drive are exhibited by sites in different radial sectors, and that similar tendencies are apparent for sites within the same radial sector.

magnitude with repetitive input drive ranged from 100 μm to 500 μm.

Since observations such as those shown in Figure 8 imply the existence of sharp functional boundaries separating aggregates exhibiting different dynamic behaviors to the same stimulus, in the two additional experiments we sought to determine if such boundaries define "columns" in the slice; that is, do the boundaries separating cortical neuronal populations exhibiting different dynamics in response to the same repetitive stimulus extend continuously from the middle layers to the upper layers? Figure 9 shows representative data bearing on this question. In this study, the changes in response evoked by repetitive drive were studied at an array of four different recording sites in the same slice using the same repetitive stimulus. As shown in Figure 9*A*, recording sites B and C were at one tangential position, while sites D and E were at another (the members of each pair of recording sites were separated in the tangential plane by 100 μm); sites B and D were in the upper layers (upper layer III), and sites C and E were both in the middle layer (IV). Two different behaviors were evoked at the four sites in response to single site repetitive stimulation: at site C the response increased (by 60%), at site E it decreased (by 20%), and parallel changes occurred at the two upper-layer sites (sites B and D, respectively). This result suggests (1) that both the upper and middle cortical layers within the same radial sector of the slice exhibit similar changes in response to repeated stimulation, and (2) that the functional boundary separating neuronal aggregates that respond with opposing changes in response magnitude to the same repetitive stimulus extends radially across cortical layers.

Layer Dependency

A consistent finding of all slice experiments described so far is that the decreases in postsynaptic neuronal response evoked by repetitive drive are larger, take

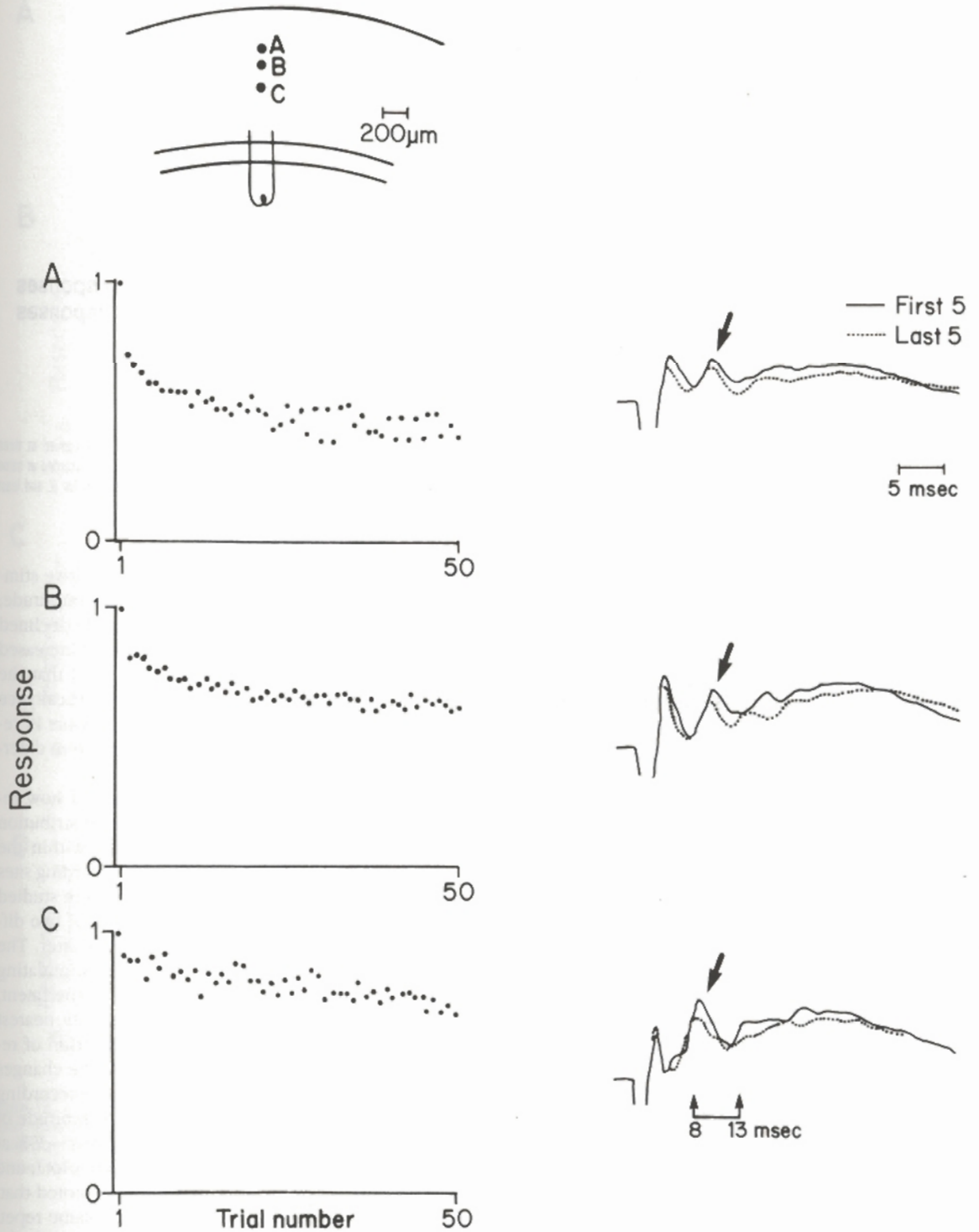

Figure 10. Behavior of evoked response at different vertical locations in same slice during 5 Hz stimulation. Schematic arrangement of recording sites and stimulating electrode is shown at the *top. Left column,* Changes in response magnitude observed at three (*A–C*) recording sites; postsynaptic activity between 8 and 13 msec was measured. *Right column,* Averaged evoked response computed for first five (*solid traces*) and for last five (*broken traces*) stimuli at each site.

place more rapidly, and occur more frequently in the upper layers than in the middle layers (Figs. 6, 9). As indicated in Figure 2, the magnitude of postsynaptic response, the ratio of postsynaptic to presynaptic response, and latency to peak postsynaptic response to white matter stimulation all change systematically with cortical layer. Figure 10 shows an example of a type of experiment (six were carried out) in which we attempted to ascertain the changes in the postsynaptic response in the different layers in the same cortical column during repetitive afferent drive. Three recording sites located in the same column were studied using the same repetitive single site stimulus (see diagram at top). The responses recorded at the dif-

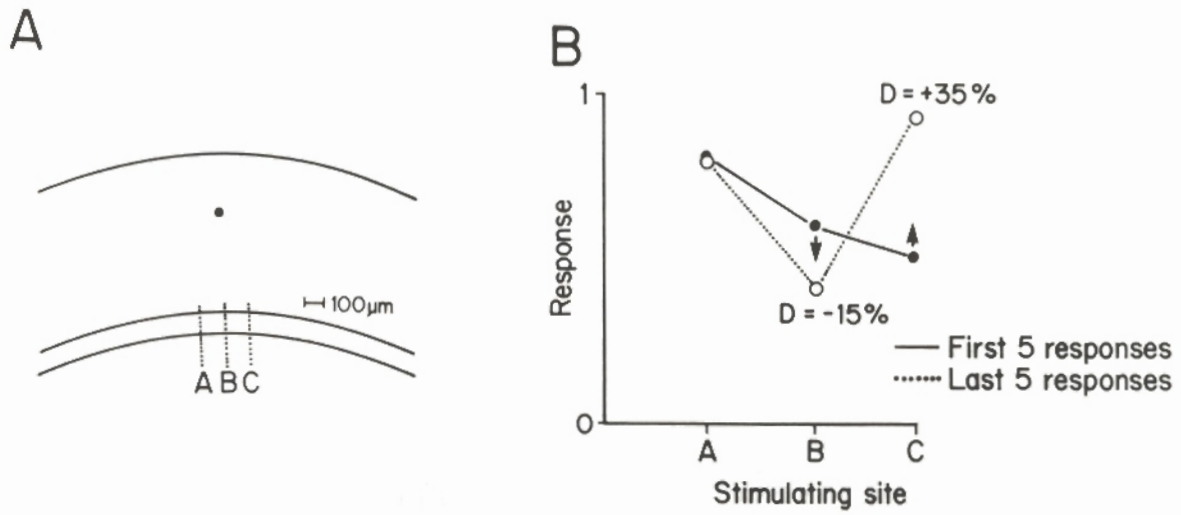

Figure 11. *A*, Arrangement of recording site and sites of stimulating electrode. All recordings were obtained at same site (layer III); repetitive stimuli were applied at three different sites (*A, B,* and *C*) in layer VI (electrode separation, 100 μm). *B*, Initial (*solid line*) and final (*broken line*) response magnitude between 6 and 11 msec recorded at same cortical site to stimuli applied at the three different locations. Note that magnitude of initial evoked response was greatest for *stimulus A*, intermediate for *stimulus B*, and least for *stimulus C*. Note also that this ordering changes in the course of repetitive drive.

ferent levels of this column were similar in form, with well-developed long-latency components being present at each site (see traces at right). Note (1) that the latency to the peak of the late postsynaptic response component increases as the upper layers are approached (8.3 msec at site A, 8.3 msec at site B, 7.1 msec at site C; indicated by arrows), and (2) that although a decrease in response occurs at all three sites with repetitive stimulation, the time course of the decrease is quite different at each site (it is most rapid in the upper two sites, and is slowest at the deepest site). In general, increases in postsynaptic response were observed relatively rarely in the upper layers, even in those experiments where increases were observed in the middle layers of the same column.

Modification of the Spatial Pattern of Cortical Response during Repetitive Stimulation

The next series of experiments ($n = 18$) sought to examine the extent to which the changes in evoked potential response observed with repetitive drive are dependent on the place at which repetitive drive is applied to the network. The design was straightforward: all recordings were obtained at a single site, and input drive was independently applied at each of three different sites; the separation between neighboring stimulus sites was 100 μm (Fig. 11*A*). Changes in response magnitude at a given recording site during repetitive stimulation were quantitatively characterized using the D-value index described previously. Figure 11*B* compares the averaged response to the first and last five stimuli applied at each of the three different stimulus sites. First, it is clear (solid line) that the magnitude of postsynaptic response was highest for site A and declined progressively as the stimulus was shifted away from site A. Second, repetitive stimulation led to dynamic behaviors that were different for each of the three different stimulus sites (summarized by broken line). Thus, repetitive stimulation at site A did not modify response amplitude; with site B stimulation, response magnitude declined substantially; and with site C stimulation, it increased dramatically. The conclusion is, therefore, that the same cortical neural population in the cortical slice can undergo very different dynamic behaviors in response to repetitive afferent drive arising from different places in the cortical white matter.

Figure 12 provides another example of how repetitive afferent drive modifies the spatial distribution of the response set up by the stimulus within the sensorimotor cortical network. At three recording sites (1–3) in layer III, changes in response were studied during repetitive stimuli applied to each of two different sites (A, B) in the cortical white matter. The relative positions of the recording and stimulating sites are shown in Figure 12*A*. In this experiment, each recording site was separated from its nearest neighbor(s) by 100 μm. Under the condition of repetitive drive applied at stimulus site A, the changes in response were quite different at the three recording sites (see Fig. 12*B*); that is, when the magnitude of the change is expressed as a D-value, it was −39% at site 1 (left plot), +87% at site 2 (center plot), and −51% at site 3 (right plot). It should be noted that, due to these very different effects of the same repetitive stimulus on the response recorded at different sites, the global pattern of network activity modifies in the course of repetitive stimulation. For the example shown in Figure 12*C*, response to the first five stimuli at stimulus site A was highest at recording site 3, and lowest at recording site 2 (solid line). With repetitive stimulation at site A, however, response magnitude at recording sites 1 and 3 decreased, while at recording site 2 it increased. As a result, with repetition of stimulus A the response at recording site 2 came to exceed the initial level of responding at sites 1 and 3.

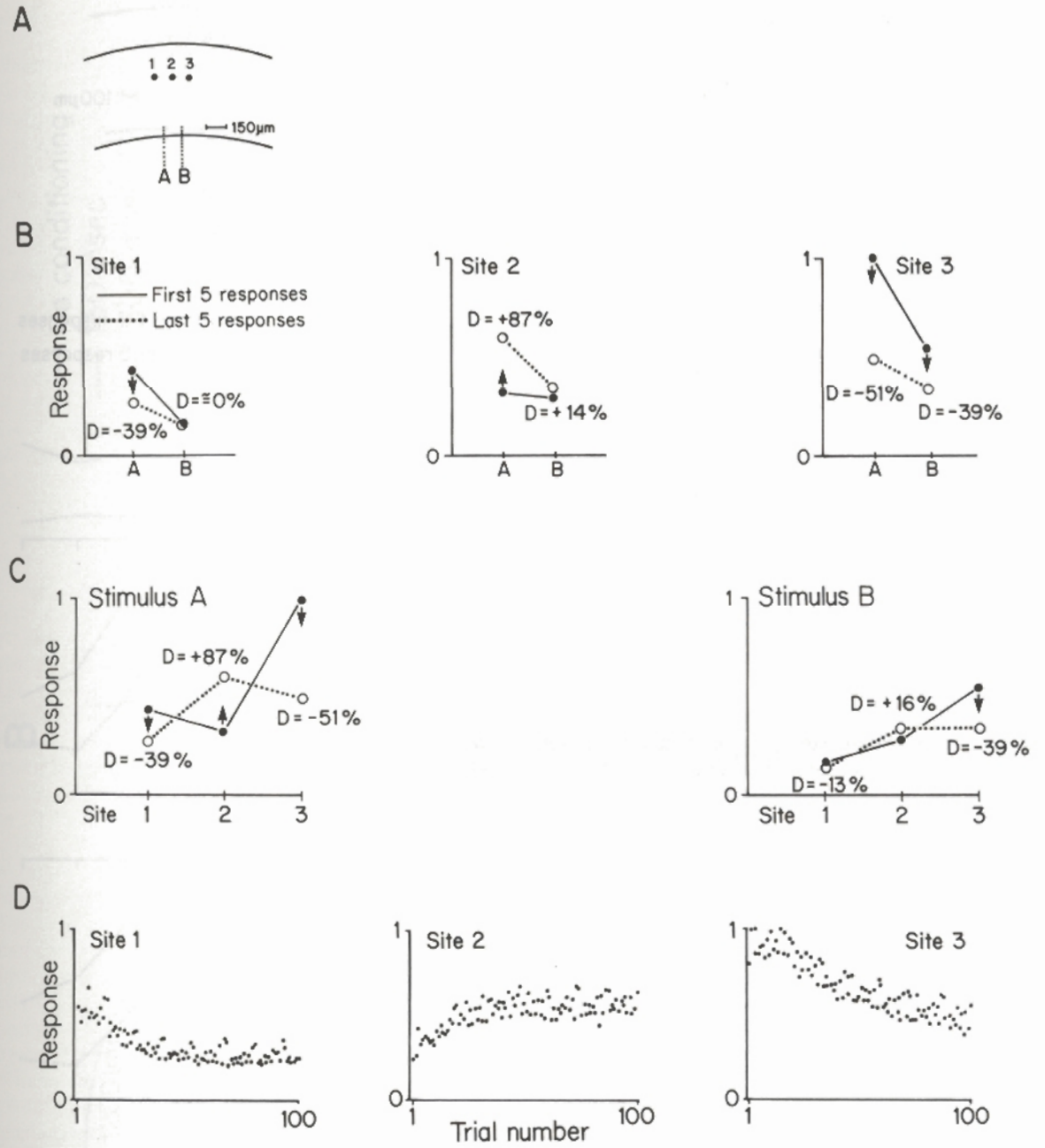

Figure 12. *A,* Arrangement of recording electrodes (*1, 2,* and *3*) and stimulating electrodes (*A* and *B*). *B,* Initial and final levels of response obtained at each site to stimuli applied independently at the two stimulus locations (format as in Fig. 11). At recording *site 2,* the response grows with repetitive stimulation (especially with *stimulus A*), whereas at *sites 1* and *3* it declines. *C,* Plots comparing the initial and final activity patterns evoked by stimulation at *sites A* (*left*) and *B* (*right*). Note that for each stimulus site the initial and final activity patterns are different. *D,* Data obtained from the three recording sites during repetitive stimulation at *location A.*

Figure 12*D* shows the "raw data" obtained at all three recording sites during repetitive input from stimulus site A. With stimulation at site B, the change in response at each recording site was small compared to that observed with repetitive stimulation at stimulus site A (see Fig. 12*C,* right). Though similar changes were observed (i.e., for both stimulus A and B, response increased at site 2 and decreased at sites 1 and 3), repetitive drive applied at site B was less effective in inducing dynamic behaviors than was stimulation at site A. As shown in Figure 12*C,* the changes in response observed at the same recording site also were different under the two different repetitive stimulus conditions.

To obtain further information about how the global spatial pattern of activity in the slice changes with repetitive drive, postsynaptic response was measured at an array of seven middle-layer sites, each separated by 100 μm; and stimuli were delivered to two different sites at the layer VI/white matter junction (see Fig. 13). The two plots at the top (Fig. 13*A,B*) show the spatial distribution of the postsynaptic response to independent stimulation of the corresponding stimulus sites, both early and late during the period of

repetitive stimulation. Despite a decrease in response magnitude at all sites during repetitive stimulation under all three conditions (stimulus A alone, Fig. 13*A*; stimulus B alone, Fig. 13*B*; and A and B applied simultaneously, Fig. 13*C*), inspection of the plots for all three conditions suggests that the local differences in the response of neighboring neuron populations to each stimulus condition are enhanced by repetitive drive. That is, under all three conditions of repetitive stimulation studied, repetitive stimulation consistently was accompanied by a spatial sharpening of the initial global cortical activity pattern.

Integration of Input Drive Arising from Different Sources

To this point, our studies of the effects of repetitive input on the response of the somatosensory cortical slice primarily, but not exclusively, addressed the question of how this network temporally integrates information provided at a single input site. In the next series of experiments, therefore, we attempted to obtain observations that would reveal the capacity of the network to integrate single inputs applied at spatially separated sites in the white matter. This possibility, already suggested by results such as those shown in Figure 13*C*, was investigated more directly in two experiments of the type shown in Figure 14.

To study whether a conditioning stimulus arriving at one site can influence the response to another input delivered to a second site, the experimental approach shown at the top of Figure 14*A* was used. Two stimulating electrodes were placed at sites in the white matter separated by 200 μm, and recordings were obtained from a locus in layer II positioned midway between the two stimulus sites. A fully developed response in layer II was elicited from either stimulus site, although the response evoked from stimulus site A was larger than that evoked from site B at the same intensity of stimulation. To measure the effect of conditioning stimulation, one of the two stimuli (hereafter referred to as the "conditioning stimulus") was delivered initially, followed by the other stimulus (the "test stimulus") after a known delay. The response to test stimulation was measured both in the presence and in the absence of the conditioning stimulus.

The typical effect of conditioning stimulation on the response to a test stimulus is demonstrated in Figure 14*A*. For the traces shown at the top, stimulator B served as the conditioning stimulus, and the test stimulus at site A was applied after a 200 msec delay. Note that the response to the test stimulus was larger when it was delivered subsequent to the conditioning stimulus (broken line). Like the previously observed effects of repetitive stimulation, the effect of a single conditioning stimulus was component selective; that is, the early part of the postsynaptic response (up to about 10 msec) was not changed, but the late component was enhanced. For the trace shown at the bottom of Figure 14*A*, stimulus A was used to provide the conditioning stimulus, and once again, selective enhancement of the late component of the response to the test stimulus was observed. Of interest is the

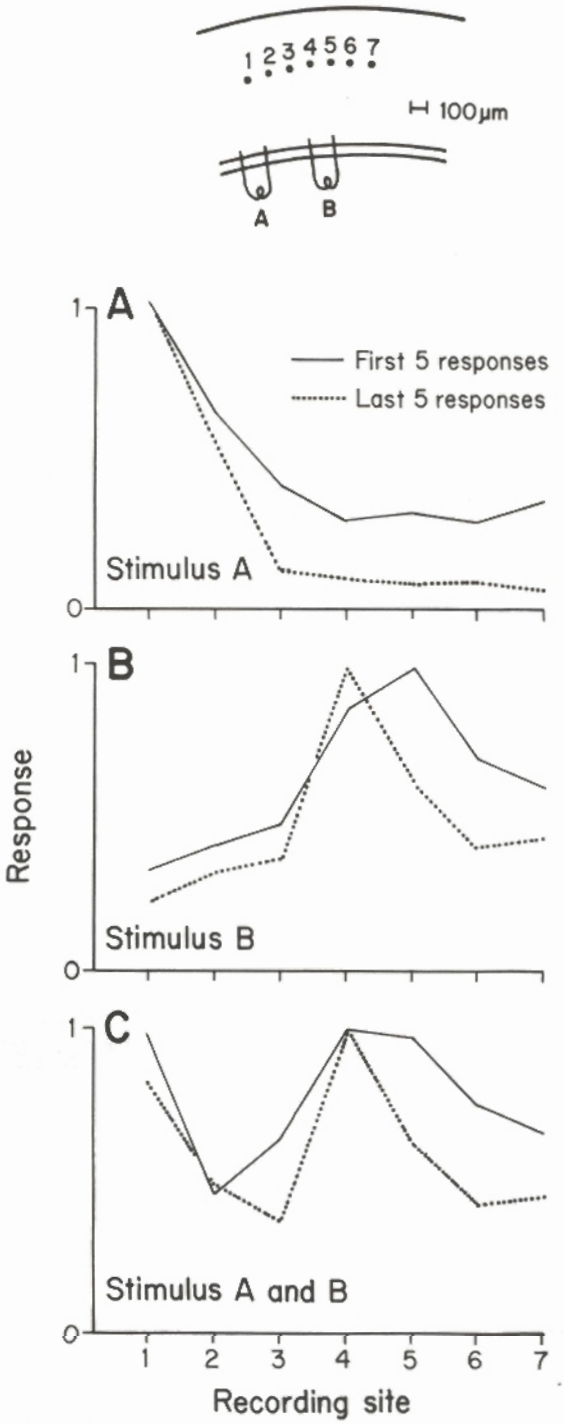

Figure 13. Modification of spatial activity pattern during repetitive stimulation. Fifty stimuli were delivered at 5 Hz. *Top*, Arrangement of recording sites and stimulating electrode. Separation of neighboring recording site was 100 μm; the two stimulating electrodes were separated by 300 μm. *A*, Initial responsivity at the seven different sites (*solid line*; averaged response to first five stimuli) evoked by stimulation at *location A* and final responsivity (averaged response for last five stimuli; *broken line*); postsynaptic response between 8 and 13 msec was measured. *B*, Format as in *A*: stimulating electrode at *location B*. *C*, Format as in *A* and *B*: simultaneous stimulation at *locations A* and *B*.

fact that stimulus A led to larger effects on a subsequent test stimulus than did stimulus B—this was interpreted to indicate the existence of asymmetric interactions among the involved cortical neuron populations.

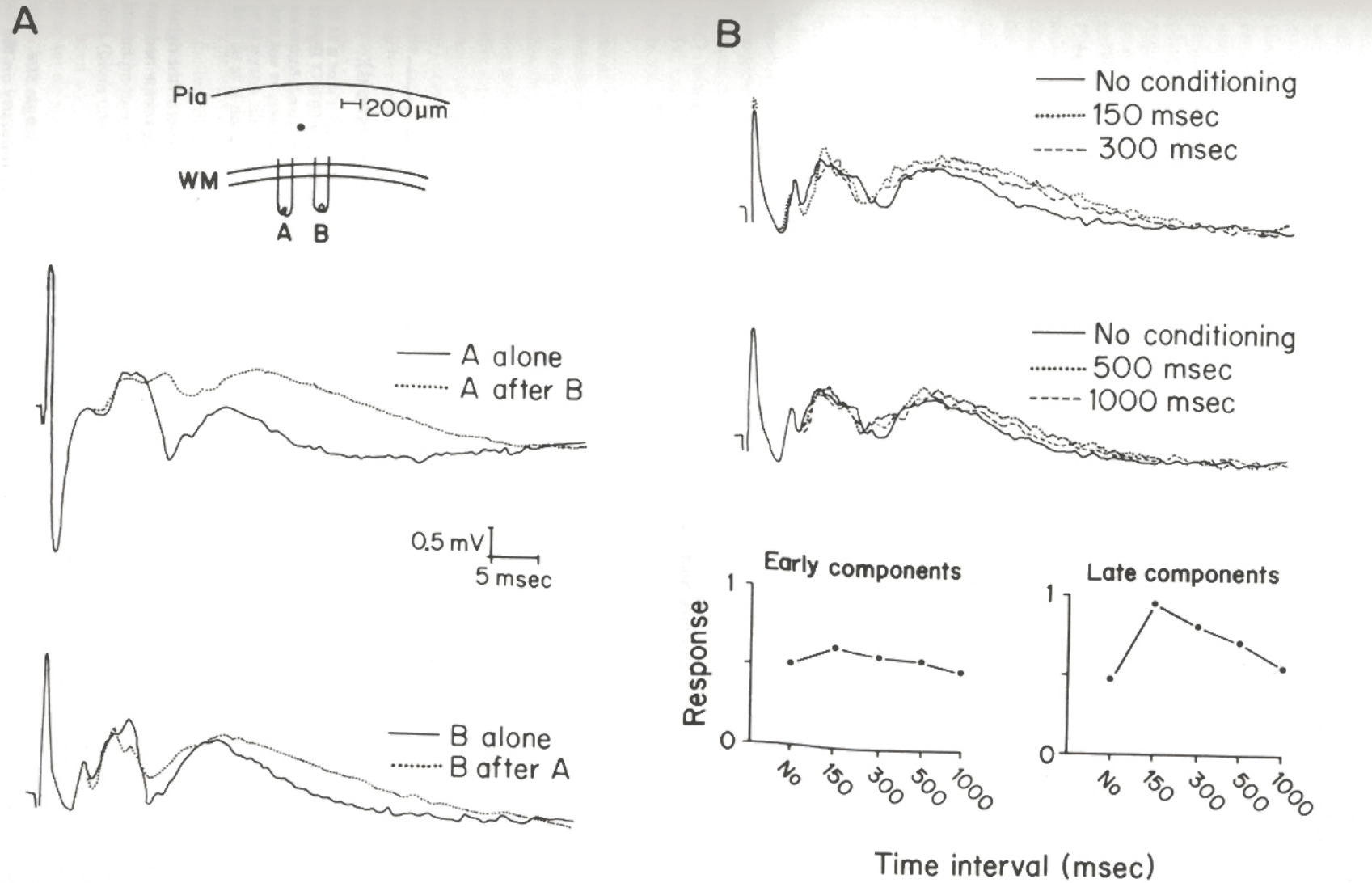

Figure 14. Effect of preceding stimulation applied to neighboring column. *A*: *Top*, Arrangement of recording site and stimulating electrodes. *Middle*, Response to *stimulus A* (test stimulus; *solid trace*), and to *stimulus A* following *stimulus B* (200 msec following conditioning stimulus; *broken trace*). *Bottom*, Same as above, except *stimulus A* served as conditioning stimulus, and *stimulus B*, as test stimulus. *B*, Effects of time interval between conditioning and test stimulus. *Top*, Response pattern to test stimulus without conditioning (*solid trace*) stimulus, and following conditioning stimulus after 150, 300, 500, and 1000 msec (*broken trace*). Each trace is averaged response of five consecutive trials applied at 0.1 Hz. *Bottom*, Amplitude of postsynaptic activity observed at 5–10 msec (*left*) and at 15–22 msec (*right*) plotted as function of time interval.

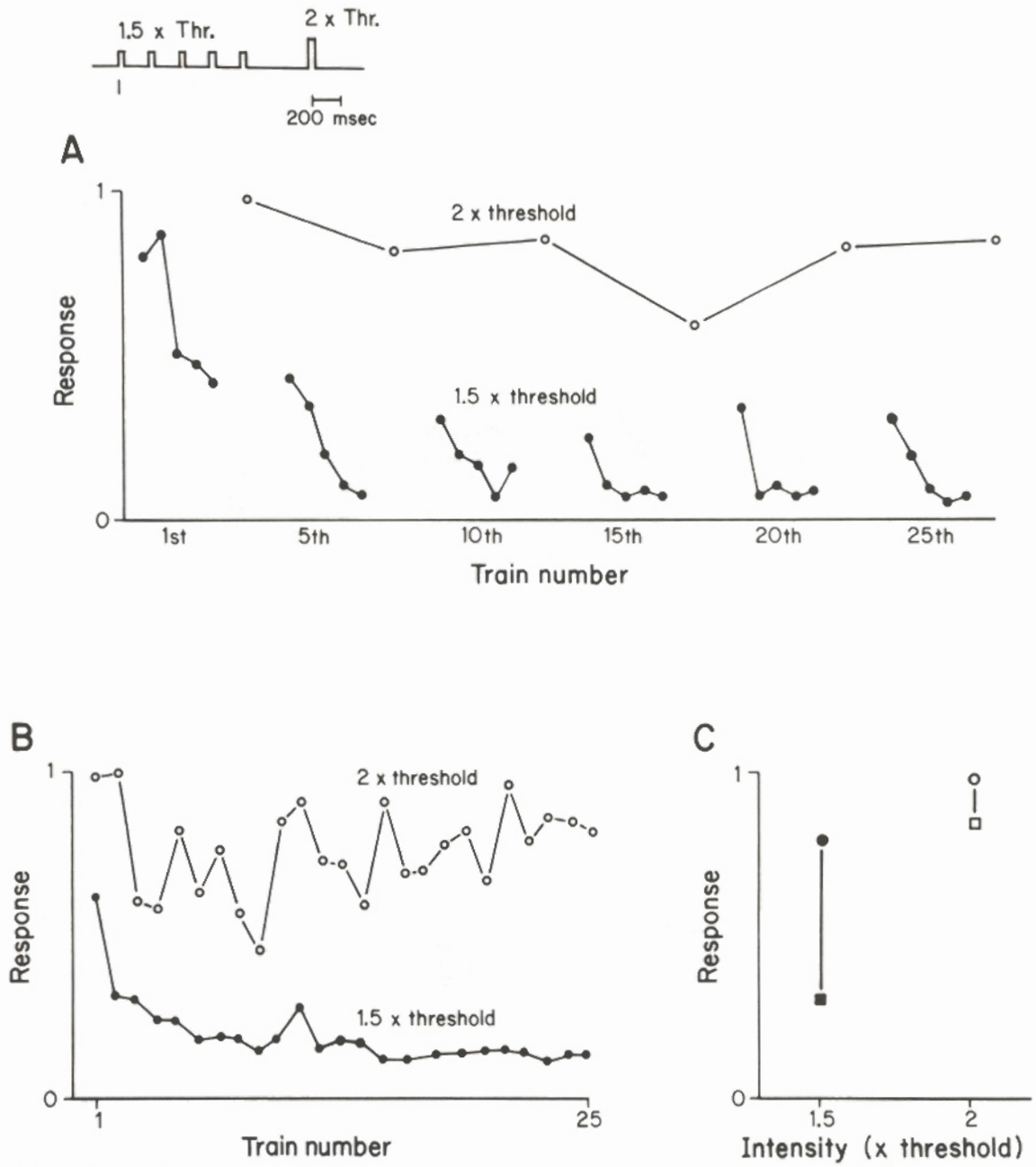

Figure 15. Dynamic behavior of responses evoked by two different intensities of single site stimulation. The temporal sequence of stimuli within a train is shown at the *top left*; a burst of weak stimuli (5/sec, 1.5x) was followed by a single pulse after a delay of 1 sec; 25 trains were applied at a single white matter site at 0.3 Hz. *A*, Magnitude of responses to the 1st, 5th, 10th, 15th, 20th, and 25th trains. *Solid circles* indicate responses to weak stimuli; *open circles* indicate responses to strong stimuli. Response was measured as magnitude of postsynaptic activity observed between 7 and 13 msec. *B*, Response to strong stimulus (*open circles*) and the averaged response to five weak stimuli in each train (*solid circles*) shown as a function of train number. *C*, Response to first and last stimuli at the two different intensities (*circles* for first stimulus; *rectangles* for last stimulus). Note that the response to both the weak and strong stimuli became smaller with repetitive drive, and that the difference between the responses to the two stimuli became greater with stimulus repetition.

The above effects subsequently were shown to be dependent on the time interval between conditioning and test stimuli. In Figure *B*, the effects of the conditioning stimulus were studied at different intervals (150, 300, 500, 1000 msec). Shorter intervals were not studied because the response to the conditioning stimulus was still evident. Again, it is the late evoked potential response components that display the effect most prominently; the early and late latency response components are plotted as a function of conditioning interval in the plots at the bottom of Figure 14*B* (early components on the left; late components on the right). It is apparent that the early responses remained unchanged at all intervals, while the late components attained a maximum at the shortest (200 msec) conditioning test interval.

Differential Effects of Repetitive, Single Site Stimulation Differing Only in Intensity on the Response of the Slice

The possible functional meaning of the nonrandom, complicated, dynamic behaviors of the cortical sen-

sorimotor network triggered by repetitive input drive was investigated in a single experiment in which two different intensities of stimuli were applied at a single site in the white matter (Fig. 15). The experimental design is depicted at the top. A 5 Hz stimulus train was applied at low intensity (1.5× threshold), and each train was followed by a single stimulus of higher intensity (2× threshold) after an interval of 500 msec. Trains and single stimulus presentations were repeated at an interval of 1.5 sec. During each train, the response to the weak stimulus progressively decreased, and recovered only partially during the interval separating successive trains (solid symbols, Fig. 15*A*). However, the response to the stronger single pulse stimulus delivered at 500 msec after each train of weaker stimuli behaved in a very different manner: the response to this more intense stimulus did not change substantially over the entire study (open symbols, Fig. 15*A*). In Figure 15*B*, the data for all 25 trains are shown (for each train of weak stimuli, the responses to the 5 Hz stimuli were averaged; indicated by solid circles). As described above, a gradual decrease in responsivity to the weak stimulus occurred throughout the entire run, while the response to the stronger stimulus (indicated by the open circles) was relatively well maintained. A major result of the above stimulus-evoked changes in responsivity is that the network's differential response to the two strengths of input drive became progressively enhanced (Fig. 15*C*). Response to weak stimulus was decreased by 62.5%, while response to strong was decreased only by 16.0%.

Cortical Activity Patterns Revealed by 2DG Metabolic Mapping Method

The view of cortical dynamics advanced in earlier studies (Whitsel et al., 1989, 1991) and in the preceding article (Lee and Whitsel, 1992) was that repetitive input drive leads to changes in the functional connectivity among large neural assemblies (columns) occupying separate locations in the network. While most conventional neurophysiological approaches possess appreciable temporal resolution, they typically fail to achieve the density of sampling needed to assemble an accurate image of the widespread changes in network status that we postulate take place with repetitive input drive. One method that has been employed successfully for the *in vivo* demonstration of global stimulus-evoked cortical activity patterns is the 2DG metabolic mapping method. This method has been used to advantage for analyzing the spatial patterns of cortical activation evoked by a range of repetitive natural stimulation, including vibrotactile and brushing stimulation (Juliano et al., 1981, 1983). To date, however, the 2DG method has not been used to map the distribution of neural activity set up by controlled stimulation in the cortical slice. Thus, in the next series of experiments ($n = 7$) we sought to employ the 2DG method to study the global activity patterns set up in the sensorimotor slice by repetitive single site stimulation.

The first questions addressed with the 2DG method were (1) whether repetitive stimuli form metabolically distinct, column-shaped areas in the slice during repetitive electrical stimulation, and (2) if such stimuli lead to a pattern of active and inactive columns, whether these columns reflect the attributes of the column-shaped neuronal populations distinguished by their differential dynamic behavior to repetitive input drive in the cortical slice. Figures 16–19 show representative 2DG labeling patterns generated in the slice using single site repetitive stimuli.

Figure 16, *A* and *B*, allows comparison of the global spatiointensive patterns of labeling in two slices that received repetitive afferent drive differing only in frequency (0.1 Hz in *A*, 5 Hz in *B*). It is evident that the distribution of label in the slice that received the higher-frequency drive (5 Hz, 2.0× threshold; Fig. 16*B*) from a central site at the layer VI/white matter junction (arrowheads indicate sites of stimulation) is much better developed, possesses more clear-cut lateral boundaries, and is more closely related to the site of stimulation than is the labeling in the slice stimulated at lower frequency (0.1 Hz, 2.0× threshold; Fig. 16*A*). The representative digitized images of slice sections shown in Figure 16*C* (these images were obtained from the slice whose map is shown in Fig. 16*B*) also reveal that the distribution of labeling in individual autoradiographs is "columnar" in nature, with the focus of above-background labeling located in immediate proximity to the site of stimulation (indicated by arrowheads). Figure 16*D* compares the radially averaged ^{14}C concentration values obtained from one of the images shown in *C* (solid line) with the values obtained from a corresponding image from the map shown in *A* (broken line). The arrowhead along the ordinate indicates the level of background labeling.

Figures 17 and 18 extend the view of the spatial pattern of 2DG incorporation in the stimulated cortical slice provided by Figure 16: shown are two-dimensional surface maps (Figs. 17*A*, 18*A*) of the distribution of label in different slices obtained with single site stimulus conditions known to evoke dynamic behaviors (stimulus condition: 5 Hz, 1 sec train per 2 sec for 15 min). Quantitative analyses of these patterns of labeling are shown at the bottom of each figure. For the case shown in Figure 17, the coherent pattern of labeling relates closely to the locus of white matter stimulation (see vertical line at arrowhead). Visual examination of the pattern reveals (1) that the area of the cortical slice activated by single site stimulation is extensive (size, 1–2 mm), and (2) that this area is composed of more or less distinct, patchlike sectors of above-background labeling. The spatiointensive pattern was analyzed (for the level indicated by the horizontal arrowhead) by measuring the spatial distribution of ^{14}C concentration values (Fig. 17*B*) and its spectral (spatial frequency) characteristics (Fig. 17*C*). From the map and the analyses presented in Figure 17, it is obvious that the activated pattern is spatially periodic, consisting of components having a center-to-center spacing of approximately 1 mm. In Figure 18, the presence of a periodic labeling pattern

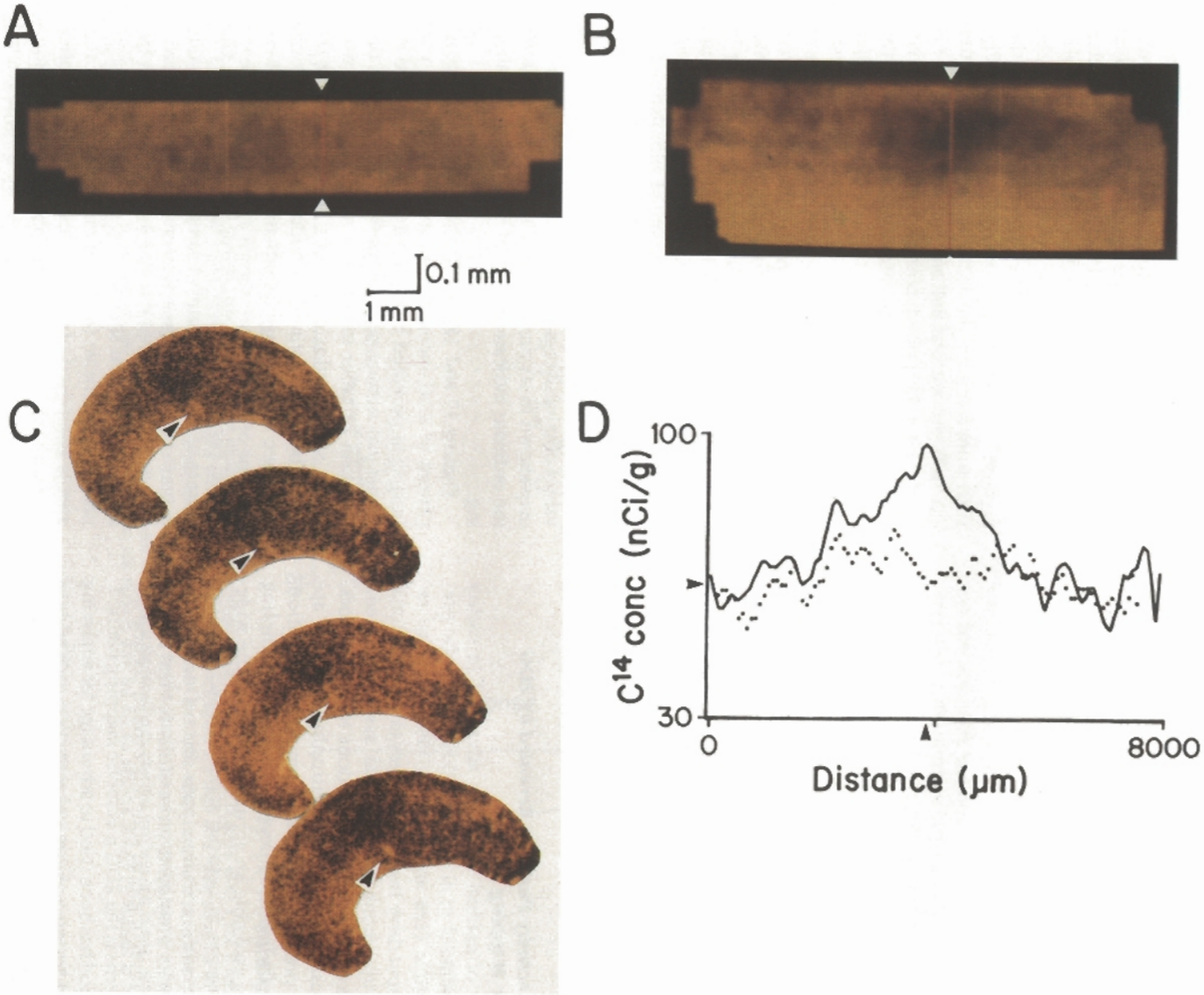

Figure 16. 2DG activity patterns established in cortical slice by repetitive single site stimulation. *A*, Surface map showing the distribution of 2DG label evoked using single site stimulation, 0.1 Hz, 1.5× threshold, for 15 min. The *darker* a region, the higher the ^{14}C-2DG concentration; map was generated from digitized autoradiographic images of 13 serial 20 μm sections. The stimulation site (*arrowheads*) served as the alignment point for all sections. *B*, Surface map of the distribution of 2DG label at 5 Hz, 1.5× threshold, for 15 min. Map was generated from the digitized images of 25 serial 20 μm sections. *C*, Digitized images of four of the autoradiographs used to reconstruct the map shown in *B*. *D*, Sequence of average ^{14}C concentration values determined for one of the images shown in *C* (*solid line*), and for a single image from the map shown in *A* (*broken line*). Each value indicates average ^{14}C concentration value for a 70-μm-wide rectangular bin that extended from layer II to layer IV. The *arrowhead* on the *ordinate* indicates background ^{14}C concentration value.

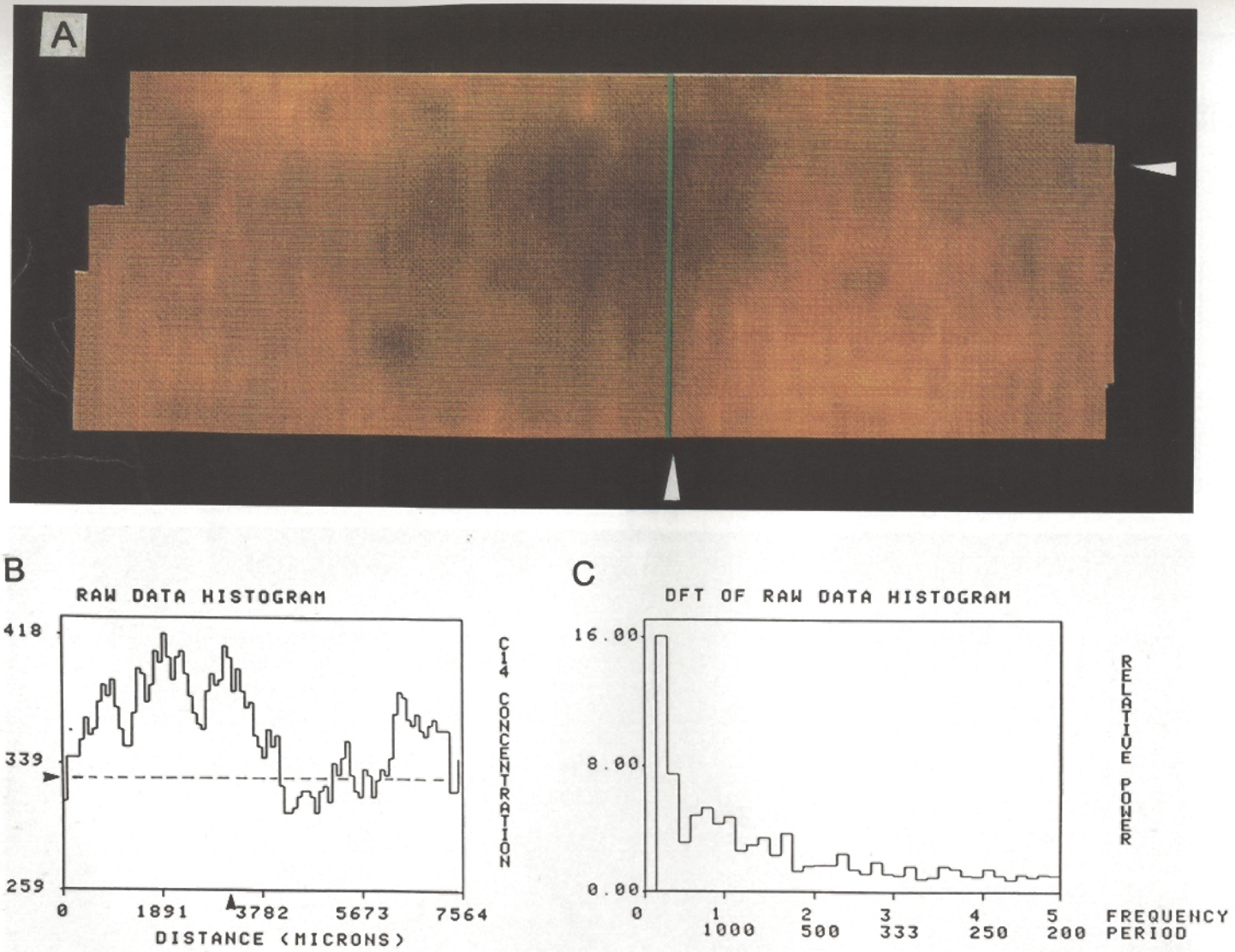

Figure 17. *A*, Surface map of the distribution of 2DG label in slice stimulated at single site (*vertical arrowhead*) using 5 Hz pulse trains of 1 sec duration delivered at 2 sec intervals for 15 min; stimulus intensity, 1.5× threshold. *B*, Sequence of average ^{14}C concentration values determined for the section at the map level indicated in *A* by the *right arrowhead*. Bin width, 50 μm. *X-axis*, tangential cortical distance (in mm). The *arrowhead* on the *ordinate* indicates background ^{14}C concentration values. *C*, Discrete Fourier transform (*DFT*) of data series in *B*.

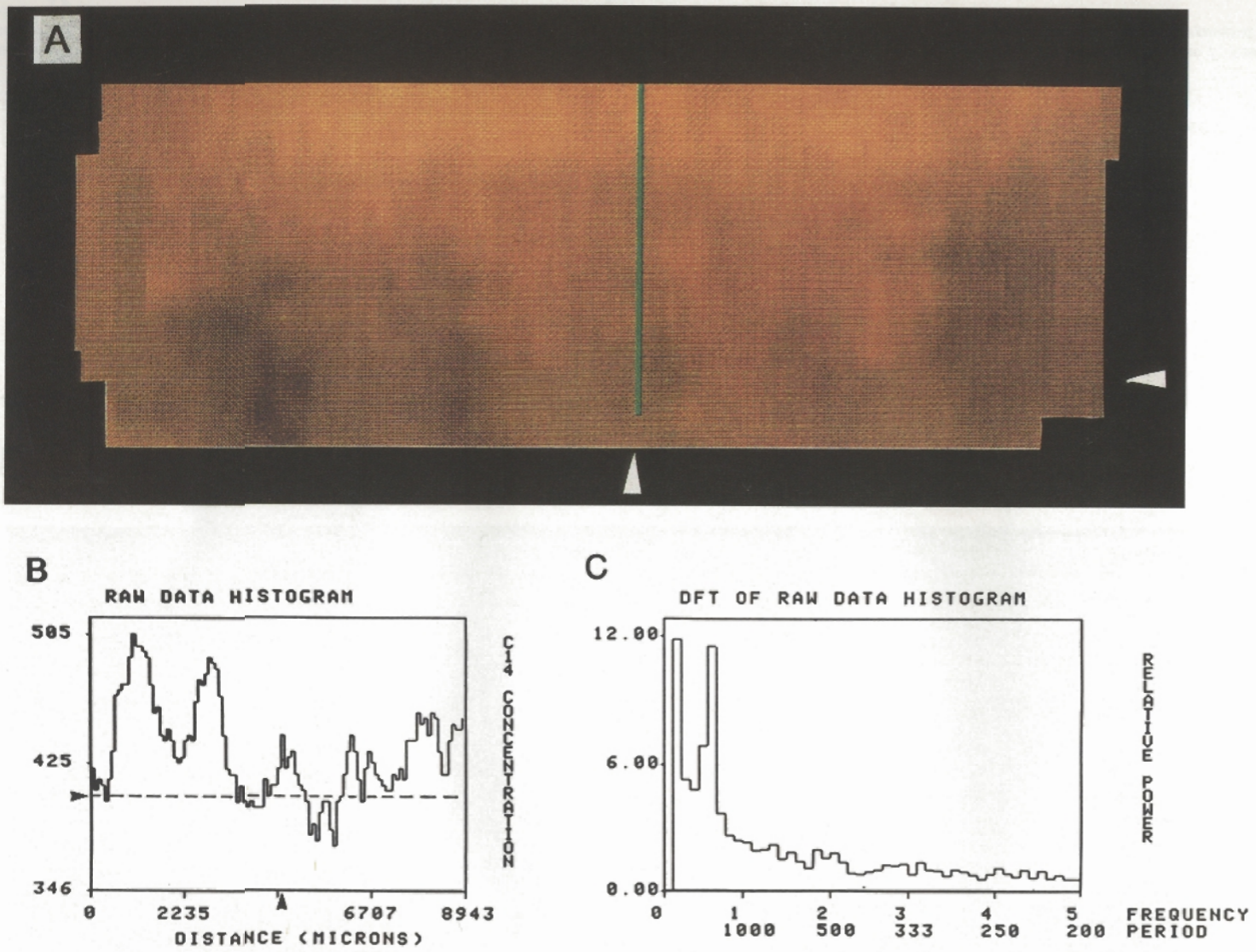

Figure 18. 2DG activity pattern of cortical slice stimulated in same way as in Figure 17. Note existence of periodicities in the labeling pattern (*A*), in the data series (*B*) for a single section taken at the level indicated by the *arrowhead* on the *left* in *A*, and in the discrete Fourier transform (*DFT; C*). In *C*, the second peak in power occurs at a spatial frequency of 0.7 cycles/mm.

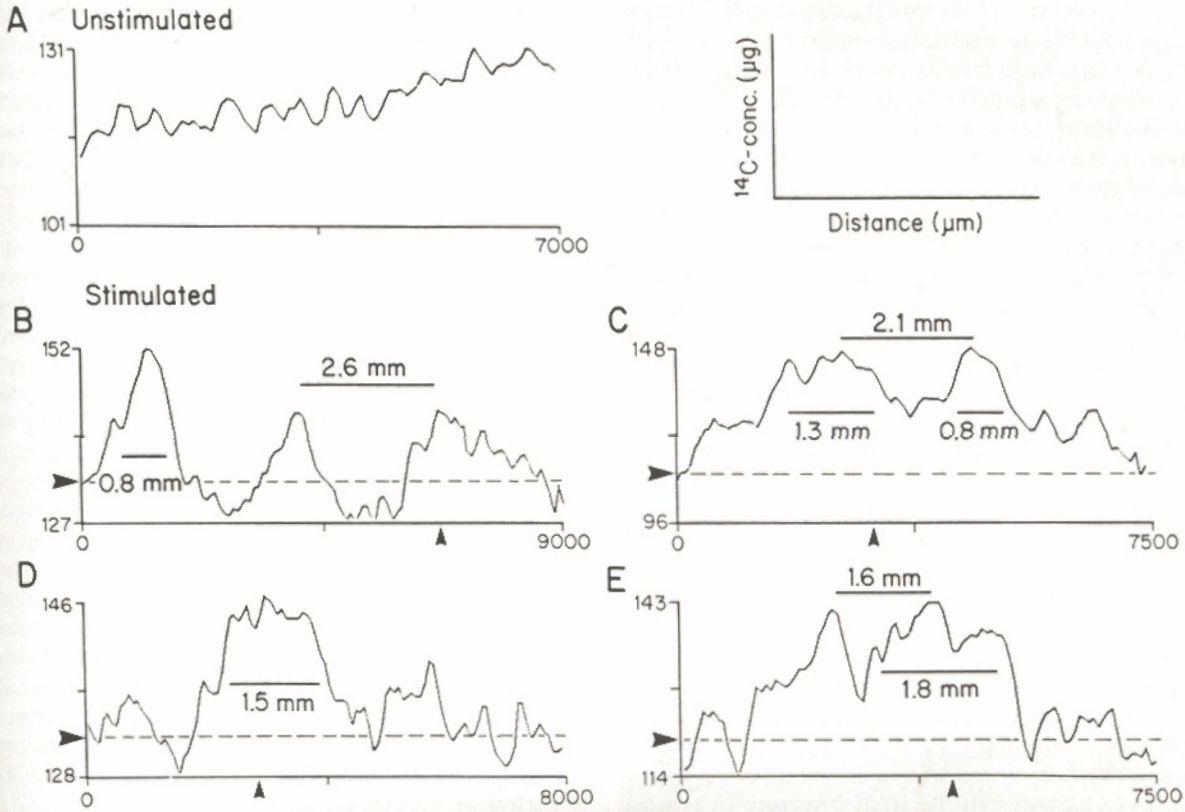

Figure 19. Sequences (*A–E*) of average ^{14}C concentration values determined for sections obtained from five different slices. *A*, Unstimulated slice (exposed to 2DG for 15 min). *B–E*, Slices stimulated at a single site with 5 Hz pulse trains of 1 sec duration delivered at 2 sec intervals for 15 min; stimulus intensity, 1.5× threshold. *Numbers over horizontal* bars indicate width of zones distinguishable on the basis of average ^{14}C concentration values. *Vertical arrowheads* along *abscissae* indicate locations of stimulating electrode.

is even clearer. However, in this case, the labeling pattern is more complex than the pattern of Figure 17; specifically, while the area immediately above the stimulating site (indicated by vertical line and arrowhead) shows above-background labeling, multiple column-shaped areas of above-background labeling located both to the left and to the right of the stimulus site also are evident (Fig. 18*A*). Discrete Fourier transformation (Fig. 18*C*) of the data series shown in Figure 18*B* reveals two prominent peaks at 0.2 and 0.7 cycles/mm. This result is fully consistent with the conclusion that the labeling pattern is periodic, comprising columnlike profiles averaging 1 mm in tangential width.

Additional quantitative analyses of the distribution of 2DG tracer were carried out on one unstimulated ("control") slice, and on four additional stimulated slices (the single site stimulus conditions used to evoke the 2DG patterns in these four slices were the same as those previously used to elicit dynamic neurophysiological behaviors). Each graph shown in Figure 19 was obtained from a single digitized image of a 20 μm section obtained from each of the above-described five slices. Each point indicates the average ^{14}C concentration value computed for a 50–70-μm-diameter rectangular field extending across the full radial width of layers II–VI. The major difference between the unstimulated slice (Fig. 19*A*) and the stimulated slices (Fig. 19*B–E*) is the lack of large differences in average ^{14}C concentration between neighboring tangential regions in the former, and the presence of larger differences between neighboring tangential territories in the latter; that is, only random, low-amplitude oscillations in local ^{14}C concentration characterize the label distribution in the unstimulated slice, whereas lower-spatial-frequency, higher-amplitude oscillations are characteristic of all the stimulated slices. The tangential distances separating neighboring regions of high ^{14}C concentration in the stimulated slices varies from 1.6 to 2.6 mm; the tangential diameter of the strongly labeled areas ranges between 0.8 and 1.8 mm.

Taken together, the 2DG observations of Figures 16–19 seem completely consistent with the central idea advanced on the basis of the neurophysiological findings of this study, that repetitive single site stimulus input drive "fractionates" the network of the sensorimotor cortical slice into a complex pattern of responding and nonresponding cortical columns.

Discussion

While the somatosensory cortical slice obviously lacks many of the structural ingredients of the intact somatosensory nervous system (e.g., peripheral receptors, projection pathways, subcortical nuclei, etc.), the observations obtained in the present study confirm that it is an appropriate model system for approaching issues (e.g., dynamic mechanisms of cor-

tical information processing capacity) formerly regarded to require the intact organism. In particular, the findings indicate that the slice is capable of expressing several of the fundamental functional characteristics of the living cortex, that is, columnar organization and dynamic pericolumnar lateral interactions.

In this study, we have attempted to exploit these properties of the slice in order to determine the mechanisms responsible for the time-dependent effects of repetitive afferent drive on somatosensory cortex. This was attempted at two very different levels: first, at the level of small local neuronal groups, and second, at the level of large, spatially distributed neuronal populations. To us it seems unlikely that the effects of different patterns of afferent drive could have been investigated using the currently available methods for *in vivo* neurophysiological experimentation. In particular, the precision with which one can control the input pattern in the cortical slice preparation cannot be approximated in *in vivo* studies.

Reconcilation of Views of the Cortical Effects of Repetitive Stimulation

Sensory cortical network plasticity conventionally has been studied by measuring changes in neural response consequent to an experimental manipulation (e.g., to a change in the level or nature of sensory drive). A wide variety of such studies of the cortical slice have been carried out, but no concensus view has emerged that ties together the diverse and, in some cases, seemingly contradictory experimental observations that have resulted. One widely publicized effect of repeated stimulation on the response of the neocortical slice has been described as a form of long-term potentiation (LTP). This form of LTP is induced in the cortical population response to white matter stimulation by the delivery of high-frequency, "burst" stimuli (e.g., by 100 msec trains of 100 Hz stimuli every 5 sec for 10 min), and is blocked by NMDA receptor antagonists (Artola and Singer, 1987; Sutor and Hablitz, 1989a,b). Once established, the LTP can persist for hours. Low frequencies of low-intensity input drive comparable to those used in the present study also have been reported to lead to increases as well as decreases in the response of neural populations in the cortical slice. For example, Teylor et al. (1984) reported a 30% decrement in the layer II postsynaptic response to brief periods of low-frequency (1 Hz) stimulation of layer VI, but no changes were observed to the same stimulation in layer IV. The response of layer II recovered within 1 min after termination of stimulation. In contrast, Thomson (1986) reported a gradual augmentation of the pyramidal cell EPSP evoked by electrical stimulation of white matter at 1 Hz, which could be blocked by NMDA receptor antagonists. No decrement in neuronal response was observed.

The failure of previous workers to detect the "two-way" (both increases and decreases) changes that have been demonstrated for the same neuron population in the experiments of this report appears to us to be a consequence of experimental design. First, different contributors to this area of research have focused on different anatomically and physiologically defined subsets of cortical neurons. Thus, in the study of Teyler et al. (1984), changes in the response of the upper cortical layers were emphasized, a locus at which their study routinely and prominently demonstrated decreased response to repetitive drive. The study of Thomson (1986), by contrast, deals solely with recordings made in "on-column" sites from pyramidal cells antidromically activated from the underlying white matter. We think it is likely that if "off-column" pyramidal cells had been sampled in that study, decreases as well as increases in cortical neuronal response to the same stimulus would have been observed. Second, few studies have mapped the spatial distribution of the effects of repetitive stimulation, a decision that (based on the findings of the present study) would restrict severely one's view of the impact of such stimulation on the entire network. The third and final difference between this and previous studies of the cortical slice is the frequency of repetitive stimulation used. For example, while 5 Hz stimulation was mainly used in the present study, Teylor et al. (1984) used 1 Hz stimulation. Although 1 Hz proved effective in some of the experiments of the present study, the most clear-cut and largest effects we observed were obtained using 5 Hz stimulation.

Based on the above considerations, the lack of consensus in the existing literature concerning the effects of repetitive input drive appears resolved. Our conclusion is that repetitive input drive should not be regarded as leading exclusively to either enhancement or depression of cortical neuronal response, but to the development of a highly structured spatial pattern of both decreases and increases in response magnitude, each occurring at different locations within the spatially distributed cortical neural population responding to the stimulus.

Frequency of Input Drive Is a Crucial Factor for the Mechanisms That Mediate Cortical Pericolumnar Interactions

The conditions required to evoke LTP in the cortical slice were avoided in the present study. Under the conditions employed here, maximum changes in response were observed with 10–20 stimulus presentations, and complete recovery occurred within 2–3 min of the cessation of repetitive stimulation. Similar changes in neuronal response have been reported with frequencies of repetitive stimuli between 1 and 12 Hz (Alger and Teyler, 1976; Teyler et al., 1985). No previous studies have reported changes in cortical response at frequencies of stimulus delivery between 0.1 and 0.3 Hz using the stimulus intensities, durations, and exposure periods used in the present study. More specifically, Alger and Teyler (1976) indicated that the magnitude of the response changes evoked by repetitive drive increases with stimulus frequencies above 1 Hz, and saturates at 8 Hz. In most experiments of the present study, therefore, 5 Hz stimulation was used with the following rationale: neither

synaptic fatigue (i.e., transmission failure due to depletion of neurotransmitter at release sites) nor changes in the membrane's ability to recover excitability following spike discharge are likely to be involved in the generation of the responsivity changes that accompany 5 Hz drive, (1) since a 200 msec interstimulus interval is long enough for synaptic neurotransmission to follow the presynaptic events, and (2) because presynaptic elements easily follow 5 Hz stimulation.

Our view of cortical dynamic pericolumnar interactions relies on the assumption that changes in cortical neural response are triggered by the accumulation of potassium in the restricted extracellular space of the cortex during repetitive stimulation (Whitsel et al., 1990; see also preceding article). Such stimulus-evoked increases in $[K^+]_o$ during repetitive stimulation have been reported in a variety of CNS structures, including somatosensory cortex (Lux and Neher, 1973; Poolos et al., 1987; Poolos and Kocsis, 1990). Furthermore, it is known that $[K^+]_o$ accumulation is frequency dependent and short lasting, and occurs to an extent sufficient to influence membrane potential and ionic driving forces (Lux and Neher, 1973; Signer and Lux, 1975; Cordingley and Somjen, 1978; Somjen, 1979; Poolos and Kocsis, 1990). At the stimulus frequencies utilized in the present study, $[K^+]_o$ is increased to two to three times its resting level. Furthermore, NMDA receptor activation has been demonstrated at the levels of $[K^+]_o$ presumed to be attained by the repetitive stimuli employed here (Poolos and Kocsis, 1990).

Layer Dependency of the Proposed Dynamic Process

The laminar and tangential position of the recording electrode determines the experimentalist's view of the cortical dynamic response to repetitive drive. In the tangential dimension, the neurons of the slice undergo complicated, nonrandom dynamic modifications of their response to afferent drive. In the vertical dimension of the cortical slice, the extent to which the process is observable varies with cortical layer; that is, the dynamic process is "layer dependent" in the sense that the changes triggered by repetitive drive occur most rapidly and prominently in the upper layers, and are least obvious in layer IV.

The data obtained in intact animals using field potential recording and current-source density analysis (Chapin, 1986; Di et al., 1990) provide a view of information flow in somatosensory cortex that fits well with the above observations of the response of the cortical slice to repetitive drive. First, it is clear that afferent processing within a cortical column originates in those layers (layer IV and lower layer III) that in somatosensory cortex are the principal direct recipients of specific thalamocortical afferent input. Second, it also is clear that a class of middle-layer cells (the spiny stellates) relays the excitatory drive expressed on layer IV to the upper layers of the same cortical columns. In the slice preparation, this information-processing sequence appears to be well preserved (Langdon and Sur, 1990; Vaknin and Teyler, in press). In basal layer III and layer IV, thalamocortical afferents are the major source of input, while layers II–III, in addition to receiving within-column input from spiny stellate cells, also are activated by axons originating from cells in other cortical columns.

The systematic shift in the latency of late evoked potential components observed as we shifted the recording electrode from the middle to the upper cortical layers fits with the above interpretation. Since the late component of the evoked response is more subject to change with repetitive stimulation, it seems reasonable to conclude that it is the connections to a column that arise from outside that column (the corticocortical connections) that undergo the major modifications during repetitive afferent drive. The layer dependency of the response modifications that accompany repetitive drive also suggests that the direct, most reliable inputs delivered to the middle layers by direct thalamocortical afferents (glutamate or a close congener is the neurotransmitter; Hicks et al., 1987; Herrling et al., 1990) come to dominate neurons with repetitive stimulation, while lateral interactions and modulations take place primarily in the superficial cortical layers.

Adaptive Properties of Somatosensory Sensory Cortex

What is the role of the adaptive cortical process demonstrated in the present studies? We have proposed that this dynamic process enables repetitive stimulation to sharpen the spatially periodic, distributed response of the network to an environmental stimulus. Based on the evidence provided in human studies of the effects on perceptual capacities of oscillatory tactile stimuli (Vierck and Jones, 1970; Hollins et al., 1990; Goble and Hollins, in press), we presume that the network becomes more efficient in extracting information about subsequent stimuli similar in form to those triggering the adaptive process in the first place. An outcome consistent with this expectation was the observed tendency for improved discriminability of the cortical patterns evoked by stimuli differing only slightly in intensity, an outcome accompanied by a preferential loss of the network's responsivity to weak but not to stronger stimulation applied at the same locus (see Fig. 15).

Modification of the Stimulus-evoked Global Activity Pattern

The available observations suggest that repetitive input drive leads to a spatial pattern of response changes that is "columnlike." That is, repetitive afferent drive leads to the formation of column-shaped neuronal aggregates, each of which undergoes a characteristic alteration of its response to input. A notable property of these patterns is that neighboring column-shaped aggregates typically exhibit opposing changes in response to the same stimulus. The neurophysiological mapping observations of this report are consistent with the existence of boundaries that extend continuously from the middle layers toward the pial surface—and with the idea that these boundaries sepa-

rate column-shaped aggregates distinguishable on the basis of the time-dependent alterations they undergo in their responses to repetitive afferent drive.

The neurophysiological results also lead us to propose (1) that repetitive drive causes the intrinsic network of the somatosensory cortical slice to modify its initial response to the applied stimulus, and (2) that as a result of this transformation, a new global spatial activity pattern emerges that enhances the local differences present in the initial response pattern (i.e., the final spatiointensive activity pattern possesses greater local contrast than the pattern evoked by the initial stimuli). Support for such a "sharpening" of the periodicity in the global network activity pattern evoked by repetitive stimulation was provided in experiments that used the 2DG metabolic mapping technique. Based on the available evidence (both neurophysiological and 2DG), it is clear that a spatially sharpened, columnar pattern of activity evoked in the cortical slice by repetitive input drive emerges even with relatively short periods of stimulation. As a result, such dynamic changes in network behavior demonstrated in the slice experiments of this article seem very well suited to respond to the information processing demands placed on somatosensory cortex during everyday life.

As will be described in detail in a forthcoming report, the neurotransmitter/receptor/connectional mechanisms underlying the capacity of the sensorimotor slice to respond dynamically to repetitive single site input drive appear similar to those represented in the model described in the preceding article (see Lee and Whitsel, 1992, their Figs. 16, 17). This conclusion is based on our finding that both the changes in neural response and the spatially periodic 2DG metabolic activity patterns set up the cortical slice by repetitive white matter stimulation are attenuated by drugs that block either the NMDA (i.e., aminophosphonovalerate or phencyclidine) or GABA/A (i.e., bicuculline) receptors (C.-J. Lee, B. L. Whitsel, M. Tommerdahl, and C. Wong, unpublished observations).

Notes

We gratefully acknowledge the contributions of the following to the work described in this article: Drs. Jay Dean and Alan Light of the Department of Physiology at The University of North Carolina at Chapel Hill provided critical assistance during the initial phase of our evoked potential studies of the cortical slice. Mr. Calvin Wong provided expert technical assistance during both the recording and 2DG experiments, and also helped with the development of the data figures and with the typing and editing of the manuscript. Ms. Carol Metz assisted with the development of autoradiographic images of 2DG-labeled slices. Drs. E. Kelly and M. Hollins provided useful recommendations for revisions of an early version of the manuscript. The work was supported, in part, by NIDR Program Project Grant DE 07509 (B. Whitsel, Program Director) and by NIMH RO1 Grant MH48654 and EPA Cooperative Agreement CR818321.

Chang-Joong Lee is now at Neural Systems Section, NIH/NINDS, Bethesda, MD 20205.

Correspondence should be addressed to Barry L. Whitsel, Department of Physiology, The University of North Carolina at Chapel Hill, Chapel Hill, NC 27599-7545.

References

Alger BE, Teyler TJ (1976) Long-term and short-term plasticity in the CA1, CA3, and dentate regions of the rat hippocampal slice. Brain Res 110:463–480.

Angel RW, Quit WM, Boylls CC, Weinrich M, Ronditzky RL (1985) Decrement of somatosensory evoked potentials during repetitive stimulation. Electroencephalogr Clin Neurophysiol 60:335–342.

Artola A, Singer W (1987) Long-term potentiation and NMDA receptors in rat visual cortex. Nature 33:649–652.

Chagnac-Amati Y, Connors BW (1988) Horizontal spread of synchronized activity in neocortex, and its control by GABA-mediated inhibition. J Neurophysiol 61:747–758.

Chapin JK (1986) Laminar differences in sizes, shapes, and response profiles of cutaneous receptor fields in the rat SI cortex. Exp Brain Res 62:549–559.

Connors BW, Gutnick MJ, Prince DA (1982) Electrophysiological properties of neocortical neurons *in vitro*. J Neurophysiol 48:1302–1320.

Cordingley G, Somjen G (1978) The clearing of excess potassium from extracellular space in spinal cord and cerebral cortex. Brain Res 151:291–306.

Delberghe X, Marvoudakis N, Zegers de Beyl D, Bruno E (1990) The effect of stimulus frequency on post- and pre-central short-latency somatosensory evoked potentials (SEPs). Electrocephalogr Clin Neurophysiol 77:86–92.

Di S, Baumgartner C, Barth DS (1990) Laminar analysis of extracellular field potentials in rat vibrissa/barrel cortex. J Neurophysiol 63:832–840.

Dingledine R, ed (1984) Brain slices. New York: Plenum.

Goble A, Hollins M (in press) Vibrotactile adaptation enhances amplitude discrimination. J Acoust Soc Am, in press.

Harrison NL, Simmonds M (1985) Quantitative studies on some antagonists of *N*-methyl D-aspartate in slice of rat cerebral cortex. Br J Pharmacol 84:381–391.

Herrling PL, Meier CL, Salt TE, Seno N (1990) Involvement of NMDA receptors and non-NMDA receptors in cortico-cortical and thalamo-cortical excitatory postsynaptic potentials in the anaesthetized cat. J Physiol (Lond) 425: 89.

Hicks TP, Lodge D, McLennan H (1987) Excitatory amino acid transmission. New York: Liss.

Hollins M, Goble AK, Whitsel BL, Tommerdahl M (1990) Time course and action spectrum of vibrotactile adaptation. Somatosens Motor Res 7:205–221.

Juliano SL, Hand P, Whitsel BL (1981) Patterns of increased metabolic activity in somatosensory cortex of monkeys (*Macaca fascicularis*) subjected to controlled cutaneous stimulation: a 2-deoxyglucose study. J Neurophysiol 46: 1260–1284.

Juliano SL, Hand P, Whitsel BL (1983) Patterns of metabolic activity in cytoarchitectural area SII and surrounding cortical fields of the monkey. J Neurophysiol 50:961–980.

Kelso SR, Nelson DO, Silva NL, Boulant JA (1983) A slice chamber for intracellular and extracellular recording during continuous perfusion. Brain Res Bull 10:853–857.

Konnerth A (1990) Patch-clamping in slices of mammalian CNS. Trends Neurosci 13:321–323.

Langdon RB, Sur M (1990) Components of field potentials evoked by white matter stimulation in isolated slices of primary visual cortex: spatial distributions and synaptic order. J Neurophysiol 64:1484–1501.

Lee KS (1982) Sustained enhancement of evoked potentials following brief, high-frequency stimulation of the cerebral cortex *in vitro*. Brain Res 239:617–623.

Lee C-J, Whitsel BL (1992) Mechanisms underlying somatosensory cortical dynamics: I. *In vivo* studies. Cereb Cortex 2:81–106.

Lee C-J, Whitsel BL, Tommerdahl M, Wong C (1990) Effects of repetitive afferent drive on responsivity of sensorimotor cortical slice. Soc Neurosci Abstr 16:227.

Lee SM, Weisskopf MG, Ebner FF (1991) Horizontal long-term potentiation of responses in rat somatosensory cortex. Brain Res 544:303–310.

Lux HD, Neher E (1973) The equilibration time course of $[K^+]_o$ in cat cortex. Exp Brain Res 19:190–205.

McCormick DA (1989) GABA as an inhibitory neurotransmitter in human cerebral cortex. J Neurophysiol 62:1018–1027.

Poolos N, Kocsis J (1990) Elevated extracellular potassium enhances synaptic activation of *N*-methyl-D-aspartate receptors in hippocampus. Brain Res 508:7–12.

Poolos NP, Mauk MD, Kocsis JD (1987) Activity-evoked increases in extracellular potassium modulate presynaptic excitability in the CA1 region of the hippocampus. J Neurophysiol 58:404–416.

Ried KH, Edmonds AL Jr, Schurr A, Tseng MT, West CA (1988) Pitfalls in the use of brain slices. Prog Neurobiol 31:1–18.

Shaw C, Teyler TJ (1982) The neural circuitry of the neocortex examined in the *in vitro* brain slice preparation. Brain Res 243:35–47.

Singer W, Lux H (1975) Extracellular potassium gradients and visual receptive fields in the cat striate cortex. Brain Res 96:378–383.

Sokoloff L, Reivich M, Kennedy C, Des Rosiers MH, Patlack CS, Pettigrew KD, Sakurada O, Shinohara M (1977) The (^{14}C) deoxyglucose method for the measurement of local cerebral glucose utilization: theory, procedure, and normal values in the conscious and anesthetized albino rat. J Neurochem 28:897–916.

Somjen G (1979) Extracellular potassium in the mammalian central nervous system. Annu Rev Physiol 41:159–177.

Sutor B, Hablitz JJ (1989a) EPSPs in rat neocortical neurons *in vitro*. I. Electrophysiological evidence for two distinct EPSPs. J Neurophysiol 61:607–620.

Sutor B, Hablitz JJ (1989b) EPSPs in rat neocortical neurons *in vitro*. II. Involvement of *N*-methyl-D-aspartate receptors in generation of EPSPs. J Neurophysiol 61:621–634.

Teyler TJ, Chiaia N, DiScenna P, Roemer RA (1984) Habituation of central nervous system evoked potentials: intrinsic habituation examined in neocortex, allocortex, and mesencephalon. In: Habituation, sensitization, and behavior (Peeke HVS, Petrinovich L, eds), pp 251–285. New York: Academic.

Thomson AM (1986) A magnesium-sensitive post-synaptic potential in rat cerebral cortex resembles neuronal responses to *N*-methylaspartate. J Physiol (Lond) 370:531–549.

Tommerdahl M (1989) Stimulus evoked activity patterns in somatosensory cortex: evidence for an opponent mechanism. PhD thesis, The University of North Carolina at Chapel Hill.

Tommerdahl M, Baker R, Whitsel B, Juliano S (1985) A method for reconstructing patterns of somatosensory cerebral cortical activity. Biomed Sci Instrum 21:93–98.

Tommerdahl M, Whitsel BL, Cox E, Diamond M, Juliano S, Kelly DG (1987) Analysis of the periodicities in somatosensory cortical activity patterns. Soc Neurosci Abstr 13:470.

Tommerdahl M, Lee C-J, Whitsel BL (1991) Stimulus-evoked 2DG labeling in SI: *in vivo* and *in vitro* studies. Soc Neurosci Abstr 17:626.

Vaknin G, Teyler TJ (in press) Major excitatory pathways in rat visual cortex: III. Intrinsic connectivity revealed by current density analysis derived from electrical activation of vertical and laminar afferents. In press.

Vierck CJ Jr, Jones MB (1970) Influences of low and high frequency oscillation upon spatio-tactile resolution. Physiol Behav 5:1431–1435.

Whitsel BL, Favorov O, Tommerdahl M, Diamond M, Juliano S, Kelly DG (1989) Dynamic processes govern the somatosensory cortical response to natural stimulation. In: Sensory processing in the mammalian brain (Lund JS, ed), pp 84–116. New York: Oxford UP.

Whitsel BL, Favorov OV, Kelly DG, Tommerdahl M (1991) Mechanisms of dynamic peri- and intra-columnar interactions in somatosensory cortex: stimulus-specific contrast enhancement by NMDA receptor activation. In: Information processing in the somatosensory system (Franzen O, Westman J, eds), pp 353–369. New York: Stockton.