Spatiotemporal Effects of Microstimulation in Rat Neocortex: A Parametric Study Using Multielectrode Recordings

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INTRODUCTION

Electrical stimulation of nervous tissue has been a tool used extensively in experimental neuroscience to elicit neuronal or behavioral responses in a large variety of preparations (Ranck 1975; Tehovnik 1996). Recently, a new interest for stimulation of neuronal tissue has arisen from the prospect that signals from machines may be transferred directly to neuronal tissue. In cat motor cortex Asanuma and colleagues showed that the current needed to evoke an action potential in corticospinal projection neurons is proportional to the square of the distance between a microelectrode and the soma (Asanuma et al. 1976; Stoney et al. 1968). In particular, it has been shown that the population of corticospinal projection neurons directly activated by a small current pulse (e.g., <20 μA and 0.2 ms) is grouped around the electrode at a distance of about 100 μm (Stoney et al. 1968) and thus excites a few thousand cortical cells directly (Tehovnik 1996). The second problem to be solved is the relative contribution of different neuronal elements, like dendrites, somata, and axons to the direct activation of neurons. There is substantial evidence from experimental work as well as from computer simulations that electrically evoked activity in gray matter is attributed mainly to the excitability of axonal elements and only to a small degree to somata and dendrites (Gustafsson and Jankowska 1976; McIntyre and Grill 2000; Nowak and Bullier 1998a,b; Ranck 1975; Rattay 1999). This insight represents the third problem to be solved: that the direct neuronal activation of somata must be expected to be masked by intense axonal excitation carrying the activation by ortho- and antidromic conveyance of spikes to areas, the spatial layout of which depends much on the interconnectivity of the structure (“indirect” effects). Because intrinsic axonal elements are abundant in neocortex and most other gray matter in the CNS, local antidromic and/or synaptic effects must be expected to carry the major part of the stimulation effect even though only a small number of neuronal somata have been stimulated directly.

The present study focuses on the “indirect” effects of intracortical electrical stimulation, which we refer to as the spatiotemporal “blur” of evoked activity. The neocortex is an important future candidate for CNS stimulation aimed at restoring brain function because important functions are localized on the neocortical surface in an easily accessible way and in many cases are functionally separated and organized in well-charac-

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terized cortical maps. On the other hand, the prototypic neocortical architecture contains several excitatory and inhibitory cell types that are complexly interconnected locally as well as over larger distances (see reviews in Braitenberg and Schüz 1991 and Peters and Jones 1984). Neocortical connectivity is strongest within cortical columns spanning the depth of the neocortex (Hubel and Wiesel 1977; Mountcastle 1957) but it is pronounced as well along the axes spanning its surface. Long horizontal fibers present in all layers (with a preponderance in layers 2/3 and 5) transmit excitatory signals over distances of up to several millimeters and contacts mainly excitatory but also to inhibitory neurons (Dalva et al. 1997; Hirsch and Gilbert 1991; Kim and Ebner 1999; McGuire et al. 1991; Schubert et al. 2001). Inhibitory signals show horizontal spread as well. Long horizontal inhibitory axons are shorter on average than the excitatory ones (about 1 mm; for review see Kisvárday 1992), but in addition, inhibition can be transmitted by electrically (and synaptically) interconnected networks of interneurons and thereby potentially may spread over a large range of neocortex as well (Galarreta and Hestrin 1999; Gibson et al. 1999). As alluded to in the previous paragraph, the high interconnectivity of the neocortex predicts that indirectly evoked activity—by retrograde, synaptic, or electrical transmission—must play an important role in determining the effect of electrical stimulation of neocortex (Nowak and Bullier 1998a). This spatiotemporal extent of indirect effects determines whether and how interaction of stimulus applied at different points in space and time occurs and will be critical for future applications because it is clear from the distributed representation of signals on the surface of the neocortex that multielectrode techniques have to be employed to reach the spatiotemporal resolution needed to evoke functional activity patterns (Nicollelis 2001). To use multielectrode stimulation in the neocortex, a mandatory step must be to gain detailed knowledge on how a single electrical pulse affects the activity of the surrounding neuronal circuit. What are the changes of neuronal activity evoked by a certain stimulation intensity in space and time and what follows from these characteristics for the interaction of two stimuli in the temporal (pulse intervals) and spatial (cross electrode) domain? As a first step, we measured these parameters using dense microelectrode arrays in primary somatosensory cortex of ketamine anesthetized rats. Responses of single unit and multunit spike trains were recorded varying electrical (charge transfer), spatial (electrode location), and temporal (pulse intervals) parameters of electrical stimulation.

METHODS

Surgery

Twenty-one Sprague–Dawley rats of both sexes (body weight 300 to 400 g) were used in the present study. All experimental and surgical procedures were performed in accordance with the policy on the use of animals in neuroscience research of the Society for Neuroscience and German Law. The rats were anesthetized with a mixture of ketamine (100 mg/kg) and xylazine (10 mg/kg) administered intraperitoneally. The depth of anesthesia was maintained with additional doses of ketamine as required to keep the hind-limb withdrawal reflex to a painful stimulus below threshold. The animal’s rectal temperature and heart rate were constantly monitored. The temperature was adjusted to 37.0°C using a controlled heating pad (Fine Science Tools, Heidelberg, Germany). For recordings from the neocortex, animals were placed in a stereotaxic apparatus and craniotomy was performed to open the primary somatosensory cortex. After removal of the dura mater a multielectrode array consisting of a row of 7 electrodes was lowered into the neocortex at a 90° angle with respect to its surface using a hydraulic micropositioner (Kopf 650; David Kopf Instruments, Tujunga, CA). After placement of the electrode array the cortex was covered with mineral oil to prevent drying. The receptive field of the recording sites was coarsely assessed using either an air puff (originating from a plastic tube; diameter about 0.5 mm) directed against the whiskers (duration 20 ms, distance to whisker pad 2–3 cm) and/or a cotton swap lightly touching parts of the body. In preliminary experiments the placement and orientation of the electrode array were varied. Stimulation of areas representing whiskers, snout, and forelimb yielded qualitatively the same results. For the recordings presented here locations were analyzed for which an air puff against the whisker pad on the contralateral side to the recorded hemisphere elicited a clear response in at least part of the electrodes. The data of the present study were thus recorded in whisker and neighboring snout representations. At the end of the experiment the recording sites were marked with electrolytic lesions. The animal was deeply anesthetized with barbiturates and perfused through the aorta using phosphate buffer (0.1 M) followed by paraformaldehyde (4% in phosphate buffer). The brain was processed using standard histological procedures. The layer of recording was assessed by investigating lesions in Nissl-stained sections oriented orthogonally to the surface of the neocortex.

Electrodes

Multielectrode arrays were custom made in our laboratory. Seven standard etched and lacquered tungsten electrodes (shaft diameter 100 μm; tip size about 10 μm; impedance >2 MΩ) were mounted inside a linear array of polyimide tubing (HV Technologies, Trenton, GA) and crimp connected on their back ends to a microplug (Bürklin, München, Germany). The tip distance was 450 μm. The electrode at one end of the row was used for electrical stimulation and was placed inside a stainless steel tube that was connected to ground during the recording sessions. This shielding reduced the stimulus artifact such that minimal latencies of 2 ms could be safely determined (see next section).

Electrophysiology

The electrophysiological recordings of extracellular signals were performed using a multichannel extracellular amplifier (MultiChannel Systems, Reutlingen, Germany; gain 5000; sampling rate 20 kHz; band-pass filter with cutoff frequencies of 200 and 5000 Hz). Action potentials were extracted from the voltage traces off-line by a threshold and stored as cutouts of 2 ms length on the hard drive of a PC. Spike sorting was performed with PCA clustering using a MATLAB-based program (Egert et al. 2002; the software is available on the internet at http://www.brainworks.uni-freiburg.de/). Through the first electrode in the row of electrodes rectangular biphasic current pulses (cathodal first) were delivered at a rate of 1 Hz using a programmable stimulator (STG 1008; MultiChannel Systems). To avoid the need to apply very low current amplitudes, the stimulus amplitude was fixed to 8 μA and the intensity was changed by varying the pulse duration between 100 to 600 μs in steps of 100 μs (charge transfer of 0.8 to 4.8 nC, varied from pulse to pulse in a pseudo-random fashion). The intervals chosen cover the range of pulse durations for which the most significant change of direct excitability of pyramidal cells was observed by Stoney et al. (1968; see their Fig. 6: the chronaxie of neocortical pyramidal cells is just above the lowest intervals used here). In double-pulse experiments the intensity of the first ("conditioning") pulse was adjusted to a charge transfer that was just sub-threshold (i.e., it did not elicit a response), or to a suprathreshold
charge transfer showing a clear response. The second (“test”) stimulus was then varied as described for the single-pulse experiments. The interpulse interval was 75 ms. In a second set of double-pulse experiments the intensities of both pulses were set to the same suprathreshold levels. The interpulse interval was 75 ms. In a second set of double-pulse experiments the intensities of both pulses were set to the same suprathreshold level and the interval between the pulses was varied in a pseudo-random fashion (25, 50, 100, and 200 ms).

Data analysis

The calculation of response parameters for the short latency response was hampered by the stimulus artifact and thus had to be restricted to single units with a good signal-to-noise ratio. Figure 1, A and B demonstrates the assessment of latencies of single units that could be performed accurately to within a minimum of 2 ms. The stimulus artifact consisted of a fast phase in which its amplitude was very high, driving the amplifiers into saturation lasting for <2 ms and followed by a slow positive wave ending at around 5 ms. Short-latency spikes residing on the slow phase of the artifact typically could not be detected using a voltage threshold (Fig. 1A). This, however, could be commonly achieved by blanking the high-amplitude phase of the stimulus artifact (0 to 2 ms after onset of the stimulus; gray area in Fig. 1, A and B), subsequent differentiation, and the usage of a threshold of voltage slope (μV/ms). Spike extraction was realized by a semiautomatic MATLAB program (MathWorks, Natick, MA). Voltage traces of all spike trains thus extracted were visually inspected by the experimenter to exclude errors.

Peristimulus time histograms (PSTHs) were computed as spike renewal densities (Abeles 1982) at a resolution of 0.1 ms (for fast excitation) and 1 ms (for inhibition and rebound response) to estimate firing rates at different points in time around the stimulation. They were used to quantify excitatory bursts and inhibitory responses after electrical stimulation. The spontaneous frequency (f spont ) was the average rate before the application of an electrical stimulus (horizontal black broken line in Fig. 1, C and D). For the analysis of fast excitatory responses, the PSTH was low-pass filtered by passing a Gaussian (kernel length 5 bins) over it. The unit was counted as generating a response if the firing rate increased by 1.25f spont (denoted “thresh” in Fig. 1C) in an interval of 2 to 10 ms after stimulation. The rebound response after the inhibitory response was quantified the same way. The duration of excitatory responses was taken as the time the firing frequency stayed above threshold (interval 1 in Fig. 1C). The strength of the response was defined as the integral of the response above the spontaneous firing rate yielding the number of excess spikes per trial (gray area in Fig. 1C). For the analysis of inhibitory responses, the PSTH was low-pass filtered by passing a Gaussian (kernel length 50 bins) over it. A unit was classified as responding if the firing rate undershot 0.75f spont (denoted “thresh” in Fig. 1D). The duration of the inhibitory response was measured from the stimulation to the point in time where the firing rate crossed the threshold again (interval 1 in Fig. 1D). The strategy to calculate the response frequency f resp and the strength of the response deviated from that used for excitation. The reason was that the minimal frequency during the inhibition in many cases was zero but the slope back to the spontaneous firing rate varied from case to case. We thus took the average frequency within the interval 12 to 80 ms after stimulus onset as f resp (see interval 2 in Fig. 1D). The strength of the inhibitory response was computed as 1−(f resp/f spont ).

Test for antidromic invasion

Testing for antidromic invasion was realized by applying spike-triggered stimulation and comparing the response strength and latencies of single units in a “collision” situation with those obtained in a “no collision” situation. The “collision” experiment was realized by triggering the stimulation (4.8 nC) on the occurrence of a single-unit action potential at a delay of 1 ms. The “no collision” experiment differed from this by a longer delay that exceeded the response latency but was used typically about 10 ms) to ensure that the axons contributing to an eventual antidromic response recovered from the refractory period before the onset of stimulation. The duration of the command pulse that triggered the stimulator was set to 1 s, thus defining a lower limit for possible interstimulus intervals. As a control for possible introduction of features of temporal patterns in spike trains by the spike-triggered nature of the recordings (i.e., features of the autocorrelagram), we performed control recordings that were identical to the “collision” and “no collision” tests in that they were recorded in a spike-triggered way but the stimulator was deactivated. The response strength and latency apparent in “collision” and “no collision” experiments was calculated after subtraction of the control histograms (Fig. 4, A and B).
RESULTS

In a preliminary set of experiments, the range of stimulus intensities that would evoke cortical responses in units at distances ≤2.25 mm was qualitatively explored. In these experiments, stimulation of 0.1 to 0.4 nC (1 to 4 μA at 0.1 ms) never induced visible neuronal responses (80 out of 80 units; Fig. 2A). Higher stimulus intensity of 0.8 nC (8 μA at 0.1 ms) evoked excitatory and/or inhibitory responses in 4 of these cases, a fraction that could be increased to 33 out of 80 cases at the highest intensity tested (4.8 nC equivalent to 8 μA at 0.1 ms; Fig. 2A). Guided by these results we set the range of stimulus intensities to be used for the quantitative analyses from 0.8 to 4.8 nC. It should be noted that this range of intensities is lower than typical intensities used in earlier studies to evoke behavioral responses in rat, cat, and monkeys by a factor of 10 to 2,000 (Tehovnik 1996; see his Fig. 10 and references therein). However, studies that deliberately sought to minimize stimulation currents showed that stimulation intensities comparable to those used here readily modulated behavior (Salzman et al. (1990) used repetitive stimulation at 2 Hz).

FIG. 2. Typical responses to microstimulation. Raster plots and PSTHs (recorded 450 μm away from stimulation electrode) are plotted on different time scales. A: range of effective stimulation intensity demonstrated by typical multunit recording (scales of leftmost histogram apply to all; bin size, 1 ms). In a set of preliminary experiments, intensities < 0.8 nC evoked neuronal responses in 4 of 80 cases. At intensities of 4.8 nC responses could be evoked in 35 of 80 cases. Based on these results range of stimulation intensities used for quantitative analyses (see Figs. 3 and 6 and Tables 1–3) was restricted from 0.8 to 4.8 nA. B: excitatory response (single-unit recording; bin size, 0.1 ms). Stimulation (4.0 nC) occurred at time 0. C: full sequence of responses consisting of fast excitatory, long-lasting inhibitory and rebound response (indicated by arrows) (multunit recording; bin size, 1 ms). Stimulation (4.0 nC) occurred at time 0. D: fast excitatory response followed stimulation frequencies of ≤40 Hz (occurrence of stimulus pulses are marked with triangles along time axis). Long-lasting inhibition shows complex interaction pattern best visible with stimulation at intervals of 100 ms (10 Hz), corresponding roughly to its duration. Inhibitory responses to individual pulses merged at stimulation frequencies of 20 and 40 Hz. Last pulse in these trains readily evoked inhibitory response.
responses obtained in the major responsive area (no visible effect and there was no sign of patchiness of the orientation of the electrode array on the cortical surface had surprisingly similar from case to case. In particular, the (Fig. 2D). The spatial pattern of neuronal responses was stronger stimulus intensity (>2.4 nC) evoked a variable rebound excitation after the long inhibition that lasted for several hundreds of milliseconds (Fig. 2C). In 10 out of 11 cases tested the short-term excitatory spikes followed stimulus trains delivered at frequencies of 5, 10, 20, and 40 Hz (Fig. 2D). The spatial pattern of neuronal responses was surprisingly similar from case to case. In particular, the orientation of the electrode array on the cortical surface had no visible effect and there was no sign of patchiness of the responses obtained in the major responsive area (≤1,350 μm) (Fig. 5 shows a representative example of an inhibitory response with a complete set of recordings from all electrodes). We thus did not try to correlate response patterns to the location of electrodes within the rat’s somatosensory body map. Also the neuronal responses at different depths corresponding to layer 2 to layer 6 (130 to 1,467 μm below the pia) were uniform, confirming earlier results in association cortex (Contreras et al. 1997) and were thus pooled for the analysis. Quantitative analysis was performed on 72 single-unit spike trains with signal-to-noise ratio >3 (see

nC in monkey area MT to bias direction estimation of the animal; Tehovnik et al. (2003) found a minimum of 0.6 nC applied repetitively to evoke saccades in monkey area V1].

Typically, suprathreshold electrical microstimulation at all cortical depths induced a short latency excitatory response containing a single spike or doublet of spikes (Fig. 2B), followed by a conspicuous inhibition, which lasted for 100 to 150 ms after the stimulation (Fig. 2C). In some cases stronger stimulus intensity (>2.4 nC) evoked a variable rebound excitation after the long inhibition that lasted for several hundreds of milliseconds (Fig. 2C). In 10 out of 11 cases tested the short-term excitatory spikes followed stimulus trains delivered at frequencies of 5, 10, 20, and 40 Hz (Fig. 2D). The spatial pattern of neuronal responses was surprisingly similar from case to case. In particular, the orientation of the electrode array on the cortical surface had no visible effect and there was no sign of patchiness of the responses obtained in the major responsive area (≤1,350 μm) (Fig. 5 shows a representative example of an inhibitory response with a complete set of recordings from all electrodes). We thus did not try to correlate response patterns to the location of electrodes within the rat’s somatosensory body map. Also the neuronal responses at different depths corresponding to layer 2 to layer 6 (130 to 1,467 μm below the pia) were uniform, confirming earlier results in association cortex (Contreras et al. 1997) and were thus pooled for the analysis. Quantitative analysis was performed on 72 single-unit spike trains with signal-to-noise ratio >3 (see

METHODS) for excitation and 126 spike trains for inhibition (multiunit: n = 84; single unit: n = 42). All spike trains used for the quantitative analysis were recorded under pseudo-random presentation of stimulus intensities. An additional 351 units were not analyzed quantitatively because the stimulus intensity was not ordered in a pseudo-random fashion; however, they qualitatively supported the main results reported in the following sections. Multiunit and single-unit spike trains were pooled for the quantitative analysis of the inhibition because they did not show obvious differences in response properties. In a first step we assessed the percentage of units that responded to the stimulus for the 2 factors stimulus intensity and distance from the stimulating electrode. From the responding units, we assessed 3 response variables: strength, latency, and duration. To assess statistically significant effects of the 2 factors on the response variables MANOVA was employed.

FIG. 3. Excitatory response. Percentage of single units at different distances from recording electrode responding to varying intensity of stimulation. Units recorded at all cortical depths were pooled. Absolute number of responding cells is indicated on columns. See also Table 1.

FIG. 4. Excitatory response, collision test for antidromic invasion. A: control recordings. Firing with respect to spontaneously occurring action potentials (arrows) of a single unit is shown. Minimal interval between action potentials that triggered recording was 1 s. Resulting histogram reflects features of autocorrelogram function introduced by these conditions of spike-triggered recording. B: spike-triggered stimulation at delay of 1 ms (“collision”) and 10 ms (“no collision”). Cell and recording conditions were identical to those shown in A with exception that stimulation device was activated (stimulation occurred at time 0). If antidromic invasion played a role in generation of response, it should lead to extinction of spikes by collision in case of short delay but not in case of long delay. This was not the case. C: response strength of 16 single units recorded under conditions shown in A and B. Measurement was taken as area under peak in test situation (multiunit: n = 84; single unit: n = 42). All spike trains used for the quantitative analysis were recorded under pseudo-random presentation of stimulus intensities. An additional 351 units were not analyzed quantitatively because the stimulus intensity was not ordered in a pseudo-random fashion; however, they qualitatively supported the main results reported in the following sections. Multiunit and single-unit spike trains were pooled for the quantitative analysis of the inhibition because they did not show obvious differences in response properties. In a first step we assessed the percentage of units that responded to the stimulus for the 2 factors stimulus intensity and distance from the stimulating electrode. From the responding units, we assessed 3 response variables: strength, latency, and duration. To assess statistically significant effects of the 2 factors on the response variables MANOVA was employed.
Figure 3 depicts the percentage of single units that showed a short excitatory response for all distances from the stimulation site and for all stimulation intensities. The probability of obtaining an excitatory response was close to one (\( \geq 90\% \)) in the higher range of stimulus intensities and the shortest distance to the stimulation site (450 \( \mu m \)). The percentage then fell rapidly with distance and decreasing stimulus intensity. Within a range of 1,350 \( \mu m \) and a charge transfer of more than 1.6 nC, about 50% of the cells responded. Farther away only 2 responding cells were found (out of 25).

Table 1 shows the response properties of the cells that were classified as responding. The strength of the fast excitatory response was most conspicuously related to the distance of the cell to the stimulation site. It was strongest at 450 \( \mu m \) with up to a mean of 0.65 excess spikes/trial (maximum 0.9 excess spikes/trial in an individual case) and declined to about half this value at 1,350 \( \mu m \). Latency was related to the distance as well. The latency at 450 \( \mu m \) was on average 2.39 ms and increased to 2.73 ms at 1,350 \( \mu m \). The average progression of activity across the first 3 electrodes occurred therefore at a velocity of 2.6 m/s. In the 2 cases observed farther away than 1,350 \( \mu m \) the latency was unusually long (about 6 ms). The duration of the response was between 1.3 and 2.8 ms, indicating that the response showed a high temporal precision. The highest precision (i.e., shortest response) was found in cells that were recorded close to the stimulation site. Significance testing was performed on the part of the data matrix that satisfied the complete design criterion of MANOVA (entries of \( n > 1 \)). The range, thus tested, was 1.6–4.8 nC and 450–1350 \( \mu m \) and contained numbers of cells as given in the first section of Table 1. It should be noted that the 2 sole cells located farther away than 1,350 \( \mu m \) showed outlying values for all 3
variables tested. They showed a strong response at a long latency of over 5 ms. Because the factors for the 2 cells fell outside the testable range they did not enter the statistical analysis. A significant dependency was determined for all 3 variables on the distance to the stimulation site (response strength: \( P = 0.003 \); latency: \( P = 0.018 \); duration: \( P = 0.01 \)). There was a certain trend in the data also for the second factor, the intensity of stimulation. The strength of the response tended to be larger with higher stimulation intensity. This was most obvious for the 5 cells close to the stimulation site (450 and 900 \( \mu \)m) that responded to the weakest stimulation intensity of 0.8 nC. They barely reached the criterion for a response and displayed weak strengths of around 0.1 excess spikes per trial. The duration of the response tended to grow longer with increasing stimulus intensity, indicating that with stronger stimulation some cells fired doublets of spikes. The latency showed a slight tendency to increase by around 0.2 ms from low to high stimulation intensity. None of these trends, however, reached significance (\( P > 0.05 \)).

To test for antidromic activation, stimulation pulses triggered by a spontaneous action potential of a responding cell were applied ("collision experiment"). The underlying (sometimes tacit) assumption for the classic version of this test (Lipski 1981) was that there is a clear dichotomy between antidromic and orthodromic conductance (i.e., the origin of the evoked spike was expected to be either antidromic or orthodromic conductance, but never both at the same time). The classic interpretation of spike-triggered stimulation is then simple: the evoked response is entirely blocked in case of antidromic invasion but it is left untouched if the conveyance is orthodromic. In the present experimental situation, however, such a dichotomy did not exist because stimulation as well as recording sites were located nearby in the cortical gray matter with rich interconnections between them running both ways. We thus resorted to a quantitative version of the collision test to detect a possible contribution of antidromic invasion. Triggering the stimulation by a spontaneously occurring action potential created a situation of possible "collision" of the spontaneous spike and an antidromically conveyed one if the delay between spike and stimulation was below the response latency. A "no collision" situation was created by increasing the spike-stimulation delay to values exceeding the response latency plus a presumed axonal refractory period (typical value used here was 10 ms; Fig. 4B). We corrected for firing rate modulations arising from temporal patterning of spikes (i.e., features of the autocorrelogram introduced by the spike-triggered nature of the recording) using a control recording obtained under spike-triggered conditions but without stimulation. These controls were subtracted from the test histograms after aligning the histograms to the spike times that served as trigger (Fig. 4A).

### Table 1. Properties of the fast excitatory response with respect to stimulation intensity and distance from stimulation electrode

<table>
<thead>
<tr>
<th>Excitation</th>
<th>0.8 nC</th>
<th>1.6 nC</th>
<th>2.4 nC</th>
<th>3.2 nC</th>
<th>4.0 nC</th>
<th>4.8 nC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of units</td>
<td>450 ( \mu )m ( n = 16 )</td>
<td>3</td>
<td>12</td>
<td>15</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>900 ( \mu )m ( n = 19 )</td>
<td>2</td>
<td>11</td>
<td>14</td>
<td>14</td>
<td>17</td>
<td>17</td>
</tr>
<tr>
<td>1,350 ( \mu )m ( n = 12 )</td>
<td>—</td>
<td>2</td>
<td>5</td>
<td>8</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>1,800 ( \mu )m ( n = 11 )</td>
<td>—</td>
<td>—</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>2,250 ( \mu )m ( n = 6 )</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>2,700 ( \mu )m ( n = 8 )</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Strength (spikes)</td>
<td>450 ( \mu )m</td>
<td>0.15 ± 0.05</td>
<td>0.52 ± 0.50</td>
<td>0.56 ± 0.45</td>
<td>0.63 ± 0.45</td>
<td>0.63 ± 0.58</td>
</tr>
<tr>
<td>900 ( \mu )m</td>
<td>0.11 ± 0.05</td>
<td>0.35 ± 0.53</td>
<td>0.49 ± 0.49</td>
<td>0.53 ± 0.53</td>
<td>0.53 ± 0.60</td>
<td>0.52 ± 0.65</td>
</tr>
<tr>
<td>1,350 ( \mu )m</td>
<td>—</td>
<td>0.12 ± 0.00</td>
<td>0.28 ± 0.17</td>
<td>0.21 ± 0.22</td>
<td>0.23 ± 0.19</td>
<td>0.32 ± 0.19</td>
</tr>
<tr>
<td>1,800 ( \mu )m</td>
<td>—</td>
<td>—</td>
<td>0.64</td>
<td>0.60 ± 0.08</td>
<td>0.72 ± 0.12</td>
<td>0.68 ± 0.03</td>
</tr>
<tr>
<td>2,250 ( \mu )m</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>2,700 ( \mu )m</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Latency (ms)</td>
<td>450 ( \mu )m</td>
<td>2.10 ± 0.14</td>
<td>2.16 ± 0.27</td>
<td>2.36 ± 0.61</td>
<td>2.30 ± 0.32</td>
<td>2.41 ± 0.31</td>
</tr>
<tr>
<td>900 ( \mu )m</td>
<td>2.05 ± 0.05</td>
<td>2.18 ± 0.30</td>
<td>2.26 ± 0.35</td>
<td>2.67 ± 1.02</td>
<td>2.93 ± 1.11</td>
<td>3.18 ± 1.01</td>
</tr>
<tr>
<td>1,350 ( \mu )m</td>
<td>—</td>
<td>2.55 ± 0.25</td>
<td>2.56 ± 0.22</td>
<td>3.03 ± 0.60</td>
<td>2.64 ± 0.26</td>
<td>2.87 ± 0.29</td>
</tr>
<tr>
<td>1,800 ( \mu )m</td>
<td>—</td>
<td>—</td>
<td>6.2</td>
<td>6.05 ± 0.15</td>
<td>5.65 ± 0.25</td>
<td>5.75 ± 0.15</td>
</tr>
<tr>
<td>2,250 ( \mu )m</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>2,700 ( \mu )m</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Duration (ms)</td>
<td>450 ( \mu )m</td>
<td>1.30 ± 0.41</td>
<td>1.44 ± 0.59</td>
<td>1.57 ± 0.70</td>
<td>1.60 ± 0.61</td>
<td>1.51 ± 0.45</td>
</tr>
<tr>
<td>900 ( \mu )m</td>
<td>1.50 ± 0.30</td>
<td>1.39 ± 0.48</td>
<td>1.72 ± 0.69</td>
<td>1.71 ± 0.52</td>
<td>1.96 ± 0.57</td>
<td>1.64 ± 0.36</td>
</tr>
<tr>
<td>1,350 ( \mu )m</td>
<td>—</td>
<td>2.20 ± 0.60</td>
<td>1.98 ± 0.59</td>
<td>1.63 ± 0.47</td>
<td>1.76 ± 0.43</td>
<td>2.54 ± 1.08</td>
</tr>
<tr>
<td>1,800 ( \mu )m</td>
<td>—</td>
<td>—</td>
<td>2.80</td>
<td>2.35 ± 0.05</td>
<td>2.60 ± 0.30</td>
<td>2.30 ± 0.10</td>
</tr>
<tr>
<td>2,250 ( \mu )m</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>2,700 ( \mu )m</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

Values are means ± SD. All statistical analysis using MANOVA was done on the section of the matrix indicated by the broken frame (satisfying the complete design requirement). Compare to Fig. 3.

*J Neurophysiol • Vol. 90 • November 2003 • www.jn.org*
The short latency excitatory response was typically followed by a widespread and long-lasting depression of firing rates. A representative multielectrode recording is shown in Fig. 5. PSTHs for multiunit spike trains recorded at all distances and for all stimulation intensities are depicted. The threshold to evoke the inhibitory response in this example was 1.6 nC and the response was detected up to a distance of 1,800 μm from the stimulation electrode. The stimulus intensity required to evoke the inhibitory response was higher for greater distances from the stimulation electrode (marked with hollow arrows in Fig. 5). A remarkable finding was that the duration of the inhibitory response was almost constant for the whole range of stimulus intensities applied. Whenever an inhibitory response was evoked, it had a fixed duration that could not be changed by higher stimulus intensities and was the same at different locations with respect to the stimulus electrode. After the end of the inhibition a rebound excitation was visible at thresholds higher than that evoking inhibition alone (marked with filled arrows in Fig. 5).

The characteristics demonstrated by the example in Fig. 5 match those observed in the population of recorded spike trains (Fig. 6). The overall distribution of units showing an inhibitory response was similar to that seen with fast excitation. At the lowest charge transfer applied (0.8 nC) 23% of the units closest to the stimulation electrode (450 μm) showed a long-lasting inhibitory response. The probability to detect it increased with increasing stimulus intensity to a maximum of 85% and decayed to very low values at 1,800 μm from the stimulation size but was observed in 2 cases even at the greatest distance (2.7 mm).

Table 2 shows the response variables of the units that were classified as generating an inhibitory response. The duration was fixed, irrespective of location and stimulation intensities, as seen in the example of Fig. 5. The part of the data matrix that contained at least n ≥ 1 spike trains to satisfy the complete design criterion of MANOVA (distance from 450 to 1,800 μm; charge transfer from 1.6 to 4.8 nC; see Table 2) confirmed this impression: no statistical significance was obtained (P > 0.05). The strength of the inhibitory response, on the other hand, was significantly dependent on intensity of stimulation as well as distance from the recording site. It was highest (0.85) in units recorded at 450 μm distance and stimulated with the highest charge transfer. The strength fell to around 0.3 in the responding cells farther away than 1,800 μm. The dependency of the response strength on both factors was statistically significant in the range of factors tested (MANOVA, stimulation intensity: P < 0.04, distance: P < 10⁻⁶; see Table 2).

Table 2. Properties of the inhibitory response with respect to stimulation intensity and distance from stimulation electrode

<table>
<thead>
<tr>
<th>Strength (%)</th>
<th>0.8 nC</th>
<th>1.6 nC</th>
<th>2.4 nC</th>
<th>3.2 nC</th>
<th>4.0 nC</th>
<th>4.8 nC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of units</td>
<td>450 μm (n = 26)</td>
<td>6</td>
<td>22</td>
<td>21</td>
<td>20</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>900 μm (n = 25)</td>
<td>5</td>
<td>10</td>
<td>12</td>
<td>13</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>1,350 μm (n = 26)</td>
<td>—</td>
<td>7</td>
<td>9</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>1,800 μm (n = 17)</td>
<td>—</td>
<td>2</td>
<td>5</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>2,250 μm (n = 16)</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>2,700 μm (n = 16)</td>
<td>—</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Duration (ms)</td>
<td>450 μm</td>
<td>76 ± 22</td>
<td>107 ± 33</td>
<td>102 ± 39</td>
<td>127 ± 26</td>
<td>122 ± 37</td>
</tr>
<tr>
<td></td>
<td>900 μm</td>
<td>57 ± 13</td>
<td>109 ± 33</td>
<td>129 ± 33</td>
<td>117 ± 49</td>
<td>133 ± 49</td>
</tr>
<tr>
<td></td>
<td>1,350 μm</td>
<td>123</td>
<td>128 ± 60</td>
<td>125 ± 47</td>
<td>138 ± 51</td>
<td>117 ± 62</td>
</tr>
<tr>
<td></td>
<td>1,800 μm</td>
<td>—</td>
<td>102 ± 0.5</td>
<td>110 ± 10</td>
<td>111 ± 60</td>
<td>124 ± 30</td>
</tr>
<tr>
<td></td>
<td>2,250 μm</td>
<td>85</td>
<td>21</td>
<td>130</td>
<td>167 ± 30</td>
<td>155 ± 19</td>
</tr>
<tr>
<td></td>
<td>2,700 μm</td>
<td>—</td>
<td>81 ± 8</td>
<td>121 ± 4</td>
<td>120</td>
<td>86</td>
</tr>
</tbody>
</table>

Values are means ± SD. All statistical analysis using MANOVA was done on the section of the matrix indicated by the broken frame (satisfying the complete design requirement). Compare to Fig. 6.

Inhibitory response

0.27 excess spikes, latency 2.87 ± 0.84, n = 16, t-test for paired samples, P > 0.05 for both parameters; Fig. 4C). These results do not point to a major role of antidromic invasion for the generation of the short latency excitatory response.

Rebound response

The rebound response was not a common observation at the stimulus intensities used in this study. The rebound response after the inhibition could be avoided (except in one case) by keeping the stimulation intensity below 2.4 nC (Table 3). The highest percentage of cells generating a rebound response (between 15 and 19%) was observed at a stimulation intensity of 4.8 nC and distances ≈1,350 μm. The rebound response showed variable shapes from unit to unit. For instance, the duration was on average 262 ms and ranged in individual cases from 86 to 520 ms. There was a slight tendency to obtain stronger and longer rebound responses at sites close to the stimulation electrode. The effect, however, did not reach significance within the part of the matrix (distance from 450 to...
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The duration of the inhibitory response of more than 100 ms must have a critical impact on the local effect of repetitive neocortical stimulation at rates higher than about 10 Hz. This was demonstrated by the complex interaction patterns of the inhibitory response observed at a stimulation frequency of 10 Hz (Fig. 2D). To test the temporal interaction more quantitatively, we employed double pulses given by the stimulation electrode at an interval of 75 ms such that the second pulse would fall halfway into the ongoing inhibitory response evoked by the first pulse. The test pulse (the single pulse in control trials or 2nd pulse in the double-pulse trials) was varied across the whole range of intensities (0.8 to 4.8 nC; Fig. 7A). The conditioning (1st) pulse was chosen to be either subthreshold (i.e., not evoking an overt inhibitory response; Fig. 7B) or just suprathreshold (Fig. 7C). The single pulse and these 2 double-pulse conditions were applied in pseudo-random order. The effect of the conditioning pulse on the inhibition evoked by the test pulse was then compared with the effect of the single pulse alone. Figure 7 demonstrates PSTHs of a typical experiment (electrode distance 450 μm). The inhibition evoked by a test pulse after a subthreshold conditioning pulse (Fig. 7B) was undistinguishable from the one evoked by a single pulse alone (Fig. 7A; for purposes of comparison, the arrow depicts the duration of the inhibition evoked by a single pulse of 1.6 nC charge transfer and has the same length throughout the plot). In the second condition when the conditioning pulse evoked an inhibitory response itself, the test pulse elongated the ongoing inhibition by maximally 25 ms at the highest stimulus intensities of 4.0 and 4.8 nC (Fig. 7C). This elongation, however, was clearly less than would have been expected from a linear superposition (75 ms).

Temporal interaction of inhibitory response

The results of the example shown in Fig. 7 are reflected in the population data. Figure 8A plots the duration of inhibition evoked by a single pulse and by the test pulse in the case of a subthreshold conditioning pulse. Measurements from spike trains recorded at different distances were pooled. For all stimulus intensities, the null hypothesis that the duration of the inhibition evoked by the test pulse was not different from the one evoked by the single pulse had to be accepted (Fig. 8A, t-test for paired samples, P > 0.05 for all stimulation intensities, 0.8 nC: n = 4; 1.6 nC: n = 19; 2.4 nC: n = 25; 3.2 nC: n = 25; 4.0 nC: n = 28; 4.8 nC: n = 28). The second graph (Fig. 8B) depicts the results from experiments in which the conditioning pulse was suprathreshold. It compares the duration of the inhibition as expected if the test pulse evoked an inhibition of normal length (duration of inhibition after a single pulse plus the interpulse interval) with the actual duration of the inhibition measured in the double-pulse experiments. For stimulus intensities higher than 1.6 nC, the observed inhibition was significantly shorter than expected by linear superposition (Fig. 8B, t-test for paired samples, P < 0.05 for stimulation intensities marked by an asterisk; 0.8 nC: n = 19; 1.6 nC: n = 19; 2.4 nC: n = 20; 3.2 nC: n = 25; 4.0 nC: n = 21; 4.8 nC: n = 21).

The double-pulse experiments described so far indicated that inhibitory responses evoked by electrical stimulation interact in a highly nonlinear way. Subthreshold conditioning pulses do not influence the characteristics of an inhibitory response, whereas, conversely, overt inhibitory responses can be modified by a second pulse only to a small degree. To investigate the temporal properties of this interaction pattern, we systematically varied the pulse interval with which a second pulse was applied after a suprathreshold conditioning pulse. The recording shown in Fig. 9 featured an inhibitory response after a single pulse lasting for somewhat longer than 100 ms (electrode distance 450 μm). The control trials (single pulse) and

<table>
<thead>
<tr>
<th>Number of units</th>
<th>0.8 nC</th>
<th>1.6 nC</th>
<th>2.4 nC</th>
<th>3.2 nC</th>
<th>4.0 nC</th>
<th>4.8 nC</th>
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<tbody>
<tr>
<td>450 μm (n = 26)</td>
<td>1</td>
<td>3</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>900 μm (n = 25)</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>1,350 μm (n = 26)</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>1,800 μm (n = 17)</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>2,250 μm (n = 16)</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>2,700 μm (n = 16)</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Strength (spikes)</th>
<th>0.8 nC</th>
<th>1.6 nC</th>
<th>2.4 nC</th>
<th>3.2 nC</th>
<th>4.0 nC</th>
<th>4.8 nC</th>
</tr>
</thead>
<tbody>
<tr>
<td>450 μm</td>
<td>0.58</td>
<td>0.50</td>
<td>0.50</td>
<td>0.46</td>
<td>0.45</td>
<td>0.45</td>
</tr>
<tr>
<td>900 μm</td>
<td>0.43</td>
<td>0.39</td>
<td>0.39</td>
<td>0.37</td>
<td>0.35</td>
<td>0.35</td>
</tr>
<tr>
<td>1,350 μm</td>
<td>0.42</td>
<td>0.43</td>
<td>0.37</td>
<td>0.39</td>
<td>0.38</td>
<td>0.38</td>
</tr>
<tr>
<td>1,800 μm</td>
<td>0.28</td>
<td>0.37</td>
<td>0.39</td>
<td>0.38</td>
<td>0.38</td>
<td>0.38</td>
</tr>
<tr>
<td>2,250 μm</td>
<td>0.22</td>
<td>0.22</td>
<td>0.22</td>
<td>0.22</td>
<td>0.22</td>
<td>0.22</td>
</tr>
<tr>
<td>2,700 μm</td>
<td>0.34</td>
<td>0.34</td>
<td>0.34</td>
<td>0.34</td>
<td>0.34</td>
<td>0.34</td>
</tr>
</tbody>
</table>

The values used in the analysis are means ± SD. All statistical analysis using MANOVA was done on the section of the matrix indicated by the broken frame (satisfying the complete design requirement).
double-pulse trials at intervals of 25, 50, 100, and 200 ms were presented in pseudo-random order. The general finding was that the second pulse, if it fell within the period of inhibition, elongated the inhibition by about 25 ms as seen before. This was true even for the trials in which the second pulse was given at a point in time just before the cessation of the inhibition (interval 100 ms). Pulses that occurred after the end of the inhibitory response evoked an inhibitory response of their own by interrupting the rebound excitation. The average effect on the duration of inhibition computed from all recording sites is shown in Fig. 10. Statistically, the duration of the compound inhibition of double pulses at all intervals smaller than 200 ms was indistinguishable (one-way ANOVA, $P > 0.05, n = 76$; unit recordings from all distances were pooled). At an interval of 200 ms the measured duration corresponded to the one observed after single pulses, given that the second pulse evoked a separate response of its own. We conclude from these experiments that the highly nonlinear mode of interaction is active throughout the entire duration of the inhibitory response.

One possibility to explain the inability of the second pulse to prolong the inhibitory response is the failure to activate fibers at the stimulation site after the second pulse. This possibility, however, was rendered unlikely by the finding that the excitatory response to the second pulse (or a train of pulses; see Fig. 2D) was largely intact. Figure 11A shows an example of a unit stimulated with a double pulse at different intervals (distance from stimulation site 900 μm). The stimulation intensity for both pulses (conditioning: gray histogram; test: black histograms) was identical and suprathreshold to evoke an inhibition (4.0 nC). Test pulses applied at interpulse intervals of 25, 50, and 100 ms fell within the inhibitory period and consistently evoked a short latency excitatory response at even a higher amplitude than that generated by the conditioning pulse. This tendency was observed also in the population average, although it did not reach statistical significance (Fig. 11B; one-way ANOVA, $P > 0.05, n = 8$; neurons recorded at different distances from the stimulation electrode were pooled for statistical analysis). The response after a test pulse at 200 ms occurred after the end of the inhibitory response and was more nearly comparable with the first response.

**DISCUSSION**

**General considerations**

The present study provides an estimate of the maximal resolution of signals that can be transferred to the neocortex by microstimulation. To this end the spatiotemporal blur of activity evoked at the stimulation site was measured. Although we consider the neocortex in many species and cortical areas to be built up according to a prototypic cortical architecture (for review, see Braitenberg and Schütz 1991), it has to be borne in mind that there are certain variations between different cortical areas in architecture and, particularly, in the layout of horizontal connections (Lund et al. 1993). Furthermore, at some sites...
on the cortical surface, within or between neighboring areas, distinct spatial discontinuities of horizontal connections exist (e.g., Manger et al. 1997). Such variability might affect the detailed profile of activity evoked by electrical stimulation and deserves attention in each case in which microstimulation is used. Nevertheless, in view of the prototypic architecture of neocortex we feel confident that the present results obtained in rat somatosensory cortex provide a correct description in general terms also for other cortical areas and/or species.

How much did the effects of ketamine anesthesia contribute to the patterns of evoked activity observed in the present study? Ketamine takes effect by selective blockade of NMDA receptors leaving fast transmission by AMPA and, notably, GABA receptors intact (Ebert et al. 1997; Sonner et al. 2003). In contrast to other anesthetics, the responsiveness of the neocortex is not reduced—spontaneous firing rate and sensory evoked cortical activity are actually enhanced (Kayama et al. 1972). The main statements of the present study are based on a sequence of fast excitation and inhibition evoked by electrical stimulation. As we discuss later in detail, both responses are presumably based on direct electrical activation of fibers around the stimulation electrode followed by antidromic and orthodromic monosynaptic conveyance. Because of this relatively direct link of these responses to the activating current we believe that their susceptibility to pharmacological effects of ketamine should be relatively small. Support for this notion comes, first, from the finding of very similar sequences of excitation and inhibition after peripheral sensory stimulation in the absence of general anesthesia (Simons 1978; Simons and Carvell 1989) as well as after electrical stimulation in vitro (Hirsch and Gilbert 1991). Second, we confirmed the existence of a comparable pattern of cortical excitation and long inhibi-

FIG. 8. Population data of double-pulse experiments. Duration of inhibitory response is plotted against intensity of test pulse. All spike trains featuring an inhibitory response were used for this analysis. Single and multiunits recorded at different distances to stimulation electrode were pooled. A: double pulse: conditioning at subthreshold stimulus intensity (compare Fig. 7B). Duration of inhibitory response evoked by test pulse (black diamonds) is not significantly different from that observed after single pulses (squares) of same intensity (t-test for dependent samples, \( P > 0.05 \) for all stimulus intensities). B: double pulse: conditioning at suprathreshold stimulus intensity (compare Fig. 7C). Inhibition (as measured from occurrence of conditioning pulse) shows small elongation at higher intensity of test pulse (black diamonds). Observed duration is significantly shorter than that expected from linear superposition of inhibitory responses (open squares, t-test for dependent samples, \( P < 0.05 \) when marked with asterisks). Mean duration observed after single pulses is plotted as well (gray).

FIG. 9. Double-pulse stimulation: variation of interstimulus interval. Example was recorded at distance of 450 mm from stimulation electrode (stimulation intensity for both pulses: 4.0 nC). Up to an interval of 100 ms test pulse induced only a small change of about 25 ms in duration of inhibitory response. Note that fast excitation was blanked. For purposes of comparison empty arrows are of same length throughout figure.
cal stimulation to activate preferentially occurring activation originates from the known bias of electrical activity after cortical microstimulation in preliminary experiments using double-pulse stimulation is elongated by about 25 ms compared with that observed with single pulses because second pulse occurred after end of inhibitory response (depicted in gray; compare Fig. 9).

Origin of electrically evoked neuronal responses: the spatiotemporal blur

A decisive difference of electrically evoked to naturally occurring activation originates from the known bias of electrical stimulation to activate preferentially fibers of passage rather than somata (Gustafsson and Jankowska 1976; McIntyre and Grill 2000; Nowak and Bullier 1998a,b; Ranck 1975; Rattay 1999). A multitude of fibers pass by the stimulation site, many of which are not directly linked to the site of stimulation because their origin as well as their target reside at randomly distributed locations around the stimulated site. Once this—in functional terms—arbitrarily composed bundle of fibers gets excited, the activity carried along the horizontal axes must be expected to be composed of a complex mixture of antidromic and orthodromic conveyance of action potentials on excitatory and inhibitory fibers that we tag the “spatiotemporal blur.” The present data reflect these expectations because the pattern of evoked activation indicated the contribution of both, excitation and inhibition. Direct antidromic evasion, however, did not play a major role in the generation of the excitatory responses, although it was reported using intracellular recordings (Contreras et al. 1997). It has to be emphasized that the collision experiment employed by us tests the contribution only of direct antidromic conveyance (i.e., on the axon of the cell under observation). Therefore the possibility remains that antidromic conveyance on axons of other, unobserved, neurons (which in turn are synaptically connected to the cell under observation) plays a role.

The notion that the spatiotemporal blur is based on activity in a conglomerate of fibers of different types rather than orderly conveyance on a homogeneous fiber tract may help to explain two unexpected findings of the present study. First, the horizontal progression of the fast excitatory response was about 5 times faster (about 2.6 m/s) than conduction velocities of horizontal axons measured in intracellular recordings (about 0.5 m/s; Nowak and Bullier 1998a). This feature is obviously incompatible with the idea that just one functionally defined type of fibers gets activated by the stimulation. To explain it, complex interactions of the functionally different parts of the activated conglomerate of fibers have to be assumed. It is noteworthy in this respect that at distances greater than 1,350 μm, excitatory responses did not match the characteristics of those typically found closer to the stimulation site. Such remote responses were rare, unusually strong, and showed latencies of more than 5 ms corresponding better to the range of conduction velocities found for individual horizontal fibers (Nowak and Bullier 1998b). It is entirely possible that these responses originated from individual fibers/somata or homogenous fiber tracts activated at the stimulation site. The second unexpected result was that the extent of horizontal spatiotemporal blur was similar at all cortical depths tested and that it did not reflect the patchiness of horizontal connections as known from morphological studies (Kim and Ebner 1999). This finding may be easily explained by the notion that it is mainly fibers of passage (and not fibers that originate or terminate at the stimulation site) that contribute to the observed effects. The spatial specificity in the vertical (i.e., with respect to cortical layers) as well as the horizontal direction (i.e., with respect to other cortical columns), which undoubtedly exist in single neuron to neuron connections, must be expected to get skewed in the compound effect originating from the activation of fibers of passage.

The present results show that the extent of electrically evoked activity (even at intensities close to threshold) was large and seemed to surpass the known physiological spread of activity (e.g., assessed by the size of receptive fields and somatotopic maps) (Ghazanfar and Nicoletis 1999; Simons 1978). However, nonclassical properties of receptive fields in primary sensory areas have been shown to originate from an area several millimeters wide on the cortical surface (Bolz et al. 1989; Moore et al. 1999). Furthermore, a study using voltage-sensitive dye imaging in rat’s somatosensory cortex has shown that sensory activity initially emerges at one cortical column but then is able to progress over large parts of the somatosensory cortex depending on the strength of input (Petersen et al. 2003). In summary then, the minimal extent of “physiological” types of activation may be in a similar range as observed here for electrically evoked activation. Additional evidence in favor of this notion comes from the fact that the overall horizontal spread observed in the present study is in general accordance with the extent of known presumptive anatomical substrates in rat’s somatosensory cortex: intrinsic horizontal fibers (≤2 mm: Gottlieb and Keller 1997; 2 to 3 barrels’ distance: Kim and Ebner 1999) or horizontal branching of afferent thalamocortical fibers (1.3 mm: Arnold et al. 1997).

Neuronal bases for long-lasting inhibition.

The long-lasting inhibition may be attributed either to an activation of inhibitory synapses or to a decrement of the gain of excitatory synapses (or intrinsic membrane excitability) after the first volley of excitation. The contribution of synaptic inhibition is strongly supported by reports demonstrating horizontal spread of inhibitory fibers (Kisvarday 1992), polysyn-

The alternative process—a reduction of excitation in the network—could in principle be carried by 2 different mechanisms. First, intrinsic membrane currents could be triggered by the first wave of excitation and lead to a long-lasting depression of firing rate. Prominent candidates for such a mechanism are long-lasting afterhyperpolarizations (AHPs) in pyramidal neurons based on calcium- or sodium-dependent potassium currents (Schwindt and Crill 1989; Schwindt et al. 1988). Second, a decrement of network excitability could be based on short-term depression of excitatory intracortical synapses (Abbott et al. 1997; Thomson et al. 1993; Tsodyks and Markram 1997). Both mechanisms, AHPs and lowered synaptic efficacy, would reduce excitatory drive from cortical neurons by suppressing firing rates in the population of postsynaptic neurons.

The decisive argument against a significant contribution of these possible mechanisms comes from our double-pulse experiments: We show that the long inhibitory response cannot be elongated, although the arrival of fast excitation at the postsynaptic neuron was undisturbed. If the inhibition were based on slow AHPs, the second pulse should have elongated an ongoing AHP because postsynaptic spikes with concomitant additional influx of calcium/sodium were readily elicited. Supporting this argument, slow AHPs have been shown to be strengthened and elongated by repetitive spiking (Schwindt and Crill 1989; Schwindt et al. 1988). Synaptic depression should be strengthened by a successful second activation of the excitatory synapses as well. In conclusion, both mechanisms, AHPs and synaptic depression, most likely would elongate the inhibitory response to a greater extent and in a more graded way than observed in our experiments.

Our finding that the duration of inhibition is virtually fixed at the low stimulation intensities studied here (see, however, effects of high-intensity stimulation in Krnjevic et al. 1966) is
consequences for electrical stimulation of neocortex

At first glance the temporal properties of the evoked activity seem not to be favorable for reliable signal transfer. The inhibitory response is very slow and leads to a complex interaction pattern at around 10 Hz determined by the nonlinear superposition, whereas it fuses at higher frequencies to form an almost uniform inhibitory background (Fig. 2D; see also Chung and Ferster 1998; Kara et al. 2002). The interaction pattern at frequencies around 10 Hz most likely does not correspond to any type of naturally occurring activity and its effect on the ability of the brain to use these frequencies deserves close attention in future behavioral experiments. Nonetheless, electrical stimulation seems to be able to transmit temporal information faithfully to the cortical substrate by the reliable fast excitatory response. At frequencies >10 Hz the continuous inhibition may even help to make the excitatory responses stand out at a favorable signal-to-noise ratio. The fact that awake behaving monkeys, indeed, were able to extract the rate of about 2.7 mm (1.35 mm to each side) around the stimulation site. —

The present results strongly suggest that the spatial spread of local activity must have been much higher than assumed in the studies cited above and challenges the view that the specific modulation of behavior was a consequence of the limitation of evoked activity to one column (Murase et al. 1993; Nichols and Newsome 2002). To account for column-specific behavioral responses to microstimulation the restriction of evoked activity to one column is not imperative. It is sufficient to assume that the geometrical center of the responding area can be estimated by integrating the activity of a distributed population of cells. Algorithms that perform such readout of population codes have been proposed to exist in different systems (e.g., Deneve et al. 1999) and appear to be well suited to focus and specify the blurred activity on the next stage of cortical processing as a basis for column-specific behavioral effects. The present finding that the percentage of responding cells as well as strength of neuronal responses fall at a monotonic slope over distance from the stimulation site, together with topographic mapping of features on the cortical surface, might be helpful for this task.

In summary, the optimal spatial resolution of multisite stimulation (i.e., electrode tip distances)—at least in some neocortical areas—may eventually be much higher than indicated by the extent of the spatial blur measured here. However, our data implicate that dense microelectrode arrays with tip distances in the order of the width of cortical columns will surely face the problem of spatial interaction of evoked activity. Also, nonlinear temporal interaction of inhibitory responses at low stimulation frequencies have to be taken into account. Future experiments must address the question of how the profile and multisite spatiotemporal interaction of electrically evoked activity and their arrangement on a cortical columnar map affect behavioral responses.

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DISCLOSURES

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