Long-term potentiation and long-term depression of horizontal connections in rat motor cortex

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Abstract. This paper reviews studies that investigated conditions resulting in long-lasting modifications of synaptic efficacy in horizontal connections within layers II/III of adult rat motor cortex using the *in vitro* slice preparation. Long-term potentiation (LTP) was induced by high frequency theta burst stimulation (TBS) when local synaptic inhibition was transiently suppressed by bicuculline methiodide application at the recording site immediately prior to TBS of the horizontal pathway. Without bicuculline, TBS failed to produce LTP. LTP could also be induced without Bic application by conjoint TBS of horizontal and vertical (ascending) inputs. By contrast, long-term depression (LTD) of horizontal transmission was induced by 10 min of 2 Hz stimulation. Depressed horizontal connections nevertheless retained the capability for synaptic strength increases. These studies indicate that synaptic modification across horizontally connected neurones is regulated both by the arrangement of their intrinsic circuits and by the availability of mechanisms for modification at individual synapses. Activity dependent forms of synaptic plasticity operating within horizontal connections may form a spatial substrate and mechanism for experience-dependent regulation of cortical representations.

INTRODUCTION

An important feature of representation patterns in adult primary motor cortex (MI) ia a capability for rapid and long-lasting reorganization. Substantial modifications in motor maps may be induced by peripheral nerve transection or sensory feedback modulation (Donoghue et al. 1990, Sanes et al. 1990). Adjustment in limb configuration (Sanes et al. 1992), repetitive limb movement (Qui et al. 1990) and forms of motor learning (Suner et al. 1993, Donoghue and Sanes 1994) may also result in reorganization of motor representations. It has been proposed that intrinsic horizontal excitatory synaptic connections form anatomical and functional substrates to restructure motor representation patterns within MI (Jacobs and Donoghue 1991, Hess and Donoghue 1994, Donoghue et al. 1996). These connections originate from surface-tangential and oblique axon collaterals of cortical pyramidal neurones and form an intrinsic connectional network within motor cortex (Landry et al. 1980, DeFelipe et al. 1986, Ghosh and Porter 1988, Huntley and Jones 1991, Jacobs and Donoghue 1991, Weiss and Keller 1994). Changes in the coupling strength of neurones located across MI could form new output arrangements so that output of one area comes to drive new output cell collections. The effectiveness of synaptic coupling could be changed by activity-dependent processes that result in long--lasting modifications in the strength of horizontal excitatory synaptic connections across the MI representation. In certain hippocampal and neocortical synapses two general forms of these modifications have been described: long-term potentiation (LTP, see Bear and Kirkwood 1993, for a review) and long-term depression (LTD, for review see Linden 1994). It has been found that the direction of the modification, i.e. increase ᴜ decrease of response, depends on the frequency and pattern of stimulation. We review here studies that tested the possibility and conditions of the induction of both LTP and LTD in horizontal pathways within superficial layers of rat MI.

HORIZONTAL SYNAPTIC CONNECTIONS

The motor cortex is synaptically linked with various subcortical and cortical areas and it contains a complex pattern of internal connectivity (reviewed in Wise and Donoghue 1986, Donoghue and Sanes 1994). Among intrinsic connections, there are horizontally running fibres forming an extensive intrinsic connectional matrix. In addition to a major efferent axon, clusters of axonal arborizations typically exist in the immediate vicinity of somata of cortical pyramidal neurones. In primates (DeFelipe et al. 1986, Ghosh and Porter 1988, Huntley and Jones 1991), cats (Landry et al. 1980, Keller and Asanuma 1993) and rats (Aroniadou and Keller 1993, Weiss and Keller 1994) intralaminar horizontal and oblique axon collaterals of pyramidal neurones may extend for several millimeters within superficial (layers II/III) and deep (layer V) cortical layers. Clusters of axon terminals have been observed along the course of horizontally oriented fibres, forming synapses on pyramidal as well as non--pyramidal, presumably inhibitory neurones (Keller and Asanuma 1993), the vast majority of which appear to use the transmitter GABA (reviewed by Benardo and Wong 1995). Interlaminar fibres form an additional intrinsic connection system for cortical pyramidal neurones; these are vertically aligned similar to extrinsic afferents originating subcortically or from remote cortical areas. In contrast to the widely distributed excitatory projections of pyramidal cells in MI, activation of non-pyramidal, fast-spiking cells provides intracortical inhibition (Thomson and Deuchars 1994) that exert their effects locally (but cf. Kang et al. 1994, who suggest that some inhibitory neurones may distribute their influence up to 1.2 mm away). These data suggest that the horizontal fibre system interconnects neurones over a wide region of cortex and, moreover, that an interplay of excitatory and inhibitory synaptic transmission and their long-term modifications within this system may regulate associations of the cells in MI, and probably in other cortical areas as well.

Horizontal fibres spread much of their influence through layer V and layers II/III. It has been shown using field potential recording and current source-density analysis in rat MI that stimulation of layer V evokes monosynaptic excitation at distances up to 1.5-2.0 mm both in layer V and "obliquely" in layers II/III. Stimuli applied to layers II/III evoke prominent horizontal monosynaptic responses confined mostly to the same layers up to 1.5-2.0 mm away (Aroniadou and Keller 1993, Hess et al. 1994). While the non-NMDA types of glutamate receptors mediate most of these connections, NMDA receptor activation contributes 15-20% to responses recorded from superficial as well as deep layers (Aroniadou and Keller 1993, Hess et al. 1994). In addition, strong activation of horizontally running projections may polysynaptically excite cells along the whole depth of the cortex (Aroniadou and Keller 1993, K.M. Jacobs, unpublished).

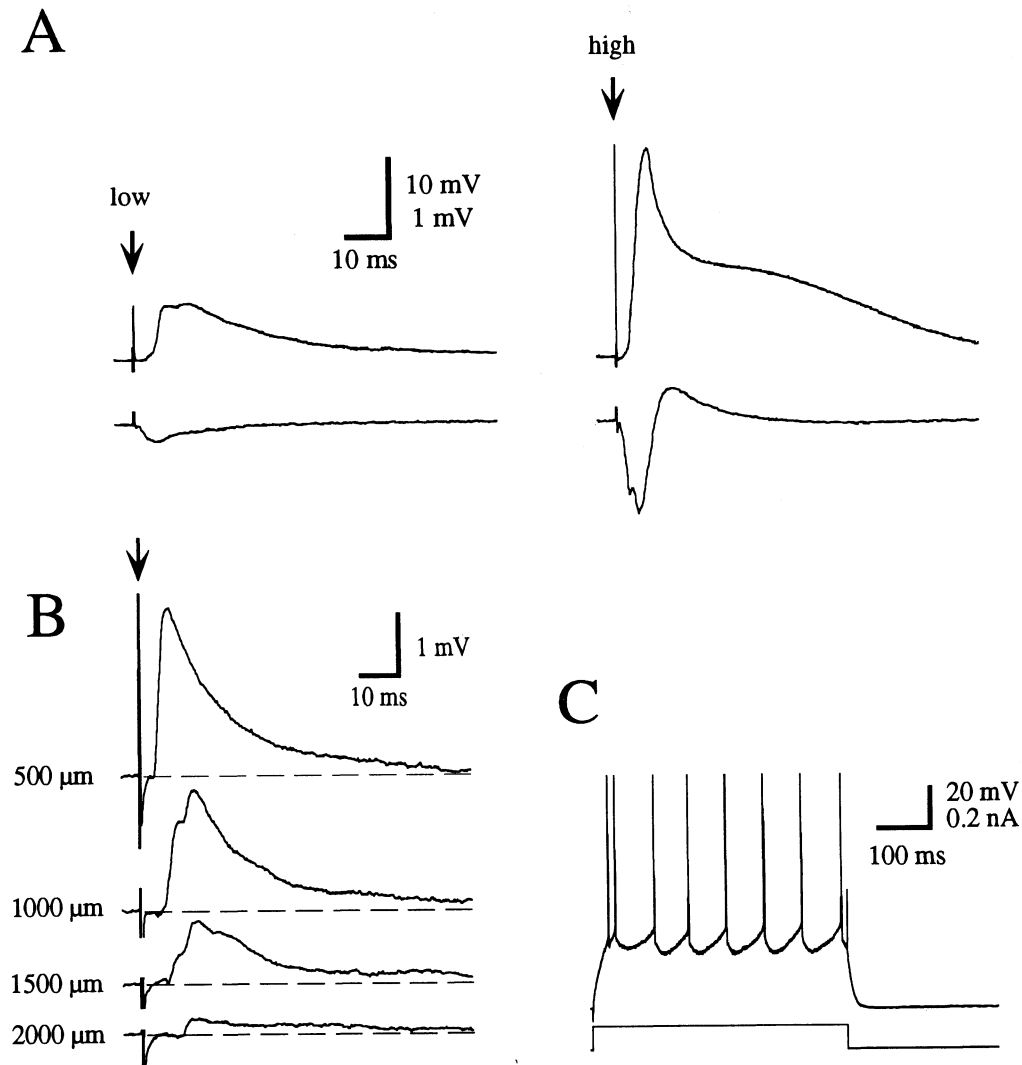Intracellular recordings have shown that the strongest excitatory connections between pyrami-

Fig. 1. Responses evoked in horizontal pathways within layers II/III. A, correspondence between intracellular postsynaptic potentials (upper traces) and field potentials recorded within 100 μm (lower traces), evoked at low (left) and high (right) stimulus intensity. Shown are averages of 5 single responses. Arrows indicate stimulus artifacts. B, postsynaptic potentials evoked at increasing distance from the recorded cell (500-2,000 μm). Shown are 10 trial averages. Note increasing latency to the onset of responses and their apparent composition of discrete components (see also A, left panel), reflecting differences in conduction time of activated fibres. C, typical regular-spiking response of a pyramidal neurone (upper trace) to 500 ms, 1.25 nA current pulse (lower trace).

dal neurones in rat motor cortex are between neighbouring cells, displaced horizontally by less than 0.1 mm and belonging presumably to a single cortical column (Thomson and Deuchars 1994). However, depolarizing postsynaptic potentials could be evoked in pyramidal neurones by extracellular stimuli applied horizontally at distances up to 2.0 mm in layer III (Fig. 1B) and at least 1.25 mm in layer V (Jacobs 1994). Activation of sites displaced horizontally by long distances usually does not suffice to generate action potentials; depolarizing post--synaptic potentials of maximum few mV in amplitude are usually recorded. With *in vitro* preparations, the resting membrane potential of layer II/III cells is below the equilibrium potential for Cl-, the principal ion mediating fast IPSPs in the neocortex (on average: -84 mV; Hess et al. 1996) and therefore hyperpolarizing IPSPs are not visible (see Fig. 1A). More classical IPSPs are usually revealed when cells are steadily depolarized with current injection. Layer V cells are more depolarized *in vitro* (on average: -69 mV; Jacobs 1994) so that small amplitude IPSPs following initial depolarizing postsynaptic potentials may be observed. Small polysynaptic excitatory contributions to the later parts of responses are also frequently encountered. Many cells exhibit enhanced depolarizing postsynaptic potentials at depolarized membrane potentials. These effects represent the activation of voltage-dependent conductances (Stafstrom et al. 1985) and the NMDA receptor-dependent current (Thomson and Deuchars 1994) which may be involved in synaptic plasticity.

LONG-TERM POTENTIATION

Although individual circuits within the motor cortex have received extensive investigation (reviewed in Donoghue et al. 1996), the potential for long-term modifications oı synaptic efficacy of horizontal connections within motor cortical areas has only recently been tested in our laboratory. The possibility for synaptic plasticity in adult rat MI was investigated in neocortical slice preparations maintained in a standard interface chamber (Hess et al.

1994). Two experimental paradigms effectively induced LTP in layers II/III horizontal connections within MI (Hess and Donoghue 1994, Hess et al. 1996). In both cases LTP induction was conditional; high frequency tetanizing stimulation of the horizontal connections alone was not sufficient to induce LTP in contrast to, for example, hippocampal cortex. Responses evoked in the superficial horizontal pathways increased in amplitude after high frequency tetanizing stimulation using a theta burst pattern (TBS), but only when synaptic inhibition was transiently decreased at the recording site immediately before tetanization. Short term reduction of inhibition was achieved by a few seconds of local application of $GABA_A$ antagonist bicuculline methiodide (Bic) immediately prior to TBS. A second approach involved simultaneous tetanization of "vertical" pathways, ascending from the deeper layers below and the horizontal pathways, without pharmacological disinhibition.

Figure 2A shows an example of LTP induced in a horizontal pathway by TBS coupled with focal Bic application. A 25 - 35% increase in response amplitude persisted for the duration of measurements, up to 60 min (Hess and Donoghue 1994). The local nature of the disinhibitory action was demonstrated by application of Bic alone next to the test recording site (Fig. 2A), which only transiently increased the amplitude of the response. In the absence of Bic, TBS of horizontal pathways consistently failed to potentiate their efficacy, showing the strong requirement for decreased inhibition for LTP induction by TBS stimulation of this pathway. LTP was not restricted to the closest horizontal connections, LTP of comparable magnitude could be produced in a longer-range pathways (approx. 1 mm) as well.

To characterize the influence of the amount of horizontal stimulation upon the incidence and magnitude of LTP, the effects of low and high intensity TBS on a single horizontal input and simultaneous TBS of two converging horizontal inputs were investigated (Hess et al. 1996). TBS delivered at one site at control stimulus intensity, evoking 50% maximum response (termed "test intensity"), was
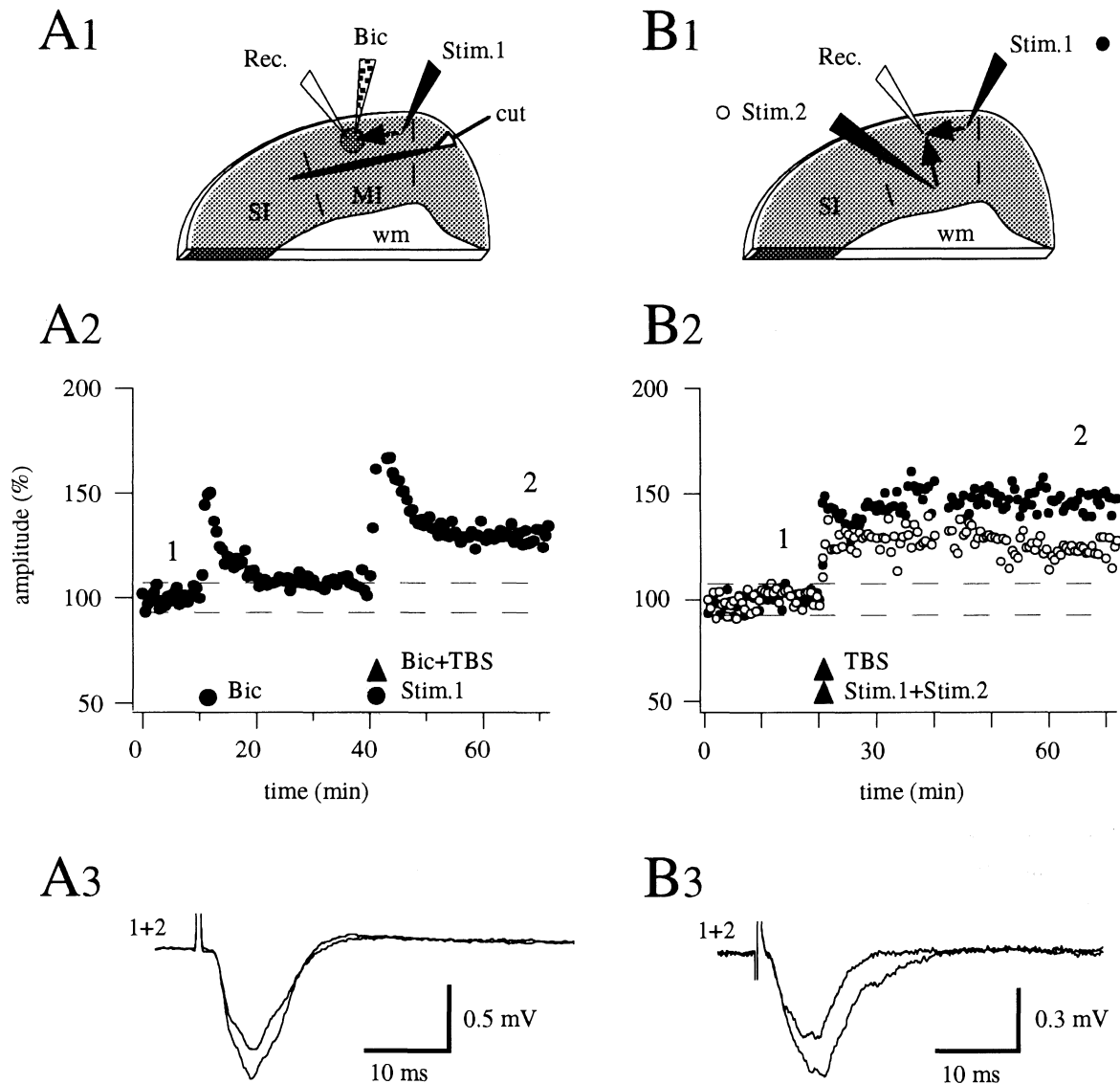
Fig. 2. Conditional LTP can be induced in the horizontal connections of rat motor cortex in two ways. A, LTP induction by theta burst stimulation (TBS) of layer II/III horizontal fibres coupled with transient pharmacological reduction of synaptic inhibition by focal application of bicuculline. A1, typical configuration of stimulating (Stim.1), recording (Rec.) and drug application (Bic) pipettes in the slice. MI, primary motor cortex; SI, primary somatic sensory cortex; wm - white matter. In a subset of slices superficial and deep cortical layers were isolated (cut). A2, time course of field potential amplitude evoked in horizontal pathway during the experiment. Filled circles denote the time of Bic delivery, triangle denotes TBS. Note the transient nature of Bic-induced response change. In this experiment a cut was made in the slice approx. at the layer III/V border (see A1). Dashed lines demarcate ± 2 SD about the mean baseline response. A3, superposition of averaged responses (*n*=5) recorded at times indicated in A2. B, LTP induction by simultaneous theta burst stimulation (TBS) of horizontal and vertical pathways. Symbols as in A. B1, arrangement of microelectrodes. B2, time course of field potential amplitude during experiment. Filled and open circles denote responses to horizontal (Stim.1) and vertical (Stim.2) stimulation, respectively (see B1). B3, superposition of averaged responses (*n*=5) recorded in the horizontal pathway at times indicated in B2.

least reliable, inducing LTP in fewer than half of the attempts. Doubling the stimulus intensity during TBS and using simultaneous two-input stimulation (combined with Bic application) proved to be most

effective in LTP induction (100% of tested cases). Simultaneous TBS of two horizontal pathways (lateral and medial to the recording site) produced slightly larger LTP magnitudes. However, despite

the reliability of LTP induction with increased stimulation strength, the requirement for local, transient disinhibition was not removed. This condition may reflect the critical placement of inhibition in the horizontal pathway so that inhibitory postsynaptic potentials arrive soon enough after excitation to prevent the postsynaptic target neurones from reaching a threshold for LTP induction (Wigström and Gustafsson 1985, Artola et al. 1990).

The complexity of cortical interconnections might implicate any of a number of pathways in the LTP evoked in the superficial layers by stimulation at horizontally displaced sites. However, the sufficiency of the circuitry of the superficial layers for LTP could be demonstrated. In slices in which tissue located deeper than 1 mm below the cortical surface (see Fig. 2A1) was removed, LTP resembling that observed in complete slices could still be obtained (Hess and Donoghue 1994) in both field potential and intracellular recordings (Hess et al. 1996).

The question arises whether more physiologically relevant neuronal activity could create the appropriate conditions for LTP induction in horizontal connections. It is known that repetitive activity can fatigue synaptic inhibition (Deisz and Prince 1989) and vertically running fibres form the most effective excitatory connections in the neocortex. As mentioned earlier, vertically ascending connections in slice preparations can comprise a mixture of thalamocortical, long cortico-cortical and locally ascending pathways. Therefore LTP induction was next attempted by simultaneous TBS of converging horizontal and vertical pathways (Hess et al. 1996). In order to activate vertical pathways, a second stimulating electrode was placed below the recording electrode, roughly at the boundary between layers III and V (Fig. 2B1). Co-activation of horizontal and vertical inputs resulted in reliable LTP induction in the horizontal pathway (Fig. 2B2), thereby replacing the need for pharmacological disinhibition. These results suggest that the potential for LTP in horizontal connections may be regulated by spatiotemporal interactions between horizontal and vertical pathways. Because horizontal LTP in

these studies was reversibly blocked by bath application of an NMDA receptor antagonist, the activation of this receptor probably forms an essential step in plasticity of these intracortical connections, as has been suggested for other forms of LTP.

LONG-TERM DEPRESSION

Existence of only a mechanism to enhance synaptic efficacy could quickly lead to saturation of all excitatory synapses. However, the obverse of LTP, long-term depression (LTD), is another form of activity-dependent synaptic plasticity available in the adult brain to decrease synaptic strength (for review and further discussion see Artola et al. 1990, Hirsch and Crepel 1990, Dudek and Bear 1993, Linden 1994). Interestingly, LTD induction requires repeated afferent activity as for LTP, but at a lower rate or intensity, suggesting that different activation patterns selectively activate different plasticity mechanisms. The chief difference between most effective stimulation patterns inducing LTD vs. LTP is frequency of stimulation (low vs. high), however, the temporal structure of stimulation has yet to be fully explored as a significant parameter.

LTD in MI horizontal connections has recently been demonstrated in our laboratories using field potential and intracellular recording. Long-term depression, lasting an hour or more, was obtained reliably by low-frequency stimulation (LFS) of the horizontal pathways within layers II/III, applied at 2 Hz for 10 min (Hess and Donoghue 1996). Figure 3 shows an example of a representative experiment. The magnitude of the effects of conditioning stimulation depended on stimulus intensity. In 77% of tested slices LTD was induced by LFS applied at the test stimulus intensity, evoking 50-70% maximum response. The depression of response amplitude to 79% of baseline values was measured 25-30 min after LFS ended. When conditioning stimulation intensity was doubled, larger LTD (59% of baseline) was induced in all tested cases. This intensity effect may reflect the requirement for a certain minimum postsynaptic depolarization to initiate processes leading to LTD and is consistent with the existence
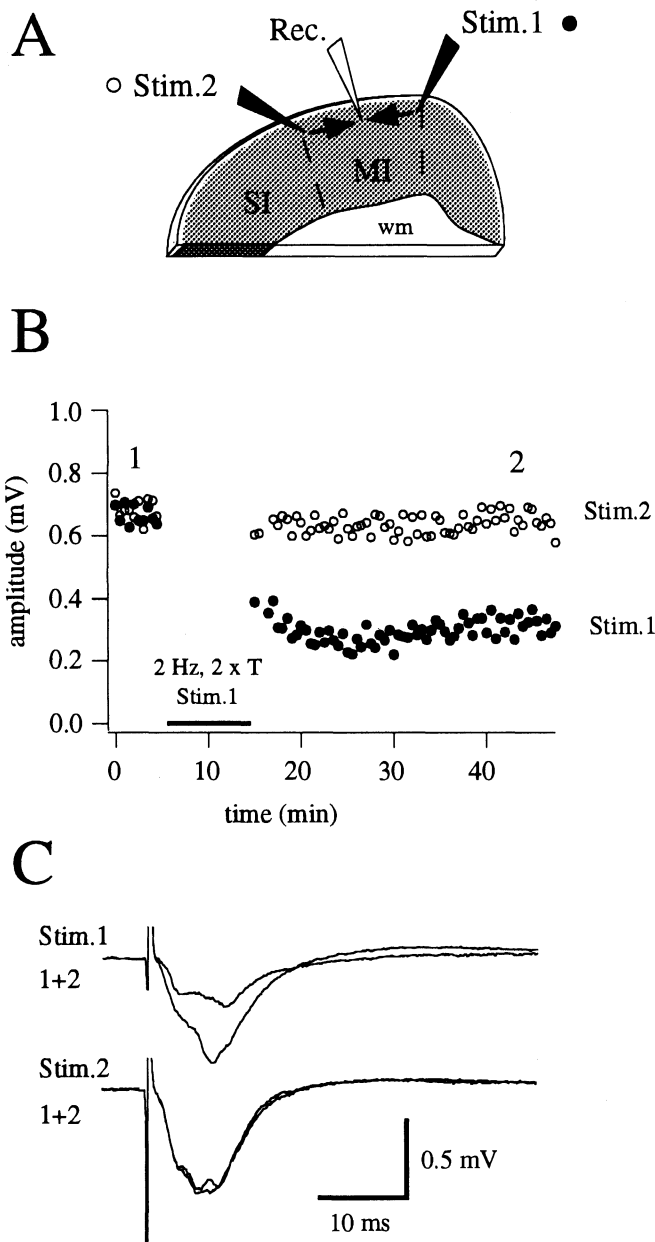
Fig. 3. LTD induction in layer II/III horizontal connections. A, typical configuration of stimulating (Stim.) and recording (Rec.) microelectrodes in the slice. B, time course of field potential amplitude in the test (filled symbols) and control input (open symbols) during the experiment. Bar denotes time of 2 Hz stimulation applied to Stim.1 at double test (2× T) intensity. Note the specificity of changes to the conditioned input. C, superposition of responses (10 trial averages) recorded at times indicated in B.

of a threshold for LTD induction (Artola et al. 1990). In most (87%) of tested cases LTD in hori-

zontal connections was specific to the LFS-activated pathway.

Depressed horizontal pathways retained the ability to increase synaptic strength at levels comparable to control, unstimulated inputs (Hess and Donoghue 1996). LTP could be obtained in these pathways using either of the two strategies described above. Two-input TBS combined with Bic application resulted in field response increase averaging 45% over baseline. Simultaneous TBS of converging horizontal and vertical inputs induced a long-lasting increase of the response amplitude averaging 31% over pre-TBS levels for the horizontal and 32% for the vertical input. These data indicate the reversible character of plastic changes in horizontal synaptic transmission.

CONCLUSIONS

Experiments on the plasticity of adult MI horizontal connections demonstrate the potential for activity-dependent modification within the superficial cortical layers. Both increases and decreases of the strength of synaptic coupling may be induced, depending on the specific activity patterns. Synaptic modification across horizontally connected neurones appears to be regulated both by the arrangement of intrinsic circuitry (particularly the placement of inhibitory neurones within the circuit) and by the availability of appropriate mechanisms for modification at individual synapses. The properties of horizontal connections indicate that they form a spatial substrate and provide activity dependent mechanisms for plasticity of cortical representation patterns. The existence of an adaptive and dynamic network existing within the motor cortex can provide immense flexibility to the motor system that could participate in motor learning or in recovery of function after damage.

REFERENCES

Aroniadou V.A., Keller A. (1993) The patterns and synaptic properties of horizontal intracortical connections in the rat motor cortex. J. Neurophysiol. 70: 1553-1569.

Artola A., Bröcher S., Singer W. (1990) Different voltage-dependent thresholds for inducing long-term depression and long-term potentiation in slices of rat visual cortex. Nature 347: 69-72.

Bear M.F., Kirkwood A. (1993) Neocortical long-term potentiation. Curr. Opin. Neurobiol. 3: 197-202.

Benardo L.S., Wong R.K.S. (1995) Inhibition in the cortical network. In: The cortical neuron. (Eds. M. Gutnick and I. Mody). Oxford University Press, Oxford, p. 141-155.

DeFelipe J., Conley M., Jones E.G. (1986) Long-range focal collateralization of axons arising from cortico-cortical cells in monkey sensory-motor cortex. J. Neurosci. 6: 3749-3765.

Deisz R.A., Prince D.A. (1989) Frequency-dependent depression of inhibition in guinea-pig neocortex in vitro by GABA$_B$ receptor feed-back GABA release. J. Physiol. (Lond.) 412: 513-541.

Donoghue J.P., Hess G., Sanes J.N. (1996) Substrates and mechanisms for learning in motor cortex. In: Acquisition of motor behavior in vertebrates (Eds. J. Bloedel, T. Ebner and S.P. Wise). MIT Press, Boston (in press).

Donoghue J.P., Sanes J.N. (1994) Motor areas of the cerebral cortex. J. Clin. Neurophysiol. 11: 382-396.

Donoghue J.P., Suner S., Sanes J.N. (1990) Dynamic organization of primary motor cortex output to target muscles in adult rats. II. Rapid reorganization following motor nerve lesions. Exp Brain Res. 79: 492-503.

Dudek S.M., Bear M.F. (1993) Bidirectional long-term modification of synaptic effectiveness in the adult and immature hippocampus. J. Neurosci. 13: 2910-2918.

Ghosh S., Porter R. (1988) Morphology of pyramidal neurones in monkey motor cortex and the synaptic actions of their intracortical axon collaterals. J. Physiol. (Lond.) 400: 593-615.

Hess G., Aizenman C.D., Donoghue J.P. (1996) Conditions for the induction of long-term potentiation in layer II/III horizontal connections of the rat motor cortex. J. Neurophysiol. (in press)

Hess G., Donoghue J.P. (1994) Long-term potentiation connections provides a mechanism to reorganize cortical motor maps. J. Neurophysiol. 71: 2543-2547.

Hess G., Donoghue J.P. (1996) Long-term depression of horizontal connections in rat motor cortex. Eur. J. Neurosci. (in press)

Hess G., Jacobs K.M., Donoghue J.P. (1994) N-methyl-D-aspartate receptor-mediated component of field potentials evoked in horizontal pathways of rat motor cortex. Neuroscience 61: 225-235.

Hirsch J.C., Crepel F. (1990) Use-dependent changes in synaptic efficacy in rat prefrontal neurons in vitro. J. Physiol. 427: 31-49.

Huntley G.W., Jones E.G. (1991) Relationship of intrinsic connections to forelimb movement representations in monkey motor cortex: a correlative anatomic and physiological study. J. Neurophysiol. 66: 390-413.

Jacobs K.M. (1994) The functional architecture of neocortical circuits. Ph.D. Thesis. Brown University, Providence, R.I., USA.

Jacobs K.M., Donoghue J.P. (1991) Reshaping the cortical map by unmasking latent intracortical connections. Science 251: 944-947.

Kalaska J.F., Crammond D.J. (1992) Cerebral cortical mechanisms of reaching movements. Science 255: 1517-1523.

Kang Y., Kaneko T., Ohishi H., Endo K., Araki T. (1994) Spatiotemporally differential inhibition of pyramidal cells in the cat motor cortex. J. Neurophysiol. 71: 280-293.

Keller A., Asanuma H. (1993) Synaptic relationship involving local axon collaterals of pyramidal neurons in the cat motor cortex. J. Comp. Neurol. 336: 229-242.

Keller A., Iriki A., Asanuma H. (1990) Identification of neurons producing LTP in the cat motor cortex: intracellular recordings and labelling. J. Comp. Neurol. 300: 47-60.

Landry P., Labelle A., Deschenes M. (1980) Intracortical distribution of axonal collaterals of pyramidal tract cells in the cat motor cortex. Brain Res. 191: 327-336.

Linden D.J. (1994) Long-term synaptic depression in the mammalian brain. Neuron 12: 457-472.

Qui X., O'Donoghue D., Humphrey D. (1990) NMDA antagonist (MK-801) blocks plasticity of motor cortex maps induced by passive limb movement. Soc. Neurosci. Abstr. 16: 178.7.

Sanes J.N., Suner S., Donoghue J.P. (1990) Dynamic organization of primary motor cortex output to target muscles in adult rats I. Long-term patterns of reorganization following motor or mixed peripheral nerve lesions. Exp. Brain Res. 79: 479-491.

Sanes J.N., Wang J., Donoghue J.P. (1992) Immediate and delayed changes of rat motor cortical output representation with new forelimb configurations. Cereb. Cortex 2: 141-152.

Stafstrom C.E., Schwindt P.C., Chubb M.C., Crill W.E. (1985) Properties of persistent sodium conductance and calcium conductance of layer V neurons from cat sensorimotor cortex in vitro. J. Neurophysiol. 53: 1153-1170.

Suner S., Gutman D., Gaal G., Sanes J., Donoghue J.P. (1993) Reorganization of monkey motor cortex related to motor skill learning. Soc. Neurosci. Abstr. 19: 775.

Thomson A.M., Deuchars J. (1994) Temporal and spatial properties of local circuits in neocortex. Trends Neurosci. 17: 119-126.

Weiss D.S., Keller A. (1994) Specific patterns of intrinsic connections between representation zones in the rat motor cortex. Cereb. Cortex 4: 205-214.

Wigström H., Gustafsson B. (1985) Facilitation of hippocampal long-lasting potentiation by GABA antagonists. Acta Physiol. Scand. 125: 159-172.

Wise S.P., Donoghue J.P. (1986) Motor cortex of rodents. In: Cerebral cortex. Functional properties of cortical cells (Eds. E.G. Jones and A. Peters). Vol. 5. Plenum Press, New York, p. 243-269.