Plasticity of the Synaptic Modification Range

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Rioult-Pedotti M-S, Donoghue JP, Dunaevsky A. Plasticity of the synaptic modification range. J Neurophysiol 98: 3688–3695, 2007. First published October 3, 2007; doi:10.1152/jn.00164.2007. Activity-dependent synaptic plasticity is likely to provide a mechanism for learning and memory. Cortical synaptic responses that are strengthened within a fixed synaptic modification range after 5 days of motor skill learning are driven near the top of their range, leaving only limited room for additional synaptic strengthening. If synaptic strengthening is a requisite step for acquiring new skills, near saturation of long-term potentiation (LTP) should impede further learning or the LTP mechanism should recover after single-task learning. Here we show that the initial learning-induced synaptic enhancement is sustained even long after training has been discontinued and that the synaptic modification range shifts upward. This range shift places increased baseline synaptic efficacy back within the middle of its operating range, allowing prelearning levels of LTP and long-term depression. Persistent synaptic strengthening might be a substrate for long-term retention in motor cortex, whereas the shift in synaptic modification range ensures the availability for new synaptic strengthening.

INTRODUCTION

Plasticity of synaptic connections is now widely believed to play an essential role in learning and memory formation. In the hippocampus, cortex, and amygdala, population measures indicate that long-term potentiation (LTP) and long-term depression (LTD) are associated with learning to modify synaptic efficacy (Martin et al. 2000). Learning generally leads to an overall enhancement of synaptic responses in the hippocampus (Gruart et al. 2006; Moser et al. 1993; Sacchetti et al. 2001; Whitlock et al. 2006), the neocortex (Hodgson et al. 2005; Monfils and Teskey 2004; Rioult-Pedotti et al. 1998; Roman et al. 1999; Ziemann et al. 2004; but see Cohen and Castro-Alamancos 2005), and the amygdala (Kernan and Shinnick-Gallagher 1997; Rogan et al. 1997). The occlusion of learning by prior LTP saturation in hippocampus (Barnes et al. 1994; Castro et al. 1989; McNaughton et al. 1986; Moser et al. 1998) and the occlusion of LTP after learning in the neocortex (Monfils and Teskey 2004; Rioult-Pedotti et al. 2000; Ziemann et al. 2004) and the hippocampus (Gruart et al. 2006; Whitlock et al. 2006) has been used to argue that LTP is the mechanism by which learning strengths synapses.

Synapses appear to have a finite ceiling and floor that define a synaptic modification range. The synaptic modification range can be experimentally defined using saturating levels of electrically induced LTP and LTD (Rioult-Pedotti et al. 2000). In a previous study, 5 days of training on a pellet reach-and-grasp task enhanced the efficacy of horizontal connections in the primary motor cortex (MI), revealed as an increase in field potential (FP) amplitude over control. At this time synapses approached the upper limit of the synaptic modification range shown by the occlusion of LTP in slices in the engaged motor cortex horizontal pathways (Martin and Morris 2001; Rioult-Pedotti et al. 2000). The saturating effect of learning-related modification predicts that unless synapses are placed closer to the midpoint of the synaptic modification range, additional strengthening through LTP-like mechanisms in vivo would be blocked. Indeed, such interference has been reported in humans (Shadmehr and Brashers-Krug 1997); learning one motor skill transiently interferes with learning another motor skill. Here, we hypothesized that the capacity for further potentiation would be restored if either overall strengthening diminished after initial learning or if the limiting modification range shifted or expanded.

The rat motor skill learning paradigm has specific advantages to test these hypotheses. First, horizontal connections in the MI forelimb area are markedly and reliably enhanced during motor skill learning, providing a robust marker of synaptic change. Second, strengthening is unilateral so that the untrained MI serves as a within-animal control for the training and other behavioral effects that may vary when data are compared across animals. Third, learning-induced changes can be evaluated in slice preparations, allowing control not only of sample site, but also of recording and preparation conditions.

In this study we present evidence that the initial learning-induced increase in synaptic strength persists and the task is retained even when task performance is discontinued for ≥2 mts. The operating range shifts upward and places increased synaptic strength back to the middle of the operating range.

METHODS

Behavior

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. Adult female Sprague–Dawley rats (180–220 g) were kept in pairs (one to three pairs per cage) 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 whereas the second rat was used as an untrained control animal. The training apparatus has been modified compared with the apparatus used for previously published work using the motor skill learning paradigm. The new task is more difficult for the rats to perform and thus the success rate in general is lower compared with the success rate previously published. For training, rats were placed in a training cage (Coulburn, Habitest), with an attached food box (custom-made) hold-
Adult rats learned to reach with one forelimb through an aperture to grasp and retrieve single food pellets. Pellet retrieval improved over the first 4–5 days, then stabilized at 27.4 ± 0.1% success (mean for days 6–23, n = 13; ETP, Fig. 1A). Retrieval success showed no further improvement with continual, daily training ≥105 successive days. The reaching efficiency, which is the reaching time per successfully retrieved pellet, improved with a similar time course and stabilized at 9.04 ± 0.8 s (mean for days 6–23, n = 13). An independent learning measure, number of dropped pellets, improved with a slower time course. The number of dropped pellets decreased over 15 training days (Fig. 1A) without further change (mean = 6.6 ± 0.8 for days 16–23, n =
Another group of animals was trained either for 5 days or until behavioral measures stabilized (14 days) and examined for task retention 78 days after training started (STP + 2 mts). Results in Fig. 1B indicate that the initially learned skill was retained for 2 mts without any task performance (% success: initial training 34.78 ± 6.04; retention 34.7 ± 4.26. Drops: initial training 11.89 ± 1.59; retention 12 ± 1.65, n = 8). Additional rats that learned and performed the task for 5 or 14 days were retested for task retention 3 or 4 mts later. All animals remembered the task (results not illustrated).

Rats that performed the learned task on a daily basis for an extended period of time (ETP) did not show further performance improvement. Rats that acquired and performed the task for a short period of time (STP + 2 mts) still performed at maximum level even though task performance was discontinued. These results indicate that once the task is acquired, daily performance is not necessary for retention. Next we determined the electrophysiological correlates of extended task performance (ETP) and task retention (STP + 2 mts) in the forelimb motor cortex. For comparison we added another group of short task performance (STP) and an untrained control group (Ctrl) (Rioult-Pedotti et al. 2000).

**Persistent synaptic strengthening**

Comparison of the three groups of trained animals demonstrated that enhancement of extracellular FP amplitudes induced by initial training (3–6 days, STP) persisted whether task performance was continued (ETP) or discontinued (STP + 2 mts). Figure 2 illustrates the larger FP amplitudes observed in the trained MI in all training conditions, whereas untrained control animals showed no interhemisphere difference. To examine whether the effects were a function of stimulation intensities used, FP amplitudes were compared for a range of stimulation intensities at multiples of threshold intensity. Similar FP enhancement was evident across stimulation intensities, as illustrated by the I/O relationships in single examples from STP, ETP, and STP + 2 mts rats (Fig. 2A). No such interhemisphere difference was observed in an untrained control rat (left). Stimulation intensities required to produce threshold responses (0.2 mV) were not significantly different in both hemispheres for all three conditions (trained MI 7.9 ± 0.3 μA; untrained MI 8.1 ± 0.28 μA; n = 28, P > 0.5), indicating that the documented learning effect did not depend on the absolute stimulation intensities applied. Figure 2B illustrates evoked responses from individual animals (same as in Fig. 2A) for all stimulation intensities and training conditions.

Group data confirmed the persistent FP enhancement with and without extended task performance. The mean FP amplitude ratios between trained and untrained hemispheres were similar for all three groups: STP 1.43 ± 0.08 (n = 31, P < 0.0001); ETP 1.44 ± 0.07 (n = 20, P < 0.0001); STP + 2 mts 1.52 ± 0.092 (n = 8, P < 0.0001) (Fig. 2C). These ratios were significantly greater than those in untrained controls, which showed no significant interhemisphere difference (0.95 ± 0.09, n = 23, P = 0.9). These results demonstrate that the synaptic strengthening of MI intracortical connections observed after 3–6 training days persisted irrespective of whether task performance was continued.
Shift of the synaptic modification range

We next determined the effect of extended task performance and retention on the synaptic modification range. Repeated high- and low-frequency stimulations were used to determine LTP and LTD saturation levels, which define the upper (ceiling) and lower (floor) limits of the synaptic modification range. Figure 3 (left) illustrates LTP saturation experiments for individual animals in the three different training conditions: 3 days (Fig. 3A, STP), 23 days (Fig. 3B, ETP), and 5 days trained and recorded 2 mts later (Fig. 3C, STP + 2 mts). As previously shown (Rioult-Pedotti et al. 2000), STP yielded small levels of LTP in the trained compared with the untrained MI (Fig. 3A, left, individual rat, traces 2 and 4). However, LTP amounts were large in both hemispheres in the ETP rat (Fig. 3B, left, traces 6 and 8), and in the STP + 2 mts rat (Fig. 3C, left, traces 10 and 12). Group data confirm the return to normal LTP (with respect to initial baseline) in ETP and STP + 2 mts rats. Figure 3 (right) illustrates maximum LTP for all three training conditions. The relative amount of LTP was similar in the trained (148.0 ± 4.5%, n = 9) and untrained MI (146.7 ± 3.9%, n = 6, P = 0.83) in the ETP group (Fig. 3B, right) as well as in the STP + 2 mts rats (untrained MI: 151.72 ± 4.13%, n = 6; trained MI: 146.3 ± 3.02%, n = 6, P = 0.74; Fig. 3C, right). This is in marked contrast to the STP group (Fig. 3A, right) where LTP was saturated at 115.6 ± 5.5 and 146.6 ± 6.7% of baseline in the trained and untrained MI, respectively (n = 5 for both hemispheres, P = 0.007).

LTD levels, like those of LTP, were affected by ETP and STP + 2 mts; less LTD was found in the trained MI than in the STP group. However, LTD saturation was similar to that of untrained rats. Figure 4 (left) illustrates LTD saturation experiments. Results from single cases show no interhemisphere difference with ETP (Fig. 4B, traces 6 and 8) and STP + 2 mts (Fig. 4C, traces 10 and 12) but greater LTD in the trained compared with the untrained MI in an STP rat (Fig. 4A, traces 4 and 2). Group data verify that LTD saturation in the ETP and STP + 2 mts groups returned to pretraining levels (maximal LTD: ETP, trained MI = 75.9 ± 2.9%, n = 7; untrained MI = 74.8 ± 2.2%; n = 5, P = 0.72; STP + 2 mts, trained MI = 65.8 ± 3.3%, n = 5; untrained MI = 69.9 ± 2.3%, n = 5, P = 0.34) (Fig. 4, B and C, right). In terms of the relative amount of LTD, the ETP trained MI was different from the STP trained MI (59.7 ± 2.7%, n = 6, P = 0.0004), but not different from the STP untrained MI (76.4 ± 3.2%, n = 7, P = 0.91; Fig. 4A, right). The same is true when STP and STP + 2 mts groups are compared (untrained, P = 0.001; trained, P = 0.84).

The difference in LTP and LTD saturation between the three experimental groups is readily seen by comparing the ratios of saturated LTP and LTD between trained and untrained MI for all training conditions (Fig. 5, A and B). Figure 5A illustrates...
that initial learning (STP) reduced the interhemisphere ratio of maximal synaptic strength (ceiling) from 1.05 ± 0.06 (left/right) in untrained controls (Ctrl) to 0.38 ± 0.09. The interhemisphere ratio recovered to control levels in the ETP group (0.99 ± 0.12) and in the STP + 2 mts group (0.99 ± 0.08). The amount of available LTD was also evident when the ratio between sides was compared. Initial training produced an increase in the interhemisphere ratio from 1.04 ± 0.08 in untrained controls (Ctrl) to 1.61 ± 0.14 (STP). The interhemisphere ratio of saturated LTD returned to pretraining levels following extended task performance (1.02 ± 0.06) as well as when performance was discontinued following initial training (1.09 ± 0.19) (Fig. 5B). Pathways that were saturated with repeated low-frequency stimulation were successfully repotentiated, demonstrating that synaptic modification mechanisms were intact when LTD was measured.

Analysis of LTP/LTD as percentage of baseline illustrates that STP leads to a transient decrease in LTP and increase in LTD, which then returns to pretraining levels with and without continued task performance. This result gives the impression that the Ctrl and ETP and STP + 2 mts synaptic modification ranges are identical. Comparing absolute FP amplitudes at saturation reveals a different picture. Figure 5C shows the same data set as used for previous figures in absolute amplitudes (in millivolts) at baseline, saturated LTP (dark gray) and saturated LTD (light gray) in all training conditions for each hemisphere. This analysis reveals that in contrast to the FP enhancement, the synaptic modification range was altered in the ETP and the STP + 2 mts rats compared with STP and control rats. A new ceiling and floor of the synaptic modification range is established irrespective of whether the animals continue to perform the task or not (ceiling Ctrl: 0.95 ± 0.04 mV, n = 16; STP + 2 mts tr: 1.37 ± 0.07 mV, n = 6; P = 0.003; floor Ctrl: 0.48 ± 0.06 mV, n = 14, STP + 2 mts tr: 0.73 ± 0.06 mV, n = 6, P = 0.02), whereas the baseline responses, once enhanced, remain enhanced (baseline Ctrl: 0.74 ± 0.035, n = 32; STP + 2 mts tr MI: 0.97 ± 0.028, n = 11, P = 0.0015). The new limits effectively place the enhanced synaptic strength back to the middle of the new operating range, regaining the ability to both increase and decrease synaptic strength. Individual amplitude measurements for controls, STP, and STP + 2 mts are illustrated in Supplementary Fig. 1.1

DISCUSSION

This study reveals that the initial, learning-related enhancement of synaptic strength is maintained for weeks and parallels the retention of the initially learned skill. Second, the synaptic modification range shifted upward and restored full dynamic operating range. The persistent change in synaptic strength could reflect the permanent storage of the skill, whereas the range shift might ensure the availability for new synaptic strengthening and suggests a novel homeostatic mechanism in vivo.

Sustained strengthening and task retention

That memories can last for a very long time, even a lifetime, leads to the question whether synaptic strengthening can last,
last for weeks (Trepel and Racine 1998). Our study shows that learning-induced LTP-like increase in synaptic strength is sustained for months while the motor skill is remembered, suggesting that persistent modification might be related to retention of the skill.

The initial enhancement with task acquisition (STP) and persistence with or without extended task performance (ETP and STP + 2 mts) could result from the same or different mechanisms. Task acquisition and/or retention could modify intrinsic properties, synaptic properties, or neuronal connectivity. Increased membrane excitability could lead to larger postsynaptic responses as has been shown during early phases of classical conditioning in hippocampus and MI (Aou et al. 1992; Moyer et al. 1996; Thompson et al. 1996). The finding that membrane excitability in animals that demonstrated long-term retention was indistinguishable from that of naïve controls suggests that this mechanism is not involved in the consolidation process (Thompson et al. 1996). It is therefore unlikely that altered excitability accounts for increased FPs with initial task learning in our experiments because absolute stimulation intensities were not modified with learning. Increased excitability would also more effectively depolarize the postsynaptic neurons during LTP inducing stimulation and thus would lead to increased LTP, rather than occluded LTP as documented in our studies. Moreover, the peak amplitudes of FPs in trained and untrained hemispheres show no temporal displacement, which is consistent with a slope change. Therefore increased FPs are most likely to be of a synaptic nature (Rioult-Pedotti et al. 1998).

Enhanced FP responses could be achieved through a change in the balance between excitation and inhibition. Because inhibition and excitation are most likely temporally overlapping (Anderson et al. 2000; Borg-Graham et al. 1998; Wehr and Zador 2003), especially if inhibitory and excitatory synapses are colocalized on dendrites (Keller 2002; Knott et al. 2002), their relative contribution cannot easily be determined with methods used in this study. We therefore cannot rule out a role for reduced inhibition as a mechanism for the learning-induced enhanced FPs observed here.

Modification and insertion of glutamate receptors to existing synapses have been established as constituting one mechanism for enhanced synaptic strength (reviewed in Derkach et al. 2002; Malenka 2003). Enhanced glutamate-receptor–mediated transmission would lead to larger FPs without affecting the modification range and thus would be consistent with our observations of enhanced population responses and unchanged modification range after initial task learning (STP). Different regulatory mechanisms are likely to be involved in the maintenance of enhanced responses. For example, sustained synaptic strengthening could be mediated by larger or more numerous synapses (reviewed in Harms and Dunaevsky 2006).

**Restoration of synaptic plasticity**

Initial task acquisition and performance improvement seem to drive population synaptic strength to the upper limit of a fixed operating range, thus creating an imbalance. The saturating effect of learning–related modification predicts that unless synapses are placed closer to the midpoint of the synaptic modification range, additional strengthening through LTP-like
mechanisms in vivo would be blocked. Further synaptic strengthening might be prevented by learning-induced general-
ized strengthening inhibition of plasticity in MI, or by homeostatic compensatory mechanisms (Roth-Alpermann et al. 2006), or by a shift of the operating range to adjust to the new enhanced synaptic strength. We have shown that the capacity for MI horizontal connections to express normal amounts of LTP and LTD restores with time after a motor skill has been acquired with or without continuous daily performance. A shift of the operating range was revealed only when absolute response amplitudes were considered (Fig. 5C), whereas the relative measurements simply indicate recovery of LTP and LTD to prelearning levels (Fig. 5, A and B).

Mechanisms involved in the restoration of plasticity are unknown. The observed shift in the modification range could reflect changed features of existing synaptic connections (Luescher et al. 2000) or changes in connectivity (i.e., formation of new synapses). Motor skill learning-dependent synapto-
genesis has been shown to occur in functionally reorganized cortex (Kleim et al. 2002). Formation of new synapses could be used for new synaptic strengthening with additional task learning. To be consistent with our observation of maintained synaptic strength newly formed synapses would have to remain inactive during normal activity. Such “silent synapses” have been detected in the adult brain (He et al. 1998; Nusser et al. 1998), but are much more prevalent during development (Isaak et al. 1995; Liao et al. 1995). We find that with ETP and STP + 2 mts the synaptic modification range shifts rather than expands. The formation of silent synapses might therefore explain restoration of the ability to induce LTP but not the reduced LTD in the ETP and STP + 2 mts animals compared with STP. It is possible that other homeostatic mechanisms keep the size of the modification range constant. Motor memories undergo a process of consolidation over time and develop even in the absence of training or task performance. These initially labile memories progress to a more durable condition and finally to a permanent memory (Shadmehr and Brashler-Krug 1997). The shift of the floor of the modification range toward larger values may represent such a process of consol-
idation where existing patterns of strengthened synapses might become permanently established.

The shift of the synaptic modification range could present an alternate interpretation of the postulate that the sum of all synaptic weights remains constant (Abbott and Nelson 2000; Fregnac 1998; Miller 1996; Turrigiano 1999; Turrigiano and Nelson 2000). Our present findings show evidence for a novel homeostatic mechanism in vivo: instead of returning the integ-
ratrice function of neurons back to within the working range as most models suggest, our results indicate that homeostasis is regained by adjusting the supposedly finite limits of the working range. Whether additional learning can take advantage of the newly available range for further learning is yet to be determined experimentally and would be a strong test of the relationship between learning, synaptic strength, and LTP. Plasticity of synaptic modification range adds a new dimension to how neural circuits change with learning and consolidation. The marked changes in MI appear to provide a useful system to test whether range plasticity is an important mechanism for the acquisition of new cortically mediated behaviors.

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