Long-term Potentiation Induces Expanded Movement Representations and Dendritic Hypertrophy in Layer V of Rat Sensorimotor Neocortex

While long-term potentiation (LTP) is currently the most widely investigated model of the synaptic mechanisms underlying learning, there is a paucity of reports on the direct effects of LTP on cortical organization. Here we show that strengthening polysynaptic potentiation correlates with an expanded neocortical area that responds to intracortical microstimulation-induced movements of rat forelimb and increased dendritic material in layer V pyramidal cells. Rats carried a stimulating electrode in the corpus callosum (midline), and a recording electrode in the right caudal forelimb area (CFA). Each rat received 15 days of either high frequency stimulation (HFS) or handling. Evoked potentials of the transcallosal pathway were recorded in the right hemisphere before and after 15 days of stimulation or handling. Following the last stimulation, movement representations were determined in the left CFA using high-resolution intracortical microstimulation (ICMS) and then the brains were processed for Golgi–Cox staining. Our results show that synaptic modification results in a recruitment of more neocortical area into movement representations and increases in several measures of dendritic morphology in layers III and V. This study sheds light on the interaction between artificial models of learning, receptive field characteristics and dendritic morphology in the sensorimotor cortex.

Keywords: caudal forelimb area, dendritic morphology, Golgi–Cox, long-term potentiation, motor cortex reorganization, neocortex

Introduction

Changes in the efficacy of synaptic transmission have attracted a great deal of attention because of their potential role in the development of neural circuitry as well as learning and memory (Chen and Tonegawa, 1997). Long-term potentiation (LTP), defined as a strengthening of synaptic efficacy, is currently the most widely investigated model of the synaptic mechanisms underlying learning (Bi and Poo, 1998). Within the cerebral cortex, learning is accompanied by changes in the topography of sensory and motor representations (Recanzone et al., 1993; Cruikshank and Weinberger, 1996; Kleim et al., 1998). This reorganization is in turn thought to be supported by an LTP-like mechanism (Hess and Donoghue, 1994).

Motor learning has been shown to induce motor map reorganization (Kleim et al., 1998), synaptogenesis (Kleim et al., 1996, 2002; Jones et al., 1999) as well as an increase in synaptic strength in vitro in layer II–III of the neocortex (Riout-Pedotti et al., 1998, 2000). High frequency stimulation (HFS) has been shown to reliably induce LTP in the rat somatomotor cortex both in vitro (Hess et al., 1996) and in vivo (Trepel and Racine, 1998), and low intensity electrical stimulation also alters the cortical representation of movements in close proximity to the stimulating electrode (Nudo et al., 1990; VandenBerg and Kleim, 2001). Recently, Teskey et al. (2002) found that a strengthening in polysynaptic efficacy following repeated epileptiform activity induced a proportional increase in movement representations in the same region. To date, however, there have been no reports on the direct effects of HFS on either, the functional organization of movement representations or layers III and V dendritic morphology in the CFA.

Because LTP can be reliably induced in the neocortex of awake, freely behaving rats (Racine et al., 1995; Trepel and Racine, 1998), we have an opportunity to investigate its effects on the organization of movement representations in the neocortex. We tested the hypothesis that strengthening polysynaptic efficacy would expand the neocortical area that responds to intracortical microstimulation-induced movements. Further, we examined the dendritic morphology of Fr1 pyramidal cells in layers III and V to evaluate in what respect these inputs contribute to changes in polysynaptic efficacy. Prior studies suggest that the polysynaptic component of the neocortical evoked potential displays the most robust change in response to theta-patterned HFS (Werk and Chapman, 2003), and that such modification is mediated by a sink in layer V (Chapman et al., 1998). Furthermore, intracortical microstimulation (ICMS) elicits movement via direct and transynaptic activation of layer V corticospinal neurons (Stoney et al., 1968; Jankowska et al., 1975). Yet the only study that examined the effects of neocortical LTP induction on dendritic morphology examined layer III pyramidal cells (Ivanco et al., 2000). In the present study, changes in synaptic efficacy were measured in the right hemisphere, following HFS, and movement representations were determined in the left hemisphere using ICMS. The brains were then processed for Golgi–Cox staining and both layers III and V pyramidal cells from the CFA were analyzed for dendritic morphology and spine density. The CFA was chosen for analysis because it has well-defined borders and the capacity for internal reorganization and expansion (Kleim et al., 1998; Teskey et al., 2002). Our study addresses the relation between synaptic efficacy and receptive field characteristics in the motor cortex.

Materials and Methods

Subjects

Twelve male Long–Evans rats, weighing between 350 and 425 g at the time of surgery, were used. The rats were obtained from the University of Calgary Breeding Colonies. All animals were housed individually in clear plastic cages in a colony room that was maintained on a 12 h on/12 h off light cycle. All experimentation was conducted during the light phase. Rats were maintained on Lab Diet no. 5001 (PMI feeds Inc., St Louis, MO) and water ad libitum, and were handled and cared for according to the Canadian Council for Animal Care guidelines.
Treatment Groups
The rats were divided into two groups, and received 15 sessions of either HFS (n = 8) or handling (n = 4). At least 24 h following the last stimulating or handling session, the rats had their movement representations determined using ICMS, and then were intracardially perfused and the brains were processed for Golgi–Cox staining.

Electrode Implantation
Twisted-wire bipolar stimulating and recording electrodes were constructed and implanted according to the methodology previously reported (Teskey et al., 1999). Animals were anesthetized with 58.85 mg/kg ketamine (85%) and xylazine (15%) at 0.5 ml/kg, injected intramuscularly. Lidocaine 2% was administered subcutaneously at the incision site. Two bipolar electrodes were chronically implanted according to the stereotaxic coordinates of Swanson (Swanson, 1992). The stimulating electrode was implanted 1.0 mm anterior to bregma, on the midline, in the callosal matter. The recording electrode was implanted +1.0 mm anterior to bregma and +4.0 mm lateral to midline in the right frontal neocortex. Electrophysiological monitoring was performed during the implantation in order to adjust the dorsal–ventral placements to maximize evoked response amplitude. Histological analysis revealed that the stimulating electrode was located in the corpus callosum and the recording electrode was located in the right CFA of motor cortex for each rat. The upper pole of the recording electrode was located in layer II/III, and the lower pole in deep layer V or upper layer VI.

The gold-plated amphenol pins connected to the electrodes were inserted into a nine-pin McIntyre connector plug, and attached to the skull with four stainless steel screws and dental cement. One of the stainless steel screws served as the ground reference. Experimental procedures commenced 7 days after surgery.

Evoked Potentials
Neocortical evoked potentials were recorded according to the methodology previously reported (Teskey et al., 2002). Briefly, baseline input–output (I–O) measures were conducted for two consecutive days prior to training/stimulation. The I–O measures were obtained by delivering pulses of increasing intensity to the callosal white matter and recording the resultant evoked potentials from the frontal neocortical electrode. The stimulation pulses consisted of biphasic rectangular waves, 200 µs in duration and separated by 200 µs. Ten stimulation pulses were delivered at each of 11 ascending logarithmic intensities (10, 32, 46, 68, 100, 147, 215, 316, 464, 681 and 1000 µA) at a frequency of 0.1 Hz. This type of recording was necessary to ensure that the evoked responses were stable, and to identify whether the stimulation threshold to induce a response was constant pre- and post-stimulation. Stimulation voltages were computer-generated and converted to amperage via a constant current and isolation unit (World Precision Instruments, Sarasota, FL). The recorded signals were filtered at half amplitude, below 1 Hz and above 100 Hz, and then amplified 1000 or 2000 times (Grass Neurodata Acquisition System Model 12). The analog signals were digitized at a sampling rate of five points per millisecond and the averaged evoked potential, at each intensity, was stored to a computer hard disk (Datawave, Denver, CO). All I–O measures were conducted while the animals were awake but immobile. This was necessary because ongoing behavior can dramatically affect the size and shape of the evoked potentials (Racine et al., 1975; Vanderwolf et al., 1987).

Stimulation
Theta-patterned HFS protocols previously found to reliably induce chronic LTP in the neocortex of awake behaving rats were used in the present study (Teskey and Valentine, 1998; Werk and Chapman, 2003). The HFS consisted of eight biphasic square wave pulses, each pulse 200 µs in duration, with a pulse intensity of 1000 µA base to peak and frequency of 100 Hz. The eight pulses were delivered as two paired trains of four pulses that were separated by 150 ms. Thirty trains per session were delivered, once daily, at 0.1 Hz. Follow-up evoked potentials were obtained 24 h after the 15th HFS or handling session. Electrographic recordings and the animal’s behavior were monitored during HFS and for 1 min thereafter. There was no evidence of epileptiform activity during or shortly after HFS. During HFS, the animals did not display any change in behavior and remained quiet and immobile.

Evoked Potential Analysis
Evoked potentials obtained pre- and post-stimulation to the callosum (or handling) were measured for change in the polysynaptic (late) component. The polysynaptic component was chosen for analysis because it displays the largest and most reliable change and reflects polysynaptic activity with a source in layer V (Chapman et al., 1998; Trepel and Racine, 1998). The polysynaptic component was defined as the surface negative component occurring between 12 and 40 ms post stimulation (see Fig. 1). The area score was calculated by summing all the difference values (sampled every 200 µs) from the two evoked potentials between 12 and 40 ms after the stimulation artifact, and then dividing by the amount of amplification (Teskey and Valentine, 1998). Only potentials evoked at 681 µA were statistically analyzed.

Intracortical Microstimulation
One day following the last stimulation or handling session, ICMS techniques were used to generate detailed maps of the motor cortex forelimb regions (Kleim et al., 1998) by experimenters that were unaware of the rats’ treatment condition. Rats were anesthetized with keta-
mine hydrochloride (70 mg/kg, injected i.p.), and xylazine (5 mg/kg, i.p.), and with ketamine (20 mg/kg, i.p.) as supplementary injections when needed. A craniotomy was performed over the left motor cortex, contralateral to the electrode assembly, and the dura was carefully removed. The exposed cortex was then covered with warm (37 °C) silicone oil. A small puncture was made in the cisterna magna to drain the cerebrospinal fluid. A glass microelectrode controlled by a hydraulic microdrive was used to make penetrations to a depth of ∼1550 µm (corresponding to cortical layer V), with an interpenetration distance of 375 µm. Stimulation consisted of 13 200 µs cathodal pulses delivered at 350 Hz from an electrically isolated stimulation circuit. Rats were maintained in a prone position, with the limb contralateral to the side being supported by placing one finger below the elbow joint and elevating the forelimb. This allowed for visual detection, by the same experimenter, of all possible forelimb movements. The end of a row was characterized by any non-forelimb movement that included head and neck, vibrissa or non-responsive sites. At each penetration site the minimal threshold required to elicit a movement was recorded. Sites where no movement was detected at ≤0.5 mA were recorded as unresponsive. The level of anesthesia was assessed by monitoring the breathing rate and by revisiting positive-response sites to check for changes in movement thresholds as mapping progressed. Each mapping session lasted ∼2.5 h.

Movement Representation Analysis

An image analysis program (Canvas v. 8) was used to calculate the aerial extent of the caudal forelimb area (CFA). The CFA was separated from the rostral forelimb area (RFA) by a band of neck/whisker representations (Kleim et al., 1998) and was chosen for analysis because previous work demonstrated its capacity to undergo reorganization following behavioral manipulations (Kleim et al., 1998; Remple et al., 2001) or stimulation (Nudo et al., 1990; Teskey et al., 2002). The RFA has proven, thus far, to be resistant to modifications (Kleim et al., 2002). The proportion of distal and proximal movements that occupied the CFA was then calculated. The mean stimulation threshold for each movement category and the mean number of dually responsive sites were also measured.

Perfusion and Histology

Following mapping, rats were intracardially perfused with 0.9% saline. Their brains were removed and immersed whole in 40 ml of Golgi–Cox solution (Glaser and van der Loos, 1981). The solution was changed after 2 days and the brains remained immersed for an additional 14 days before being placed in a 30% sucrose solution for 2 days, cut on a vibratome at 200 µm, and developed using a procedure outlined by Gibb and Kolb (1998).

Analysis of Dendritic Branching, Length and Spine Density

Layer III pyramidal cells in the frontal neocortex (Frl1), at the electrode tip site in the ipsilateral and in the un-implanted contralateral hemispheres, were traced using a camera lucida at 250x. While there were no apparent differences in the peripheral portions of the field, tissue from both control and stimulated animals were treated in exactly the same manner, under blind conditions, which should have eliminated any systematic bias. Dendritic trees had to maintain the following criteria to be included in the data analysis: (i) the cells had to be well impregnated and in full view, unblocked by blood vessels, astrocytes, or clustering of dendrites from other cells; (ii) the apical and basilar arborizations had to appear intact and visible in the plane of section. Cells were chosen by first locating area Fr1 (Zilles, 1985) and then drawing each cell in the section that maintained the above criteria. Following drawing of the cells, each branch segment was counted and summarized by branch order according to the methods of Coleman and Riesen (1968). As such, basilar dendrites were determined to be first order if the branch originated from the cell body increasing in order with every bifurcation. The branches were determined for the apical dendrites such that branches originating from the primary apical dendrite were first order. Five cells were drawn from each hemisphere for each rat. The mean of the measurements on 10 cells per rat was used for statistical analyses. Apical and basilar branches were separated for the statistical analysis, since basilar branches are believed to receive input from proximal neurons whereas apical dendrites may receive input from more distal neurons (Feldman et al., 1998).

A Sholl analysis (Sholl, 1956) of ring intersections was used to estimate dendritic length. The number of intersections of dendrites with a series of concentric spheres at 20 µm intervals from the center of the cell body was counted for each cell. Total dendritic length (in µm) was estimated by multiplying the number of intersections by 20x. Spine density was measured from one apical dendritic branch in the terminal tuft, and one from the secondary branch proximal to the cell body for one basilar branch, following the procedure of Woolley et al. (1990). Spine density measures were made from a segment between 10 and 50 µm in length. The dendrite was traced (1000x) using a camera lucida drawing tube and the exact length of the dendritic segment was calculated by placing a thread along the drawing and then measuring the thread length. Spine density was expressed as the number of spines per 10 µm. No attempt was made to correct for spines hidden beneath or above the dendritic segment, therefore the spine density values are likely to underestimate the actual density of the dendritic spines.

Statistical Analyses

Independent samples t-tests were conducted between the HFS and control groups to evaluate whether they were different for their map size and the late component of their evoked potential. t-tests were also conducted between the groups to evaluate whether their thresholds to elicit an evoked potential, their threshold to elicit a movement, or the amount of ketamine administered were consistent. Planned comparisons (t-tests) were also performed, comparing the HFS animals to the control animals for the basilar and apical dendritic measures (branch complexity, dendritic length, and spine density).

Since we had a priori hypothesized that HFS would induce an increase on all dendritic measures, one-tailed t-tests were conducted. Correlations were performed between the polysynaptic component and the CFA measures, because previous findings suggest that a change in polysynaptic efficacy might account for a significant amount of variance in CFA size (Teskey et al., 2002). All statistical analyses were performed using StatView.

Results

Effects of HFS on the Evoked Responses

Representative responses evoked in the right motor cortex by stimulation of the corpus callosum following handling or HFS are shown in Figure 1B and C, respectively. Examination of the field potentials elicited with the ascending series of stimulation intensities showed that the mean (±SEM) minimum amount of current (µA) to obtain the evoked potentials in the handling (51.5 ± 5.5) and HFS (54.8 ± 8.5) groups did not change. Furthermore, proportionately the largest changes in the late component area were elicited by 681 µA of current. For this reason the potentials evoked by 681 µA are the only ones reported here.

HFS resulted in LTP that was characterized by an increase in the size of the polysynaptic component of the evoked potential generated in layer V (Chapman et al., 1998; Trepel and Racine, 1998; Werk and Chapman, 2003). A t-test conducted between the two groups revealed a significant [(8) = −6.607, P < 0.0001] effect of HFS on the size of the evoked potential late component (see Fig. 1C).

Effects of LTP Induction on Motor Representations

The mean (±SEM) amounts of ketamine, as a function of body weight and duration of surgery (expressed in ml/kg per hour), administered to the control (0.48 ± 0.04) and LTP (0.52 ± 0.05) rats were not significantly different (P = 0.79). Furthermore, the mean (±SEM) current intensities (thresholds) in µA required
to elicit forelimb motor responses were not significantly different across the control (29.74 ± 2.53) and LTP (27.88 ± 1.56) groups (P = 0.2451).

The mean cortical area that elicited digit, wrist, elbow, and shoulder movements in the forelimbs was analyzed in the LTP and control groups. We observed a significant effect of HFS on the size of the total CFA between the two groups. An independent samples t-test between the control and LTP animals revealed that HFS induced an increase in the mean size of the total CFA [(8) = –2.72, P < 0.05] (Fig. 2A). When comparing the subcomponents of the CFA, no significant differences were found between the mean proportion of proximal (shoulder and elbow) and distal (wrist and digit) movement representations between the LTP and control groups (Fig. 2B). This suggests that LTP did not result in a reorganization of the relative proportions of the CFA devoted to proximal and distal representations.

The total mean neocortical area of forelimb movement representations in potentiated rats was ~36% larger than control. This expansion of the forelimb movement area occurred in the medial, lateral, anterior and posterior directions relative to the control area, indicating that the expansion of the movement representations was not attributable simply to an invasion of the RFA (Fig. 3).

**Effects of HFS on Layer III Dendritic Length, Branch Complexity, and Spine Density**

There were no differences in the mean (± SEM) dendritic length between the HFS animals and the control animals for either the layer III apical [1327 (±113) vs 1399 (±135)] or basilar dendrites [1386 (±92) vs 1377 (±112)], t(16) = 0.65, P = 0.26 and t(16) = 0.915, P = 0.18, respectively. There were no differences between the HFS animals and controls [t(16) = 1.19, P = 0.25; t(16) = 0.324, P = 0.7] on apical or basilar branch complexity in layer III. The mean (± SEM) number of apical and basilar branches for the control and HFS animals were 24 (±1.57) and 39 (±2.4), and 26 (±1.49) and 40.9 (±2.43), respectively. A significant difference in spine density was found between the HFS and sham-implanted controls for both the layer III apical and basilar dendrites, t(16) = 4.69, P < 0.0001 and t(16) = 7.09, P < 0.0001, respectively. The apical and basilar spine density for the HFS animals [11.32 (±0.25) and 10.99 (±0.34)] was, on average, greater than for the control animals [8.94 (±0.2) and 8.88 (±0.25)] (see Fig. 4A–D).

**Effects of HFS on Layer V Dendritic Length, Branch Complexity, and Spine Density**

There was a significant difference in the mean (± SEM) dendritic length between the HFS animals and the control animals for the layer V basilar dendrites [2278.5 (±96.77) vs 2641.5 (±91.66)], t(16) = –2.72, P = 0.008 (see Fig. 5A). As is shown in Figure 5B, there also was a significant difference between the HFS animals and controls [(16) = 1.73, P = 0.05] on basilar branch complexity in layer V. The mean (± SEM) number of basilar branching for the control and HFS animals were 46.88 (±1.71) and 51.78 (±1.74). A significant difference in spine density was found between the HFS and sham-implanted controls for the layer V basilar dendrites, t(16) =

![Diagram](http://example.com/diagram.png)

**Figure 3.** Threshold forelimb motor representations. Shade-coded topography of movement representations of the rat CFA for the (A) threshold map of an implanted control (k12), and (B) threshold map of an animal that received HFS (k17). The stimulating microelectrode was repeatedly lowered 1550 µm into the left motor cortex, and stimulation was applied (up to 60 µA) until movement was elicited. External map boundaries were always defined as electrode penetrations that failed to elicit movement, or non-CFA movements. A–P refers to the direction of the caudal–posterior axis. M–L refers to the direction of the medial–lateral axis and also defines the coronal plane in which the stimulating electrode was situated (+1.0 anterior to bregma).
3.259, P < 0.01. The layer V basilar spine density for the HFS animals [10.06 (±0.52)] was greater than for the control animals [8.01 (±0.38)] (see Fig. 5C). Representative examples of the effects of handling (Fig. 6A,C) and HFS (Fig. 6B,D) on dendritic length, branching complexity and spine density are shown.

**Relationship Between Polysynaptic Efficacy and CFA Size**

A linear regression revealed a significant (P = 0.0378) relationship between the evoked potential polysynaptic component and the size of the CFA that is described by the equation 

\[ Y = 11.67X - 34.57 \]  

(Fig. 7). An r value of 0.523 indicated a moderately high positive correlation between the polysynaptic component and the size of the CFA. Thus, knowledge of the polysynaptic component accounted for 27.3% of the variance in forelimb motor map area.

**Discussion**

We examined the plasticity of the CFA in response to HFS by measuring polysynaptic efficacy, neocortical motor representations, and dendritic morphology of layers III and V pyramidal cells. To our knowledge, this represents the first report of the effects of neocortical LTP induction on the cortical distribution of movement representations, as well as the first study to examine the effects of LTP induction on Layer V Fr1 dendritic morphology. We found that repeated sessions of HFS to the corpus callosum not only induced neocortical LTP, which confirmed previous reports (Racine et al., 1995; Chapman et al., 1998; Trepel and Racine, 1998; Werk and Chapman, 2003), but also led to an increase in the area of neocortex that elicits forelimb movement representations, and an increase in branch complexity, dendritic length and spine density in layer V, as well as spine density in layer III.
The central observation of this study is that under identical stimulation conditions, the area of neocortex from which forelimb movements could be elicited was related to the extent of LTP induction measured in neocortical layer V. This is not to say that the expanded areas necessarily represent 'new' areas of forelimb motor cortex. Rather, these areas have undergone some functional change due to HFS. This functional change facilitates the ability to elicit forelimb movements from regions of cortex that would not previously produce movements in response to similar stimulation. It is important to point out that the area of neocortex defined as the CFA is highly dependent upon the methods used during mapping.

Stimulation parameters such as current intensity, pulse train, stimulation frequency, as well as the anesthetic level can all affect the motor cortex maps (Donoghue and Wise, 1982; Graziano et al., 2002). Although the baseline maps reported in any experiment are dependent upon the techniques being used, the topography of movement representations for control animals in the present study are consistent with those of previous reports that used similar stimulation parameters (Gioanni and Lamarche, 1985; Castro-Alamancos and Borrell, 1993; Kleim et al., 1998; Teskey et al., 2002). Furthermore, in the present study the map expansion was not dependent on anesthetic levels, stimulation sensitivity (thresholds), or a simple invasion into the rostral forelimb region.

Because the thresholds for obtaining an evoked response and ICMS-induced movements were equivalent in the LTP and control groups, the changes in synaptic efficacy and map size are not the result of a nonspecific sensitization of the cortical fibers (de Jonge and Racine, 1987; Kleim et al., 1998). A previous study, in which a current-source density analysis of the CFA region was performed, demonstrated that the late component of the evoked potential has a sink in upper layer V and a source in deep layer V (Chapman et al., 1998). This component was also found to be polysynaptic in nature, because it failed to be expressed when stimulations were administered at high frequencies (Chapman et al., 1998). ICMS-induced movements are most easily elicited when the stimulation electrode is positioned in layer V with the applied current likely exciting horizontal fibers rather than via direct excitation of a few corticospinal neurons (Jankowska et al., 1975; Lemon et al., 1987). Thus, it seems likely that potentiation of synaptic efficacy within layer V mediates the expansion in the motor map.

Prior studies have shown that changes could be induced within the CFA following skilled motor training (Kleim et al., 1998) and repetitive ICMS (Nudo et al., 1990). Skilled motor training was found to induce an increase in wrist at the expense of elbow representations, but the overall size of the CFA was not altered. Repetitive ICMS was found to induce a short-term expansion within CFA, but subsequent retraction of the same boundaries occurred within tens of minutes (Nudo et al., 1990). Our results reported here, however, suggest that HFS yielded two important differences from skilled training...

Figure 6. Individual examples of basilar arborizations and spine density of layer V pyramidal cells from area Fr1 following handling (A and C) or HFS (B and D). As shown in B, HFS induced an increase in dendritic length and branch complexity when compared with controls (A). HFS also induced an increase in spine density (D) when compared with controls (C).

Figure 7. Scatterplot of the polysynaptic component of the evoked potential with forelimb motor map size. Graphical representation of the relationship between neocortical polysynaptic component and CFA for all rats. Diamonds represent values from the rats that received callosal HFS, and circles represent the values from the controls. A strong positive correlation was observed between the polysynaptic component of the evoked potential and the CFA size. The regression line described by the equation $Y = 11.87X - 34.57$ is shown.
and repetitive ICMS: expansion of the boundaries of the CFA and maintenance of the ratio of proximal and distal representations. Furthermore, changes in evoked potentials, similar to those found in the present study, have previously been found to last for weeks following chronic LTP-induction in the neocortex (Racine et al., 1995), suggesting that we may have induced a much more persistent alteration in movement representations.

ICMS-derived maps are believed to depend upon the synaptic activity of horizontal afferents (Jankowska et al., 1975). In the present experiment the stimulated afferents were callosal but the potentiation effects probably occurred in multiple intracortical fiber systems (Werk and Chapman, 2003). This may explain why the synaptic potentiation led to an expansion rather than a reorganization of movement representations. Further, motor skill training may act to strengthen a specific subset of synapses in contrast to the relatively widespread potentiation of a large set of afferents such as was done here.

Changes in GABAergic and glutamatergic transmission may underlie both LTP and alterations in movement representations. The callosal-neocortical evoked potential is primarily a glutamate-mediated EPSP to GABA-mediated IPSP sequence (Metherate and Ashe, 1994; Trepel and Racine, 1998). With respect to glutamate, induction of neocortical LTP in vitro can be blocked by the presence of an NMDA-antagonist (Hess et al., 1996). In the chronic freely behaving preparation, NMDA blockade was found to prevent the induction of LTP (Trepel and Racine, 1998). Application of NMDA antagonists has also been shown to block motor cortex map plasticity (Qiu et al., 1996), although a reduction of NMDA mediated inhibition following neocortical LTP has yet to be demonstrated. Jacobs and Donoghue (1991) demonstrated that by decreasing intracortical inhibition through application of GABA antagonists, cortical representations can be reshaped. A potential neurochemical mechanism for the CFA expansion is that adjacent cortical regions expand when pre-existing lateral excitatory connections are unmasked by a decrease in intra-cortical inhibition (Jacobs and Donoghue, 1991). Thus, both suppression of GABA and enhancement of glutamatergic-mediated currents may underlie the synaptic potentiation, as well as the changes in movement representation.

The increase in dendritic material in layer V suggests a greater number of excitatory synapses (Shepherd, 1990) in the potenti- ated animals. An increase in spine density has previously been found to occur in the hippocampus following LTP induction (Trommald et al., 1996; for a review, see Muller et al., 2000; Gazzaley et al., 2002). In the present study, whereas HFS induced a significant increase in spine density in layer III pyramidal cells, we did not find an effect on branching complexity or dendritic length. These results differ slightly from those of Ivanco et al. (2000) who reported that dendritic length was also significantly increased following LTP induction. Different stimulation parameters used in each study may account for the discrepancy. In contrast to the layer III results, dendritic length, branching and spine density increased in layer V pyramidal cells. The anatomical results reported here provide a physical instantiation for the increase in polysynaptic efficacy. Effectively, increased layer V dendritic material is consistent with increased polysynaptic efficacy at layer V pyramidal neurons (Chapman et al., 1998).

Collectively, the present study sheds light on mechanisms of synaptic efficacy that may underlie the functional reorganization of the neocortex. Given the pliable nature of movement representations reported in this study, future work should assess the effects of LTP on the animals’ behavior in a task that specifically depends on the CFA. Lastly, given that LTP is thought to model the synaptic mechanisms underlying learning and memory, the present results also raise the possibility that LTP provides a common mechanism of learning, memory, and motor map organization operating within the sensorimotor neocortex.

**Notes**

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