14. A. Majumdar *et al.*, *Nature Genet.* **20**, 212 (1998).
15. F. X. Barre *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **97**, 3084 (2000).
16. P. C. Hanawalt, *Science* **266**, 1957 (1994).
17. A. F. Faruqi, H. J. Datta, D. Carroll, M. M. Seidman, P. M. Glazer, *Mol. Cell. Biol.* **20**, 990 (2000).
18. U. Galderisi, A. Cascino, A. Giordano, *J. Cell Physiol.* **181**, 251 (1999).
19. B. Bramlage, E. Luzi, F. Eckstein, *Trends Biotechnol.* **16**, 434 (1998).
20. L. Good, P. E. Nielsen, *Antisense Nucleic Acid Drug Dev.* **7**, 431 (1997).
21. J. M. Gottesfeld, L. Neely, J. W. Trauger, E. E. Baird, P. B. Dervan, *Nature* **387**, 202 (1997).
22. A. Cole-Strauss *et al.*, *Science* **273**, 1386 (1996).
23. M. Famulok, *Curr. Opin. Struct. Biol.* **9**, 324 (1999).
24. T. L. Chen, P. S. Miller, P. O. Ts'o, O. M. Colvin, T. L. Chem, *Drug Metab. Dispos.* **18**, 815 (1990).
25. S. Agrawal, J. Temsamani, J. Y. Tang, *Proc. Natl. Acad. Sci. U.S.A.* **88**, 7595 (1991).

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Learning-Induced LTP in Neocortex

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The hypothesis that learning occurs through long-term potentiation (LTP)– and long-term depression (LTD)–like mechanisms is widely held but unproven. This hypothesis makes three assumptions: Synapses are modifiable, they modify with learning, and they strengthen through an LTP-like mechanism. We previously established the ability for synaptic modification and a synaptic strengthening with motor skill learning in horizontal connections of the rat motor cortex (MI). Here we investigated whether learning strengthened these connections through LTP. We demonstrated that synapses in the trained MI were near the ceiling of their modification range, compared with the untrained MI, but the range of synaptic modification was not affected by learning. In the trained MI, LTP was markedly reduced and LTD was enhanced. These results are consistent with the use of LTP to strengthen synapses during learning.

Most cortical excitatory synaptic connections appear to be capable of persistent bidirectional modification. The ability for LTP or LTD to modify individual synapses has made LTP or LTD the most widely held candidate mechanism for learning. Experimental evidence supports this view but has not demonstrated that synaptic modifications that occur during learning use LTP or LTD (*1*). Saturation of synaptic efficacy before learning, using LTP-inducing stimuli in vivo, interferes with hippocampally mediated spatial learning (*2*–*5*). This result suggests that saturating synapses impairs learning but does not demonstrate that this same modification mechanism is used when natural learning occurs. In addition, it has been shown that synapses likely to be activated during learning change their efficacy after learning occurs. In the amygdala (*6*, *7*) and the motor cortex (*8*), candidate pathways capable of LTP are stronger after learning, but it has not been tested explicitly whether LTP strengthened these pathways.

We have previously demonstrated that layer II/III horizontal connections in rat primary motor cortex (MI) are capable of LTP and LTD (*9*, *10*) and are strengthened with forelimb motor skill learning (*8*). Strengthening is present in MI opposite to the trained forelimb ("trained MI") but not in the hindlimb area of MI or in the same rats' ipsilateral "untrained MI" (*8*). If LTP was used to achieve synaptic enhancement during learning and this process is saturable, then subsequent attempts at electrically induced LTP after learning should produce less LTP. Critical measures necessary to test this prediction are the identification of baseline synaptic strength, measured as field potential amplitude (*11*), and the upper and lower limits of modification, termed the "synaptic modification range," in trained and untrained pathways.

We defined the synaptic modification range using repeated LTP or LTD induction until saturation was reached. We used this synaptic modification range model to test whether LTP is a mechanism engaged in learning and to examine whether learning affects this range. Theoretically, the range could remain unchanged, shift, or expand as a result of learning. We know that learning increases the strength of horizontal connections because there is an absolute change in field potential amplitude in these pathways after learning (*8*). An unchanged synaptic modification range predicts less LTP but more LTD in trained pathways compared with controls. Thus, synapses closer to the ceiling of their modification range cannot express much additional LTP, and this would be consistent with the use of LTP in learning.

Rats were trained for five successive days to reach with their preferred forelimb into a box and retrieve small food pellets (*12*). Grasp attempts began during the first session, and success rate improved during the first three training days, when it became asymptotic (Fig. 1A). After training, field potentials evoked across layer II/III horizontal connections in the MI forelimb region were recorded simultaneously from both hemispheres (*13*) in slice preparations (*14*). Field potential amplitudes were 1.59 ± 0.10 times larger (N = 32) in the trained MI compared with the control, untrained MI forelimb region. There was no interhemisphere difference in paired control animals and in the hindlimb MI of trained animals (Fig. 1B) (*15*). Stimulation intensities producing half maximal response amplitudes were not significantly different in the trained and untrained MI (22.0 ± 1.24 μA and 21.1 ± 1.22 μA, respectively; N = 21; P = 0.44), indicating that the differences in field potential amplitude could not be explained by the use of different stimulating intensities.

After 5 days of training, repeated theta burst stimulation (TBS) (*16*) produced less LTP in the trained MI than in the opposite, untrained MI. In a striking example shown in Fig. 2A (top), no LTP could be produced in the trained MI horizontal connections despite repeated induction attempts. Simultaneous recordings in the untrained MI of the same slice resulted in normal amounts of LTP, with complete saturation at 163% of baseline (Fig. 2A, bottom). We examined whether these apparently saturated synapses in the trained MI retained the capability to undergo potentiation by first bringing them to lower strength using low-frequency stimulation (LFS) (*17*) (66% of baseline, Fig. 2A, top). Subsequent TBS potentiated these synapses, demonstrating their capacity for LTP (124% of renormalized baseline). These data suggest that the large field potential amplitude that appears in MI horizontal connections after learning reflects a population of strengthened synapses that retains the mechanism for LTP.

Both the amount of LTP and the number of attempts to reach saturation were lower in the trained MI. Considered as a group, LTP in animals trained for 5 days was saturated at 114.5 ± 3.6% of baseline in the trained MI. By comparison, the untrained MI was saturated at 152.1 ± 9.9% of baseline (N = 11, P < 0.001), comparable to levels seen in control rats (Fig. 2B). In 3 of these 11 cases, no LTP could be induced in the trained MI (Fig. 2A, top). In these cases, LTP was nev-

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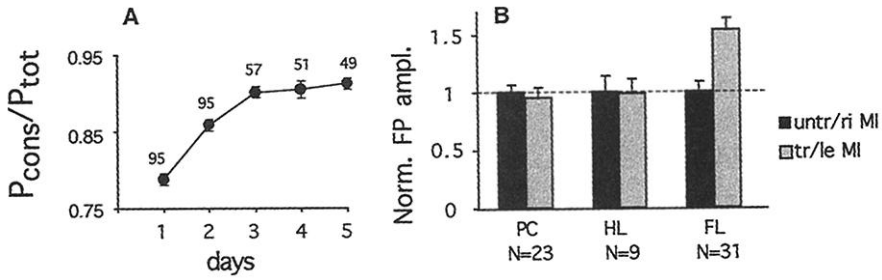

Fig. 1. Learning a new motor skill potentiates synaptic responses in MI horizontal connections. (**A**) Learning was defined as the success rate over time, which is the ratio of the number of consumed pellets and the number of retrieved pellets (P_{cons}/P_{tot} ± SEM). Numbers above data points represent the number of animals trained for various numbers of days. A randomly chosen subset of animals trained for 5 days was used for subsequent electrophysiological recordings of the present project. (**B**) Learning specifically strengthened extracellular field potentials (FP) in the MI forelimb region (FL). There were no interhemispheric differences in the hindlimb region (HL) or in paired controls (PC) (*12*). Means ± SEM are from four times threshold intensity (*42*) and were normalized to the untrained (untr) or right (ri) hemisphere. ampl., amplitude; tr, trained; le, lett.

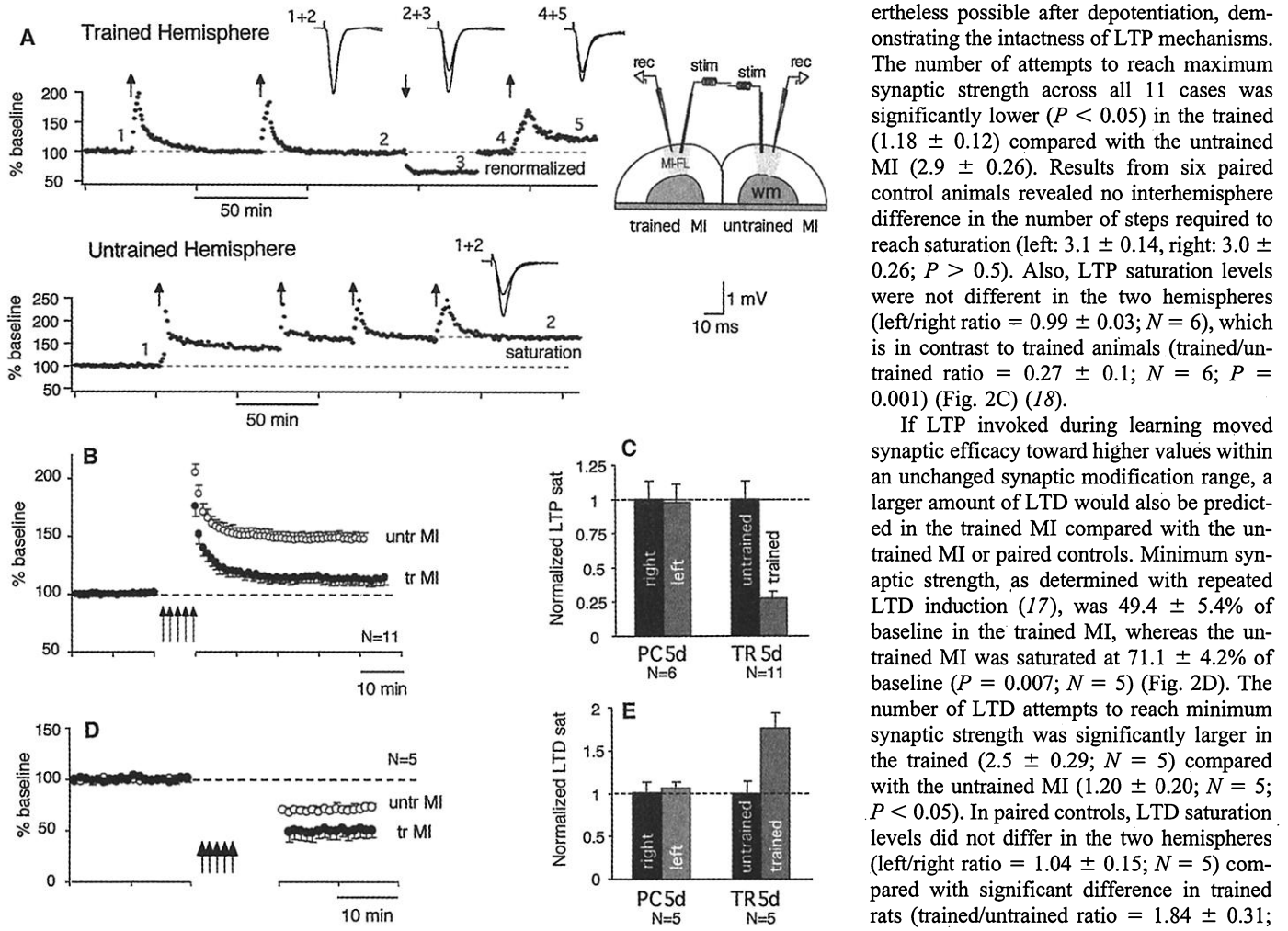

Fig. 2. Learning has complementary effects on LTP and LTD. (**A**) Simultaneous LTP saturation in the trained and untrained MI in the same slice. Stimulation (stim) and recording (rec) conditions as illustrated in the inset (wm, white matter; MI-FL, forelimb region of the primary motor cortex). Field potential waveforms (averages of five traces) were taken at times indicated by numbers. Up arrows, LTP induction; down arrow, LTD induction. (**B**) Group data demonstrate reduced LTP in the trained MI. The five arrows represent the use of a number of LTP inductions. Averages were taken from the final LTP attempt (at saturation as compared with pre-TBS baseline), which confirmed that no further increase of the field potential peak amplitude occurred. (**C**) Less LTP was possible in the trained MI compared with the untrained MI and either hemisphere of paired control animals (PC). Mean values were normalized to the right or untrained MI. TR 5d, animals trained for 5 days. (**D**) Group data ($N = 5$) show more LTD in the trained MI. The five arrows represent multiple LTD inductions. Averages were taken from the LTD saturation levels. (**E**) LTD saturation of five trained (TR) and five paired control rats (PC). The trained MI showed more LTD than the untrained MI and both hemispheres of paired controls. Values were normalized to the right or untrained MI.

ertheless possible after depotentiation, demonstrating the intactness of LTP mechanisms. The number of attempts to reach maximum synaptic strength across all 11 cases was significantly lower ($P < 0.05$) in the trained (1.18 ± 0.12) compared with the untrained MI (2.9 ± 0.26). Results from six paired control animals revealed no interhemisphere difference in the number of steps required to reach saturation (left: 3.1 ± 0.14, right: 3.0 ± 0.26; $P > 0.5$). Also, LTP saturation levels were not different in the two hemispheres (left/right ratio = 0.99 ± 0.03; $N = 6$), which is in contrast to trained animals (trained/untrained ratio = 0.27 ± 0.1; $N = 6$; $P = 0.001$) (Fig. 2C) (*18*).

If LTP invoked during learning moved synaptic efficacy toward higher values within an unchanged synaptic modification range, a larger amount of LTD would also be predicted in the trained MI compared with the untrained MI or paired controls. Minimum synaptic strength, as determined with repeated LTD induction (*17*), was 49.4 ± 5.4% of baseline in the trained MI, whereas the untrained MI was saturated at 71.1 ± 4.2% of baseline ($P = 0.007$; $N = 5$) (Fig. 2D). The number of LTD attempts to reach minimum synaptic strength was significantly larger in the trained (2.5 ± 0.29; $N = 5$) compared with the untrained MI (1.20 ± 0.20; $N = 5$; $P < 0.05$). In paired controls, LTD saturation levels did not differ in the two hemispheres (left/right ratio = 1.04 ± 0.15; $N = 5$) compared with significant difference in trained rats (trained/untrained ratio = 1.84 ± 0.31; $N = 5$; $P < 0.05$) (Fig. 2E) (*18*). To ensure that depressed pathways retain plasticity, we attempted LTP induction with a single TBS in all experiments after LTD saturation. Repotentiation was possible in 5 of 11 animals, which were therefore included in the LTD group analysis. The remaining six animals could not be repotentiated (as sometimes seen in prolonged slice experiments) and were therefore excluded from group analysis. In summary, learning enhanced the relative amount of LTD in the trained MI, consistent with the hypothesis of a shift of baseline synaptic strength away from its floor and

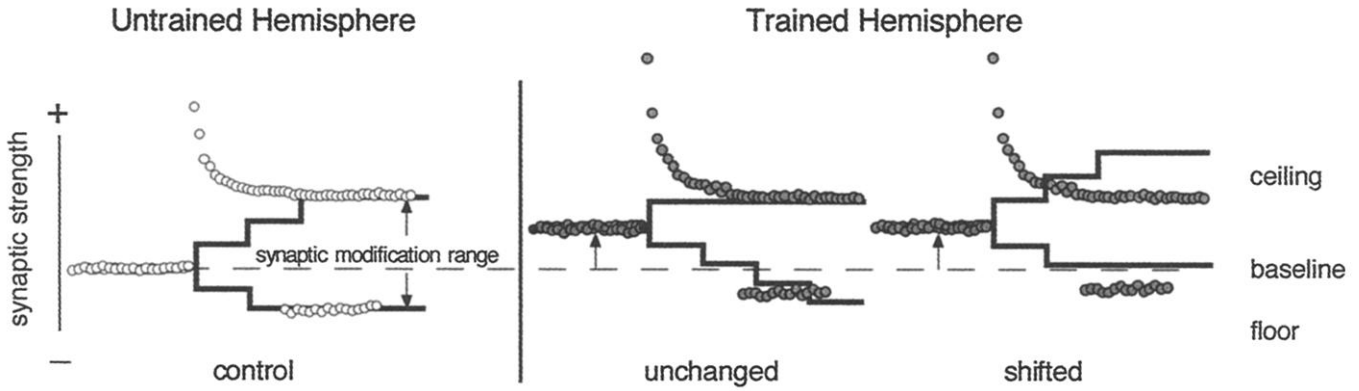

Fig. 3. Saturation data most closely fit a model in which strengthening occurs without a shift in the population overall modification range. Steps reflect each induction of LTP or LTD until saturation was achieved. Group data from LTP and LTD saturation experiments in the untrained MI (control, open circles) were superimposed and served as the basis for defining the synaptic modification range. On the right side (trained hemisphere), average results from LTP and LTD saturation (filled circles) of trained MIs (Fig. 2, B and D) are superimposed on the alternate models of an unchanged and a shifted range of synapticmodification. Models with altered range are not compatible with the data and support the conclusion that the synaptic modification range remains fixed. Our results could also suggest that learning results in a shift in synaptic modification threshold. Larger LTD and smaller LTP could occur with a rightward shift in theta according to the Bienenstock-Cooper-Munro (BCM) theory of synaptic modification, as has been reported in developing neocortex (*43*).

toward the upper modification range limits.

Although LTP and LTD levels were modified with training, the overall synaptic modification range was the same in trained and untrained pathways as summarized in Fig. 2, B and D. Figure 3 presents the combined results from LTP and LTD experiments and compares it with outcomes predicted in our initial model. Each individual step in the solid line depicts the result of a single conditioning stimulation. In the untrained MI (control), the model reflects the baseline measurements with its experimentally identified upper and lower limits of synaptic efficacy under control conditions. Skill learning increased the strength of horizontal connections in the trained MI (*8*), which is shown by the upward shift of the baseline relative to the untrained condition (Fig. 3, upward arrows in the trained MI). The model of an "unchanged" and "shifted" synaptic modification range has been superimposed on our LTP and LTD saturation data from the trained MI. The "shifted" model, as well as any expanded range model, can be rejected because much less LTP occurred experimentally than predicted by those models. The "unchanged" model, in which synapses retain their normal operating range, is most consistent with our finding of less LTP and more LTD after learning.

The reduction in LTP and increase in LTD in MI layer II/III horizontal connections associated with learning provide strong evidence that synaptic strengthening engages LTP during skill learning and that potentiation during learning moves the overall population synaptic weight closer to the maximum of its operating range. Previous work has pointed out close ties between pathways that are capable of LTP and that also support learning. In the amygdala, fear conditioning increases synaptic strength in vivo (*6*) and in vitro (*7*), and the same pathways appear to be capable of LTP. However, LTP occlusion in the amygdala has not been tested (*19*). Hippocampal circuits are capable of LTP and support various forms of learning, but the connection between LTP and learning has been controversial (*2–5*, *20–26*), partly because attempts to block learning by first saturating synapses using LTP has been difficult to achieve. Our approach differed because we attempted to saturate LTP after learning had modified synapses.

One reasonable prediction of our finding is that near saturation of LTP would prevent further learning. If true, the cortex would seem to have a limited capacity to contribute to learning. However, recent work in the hippocampus has shown that spatial learning is not impaired until pathways are >90% saturated (*5*). If this result extends to MI, small residual LTP could be sufficient to support further learning. Why do we see such large effects of reach and grasp learning in MI synapses? Exceptionally large modifications may appear only in rats naïve to any complex motor skills; subsequent learning might lead to more subtle, but highly meaningful enhancements and decrements beyond the sensitivity of the current methodology. Thus, one cannot equate the amount of LTP with the amount of learning because the relation may depend on prior history. It is noteworthy that learning motor skills does transiently interfere with learning other motor skills in humans (*27*); if such interference occurs in rats, the learning capacity may be restored by changes in synaptic modification range after prolonged task training. This remains to be examined.

The most reasonable explanation for an increase in field potential amplitude associated with motor skill learning is that synapses were potentiated by an LTP-like mechanism. Other mechanisms have been suggested to act during learning but are less plausible. A generalized increase in excitability has been recognized at early phases of classical conditioning in the hippocampus and MI (*28–30*). Our data are not consistent with excitability changes because absolute stimulation intensities were not altered by training. In addition, increased excitability would be expected to produce more LTP in the trained MI because stimulation would more effectively drive postsynaptic cells. Rather than using LTP, increases in synaptic efficacy could also occur if learning induced the formation of new synapses, as has been reported after lesions in the visual and somatosensory cortex (*31*, *32*), and learning in the motor cortex (*33*). These effects appear to require more than 5 days to develop, although new synapses can be formed rapidly subsequent to LTP induction [(*34–36*), but compare (*37*)]. However, if synapses were newly formed or preexisting silent synapses were revealed (*38*) after motor skill training, and they were functional, larger than normal LTP would be expected from the larger complement of synapses, and the synaptic modification range would appear to expand because the stimulated and recorded population of synapses would be greater. This is in marked contrast to our findings of less LTP and an unchanged synaptic modification range subsequent to learning. Adding new receptors to existing synapses (*39*, *40*) would lead to larger field potentials and would not change the synaptic modification range and therefore is one favored mechanism to explain the present data. Whatever cellular mechanism enhances synaptic efficacy, the data presented here establish a strong link between LTP and learning-induced synaptic plasticity.

References and Notes

1. S. J. Martin, P. D. Grimwood, R. G. M. Morris, *Annu. Rev. Neurosci.* **23**, 649 (2000).
2. B. L. McNaughton, C. A. Barnes, G. Rao, J. Baldwin, M. Rasmussen, *J. Neurosci.* **6**, 563 (1986).
3. C. A. Castro, L. H. Silbert, B. L. McNaughton, C. A. Barnes, *Nature* **342**, 545 (1989).
4. C. A. Barnes *et al.*, *J. Neurosci.* **14**, 5793 (1994).
5. E. I. Moser, K. A. Krobert, M.-B. Moser, R. G. M. Morris, *Science* **281**, 2038 (1998).
6. M. T. Rogan, U. V. Staeubli, J. E. LeDoux, *Nature* **390**, 604 (1997).
7. M. G. McKernan, P. Shinnick-Gallagher, *Nature* **390**, 607 (1997).
8. M.-S. Rioult-Pedotti, D. Friedman, J. P. Donoghue, *Nature Neurosci.* **1**, 230 (1998).
9. G. Hess, C. D. Aizenman, J. P. Donoghue, *J. Neurosci.* **75**, 1765 (1996).
10. G. Hess, J. P. Donoghue, *Eur. J. Neurosci.* **8**, 658 (1996).
11. Baseline synaptic strength was defined as half maximal field potential amplitude measured in a slice preparation before any conditioning stimulation was applied.
12. Animals were cared for in accordance with National Institutes of Health guidelines for laboratory animal welfare. All experiments were approved by the Brown University Institutional Animal Care and Use Committee. Methods have been described in detail elsewhere (*8*). Briefly, adult female Sprague-Dawley rats (170 to 220 g) were housed in pairs and were food-restricted to maintain their body weight at 85% of their free feeding weight. One rat of each pair was trained in a skilled reaching task, and the second one was used as a paired control and received a similar amount of handling and spent a similar amount of time in the training cage but did not have to reach for the pellets. Rats that received training were placed in a training cage containing a plastic box with a small opening (diameter 13 mm) that gave access to a pile of small food pellets (45 mg, Noyes Precision Food Pellets). Rats had to learn to reach through a hole into the food box and grasp and retrieve pellets with their preferred forelimb. Animals received one training session per day, which lasted 1 hour. Rats were trained for five successive days.
13. In an in vitro slice preparation (*14*), concentric bipolar stimulating electrodes were placed in layer II/III, 200 to 300 μm below the pial surface in the region of the MI forelimb representation (2 mm lateral to the midline). Field potentials were recorded with glass micropipettes that were displaced laterally by 500 μm from each stimulation electrode. The electrodes were placed mirror symmetrically in both hemispheres. Slices anterior to the corpus callosum were used for recordings. The hemisphere ipsilateral to the trained forelimb, the untrained MI, served as internal control. 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 as a measure of the population excitatory synaptic response because in the neocortex it reflects a monosynaptic current sink (*41*) and correlates well with intracellular excitatory postsynaptic potentials evoked in this pathway.
14. Twenty to 45 hours after the last training session, brains were removed, and coronal slices including the region of the MI forelimb representation, 1 to 2 mm anterior to the bregma, were prepared and superfused with artificial cerebrospinal fluid (ACSF) of the following composition: 126 mM NaCl, 3 mM KCl, 1.25 mM NaH_2PO_4, 26 mM $NaHCO_3$, 1 mM $MgSO_4$, 2 mM $CaCl_2$, and 10 mM glucose, bubbled with a 95% O_2/5% CO_2, at 35.0 ± 0.5°C. The humidified atmosphere over the slices was saturated with 95% O_2/5% CO_2. Slices remained attached to each other during tissue slicing. All electrophysiology experiments were done blind to the experimental condition: The person who performed the dissection, the electrophysiology, and the analysis was blind to the hemisphere or the hemisphere and training history.
15. To examine the synaptic strength in each hemisphere, we recorded input-output curves for a range of stimulation intensities (one to five times threshold intensity) at the beginning of each experiment and calculated the interhemisphere ratios. The amplitude enhancement was independent of the stimulation intensity used (*8*). For all statistical analysis, paired two-tailed *t* tests were used.
16. After establishing at least a 20-min period of stable response amplitudes using the stimulation intensity that produced 50% maximal response amplitude, repeated TBS preceded by local transient touch application of bicuculline (*8*, *9*) was applied until responses reached a maximum strength, i.e., were saturated. The standard LTP induction protocol was five TBS delivered every 10 s at twice test stimulation intensity (*9*). Each TBS consisted of 10 bursts (1 burst = 5 pulses at 100 Hz) delivered at 5 Hz. Pathways were considered saturated if the difference between two states of LTP inductions was not significantly different ($P > 0.5$). Maximum LTP was expressed as percentage of baseline. For interhemisphere ratios, values above 100% were used.
17. Low-frequency stimulation (2 Hz for 15 min at twice test stimulation intensity) was applied until responses were saturated at minimum strength. Pathways were considered saturated if the difference between two states of LTD inductions was not significantly different ($P > 0.5$). Maximum LTD values are expressed as percentage of baseline. To calculate interhemisphere ratios, we used values below 100%.
18. LTP or LTD saturation in the trained MI was significantly different from either hemisphere of paired control animals ($P < 0.05$), and LTP or LTD saturation of paired controls was not different from the untrained MI ($P > 0.5$).
19. S. Maren, *Trends Neurosci.* **22**, 561 (1999).
20. D. L. Korol, T. W. Abel, L. T. Church, C. A. Barnes, B. L. McNaughton, *Hippocampus* **3**, 127 (1993).
21. K. J. Jeffrey, R. G. M. Morris, *Hippocampus* **3**, 133 (1993).
22. R. J. Sutherland, H. C. Dringenberg, J. M. Hoesing, *Hippocampus* **3**, 141 (1993).
23. G. B. Robinson, *Hippocampus* **2**, 389 (1992).
24. D. P. Cain, E. L. Hargreaves, F. Boon, Z. Dennison, *Hippocampus* **3**, 153 (1993).
25. K. J. Jeffery, *Hippocampus* **7**, 95 (1997).
26. T. J. Shors, L. D. Matzel, *Behav. Brain Sci.* **20**, 597 (1997).
27. R. Shadmer, T. Brashers-Krug, *J. Neurosci.* **17**, 409 (1997).
28. J. R. Moyer, L. T. Thompson, J. D. Disterhoft, *J. Neurosci.* **16**, 5536 (1996).
29. S. Aou, C. D. Woody, D. Birt, *J. Neurosci.* **12**, 560 (1992).
30. J. P. Donoghue, *Curr. Opin. Neurobiol.* **5**, 749 (1995).
31. S. C. Darian-Smith, C. D. Gilbert, *Nature* **368**, 737 (1994).
32. S. L. Florence, H. B. Taub, J. H. Kaas, *Science* **282**, 1117 (1998).
33. J. A. Kleim, E. Lussnig, E. R. Schwarz, T. A. Comery, W. T. Greenough, *J. Neurosci.* **212**, 76 (1996).
34. M. Maletic-Savatic, R. Malinow, K. Svoboda, *Science* **283**, 1923 (1999).
35. F. Engert, T. Bonhoeffer, *Nature* **399**, 66 (1999).
36. N. Toni, P.-A. Buchs, I. Nikonenko, C. R. Bron, D. Muller, *Nature* **402**, 421 (1999).
37. K. E. Sorra, K. M. Harris, *J. Neurosci.* **18**, 658 (1998).
38. R. C. Malenka, R. A. Nicoll, *Neuron* **19**, 473 (1997).
39. R. C. Carroll, D. V. Lissin, M. von Zastrow, R. A. Nicoll, R. C. Malenka, *Nature Neurosci.* **2**, 454 (1999).
40. S.-H. Shi *et al.*, *Science* **284**, 1811 (1999).
41. V. A. Aroniadou, A. Keller, *Cereb. Cortex* **5**, 353 (1995).
42. Threshold intensity is the stimulation intensity used to evoke a response of 0.2-mV amplitude.
43. A. Kirkwood, M. G. Rioult, M. F. Bear, *Nature* **381**, 526 (1996).

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