Strengthening of horizontal cortical connections following skill learning

Mengia-S. Rioult-Pedotti[1], Daniel Friedman[1], Grzegorz Hess[1,2] and John P. Donoghue[1]

[1] *Department of Neuroscience, Brown University, Providence, Rhode Island 02912, USA*

[2] *Permanent address: Jagiellonian University, Institute of Zoology, 31-102 Krakow, Poland*

Correspondence should be addressed to M.-S. R.-P. (mengia_rioult@brown.edu)

Learning a new motor skill requires an alteration in the spatiotemporal pattern of muscle activation. Motor areas of cerebral neocortex are thought to be involved in this type of learning, possibly by functional reorganization of cortical connections. Here we show that skill learning is accompanied by changes in the strength of connections within adult rat primary motor cortex (M1). Rats were trained for three or five days in a skilled reaching task with one forelimb, after which slices of motor cortex were examined to determine the effect of training on the strength of horizontal intracortical connections in layer II/III. The amplitude of field potentials in the forelimb region contralateral to the trained limb was significantly increased relative to the opposite 'untrained' hemisphere. No differences were seen in the hindlimb region. Moreover, the amount of long-term potentiation (LTP) that could be induced in trained M1 was less than in controls, suggesting that the effect of training was at least partly due to LTP-like mechanisms. These data represent the first direct evidence that plasticity of intracortical connections is associated with learning a new motor skill.

The primary motor area (M1), a cortical region necessary for skilled voluntary movements, seems also to participate in learning motor skills. This conclusion is based largely upon findings that adult M1 representations are modifiable[1–4], that dendritic morphology of M1 pyramidal neurons is altered by experience[5] and that connections among M1 neurons are capable of activity-dependent, long-term changes in efficacy[6–11]. Despite these suggestive findings, there is no direct evidence that learning is accompanied by functional modifications of M1 circuits. Modifications outside of the cortex, which have been repeatedly documented, might account for cortical motor or sensory map changes that can be produced by experience or nerve lesions[12,13]. Further, there is no evidence that morphological changes, such as an increase in the number of dendritic branches, actually alter functional interactions in the cortex. Finally, there has been no definitive evidence that LTP-like mechanisms are engaged within cortex during any form of learning. Demonstration of synaptic modification in conjunction with learning is an essential step towards understanding how cortical circuits support motor skills or other forms of learning.

The intrinsic horizontal pathways are a potential substrate for experience-dependent reorganization of relationships among M1 neurons. Layer II/III pyramidal cells form a broad, intrinsic horizontal projection system in M1, and their intracortical pattern correlates with sites that reorganize after nerve lesions[14,15]. Pharmacological adjustments of the excitatory-inhibitory balance within M1 restructures motor representations, apparently by uncovering latent horizontal pathways[16]. In addition, horizontal connections are capable of LTP, providing a potential activity-dependent mechanism for synaptic modification[3,7,9,10,17]. These findings raise the possibility that changes in horizontal connection strength may accompany motor learning. Here we show that field potentials evoked by stimulation of rat M1 horizontal connections increase after learning and practicing a skilled reaching task. The amount of LTP that could be induced by electrical stimulation was also reduced after learning, implying that the observed strengthening of horizontal connections may involve an LTP-like mechanism. Plasticity of M1 connections may therefore create cortical circuits needed to acquire or perform new motor behaviors.

Results

Rats were trained to reach through a hole in a food box with a single forepaw in order to retrieve small food pellets using a grasping motion. Training and subsequent practice lasted three (n = 1) or five (n = 13) successive days with one training session (approximately one hour) per day. Successful performance of this skill occurred in the first one or two sessions, and the remaining sessions consisted of repeated practice and refinement of the skill. By the final two days of training, all rats achieved a performance of about 1.5 pellet retrievals per minute, with few errors in the reach, grasp or retrieval actions. A group of comparably handled, age- and sex-matched cage mates (termed 'paired controls', n = 14) and another group of naive rats ('unpaired controls', n = 12) served as controls.

The strength of intrinsic horizontal synaptic connections within layers II/III was evaluated *ex vivo* using coronal brain slices containing both hemispheres (Fig. 1a); experimenters were blind to whether the animal was trained, and if so, which forelimb was trained. Slices were taken 20–45 hours after the last training session to rule out effects that might persist immediately after practice of the task. Field-potential responses evoked in the horizontal pathway by electrical stimuli were examined simultaneously through stimulating and recording electrodes that were mirror symmetrically positioned in layer II/III of the left and right M1 (Fig. 1a). Thus, one side in each trained rat

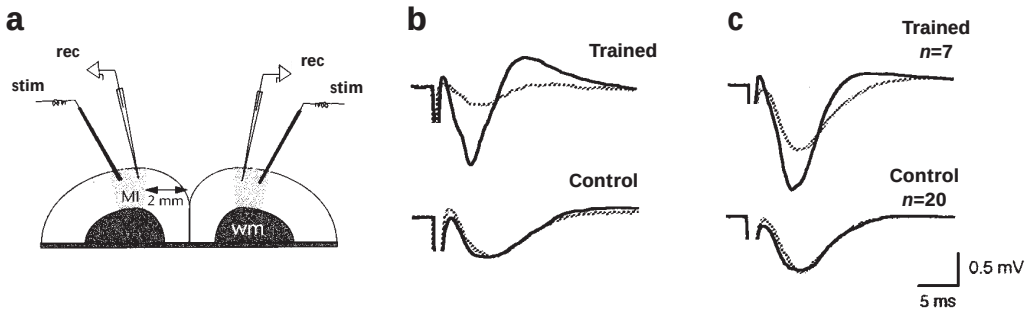

Fig. 1. Consequences of motor skill learning on field-potential responses evoked in layer II/III horizontal connections of M1. **(a)** Mirror-symmetric placement of stimulating (stim) and recording (rec) microelectrodes bilaterally in layers II/III of M1 in a coronal slice containing both hemispheres. wm, white matter. **(b)** Single-case examples of field potentials (averages of five sweeps), evoked at 60% maximum stimulation intensity from a single trained (top) and a single paired-control (bottom) animal. Dark lines represent the trained M1 or left M1, hatched lines, the untrained M1 or right M1. **(c)** Group average responses for trained (top, $n = 7$) and control (bottom, $n = 20$, paired and naive) rats at 60% maximal stimulation intensity, illustrating enhanced field potential in the horizontal pathway of M1 contralateral to the limb used in the reaching task. Same format as (b).

provided a within-animal control because the majority of engaged M1 neurons are located in the hemisphere contralateral to the limb they influence[18]. For trained animals, we term M1 contralateral to the trained limb the 'trained M1', and its counterpart on the other side the 'untrained M1'. In all control animals, the terms 'left M1' and 'right M1' are used.

Stimulation evoked an initially negative-going field potential of similar shape in all rats as previously described[7]. However, for each rat that had learned the skilled-reaching task, field potentials evoked in the trained M1 were consistently larger in amplitude than in the untrained M1 (Fig. 1b and c). Amplitudes in the trained M1 were also larger than those observed for the control animals. Amplitude differences between trained and untrained M1 were not a result of stimulus intensity because absolute current intensities used on the two sides were not significantly different ($p = 0.23$). Indeed, in 71% of the cases, the stimulation intensity was slightly larger ($27.24 \pm 3.38\%$, $n = 10$) on the untrained side.

The amplitude differences between trained M1 and untrained M1 were specific to the region of the M1 forelimb representation. Layer II/III field-potential measurements from the hindlimb region of the trained M1 and untrained M1 in an additional group of trained rats showed no significant side-to-side amplitude differences at any stimulation intensity ($p = 0.2$–0.8, $n = 9$), whereas slices taken from the forelimb region of the same animals showed a larger response in the trained than untrained M1. Field potentials in the hindlimb and forelimb areas were similar in shape. Peak amplitudes for the hindlimb at 2.5 and 5 times threshold intensity were 0.92 ± 0.12 mV and 1.47 ± 0.19 mV in the trained M1 and 0.97 ± 0.13 mV and 1.45 ± 0.19 mV in the untrained M1.

The relationship between stimulus intensity and response amplitude was evaluated systematically using two different approaches (Fig. 2a–c) to rule out the possibility that these effects were a consequence of the particular intensities used. One series of 7 trained and 20 control (8 paired, 12 unpaired) rats was tested with stimuli that were a constant fraction of the stimulus intensity evoking a maximum response (absolute intensity less than or equal to 220 μA). A second series of seven trained and six paired control rats was tested with stimuli that were a constant multiple of the stimulus intensity evoking a minimal response of about 0.1 mV (absolute threshold intensity 12–30 μA). In both series, the average responses for the trained M1 were larger than the untrained M1 at every stimulation intensity, whereas there were no differences between left and right sides in the control groups (Fig. 2a and b). To compare the relative change between trained and untrained M1, the common logarithm of the response-amplitude ratio between sides was calculated for each animal at each stimulation intensity. The average log ratio obtained for trained animals was significantly different from zero ($p < 0.05$) over a broad range of stimulus intensities, reflecting larger peak amplitudes in the trained M1. This ratio was not different in control animals ($p > 0.05$; Fig. 2c). Figure 2d plots the distribution of the entire data set; the trained animals show a marked skew toward larger responses on the trained side, whereas untrained controls show no difference between the two hemispheres.

Amplitude differences observed in the trained M1 might arise by several mechanisms. Larger responses in the horizontal pathways could result from newly formed synapses, from postsynaptic excitability increase or from engaging an LTP-like mechanism that increased the strength of existing synapses. A larger initial slope for field potentials evoked at 60% maximal stimulation intensity in the trained M1 (trained M1, 0.735 ± 0.079 mV per ms; untrained M1, 0.439 ± 0.079 mV per ms; $p < 0.025$) suggested that modifications occurred by a change in synaptic efficacy, rather than an excitability change. Because layer II/III horizontal connections are capable of LTP, we compared the ability to potentiate this pathway in trained M1 and untrained M1. We postulated that if learning recently engaged an LTP-like process in the trained pathway, further electrically induced potentiation of this pathway might be occluded. LTP induction was attempted by theta-burst stimulation simultaneously delivered during a transient reduction of $GABA_A$-receptor-dependent synaptic inhibition by focal application of small amounts of bicuculline at each of the two recording sites[6,7], an established method to obtain LTP reliably in neocortex. In accord with previous results[7], LTP was readily induced in the untrained M1 ($24.1 \pm 11.7\%$ increase, $n = 6$) and in both hemispheres of control rats (left M1, $39.7 \pm 10.5\%$; right M1, $36.1 \pm 12.8\%$; $p = 0.8$, $n = 7$). The difference between untrained M1 and left and right M1 of controls was not significant ($p = 0.35$). In contrast, the average increase in field potential amplitude in the trained M1 was only $6.5 \pm 4.2\%$ ($n = 6$; Fig. 2e), which was significantly less than the untrained M1 ($p<0.035$, $n = 6$). Thus, although identical stimulation and recording procedures were applied to both hemispheres, the amount of LTP that could be induced in the trained hemisphere was reduced.

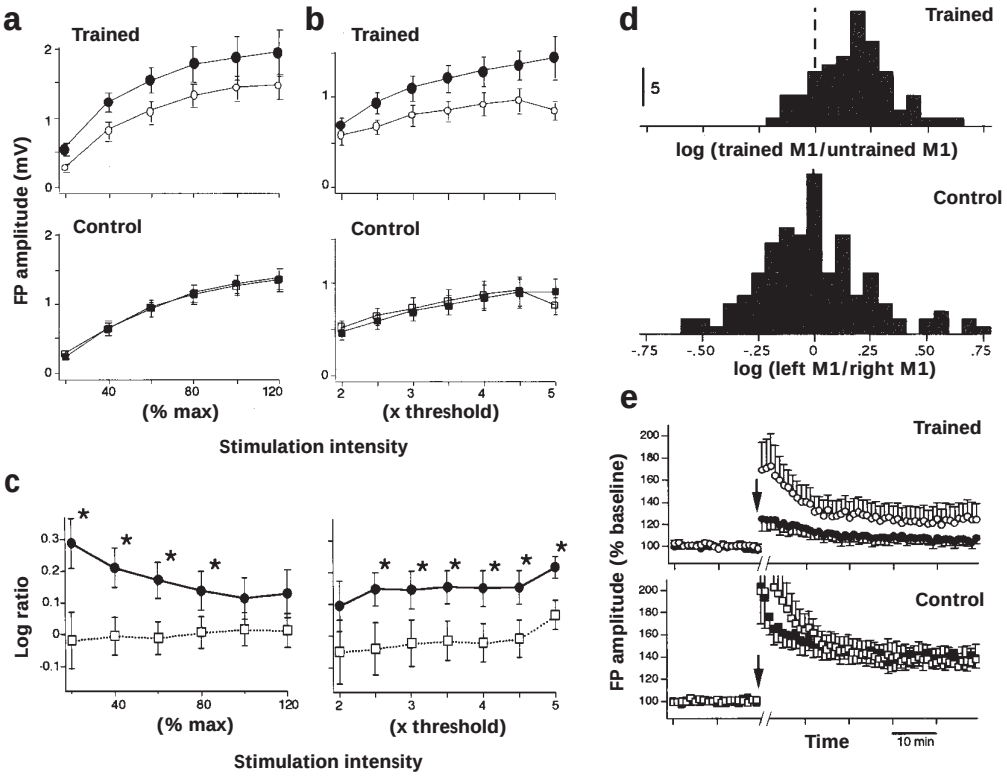

Fig. 2. Response differences in trained and control rats. **(a)** Field-potential amplitudes for trained (top, $n = 7$) and paired- and naive-control (bottom, $n = 20$) animals. Stimulation intensities are plotted as a percentage of the stimulus evoking the maximal response. Filled symbols represent the trained M1 or left M1, open symbols the untrained M1 or right M1. Note the larger response magnitude in the trained M1 across intensities. **(b)** Field-potential amplitudes for trained (top, $n = 7$) and paired-control (bottom, $n = 6$) animals. Stimulation intensities were varied as multiples of threshold intensity inducing a minimal response (about 0.1 mV). Symbols as in (a). **(c)** Comparison of average (± standard error) log-response ratios for trained/untrained M1 (filled symbols) and left/right M1 (open symbols) for series in (a) (left) or series in (b) (right). Asterisks indicate statistical differences between control and experimental ratios. **(d)** Log ratio of field-potential amplitudes for trained (top) and control (bottom) groups. The histograms show the distributions of all log ratios for all rats at all stimulation intensities (entire data set). The mean value for trained animals (0.17) is significantly different from zero ($p<0.001$), demonstrating a shift towards the trained M1. **(e)** Effect of training on electrically induced LTP. Each point indicates the relative field-potential amplitude before and after LTP induction in both hemispheres (arrow) in trained (top, $n = 6$) and paired control (bottom, $n = 7$) animals. Averages after LTP induction are compiled from the last 50 min of recordings because of a variable duration of the transient bicuculline effect (interruption in the x-axis). Open symbols (+ standard error) for right M1 or untrained M1, filled symbols (- standard error) for left M1 or trained M1.

Discussion

These results demonstrate that learning and practicing a motor skill is accompanied by an increased efficacy of horizontal connections in motor cortex. Although there is extensive data showing that motor skill learning modifies cortical representations[1,2,19,20] and alters dendritic morphology[5] and that cortical connections are capable of activity-dependent strength changes, our results provide the first direct evidence for a functional change of a cortical connection associated with motor skill learning. Our results compare with changes recently observed in the amygdala following fear conditioning, a markedly different form of learning[21,22] and might provide a basis for correlation-strength changes that have been observed during auditory conditioning[23]. Plasticity of horizontal connections could contribute to the reorganization of motor cortical representations that accompanies motor skill learning, because information from one region of M1 would be spread more effectively to other regions. This hypothesis is consistent with recent findings demonstrating that only the parts of M1 receiving strong horizontal inputs reorganize immediately after nerve lesions in the rat[14] and that M1 representations in monkeys and humans enlarge or rearrange during motor skill learning[4,24–26]. Our data cannot differentiate between changes that result from the early modifications in behavior that occur when the task is first achieved and the slower improvements in skill that occur with subsequent practice. Both can be considered as forms of learning. Increased efficacy of horizontal connections would not seem to be a consequence of movement alone. Although the number of movements might be different for the practiced limb, the actual number is small when considered as a fraction of the total number of movements made over the one to two days after training ended. The movements made were at a low rate (about 1.6 per minute), required little force and were mixed with many overlearned bilateral movements in consummatory actions, yet enhanced horizontal-connection strength persisted in M1 in the region of the forelimb. Changes in horizontal connections are also not widespread in M1 because learning did not modify the layer II/III horizontal pathway in the hindlimb area.

The marked effect of learning upon field-potential amplitude within the M1 forelimb region suggests that a large number of connections within this area have been modified in conjunction with skill learning. It is difficult to conceptualize how such a generalized effect can provide a substrate for implementing the detailed pattern of movements acquired. Studies of human motor-skill learning using transcranial magnetic stimulation suggest that motor-cortical maps shrink again after explicit knowl-

edge of the task is gained[25] . Thus, the seemingly generalized changes we observe after five days of training may eventually lead to more specific circuits suitable for producing the skill. By contrast, fMRI studies during human motor-skill learning indicate that representations continue to enlarge with repeated practice[24,26]. We will need to examine additional time points to determine what happens to field-potential increases and decrements in LTP before we can make more definitive statements of the role of these modification in acquiring new motor skills.

Modifications in horizontal connections seem to result from changes in synaptic efficacy, perhaps through LTP-like mechanisms, rather than other means. Learning seems to occlude LTP induction, suggesting they share a similar mechanism. The increase in the initial slope of field potentials from the trained M1 is consistent with the hypothesis that learning occurred through synaptic modification. Although compelling evidence is lacking, LTP is a strong candidate mechanism for many forms of learning because it can lead to long-lasting modification of activated synapses[27] at a number of sites, including horizontal cortical connections[7,17]. Other mechanisms are potentially plausible but are not fully consistent with our results. For instance, a different form of learning, involving classical conditioning, leads to increases in the membrane excitability of M1 neurons[28], rather than synaptic modification. If increases in horizontal field potentials were due to excitability changes in postsynaptic neurons, no initial slope changes would be expected and tetanization would likely produce greater postsynaptic depolarization and hence, a larger amount of LTP[29]. Growth of new synaptic connections, which has been suggested to occur in adult cortex[30,31], could also lead to larger responses after learning. However, these new synapses would have to be unable to undergo LTP to be consistent with the finding of less LTP in the trained M1. We therefore think it unlikely that these mechanisms underlie the increased field-potential amplitudes seen after skill learning, although additional studies are required to identify the exact mechanism involved.

We only examined the horizontal pathway after three or five days of practice following skill acquisition. Although synaptic modification seems to occur during this period, other mechanisms may operate during initial skill acquisition or during later skill improvement. Intracellular studies and measurements of the time course of change may help to clarify what leads to such dramatic increases in this pathway's efficacy. It is likely that this effect is not peculiar to M1. Plasticity of synapses formed by horizontally oriented axon collaterals may operate throughout many areas of the cerebral cortex to restructure various representation patterns. For example, within visual cortex, filling-in phenomena and reorganization of visual receptive fields after lesions or other perturbations appear to be mediated via horizontal connections[32]. The common occurrence of these connections in all cortical areas suggests that plasticity of synapses formed by horizontal pathways may be an important contributor to learning-related processes throughout the cerebral cortex.

Methods

Animals were cared for in accordance with National Institute of Health guidelines for laboratory animal welfare. All experiments were approved by the Brown University Institutional Animal Care and Use Committee. Forty adult female Sprague-Dawley rats (150–225 g) housed on a normal 12:12 light-dark cycle were used for this study. Twenty-eight animals were housed in pairs and were food restricted to maintain their body weight at roughly 85% of their free-feeding weight. Water was provided ad libitum. One rat from each pair was placed in an operant test cage (22.8 cm cube), which contained a Plexiglas food box (3.2 x 4.5 x 5 cm) with a 1.3 cm diameter hole through which the food was retrieved. Small food pellets (45 mg; Noyes Precision Food Pellets) were placed on the floor of this food box within reaching distance. Rats learned to reach into the food box with their preferred paw to retrieve food pellets using a grasping action. It was impossible for the rats to reach in the food box with both paws, although initial attempts sometimes involved trying to reach with both paws or attempting to place the snout in the food box and reach with the tongue. All but one animal, which was excluded from the analysis, selected the right forelimb to perform the task. Animals received one training session per day lasting for one hour. The training and practice period lasted five days for thirteen rats and three days for one rat. Because there was no readily apparent difference in the reaching behavior nor in the electrophysiological results after the three- and five-day training, data were grouped together. The second animal from a pair served as a paired control, received a comparable amount of handling and was given similar numbers of food pellets. The remaining twelve animals were used as naive controls.

The experimenter was unaware of the rats' training condition until data analysis of the pair was completed. Coronal brain slices containing the region of the M1 forelimb representation[33], 1–2 mm anterior to the bregma, were prepared as described[7] and superfused with artificial cerebrospinal fluid (ACSF) of the following composition (in mM): 126 NaCl, 3 KCl, 1.25 NaH_2PO_4, 26 $NaHCO_3$, 1 $MgSO_4$, 2 $CaCl_2$, and 10 glucose, bubbled with a 95% O_2, 5% CO_2 mixture at 35 ± 0.5°C. The humidified atmosphere over the slices was saturated with 95% O_2, 5% CO_2. Coronal slices from the hindlimb region were cut at the level of the anterior part of the hippocampal formation.

Field potentials were recorded using glass micropipettes placed in layer II/III, 200–350 µm below the pial surface in the region of the M1 forelimb representation (2–2.2 mm lateral to the midline). Concentric bipolar stimulating electrodes were displaced horizontally by 500 µm from each recording electrode (Fig. 1a). Consistent mirror-symmetrical placement of the electrodes at identical locations in both hemispheres was achieved with a reticle. Slices were not attached by the corpus callosum, but remained attached to each other during tissue slicing. For stimulation, constant current pulses (0.2 ms) were delivered at 0.033 Hz. We used the amplitude of the field potential evoked in the layer II/III horizontal pathway to measure of the population excitatory synaptic response because it reflects a monosynaptic current sink[9] and correlates well with intracellular excitatory postsynaptic potentials evoked in this pathway[7]. Input–output curves for a range of stimulation intensities were constructed for each stimulation–recording pair. In the first set of experiments, we averaged three to five sweeps evoked at 20, 40, 60, 80, 100 and 120% of the intensity inducing a maximum response. In the second set, we averaged three responses to stimuli of 2, 2.5, 3, 3.5, 4, 4.5 and 5 times the intensity that evoked a 0.1 mV (threshold) response. Stimulation did not influence the contralateral hemisphere because the slices used did not contain the corpus callosum. Field-potential peak amplitudes were calculated from averages of three to five waveforms, and the common logarithm of the left /right ratio was calculated for each animal. Because the log of this ratio fit a Gaussian distribution, parametric testing was used (paired *t*-test). Similar input-output curves were constructed for field potentials recorded from the hindlimb area of trained animals.

After establishing a 20 min period of stable response amplitudes using a stimulation intensity 50-60% of maximum, LTP induction was attempted with an established and reliable protocol for MI[7]. Prior to tetanic stimulation, the $GABA_A$ receptor antagonist bicuculline (3.5 mM) was applied within 100 µm from the recording electrodes using a glass pipette; the time of application on each side was separated by less than two minutes, and the same pipette was used for each side of a slice. The bicuculline pipette was retracted as soon as field-potential responses to test stimulation increased to about 150–200% of baseline (typically within 10–30 s). Immediately following bicuculline application, LTP was attempted by delivering theta-burst stimulation (5 sequences of 10 bursts 10 seconds apart; 1 burst is 5 pulses at 100 Hz) at double test intensity simultaneously through both stimulating electrodes[7]. The LTP effect was recorded for at least 30 min after it reached a stable plateau.

Acknowledgements

We thank Drs. Barry Connors, Mark Bear, and Marc G. Rioult for critical comments. This work was supported by NIH grant NS22517. G. H. is an international scholar of the Howard Hughes Medical Institute.

RECEIVED 21 JANUARY; ACCEPTED 26 MAY 1998

1. Cohen, L.G., Brasil, N.J., Pascual-Leone, L.A. & Hallett, M. Plasticity of cortical motor output organization following deafferentation, cerebral lesions, and skill acquisition. *Adv. Neurol.* 63, 187–200 (1993).
2. Donoghue, J.P. Plasticity of sensorimotor representations. *Curr. Opin. Neurobiol.* 5, 749–754 (1995).
3. Donoghue, J.P., Hess, G. & Sanes, J.N. in *Acquisition of Motor Behavior* (eds. Bloedel, J., Ebner, T. & Wise, S.P.) 363–386 (MIT Press, Cambridge, 1996).
4. Nudo, R.J., Milliken, G.W., Jenkins, W.M. & Merzenich, M.M. Use-dependent alterations of movement representations in primary motor cortex of adult squirrel monkeys. *J. Neurosci.* 16, 785–807 (1996).
5. Greenough, W.T., Larson, J.R. & Withers, G.S. Effects of unilateral and bilateral training in a reaching task on dendritic branching of neurons in the rat motor-sensory forelimb cortex. *Behav. Neural Biol.* 44, 301–314 (1985).
6. Hess, G. & Donoghue, J.P. Long-term potentiation of horizontal connections provides a mechanism to reorganize cortical motor maps. *J. Neurophysiol.* 71, 2543–2547 (1994).
7. Hess, G., Aizenman, C.D. & Donoghue, J.P. Conditions for the induction of long-term potentiation in layer II/III horizontal connecitons of the rat motor cortex. *J. Neurophysiol.* 75, 1765–1778 (1996).
8. Hess, G. & Donoghue, J.P. Long-term depression of horizontal connections in rat motor cortex. *Eur. J. Neurosci.* 8, 658–665 (1996).
9. Aroniadou, V.A. & Keller, A. Mechanisms of LTP induction in rat motor cortex in vitro. *Cereb. Cortex* 5, 353–362 (1995).
10. Castro-Alamancos, M.A., Donoghue, J.P. & Connors, B.W. Different forms of synaptic plasticity in somatosensory and motor areas of the neocortex. *J. Neurosci.* 15, 5324–5333 (1995).
11. Asanuma, H. & Pavlides, C. Neurological basis of motor learning in mammals. *Neuroreport* 8, i–vi (1997).
12. Kaas, J.H. Plasticity of sensory and motor maps in adult mammals. *Annu. Rev. Neurosci.* 14, 137–167 (1991).
13. Merzenich, M.M., Recanzone, G., Jenkins, W.M., Allard, T.T. & Nudo, R.J. in *Neurobiology of Neocortex* (eds Rakic, P. & Singer, W.) 41–67 (Wiley, New York, 1988).
14. Huntley, G.W. Correlation between patterns of horizontal connectivity and the extent of short term representational plasticity in rat motor cortex. *Cereb. Cortex* 7, 143–156 (1997).
15. Donoghue, J.P. Limits of reorganization in cortical circuits. *Cereb. Cortex* 7, 97–99 (1997).
16. Jacobs, K. & Donoghue, J. Reshaping the cortical map by unmasking latent intracortical connections. *Science* 251, 944–947 (1991).
17. Hirsch, J. & Gilbert, C. Long-term changes in synaptic strength along specific intrinsic pathways in the cat's visual cortex. *J. Physiol. (Lond)* 461, 247–262 (1993).
18. Price, A.W. & Fowler, S.C. Deficits in contralateral and ipsilateral forepaw motor control following unilateral motor cortical ablation in rats. *Brain Res.* 205, 81–90 (1981).
19. Garraghty, P.E. & Kaas, J.H. Dynamic features of sensory and motor maps. *Curr. Opin. Neurobiol.* 2, 522–527 (1992).
20. Sanes, J.N. & Donoghue, J.P. Motor areas of the cerebral cortex. *J. Clin. Neurophysiol.* 11, 382–396, (1994).
21. Rogan, M.T., Staeubli, U.V. & LeDoux, J.E. Fear conditioning induces associative long-term potentiation in the amygdala. *Nature* 390, 604–607 (1997).
22. McKernan, M.G. & Shinnick-Gallagher, P. Fear conditioning induces a lasting potentiation of synaptic currents in vitro. *Nature* 390, 607–611 (1997).
23. Ahissar, E. *et al.* Dependence of cortical plasticity on correlated activity of single neurons and on behavioral context. *Science* 257, 1412–1415 (1992).
24. Grafton, S.T. *et al.* Functional anatomy of human procedural learning determined with regional cerebral blood flow and PET. *J. Neurosci.* 12, 2542–2548 (1992).
25. Pascual-Leone, A., Grafman, J. & Hallett, M. Modulation of cortical motor output maps during development of implicit and explicit knowledge. *Science* 263, 1287–1289 (1994).
26. Karni, A. *et al.* Functional MRI evidence for adult motor cortex plasticity during motor skill learning. *Nature* 377, 155–158 (1995).
27. Morris, R.G.M., Davis, S. & Butcher, P. in *Long Term Potentiation: A Debate of Current Issues* (eds Baudry, M. & Davis, J.L.) 267–300 (MIT Press, Cambridge, 1991).
28. Woody, C.D., Gruen, E. & Birt, D. Changes in membrane currents during Pavlovian conditioning of single cortical neurons. *Brain Res.* 539, 76–84 (1991).
29. Yoshimura, Y. & Tsumoto, T. Dependence of LTP induction on postsynaptic depolarization: a perforated patch-clamp study in visual cortical slices of young rats. *J. Neurophysiol.* 71, 1638–1645 (1994).
30. Darian, S.C. & Gilbert, C.D. Axonal sprouting accompanies functional reorganization in adult cat striate cortex. *Nature* 368, 737–740 (1994).
31. Kleim, J.A., Lussnig, E., Schwarz, E.R., Comery, T.A. & Greenough, W.T. Synaptogenesis and Fos expression in the motor cortex of the adult rat after motor skill learning. *J. Neurosci.* 16, 4529–4535 (1996).
32. Gilbert, C.D. Rapid dynamic changes in adult cerebral cortex. *Curr. Opin. Neurobiol.* 3, 100–103 (1993).
33. Donoghue, J.P. & Wise, S.P. The motor cortex of the rat: cytoarchitecture and microstimulation mapping. *J. Comp. Neurol.* 212, 76–88 (1982).