Simultaneous Induction of Pathway-Specific Potentiation and Depression in Networks of Cortical Neurons

Y. Jimbo,* T. Tateno,* and H. P. C. Robinson#

*NTT Basic Research Laboratories, Kanagawa 243-0198, Japan, and #Physiological Laboratory, University of Cambridge, Cambridge CB2 3EG, England

ABSTRACT Activity-dependent modification of synaptic efficacy is widely recognized as a cellular basis of learning, memory, and developmental plasticity. Little is known, however, of the consequences of such modification on network activity. Using electrode arrays, we examined how a single, localized tetanic stimulus affects the firing of up to 72 neurons recorded simultaneously in cultured networks of cortical neurons, in response to activation through 64 different test stimulus pathways. The same tetanus produced potentiated transmission in some stimulus pathways and depressed transmission in others. Unexpectedly, responses were homogeneous: for any one stimulus pathway, neuronal responses were either all enhanced or all depressed. Cross-correlation of responses with the responses elicited through the tetanized site revealed that both enhanced and depressed responses followed a common principle: activity that was closely correlated before tetanus with spikes elicited through the tetanized pathway was enhanced, whereas activity outside a 40-ms time window of correlation to tetanic pathway spikes was depressed. Response homogeneity could result from pathway-specific recurrently excitatory circuits, whose gain is increased or decreased by the tetanus, according to its cross-correlation with the tetanized pathway response. The results show how spatial responses following localized tetanic stimuli, although complex, can be accounted for by a simple rule for activity-dependent modification.

INTRODUCTION

Activity-dependent modification of synaptic strength plays a central role in the formation of correct connections between neurons during development (Meister et al., 1991; Wong et al., 1993; Katz and Shatz, 1996) and in the processes of learning and memory in the mature central nervous system (Bliss and Collingridge, 1993; Artola and Singer, 1994). Long-term potentiation (LTP) (Bliss and Lømo, 1973) and depression (LTD) (Linden, 1994) are two important cellular mechanisms of synaptic modification that have been studied mainly as changes in the response of single neurons, or in local field potentials at single sites, after a strong inducing stimulus; they are particularly well characterized in the hippocampus (Bashir et al., 1994; McNaughton, 1993). Little is known, however, about network-wide changes in firing after localized inducing stimulation. Both LTP and LTD occur in the cerebral cortex, where they can be produced by rather similar protocols of repetitive stimulation. In general, LTP appears to require higher frequency stimulation than does LTD and results in a higher intracellular calcium level in postsynaptic cells (Hansel et al., 1996; Castro-Alamancos et al., 1995). Recently it has been reported that activity-dependent potentiation of synaptic efficacy is not restricted to the activated synapses, but can spread to nearby synaptic sites (Engert and Bonhoeffer, 1997). A similar phenomenon has been reported for LTD (Fitzsimonds et al., 1997). Thus the spatial effects of stimulus-induced synaptic modification are hard to predict from present knowledge.

Here we attempt to characterize some of the principles that govern spatial changes in activity after synaptic modification, by examining the activity of a large set of cortical neurons in a uniform network, before and after a single-site tetanic stimulus, in response to stimulation through a large set of different pathways. To do this, we cultured networks of dissociated cortical neurons, which form extensive functional synaptic connections and display synchronized spontaneous activity and synaptic plasticity (Robinson et al., 1993; Otsu et al., 1995), on the surface of planar electrode arrays, allowing extracellular single-unit recording and stimulation at 64 different sites.

MATERIALS AND METHODS

Cell culture

The method for preparing dissociated cortical cell cultures was based on a slightly modified version of the method of Muramoto et al. (1988). Cortical tissue was taken from E17-18 Wistar rat embryos and dissociated by trituration after digestion with 0.02% papain (Boehringer). Cells were plated on laminin and poly-D-lysine-coated (Sigma) electrode array substrates. The culture medium consisted of Dulbecco's modified Eagle's medium (DMEM) (Gibco) containing 5% FBS (Hy-clone), 5% heat-inactivated horse serum (Gibco), 2.5 μg/ml insulin (Sigma), and penicillin/streptomycin (5–40 U/ml; Sigma), conditioned overnight in glial cell cultures (Baughman et al., 1991). Half of the culture medium was exchanged twice a week. Recordings were carried out after 30–50 days in vitro (DIV). The electrophysiological properties of cortical neurons change during development, with morphological differentiation and expression of ion channels and receptors (Luhmann and Prince, 1991; Burgard and Hablitz, 1993). At the stage of culture used here, the spontaneous firing

Received for publication 5 January 1998 and in final form 12 October 1998.

Address reprint requests to Dr. Yasuhiko Jimbo, NTT Basic Research Laboratories, 3-1 Morinosato Wakamiya, Atsugi-shi, Kanagawa 243-0198, Japan. Tel.: 81-462-40-3524; Fax: 81-462-40-4728; E-mail: jimbo@will.brl.ntt.co.jp.

0006-3495/99/02/670/09 $2.00

patterns of neurons have reached a developmentally stable period (Kamioka et al. 1996; Watanabe et al., 1996).

Recording evoked responses through electrode array substrates

Culture substrates containing 64 embedded electrodes were used to stimulate and record activity in the network. The 64 electrode terminals were arranged in a grid covering an area of 1.6 × 1.3 mm (Fig. 1). A test stimulus pulse was applied from each of the 64 sites and scanned sequentially, and the extracellular spike responses to each test stimulus were recorded at all 64 sites for 160 ms. Recording of these 64 evoked network responses was repeated 10 times. After the stability of the responses was confirmed, tetanic stimulation was applied to a single site showing a moderate local response. The test stimulus consisted of a single bipolar pulse (100 μs at +0.6 V, followed by 100 μs at −0.6V). The stimulus was applied at 3-s intervals from sequential stimulation sites. For tetanic stimulation, 20 trains of 10 pulses of the same intensity and duration at 20 Hz were applied at 5-s intervals. The same 64 × 10 evoked responses were then recorded again. The total experiment thus included 1280 test-evoked responses and 20 tetanus-evoked responses and lasted ~70 min. Stimulation sites were scanned and individual sites were rapidly switched between stimulation and recording with custom programmable circuits containing the recording preamplifiers and TTL-controlled analog switches. Constant voltage stimulation was used, and the stimulation pulses were added to the DC offsets at each electrode, which were tracked and stored by sample/hold circuits. This avoided the problem of drift in the properties of the electrode/electrolyte interface and allowed a stable stimulus to be applied. The input levels of all of the recording preamplifiers were held at the stored DC offset levels during the stimulation period to avoid saturation, thereby allowing recording of action potentials as soon as 5 ms after stimulation at the same site. To examine tetanus-induced changes in synaptic currents, whole-cell patch-clamp recording was carried out, using an intracellular solution of the following composition (mM): 125 potassium methylsulfonate, 5 $MgCl_2$, 10 HEPES, 10 glucose, 0.2 EGTA, 4.5 Na-ATP, 0.5 Na-GTP, pH 7.2. The bath solution consisted of (in mM) 148 NaCl, 2.8 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 10 HEPES, and 10 glucose (pH 7.2).

Data analysis

Spikes were detected on-line as excursions above a threshold of 5 × σ, the standard deviation of the signal during quiescent periods, and stored on hard disk. A sampling rate of 25 kHz per channel was used. Spikes corresponding to individual cells were separated from the set of detected spikes, as clusters in the amplitude versus width distributions (Meister et al. 1994). Cross-correlation functions between the activity evoked through single pathways and that through the tetanic pathway were calculated by the following procedure. For the kth trial (k = 1, . . . , 10), for neuron i (i =

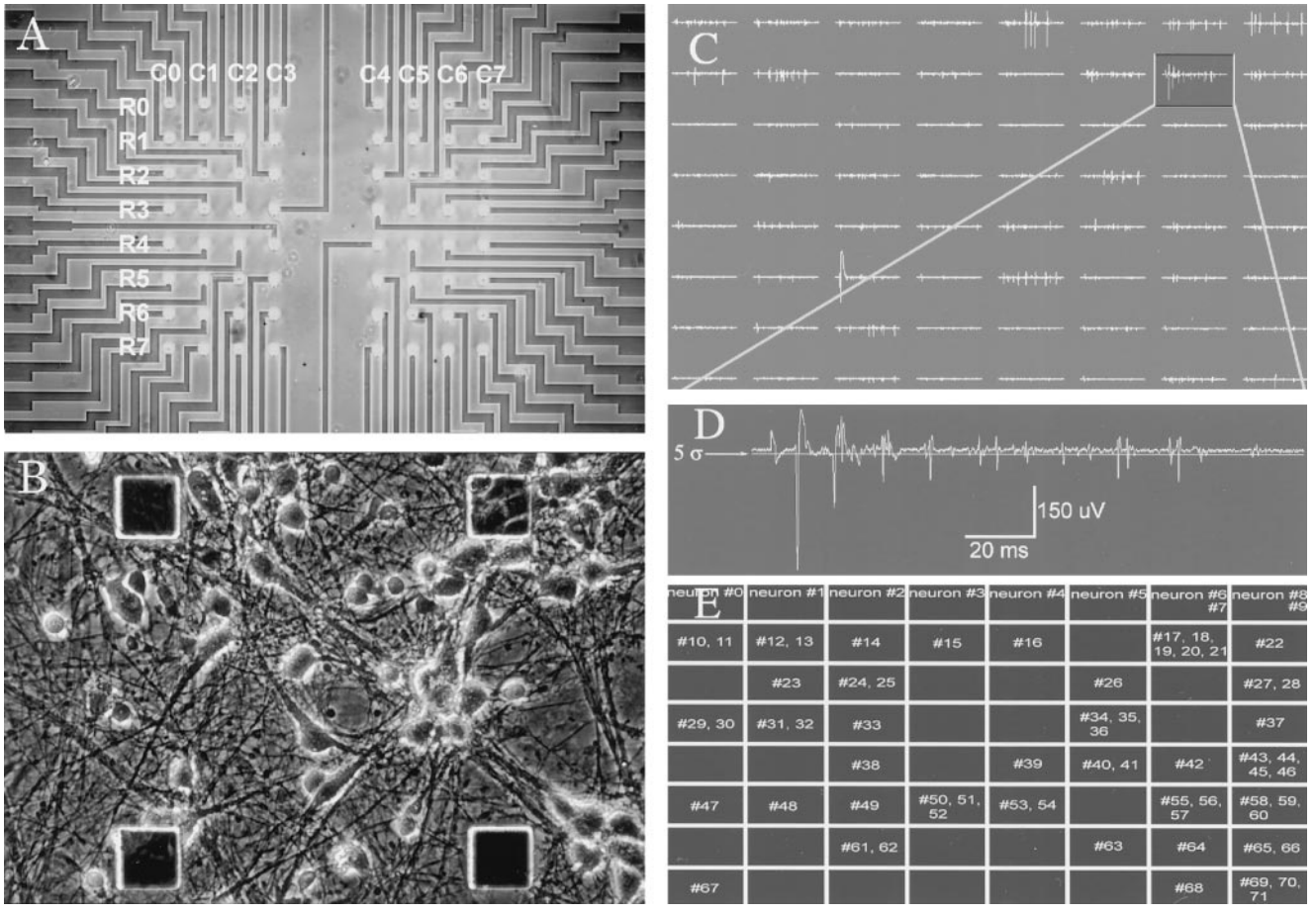

FIGURE 1 Multisite spike recording method. (*A*) A transparent electrode array substrate. The recording area consists of two blocks of 32 embedded electrodes, separated by 500 μm. Electrode terminals had an area of 30 × 30 μm, and the distance between neighboring terminals was 180 μm (center to center). Each site is identified by row and column number. (*B*) Network of cortical neurons cultured on the substrate. The four dark squares are platinized substrate electrodes. (*C*) Extracellular potential traces measured at the 64 sites in response to stimulation through the site (R5, C2), where a large artefact is observed. (*D*) An enlarged view of the activity at one site, showing the spike detection threshold of five times the standard deviation of the baseline noise (σ). (*E*) Locations of neurons identified at the 64 sites. The total number of identified neurons was 72.

1, . . . , 72) and stimulation site j ($j = 1, \ldots, 64$), let the sequence of spike times in units of the sampling interval (1/25 ms) be $\{n_{i,j}^{(k)}(u)\}$ ($u = 1, 2, \ldots, N_{i,j}^{(k)}$), where $N_{i,j}^{(k)}$ is the number of spikes recorded. The recorded spike trains, denoted by $sp_{i,j}^{(k)}(n)\}$ ($n = 1, 2, \ldots, N_{samp}$), where N_{samp} is the number of samples, were also represented in 1/25-ms time bins, but individual spikes were spread over ±1 ms n to smooth the cross-correlation function:

$$sp_{i,j}^{(k)}(n) = \sum_{u=1}^{N_{i,j}^{(k)}} \delta(n - n_{i,j}^{(k)}(u)) \quad (1)$$

where $\delta(t)$ is defined by

$$\delta(t) = \begin{cases} 1 & (-25 \le t < 25) \\ 0 & (\text{otherwise}) \end{cases} \quad (2)$$

The aggregate of 10 trials for pre- or posttetanus was calculated as

$$SP_{i,j}(n) = \sum_{k=1}^{10} sp_{i,j}^{(k)}(n) \quad (3)$$

Using this representation, the cross-correlation function of $SP_{i,j}(n)$ with the tetanus-site activated sequence $SP_{i,tet}(n)$ was calculated as

$$crl_{i,j}(m) = \sum_{n} SP_{i,j}(n) SP_{i,tet}(n + m) \quad (4)$$

and normalized by the value of their autocorrelation functions at $m = 0$ to be $Crl_{i,j}(m)$. The average of this correlation function was calculated as

$$C_j(m) = \frac{1}{Na_j} \sum_{i=1}^{Na_j} Crl_{i,j}(m) \quad (5)$$

where Na_j is the number of neurons that were activated by both site j and the tetanus site. This was calculated for each of the 64 stimulation sites for the pre- and posttetanus trials.

RESULTS

Network responses from multiple stimulation sites

The electrode-array culture substrate is shown in Fig. 1 *A*. The 64 electrode terminals were arranged in a grid covering an area of 1.6 × 1.3 mm. Rat cortical neurons were cultured on the substrates (Fig. 1 *B*), and recordings were carried out after 30–50 days in vitro (DIV) cultures. Our conclusions are based on data from eight different cultures recorded under the same conditions. Of these, data from a single culture (41 DIV), which demonstrates our conclusions most clearly, are described below in detail. Firstly, a test stimulus pulse was applied from each of the 64 sites and scanned sequentially, and the extracellular spike responses to each test stimulus were recorded at all 64 sites. Spikes corresponding to individual cells were separated from the set of detected spikes (Fig. 1, *C* and *D*) as clusters in the amplitude versus width distributions (Meister et al., 1994). A total of 72 neurons, with a spatial distribution as shown in Fig. 1 *E*, were identified in this way.

The amplitude of the stimulus was set to produce a restricted local activation of neurons, rather than a regeneratively propagating wave of activity. With the stimulus used here, less than half of the 72 identified neurons were activated in 31% of all of the trials. In 72% of the trials, at least one-third of the neurons were silent. From the density of neurons and the extent of spread of activity, we estimate that the functional networks involved in these responses comprise ~200–300 neurons.

Tetanus-induced changes in network activity and synaptic currents

These 64 evoked network responses were recorded 10 times. After the stability of the responses was confirmed, tetanic stimulation was applied to a single site showing a moderate local response, in this case from electrode (R6, C4). The same 64 × 10 evoked responses were then recorded again. Fig. 2 shows tetanus-induced changes in the numbers of evoked spikes, displayed as a matrix of the responses of 72 neurons for each of 64 stimulation pathways, coded by color to show no change (*green*), an increase (*red-yellow*), or a decrease (*blue-black*) in the number of spikes. Three aspects of the effects of the single-site tetanus are demonstrated in this figure. First, both enhancement and depression of activity are produced by the same tetanic stimulus, in different pathways. Second, neurons activated by a particular pathway show changes in the same direction (changes along any row in Fig. 2 are either increases or decreases, but not a mixture). Examples of this are shown in more detail for two pathways at the top of Fig. 2. Third, individual cells (columns in Fig. 2) show a mixture of enhancement and suppression of activity when activated by different stimulation pathways. An example of the profile of change in a single cell activated by different pathways is shown at the right of Fig. 2. This observation is consistent with the ability of single cortical neurons to express both LTP and LTD simultaneously for different synaptic inputs (Artola and Singer 1994). Thus potentiation or depression is pathway-specific, not neuron-specific. Both potentiation and depression effects lasted throughout the 32-min period of recording posttetanus, as can be observed in Fig. 4. Of the eight different cultures tested, four showed a mixture of enhancement and depression in different pathways, and four showed almost exclusively potentiation or no change, as summarized in Table 1. Homogeneity of potentiation or depression in individual pathways was observed in all cultures.

Tetanic stimulation also produced a modification of synaptic currents recorded in whole-cell patch-clamp mode. An example of potentiation is shown in Fig. 3, where a maintained increase in the amplitude, particularly of the late phase, of the synaptic current is seen after tetanus. This change appears to correspond to a higher number of unitary components in the synaptic current. Whole-cell recording was carried out in 15 cells, with eight cells showing clear potentiation of synaptic currents, which was more marked in the late components of the current. These changes in whole-

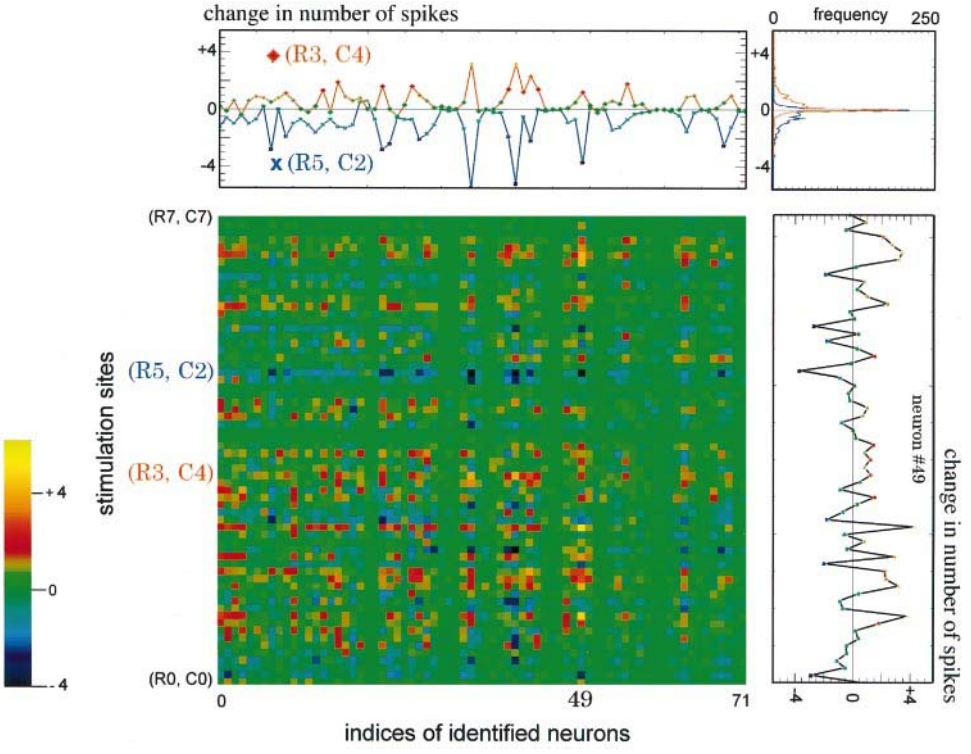

FIGURE 2 Network activity changes induced by tetanus. A stimulus pulse was applied through each of the 64 sites sequentially, and the total number of spikes generated in each of the 72 neurons was counted. This procedure was repeated 10 times before and after tetanus, and the average was computed for each case. The differences in the averages are displayed using a color map in a 72 × 64 matrix, with green indicating no change, and red-yellow and blue-black corresponding to increased and decreased activity, respectively (*color scale at left*). The profiles of two example responses to stimulation at sites (R3, C4) and (R5, C2) are plotted in the upper panel, showing that the population of neurons responds homogeneously with increased or decreased activity, respectively. The upper right inset shows the distribution of changes in two groups (*red and blue*) of 10 selected stimulus pathways, showing that almost all stimulus pathways fall into one of two one-sided distributions: increased activity or decreased activity. In contrast, the responses of a single cell (cell 49) to the 64 stimulus pathways show a mixture of enhancement and depression.

cell currents were maintained for the duration of whole-cell recording: for example, in one cell, the total synaptic charge flux at 10 min after tetanus was 205% of the pretetanus level and 220% at 30 min after tetanus.

Triggering of potentiation and depression

To attempt to understand the factors that control the direction of the change in activity, we examined whether the changes displayed by different cells in a pathway were correlated with their firing level during the tetanic stimulus. Fig. 4, *A* and *C*, shows the changes produced in two pathways, one with increased activity in its output cells (R3, C4) and the other with decreased activity (R5, C2), both over 400 μm from R6, C4, the site of tetanus. To clarify the relationship between the change in spike number in each pathway and the level of firing during tetanus, the responses of cells were sorted according to the level of pretetanic activity, and the corresponding activity during tetanus was plotted below. As can be seen for the pathway with tetanus-enhanced activity (Fig. 4 *A*), there is a clear correlation, which is reflected in a positive correlation coefficient of 0.65. The same is true for the pathway showing decreased posttetanic activity (Fig. 4 *C*). However, the average correlation coefficients ($n = 10$) were 0.49 for pathways with increased activity and 0.50 for pathways with depressed activity. This suggests that triggering of either enhancement or depression in a pathway requires a correlation with the number of spikes elicited during tetanus—this by itself, though, does not determine whether the pathway is enhanced or depressed. However, a different pattern was observed in the individual spike trains in the two cases. Fig. 4 *B* shows the spike trains generated in neuron 9 in response

TABLE 1 Summary of tetanus-induced changes in stimulus pathways in eight cultures

Sample	[DIV]	Potentiated	Depressed	No change
A	41	19	9	36
B	44	20	7	37
C	44	21	1	42
D	44	37	0	27
E	47	13	13	38
F	48	10	14	40
G	52	20	1	43
H	53	33	0	31

The numbers of potentiated pathways, depressed pathways, and pathways showing no change after tetanus are shown. A change is defined as four or more neurons in the pathway showing a shift of at least one in the mean number of spikes in each sweep, averaged over 10 trials.

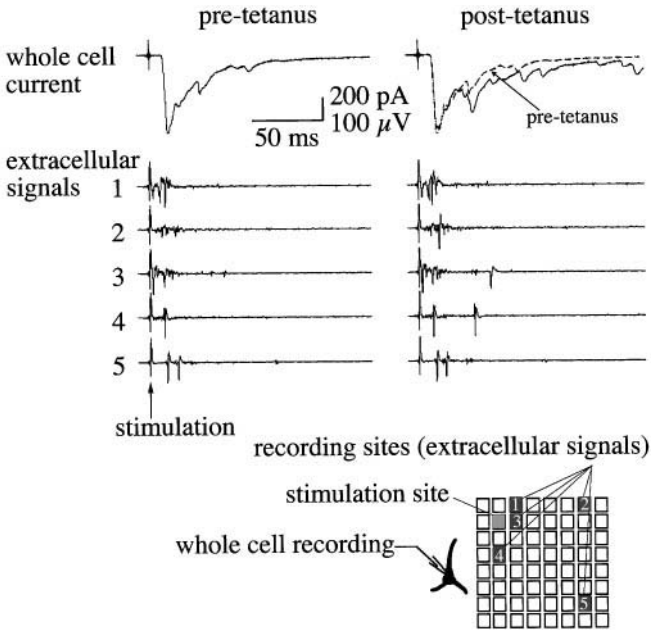

FIGURE 3 Potentiation of synaptic currents induced by tetanus. Whole-cell current recorded in a single cell (*top*) and activity in five extracellular channels recorded in response to a single stimulus delivered at (R1, C1), before tetanus (*left*) and after tetanus (*right*). The cell was located near the outside of the substrate electrode region. Membrane potential was clamped at the resting level (−60 mV). Synaptic currents after tetanus showed a maintained increase in amplitude, and in the number of unitary components, which paralleled an increase in the number of spikes evoked extracellularly.

to the (R3, C4) stimulus. In this case, the spikes were concentrated in the first 50 ms, both before and after tetanus, but the reliability of spikes is clearly seen to increase after potentiation. For example, a spike is elicited at 20 ms after the stimulus in five of 10 trials before tetanus, and in seven of 10 trials after tetanus, and at 45 ms in four of 10 trials before tetanus and in nine of 10 trials after tetanus. The responses of the same neuron to stimulation of the (R5, C2) pathway are shown in Fig. 4 *D*. In this case, late spikes were significantly depressed after tetanus, although the early part (<50 ms) remained almost unchanged. This was a general phenomenon in the set of 72 neurons, as shown clearly by raster plots of the pretetanus and posttetanus spike trains of all neurons (Fig. 5) activated by stimulation of the potentiated (R3, C4) pathway (Fig. 5 *A*) or the depressed (R5, C2) pathway (Fig. 5 *B*), and was found in the other stimulus pathways examined (not shown).

Correlations with activity in the tetanized pathway

What factors govern whether potentiation or depression of a pathway is produced by tetanic stimulation? Clearly, for any given stimulus pathway, there is a wide variation in the number of spikes produced in different cells, and so the absolute numbers of spikes do not determine the direction of change for a specific pathway, as already noted. We therefore examined the influence of spike timing, by computing the cross-correlation function of spike trains during stimulation through a particular pathway with the spike trains evoked by single pulses in the tetanized pathway (Fig. 6). The cross-correlation function is a measure of the frequency with which one cell fires, as a function of time relative to firing of an action potential in another cell. In the case of a potentiated stimulus pathway (Fig. 6 *A*), the spikes show a relatively tight correlation, concentrated within ±50 ms, to tetanic pathway spikes. After tetanus, the peak is narrower still. In a depressed stimulus pathway (Fig. 6 *B*), spikes before tetanus are much more loosely correlated to tetanic pathway spikes, but the effect of tetanus is qualitatively the same: a pronounced contraction of the cross-correlation function around its central peak, enhancing the frequency of spikes that coincide closely with spikes in the tetanized pathway and depressing the frequency of those that do not. This is demonstrated in Fig. 6 *C*, which compares the ratio of the area in the central 16 ms of the average cross-correlation function after tetanus to that before, for all 64 stimulation pathways. For 60 of 64 cases, this ratio increased. The average factor of increase was 1.35 ± 0.29. The maximum of the correlation coefficient increased from 0.23 ± 0.11 to 0.26 ± 0.10. Thus tetanic stimulation caused a relative strengthening of the parts of each stimulus pathway that are closest in correlation to the tetanus-activated pathway and depression of the rest. If the stimulus-activated pathway is initially closely correlated in time, then an overall enhancement of firing is produced, whereas if it is loosely correlated, a depression in overall number of spikes results. The closeness of correlation may reflect the number of synapses that a pathway has in common with the tetanically activated pathway.

DISCUSSION

In this study we have examined how a large number of different synaptic pathways in a network of cortical neurons are affected by a single, localized tetanic stimulus, to characterize how a complex network responds to a single stimulus event, and whether the response can be understood or predicted from our current knowledge of LTP and LTD in the cortex. We have used a preparation in which this goal is experimentally feasible, a dissociated culture of cortical neurons on the surface of an electrode array. The electrophysiological characteristics and synaptic physiology of cortical neurons in culture appear to closely parallel those in intact tissue of the same age (McCormick and Prince, 1987), and the structure of these cultured networks in many ways resembles the uniform and highly divergent synaptic connections of the local circuitry of intact cortex. Our results, therefore, should give some insight into the changes that might occur in the network in intact developing cortex after a localized tetanic stimulation.

We found that a single, localized tetanic stimulus produces long-lasting changes in the network responses to single stimuli delivered at many different sites in the net-

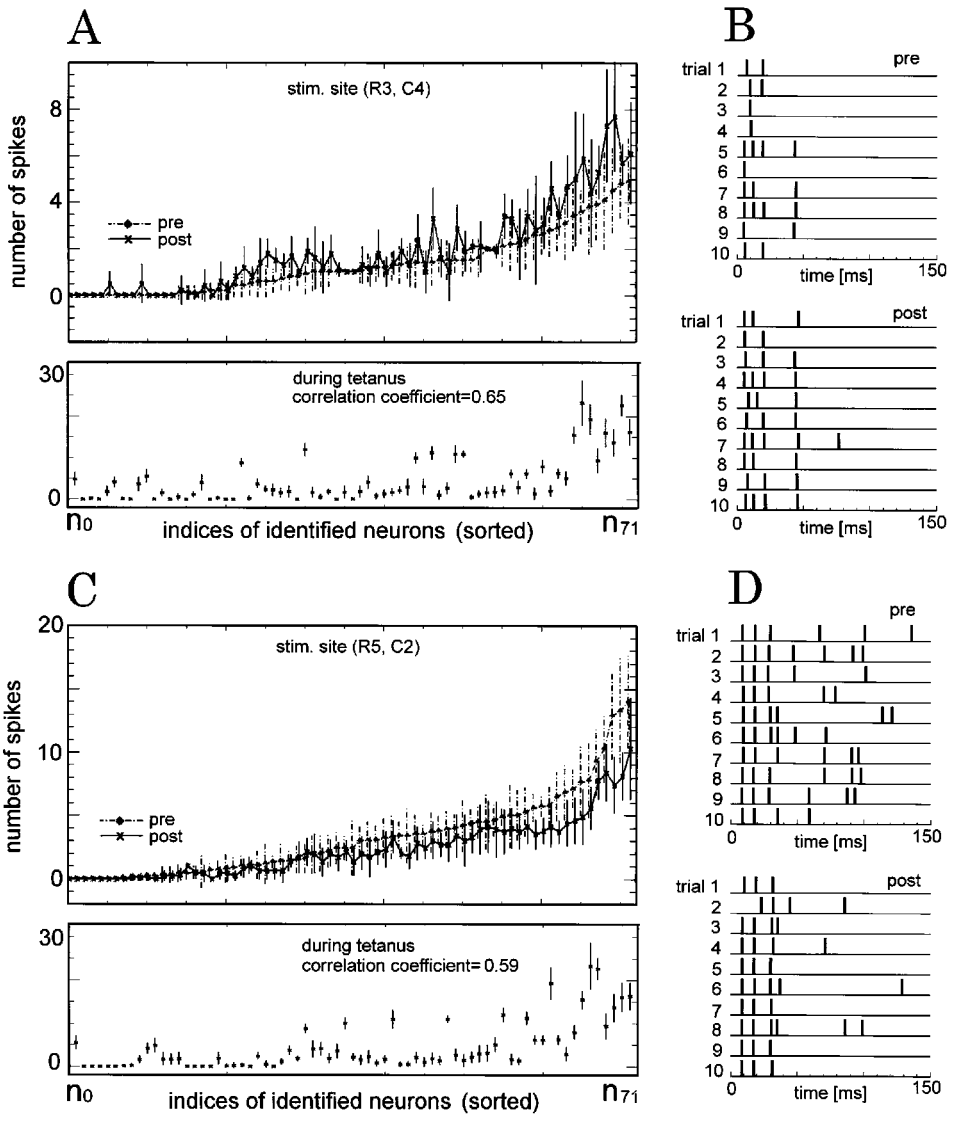

FIGURE 4 Correlation of activity change with activity in the tetanic pathway. (*A*) A pathway (R3, C4) showing potentiated activity. The activity of cells before (– · – ◆ – · –) and after (— × —) tetanus is plotted in order of increasing pretetanic activity in the top panel, and activity during tetanus is plotted with the same order in the lower panel. The solid vertical bars represent the standard deviations for 10 trials. A clear correlation between the degree of increase and the amount of activity during tetanus is seen (correlation coefficient = 0.65). (*B*) Individual spike trains of neuron 9 in response to the (R3, C4) stimulus, showing an increased reliability of early spikes. (*C*) As in *A*, but for stimulus pathway (R5, C2), which showed a homogeneous decrease in activity. The correlation coefficient between the absolute amount of decrease in activity and the amount of activity during tetanus was 0.59. (*D*) Spike trains of neuron 9 in response to stimulus pathway (R5, C2), showing depressed late spikes.

work (Fig. 2). Because of the nature of the recording technique, long-term modifications in pathways in the culture were measured as changes in the number of extracellular spikes recorded from resolved neurons. However, we confirmed that these changes were associated directly with modifications of synaptic currents elicited in single neurons, using simultaneous whole-cell voltage clamp from single neurons (Fig. 3). Larger numbers of spikes recorded extracellularly corresponded to longer-lasting bursts of unitary synaptic currents in individual neurons, with a greater integrated amplitude or total charge flux. The fact that this potentiation of synaptic current was maintained throughout the time course of whole-cell recording (~30 min) supports the idea that the changes in network response before and after tetanus reflect processes of long-term synaptic modification.

It could be argued that tetanus could induce a long-lasting electrochemical change in the stimulated electrode, which could lead to modifications in subsequent network responses. However, this is extremely unlikely, as the network responses are in general elicited by the stimulation of electrodes different from those used to elicit the tetanus. Another possibility might be that tetanus produces release of a diffusible substance that directly affects neuronal excitability or modifies synaptic strength. However, this would not explain the heterogeneity of the changes, or their long-lasting nature. Thus we believe that the most likely explanation for the tetanus-induced changes in network response is the summed effect of long-term potentiation (Otsu et al., 1995) and long-term depression at synapses in the culture.

It is noticeable that the potentiation of synaptic currents is more pronounced at later stages of the response, ~20 ms after the stimulus, than in the earlier phase. This could be explained if the early components are monosynaptic, whereas later components are polysynaptic, if a small change in the amplitude or reliability of the monosynaptic component is amplified in its effect on recruitment of polysynaptic pathways. Changes in the numbers of spikes observed through the electrode array show a similar tendency.

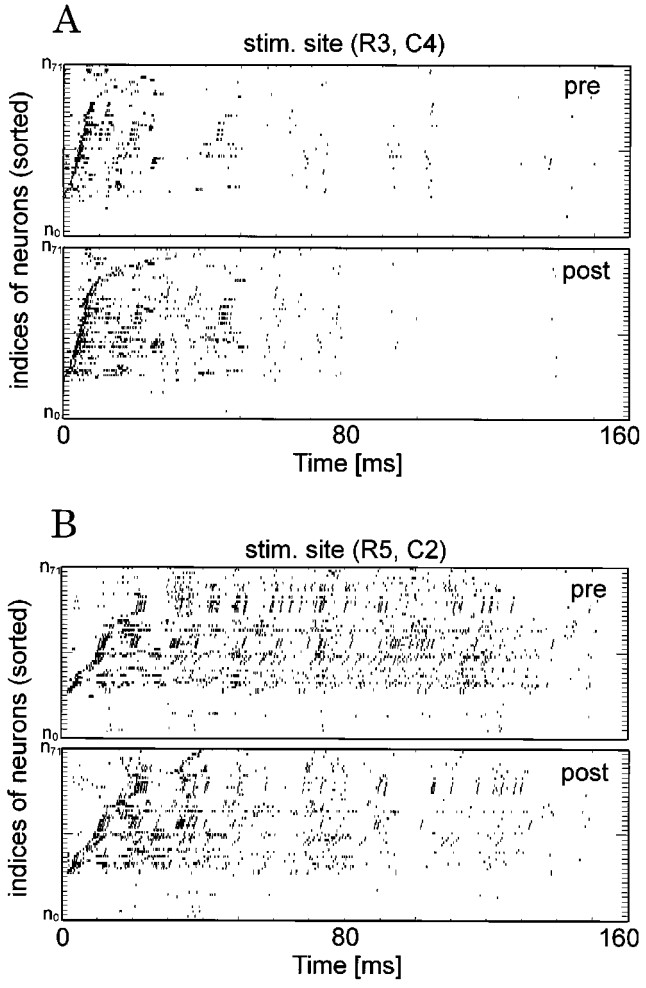

FIGURE 5 Raster representation of spike trains in the 72 neurons. All spikes from 10 trials are superimposed on individual traces, for pre- and posttetanus. The order of neurons was sorted by the latency to the first spike. (*A*) (R3, C4) response as an example of activity increase. The number of spikes increased significantly after tetanus in the first half of the recording. In the second half, although the number of spikes was small, tetanus produced a slight decrease. (*B*) (R5, C2) response as an example of activity decrease. No significant changes are seen in the early component, but a clear, population-wide decrease in activity is seen in the late component.

The fact that tetanus delivered at one site affects responses elicited from a widely separated site implies that the pathways activated by the two sites share common neurons. This is not surprising, in view of the wide and random divergence in these cultures (Maeda et al., 1995). A surprising feature of these changes was that the direction, or sign, of the change in activity was homogeneous for each stimulus pathway. A stimulus pathway originates in a set of neurons that are excited directly by a single stimulus pulse and then fire repetitively, presumably as a consequence of recurrent excitation (Douglas et al., 1995). From whole-cell recordings of synaptic currents elicited by single stimulus pulses at multiple sites, we estimate, very roughly, that neurons have a 10–20% chance of forming a monosynaptic connection with nearby neurons. Assuming that connections are random, there would be a large number of recurrent polysynaptic connections, as in the intact cortex. It is reasonable to speculate, therefore, that the firing of all neurons in the group will be determined by whether the overall synaptic gain of a central, reverberating circuit of cells, specific for each stimulus pathway, has been increased or decreased, according to how closely correlated the spikes generated by the circuit are with those generated by a single pulse in the tetanized pathway. This would then imply that the repetitive tetanic stimulation, in addition to strongly activating the circuit associated with the tetanized pathway, also produces a widespread weaker activation of those reverberating circuits that become modified.

The fact that the same tetanus produced potentiation in individual neurons activated through some pathways and depression in the same neurons when activated through other pathways is evidence that long-term modifications are synapse-specific rather than cell-specific. It may also explain why modifications of the responses of single cortical neurons or single sites after tetanic input stimulation in the cortex in the literature are so variable, in both magnitude and direction (Tsumoto, 1992). The relatively diffuse and widely divergent connections in the cortex and in these cortical cultures, for example, in comparison to the hippocampus, mean that a single tetanus could lead to a wide range of degrees of excitation in many different areas and therefore possibly to different levels or directions of synaptic modification in different locations. We found that we could account for this variation by analyzing the aggregate cross-correlation of firing in individual pathways with firing in the tetanized pathway. This showed that both net potentiation and net depression involve an increase in the proportion of spikes in close synchrony ($<$40 ms difference) to spikes in the tetanized pathway and a decrease in the proportion of spikes at larger time differences—a "contraction" of the cross-correlation function. This emphasizes that potentiation and depression represent two different possible outcomes of the same process. Which actually occurs in a pathway is determined by the initial degree of spread in the cross-correlation: tightly correlated pathways become potentiated, loosely correlated pathways become depressed. The same principle has also been inferred from cross-correlations of activity between pairs of neurons in the auditory cortex of behaving monkeys by Ahissar et al. (1992), who suggested that it acts to select behaviorally relevant connections between cortical neurons. The requirement for precise coincidence to produce synaptic-specific enhancement has recently been demonstrated in single cortical pyramidal neurons by Markram et al. (1997), who showed that spikes need to occur within 100 ms of synaptic inputs to produce potentiation. The present results thus demonstrate some consequences of this principle in a network: namely, that enhancement and depression of different pathways occur simultaneously, according to their correlation with the tetanically activated pathway, and can be routed in parallel through the same neurons.

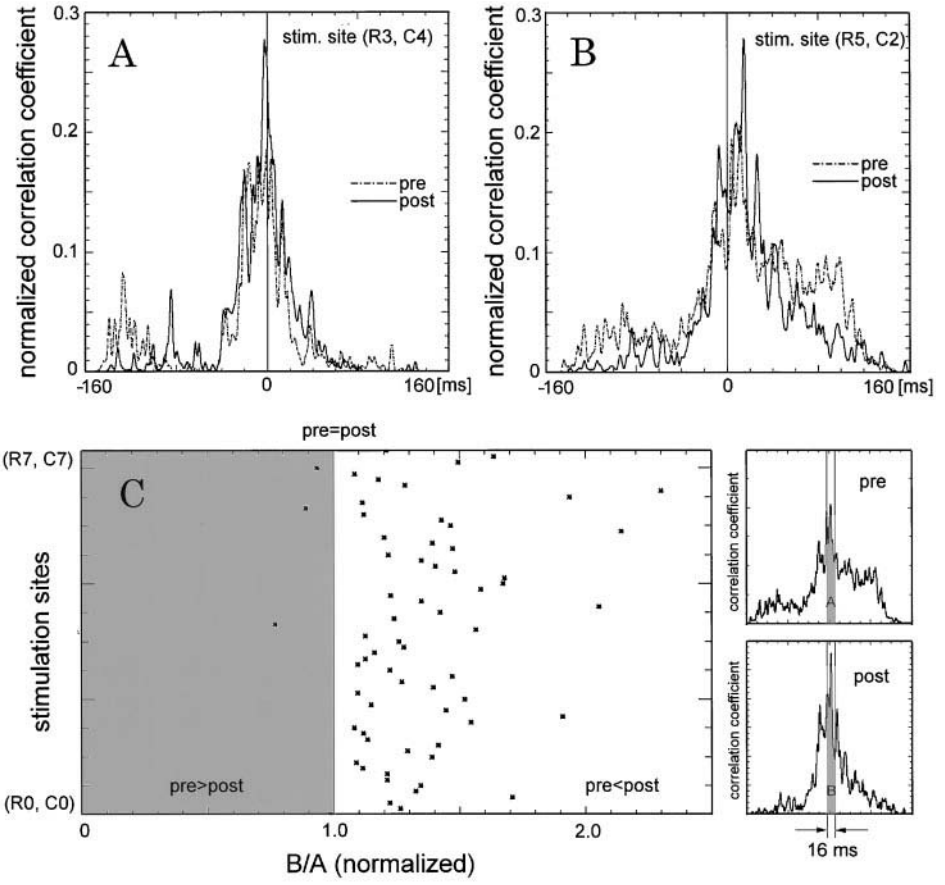

FIGURE 6 Cross-correlation of activity in stimulus pathways and in the tetanus-activated pathway. Cross-correlation was calculated by the procedure described in Materials and Methods. (*A*) Average cross-correlation functions for the (R3, C4) pathway as an example of activity increase. The solid line indicates posttetanus and the broken line pretetanus. The maximum increased posttetanus by ~50%. (*B*) Average cross-correlation functions for the (R5, C2) evoked response as an example of activity decrease. After tetanus, firing at large delays from the central peak of correlation is suppressed, whereas the most strongly correlated firing is elevated. (*C*) Distribution of an index of contraction of the average cross-correlation function for different stimulus pathways. The ratio of the proportion of spikes lying within ±8 ms of the peak after tetanus (*lower inset, shaded area*) to that before tetanus (*upper inset*) is plotted for each stimulus pathway. In 60 of 64 pathways, this index of contraction showed a clear increase. The average index was 1.35, and the standard deviation was 0.29.

We thank Dr. A. Kawana of NTT Basic Research Laboratories for useful discussions.

This work was supported by a grant from the Human Frontiers Science Program (RG 89/94B) and from the EC Biotech Programme (CT962011).

REFERENCES

Ahissar, E., E. Vaadia, M. Ahissar, H. Bergman, A. Arieli, and M. Abeles. 1992. Dependence of cortical plasticity on correlated activity of single neurons and on behavioral context. *Science.* 257:1412–1415.

Artola, A., and W. Singer. 1994. NMDA receptors and developmental plasticity in visual neocortex. *In* The NMDA Receptor. G. L. Collingridge and J. C. Watkins, editors. Oxford University Press, London. 313–339.

Bashir, Z., N. Berretta, Z. Bortolotto, K. Clark, C. Davis, B. G. Freguelli, J. Harvey, B. Potier, and G. L. Collingridge. 1994. NMDA receptors and long-term potentiation in the hippocampus. *In* The NMDA Receptor. G. L. Collingridge and J. C. Watkins, editors. Oxford University Press, London. 295–312.

Baughman, R. W., J. Huettner, K. A. Jones, and A. A. Khan. 1991. Cell culture of neocortex and basal forebrain from postnatal rats. *In* Culturing Nerve Cells. G. Banker and K. Goslin, editors. MIT Press, Cambridge, MA. 227–249.

Bliss, T. V., and G. L. A. Collingridge. 1993. A synaptic model of memory: long-term potentiation in the hippocampus. *Nature.* 361:31–39.

Bliss, T. V., and T. Lømo. 1973. Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. *J. Physiol. (Lond.).* 232:331–356.

Burgard, E. C., and J. J. Hablitz. 1993. Developmental changes in NMDA and non-NMDA receptor-mediated synaptic potentials in rat neocortex. *J. Neurophysiol.* 69:230–240.

Castro-Alamancos, M. A., J. P. Donoghue, and B. W. Connors. 1995. Different forms of synaptic plasticity in somatosensory and motor areas of the neocortex. *J. Neurosci.* 15:5324–5333.

Douglas, R. J., C. Koch, M. Mahowald, K. A. Martin, and H. H. Suarez. 1995. Recurrent excitation in neocortical circuits. *Science.* 269:981–985.

Engert, F., and T. Bonhoeffer. 1997. Synapse specificity of long-term potentiation breaks down at short distances. *Nature.* 388:279–284.

Fitzsimonds, R. M., H. Song, and M. Poo. 1997. Propagation of activity-dependent synaptic depression in simple neural networks. *Nature.* 388: 439–448.

Hansel, C., A. Artola, and W. Singer. 1996. Different threshold levels of postsynaptic $[Ca^{2+}]_i$ have to be reached to induce LTP and LTD in neocortical pyramidal cells. *J. Physiol. (Paris).* 90:317–319.

Kamioka, H., E. Maeda, Y. Jimbo, H. P. C. Robinson, and A. Kawana. 1996. Spontaneous periodic synchronized bursting during formation of mature patterns of connections in cortical cultures. *Neurosci. Lett.* 206: 109–112.

Katz, L. C., and C. J. Shatz. 1996. Synaptic activity and the construction of cortical circuits. *Science.* 274:1133–1138.

Linden, D. J. 1994. Long-term synaptic depression in the mammalian brain. *Neuron.* 12:457–472.

Luhmann, H. J., and D. A. Prince. 1991. Postnatal maturation of the CABAergic system in rat neocortex. *J. Neurophysiol.* 65:247–263.

Maeda, E., H. P. C. Robinson, and A. Kawana. 1995. The mechanisms of generation and propagation of synchronized bursting in developing networks of cortical neurons. *J. Neurosci.* 15:6834–6845.

Markram, H., J. Lübke, M. Frotscher, and B. Sakmann. 1997. Regulation of synaptic efficacy by coincidence of postsynaptic APs and EPSPs. *Science.* 275:213–215.

McCormick, D. A., and D. A. Prince. 1987. Post-natal development of electrophysiological properties of rat cerebral cortical pyramidal neurons. *J. Physiol. (Lond.).* 393:743–762.

McNaughton, B. L. 1993. The mechanism of expression of long-term enhancement of hippocampal synapses: current issues and theoretical implications. *Annu. Rev. Physiol.* 55:375–396.

Meister, M., J. Pine, and D. A. Baylor. 1994. Multi-neuronal signals from the retina: acquisition and analysis. *J. Neurosci. Methods.* 51:95–106.

Meister, M., R. O. Wong, D. A. Baylor, and C. J. Shatz. 1991. Synchronous bursts of action potentials in ganglion cells of the developing mammalian retina. *Science.* 252:939–943.

Muramoto, K., K. Kobayashi, S. Nakanishi, Y. Matsuda, and Y. Kuroda. 1988. Functional synapse formation between cultured neurons of rat cerebral cortex. Block by a protein kinase inhibitor which does not permeate the cell membrane. *Proc. Jpn. Acad. Ser. B Phys. Biol. Sci.* 64:319–322.

Otsu, Y., F. Kimura, and T. Tsumoto. 1995. Hebbian induction of LTP in visual cortex: perforated patch-clamp study in cultured neurons. *J. Neurophysiol.* 74:2437–2444.

Robinson, H. P. C., M. Kawahara, Y. Jimbo, K. Torimitsu, Y. Kuroda, and A. Kawana. 1993. Periodic synchronized bursting and intracellular calcium transients elicited by low magnesium in cultured cortical neurons. *J. Neurophysiol.* 70:1606–1616.

Tsumoto, T. 1992. Long-term potentiation and long-term depression in the neocortex. *Prog. Neurobiol.* 39:209–228.

Watanabe, S., Y. Jimbo, H. Kamioka, Y. Kirino, and A. Kawana. 1996. Development of low magnesium-induced spontaneous synchronized bursting and GABAergic modulation in cultured rat neocortical neurons. *Neurosci. Lett.* 210:41–44.

Wong, R. O., M. Meister, and C. J. Shatz. 1993. Transient period of correlated bursting activity during development of the mammalian retina. *Neuron.* 11:923–938.