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RESEARCH ARTICLE

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A repetitive intracortical microstimulation pattern induces long-lasting synaptic depression in brain slices of the rat primary somatosensory cortex

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Abstract Repetitive intracortical microstimulation (ICMS) applied to the rat primary somatosensory cortex (SI) in vivo was reported to induce reorganization of receptive fields and cortical maps. The present study was designed to examine the effect of such an ICMS pattern applied to layer IV of brain slices containing SI on the efficacy of synaptic input to layer II/III. Effects of ICMS on the synaptic strength was quantified for the first synaptic component (*s1*) of cortical field potentials (FPs) recorded from layer II/III of SI. FPs were evoked by stimulation in layer IV. The pattern of ICMS was identical to that used in vivo. However, stimulation intensity had to be raised to induce an alteration of synaptic strength. In brain slices superfused with standard ACSF, repetitive ICMS induced a short-lasting (60 min) reduction of the amplitude (–37%) and the slope (–61%) of *s1* evoked from the ICMS site, while the amplitude and the slope of *s1* evoked from a control stimulation site in cortical layer IV underwent a slow onset increase (13% and 50%, respectively). In brain slices superfused with ACSF containing 1.25 µM bicuculline, ICMS induced an initial strong reduction of the amplitude (–50%) and the slope (–79%) of *s1* evoked from the ICMS site. These effects decayed to a sustained level of depression by –30% (amplitude) and –60% (slope). In contrast to experiments using standard ACSF, *s1* evoked from the control site was not affected by ICMS. The presynaptic volley was not affected in either of the two groups of experiments. A conventional high frequency stimulation (HFS) protocol induced input-specific long-term potentiation (LTP) of the amplitude and slope of *s1* (25% and 76%, respectively). Low frequency stimulation (LFS) induced input-specific long-term depression (LTD) of the amplitude and slope of *s1* (24% and 30%, respectively). Application of common forms of conditioning stimulation (HFS and LFS) resulted in LTP or LTD of *s1*, indicating normal susceptibility of the brain slices studied to the induction of common forms of synaptic plasticity. Therefore, the effects of repetitive ICMS on synaptic FP components were considered ICMS-specific forms of short-lasting (standard ACSF) or long-lasting synaptic depression (ACSF containing bicuculline), the latter resembling neocortical LTD. Results of this study suggest that synaptic depression of excitatory mechanisms are involved in the cortical reorganization induced by repetitive ICMS in vivo. An additional contribution of an ICMS-induced modification of inhibitory mechanisms to cortical reorganization is discussed.

Keywords Brain slices · Repetitive ICMS · Synaptic plasticity · LTD · Cortical reorganization

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Introduction

Changes of the shape and size of receptive fields (RFs) within the representational maps of the somatosensory cortex are observed not only during the critical developmental periods, but also in adult animals after manipulation of the activity of sensory afferents (for review see Merzenich et al. 1988). Studies on the plasticity of representational maps in behaving animals revealed that changes of cortical representations and changes of RF size develop in parallel with changes of behavioral performance, implying a direct relationship between cortical organization and behavior (Recanzone et al. 1992b). Using functional imaging techniques, this relationship has been demonstrated to hold for humans in case of Braille readers and stringed instrument players (Elbert et al. 1995; Pascual-Leone and Torres 1993). Although neuronal plasticity can be induced on each level of the ascending somatosensory system, studies suggest a crucial role of cortical contributions in mediating reorganizational changes (Darian-Smith and Gilbert 1995; for review see Buonomano and Merzenich 1998). Several lines of

evidence stress the importance of cortico-cortical connections in the induction of cortical plasticity (Florence et al. 1998; Hess and Donoghue 1994; Jacobs and Donoghue 1991).

The significance of intracortical mechanisms for the induction of cortical plasticity in adult animals was examined in studies utilizing long-lasting (1–4 h) repetitive intracortical microstimulation (ICMS) in sensory and motor cortices (Dinse et al. 1993; Maldonado and Gerstein 1996; Nudo et al. 1990; Recanzone et al. 1992a; Sil'kis and Rapoport 1995; Spengler and Dinse 1994). The experimental protocol of repetitive ICMS offers the advantage of evoking synchronized pre- and postsynaptic discharges independent of the activity of the ascending sensory pathway. Repetitive ICMS applied in the hind paw field of the adult rat primary somatosensory cortex (SI) caused an overall but selective expansion of the RF size (Dinse et al. 1993; Recanzone et al. 1992a; Spengler and Dinse 1994). In these studies, the RF at the microstimulation site (msRF) enlarged due to integration of surrounding fields. As a result, the fine-grained topography of the hind paw representation was replaced by a coarse map containing predominantly the skin representation of the msRF. This ICMS-related reorganization of RFs could be detected after 15 min of ICMS, but much greater effects were observed after 2–3 h of stimulation. All changes were reversible within 6–8 h after termination of ICMS.

The protocol of repetitive ICMS differs from stimulation protocols utilized to induce long-term potentiation (LTP) or long-term depression (LTD) of synaptic efficacy in respect to the stimulation pattern and the duration of conditioning. Repetitive ICMS consists of short high frequency pulse trains (40 ms at 300 Hz) that are delivered at 1 Hz and thus are separated by an interval of 960 ms. In contrast, common forms of conditioning stimulation applied to induce synaptic plasticity in slices of the hippocampus or the neocortex are either high frequency stimulation (HFS) to induce LTP (for review see Bear and Kirkwood 1993; Bliss and Collingridge 1993; Tsumoto 1992) or low frequency stimulation (LFS) to induce LTD (for review see Bear and Abraham 1996; Linden and Connor 1995; Tsumoto 1992) which are delivered for periods in the range of a few minutes.

Although many studies demonstrated the potential capacity of repetitive ICMS to induce cortical reorganization in vivo, little is known about underlying synaptic mechanisms. The present study aimed at elucidating the type of synaptic plasticity possibly involved in the cortical reorganization of skin field representation induced by repetitive ICMS in vivo. Therefore, the stimulation protocol used to induce synaptic plasticity in brain slices of the rat somatosensory cortex was designed to match as closely as possible that used in the in vivo studies. However, presumably due to the low levels of activity in brain slices compared to in vivo preparations, a higher stimulation intensity had to be used for the induction of synaptic plasticity. To demonstrate the susceptibility of brain slices of the somatosensory cortex to the induction of LTP or LTD, the effects of conventional stimulation protocols, i.e. HFS and LFS, were examined for comparison.

Materials and methods

Experiments were performed on rat brain slices containing SI. The preparation procedures are described elsewhere (Greffrath et al. 1998) and are in accordance with the current version of the "German Law on the Protection of Animals" and the "Principles of Laboratory Animal Care". Briefly, young adult male Sprague-Dawley rats (160–250 g; Charles River, Germany) were anesthetized with diethyl ether and then decapitated. The brain was rapidly removed from the skull and immersed in chilled ACSF. Throughout the experiment, including the preparation period, ACSF was saturated with carbogen gas (95% O_2, 5% CO_2). Coronal brain slices (400–500 µm thick) were cut using a Vibroslice (Campden Instruments, UK). During the cutting procedure the brain was covered with chilled ACSF. Brain slices were transferred to a beaker containing ACSF and were allowed to equilibrate for at least 2 h at room temperature.

After equilibration, a brain slice was transferred to the recording stage of a Haas-type tissue chamber. In this chamber the slice was superfused with carbogen-saturated ACSF at a temperature of 33±1°C and at a rate of 2–3 ml/min. Field potentials (FPs) were extracellularly recorded from cortical layers II/III of SI using glass microelectrodes. Microelectrodes were filled with ACSF. After breaking the tip by gently touching the surface of the recording stage, microelectrodes had a tip resistance of approximately 2 MΩ. The position of the electrode tip was adjusted to conform approximately to the coordinates of the hind paw representational field as given by the atlas of Paxinos and Watson (1986). FPs were evoked by unipolar stimulation with rectangular electrical pulses (0.1 ms, 15–70 V, 0.1/s) applied with insulated steel wire electrodes (A-M Systems, USA). Stimulation intensity was adjusted to yield synaptic FP components with an amplitude of 65–70% of the maximal response. FPs were evoked from either of two stimulation sites in cortical layer IV (Fig. 1). One stimulation site was located on a perpendicular line from the cortical surface through the recording site (Fig. 1 *filled circle*) or up to 250 µm lateral to that line. The distance of the second stimulation electrode medial from the perpendicular line was 100–400 µm (Fig. 1 *open circle*). The distance between the two stimulation electrodes was 350–400 µm. The first stimulation electrode was used for repetitive ICMS, whereas both stimulation electrodes were used to evoke FPs throughout the experiment.

In an extensive pilot study, the ICMS intensity necessary to induce plastic changes was explored. Stimulation intensities of 5–100 µA did not result in changes of synaptic responses to test pulses. In the present study, ICMS intensities of 200–400 µA were used. ICMS consisting of repetitive pulse trains of 40 ms duration (13 pulses, 0.1 ms, 300/s) was applied for 100–150 min at a rate of 1 train/s. After ICMS, FP measurements were performed during a period of at least 95 min. The period of repetitive ICMS was preceded by a control period during which the amplitude of FPs had to be stable for at least 45 min. For each measurement, five consecutive FPs were evoked at a rate of 0.1/s. FPs were averaged online using a PC-based computer system, including an AD/DA interface (TL-1-125; Axon Instruments, USA) and the appropriate acquisition software (Axotape; Axon Instruments).

LTP was induced by HFS using brief bursts of four 0.2-ms pulses at 100 pulses/s delivered for 1 s at a repetition rate of 10 bursts/s. This sequence of stimuli was repeated 6 times at 10-s intervals. For the induction of LTD, LFS with trains of 0.2-ms pulses delivered at 1/s was applied for 30 min (a total of 1,800 pulses). The conditioning with LFS or HFS was preceded by a control period during which the amplitude of FPs had to be stable for at least 45 min. After application of the conditioning stimulation, FPs were recorded during a period of at least 65 min. In all LTD and LTP experiments, brain slices were superfused with standard ACSF.

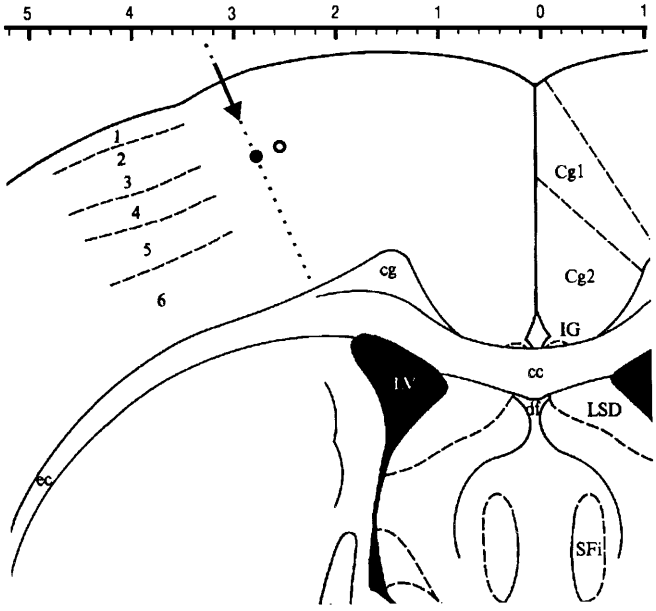

Fig. 1 Recording and stimulation sites. The recording site is indicated by the *tip of the arrow*. The perpendicular reference line from the cortical surface through the recording site is indicated by a *dotted line*. The intracortical microstimulation (ICMS) site is indicated by the *filled circle*, while the control stimulation site is indicated by the *open circle*. The margins between cortical layers *1–6* are indicated by *dashed lines*. *Cg* Cingulate cortex, *cc* corpus callosum, *cg* cingulum, *df* dorsal fornix, *ec* external capsule, *LV* lateral ventricle, *IG* indusium griseum, *LSD* lateral septal nucleus, dorsal, *SFi* septofimbrial nucleus. The *scale on top* is in millimeters relative to the medial fissure. Adopted from the atlas of the rat brain by Paxinos and Watson (1986) and modified

The standard ACSF was composed of (in mmol/l): 124 NaCl, 2 KCl, 1.25 KH_2PO_4, 1.3 $MgSO_4$, 2.5 $CaCl_2$, 26 $NaHCO_3$, 10 glucose. Synaptic FP components were depressed or eliminated by superfusion of brain slices with low Ca^{2+}/high Mg^{2+} ACSF which was composed of: 124 NaCl, 6.25 KCl, 4 $MgCl_2$, 0.2 $CaCl_2$, 26 $NaHCO_3$, 10 glucose. In one series of experiments 1.25 µmol/l bicuculline (Sigma, Germany) was added to the ACSF. FPs evoked in the present study during superfusion of slices with ACSF containing bicuculline most probably do not reflect epileptiform discharge, since the shape and sequence of FP components closely resembled those of studies using lower concentrations of bicuculline (Chagnac-Amitai and Connors 1989) or no bicuculline (Langdon and Sur 1990). Furthermore, the stable latency of FP components, the lack of irregularly generated activity of long latency, and the lack of long-lasting discharge activity (Langdon and Sur 1990) speak against epileptiform activity generated in brain slices of the present study. The contribution of NMDA receptors and AMPA receptors to the synaptic FP components was examined by application of 2-amino-5-phospho-pentanoic acid (AP5; Tocris Cookson, UK; 30 µM, 20 min) or 6-nitro-7-sulfamoylbenzo(f)quinoxaline-2,3-dione (NBQX; donated by Hoechst, Germany; 40 µM, 20 min), respectively.

Effects of ICMS were evaluated as relative change of the amplitude (as measured between baseline and peak). Additionally, the slope of the first synaptic FP component was determined between 10% and 90% of its total amplitude. Values given are the mean ± SEM. Statistical significance of effects was examined using the paired Student's *t*-test. For each set of measurements after repetitive ICMS, the amplitude and the slope of FP components were compared to the respective control measurements immediately before application of repetitive ICMS. Analysis of effects across experimental groups were performed by repeated measures two-way analysis of variance (ANOVA) with the main factors: experimental groups and time after ICMS. The latter was the repeated measures factor. Differences across groups at various times after ICMS were further analyzed in detail using the *post hoc* Newman-Keuls test. In all statistical tests, $P<0.05$ for differences between mean values was considered statistically significant, while $P<0.01$ and $P<0.001$ were considered highly significant.

Results

Characterization of FP components

The FPs evoked in cortical layers II/III of the SI by electrical stimulation in cortical layer IV were composed of fast negative peaks and a slow positive wave (Fig. 2A). This holds true for FPs evoked from both the ICMS site and the non-ICMS site. Typically the first component of an FP (Fig. 2A, component 1) was a sharp negative peak with a latency of 2.23±0.06 ms (n=46). The amplitude of this peak varied between FPs recorded from different brain slices (0–4.61 mV). This short latency peak was succeeded by a sequence of negative peaks with slower onset and slower decay (Fig. 2A, components 2 through 4). When present, the amplitude of these peaks decreased with increasing latency. In many cases the negative peaks were superposed or followed by slow positive and negative waves (latency of onset >10 ms). During superfusion of brain slices with low-Ca^{2+} ACSF the amplitude of the first negativity was increased by 30.0±6.1% (mean ± SEM; n=10), whereas the succeeding peaks and waves were strongly attenuated or blocked (Fig. 2B). Consequently, the first negativity was considered a presynaptic volley (*p*), while the later FP components were considered to be of synaptic origin. A direct activation of cortical neurons may also contribute to the component *p*. The first FP negativity followed the stimulation frequency of 50 Hz with no detectable reduction in amplitude (the steady state amplitude of the presynaptic component to 100 Hz was 80.7±2.4%; n=11). In contrast, the first synaptic FP component (*s1*) followed only stimulation frequencies of 1–2 Hz (after 30 s of stimulation at 2 Hz the amplitude of *s1* was 92.9±0.9%; n=7). The latency to peak of *s1* was 4.5±0.11 ms and its amplitude was 1.89±0.11 mV. Synaptic components with longer latency were much less prominent and stable. In slices superfused with standard ACSF, a second synaptic component (Fig. 2A, component 3 = *s2*) occurred during the control period in 24 of 46 examined cases (52.2%), and occurred in 46 of 51 cases (90.2%) when brain slices were superfused with ACSF containing bicuculline. Due to the relatively high variability of *s2* and other synaptic FP components with latencies exceeding that of *s1*, effects of repetitive ICMS on these FP components were not quantitatively evaluated.

The contribution of the AMPA and NMDA types of glutamate receptor to the synaptic components was examined in 31 and 57 brain slices, respectively (including brain slices examined in a pilot study on the stimulation

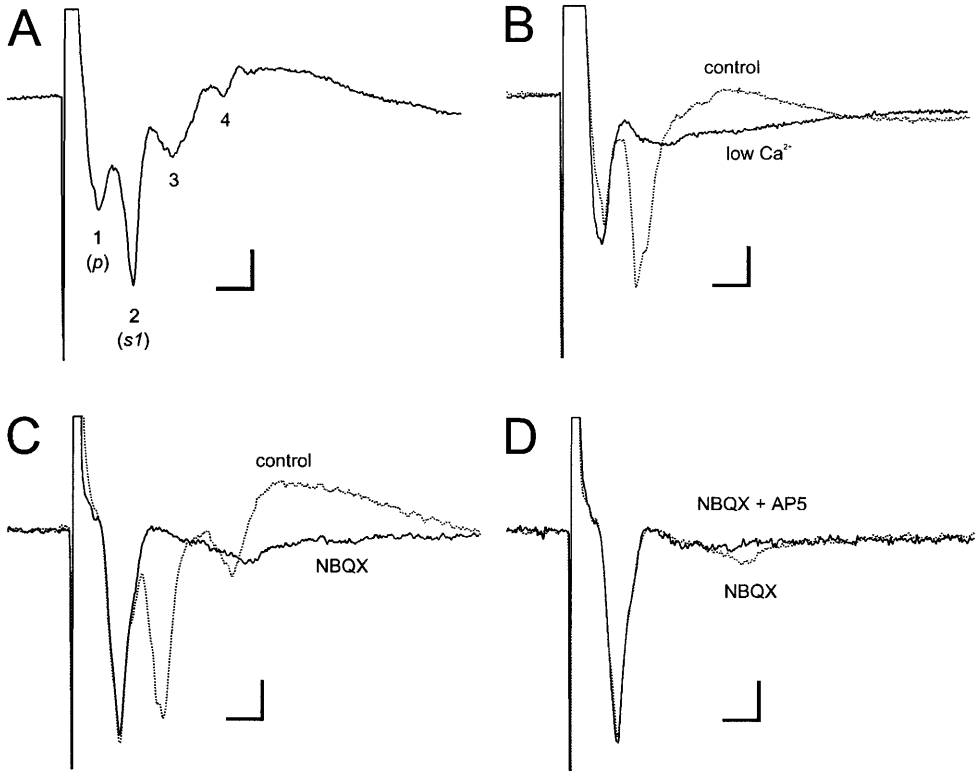

Fig. 2A–D Characteristics of field potentials (FPs) recorded from brain slices of the rat primary somatosensory cortex. **A** FPs evoked in cortical layers II/III by electrical stimulation in layer IV were composed of various fast negative peaks and a slower positive wave. **B** During superfusion of a brain slice with low-Ca^{2+} ACSF the first negative peak was slightly enhanced while the other FP components were strongly attenuated or blocked. **C** During application of NBQX the synaptic FP components and the slow positive wave were strongly attenuated or blocked; the first negative peak (i.e. the presynaptic volley) was not significantly affected. **D** The NBQX-insensitive synaptic event was blocked when AP5 was applied in addition to NBQX. Tests shown in panels **C** and **D** are performed in the same brain slice. *Dotted lines* Control FP, *solid lines* affected FP. *Scale bars* represent 0.5 mV on ordinate and 2.5 ms on abscissa. *Numbers* in **A** represent FP negativities in temporal sequence, *p* indicates the presynaptic volley, and *s1* indicates the first synaptic FP component

intensity necessary to induce plastic changes). The application of AP5 (30 µM) resulted in a reduction of the amplitude of *s1* by 7.2±1.1%, while *s2* was attenuated by 20.3±4.3%. In contrast, all synaptic components were strongly attenuated or blocked by the AMPA receptor antagonist NBQX (40 µM; Fig. 2C); the mean reduction induced by NBQX was 81.3±4.5%. The remaining synaptic component was reduced by 74.1±9.3% when AP5 was applied in addition to NBQX (n=16; Fig. 2D). In contrast to synaptic components, the presynaptic volley was not significantly affected by NBQX (mean reduction in amplitude: 0.4%) or AP5 (mean increase in amplitude: 1.1%). Thus, the major portion of synaptic events was dependent on glutamate receptors of the AMPA subtype, whereas the NMDA receptor-mediated portion was relatively small.

Effects of repetitive ICMS; standard ACSF

In brain slices superfused with standard ACSF, synaptic components of FPs evoked from the ICMS site in layer IV and recorded from cortical layers II/III were depressed after ICMS. The amplitude and slope of synaptic FP components were affected. Characteristic examples are shown in Fig. 3. Five minutes after termination of repetitive ICMS, *s1* was strongly reduced in amplitude and slope (Fig. 3A). The same holds true for effects of repetitive ICMS on *s2*. Sixty-five minutes after termination of ICMS, *s1* and *s2* had recovered from depression (Fig. 3B). The depression of synaptic components did not depend on alterations of the presynaptic component *p*, which was moderately increased or decreased after ICMS (Fig. 3A–D). The mean effects of repetitive ICMS on *s1* of the FP evoked from the ICMS site (Fig. 4; n=6) show that the initial strong reduction in amplitude by 37.1±3.2% (P<0.001) decayed during the 1st h after termination of ICMS and reached a level of depression by about 10% (P<0.05) 65 min after termination of ICMS (Fig. 4A). After 95 min the depression was not statistically significant. The time course of the ICMS-induced reduction of the slope of *s1* (Fig. 4B) resembled that of the ICMS-induced effect on the amplitude of *s1*. Immediately after termination of repetitive ICMS the slope of *s1* was reduced by 60.9±3.34% (P<0.001), an effect that decayed during 65 min of the post-ICMS period to a reduction by about 17% (n.s.). The presynaptic volley evoked from the ICMS site essentially remained unaffected by ICMS; an initial mean reduction of about 4% was not statistically significant.

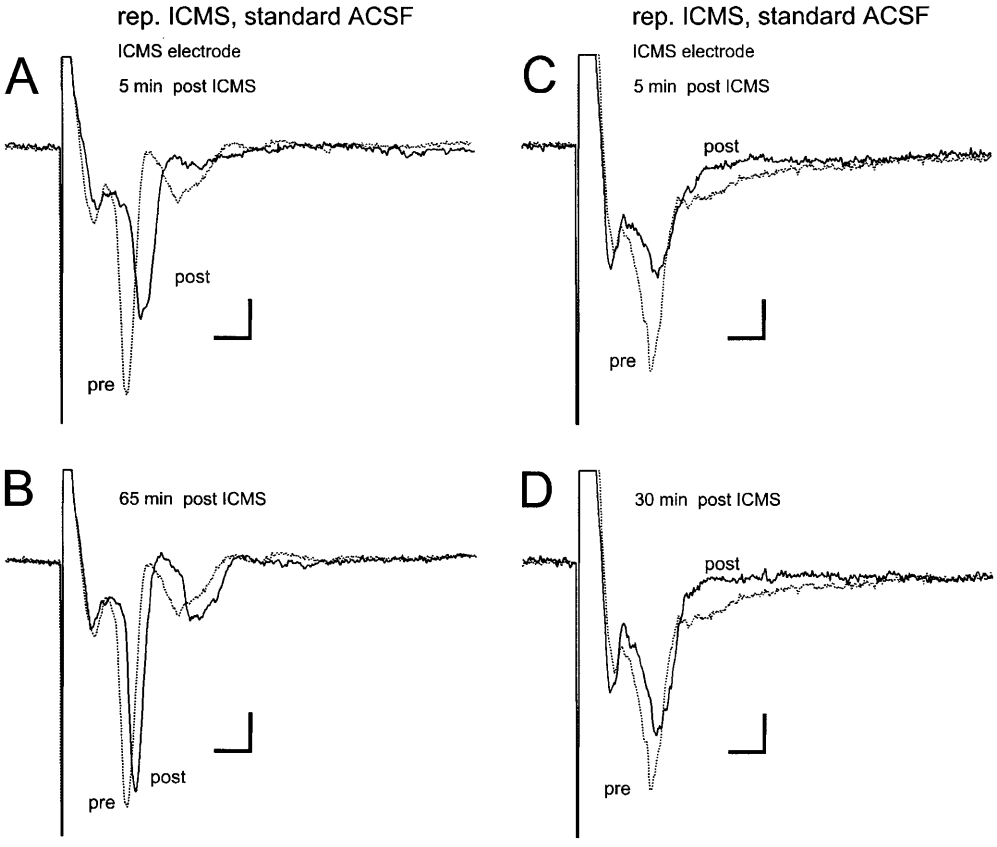

Fig. 3A–D Effects of repetitive ICMS on FPs during superfusion of the brain slice with standard ACSF. Initially the amplitude and slope of *s1* evoked from the ICMS site were reduced (**A, C**). After 30 min (**D**) or 65 min (**B**) these effects were strongly attenuated. The same was true for effects on *s2* (**B**). The depression of synaptic components did not depend on the effect of ICMS on *p* (compare **A, B** with **C, D**). *Dotted lines* Control FP, *solid lines* FP after repetitive ICMS. *Scale bars* represent 0.5 mV on ordinate and 2.5 ms on abscissa

ICMS-induced effects on the amplitude and slope of *s1* evoked from the control stimulation site differed from those on *s1* evoked from the ICMS site. After termination of ICMS, the amplitude and the slope of *s1* evoked from the control stimulation site initially were slightly but not significantly reduced (Fig. 4A, B). Thereafter, both the amplitude and the slope gradually increased to reach a statistically significant level of enhancement at 65 and 95 min after termination of ICMS: the amplitude of *s1* was increased by 10.9±3.6% and 12.7±4.3%, respectively ($P<0.05$ each), while the slope of *s1* was increased by 48.8±16.4% and 51.7±22.9%, respectively ($P<0.05$ each). This slow onset potentiation occurred in five of six slices examined. In contrast, the presynaptic volley of FPs evoked from the non-ICMS site was not significantly affected by ICMS.

Effects of repetitive ICMS; ACSF containing bicuculline

It has been demonstrated in studies on synaptic plasticity in the visual and motor cortex (Artola and Singer 1987, 1990; Hess et al. 1996) that the induction of synaptic plasticity was facilitated by a reduction of intracortical GABAergic inhibition. We therefore applied repetitive ICMS during superfusion of brain slices with ACSF containing 1.25 µM bicuculline. In these experiments, repetitive ICMS induced a strong depression of *s1* evoked from the ICMS site in layer IV. As demonstrated in a typical example (Fig. 5), the synaptic depression was characterized by a strong reduction of the amplitude and the slope of *s1* immediately after termination of repetitive ICMS (Fig. 5A). In contrast to the experiments using standard ACSF, this reduction was only slightly diminished after 65 min (Fig. 5B). On average, the amplitude of *s1* evoked from the ICMS site (Fig. 6A; $n=5$) was initially reduced by 49.5±5.5% ($p < 0.001$). After 30 min *s1* partially recovered; the remaining depression of about 30% was maintained until the end of the post-ICMS observation period of 95 min ($P<0.001$). Essentially, the same time course was observed for the ICMS-induced reduction of the slope of *s1* (Fig. 6B). Immediately after termination of repetitive ICMS, the slope was reduced by 79.1±5.4% ($P<0.001$). After 30 min the slope had partially recovered from depression, but the remaining strong reduction of the slope by about 60% ($P<0.01$) outlasted the post-ICMS observation period. Effects on the amplitude of the presynaptic volley evoked from the ICMS site were not statistically significant. The presynaptic volley initially was reduced by about 4%. It recovered thereafter and was slightly increased toward the end of the observation period.

In contrast to the effects of repetitive ICMS on *s1* evoked from the ICMS site, the amplitude of *s1* evoked from the non-ICMS site was not significantly affected by ICMS (Fig. 6A). The same was true for the slope of *s1* (Fig. 6B). A moderate increase of the presynaptic volley evoked from the non-ICMS site observed during the entire post-ICMS period was not statistically significant.

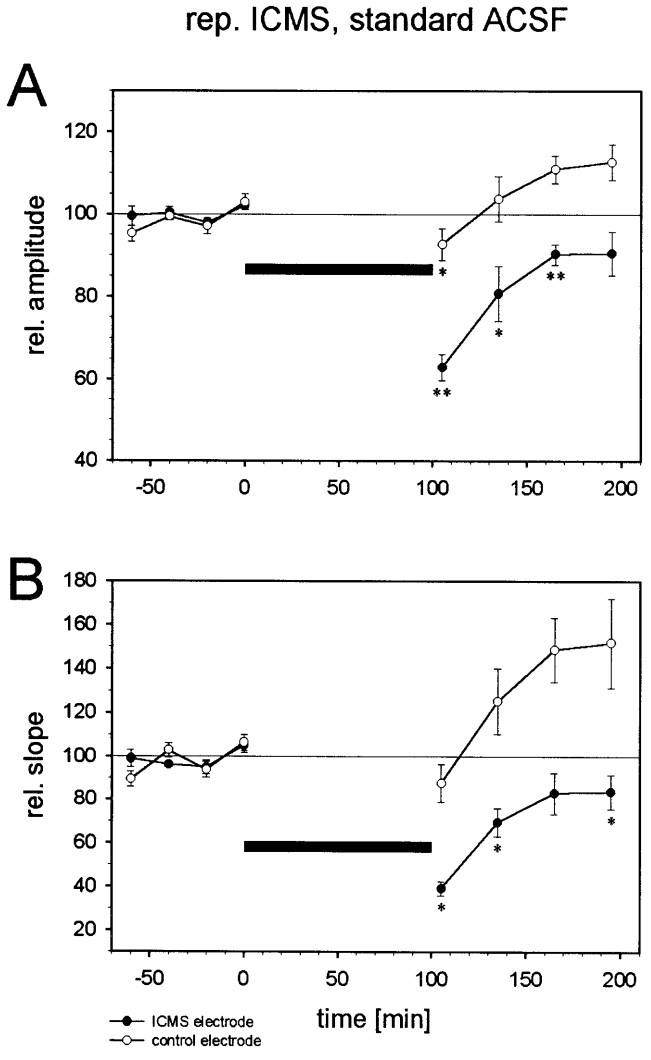

Fig. 4 Mean effects of repetitive ICMS on the amplitude (**A**) and the slope (**B**) of *s1* evoked in brain slices superfused with standard ACSF. *Filled symbols* represent mean values of the amplitude and the slope of *s1* evoked from the ICMS site, *open symbols* represent values of the amplitude and the slope of *s1* evoked from the non-ICMS site (control electrode). *Vertical bars* represent the standard error of the mean, while the *horizontal bar* represents the ICMS period. *Asterisks* indicate probability of error: * $P<0.05$, *** $P<0.001$; $n=6$ in **A** and $n=5$ in **B**. Please note the different scales on ordinate

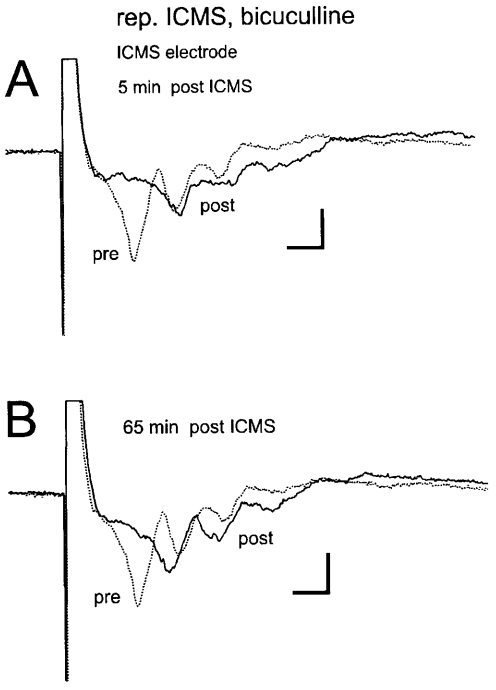

Fig. 5A,B Effects of repetitive ICMS on an FP induced in a brain slice superfused with ACSF containing 1.25 µM bicuculline. **A** Five minutes after termination of repetitive ICMS, the amplitude and the slope of the synaptic FP components evoked from the ICMS site were strongly reduced. **B** After 65 min the ICMS-induced effects on the synaptic FP components were attenuated, but a prominent reduction in amplitude and slope was still present. *Dotted lines* Control FP, *solid lines* FP after repetitive ICMS. *Scale bars* represent 0.5 mV on ordinate and 2.5 ms on abscissa

For a further analysis of the ICMS-induced effects on *s1* evoked from the ICMS site with respect to different experimental conditions, effects on the amplitude and slope induced during superfusion with ACSF containing bicuculline were compared with those induced during superfusion with standard ACSF (Fig. 7A, B). ANOVA revealed highly significant differences ($P<0.001$) for the effects on the amplitude as well as on the slope of *s1* between both groups of experimental conditions. This difference was most prominent during the later phase of the post-ICMS observation period (65 and 95 min after termination of ICMS): initially, the difference in ICMS-induced effects between both groups of experiments was significant ($P=0.015$ for the amplitude) or failed to be significant ($P=0.101$ for the slope), whereas it became highly significant at 65 and 95 min after termination of ICMS ($P<0.001$ in all cases).

Effects of LFS and HFS

In two additional series of experiments we induced common forms of synaptic plasticity in order to examine the susceptibility of the brain slices to the induction of LTP and LTD under experimental conditions identical with those used for the ICMS experiments. In these experiments the conventional stimulation paradigms, i.e. LFS or HFS, were utilized. Delivery of HFS to layer IV of the somatosensory cortex induced a potentiation of the synaptic FP component *s1* evoked from the HFS site. The mean effects of HFS ($n=6$) show that the amplitude of *s1* was increased by 22% to 28% (Fig. 8A). The slope of *s1* was increased after HFS by about 76% (results not shown). Both effects outlasted the post-HFS observation period of at least 75 min and were of high statistical significance ($P<0.01$). In contrast to these effects of HFS on *s1* evoked from the HFS site, *s1* evoked from the non-HFS site were only moderately affected in amplitude (Fig. 8A; +2.8%) and slope (+7%; results not shown). Both effects were not statistically significant.

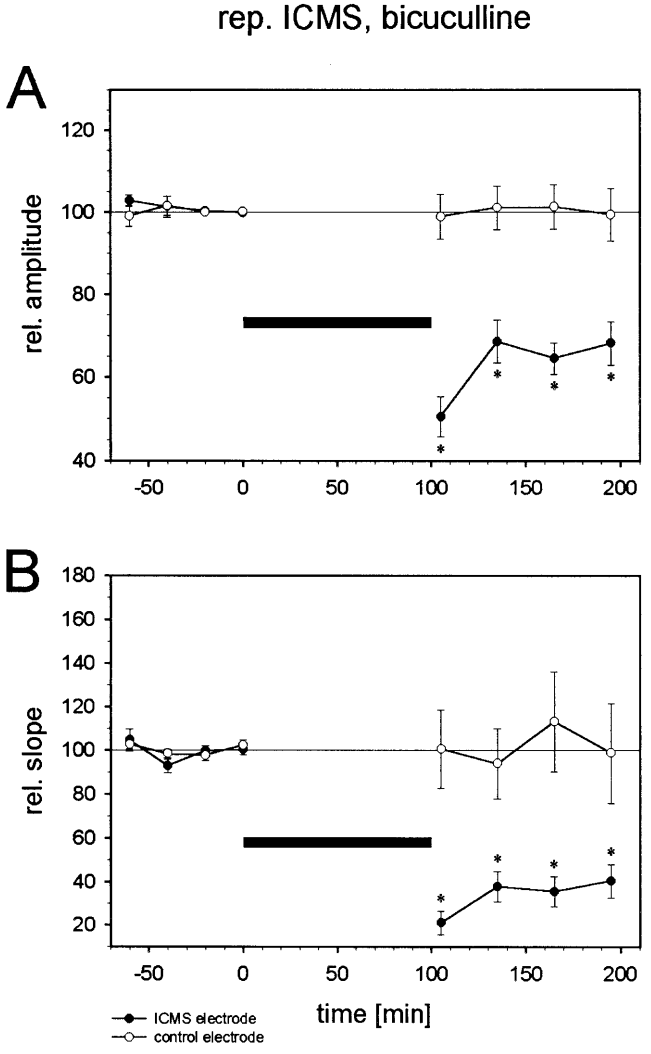

Fig. 6 Mean effects of repetitive ICMS on the amplitude (**A**) and the slope (**B**) of *s1* during superfusion of brain slices with ACSF containing 1.25 µM bicuculline. *Filled symbols* represent mean values of the amplitude and the slope of *s1* evoked from the ICMS site, *open symbols* represent values of the amplitude and the slope of *s1* evoked from the non-ICMS site (control electrode). *Vertical bars* represent the standard error of the mean, while the *horizontal bar* represents the ICMS period. *Asterisks* indicate probability of error: ** $P<0.01$, *** $P<0.001$; $n=5$. Please note the different scales on ordinate

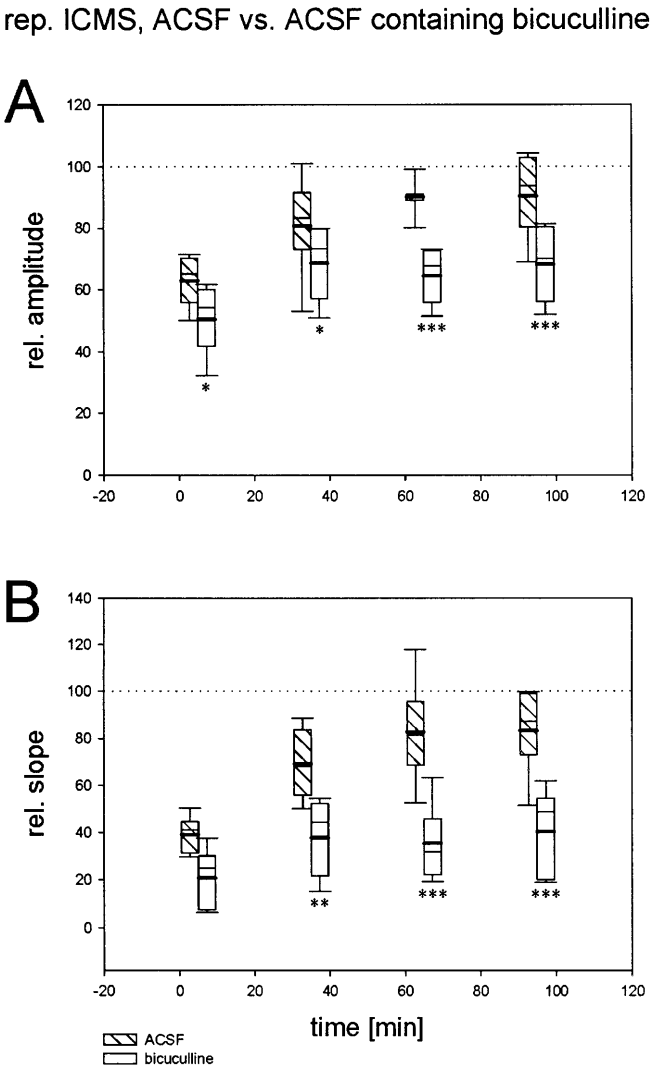

Fig. 7 Effects of repetitive ICMS on the amplitude (**A**) and the slope (**B**) of *s1* evoked from the ICMS site in brain slices superfused with standard ACSF (*hatched boxes*; $n=6$) or with ACSF containing bicuculline (*open boxes*; $n=5$). *Boxes* show the top and bottom quartiles. *Whisker caps* indicate the 5th/95th percentiles. *Thin horizontal lines within boxes* represent the medians, while *bold lines* represent the means. The *dotted line* represents the control level. Statistical significance for differences between groups were calculated using the *post hoc* Newman-Keuls test. *Asterisks* indicate probability of error: * $P<0.05$, ** $P<0.01$, *** $P<0.001$

LFS applied to layer IV of the somatosensory cortex ($n=7$) induced a depression of the synaptic FP component *s1* evoked from the LFS site. In five brain slices, the duration of the LFS-induced synaptic depression exceeded the post-LFS observation period of at least 65 min. On average, the initial depression of about 34% decayed within 20 min to a level of approximately 24% (Fig. 8B). This level of depression remained constant during the remaining observation period. The same time course was observed for the LFS-induced reduction of the slope of *s1* (results not shown). Immediately after termination of LFS, the slope of *s1* was reduced by about 39%. This reduction decayed to about 30% 45 min after termination of LFS and remained constant for the remaining observation period. The reduction of both amplitude and slope of *s1* was statistically significant ($P<0.05$ or $P<0.01$) throughout the post-LFS period. In contrast to the effects of LFS on *s1* evoked from the LFS site, the amplitude of *s1* evoked from the control site (non-LFS electrode) was not significantly affected by LFS (Fig. 8B). The same was true for the slope of *s1* evoked from the non-LFS site (results not shown).

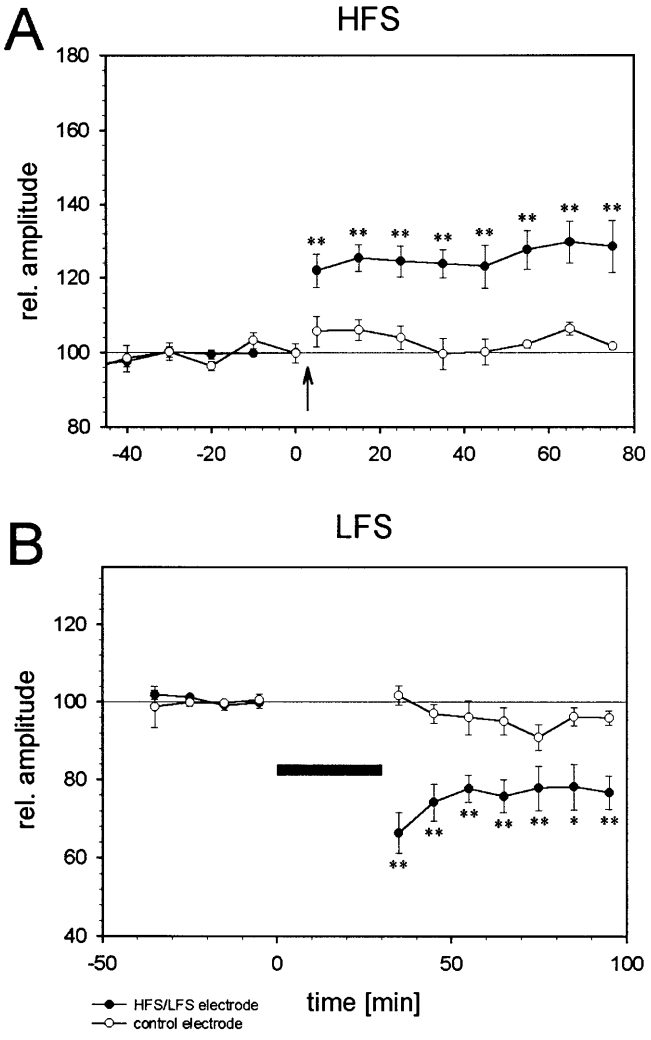

Fig. 8 Mean effects of high frequency stimulation (HFS; **A**) or low frequency stimulation (LFS; **B**) on the amplitude of the synaptic FP component *s1* evoked in brain slices superfused with standard ACSF. *Filled symbols* represent mean values of the amplitude of *s1* evoked from the HFS or LFS site, respectively, *open symbols* represent values of the amplitude of *s1* evoked from the non-HFS or non-LFS site (control electrodes). *Vertical bars* represent the standard error of the mean. The HFS period in **A** is indicated by an *arrow*, while the LFS period in **B** is indicated by a *horizontal bar*. *Asterisks* indicate probability of error: * $P<0.05$, ** $P<0.01$; $n=6$ in **A** and $n=5$ in **B**. Please note the different scales on ordinate

Discussion

Characterization of FPs

FPs evoked in cortical layers II/III by stimulation in layer IV of rat brain slices including the somatosensory cortex closely resembled those described for various neocortical regions (Aroniadou and Teyler 1992; Kudoh and Shibuki 1997; Langdon and Sur 1990; Shaw and Teyler 1982). Different sensitivity to low Ca^{2+}/high Mg^{2+} ACSF and to glutamate receptor-antagonists as well as the reliable response to different stimulation frequencies suggest that the first FP negativity was a presynaptic volley whereas the subsequent FP components were synaptically induced. The stimulation frequency for reliable response of the second FP negativity was 1–2 Hz, suggesting this component to be monosynaptically induced (Hess et al. 1994). Polysynaptic FP components were demonstrated to follow stimulation frequencies of less than 0.5 Hz (Aroniadou and Teyler 1991, 1992). The conclusion that *s1* is a monosynaptically induced FP component was further supported by its mean latency of 4.5 ms, since polysynaptic FP components were reported to occur at latencies exceeding 10 ms (Aroniadou and Teyler 1991, 1992; Hwa and Avoli 1992). The presynaptic volley presumably does not contain a prominent contribution of synaptic events, since it was reduced by only 0.4% during the application of NBQX, while the subsequent negative FP components were almost completely suppressed.

Repetitive ICMS induces synaptic depression

Application of repetitive ICMS to layer IV of the rat SI in vitro resulted in an attenuation of synaptic responses of layers II/III neuronal populations to stimulation at the ICMS site. For brain slices superfused with standard ACSF, the duration of this attenuation of synaptic efficacy was less than 65 min. A similar type of transient synaptic depression was observed after LFS in the hippocampus, the visual cortex, and the somatosensory cortex (Castro-Alamancos et al. 1995; Dudek and Bear 1992; Kirkwood and Bear 1994). Thus, the observed attenuation of synaptic efficacy can be regarded as an ICMS-induced short-lasting depression. However, the duration of this short-lasting depression exceeded that reported for the neocortex (Castro-Alamancos et al. 1995). Therefore, this short-lasting synaptic depression in SI alternatively may be interpreted in terms of a weakly expressed long-lasting reduction of synaptic efficacy, an assumption that is confirmed by the results of an ANOVA. Indeed, when intracortical GABAergic inhibition was reduced by application of the $GABA_A$ receptor-antagonist bicuculline, the ICMS-induced synaptic depression was augmented. In the latter experiments, the reduction of the first synaptic FP component in amplitude and slope outlasted the post-ICMS observation period of 95 min. The ICMS-induced depression is unlikely to result from a reduction of non-synaptic activity since the presynaptic volley was not significantly affected by ICMS. Alternatively, presynaptic depression may be caused by an activity-dependent depletion of a readily releasable neurotransmitter pool (Betz 1970). In the visual cortex, two forms of presynaptic depression by electrical stimulation were observed, a stronger form that decayed exponentially with a time constant of less than a second and a weaker form that decayed with a time constant of several seconds (Varela et al. 1997). The amount of depression was inversely related to the stimulation frequency. However, this depression may be overestimated for HFS

(Varela et al. 1999). In the medial nucleus of the trapezoid body, after stimulation with 100-ms trains at 300 Hz a rapid refilling phase within the first 300 ms was observed, which surpassed the recovery level seen at 100 Hz (Wang and Kaczmarek 1998). In the somatosensory cortex, an almost full recovery of the efficacy of excitatory connections was observed 24 s after termination of a train of 1,000 pulses at 20 Hz (Galarreta and Hestrin 1998). On the basis of these results, it may be assumed that for the given pattern of ICMS, presynaptic depression occurred during each 40-ms train of 13 pulses, but that the 960-ms intertrain interval allowed strong recovery from depression. Thus, this type of short-term plasticity may account for an initial phase of synaptic depression after termination of ICMS. However, it seems unlikely that presynaptic depletion importantly contributed to the later phase of ICMS-induced depression since the duration of the post-ICMS observation period greatly exceeded that of the reported time constants of recovery from presynaptic depletion.

In addition to the ICMS-induced long-lasting depression of *s1* evoked from the ICMS site, a potentiation of both the amplitude and the slope of *s1* evoked from the non-ICMS electrode was induced in five of six slices. This effect may indicate that in these slices the two stimulation electrodes activated some fibers common to both stimulation sites. The mechanism underlying the slow onset potentiation of synaptic input from the non-ICMS site can not be elucidated by the findings of the present study. However, it may be speculated that an activity-dependent alteration of the balance between excitation and inhibition, as observed in rat neocortical slices (Galarreta and Hestrin 1998), may have allowed the input from the non-ICMS site to potentiate. An ICMS-induced effect on inhibitory interneurons may have been involved, since the LTD induced by ICMS during application of bicuculline clearly was input-specific. Taken together, the characteristics of the ICMS-induced long-term synaptic depression in many respects resemble those of neocortical LTD induced by conventional LFS (see, for example, Castro-Alamancos et al. 1995; Kirkwood and Bear 1994). Hence, we consider the observed long-term synaptic depression an ICMS-induced form of LTD.

The role of $GABA_A$ receptor-mediated inhibition in ICMS-induced LTD

The observation that the reduction of $GABA_A$ receptor-mediated inhibition was essential for the induction of a persistent modification of synaptic strength is in accordance with results of studies on synaptic plasticity in the visual cortex (Artola and Singer 1987, 1990) and the agranular cortex (Castro-Alamancos et al. 1995). The reduction of GABAergic inhibition may facilitate mechanisms involved in maintenance of synaptic plasticity since sufficient depolarization of the postsynaptic neuron is a prerequisite for the induction of synaptic plasticity (Baranyi and Szente 1987). The induction of either LTP or LTD was demonstrated to depend on the experimental conditions (Artola et al. 1990; Dudek and Bear 1992; Kirkwood and Bear 1994; Mulkey and Malenka 1992; Yasuda and Tsumoto 1996). LTD is induced by a moderate depolarization of the postsynaptic membrane, by a low frequency of conditioning stimulation, and by a moderate increase of the intracellular calcium concentration, while a stronger depolarization of the postsynaptic membrane, a high frequency of conditioning stimulation, and a strong increase of the intracellular calcium concentration results in the induction of LTP.

In the present study ICMS failed to induce LTP, although GABAergic inhibition was strongly reduced by bicuculline, and the stimulation frequency of repetitive ICMS within each train of pulses was high (300/s). It is unlikely that this is an artifact of the slice conditions, since HFS or LFS, when applied under experimental conditions comparable to those in ICMS experiments, resulted in the induction of robust LTP or LTD even in the absence of bicuculline. The latter result suggests that the induction of LTD by ICMS was not due to insufficient reduction of intracortical inhibition. As an alternative explanation we assume that the intervals of 960 ms duration between two ICMS pulse trains may have allowed the neuronal depolarization and the Ca^{2+} influx induced by each pulse train to decline again. Thus, the level of depolarization and the intracellular Ca^{2+} concentration may have been insufficient to induce LTP. The possibility that the ICMS-induced depression was due to an unspecific effect caused by the relatively high stimulation intensities compared to those applied in vivo is unlikely, since an extensive pilot study revealed that stimulation intensities of 5–100 μA did not result in any detectable modification of synaptic efficacy. The difference between the present in vitro study and the ICMS in vivo studies with respect to stimulation intensities necessary to induce plastic changes may be due to the lack of specific and unspecific synaptic drive in brain slices compared to the somatosensory cortex in vivo.

Synaptic plasticity and cortical reorganization

Comparison of results of in vitro studies with results of in vivo studies is further complicated by the fact that in vitro FP measurements reflect the magnitude of neuronal activity recorded from a restricted cortical area, whereas in vivo RF measurements are based on a functional description of the spatial distribution of effective afferent activity in terms of the skin surface to an extended cortical area. Effects of ICMS observed in vivo, including an expansion of RFs surrounding the ICMS site (Dinse et al. 1993; Recanzone et al. 1992a; Spengler and Dinse 1994), suggest that an increase of horizontal spread of activity may be involved in this form of reorganization of SI representational maps. In fact, learning of a new motor skill was accompanied by strengthening of horizontal connections within layer II/III of the motor cortex

as revealed by examination of brain slices taken from these animals (Rioult-Pedotti et al. 1998). Furthermore, application of HFS or LFS to layer II/III of the motor cortex in vitro resulted in LTP or LTD of horizontal connections (Hess and Donoghue 1994, 1996). The possibility of an involvement of horizontal connections to the ICMS-induced reorganization can not be judged on the basis of the present results. However, as the present study revealed that repetitive ICMS in layer IV of brain slices induced synaptic depression in cortical layer II/III, a simultaneous induction of strengthening of horizontal connections within layer II/III seems improbable. Thus, we assume that synaptic depression may be involved in ICMS-induced cortical reorganization.

Concerning the shaping of RFs, GABAergic interneurons are suggested to be of high functional relevance (Dykes et al. 1984). It is generally accepted that the RF size is determined by inhibitory surrounds and subthreshold contributions, i.e. the RF size seems to be determined by a dynamically maintained balance between excitatory and inhibitory inputs. In fact, in SI of the alert monkey, large RFs were associated with small inhibitory surrounds, while small excitatory RF areas were associated with large inhibitory RF surrounds (DiCarlo et al. 1998). Since excitatory FP components were depressed by ICMS in vitro, an attenuation of inhibitory action may provide a mechanism involved in the expansion of RFs observed in SI in vivo. This reduction of intracortical inhibition could be the result of a reduced drive to inhibitory interneurons or of the induction of LTD in inhibitory intracortical connections. The induction of LTD in inhibitory interneurons was demonstrated to occur in the developing visual cortex (Komatsu and Iwakiri 1993). In adult rat brain slices, LTD of inhibitory neocortical synapses is not unequivocally demonstrated, but is suspected to occur if metabotropic glutamate receptors are involved (Komatsu 1996). In an in vivo study, repetitive ICMS was demonstrated to result in both LTP and LTD of inhibitory neurotransmission in thalamocortical neuronal networks (Sil'kis 1996). Since inhibitory events are not reflected in FPs (Aroniadou and Keller 1993), results of the present study do not provide experimental evidence for an ICMS-induced alteration of inhibitory actions. However, the assumption that the cortical reorganization induced by repetitive ICMS may be associated with a depression of inhibitory mechanisms is supported by recent in vivo results. ICMS-induced effects on RF size depended on the initial control RF size (Dinse and Churs, submitted; Haupt et al. submitted): small RFs were almost exclusively enlarged, while large RFs tended to be reduced in size. Since large RFs are characterized by the relative weakness of the inhibitory surround (DiCarlo et al. 1998), the ICMS-induced reduction in size may reflect depression of excitatory synaptic events. The observed expansion of small RFs may be the result of an ICMS-induced attenuation of the preponderance of the inhibitory surround.

In conclusion, our in vitro results provide evidence that a repetitive ICMS pattern applied to layer IV of the rat SI induced a transient depression of synaptic responses of layers II/III neurons to activation of afferents from the ICMS site. Sustained reduction of synaptic efficacy could be induced by repetitive ICMS when intracortical inhibition was attenuated by bicuculline. These forms of synaptic depression resemble neocortical STD and LTD in many respects and are suggested to be involved in the reorganization of cortical maps observed after ICMS in vivo. Further functional evidence concerning the type of synaptic plasticity involved in ICMS-induced reorganization of cortical maps can be provided by reexamination in an in vivo study.

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