Spatiotemporal Properties of an Evoked Population Activity in Rat Sensory Cortical Slices

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Wu, Jian-young, Li Guan, Li Bai, and Qian Yang. Spatiotemporal properties of an evoked population activity in rat sensory cortical slices. *J Neurophysiol* 86: 2461–2474, 2001. We have examined the spatiotemporal properties of ensemble activity, an evoked all-or-none polysynaptic activity in rat neocortical slices. Ensemble activity occurred in cortical slices bathed in normal artificial cerebrospinal fluid (ACSF) and was evoked by a single electrical shock either to the white matter or directly to the cortical tissue. This activity was seen in slices of somatosensory and auditory cortices; in other cortical areas we have not been able to evoke it. The activity developed 10 to 250 ms poststimulus and lasted 280 ± 120 ms in local field potential (LFP) recordings. Voltage-sensitive dye imaging showed that this activity was an area of activation 0.8 ± 0.4 mm wide that propagated slowly (11.4 ± 6.2 mm/s, $n = 60$, 6 animals) in the horizontal direction. Due to this propagation, the actual duration in the whole tissue may be longer ($\sim$400 ms) than that recorded by a single LFP electrode. Ensemble activity produced a low-amplitude optical signal (7–14% of the interictal-like spikes in the same tissue), suggesting a moderate net depolarization of the population. These were very different from hyperexcitable (epileptiform) events in the same tissue that had about 10 times the optical signal amplitude and propagated at 125 ± 24 mm/s ($n = 21$, 6 animals). On a global spatial scale ($\sim$0.8 mm wide in layers II–III) ensemble activity had a smooth waveform in voltage-sensitive dye signals (population transmembrane potential). On a local scale, field potential recordings showed large fluctuations with complex oscillations and substantial trial-to-trial variation. This suggests that oscillations in cortical circuits occurred only in small clusters of correlated neurons. Ensemble activity was sensitive to the excitation-inhibition balance of the local network. Antagonists of *N*-methyl-D-aspartate, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid, and GABAa receptors, and muscarinic agonists and other modest manipulations such as increasing bath concentration of Mg^{2+} to 2.5–4 mM (normally at 2 mM), or K^+ to 5–7 mM (normally 3 mM), all significantly reduced the probability of evoking the activity. The metabotropic glutamate receptor agonist, aminocyclopentane-1,3-dicarboxylic acid, blocked the activity at a low concentration (10–15 μM), while the antagonist (R,S)-α-methyl-4-carboxyphenylglycine had no effect even at high concentration (240 μM). Our data suggest that locally organized neuronal clusters may play a role in the organization of oscillatory activities in the gamma band and may participate in cortical integration/amplification occurring on a scale of $\sim$1 mm $\times$ 300 ms.

INTRODUCTION

Correlated population activity (changing of the population firing rate over a duration of 10–100 ms) has been observed during many cortical processes, including auditory (Maldonado and Gerstein 1996), somatosensory and motor processes (Nicolelis et al. 1995, 1997a, 1998; Rioult-Pedotti et al. 1998; Usher et al. 1999), visual object recognition (Miyashita 1988; Sakai and Miyashita 1991; Wang et al. 1996), and during the silent period between two testing stimuli in the delayed match-to-sample tasks (Chelazzi et al. 1993; Fuster and Alexander 1971; Miller and Desimone 1994). The common characteristics of these correlated activities are that a large portion of the participating neurons are not monosynaptically activated/inhibited by subcortical input, and that the duration of the activations and their temporal patterns (e.g., oscillations) also may be very different from the input patterns. These features suggest that even during an evoked activity the active population is largely organized by the intrinsic cortical circuits instead of passively driven by the afferent pattern.

The details of the spatiotemporal organization of correlated population activity are not well understood. One possibility is that neurons of a correlated population are concentrated in local clusters, within which a large fraction of the neurons are active in each time window. Another possible organization is that correlated neurons are scattered among nonactive and noncorrelated firing populations, and different correlated groups spatially overlap. The connectivity of neocortical local circuits is sparse and distributed (Douglas and Martin 1998; Markram et al. 1997). It is unknown how a correlated neuronal group is sustained in such a network and whether constant firing of the active neurons is required to maintain the correlation. Analysis of the spatiotemporal distribution of correlated neuronal groups may help to answer these questions.

This report studies the spatiotemporal pattern of a correlated population event in neocortical slices. This newly described activity is an evoked all-or-none event occurring in cortical tissue bathed in normal artificial cerebrospinal fluid (ACSF). Local field potential (LFP) recordings showed complex oscillations in the 20- to 80-Hz range (Metherate and Cruikshank 1999), and optical recordings showed the activity propagating horizontally (Wu et al. 1999b). This all-or-none population event can be evoked by stimulating thalamocortical afferents (Metherate and Cruikshank 1999) or by directly stimulating the cortex (Hsieh et al. 2000; Wu et al. 1999b). In the literature, similar polysynaptic activities in cortical slices have been described with nonspecific names, e.g., "multiphasic, long latency response" (Luhmann and Prince 1990), "slow potentials" (Metherate and Cruikshank 1999), or "slow oscillations"

(Sanchez-Vives and McCormick 2000). In this report we will use the name "ensemble activity" to refer to the activity that we are studying, following the convention of calling a correlated cortical neuronal group a "neuronal ensemble" (Buzaki and Choroback 1995; Nicolelis 2001).

In this report we examine the spatiotemporal organization of ensemble activity. We will also test whether this activity can be evoked in different cortical areas and examine the network mechanisms that underlie it. Other types of population events in cortical slices are seizure-like and interictal-like activities (Anderson et al. 1986; Chagnac-Amitai and Connors 1989; Chervin et al. 1988; Fleidervish et al. 1998; Prince and Tseng 1993; Sutor et al. 1994). In this report we also present evidence showing the difference between interictal-like spikes and ensemble activity.

Voltage-sensitive dye imaging and LFP recordings were used. These two methods measure activity on different spatial scales, allowing us to explore the local dynamics of the population correlation.

METHODS

Preparations

Sprague-Dawley rats of both sexes from *postnatal day 14 to 35* (*P14* to *P35*) were used for the experiments. Following National Institutes of Health guidelines, the animals were deeply anesthetized with halothane and quickly decapitated using a Stoelting small animal decapitator. The whole brain was chilled in cold ($0-4°C$) ACSF for 90 s before slicing. The ACSF used in this report contains (in mM) 132 NaCl, 3 KCl, 2 $CaCl_2$, 2 $MgSO_4$, 1.25 NaH_2PO_4, 26 $NaHCO_3$, and 10 dextrose, saturated with 95% O_2-5% CO_2 (pH 7.4), unless otherwise stated.

A stereotaxic atlas for rat brain (Paxinos and Watson 1986) was used to locate the cortical areas. Cortical slices (400 μm thick) were cut in coronal, horizontal sections by a vibratome stage (752 M vibroslice, Campden Instruments, Sarasota, FL). For some experiments, slices were cut with special angles, following the methods described by Agmon and Connors (1991) for somatosensory (barrel cortex) areas and by Metherate and Cruikshank (1999) for auditory areas. In these slices the thalamocortical afferent fibers between internal capsule and cortex were preserved. The slices were transferred from the vibratome to a holding chamber and immersed in oxygenated ACSF at room temperature (24°C) for at least 2 h. For voltage-sensitive dye imaging experiments the slices were stained with 0.02 mg/ml of an oxonol voltage-sensitive dye, NK3630 (first synthesized by R. Hildesheim and A. Grinvald as RH482, available from Nippon Kankoh, Okayama, Japan), for 1 h. The stained preparation was then perfused with dye-free ACSF at 27°C for >30 min before optical imaging. In other experiments the slices were not stained. During all experiments the preparation was continuously perfused in a submerged chamber with oxygenized ACSF at 30–35°C.

Optical imaging and electrode recordings

Optical imaging was performed with a 124-element photodiode array (Centronics, Newbury Park, CA) at a frame rate of 1,000 frames/s. A ×5 (NA 0.12, Zeiss) objective was used to project the image of the preparation onto the array where each photodetector received light from a 0.33×0.33 mm^2 area of the slice. The photocurrent of dye-related absorption signals (705 ± 15 nm) from each photodetector was individually amplified through a two-stage amplifier system. The amplifiers performed a current to voltage conversion using a feedback resistor of 20 MΩ and then a voltage gain of 200, and a high-pass filter with a time constant of 1 s (0.16-Hz corner frequency). The photocurrent from the photodiode was about 100 nA. The optical signal size (dI/I) for our signals was about 1×10^{-3} to 5×10^{-4} of the illumination intensity. Optical recording trials were usually 2 s long; we recorded 30–50 trials (with a total exposure of <120 s) from each slice. At this light intensity the activity pattern did not change noticeably after 1,000 s of total exposure (W.-J. Jin and J.-Y. Wu, unpublished observations), indicating that dye bleaching and phototoxicity were insignificant in our experiments. Signals from the photodiode array and electrodes were digitized with a 12-bit data acquisition board (Microstar Laboratories, Bellevue, WA) installed in a Pentium PC. Before digitization a 4-pole Bessel analog low-pass filter with a 333-Hz corner frequency was applied to all optical channels, to ensure that the frequency of the analog signals was lower than the Nyquist frequency of the sampling. Digitized data were directly transferred to the hard disk of the computer. A similar optical recording apparatus with 464 detectors is commercially available from WuTech Instruments (www.wutech.com), or as NeuroPlex II from RedShirtImaging, LLC, Fairfield, CT (www.redshirtimaging.com). For additional details about the apparatus, see Wu et al. (1999a) and Wu and Cohen (1993).

Glass micropipette or tungsten lacquer-coated microelectrodes (FHC, Bowdoinham, ME) were used to simultaneously sample LFPs. The tip resistance of the electrodes ranged from 500 to 2,000 KΩ for the glass pipettes (filled with 1 M NaCl) and 75 to 200 KΩ for the tungsten electrodes. To minimize damage to the cortical tissue, the electrodes were carefully inserted about 100 μm into the slice in cortical layers II–III or IV. Electrical signals were filtered at 1–500 Hz before digitizing. During optical recording the electrical signals were digitized and stored concurrently with the optical signals at a sampling rate of 1,000 Hz.

Two kinds of stimulation were used for evoking the activity. One was bipolar stimulation via a twisted pair electrode with 12.5 μm wires, which delivered a strong electrical field in a small focus between the wires. Another was monopolar stimulation with a silver ball electrode placed in the ACSF 200–1,000 μm above the tissue. Because stimulation intensity is inversely proportional to the square of the distance between the electrode tip and the tissue, a single-pole electrode allows a much larger area of tissue to be excited (compared with the twisted pair electrode).

The optical data were analyzed using the program NeuroPlex (RedShirtImaging, LLC). Data were displayed in the form of traces for numerical analysis, or pseudocolor images for visualizing the spatiotemporal patterns. Digitized data were not further filtered by NeuroPlex before displaying. The pseudocolor display was made using Variable scaling and Contour display. In the Variable scaling mode the data from each diode is first normalized to its maximum, and then each normalized data point is assigned a color according to a linear color scale (0 = deep blue and 1 = red). This linear scaling of the pseudocolor display best preserves the timing information in the signals (Lam et al. 2000; Tsau et al. 1999).

Numerical analysis, fast Fourier transform (FFT), and auto- and cross-correlation were performed using MatLab (Mathworks, Natick, MA) with the help of Dr. T. Kiemel (Univ. of Maryland).

RESULTS

Evoking ensemble activity

Ensemble activity (Fig. 1) was elicited by a single shock of moderate intensity (e.g., 10 V × 0.05 ms) to cortical slices perfused with normal ACSF ([K] = 3 mM, [Ca] = 2 mM, and [Mg] = 2 mM). This activity was an all-or-none event. Identical stimuli either elicited the activity or failed (Fig. 1, *A* and *B*, *traces I* and *II*), and the amplitude of the activity was independent of the stimulation intensity (Fig. 1*A*, *traces I–IV*).

A Auditory cortex
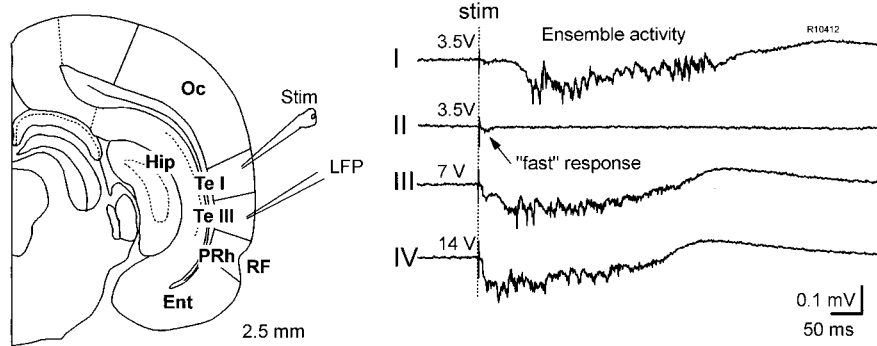
B Somatosensory cortex
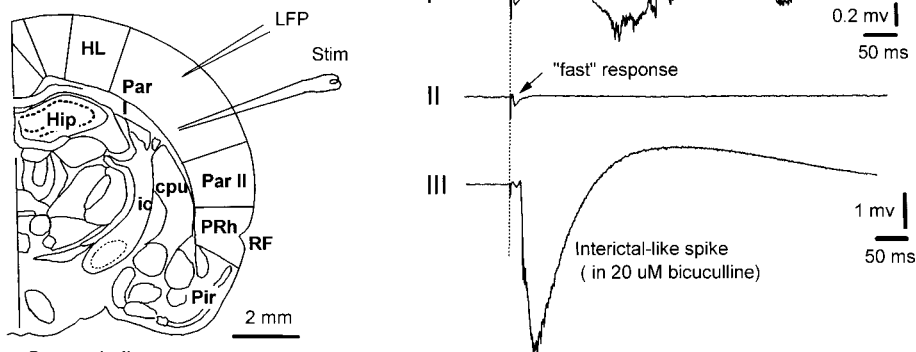
FIG. 1. Local field potential (LFP) recordings of cortical population activities. *A*: auditory cortex. *Left*: schematic diagram of stimulation-recording arrangement. The slice was perfused with normal artificial cerebrospinal fluid (ACSF), and the LFP was recorded with a tungsten microelectrode (LFP) placed in layer II–III of the auditory area (Te III). The stimulation electrode (Stim) was a 12.5 μm twisted pair electrode placed in the same layer, ~1.5 mm from the recording electrode. *Right, trace I*: an episode of ensemble activity was elicited by a single electrical shock (3.5 V $\times$ 250 μs). The stimulus first elicited a fast response; ensemble activity developed after a latency of ~120 ms. In *trial II* a stimulus of the same intensity elicited a similar fast response, but ensemble activity failed to develop. For *traces III* and *IV,* the stimulation intensities were 7 and 14 V, respectively (250 μs). Note that the amplitude of fast response increased and the latency for ensemble activity decreased. Hip, hippocampus; Occ, occipital areas (visual cortex); Te I and III, temporal area I and III (auditory cortex); RF, rhinal fissure; PRh, perirhinal cortex; Ent, entorhinal cortex. *B*: somatosensory areas. In this recording the LFP electrode was a glass electrode placed in layer II–III of somatosensory area (Par I). A stimulation electrode was placed in deep layers, 0.3 mm from the white matter, 1.2 mm lateral to the recording site. In this recording the fast response was more clearly seen. In *traces I* and *II* the slice was bathed in normal ACSF, and stimulation intensity was 12.5 V $\times$ 500 μs. In *trace III* the same preparation was perfused with ACSF containing 20 μM bicuculline; the same stimulus elicited a fast response and triggered an interictal-like spike. Interictal-like spikes had a much higher amplitude. Note that the voltage scale for *trace III* is 5 times that of *I* and *II*. cpu, caudate putamen; ic, internal capsule; HL, hind limb cortex; Par I and II, parietal cortex area I and II.

At a low stimulus intensity, the latency between the stimulus and the onset of an ensemble activity (range from 10 to 200 ms, Fig. 1) and the duration of the activity (280 $\pm$ 120 ms, mean $\pm$ SE, $n = 1{,}194$ ensembles from 6 animals) were long and variable. Prior to the development of ensemble activity, the stimulus first evoked a "fast" response (Fig. 1). This fast response had a short and fixed latency (2–5 ms) and a short-duration (~10 ms). Its waveform was similar from trial to trial, and the amplitude increased with the stimulus intensity (Fig. 1A, traces I–IV). When a low-intensity stimulus was used, the amplitude of the fast response was smaller than that of the following ensemble activity. The amplitude, waveform, and duration suggest that ensemble activity and the fast response have different neuronal substrates. The neurons activated in the fast response were likely to be activated by the stimulus either directly or after one or a few synapses; the polysynaptic (>3) contribution was less important. In contrast, at the site of the recording electrode the overall signal magnitude during ensemble activity was much larger and longer than that of the fast response, suggesting that the ensemble activity is a self-sustained polysynaptic (emerging) population activity of the local cortical network. The term "ensemble activity" is used to emphasize its population nature. This activity can be reliably evoked in slices from temporal (auditory cortex, 173 slices) and parietal (somatosensory cortex, 28 slices) areas. These slices were from 122 animals, in which 33 were 2–3 wk old, 67 were 3–4 wk old, 17 were 4–5 wk old, and 5 were older than 5 wk. The activity was seen in every animal in the age range of 2–4 wk.

Auditory cortex

Most of our experiments were done in slices from auditory area Te 1–3 (173 slices). In coronal slices at bregma -3.6 to -7.3 mm, ensemble activity was evoked by shocking the cortex directly. The LFP electrode was placed in layers II–III or IV, and the stimulating electrode was placed in cortical layers I–VI, at a lateral distance of 0.5–2 mm from the recording electrode (cf. Fig. 1A, left). An optimum stimulus-recording arrangement was to use a coronal slice at approximately bregma -5 mm, placing the twisted pair stimulation electrode in layer II–III at 2 mm dorsal to the rhinal fissure, and placing the recording electrode in layer II–III at 1 mm dorsal to the rhinal fissure. With this stimulation-recording arrangement, a single pulse stimulation at 1–2 times the threshold intensity (e.g., 4–10 V $\times$ 0.05 ms) applied at 30-s intervals had more than a 90% (calculated in 5 slices, $n = 500$ events) chance to elicit ensemble activity. Increasing the stimulus intensity often

reduced the probability of evoking ensemble activity (consistent with Luhmann and Prince 1990). In 82% of the 173 slices in auditory areas, ensemble activity was evoked by stimuli at <200% of the threshold intensity.

Ensemble activity was also evoked in slices with different slicing angles from coronal to horizontal planes. In a near horizontal section a portion of thalamocortical fibers was preserved between the cortex and internal capsule (Metherate and Cruikshank 1999, Fig. 3). In such horizontal slices stimulating the white matter and the fiber track leading to the internal capsule could both evoke ensemble activities (Metherate and Cruikshank 1999, Fig. 2). Intact thalamocortical loops are not required for sustaining ensemble activity because the thalamocortical loop was not preserved in this preparation.

Somatosensory areas (barrel cortex)

In somatosensory areas ensemble activity can be evoked with a similar stimulation-recording arrangement [i.e., using coronal slices from bregma 0 to −3.8 mm plans, placing LFP electrode in layer II–III or IV, and stimulating electrode in layer IV–VI, 0.5–2 mm lateral to the recording electrode (cf. Fig. 1B, left)]. However, evoking ensemble activity in somatosensory cortex was somehow more difficult than in auditory areas. In auditory areas, stimulating almost anywhere in the cortex could evoke ensemble activity. In somatosensory slices from the same animal, however, stimulating superficial layers had a lower chance of evoking the activity; usually we needed to adjust the stimulation intensity and the location of the stimulating electrode several times until the activity was stably evoked. Using a diffused field stimulus with the unipolar stimulation to activate a large area including several layers (~0.5 mm diam) was an effective way to evoke ensemble activities in somatosensory area (Wu et al. 1999b). With this kind of stimulation, fine adjustment of stimulus location was no longer necessary.

Stimulating some spots in the white matter could also evoke ensemble activity in somatosensory areas. Under transmitted illumination we could see that some of these spots were fiber bundles leading to the cortex. With a certain slicing angle, fibers between thalamus and cortex were better preserved (Agmon and Connors 1991). In these slices stimulating along thalamocortical fiber tracks also evoked ensemble activity. However, our stimulation was likely to activate many fibers near the electrode tip; we were thus not able to verify whether the activity was evoked by thalamocortical afferent fibers or by axon-colaterals of the output fibers.

Ensemble activity evoked in auditory and somatosensory areas had similar features (all-or-none; long and variable latency, duration, and amplitude; complex waveform; see Fig. 1). Probably because of the large trial-to-trial variation in the LFP recordings (Fig. 5), we were unable to distinguish the difference in the waveforms from the two areas.

Other cortical areas

In seven coronal slices from four animals we have searched for ensemble activity in the cortex outside of somatosensory and auditory areas. In this limited effort we applied a range of stimulation intensities to the white matter or to cortical layers I–VI and also used diffused field stimulation for a large area including multiple cortical layers. An LFP recording electrode was placed one-third of the distance from the pia to the white matter, 0.5–2 mm lateral to the stimulation. The cortical areas tested were visual cortex (occipital cortex area I; 3 slices, coronal section, bregma −5.8 to −7.2 mm), front and hind limb area of cortex (2 slices, coronal section, bregma −1.3 and 1.7 mm), and frontal cortex (2 slices, coronal section, bregma −0.8 and −1.2 mm). In these slices we first verified that ensemble activity could be evoked in the somatosensory or auditory areas before moving the electrode to other cortical areas. In the three test areas we could not evoke ensemble activity with any of our stimulation parameters. However, we do not conclude that ensemble activity only occurs in somatosensory and cortical areas. We noticed that many variables are involved in evoking ensemble activity and that in different cortical areas the optimum parameters may not have been found. For example, because ensemble activity does not develop at the same location as the stimulus, it is possible that in our tests the relative distances between the stimulation and LFP electrodes were not correct.

Amplitude and density of active neurons

In LFP recordings the amplitude of ensemble activity was significantly lower than that of hyperexcitable events in the same cortical tissue. A typical hyperexcitable event seen in cortical slices was an interictal-like spike (Fig. 1B, trace III), which could be elicited when the inhibitory neuronal network was suppressed (e.g., the slice bathed in >10 μM bicuculline) (Chagnac-Amitai and Connors 1989). Compared with the ensemble activity, interictal-like spikes had a substantially shorter latency and larger amplitude, suggesting that the activation was more synchronized and also that a significantly larger fraction of neurons (a higher population density) was activated.

To estimate the density of active neurons in ensemble activity and interictal-like spikes, we measured the amplitude of the voltage-sensitive dye signals in the same tissue during ensemble activity, interictal-like spikes, and the fast response (Fig. 2). The amplitude of the dye signals is proportional to the depolarization of all the stained membranes under each optical detector (reviewed by Wu et al. 1999a). Thus the amplitude of the dye signals is an indicator for the population activation, i.e., the combined effect of action potentials, synaptic depolarizations, and hypopolarizations of all the neurons monitored by an optical detector (each detector covers ~330 × 330 × 400 μm^3 of cortical tissue). In a fully disinhibited slice (bathed in 30 μM bicuculline) we used a large electrical shock to elicit a "maximum activation." The amplitude of the dye signals did not increase further with an increase of stimulus intensity once maximum activation was reached. We used this amplitude of maximum activation as a standard to normalize the signals of other activities elicited in the same slice. The amplitude of the fast response "on beam" to the stimulation electrode was proportional to the stimulus intensity (Fig. 2, ■). The amplitude of interictal-like spikes was about 70% of the maximum activation. This amplitude was relatively stable from trial to trial and was independent of the stimulus intensity (Fig. 2, ●). The activation during an ensemble activity was about 5–10% of the maximum activation, which was also independent of the stimulus intensity. The circles in Fig. 2 show that with large
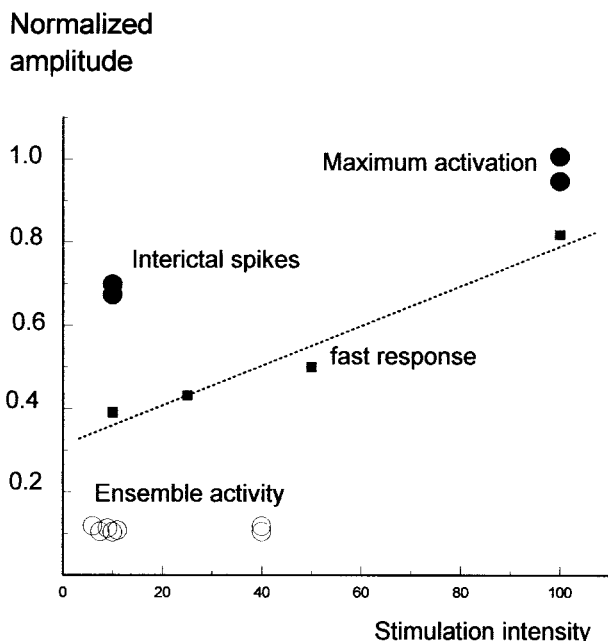
Normalized amplitude

FIG. 2. The amplitude of the 3 population activities. The absorption change at 705 nm was measured during different activities from the same tissue ($330 \times 330 \times 400 \ \mu M^3$ of cortical layers II–III in auditory area), with the stimulation electrode placed in a deep layer. The slice was stained with voltage-sensitive dye NK3630. The amplitude of the optical signals was normalized to the maximum relative absorption change in the tissue elicited by a high-intensity shock ($\sim$100 V $\times$ 0.1 ms) to the tissue perfused with 30 μM bicuculline. The amplitude of the fast response was relatively linear to the stimulation intensity. However, the amplitudes of interictal-like spikes and ensemble activity were independent from the stimulus intensity. Interictal-like spikes evoked by a small stimulus were about 70% of the maximum activation, while the amplitude of ensemble activity evoked by a large stimulus was only about 10%.

stimuli the amplitude of the fast response was much larger than that of the ensemble activity that developed later (see also images in Fig. 3C, bottom). This is consistent with the low amplitude in LFP recordings (Fig. 1A) and low spiking rate in intracellular recordings (Metherate and Cruikshank 1999), suggesting that the ensemble activity was an area with moderate net depolarization across the population and that the participating neurons might have a low firing probability.

Spatiotemporal patterns of ensemble activity

With voltage-sensitive dye imaging, ensemble activity appeared as a localized hot spot. The spot propagated relatively slowly along the horizontal direction in layers II–III. Figure 3 shows a typical event in a temporal cortical slice elicited by shocking the white matter. Figure 3A shows simultaneous recordings of the LFP and dye signals from two optical detectors. One optical detector (red trace) and the LFP electrode (black trace) were from the same location, and the activity occurred at the same time. Another optical detector (the blue trace) was 1.4 mm away from the LFP electrode; it detected activity much later (the relative locations of these recording sites are illustrated in Fig. 3B, left). These results indicate that the activity was slowly propagating and that the actual duration of the ensemble activity was longer than the duration detected by the LFP electrode.

The spatial map of the ensemble activity is shown in Fig. 3B.

Stimulation activated a column of the cortex (Fig. 3B, images a and b). This initial activation was seen in the LFP recordings as the fast response and has the same characteristics as voltage-sensitive dye signals reported by Tanifuji et al. (1994). Ensemble activity developed after this initial activation and became self-sustained in the superficial layers (Fig. 3B, images c–e). Over a duration of $\sim$400 ms, the self-sustained activity propagated slowly in the temporal area 1–3 (Fig. 3B, images f–k).

Voltage-sensitive dye images also showed that ensemble activity was an all-or-none event, independent of the stimulus intensity, and that the majority of the neurons activated during ensemble activity was not directly activated by the stimulus. In Fig. 3C the images from two recording trials, evoked by different stimulus intensities, are shown from the same preparation. In one trial (top row of images in Fig. 3C) the stimulus was at the threshold intensity, at which the fast response was barely visible to the optical detectors near the stimulating electrode (not visible in the LFP). An ensemble activity developed afterward (top row, 2nd image from left), with many more neurons activated than when activated directly by the stimulus. In another trial a stimulus of 5 times the intensity evoked a much larger fast response (bottom row of Fig. 3C, 1st image from left), but elicited an ensemble activity with similar size, duration, and active neuron density as the previous trial. When adjusting the stimulus intensity, we often found that strong stimuli increased failure to evoke ensemble activity (31 of 32 slices tested) (also consistent with Luhmann and Prince 1990). Using the imaging method we found that strong stimuli caused a large area of synchronized activity (fast response) in the cortex. This large and synchronized activation appeared to prevent the development of ensemble activity in the same area. In optical imaging (e.g., Figs. 3 and 6) ensemble activity always developed later than the fast response at a location lateral to the center of the fast response.

When the ensemble activity became self-sustaining and no longer overlapped with the fast response (images g–k of Fig. 3B), it was about 0.8 $\pm$ 0.4 mm wide in layers II–III ($n = 8$ slices; measured from the contour map using the 50% amplitude line). This size was relatively stable from preparation to preparation and in different cortical regions.
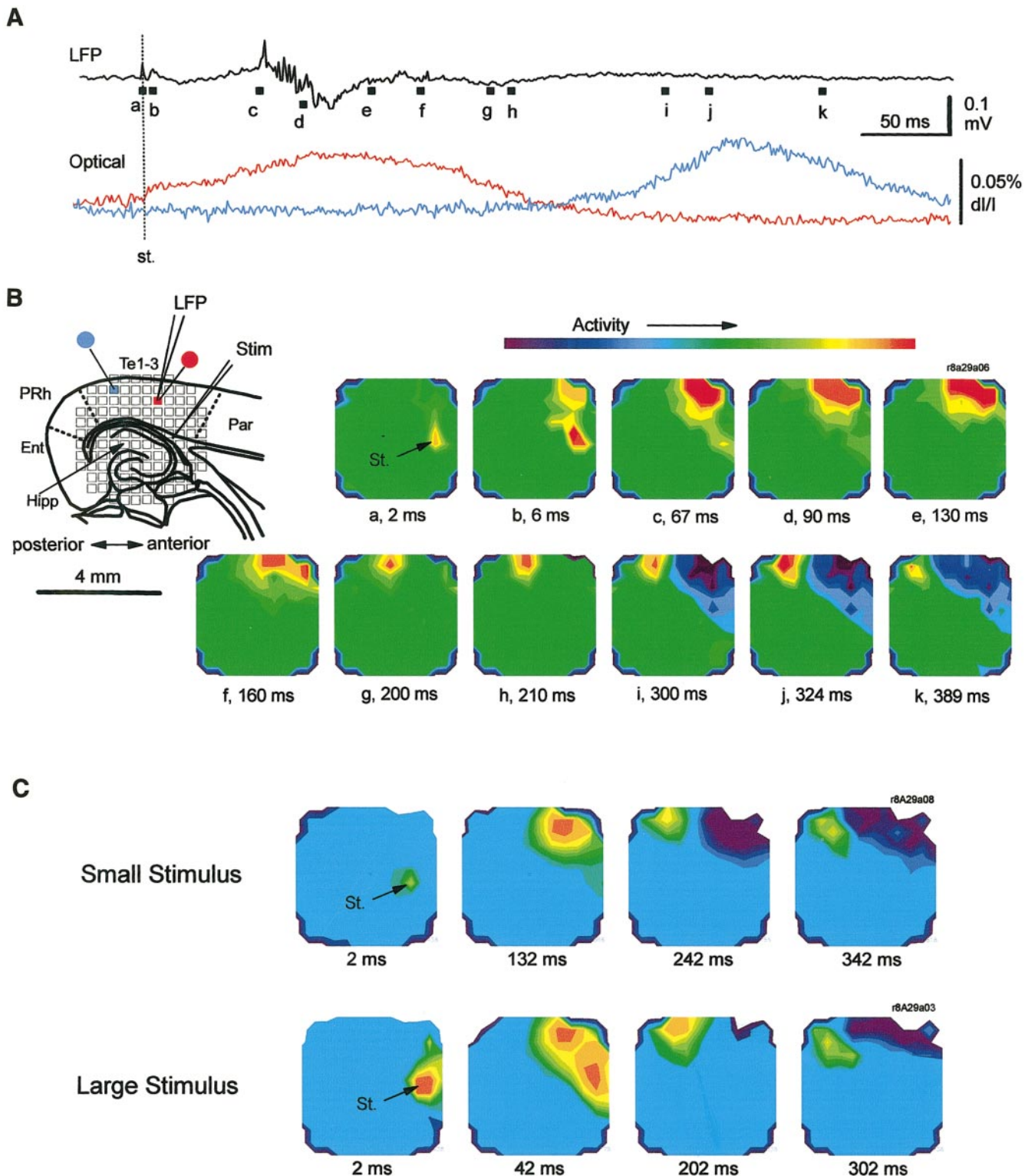
Horizontal propagation

Ensemble activity propagated horizontally in the cortex. Voltage-sensitive dye imaging revealed that the propagation velocity of ensemble activity varied from 1 to 20 mm/s ($n = 15$ slices). This velocity was substantially slower than that of the fast response (100–160 mm/s; data not shown) and interictal spikes (40–160 mm/s) (Chervin et al. 1988; Demir et al. 1999; Golomb and Amitai 1997; Golomb et al. 2000; Tanifuji et al. 1994; Tsau et al. 1998), and distinguishable from the all-or-none events when slices were partially disinhibited with 0.1–5 μM bicuculline (20–100 mm/s) (Golomb et al. 2000; Tanifuji et al. 1994).

In different slices the propagation velocity varied significantly. To minimize the variations caused by anatomical substrate (e.g., slices from different cortical areas, cutting location, and angle), we measured optically the propagation of the ensemble activity and interictal-like spikes in the same tissue (coronal sectioning of the auditory areas). In these experiments ($n = 7$ slices from 6 animals) we first perfused the slices with

normal ACSF to image the propagation of the ensemble activity. Then the slices were perfused with a high concentration (10–20 μM) of bicuculline for 20–30 min, until the same stimulus evoked interictal-like spikes. We found interictal-like spikes propagated at 125 ± 24 mm/s (measured from 21 spikes from 7 slices), significantly faster (>10 times) than ensemble activity in the same tissue (11 ± 6 mm/s). Figure 4 shows an example of the propagation measurement.

The propagation range of the interictal-like spikes was much larger than that of ensemble activity in the same tissue. In another set of experiments we used coronal slices at bregma −6.5 mm. The stimulating electrode was placed in layer I of the temporal cortex at about 2 mm dorsal to the rhinal fissure. At an optimized stimulus intensity, an LFP electrode placed in layers II–III 0.5 mm lateral to the stimulation site reliably detected the ensemble activity (more than 90% of >160 trials
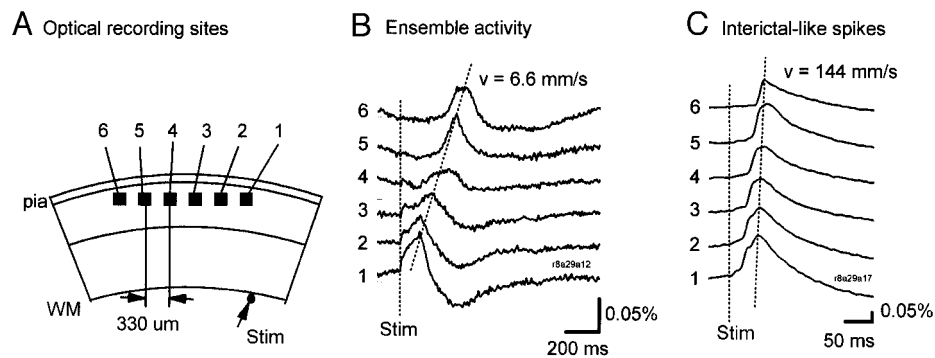
FIG. 4. Propagation of the ensemble activity and interictal-like spikes. *A*: propagation of the activities was measured with voltage-sensitive dye signals from a temporal cortical slice (horizontal section). The activity detected by 6 photodiodes (330-μm spacing) was used to calculate the velocity of the 2 forms of activity. A stimulation electrode was placed in the white matter (Stim). *B*: an ensemble activity was evoked by the stimulation and propagated horizontally. The velocity was calculated using the times at which the peak arrived at the different photodiodes. The fast response can be seen in the detectors close to the stimulation electrode (*traces 1–4*). *C*: the same preparation after perfusion with 20 μM bicuculline. The stimulus evoked an interictal-like spike with much larger amplitude and faster velocity. Note that the interictal-like spike is larger and faster.

in all 8 slices from 5 animals), while another electrode 2 mm away from the stimulation site failed completely (in all 8 slices, 0 of >160 cases). After perfusing the same slices with 5–10 μM bicuculline, the stimulus evoked interictal spikes. The evoked interictal-like spikes could be detected by an LFP electrode placed anywhere in the cortex, throughout the preparation.

Using horizontal slices the ensemble propagation range was longer e.g., ~3 mm (Fig. 3). This difference in propagation range appeared to be related to the width of the temporal cortex in these two section angles. Auditory cortex areas (Te I–III) in the coronal slices at bregma −6.5 was <2 mm wide, while in the horizontal slices it was about 4 mm wide. It thus appeared that the propagation range of the ensemble activity was related to the size of the temporal cortex rather than to the distance from the initiation site.

Trial-to-trial and location-to-location variations

The LFP activity of the ensembles had a large trial-to-trial variability (Fig. 5). This trial-to-trial variation occurred on two temporal scales. On a short temporal scale of 1–20 ms, the LFP waveform varied significantly in different trials evoked by identical stimuli (Fig. 5*A*). The waveform covered a wide spectrum of frequencies with noticeable peaks around 12, 40, and 80 Hz (Fig. 5*B*). The peaks in the spectrum also varied

from trial to trial. On a longer temporal scale of 50–100 ms, in some trials, the activity was apparently organized as bursts; identical stimuli evoked different numbers of bursts (arrows in Fig. 5*C*). The duration of the bursts and intervals between bursts were also variable. The frequency of the bursts varied from 4 to 10 Hz ($n = 20$).

With optical signals the waveform was smooth (see Figs. 3*A* and 7*B*) with no rapid fluctuations. The bursts that appeared in the LFP could not be seen in the optical signals. The trial-to-trial variation on the short temporal scale (1–20 ms) was small. However, the duration of the activation in the imaging was different from trial to trial (Fig. 6), suggesting that there might be a different number of bursts during each trial. In Fig. 6 the signals of four optical detectors located at different distances from the stimulation electrode (I–IV in Fig. 6*A*) were plotted in four consecutive trials (the 4 color traces in each plot). The onset of the traces was more consistent while the waveforms after the onset became more variable (compare the 2 time markers, 70 and 100 ms, in each plot in Fig. 6*A*). Figure 6*A* also indicates that at locations close to the stimulation site the waveforms were more consistent among trials (Fig. 6*A*, I), while at locations more distant from the stimulation site the trial-to-trial variability became larger (Fig. 6*A,* IV). The images also suggest that ensemble activity was more variable from trial

FIG. 3. Voltage-sensitive dye images of the ensemble activity. *A*: simultaneous LFP (*top trace*) and voltage dye signals from 2 optical detectors (red and blue traces). The recording positions for the electrode (LFP) and optical detectors (red and blue boxes) are shown in *B*. The letters *a–k* under the LFP trace indicate the time of the dye images in the *right section* of *B*. *B, left*: recording arrangement. The preparation was a horizontal slice of temporal cortex (Metherate and Cruikshank 1999). Temporal areas I–III (Te1–3) were imaged by the diode array (the diodes are shown as boxes). The stimulus electrode (Stim) was placed at the thalamocortical fiber track in the white matter. Par, parietal cortex; Te 1–3, temporal area I, II, and III; PRh, perirhinal cortex; Ent, entorhinal cortex; Hipp, hippocampus. *B, right*: selected images from the optical recording series. Pseudocolor was used to represent the amplitude of the voltage-sensitive dye signals. Here warm colors represent higher amplitude (depolarization). The color coding is linear with the amplitude. Two traces from the optical data are shown in *A;* the signal-to-noise ratio was large. No digital filter was applied to the raw data before color conversion. Each image represents the activity during 1 ms. The letter and the time under each image indicates the relative timing referenced to the LFP waveform (*A, top trace*). The ensemble activity did not develop at the stimulation site (st). It developed in the superficial layers and slowly propagated horizontally. The activity in the image was of longer duration than that in the LFP recordings, apparently because the electrode only detected activity in a small area near its tip. *C*: images of 2 recording trials with different stimulus intensities, showing that ensemble activity was independent of stimulus intensity. The preparation is shown in *B, left. Top row*: a threshold stimulus (4 V × 0.05 ms) evoked a barely visible fast response and an ensemble that developed later (only a snap shot at 130 ms was shown) and lasted >200 ms. *Bottom row*: a stimulus 5 times larger (20 V × 0.05 ms) evoked a larger fast response but a similar ensemble activity. Note that the latency of the ensemble activity in the 2 trials differed significantly.
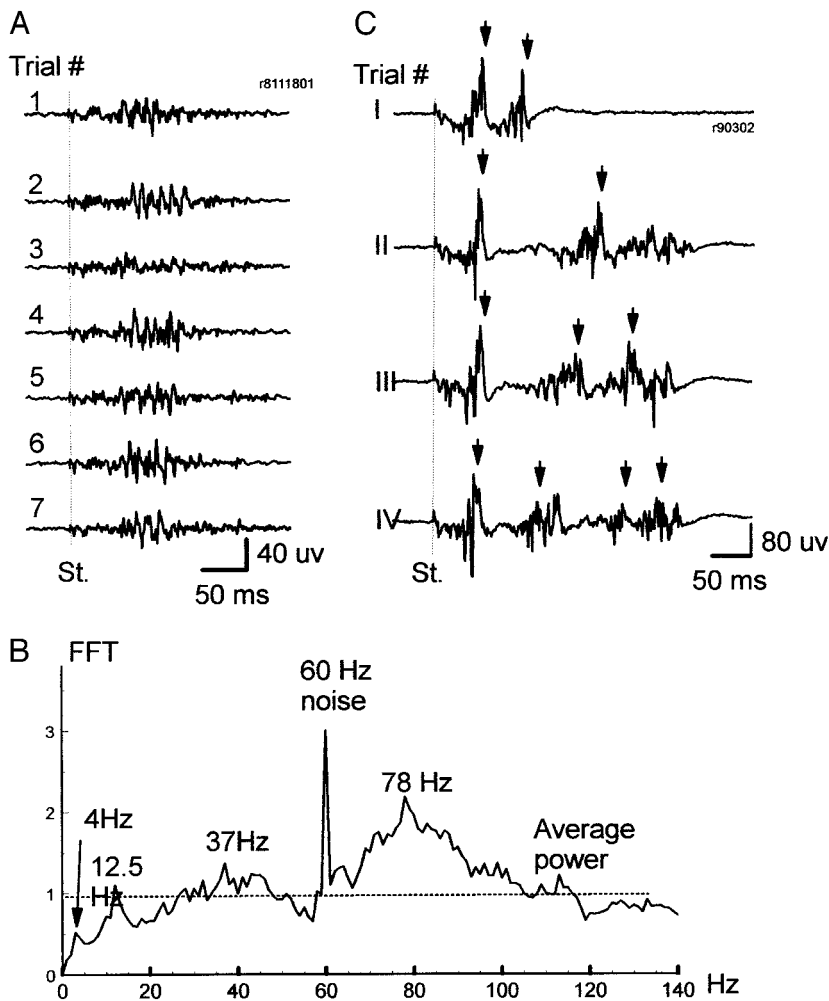
FIG. 5. Trial-to-trial variations; LFP recordings. *A*: 7 consecutive trials of LFP recordings of ensemble activity (evoked at intervals of 30 s). Large and irregular oscillations occurred in the LFP signals. The waveform varied from trial to trial. A tungsten microelectrode (tip resistance ~1 MΩ) was used and filtered between 10 and 1,000 Hz. The fast response had a regular latency and waveform and was seen as a small downward peak just after the stimulus mark (St.). *B*: average power spectrum of the waveform fluctuation. The signals from 10 consecutive trials were converted to the power spectrum (fast Fourier transform) individually, and the average of the 10 spectra is plotted. The 60-Hz peak was the line frequency noise in the recording system. Apparent peaks were seen around 12, 40, and 80 Hz. *C*: another kind of trial-to-trial variation. Four selected trials are presented, in which distinguishable bursts can be seen (arrowheads). The 1st burst was more similar from trial to trial. Later bursts were more variable in amplitude, interval, waveform, and number per trial. This kind of multiburst pattern was seen in about 20% of the trials in this slice.

to trial when it was spatially and temporally distant from the stimulation site (Fig. 6*B*).

Comparing optical and LFP signals, it appeared that the consistent onset in the optical signal correlated to the first burst in the LFP signals (Fig. 5*C*), while the more variable waveforms in optical signals might be related to the later bursts in the LFP (arrowheads in Fig. 5*C*).

The LFP signals had substantially more fluctuations than the optical signals recorded from the same location. Figure 7 compares the location-to-location variation in the LFP and optical signals. In LFP recordings, later bursts were often seen in one electrode but were missing in another electrode 200 μm away (Fig. 7*A*). This suggests that there may be smaller and more synchronized subunits (<200 μm) in the activated area of an ensemble activity (~800 μm wide). When comparing waveforms from two locations, the waveform variation detected by optical signals was much smaller than the variation recorded by LFP electrodes separated by a similar distance (Fig. 7, *A–C*). This suggests that the LFP waveform fluctuations only occurred on a local level. The signal on each optical detector was an average of the population activity in a volume of $330 \times 330 \times 400$ μm^3 of cortical tissue. The tungsten LFP electrodes, in contrast, mainly measured the sum of multidirectional current flows on a smaller spatial scale near the electrode tip. Thus on a global spatial scale, an ensemble activity appeared to be an area of moderate depolarization with an overall low population firing rate. Within this area of moderate depolarization, there may be localized neuronal clusters with different activity patterns.

Pharmacological manipulations

The excitability of the cortical tissue was crucial for sustaining ensemble activity. Metherate and Cruikshank (1999) reported that glutamate receptor antagonists [2-amino-5-phosphonovaleric acid (APV) at 50 μM or 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) at 20 μM] completely blocked ensemble activity. However, at these levels of blockade, local recurrent excitation of the intracortical network was substantially reduced. In this report we use modest manipulations to test whether ensemble activity is sensitive to the balance between local excitation and inhibition.

Ensemble activity was completely blocked when glutamate receptors were only partially blocked (APV at 25 μM or CNQX at 0.25 ~0.5 μM, $n = 4$ slices each, Fig. 8, *A* and *B*). When the preparation was perfused in ACSF with elevated [K$^+$] of 5–7 mM (normal ACSF at 3 mM), the probability of evoking ensemble activity was significantly reduced (from >90% to 30 ± 20%, $n = 115$ cases in 4 slices, Fig. 8*C*). Carbachol, even at a very low dose (<5 μM), completely blocked the ensemble activity ($n = 3$ slices) (also reported by Hsieh et al. 2000). When bath [Mg^{2+}] was elevated from 2 to
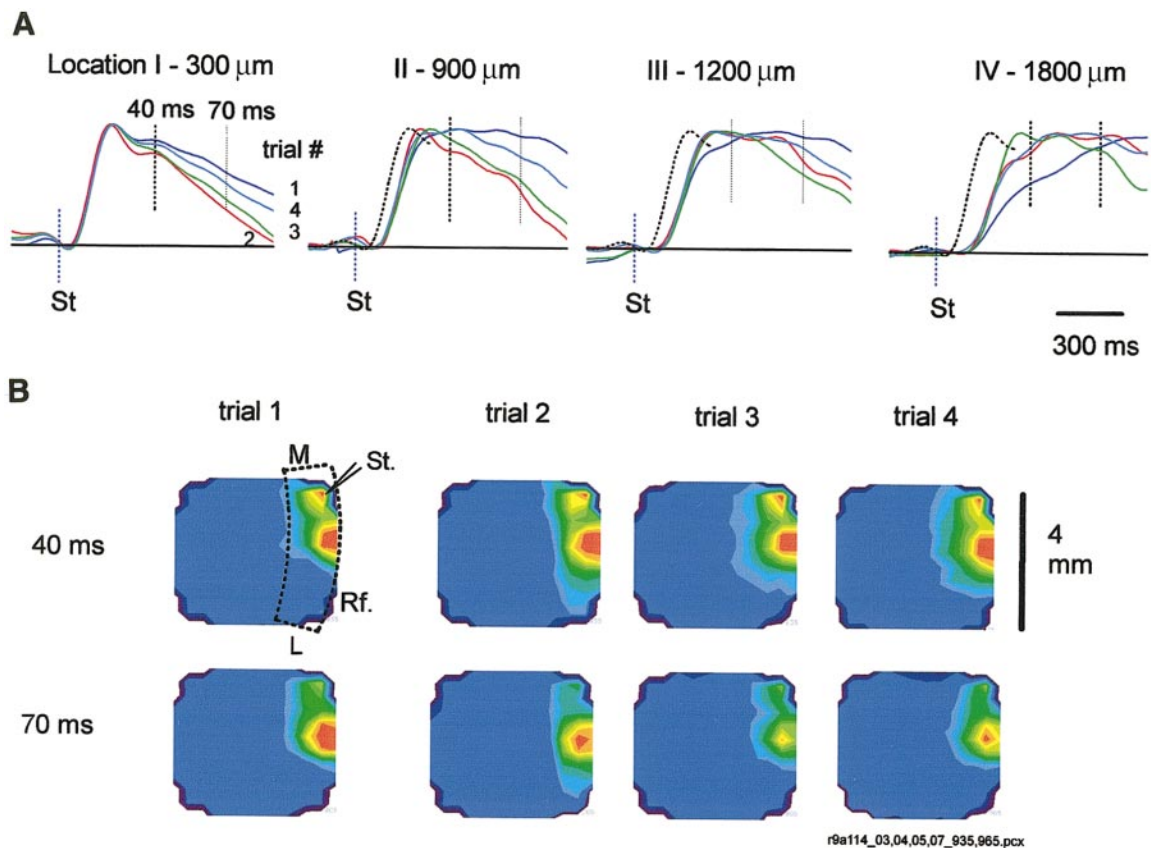
FIG. 6. Trial-to-trial variations; dye imaging. *A*: optical signals from 4 photodiodes located at 300, 900, 1,200, and 1,800 μM from the stimulation electrode were plotted (*I–IV*). In each plot signals from 4 consecutive trials (*lines 1–4;* black, red, green, and blue) are shown. The traces were normalized to show the relative change in different trials. The dotted curves in *plots II–IV* are the onset of the signal from *plot I,* showing the time delay at the different locations. The dotted vertical lines in the plots show the times (40 and 70 ms) when the snapshot images of the activity are shown in *B.* St., time of stimulation. *B*: the images of the 4 trials at 40 and 70 ms poststimulus time. The 1st image includes a schematic drawing of the preparation (M, medial; L, lateral; Rf, rhinal fissure). The preparation was a coronal slice of temporal cortex. The stimulation electrode was placed in cortex layer I (St). An ensemble activity was evoked 1 mm laterally from the stimulation electrode. The activity at 40 ms poststimulus (*top row of images*) was relatively consistent from trial to trial, while the activity at 70 ms poststimulus (*bottom row of images*) was more variable.

2.5–4 mM, the ensemble activity was completely blocked (*n* = 5 preparations, about 100 trials each, Fig. 8*D*). The effects these manipulations were reversible.

Ensemble activity was also sensitive to metabotropic glutamate receptor agonists. 1-Aminocyclopentane-1,3-dicarboxylic acid (ACPD) at 12.5–25 μM blocked the activity completely (*n* = 4 slices, Fig. 8*E*). In contrast, the metabotropic glutamate receptor antagonist (R,S)-α-methyl-4-carboxyphenylglycine (MCPG) did not have any remarkable effect on the activity (*n* = 200 events from 6 somatosensory preparations, at 100–240 μM MCPG). Perfusion with 240 μM MCPG significantly antagonized the blockade of 12.5 μM ACPD (*n* = 2 slices, Fig. 8*F*). This result suggests that ensemble activity is different from the network oscillations mediated by metabotropic glutamate receptors (Traub et al. 1996; Whittington et al. 1995).

Ensemble activity was also sensitive to changes in the inhibitory network caused by a low concentration of bicuculline (0.1–0.5 μM). When perfused with low concentrations of bicuculline, the duration of the activity became shorter, and in the LFP waveform there were larger peaks (P in Fig. 9*C*), suggesting an increased correlation in the population. When perfused with 0.5 μM bicuculline for 1 h and then switched back to normal ACSF, ensemble activity was completely

blocked while the fast response remained unchanged (*n* = 7 slices, Fig. 9*D*). This blockade was only partially reversible in two of the seven slices after a long wash (Fig. 9*E*), suggesting that the effect of bicuculline was different from other manipulations shown in Fig. 8, possibly due to long-term changes in the local network. When the preparation was perfused with a higher concentration (>5 μM) of bicuculline, interictal-like spikes (larger amplitude, less variable waveform and fast propagation velocity) began to occur. The threshold concentration of bicuculline varied from preparation to preparation. At an even higher concentration of bicuculline (>10 μM), interictal-like spikes became more stable and propagated across cortical boundaries (Fig. 1*C*). The effects of all of our manipulations are summarized in Table 1.

Since ensemble activity could only be evoked through optimized stimulation in a narrow window of intensity, our results do not rule out the possibility that in an altered excitability situation ensemble activity could be evoked using other stimulation parameters. Although at each altered condition we confirmed that ensemble activity could not be evoked using a range of stimulation intensities, we did not exhaustively search for other parameters and methods that might evoke dynamic ensembles in altered conditions.

A 200 um apart

LFP

LFP

B Same location

Optical

LFP

C 330 um apart
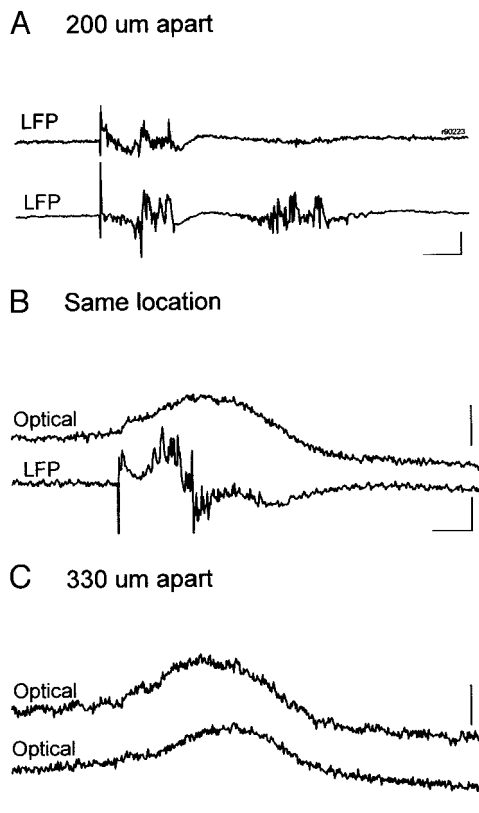

Optical

Optical

FIG. 7. Location-to-location variations. *A*: at different locations the LFP electrodes recorded very different waveforms from the same ensemble activity. In this example 2 electrodes placed in layer III were 200 μM apart from each other. The difference in waveforms occurred on 2 temporal scales: *1*) the detailed waveforms (high-frequency) were significantly different (however, the envelopes were similar), and *2*) the later bursts might appear in one location but not in the other. *B*: when the LFP electrode and optical detector were recording from the same location, LFP waveform showed more fluctuations than the optical trace. The optical waveform was always smooth (see also Fig. 3*A*). Note that the stimulus artifact is not seen in the optical signals because it is an artifact of a remote current source that does not affect transmembrane potentials. *C*: signals from 2 optical detectors have much less location-to-location variation. This is apparently because our optical signals reflect an average population activity in a larger volume of the cortical tissue (see main text). The bursts seen in the LFP signals may be generated by smaller clusters (smaller than 200 × 200 μM). In *B*, the LFP and optical signals were both low-pass filtered at 500 Hz before digitizing and were sampled simultaneously. Scale bars: *A,* 100 μV/50 ms; *B,* 0.05%, 100 μV/50 ms; *C,* 0.04%/50 ms.

DISCUSSION

In this report we describe spatiotemporal characteristics of an all-or-none activity in cortical slices. Using in vitro cortical slices allows a high signal-to-noise ratio for voltage-sensitive dye imaging (Demir et al. 1999; Tanifuji et al. 1994; Tsau et al. 1999; Wu et al. 1999b) of the spatiotemporal distribution of population activity. Because there are additional sources of noise in in vivo imaging, the sensitivity in this kind of measurement is not as high. Our in vitro data may be useful for understanding single trial dynamics of weak population activity (e.g., oscillations). However, in cortical slices, thalamocortical loops and long-range corticocortical connections are eliminated. Also, under in vitro conditions, activity is not evoked by a normal sensory input, and the evoked pattern does not interact with upcoming sensory input and ongoing spontaneous cortical activity. These differences may substantially change the spatiotemporal pattern of an evoked response. Despite

these drawbacks, in neocortex, about 80% of synapses belong to local circuits (Douglas and Martin 1998) that are preserved in cortical slices on a local scale (<0.5 mm). Intracortical mechanisms, including local recurrent excitation and its interplay with the local inhibitory network, might thus be similar to that which occurs in vivo. Ensemble activity may serve as an in vitro model for examining intracortical all-or-none population events.

Evoking ensemble activity

Ensemble activity was a long-duration, polysynaptic response elicited by a single electrical shock. Voltage-sensitive dye imaging showed that the ensemble activity was not a simple response to the stimulus (Figs. 3 and 6). The activity did not develop at the center of the fast response, and the properties of ensemble activity, i.e., all-or-none, self-sustainability, large trial-to-trial variation, and slow propagation, were also not controlled by the stimulus parameters. Strong stimulation had a reduced probability of evoking ensemble activity, suggesting that the activity pattern of the fast response (high synchrony) is incompatible with the polysynaptic process that sustains ensemble activity.

Ensemble activity can be elicited by shocking different cortical layers, white matter, or thalamocortical fiber tracks (Metherate and Cruikshank 1999). In slices with different cutting angles the activity was not noticeably different. These results suggest that the activity may be sustained by nonspecific intracortical connections.

Local recurrent excitation is dominant in neocortical circuits (Braitenberg and Schutz 1991; McGuire et al. 1984); the feedback gain of the excitation may vary substantially, depending on the input source and activity patterns (Douglas et al. 1989, 1995). It appears that ensemble activity could only develop in proper network states with a particular feedback gain; a high gain may be needed to recruit many neurons during the development of the ensemble activity (Fig. 3*C, top row*). This initial recruiting stage may be similar to the initiation of an interictal-like spike. However, after the initiation, the ensemble activity and interictal-like spike differed significantly: during ensemble activity the network gain apparently decreased, maintaining an overall low level of population activity for several hundred milliseconds. During interictal-like spikes, the feedback gain could continue to be high, which recruited a much larger portion of the neurons within a short time, resulting in a large and more synchronized population spike (Figs. 2 and 4). Inhibitory circuits might contribute to this gain control: manipulating the GABAa circuit seemed to influence the recruiting rate (the rising slope of the activity, Fig. 9) and the fraction of the population to be activated (the amplitude in optical and LFP signals).

Manipulations to the network showed that either elevating or suppressing the excitability of the cortical circuitry substantially reduced the probability of evoking ensemble activity (Figs. 8 and 9). This suggests that ensemble activity was unlikely to be an activity mediated only by particular excitatory transmitters (e.g., *N*-methyl-D-aspartate). Instead, the activity was likely to be an interplay of excitatory and inhibitory networks.
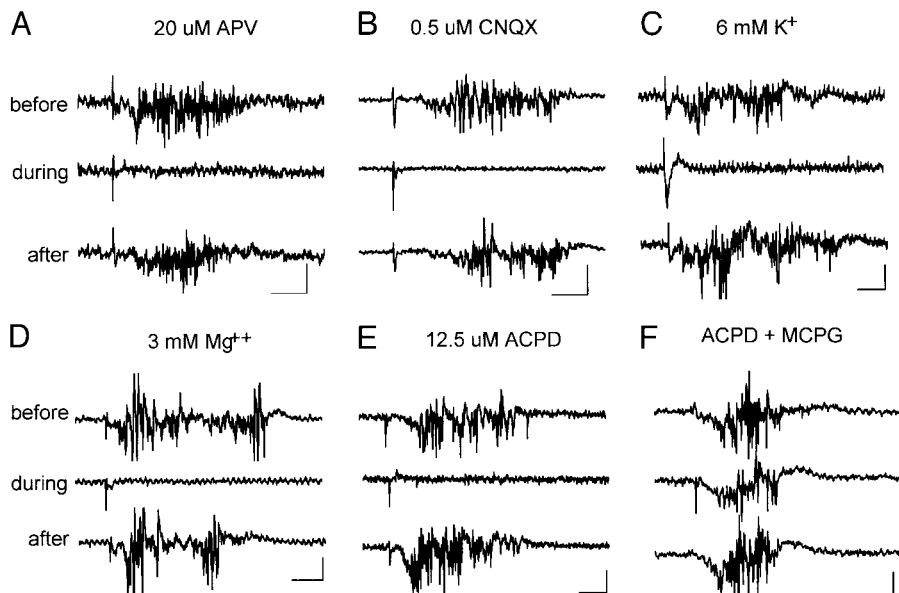
FIG. 8. Pharmacological manipulations. The *top trace* in each panel shows the activity recorded under control conditions (slice in ACSF). The *middle traces* are from the same tissue perfused with drugs or altered ionic concentrations, and the *bottom traces* show the activity after washing-out of the manipulation agents. *F, top trace*: in ACSF. *Middle trace*: 5 min in 12.5 μM 1-aminocyclopentane-1,3-dicarboxylic acid (ACPD) and 240 μM (R,S)-α-methyl-4-carboxyphenylglycine (MCPG). *Bottom trace*: washed in ACSF for 7 min. Scale bars: 0.1 mV/100 ms for all panels. APV, 2-amino-5-phosphonovaleric acid; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione.

Which cortical areas?

In our limited attempts (7 slices in 4 animals), we were unable to evoke ensemble activity outside the auditory and somatosensory areas. However, we do not conclude that ensemble activity is only associated with somatosensory and auditory cortices. We note that the method for evoking ensemble activity (i.e., proper stimulus intensity at particular locations) may differ significantly in different cortical areas. It is possible that we have not yet found the proper method to activate the local circuits in other cortices. In rat neocortex there are at least 24 anatomically defined areas (Paxinos and Watson 1986). It would be interesting to determine whether

this activity is a universal form of local population activity, given the fact that the organization of local cortical circuitry is similar in many neocortical areas (Douglas and Martin 1998).

Neuronal nature; moderate global depolarization and localized clusters

Ensemble activity was a self-sustained activity (activity without tonic input). The physical dimensions of the activity remained relatively stable for the entire duration. Voltage-sensitive dye signals showed that ensemble activity has a low amplitude, about 5–10% that of the maximum activation and about 10% that of an interictal-like spike (Fig. 2, Wu et al. 1999b). The voltage-sensitive dye signal is a linear summation of the population spike density and subthreshold potentials (Wu et al. 1999a). Assuming that in the maximum activation all excitable membranes were depolarized to 100 mV from the resting potential (sodium spike in all branches of the dendritic tree) (e.g., Stuart and Sakmann 1994; Stuart et al. 1997), then 5–10% of this depolarization should be equivalent to an average 5- to 10-mV depolarization of all the neurons, on a spatial scale ~0.8 mm wide. The contribution of the spikes is ignored
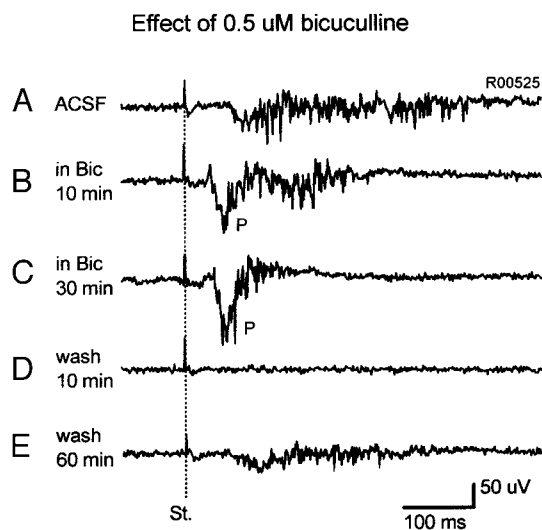
Effect of 0.5 uM bicuculline

FIG. 9. Effect of low concentrations of bicuculline. *A*: the preparation was perfused in normal ACSF, and ensemble activity was evoked by a threshold stimulus to cortical layer II. The duration of the activity was 344 ± 52 ms (*n* = 30 trials). *B*: the preparation was perfused by 0.5 μM bicuculline for 10 min. The duration and amplitude of the activity started to show noticeable changes. Peaks (P) occurred in LFP suggesting higher synchrony. *C*: after 30 min of perfusion, the duration of the activity was significantly reduced (70 ± 11 ms, *n* = 40 trials). *D*: switching back to normal ACSF for 10 min, ensemble activity could not be evoked. *E*: after 1 h of wash out with ACSF, the ensemble activity showed partial recovery.

TABLE 1. *Effects of pharmacological manipulations*

Manipulations	Effect on Ensemble Activity
5–7 mM [K]	Partially (>60%) blocked
5 μM carbachol	Completely blocked
0.5 μM bicuculline	Altered waveform; blocked when switched back to ACSF
10 μM bicuculline	Changing waveform and increasing propagation speed
2.5–4 mM [Mg]	Completely blocked
25 μM APV	Completely blocked
0.2–0.5 μM CNQX	Completely blocked
12.5–25 μM ACPD	Completely blocked
100–240 μM MCPG	No remarkable effect
12.5 μM ACPD and 240 μM MCPG	No remarkable effect

ACSF, artificial cerebrospinal fluid; APV, 2-amino-5-phosphonovaleric acid; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione; ACPD, 1-aminocyclopentane-1,3-dicarboxylic acid; MCPG, (R,S)-α-methyl-4-carboxyphenylglycine.

on this global scale because intracellular measurement showed that neurons in the active area fire only ~1.3 spikes per ensemble (Hsieh et al. 2000; Metherate and Cruikshank 1999).

On a smaller spatial scale, LFP recordings showed localized bursts (Figs. 5*C* and 7*A*), suggesting the existence of small and more active neuronal clusters in the activated area. The clusters were likely to be dynamically organized, because the recordings of many trials did not reveal fixed LFP patterns (Fig. 5). These local clusters should be smaller than 200 μM, since different burst patterns appeared on two electrodes separated by 200 μM (Fig. 7*A*). In cortical tissue local clusters may be organized by a variety of mechanisms. In addition to chemical synapses, recently described gap junction networks might also be a candidate (Draguhn et al. 1998; Gibson et al. 1999). Voltage-sensitive dye signals from the same location had a smooth waveform (Figs. 3 and 7), suggesting that the large fluctuations in LFP recording occurred in a much smaller volume of cortical tissue, which might also have a higher degree of depolarization. However, at a volume of $330 \times 330 \times 400$ μM (monitored by an optical detector), the net depolarization across the population was moderate and smooth.

Taken together, our data suggest that ensemble activity is composed of a moderate depolarization on a spatiotemporal scale of ~1 mm $\times$ 300 ms. This depolarization increases the spiking probability to approximately 1 spike/neuron during each ensemble. Within the depolarized area, there are apparently small (<200 μM $\times$ 100 ms) and more synchronized clusters.

Velocity of horizontal propagation

Ensemble activity propagates at a slow velocity (Fig. 4), significantly slower than that of all-or-none activities occurring in disinhibited cortical tissues (Fig. 4) (Chervin et al. 1988; Golomb and Amitai 1997; Golomb et al. 2000; Wadman and Gutnick 1993). This suggests that inhibition is an essential factor controlling the propagation velocity. Indeed, even when inhibition is only partially suppressed, the propagation velocity increases (Golomb et al. 2000) as well as the synchrony of the event (Fig. 9). An exceptionally slow propagation velocity of an epileptiform event was observed in a tangential layer IV slice (Fleidervish et al. 1998). In this tangential preparation the activity propagated in a two-dimensional plane, and the velocity observed between two points was only about 4 mm/s (Fleidervish et al. 1998). Comparing the Fleidervish's paper to our data, we found that their event was different from the ensemble activity described here. First, their event occurred only in a fully disinhibited tissue (in >10 μM bicuculline); in contrast, the ensemble activity was very sensitive to bicuculline (Figs. 1, 4, and 9). Second, their slow propagation velocity only occurred in the tangential layer IV slice; when other layers are intact (coronal slice) the propagation velocity is much higher using the same concentration of bicuculline (Fleidervish et al. 1998). In addition, the waveform of their event had much less fluctuation than that of ensemble activity (comparing Fig. 2 of Fleidervish et al. 1998 and Figs. 1, 5, 8, and 9 of this paper), suggesting that their event had a higher fraction of the neuronal population firing simultaneously. It would be interesting to apply voltage imaging to the tangential layer IV slice to examine the spatiotemporal organization and the actual propagation route in the two-dimensional plane of layer IV.

Oscillations and propagating waves

Population oscillations in cortical tissue often appear as propagating waves, and oscillation frequency appears to be correlated to the propagation velocity (Lam et al. 2000; Prechtl et al. 1997; Wu et al. 1999b). Ensemble activity has a slow propagation velocity and complex LFP oscillations with frequencies mainly distributed at the high end of the gamma band (Fig. 5). These oscillations might occur only in the localized neuronal clusters and not propagate. Coexistence of two or more oscillations has been observed both in vivo and in vitro (Fisahn et al. 1998; Lam et al. 2000; Prechtl et al. 1997), suggesting that the same active neuron population can sustain multiple oscillations. More analysis is needed to understand the correlation between locally organized clusters and the propagation pattern of a population event.

In LFP recordings, the waveform of ensemble activity was complex and varied considerably from trial to trial (Fig. 5). Complex local activity patterns can sometimes be determined by simple dynamic organizations. In fibrillating heart tissue, complex and apparently random temporal patterns in the electrocardiogram can be explained by the variation of a single spiral-like rotor (Jalife 1999). Similar dynamics have also been suggested during seizure-like activities (Gluckman et al. 1998). However, ensemble activity is an evoked event and its duration is short. This significantly limited our ability to analyze its dynamic composition.

Similar activities seen in vivo

Prolonged population activity has been extensively observed in vivo. A single electrical shock to the geniculocortical afferent can evoke a prolonged polysynaptic event in cat visual cortex (Douglas and Martin 1991). Recordings from the neurons in primary auditory cortex of anesthetized rat also showed prolonged discharges, occurring at 50–100 ms after tonal stimulus and lasting for several seconds (Maldonado et al. 1998). These in vivo activities have characteristics similar to the ensemble activity we observed in vitro. More generally, correlated firing of cortical neurons has been observed in behaving animals (Maldonado and Gerstein 1996; Nicolelis et al. 1995, 1997a, 1998; Usher et al. 1999), implying locally organized neuronal ensembles. Recordings during visual recognition tasks also suggest the existence of a self-sustained localized coactivation (Miyashita 1988; Miyashita and Chang 1988; Sakai and Miyashita 1991). During the silent period between two testing stimuli in the delayed match-to-sample tasks, many cortical neurons maintain a certain firing rate (Chelazzi et al. 1993; Fuster and Alexander 1971; Miller and Desimone 1994). Local recurrent excitation was suggested to explain this observation (Lisman et al. 1998). Correlated population activities also spontaneously occur in developing neocortex (Yuste 1997; Yuste et al. 1992), retina (Wong et al. 1995), locus coeruleus (Christie et al. 1989), spinal cord (O'Donovan et al. 1998), and hippocampus (Ben-Ari et al. 1989), suggesting that local recurrent excitation also plays a role in developing systems.

Voltage-sensitive dye imaging on intact turtle visual cortex and olfactory bulb showed that evoked response is organized as propagating domains of activation (Lam et al. 2000; Prechtl et al. 1997), which is strikingly similar to the ensemble activity we have seen in cortical slices. This suggests that the mecha-

nism(s) sustaining the ensemble activity may also be used in these in vivo population activities.

Functional implications

Our results suggest that cortical neurons can be activated in such a way that a large portion of the active neurons are not directly activated by the input. The duration and pattern of the population activity (e.g., oscillations) may also be substantially different from the input pattern. Correlated activation of multiple neurons may help to spatially integrate information coded by neighboring neurons. The prolonged duration may provide a time window of several hundred milliseconds, allowing an evoked activation to interact with the following input. Correlated population activity may also be a more reliable way of carrying information (Lisman 1997).

An influential hypothesis proposed that some cortical processing is executed in task-related neural assemblies, in which a local correlated activation is mutually maintained by the active neuron population (Hebb 1949; reviewed by Amit 1995; Nicolelis et al. 1997b). The membership of the neural assemblies may vary with the activity pattern in the network, and one neuron can also join multiple assemblies, depending on the context. Prolonged depolarization at a 1 mm $\times$ 300 ms scale may provide a preferred location for developing task-related cortical assemblies, because neurons within the depolarized area may have a lower threshold to afferent input and/or lateral excitation from neighboring neurons.

Finally, our data suggest that cortical local circuits work in an "episodic" mode. Afferent to the cortex elicits episodes of activation, and the duration of the episodes is relatively independent of the input. Such episodic activation may provide an elevated baseline excitation for context-related neuronal assemblies to be sustained and to interact with each other in a defined time and space.

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