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LONG-TERM CHANGES IN SYNAPTIC STRENGTH ALONG SPECIFIC INTRINSIC PATHWAYS IN THE CAT VISUAL CORTEX

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SUMMARY

1. The dense system of horizontal connections that arise and course within the striate cortex are thought to inform single cells about stimuli arising in disparate points in visual space and to modulate responses evoked from within the receptive field. To learn whether or not the strength of the horizontal connections could vary over the long term, and if such changes could affect the integration of vertical, interlaminar inputs, we have recorded intracellularly from the superficial layers in slices of the adult cat's visual cortex.

2. The monosynaptic EPSP evoked by stimulating horizontal fibres showed long-term facilitation in twelve of the twenty cells that were conditioned by repetitively pairing synaptic responses with depolarizing pulses of current; the maximum increase observed was 200%. Strong inhibition present in the postsynaptic response usually indicated that facilitation would not occur.

3. In instances where horizontal input evoked both mono- and polysynaptic EPSPs, both early and late events showed facilitation, with the most dramatic enhancement contributed by the polysynaptic components.

4. For the twenty-eight cells whose responses to stimulation of interlaminar as well as horizontal pathways were assessed, all were found to receive non-overlapping inputs from each source. Conditioning produced long-term changes in the strength of the interlaminar inputs.

5. Changes in synaptic strength were usually confined to the conditioned pathway, though in four out of twenty-six times we observed heterosynaptic facilitation of polysynaptic EPSPs.

6. The conditioning protocol led to lasting depression rather than facilitation in three out of eleven instances; the reduction was only observed in the multisynaptic components.

7. We suggest that the synaptic changes observed here may be related to certain dynamic changes in receptive field properties that have been characterized *in vivo*.

INTRODUCTION

Neuronal response properties in the visual cortex vary with changes in stimulus context. Recordings from single cells made *in vivo*, for example, show that illumination of the surround, though alone unable to evoke action potentials, can

augment or reduce the level of activity driven from within the boundaries of the receptive field (Maffei & Fiorentini, 1976; Nelson & Frost, 1985). Moreover, contextual stimulation can lead to lasting as well as acute changes in neuronal responses (Gilbert & Wiesel, 1990).

The system of horizontal connections is a likely conduit for information between disparate points in visual space. These fibres arise mainly from pyramidal neurons and course laterally, parallel to the brain's surface, extending across the cortical columns (Gilbert & Wiesel, 1979, 1983; Rockland & Lund, 1983; Martin & Whitteridge, 1984) to link cells with similar functional properties (Ts'o, Gilbert & Wiesel, 1986; Gilbert & Wiesel, 1989). Within individual columns, vertically directed axons travel across layers providing communication among cells that represent the same part of the visual field (Lorente de Nó, 1944; Hubel & Wiesel, 1962; Toyama, Kimura & Tanaka, 1981). The juncture of the vertical and horizontal collaterals is thought to allow the integration of stimuli arriving inside the receptive field with those falling outside its borders (Gilbert & Wiesel, 1990).

To examine the actions of the horizontal connections on their postsynaptic targets, we have used the slice preparation to enable surgical isolation of the various pathways and to permit intracellular characterization of their postsynaptic effects. Previously, we examined the capacity of horizontal fibres to exert short-term excitatory and inhibitory influences on the cells they contact (Hirsch & Gilbert, 1991). Recent demonstrations that some connections in the mature neocortex can express long-term potentiation (LTP) or depression (LTD) (Artola & Singer, 1987; Baranyi & Szente, 1987; Bindman, Murphy & Pockett, 1988; Artola, Brochner & Singer, 1990) similar to that originally described in the hippocampus (Bliss & Lømo, 1973; Abraham & Goddard, 1983; Abraham, Gustafsson & Wigström, 1987) led us to test whether the horizontal connections themselves can be modified by use and if such changes tend to weaken or strengthen the involved synapses. Further, since the probable role of horizontal collaterals is modulatory, we additionally investigated whether changes in the efficacy of this pathway could influence the responses to interlaminar input. An abstract summarizing some of the experiments described here has been published previously (Hirsch & Gilbert, 1990).

METHODS

Anaesthesia and surgery

Fifteen adult cats, 1·8–3·5 kg, were first anaesthetized with an intramuscular injection of ketamine (10 mg kg^{-1}) to permit the insertion of a cannula for the venous delivery of sodium thiopentone (20 mg kg^{-1}). The electrocardiogram and temperature were continually monitored; temperature was controlled with a heating pad.

The animal was placed in a stereotaxic device and the skull overlying the occipital cortices was removed. The dura was incised along the anteroposterior axis and reflected to expose the lateral gyrus. A block from the gyrus was then cut with a scalpel and removed with a spatula.

Preparation and maintenance of slices

After gently removing the pia from the excised gyrus, 400 μm slices were cut coronally with a tissue chopper designed by Katz (1987) or, in one instance, tangentially with a vibratome. The slices were held for 2–28 h in a closed-interface chamber where their lower surfaces rested on nylon nets placed over a bath containing artificial cerebrospinal fluid (ACSF) and their upper surfaces were exposed to an atmosphere of 95% O_2–5% CO_2 saturated with H_2O and warmed to 25–30 °C.

For recording, a slice was submerged in a glass-bottomed chamber with a volume of 0·4 ml and

was held stable by a weighted nylon net. Control and test solutions are perfused through the chamber at a rate of ~ 0·5 ml min^{-1} and heated to 34–35 °C. The chamber was illuminated from below and viewed from above through a dissecting microscope. In some experiments slices were used intact, and in others the superficial layers of the slices were isolated by undercutting with fragments of razor blade before the net was put in place. Figure 1 shows schematic diagrams of the intact (*A*) and undercut (*B*) preparations.

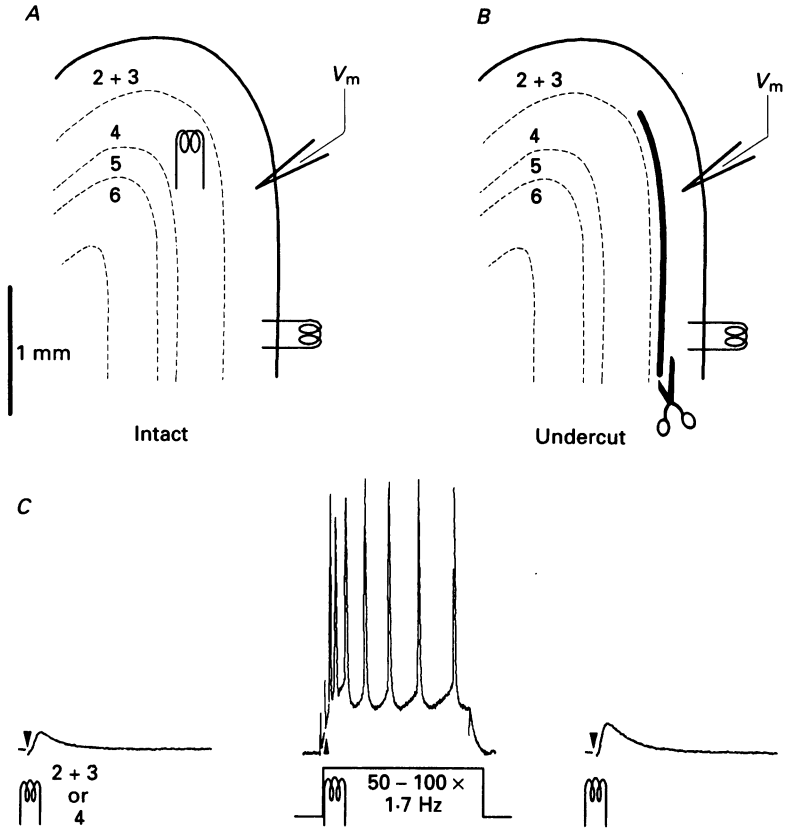

Fig. 1. Diagram of the preparations and the protocol employed to effect use-dependent change. *A*, sketch of the typical preparation, a coronal slice cut through area 17. In some experiments, the slice was undercut by making an incision along the border between layer 3 and 4 (*B*). Inputs from either layer 2+3, about 1·2 mm away from the recording site, or from layer 4, about 1 mm below the recording site, were conditioned by pairing synaptic activation with an injected pulse of current itself sufficient to make the cell fire several action potentials (*C*). The pairs were typically delivered 50 to 100 times at 1·7 Hz. V_m, membrane potential. Arrowheads point to the stimulus artifacts.

Recording and stimulation

Intracellular electrodes were pulled from borosilicate, capillary glass tubing with a 1·2 mm o.d. and 0·6 mm i.d. Electrodes had resistances of 40–100 MΩ when filled. Transmembrane voltage was monitored, and intracellular current delivered through a bridge circuit and displayed on an oscilloscope and computer terminal. Data were digitized on-line and stored on video tape and on disk for future analysis.

Electrical stimuli consisted of shocks lasting from 50 to 150 μs, of between 1 and 100 V, that were delivered at 0·1 Hz through bipolar electrodes made by gluing together two sharpened insulated

tungsten wires so that the tip separation was ~ 50 μm. Shocks were applied to either horizontal fibres in layer 2+3 at a distance of 1 mm or more, or to layer 4 at a site about 1 mm below the impalement. Figures 1*A* and *B* show the configuration of the recording and stimulating electrodes in the experimental preparation.

Protocols for conditioning

To evoke long-term changes we adapted a technique developed by Baranyi & Szente (1987). Sample traces from an experiment where conditioning led to an increase in synaptic strength illustrate the procedure (Fig. 1*C*). At first, test shocks were applied at the control frequency of 0·1 Hz while the membrane was at rest. After collecting the baseline information, we delivered a shock in coincidence with the injection of a pulse of current that was itself sufficient to make a cell fire several action potentials. Typically, fifty to one hundred such pairs were delivered at a frequency of 1·7 Hz. In a few instances, the delivery of the pairs was slowed to 0·2 Hz or we combined shocks with hyperpolarizing pulses of various amplitudes. After conditioning, the responses to the test shocks were again obtained while the membrane was at rest, and compared with the control records; input resistance, spike height, threshold for firing, and resting potential were continually monitored for consistency. A change in synaptic strength of a monosynaptic EPSP was defined as a difference of more than 20% and two standard deviations in the peak of the test response obtained before and at least 15 min after conditioning; three to six trials were averaged for each measurement. When the control and test responses comprised multiple EPSPs, an additional later timepoint in the compound potential was analysed likewise. If polysynaptic responses appeared only after conditioning they were verified as such with methods described in Fig. 4.

For single cells in the intact preparation we stimulated both interlaminar and horizontal pathways. To establish that the pathways were separate we determined that summation occurred with the synchronous activation of both presynaptic sites or we exhausted transmission along one route by delivering shocks at 10 Hz while testing the response evoked by stimulating the second set of inputs at 0·1 Hz.

Solutions

The standard ACSF contained (mM): NaCl, 125; KCl, 5; $NaHCO_3$, 26; $CaCl_2$, 2·4; $MgCl_2$, 1·3; and dextrose, 10. It was saturated with 95% O_2–5% CO_2 and adjusted to pH 7·4. When D-5-amino-phosphonovalerate (APV; Tocris Neuramin, Bristol), was used it was dissolved in the ACSF and delivered in the bath.

The recording electrodes were first backfilled with 2% biocytin (Molecular Probes) and dissolved in 3 M potassium acetate, pH 7·2 before the shanks were filled with the electrolyte. The biocytin labelled the cells during recording so that they could be identified after histological processing.

Histology

The tissue containing biocytin-labelled cells was processed using a modification of the technique described in Horikawa & Armstrong (1988). Briefly, slices were fixed overnight in 4% paraformaldehyde, cut into 70–100 μm sections with a freezing microtome and rinsed twice with phosphate-buffered saline (PBS). The sections were then incubated for 45 min in PBS containing 0·25% triton and 2% bovine serum albumin (BSA). After rinsing in PBS with 2% BSA they were incubated in avidin–HRP (Vector Laboratories, diluted 1:100 in PBS and 2% BSA) for 2 h at room temperature or overnight at 4 °C. The tissue was then washed in PBS (3 × 15 min) and incubated for 1 h in 0·05% diaminobenzidine (DAB) in PBS. Hydrogen peroxide, 0·3%, was then added at a ratio of 1:100 to the DAB solution. About 15 min later the sections were rinsed three times in PBS and were mounted, dehydrated and coverslipped.

RESULTS

We recorded from thirty-four neurons in thirty slices for periods of time lasting from 30 min to 5 h; twenty-one of these cells were conditioned by repetitively pairing a synaptic volley with a pulse of depolarizing current, as described in the Methods section and illustrated in Fig. 1. Five neurons were studied in preparations that were undercut, one in a tangential slice and the remaining cells were in slices that were

intact. The average input resistance was 38·0 ± 19·4 MΩ (mean + S.D.) and the average resting potential was 76·9 ± 6·6 mV. All the neurons we studied had action potentials whose width at half the maximum amplitude was > 1 ms; by this criterion they were identified physiologically as pyramids according to Schwartzkroin &

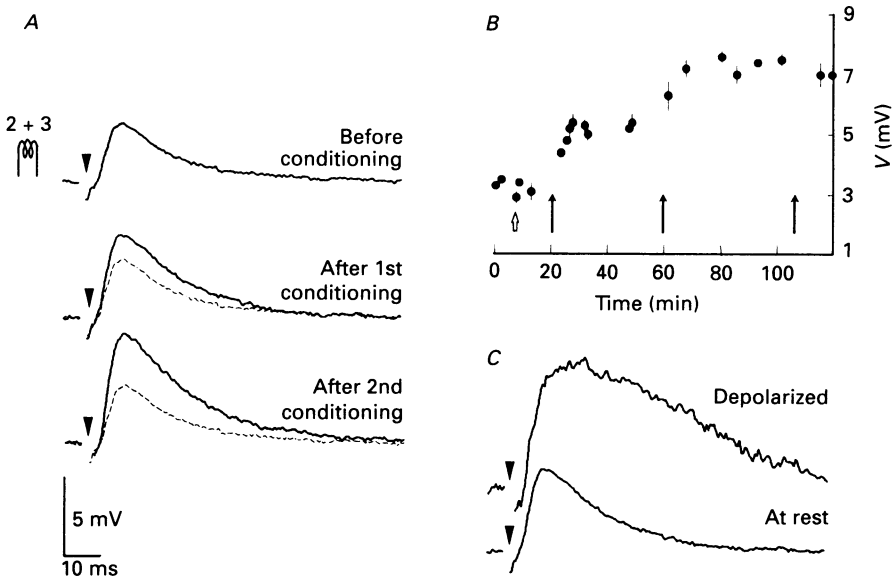

Fig. 2. The laterally evoked EPSP could be strengthened by use. The amplitude of the EPSP, measured at rest, increased following the presentation of one hundred pairs at 1·7 Hz, (*A*, middle trace) and grew further after a second round of conditioning. The dashed traces represent the original, baseline response and the arrowheads point to the stimulus artifact. The traces are averages of three trials recorded from an anatomically identified pyramidal cell in an undercut preparation. The peak of the response *versus* time is plotted in *B*. In this and subsequent figures, each point is the average of three trials ± S.D., each filled arrow denotes one round of pairing, and the open arrow marks synaptic activation at 1·7 Hz without concomitant current injection. *C*, EPSP at rest, −83 mV, (bottom trace) and while the membrane was depolarized to −63 mV (top trace) to demonstrate that no inhibition was apparent.

Mathers (1978) and McCormick, Connors, Lighthall & Prince (1985). In accord with this physiological standard, all nineteen cells that were filled with dye were pyramidal.

Undercut preparations

Our first step was testing to see if the strength of the isolated, horizontally evoked response could be modified by use. For these initial experiments we recorded from slices where the superficial layers were undercut to remove interlaminar input. Previously, in the undercut preparations, we found that about half of our sample responded to stimulation of the horizontal fibres with inhibition as well as excitation (Hirsch & Gilbert, 1991). These two cell types, as defined by postsynaptic response, displayed no obvious difference in laminar position or morphology. A total of five cells were tested in undercut preparations, and one in a tangentially cut slice that contained only the superficial layers. Of these, three, whose responses were only

excitatory, showed facilitation averaging 196±20% (174, 201, 213%). The remaining three, for which the excitation was followed by a clear-cut IPSP, were unchanged by conditioning. Examples of responses from both populations are shown in Figs 2 and 3.

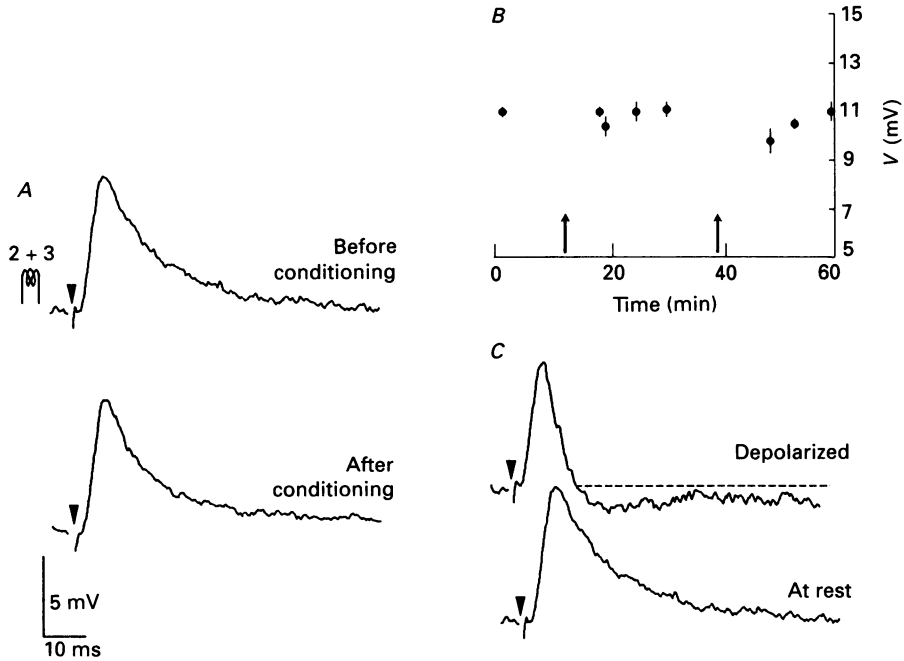

Fig. 3. Laterally evoked responses that included strong inhibition frequently remained unaltered by use. *A* and *B* show that conditioning with two volleys of one hundred pairs each failed to alter the synaptic response. The traces are averages of three trials recorded from an anatomically identified pyramidal cell in an undercut preparation. Depolarization of the cell from its resting potential of −79 mV (*C*, bottom trace) to −62 mV revealed the presence of a strong IPSP. Arrowheads point to the stimulus artifact.

Recordings from a cell that responded to stimulation of the horizontal fibres with an EPSP are presented in Fig. 2*A* (middle trace). Stimulation of the horizontal fibres at the conditioning frequency, without the concomitant injection of depolarizing current caused no change (traces not shown). After pairing the EPSP with a pulse depolarizing pulse of current, however, the peak of the EPSP, measured at the resting potential, was increased to 130%. A second round of conditioning yielded a further increase to twice the amplitude of the control (bottom). The full extent of facilitation lasted for the remaining hour of the recording but was not augmented by additional pairings. These results are presented graphically in Fig. 2*B*. Note that no trace of an IPSP appeared when the membrane was at rest or was depolarized to a voltage above the reversal potential for inhibition (Fig. 2*C*, top). Rather, the characteristic voltage-dependent enhancement of the EPSP described in previous reports (Gilbert, Hirsch & Wiesel, 1990; Hirsch & Gilbert 1991) was seen.

Traces recorded from the other kind a cell, whose response to lateral input included inhibition, are shown in Fig. 3. The IPSP was visible when the membrane was

depolarized from rest to a voltage above the reversal potential for inhibition (Fig. 3*C*, top). Even after three rounds of conditioning (Fig. 3*A*) the synaptic response of this cell remained unchanged. Thus cells of this type had stable synaptic responses that could not be facilitated or depressed by the conditioning stimuli we employed.

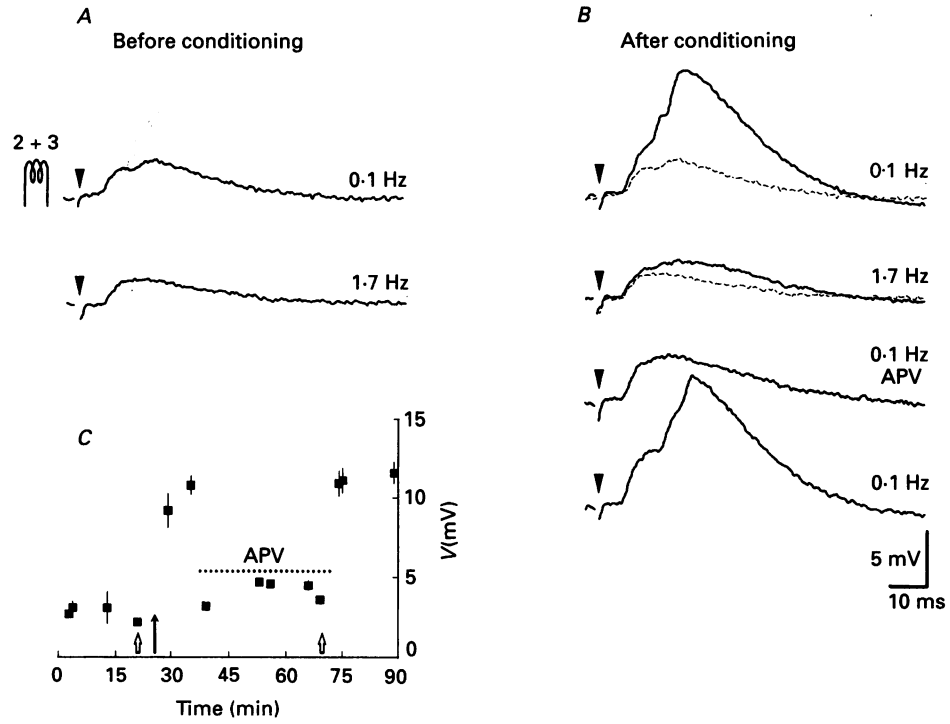

Fig. 4. Often, the largest amount of enhancement was observed in the late, polysynaptic component of the response. *A* (top trace) shows that the laterally evoked response consisted of two serial EPSPs. The second EPSP failed when the stimulus rate was increased from 0·1 to 1·7 Hz suggesting that it was di- or polysynaptic (bottom trace). After conditioning the later part of the response showed the most prominent facilitation (*B*, top and bottom); the dashed lines represent the original, baseline responses. The late components were reversibly removed by increasing the stimulus rate, or by application of 50 μM APV. The traces are averages of three trials recorded from a labelled pyramidal cell and are presented sequentially in each panel. The resting potential was −75 mV. The points in *C* were measured at the most depolarized aspect of the response.

Intact preparations

Facilitation within single pathways

Most cells in the intact preparations responded to lateral inputs with either a monosynaptic EPSP or an EPSP followed by an IPSP, as in the undercut slices. It was not uncommon (six out of twenty-eight times), however, to find a third type of response in the intact preparations, where serial EPSPs were evoked by stimulating horizontal inputs. An example of such a response is seen in the top left trace of Fig. 4; the recordings were made while the shocks were delivered at the control frequency, 0·1 Hz. The late EPSP was probably di- or multisynaptic since it failed when the

stimulus rate was rapid, 1·7 Hz (Fig. 4*A*, left). After conditioning (Fig. 4*B*), the polysynaptic component of the response to stimulation at 0·1 Hz had grown dramatically, apparently the effect of newly recruited or unmasked inputs. The middle two traces in Fig. 4*B* show that the monosynaptic EPSP was also slightly

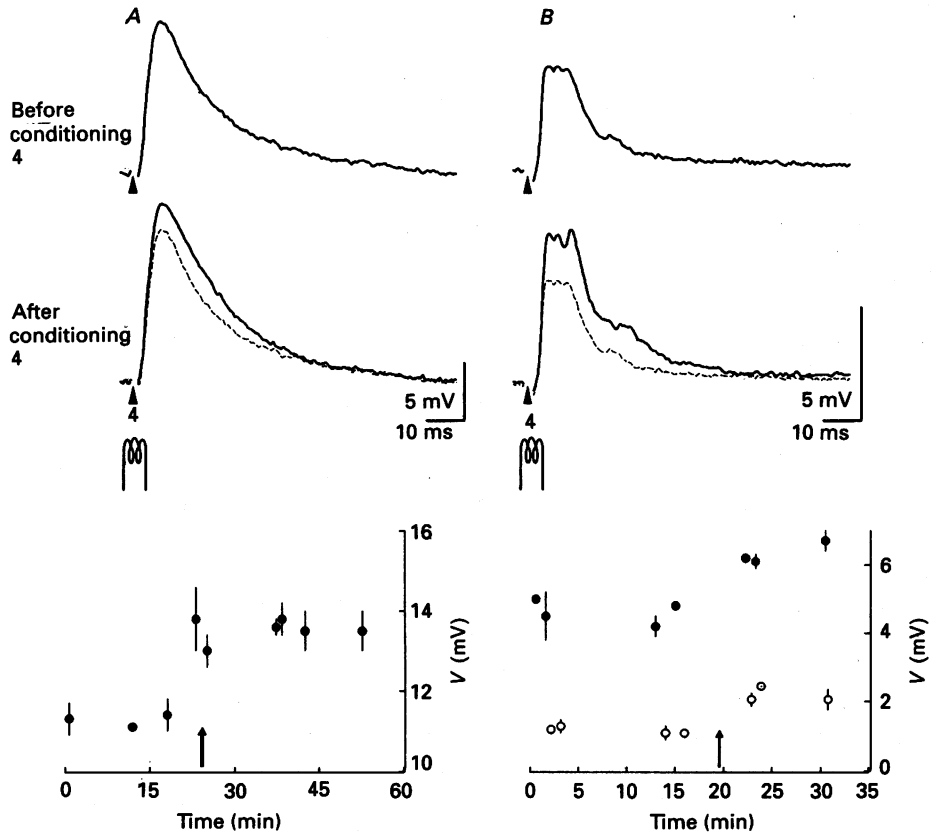

Fig. 5. Similar to the horizontally evoked response, conditioning ascending, interlaminar inputs from layer 4 could lead to the enhancement of the monosynaptic EPSP alone, as was the case for the cell represented in *A*, or include pronounced enhancement of later multisynaptic components, as was the case for the cell represented in *B*. In this figure the difference between the control and unconditioned are presented in the bottom traces. Traces are the averages of four trials each, and were obtained from pyramidal cells identified physiologically (*B*) or anatomically (*A*). The resting potential of the cell in *A* was −82 mV and of the cell in *B*, −73 mV. The filled symbols indicate points measured at the first peak of the response and the open ones chart the amplitude of the late component (*B*) measured 19 ms later. The dashed lines represent the original, baseline responses.

enhanced; it is shown in isolation, when the late components had been reversibly blocked. The late components were suppressed by two separate methods, by rapid stimulation (second trace from top) or by application of APV (third trace from top), an antagonist of *N*-methyl-D-aspartate receptors; the bottom trace shows recovery. Sutor & Hablitz (1989*a*) have demonstrated that polysynaptic events are more

susceptible to APV than monosynaptic ones. In most experiments involving composite responses, we verified that the late EPSPs were multisynaptic by using the rapid stimulation and/or APV method. The average increase in the monosynaptic EPSP was $157 \pm 26\%$ (range 121–191%, $n = 9$). This value is lower than that obtained from undercut preparations, probably because we rarely attempted multiple rounds of conditioning. Since enhancement of the polysynaptic portion of the response often involved the addition of novel EPSPs that occurred after the control response had decayed to the resting potential, expression of percentage change from baseline is not possible. After potentiation had occurred, the peak of the novel late components compared to the monosynaptic EPSP, for three cells, averaged $426 \pm 151\%$ (328, 350, 600%) larger.

Use-dependent interactions along convergent pathways

All the cells ($n = 28$) that we tested received both horizontal and vertical inputs. The patterns of facilitation observed following conditioning of fibres from layer 4 appear similar to those characteristic of the lateral pathway (Fig. 5). There could be facilitation of the monosynaptic EPSP (Fig. 5*A*) as well as of later ones (Fig. 5*B*). The average increase of the monosynaptic EPSP was $131 \pm 5{\cdot}5\%$ (125, 131, 136%, $n = 3$).

Having characterized use-dependent changes in synaptic strength along single pathways, we were prepared to ask whether changes in the synaptic strength of one pathway could alter the effects of the other. Specifically, we explored the possibility of heterosynaptic interactions between the horizontal and interlaminar circuits that converge on a single cell. The records displayed in Fig. 6 were collected from a single cell in an intact slice; the traces are presented, from top to bottom, in the order recorded. Responses to horizontally displaced shocks are shown in Fig. 6*A* while responses to stimulation of layer 4, 1 mm below the impalement, are depicted in Fig. 6*B*. Conditioning the horizontal pathway led to an increase in the strength of its postsynaptic response, but did not alter the efficacy of the interlaminar inputs (middle traces, Fig. 6*A* and *B*). Subsequently, pairing the interlaminar inputs caused a reduction rather than an enhancement along that pathway without affecting the horizontally evoked response (bottom traces, Fig. 6*A* and *B*). The expression of the facilitation and the depression seen in the last panel of the figure continued until the end of the recording session; these results are summarized graphically in Fig. 6*C*. Thus, it seems that distinct sets of synapses made with a single cell can show different patterns of use dependence. In three out of eleven instances, conditioning led to a suppression of the late response to stimulation of layer 4. Depression of late responses evoked from laterally displaced sites has not been observed, possibly due to the small sample of only four cells. Of the total of fifteen instances in which we conditioned the horizontal pathway in intact slices, ten showed facilitation and five were unchanged.

For the cell represented in Fig. 6, the effects of conditioning, whether facilitatory or suppressive, were confined to the treated pathway. For a minority of cells, however, pairing of one pathway influenced the second. The top traces in Fig. 7 show responses of a single cell to lateral and interlaminar input before delivering the pairs and the bottom records were obtained afterwards. After pairing, the horizontally evoked EPSP was enlarged. What is noteworthy here is that the interlaminar

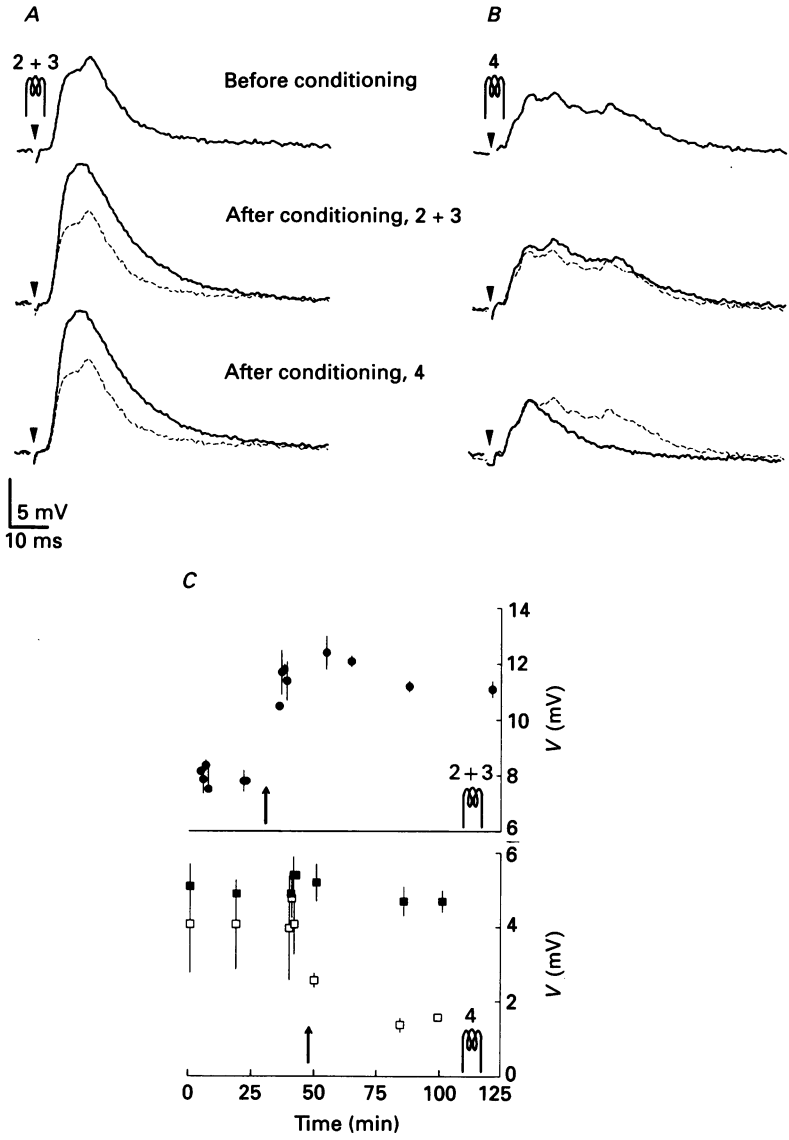

Fig. 6. Conditioning different pathways converging on the same cell may have opposite effects. For the cell represented in this figure the response to both horizontal and interlaminar stimulation was polysynaptic (*A* and *B*, upper). Conditioning the lateral inputs increased their synaptic strength but left the interlaminar response unchanged (*A* and *B*, middle). Pairing of the interlaminar inputs led to a reduction of the polysynaptic response that they evoked without affecting transmission along the horizontal pathway (*A* and *B*, lower). Each trace is the average of three trials and resting potential of the cell, a labelled identified pyramid, was −77 mV. For *C*, the top graph plots the peak response evoked from layer 2+3. In the lower plot, the filled symbols indicate the earliest peak and the open symbols mark the response amplitude 19 ± 0·5 ms later of the response from layer 4. The dashed lines represent the original baseline responses.

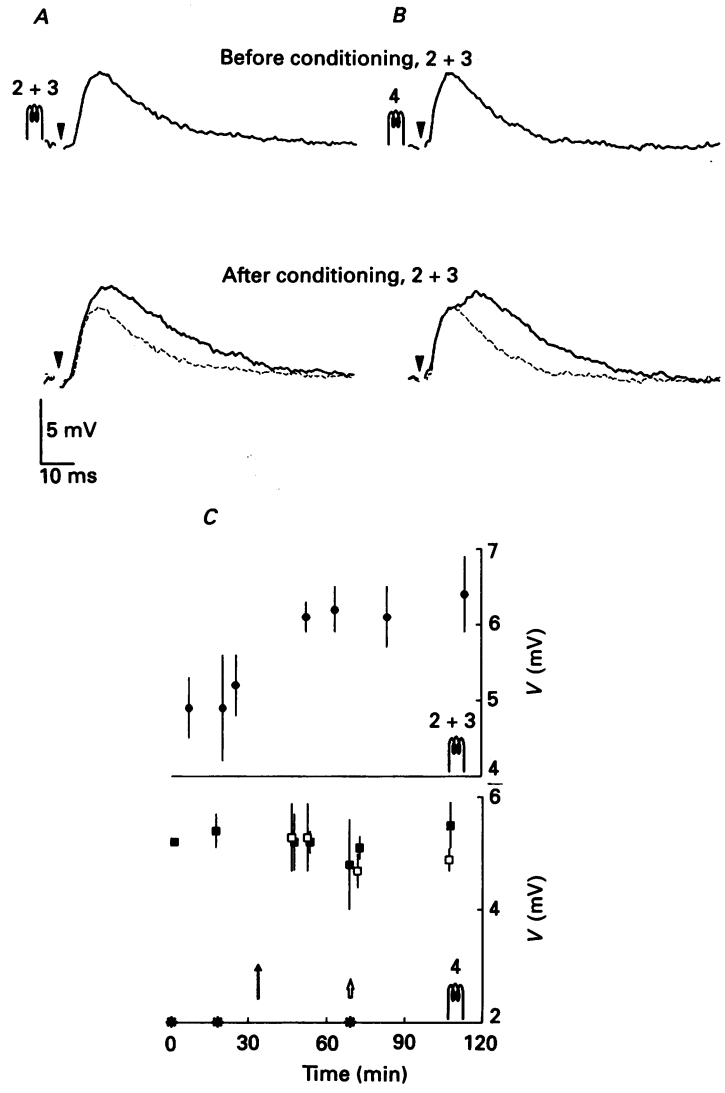

Fig. 7. At times, conditioning the response evoked by one pathway led to an enhancement of the late components evoked by the second. For the cell represented here, pairing of the synaptic response evoked by stimulating the superficial layer was followed by enhancement of the laterally evoked, monosynaptic EPSP (*A*) as well as the appearance, in the response to stimulation of the interlaminar pathway (*B*), of a late EPSP. Each trace is an average of four trials. *C*, the points in the upper plot were measured at the peak of the response evoked from layer 2+3. In the lower graph the filled symbols are for the early peak and the open ones for the amplitude of the second peak, when present, of the response evoked from layer 4; asterisks mark instances where a second peak was absent, that is, at all times before conditioning and afterwards when polysynaptic transmission was interrupted by means of rapid stimulation. The recordings were made from an anatomically identified pyramidal cell whose resting potential was −71 mV. The dashed lines represent the original baseline responses.

response was also enhanced even though it had not been conditioned. This crossed-path effect took the form of an addition of a late EPSP to the postsynaptic response. The failure of this late EPSP at rapid stimulus rates (Fig. 7*C*) shows that it was mediated by more than one synapse. Three times out of fifteen, pairing the horizontal response produced heterosynaptic facilitation of the interlaminar pathway and in one out of eleven cases conditioning the interlaminar inputs produced facilitation of the lateral response.

DISCUSSION

Directed by observations that responses in the primary visual cortex, *in vivo*, can be lastingly altered by patterns of stimulation that probably involve the horizontal connections, we set out to examine whether or not the synapses in this pathway could express long-term change. Towards that aim, we have shown that, in fact, the efficacy of synaptic connections made by horizontal fibres onto their target pyramidal cells can be strengthened by use. This facilitation is long lasting and is more likely to occur if the laterally evoked response does not include strong inhibition. In instances in which serial EPSPs were present, whether or not these were elicited from lateral or intracolumnar sites, the enhancement of the early, monosynaptic component was typically less than the summed potentiation of the later events. Additionally, we sometimes observed long-lasting depression, or heterosynaptic facilitation of multisynaptic pathways.

Monosynaptic connections

The use-dependent behaviour of various neocortical pathways differ from one another (Sutor & Hablitz, 1989*b*, Sah & Nicoll, 1991; Hirsch & Crepel, 1991). The experiments that originally established the occurrence of LTP in the mature cortex also revealed that not all responses could be changed by use (Artola & Singer, 1987; Bindman *et al.* 1988; Kimura, Nishigori, Shirokawa & Tsumoto, 1989; Sutor & Hablitz, 1989*b*). Later experiments showed that certain defined sets of connections express a decaying, rather than a sustained form of LTP (Sah & Nicoll, 1991), while others are more likely to be depressed than enhanced by conditioning (Hirsch & Crepel, 1991). Our specific demonstration that LTP can occur at contacts made between lateral fibres and their postsynaptic targets is necessary for understanding the dynamic behaviour of the horizontal circuit.

One factor thought to regulate long-term change is inhibition (Wigström & Gustafsson, 1985). Sutor & Hablitz (1989*b*) report that enhancement of the late components is prevented if the conditioning stimulus evokes IPSPs. Our results show that this trend appears to hold for the facilitation of monosynaptic EPSPs as well, and agree with the findings that blocking GABA receptors favours the emergence of long-term change (Wigström & Gustafsson, 1985; Artola & Singer, 1987; Bindman *et al.* 1988; Davies, Starkey, Pozza & Collingridge, 1991).

Polysynaptic connections

The complexity of the cortical circuit is highlighted by the observation that activating the horizontal connections produces monosynaptic EPSPs in the undercut preparations but can evoke serial EPSPs in the intact slices. These later EPSPs

probably originate from cells lying in the home column, possibly activated via a loop involving the deep layers (Gilbert & Wiesel, 1979; Lund, Henry, Macqueen & Harvey, 1979; Martin & Whitteridge, 1984). The greater amplification of the compound polysynaptic response compared to the monosynaptic one that was demonstrated following conditioning of the horizontal connections in the intact slices may emerge as a common neocortical feature. It has been indicated, though not tested, by others who studied responses to various intracolumnar inputs (Bindman *et al.* 1988; Kimura, Nishigori, Shirokawa & Tsumoto, 1989; Sutor & Hablitz, 1989*b*).

We believe that the prominence of the multisynaptic events might reflect the structure of neocortical circuitry as follows: exciting a set of afferents with a stimulating electrode produces a synaptic response in a group of cells embedded in a network of diverging and converging paths. The compound EPSP produced in a given cell, as the components of these networks are activated in turn, is larger than the monosynaptic one driven by the initial volley. Further, the late, composite EPSP will be enhanced if transmission along even a fraction of the pathways that contribute to it are potentiated, whether this occurs at early stages or at contacts made directly with the impaled neuron. This scheme depends on net gains in excitation outweighing those in inhibition.

It is easier to imagine why effecting use-dependent change by tetanizing rather than by pairing shocks and current injection recruits late EPSPs. The former method provides effective depolarization of many cells while the latter directly influences just one neuron. One explanation of the potentiation of the polysynaptic EPSP described in this study is that, during pairing, firing driven by current injection into the impaled neuron produced a depolarization in its postsynaptic targets (Mason, Nicoll & Stratford, 1991) that was sufficient to elicit LTP when combined with the shock-evoked response. Furthermore, the current pulse drives firing at a rate thought fast enough to cause fatigue of interposed inhibitory inputs (Deisz & Prince, 1989; Davies *et al.* 1991). Alternatively, Bonhoeffer, Staiger & Aersten (1989), propose that pairing can lead to an overall enhancement of transmission along many collaterals of the presynaptic fibres synapsing with the test neuron; nitric oxide may serve as the interneuronal messenger (Schuman & Madison, 1991).

The heterosynaptic facilitation that we recorded was in the polysynaptic components of the response and thus probably occurred at sites presynaptic to the impaled cell. One need not imagine a new mechanism for such crossed-path interactions to occur. Conditioning might have caused the strengthening of a synapse made by a nearby pyramid with the impaled neuron, as seems to have been the case when pairing led to the appearance of polysynaptic EPSPs within a single pathway. If interlaminar fibres contacted both the impaled cell and that neighbour, then a heterosynaptic effect would be seen on stimulation of layer 4.

We observed distinct long-term reduction in synaptic strength only for multisynaptic events. This depression could have come about either by enhanced inhibition or by attenuation of excitatory inputs, changes that could have taken place at the level of the impaled cell or at prior stages. Others have observed LTD in the early components of the response following stimulation of pathways that were homosynaptic (Artola *et al.* 1990; Hirsch & Crepel, 1991) or heterosynaptic

(Abraham *et al.* 1987; Bindman *et al.* 1988). Perhaps, differences among conditioning protocols or among the involved circuits account for the discrepancies in the various studies.

Roles for facilitation in vivo

Long-term potentiation and depression are typically associated with the processes of learning and memory, yet it is difficult to imagine these as the principal roles in the primary visual cortex. In area 17, the receptive field properties of a given cell are not fixed, but vary with changes in stimulus context. For example, conditioning a given cell by presenting variously oriented bars in the surround while shining an optimally oriented bar in the receptive field can reshape the tuning curve (Gilbert & Wiesel, 1990). These modified response properties often persist, later being seen with stimulation of the receptive field alone. The use-dependent changes in synaptic strength that we have described, both homo- and heterosynaptic, could account for some of the lasting effects resulting from contextual stimulation.

That strong inhibitory input reduces the chance of facilitation along the horizontal pathway suggests that visual patterns evoking strong inhibition would be unlikely to cause persistent modifications. Additionally, any other factors that could influence the net sign of synaptic response, such as extra-areal inputs (Cruetzfeldt, Garey, Kuroda & Wolff, 1977), could play a role in determining when various stimuli lead to lasting change.

A separate candidate for a functional correlate of the LTP is the remodelling of visuotopic organization occurring after focal retinal lesions. Here, the area of cortex that is initially rendered insensitive to light becomes responsive over the subsequent weeks and months to stimuli falling just outside the lesions' borders (Gilbert *et al.* 1990; Kaas, Krubitzer, Chino, Langston, Polley & Blair, 1990; Heinen & Skavenski, 1991; Gilbert & Wiesel, 1992). This recovery apparently occurs intracortically since the denervated area of the lateral geniculate nucleus remains silent (Gilbert *et al.* 1990). Our results raise the possibility that the cortical 'filling in' involves the strengthening of synapses made by horizontal fibres on their targets.

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