Dynamic rewiring of neural circuits in the motor cortex in mouse models of Parkinson’s disease

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Dynamic adaptations in synaptic plasticity are critical for learning new motor skills and maintaining memory throughout life, which rapidly decline with Parkinson’s disease (PD). Plasticity in the motor cortex is important for acquisition and maintenance of motor skills, but how the loss of dopamine in PD leads to disrupted structural and functional plasticity in the motor cortex is not well understood. Here we used mouse models of PD and two-photon imaging to show that dopamine depletion resulted in structural changes in the motor cortex. We further discovered that dopamine D1 and D2 receptor signaling selectively and distinctly regulated these aberrant changes in structural and functional plasticity. Our findings suggest that both D1 and D2 receptor signaling regulate motor cortex plasticity, and loss of dopamine results in atypical synaptic adaptations that may contribute to the impairment of motor performance and motor memory observed in PD.

The loss of midbrain dopaminergic neurons is the hallmark of PD, a condition characterized by deficits in both fine movement control and motor learning. While the decline in movement control in PD has been linked to alterations in basal ganglia plasticity, the deficiencies in motor learning remain largely unexplored. The basal ganglia receive glutamatergic inputs from the primary motor cortex (M1), a region that is essential for motor control and the acquisition of motor skills. M1 cortical neurons have both D1 and D2 classes of dopamine receptors and receive direct dopaminergic projections from the ventral tegmental area and substantia nigra pars compacta via mesocortical pathways. Dopaminergic terminals from the mesocortical pathway richly innervate M1 dendritic processes in both superficial and deep layers of the rodent and primate cortex, and activation of this pathway could directly modulate M1 cortical activity. Stimulation of dopaminergic projections induces activity-dependent Fos expression in M1 (ref. 2), and, using voltage-sensitive dye imaging, this evoked neuronal activity has been shown to extend throughout the M1 region. In addition, dopaminergic signaling within M1 modulates the synaptic plasticity of horizontal, intracortical connections and is important for optimizing motor skill learning. Because proper M1 processing is essential for motor learning, altered M1 plasticity may be the key mechanism underlying the severe motor learning deficits observed in PD. However, very little is known about dopamine depletion–induced synaptic adaptations in the motor cortex in vivo, particularly in relation to structural and functional plasticity in M1 during the progression of PD.

The formation and maintenance of memories involve long-term synaptic structural and functional plasticities. Structural synaptic plasticity generally occurs at dendritic spines, and recent studies suggest that functional synaptic plasticity, such as long-term potentiation (LTP) and long-term depression (LTD), can be accompanied by these structural changes at dendritic spines. For example, studies that use stimulation protocols to produce LTP can induce de novo spine formation and enlargement of spine volume. Conversely, LTD induction protocols can induce spine shrinkage and spine elimination. In the motor cortex, synaptic structural changes are associated with enhancement (LTP) or reduction (LTD) of synaptic efficacy. The learning of new motor skills can lead to spine genesis and remodeling, and these de novo spines are preferentially stabilized during subsequent training, ensuring long-term memory storage. In addition, there is strong evidence that blocking dopamine receptors in M1 can abolish LTD induction in M1 superficial layers and can impair motor learning. Despite substantial support for direct, pathological changes in M1 in PD, little is known about how dopamine regulates motor cortex physiology—specifically, how dendritic spine dynamics and synaptic functional plasticity are altered by the loss of dopaminergic innervation, and what the relationship is between them.

To address these fundamental questions, we investigated the process of dopamine depletion–induced synaptic remodeling in the intact motor cortex by repeatedly imaging the apical dendrites of layer V pyramidal neurons. Neurons were identified by expression of yellow fluorescent protein in the Thy1-YFP-H mouse line using transcranial two-photon laser scanning microscopy, and dendritic spines were studied over time. We found marked increases in both spine elimination and formation in PD mouse models. In addition, we elucidated...
distinct roles for D1 and D2 dopamine receptors in M1: D1 receptor signaling regulates spine elimination, while D2 dopamine receptor signaling was linked to spine formation. We also found evidence for the dissociation between functional LTP/LTD and motor cortex spine elimination/de novo spine formation, pointing toward a new mechanism by which neuronal activity and dopamine modulate functional and structural plasticity at excitatory synapses. Lastly, we found that dopamine depletion impaired performance during new motor skill learning and impaired learning-induced spine dynamics. Together, our studies reveal distinct roles for D1 and D2 dopamine receptor signaling in regulating spine dynamics and functional plasticity in M1. Given that these roles are disrupted by dopamine depletion, our study suggests that abnormal spine turnover in the motor cortex may contribute to motor deficits observed in PD.

RESULTS

Dopamine depletion enhances spine dynamics in motor cortex

Most excitatory synapses are located at dendritic spines, and changes in spine morphology and dynamics reflect synaptic plasticity. Structural remodeling is enhanced during experience-dependent learning and memory, as well as during pathological changes associated with neurodegenerative and neurological diseases. These structural changes in both normal and disease states highlight massive adaptations that result in the constant rewiring of neural circuits. To investigate the process of dopamine depletion-induced synaptic remodeling, we used transcranial, two-photon laser scanning microscopy to repeatedly image the same apical dendrites of layer V pyramidal neurons labeled by expression of yellow fluorescent protein in the Thy1-YFP-H line in the forelimb area of the motor cortex. By comparing images taken from two time points in the superficial layers of the motor cortex, we identified spines as newly formed, eliminated, filopodial or stable.

Figure 1 Spine turnover is increased in the dendritic spines of layer V pyramidal neurons of M1 in two mouse models of PD. (a) M1 vasculature underneath the thinned skull of a Thy1-YFP-H control mouse. Red square indicates a region of interest, which is visualized and expanded in b to reveal layer V pyramidal neurons. (c–e) Repeated imaging of the same dendritic regions reveals spine elimination (arrows) and spine formation (arrowheads). Dendritic stretches are from control (c, expanded from b), MPTP-treated (d) and reserpine-treated (e) mice. Images taken before (day 0) or 4 d after (day 4) drug injection. Scale bars represent 100 μm (a), 20 μm (b) or 2 μm (c–e). (f) Spine elimination in >2-month-old control, MPTP-injected and reserpine-injected mice (control: 6.2 ± 0.1%, n = 21 mice; MPTP: 10.9 ± 0.3%, n = 30 mice; P < 0.0001; reserpine: 10.0 ± 0.3%, n = 6 mice; P = 0.0003). (g) Spine formation in >2-month-old control, MPTP-injected and reserpine-injected mice (control: 4.8 ± 0.1%, n = 21 mice; MPTP: 8.5 ± 0.2%; n = 30 mice; P < 0.0001; reserpine: 9.8 ± 0.3%; n = 6 mice; P = 0.0003 when compared to control). (h) Spine elimination in 1-month-old control and MPTP-injected mice (control: 9.0 ± 0.4%, n = 4 mice; MPTP: 14.2 ± 0.5%, n = 5 mice; P = 0.0159, Mann-Whitney). (i) Spine formation in 1-month-old control and MPTP-injected mice (control: 6.4 ± 0.5%, n = 4 mice; MPTP: 9.8 ± 0.4%; n = 5 mice; P = 0.0159, Mann-Whitney). (j) Spine elimination in the barrel cortex of control and MPTP-injected mice (>2 months old) (control: 6.5 ± 0.2%, n = 5 mice; MPTP: 6.6 ± 0.2%, n = 7 mice; P = 0.6237, Mann-Whitney). (k) Spine formation in the barrel cortex of control and MPTP-injected mice (>2 months old) (control: 5.6 ± 0.2%, n = 5 mice; MPTP: 5.9 ± 0.4%; n = 7 mice; P = 0.5303, Mann-Whitney).

Box-and-whisker plots indicate the minimum, 25th, 50th and 75th percentiles, and maximum. Numbers in parentheses indicate numbers of animals used for analysis. *P < 0.05, ***P < 0.001; n.s., nonsignificant; Mann-Whitney.
models, pre-existing spines became significantly less stable than those in control mice over the same time periods: fewer pre-existing spines remained in MPTP-treated mice. During the same period of time in control mice, most pre-existing spines remained stable (Fig. 2h). Taken together, these data indicate that dopamine depletion triggered rapid and persistent remodeling of neural circuitry in the motor cortex. The net effect is that the pre-existing synaptic connections became unstable.

**D1 and D2 signaling differentially regulate spine dynamics**

Exogenously administered 1-3,4-dihydroxyphenylalanine (L-DOPA), the endogenous precursor of dopamine, is the most widely used drug for treating PD. To confirm the involvement of dopamine in structural plasticity in the motor cortex, we examined whether L-DOPA could reverse the enhanced spine dynamics seen in MPTP-treated mice. We treated mice with MPTP once daily for 4 consecutive days, followed by another 4 d of treatment with L-DOPA (i.p., 100 mg/kg, once daily) combined with MPTP (Fig. 3a). We found that L-DOPA could partially rescue the enhancement of spine elimination and formation caused by MPTP. Although the rate of spine turnover was still larger in animals treated with MPTP and L-DOPA compared to that in saline injected controls, the increases in the elimination and formation rates induced by MPTP with L-DOPA administration were significantly lower than those induced by MPTP injection alone (Fig. 3b). These results confirm that the enhancement in motor cortex spine dynamics observed in MPTP mice is dependent on dopamine depletion.

Dopamine exerts its function through intracellular G protein–coupled receptor signaling cascades. There are two main classes of coupled receptor signaling cascades. There are two main classes of...
The D1 class, which is coupled to G\textsubscript{s} (stimulatory) signaling, and the D2 class, which is coupled to G\textsubscript{i/o} (inhibitory or other) signaling. Activation of D1 receptors leads to increases in cytosolic cyclic AMP levels and activation of protein kinase A (PKA), while activation of D2 receptors leads to inhibition of cAMP. To further understand how distinct dopamine receptor signaling regulates spine dynamics in the motor cortex, we examined spine elimination and formation in adult mice treated with the D1 receptor antagonist SCH23390 or the D2 receptor antagonists haloperidol and raclopride (Fig. 3a and Supplementary Fig. 3). Unexpectedly, we found that D1 receptors were uniquely involved in spine elimination and D2 receptors were uniquely involved in spine formation. SCH23390 (0.25 mg/kg, i.p., twice daily) significantly promoted spine elimination but had no effect on spine formation in the same animals (Fig. 3c,d,f and Supplementary Table 1). In contrast, haloperidol (3 mg/kg, i.p., once daily) and raclopride (0.4 mg/kg, i.p., twice daily) did not change spine elimination rates, but significantly increased the rate of spine formation (Fig. 3e,f, Supplementary Fig. 3 and Supplementary Table 1). The increase in spine elimination by D1 antagonist mimicked the changes in spine elimination observed in MPTP-treated mice (P = 0.9197 compared to control; haloperidol, Mann-Whitney), while the increase in spine formation by D2 antagonist resembled the alterations in spine formation rate in MPTP-treated mice (P = 0.8016 compared to control, Mann-Whitney). Because D1 antagonists promoted spine elimination without affecting formation, SCH23390 treatment resulted in a reduction in spine density compared to MPTP treatment. In contrast, haloperidol treatment resulted in a significant increase in spine density (Fig. 3g). In addition, prolonged treatment with D1 or D2 antagonists also promoted consistent and cumulative increases in spine elimination or formation, respectively (Fig. 3h and Supplementary Table 1). As expected, the consistent increases in spine elimination and formation with these treatments resulted in further alterations in spine density (Fig. 3i). Taken together, these results suggest that l-DOPA can partially...
rescue the enhanced spine turnover seen in PD mouse models and that distinct dopamine receptor activity is critical in maintaining proper spine dynamics.

Direct dopaminergic projections regulate M1 spine turnover
Pyramidal neurons in M1 express both D1 and D2 classes of dopamine receptors. Dopaminergic modulation of M1 can arise from two anatomically and functionally distinct pathways: mesocortical or nigrostriatal projections. Mesocortical afferents project directly to M1, function in motor learning and have the potential to critically modulate synaptic and structural plasticity in M1 (refs. 2,9). In contrast, nigrostriatal afferents indirectly project to M1 via the basal ganglia and function in movement execution26. The nigrostriatal system exerts powerful modulation on the direct and indirect pathways of the basal ganglia, in which direct pathway striatal projection neurons express D1 receptor and indirect pathway striatal projection neurons express D2 dopamine receptor29. The substantia nigra reticulata is the output nucleus of these two striatal pathways, and it provides inhibition to the thalamus, which subsequently excites the cortex through glutamatergic synapses. Thus, nigrostriatal projections can indirectly modulate structural plasticity in the motor cortex through cortex–basal ganglia–thalamus closed-loop feedback.

To directly test these two possibilities, we combined in vivo imaging experiments through open-skull chronic cranial windows with subsequent focal dopaminergic terminal lesions. We turned to open-skull cranial windows because intracranial injections compromise the bone structure, which prevents us from using the thinned-skull preparation for in vivo imaging. After imaging dendritic structures through a chronic cranial window, we selectively eliminated dopaminergic terminals either in the forelimb area of M1 or the dorsal lateral striatum by stereotaxic local injection of the neurotoxin 6-hydroxydopamine (6-OHDA) (Fig. 4a,b). The denervation of dopaminergic terminals in M1 and the striatum was verified by immunostaining for tyrosine hydroxylase (TH). TH immunofluorescence in M1 cortical layers was nearly absent in the ipsilateral hemisphere 8 d after M1 6-OHDA injections, while TH signals in the striatum were comparable to those on the contralateral side (Fig. 4c and Supplementary Fig. 4). In contrast, TH signal in M1 was only slightly reduced (~30% reduction in TH immunofluorescence intensity, n = 5 mice each in control and 6-OHDA groups) as compared to the un.injected contralateral hemisphere following striatal 6-OHDA injection (Fig. 4d and Supplementary Fig. 4).

We imaged the same dendrite segments through the cranial window before and 8 d after 6-OHDA intracranial injection. Both spine elimination and formation in the motor cortex were significantly increased following M1 6-OHDA lesion. To assess the effect of eliminating dopaminergic terminals in the dorsal lateral striatum, we injected 6-OHDA into both M1 and the striatum. The results showed no further enhancement in either spine elimination or formation compared to those in M1 6-OHDA lesion models (Fig. 4c and Supplementary Table 1). Taken together, these data suggest that loss of dopamine promotes spine elimination and formation in M1 primarily through local mechanisms involving direct mesocortical projections, although we cannot exclude the possibility that basal ganglia pathways may also contribute to the effect.

Dopamine regulation of LTP and LTD
Our data from the 6-OHDA local lesion experiments suggested that primarily local synaptic mechanisms are involved in spine remodeling in M1. Therefore, we next tested whether synaptic plasticity at glutamatergic synapses in layer V pyramidal neurons is compromised following dopamine depletion. Many lines of evidence suggest that structural plasticity is a reflection of, or is caused by, functional, long-term changes in synaptic efficacy. A simple, parsimonious model is that long-term potentiation (LTP) stimulates spine formation, while long-term depression (LTD) promotes spine elimination12–16. In addition, LTD is important for stabilizing newly formed spines, along with inducing spine growth30. Our data demonstrated that dopamine depletion enhanced both spine elimination and formation by reduced

![Figure 4](image-url)
D1 and D2 receptor activity, respectively. This suggests that dopamine may control synaptic long-term plasticity through these two different classes of receptors in the motor cortex.

To directly test this hypothesis and to determine whether D1 and D2 signaling is involved in LTP and LTD induction, we performed whole-cell patch clamp recordings from identified neurons in M1 from young adult Thy1-YFP-H mice. We placed stimulation electrodes in the superficial layer of the cortex and used minimal stimulation to activate superficial layer inputs on layer V pyramidal neurons (Fig. 5a). Consistent with previous findings\(^2\), presynaptic stimulation paired with postsynaptic depolarization (pairing protocol) reliably induced LTP in layer V pyramidal neurons in the motor cortex, and this LTP induction was completely blocked by the NMDA-receptor blocker 3-[(R)-2-carboxypropyl]azetidin-4-yl]propyl-1-phosphonic acid (R-CPP, 10 μM) (Fig. 5b–f). To our surprise, LTP induction was impaired in dopamine-depleted mice by both reserpine injections and 6-OHDA lesions (Fig. 5g–i). To test whether the changes in spine dynamics were M1 specific, we assessed whether LTD induction in the neighboring barrel cortex would also be affected by dopamine depletion. Consistent with our data on structural plasticity in the barrel cortex, we found that LTD induction in the barrel cortex was not impaired in reserpine-injected mice (Supplementary Fig. 5).

To further understand how dopamine receptor signaling is involved in LTP in the motor cortex, we perfused brain slices with D1 and D2 receptor blockers. We found that LTP was specifically blocked by the D1 receptor antagonist (SCH23390, 3 μM) (Fig. 5j), but was not affected by the D2 receptor antagonist (sulpiride, 5 μM) (Fig. 5k). By contrast, LTD could not be elicited by pairing low-frequency stimulation with slight membrane depolarization (Fig. 6a), which was consistent with previous findings that LTD induction is age dependent\(^2\). LTD induction was also absent in dopamine-depleted mice (Fig. 6b–d). Notably, the same LTD pairing protocol successfully induced LTD in brain slices from young adolescent mice (3 weeks old). This form of LTD in young adolescent animals was impaired after reserpine injection (Fig. 6e,f). Taken together, our results indicate that D1 and not D2 receptor activation is required for LTP and that dopamine depletion impairs LTP induction at the superficial layer synapses on layer V pyramidal neurons. LTD in young adolescent mice was also affected by dopamine depletion. However, LTD elicited by the pairing protocol was absent in the motor cortex of adult mice, suggesting LTD may not directly contribute to changes in spine dynamics in adult mice.

Dopamine regulates structural and functional plasticity

What could promote extensive spine elimination and formation without enhancing LTP and LTD in parallel? One possibility is that LTP and LTD may be occluded following dopamine depletion, as spines are already at their maximum capacity for functional plasticity.
Glutamate uncaging

**maximum.**

**mice;** formation: control: 5.3 ± 0.4%,

n.s., nonsignificant, comparing to control

10.1 ± 0.5%,

n = 5 mice; P = 0.4127, Mann-Whitney). Inset: average EPSCs

before (1) and after (2) LTD induction.

Box-and-whisker plots indicate the minimum, 25th, 50th and 75th percentiles, and maximum. Error bars represent mean ± s.e.m. Numbers in parentheses indicate numbers of neurons recorded. *P < 0.05, **P < 0.01; n.s., nonsignificant, comparing to control group; Mann-Whitney. Insets: average EPSCs before (1) and after (2) LTD induction.

If this were true, then aberrant synaptic plasticity could prevent spines from undergoing further formation and elimination. However, the sustained and cumulative change in spine turnover during prolonged dopamine depletion (Fig. 2) argues against this hypothesis. Instead, our data imply that spines represent readily available substrates for structural plasticity that do not necessarily correlate with functional long-term synaptic plasticity.

This points to a new model for dopamine regulation of structural and functional plasticity in the adult motor cortex: de novo spine formation and elimination are dissociated from functional long-term plasticity and instead are distinctly regulated by D1 and D2 receptor signaling (Supplementary Fig. 6). D2 receptor signaling is selectively linked to spine formation, while D1 receptor signaling is selectively linked spine stabilization and elimination. Furthermore, this model emphasizes that LTP may share a common mechanism with stabilization of dendritic spines but is not necessary for spine formation, and, conversely, lack of LTP may promote spine elimination.30

Our results support this model: we find that dopamine signaling, specifically through D1 receptor activation, is required for LTP.

**Figure 6** LTD following dopamine depletion. (a,b) Representative experiments showing LTD induction in control (a) and reserpine-injected (b) mice. (c) Summary data showing LTD induction in control and reserpine-injected mice (LTD in control: 91.9 ± 6.54%, n = 5 cells from 3 mice; reserpine: 103.4 ± 13.22%, n = 5 cells from 5 mice, P = 0.4127, Mann-Whitney). (e) Summary data showing LTD inductions in control, R-CPP-treated and reserpine-injected conditions in young (3-week-old) mice. (f) Average EPSC amplitude 25–30 min after LTD induction in control, R-CPP and reserpine groups. Black bar indicates LTD induction (control: 69.1 ± 7.04%, n = 5 cells from 3 mice; R-CPP: 100.2 ± 4.52%, n = 5 cells from 3 mice, P = 0.0159 compared to control; reserpine: 107.3 ± 4.86%, n = 5 cells from 3 mice, P = 0.0079 compared to control, Mann-Whitney).

**Figure 7** Spine formation and elimination are separately regulated. (a) Schematic showing de novo spine formation (arrowhead) using combined two-photon imaging and two-photon glutamate uncaging (red circle). (b) Time-lapse images show de novo spine formation induced by photolytic glutamate release (left). Fluorescence intensity profiles along the yellow line show increase of spine head fluorescence (arrowhead) (right). Scale bar, 1 µm. (c) Summary statistics for de novo spine formation in various conditions. The dotted line indicates the percentage of successful spine formation in the control condition. Numbers in parentheses indicate number of induction attempts for each condition (SCH23390: 8.9%, n = 45 trials, P = 1; sulpiride: 29.6%, n = 54 trials, P = 0.0049; haloperidol: 31.43%, n = 35 trials, P = 0.0083; R-CPP: 2.5%, n = 80 trials, P = 0.0302; MK801: 4.9%, n = 41 trials, P = 0.3624; all Fisher’s exact test comparing to control group). (d) Spines eliminated (left) and formed (right) over 4 d in control and MK801-treated mice (spine elimination: control: 6.6 ± 0.3%, n = 7 mice; MK801: 10.1 ± 0.5%, n = 5 mice; P = 0.0025 compared to control; spine formation: control: 5.3 ± 0.3%, n = 7 mice; MK801: 4.7 ± 0.4%, n = 5 mice; P = 0.1490 compared to control; Mann-Whitney). Box-and-whisker plots indicate the minimum, 25th, 50th and 75th percentiles, and maximum. **P < 0.01; n.s., nonsignificant; Mann-Whitney.
Functionally, chronic dopamine depletion or blockade of D1 receptors abolished LTP induction, while structurally, it promoted spine elimination. In parallel, chronic dopamine depletion or D2 receptor blockade promoted spine formation without involvement of functional synaptic potentiation. This model predicts that LTD is not required for spine elimination, which is consistent with previous findings that LTD induction in many cortical regions is reduced or absent in the adult brain. This model also highlights the important function of LTP in spine remodeling during motor learning. New experiences and activities resulting in new spine formation, together with LTP-induced spine stabilization and experience-dependent pruning, represent a stable structural and functional synaptic mechanism for memory storage. In PD, owing to loss of both D1 and D2 receptor activation, both synapse elimination and formation are greatly enhanced.

This model highlights the idea that structural plasticity following dopamine depletion is mediated by local changes in M1. If we can precisely induce spine formation in M1 layer V pyramidal neurons without involving feedback from basal ganglia output, we should be able to enhance spine formation by blocking D2 receptors. By triggering glutamate release via two-photon laser uncaging, a recent study showed that de novo spine growth from the dendritic shaft can be induced with high spatial and temporal precision, and furthermore, these newly formed spines can form functional synapses with nearby axonal boutons. To determine whether de novo spine formation is regulated by D1 and D2 receptors in M1, we used both two-photon imaging...
microscopy to image the apical dendrites of layer V pyramidal neurons and two-photon uncaging of 4-methoxy-7-nitroindolinyglutamate to release glutamate at specific dendritic locations (Supplementary Fig. 7). Because dopamine is critical to spinogenesis during the second postnatal week (postnatal days 7–14) in vivo, we performed our analysis in acute cortical brain slices from young Thy1-YFP-H mice at postnatal days 15–18. Stimulation was applied to aspiny stretches of secondary and tertiary dendrites (Fig. 7a). Stimulation by 40 pulses (0.5 ms) of uncaging laser at 5 Hz induced growth of a new spine with a success rate of ~12% (n = 121 trials) (Fig. 7b,c and Supplementary Movies 1 and 2). We found that blocking D1 receptor activation by bath application of SCH23390 (3 µM) did not affect the success rate of spinogenesis (Fig. 7c), which is consistent with our in vivo imaging results. Conversely, inhibiting D2 receptor activation by sulpiride (5 µM) and haloperidol (2 µM) significantly enhanced the success rate of spinogenesis (Fig. 7c), indicating that D2 receptor signaling serves as a checkpoint for preventing excessive spinogenesis. Consistent with previous findings, we found that preventing NMDA receptor activation by R-CPP (10 µM) nearly abolished spinogenesis (Fig. 7c). Blocking LTP by a use-dependent NMDA receptor blocker, MK801, did not abolish the spinogenesis, although there was a trend suggesting a decreased success rate (Fig. 7c). This suggests that the initial Ca 2+ entry through NMDA receptors in the presence of MK801 is sufficient to induce spinogenesis. Taken together, these data indicate that D2 receptor signaling in the motor cortex is indeed directly associated with spinogenesis without requiring LTP: blocking D2, but not D1, receptor activation markedly enhanced the rate of successful spinogenesis.

Our proposed model also suggests that the lack of LTP could promote spine elimination and lower survival rates of newly formed spines. To investigate this, we tracked newly formed spines following MPTP and dopamine receptor antagonist injections and analyzed their survival rates (Supplementary Fig. 8). The survival rate of newly formed spines in MPTP-injected mice was indeed significantly lower than that in haloperidol-injected mice, which could be attributable to loss of LTP in MPTP-injected mice. To directly test whether blocking LTP could lead to changes in spine dynamics in the motor cortex, we examined spine elimination and formation in mice treated with MK801 (0.5 mg/kg, twice daily), an NMDA receptor blocker that crosses the blood-brain barrier and potently blocks LTP induction. We found that MK801 significantly promoted spine elimination, but it had no significant effect on spine formation in the same animals (Fig. 7d and Supplementary Table 1). Taken together, our data demonstrate the distinct role of long-term synaptic plasticity in spine dynamics in vivo.

These aberrant synaptic adaptations resulting from the degeneration of mesocortical projections to M1 may explain why PD patients have impaired skill learning and memory retention. We directly tested this hypothesis by investigating the relationship between performance on motor skill tasks and structural plasticity in the motor cortex following dopamine depletion. To do so, we trained mice to perform a food-reaching task. During training, control 2-month-old mice gradually increased their reaching success rates in the initial 4 d, after which their success rates plateaued. In comparison, mice pretreated with 4 d of MPTP injections exhibited significantly lower success rates (from day 5 to day 8 when compared to saline-injected control mice, Fig. 8a–c). We housed the same cohort of mice in their home cages for 1 month after their 8-d training period and then tested their performance in the food reaching task. We found that pretrained control mice maintained skillful performance, with high success rates on day 38. However, pretrained MPTP-injected mice showed lower success rates than saline-injected control mice on day 38 (Fig. 8c). Together, these data suggest that performance on a newly learned motor skill is indeed impaired following dopamine depletion.

To investigate the process of learning-induced remodeling of neural circuits in M1 and the adaptation of synaptic mechanisms in PD mouse models, we repeatedly imaged the same dendritic regions of layer V pyramidal neurons through a cranial skull window. Motor learning induced a transient increase in the formation of dendritic spines, accompanied by enhanced spine elimination in the motor cortex contralateral to the reaching forelimb in control mice (Fig. 8d,e and Supplementary Table 2). These data are consistent with previous reports demonstrating that motor skill learning can induce rapid formation of dendritic spines followed by selective pruning. MPTP-injected mice that underwent the same motor skill training did not show significant increases in spine formation when compared to naive MPTP-injected mice; in addition, enhancement of spine elimination after training was also impaired (Fig. 8f,g and Supplementary Table 2).

Previous studies have shown that newly formed, learning-induced spines are preferentially stabilized during training and endure even after training stops, thus providing neural substrate for long-lasting motor memory. LTP is critical in stabilizing dendritic spines, and it is possible that, in MPTP-injected mice, LTP is impaired following loss of dopamine, leading to failures in stabilizing the newly formed, learning-induced spines. To directly test whether spine stabilization is indeed impaired, we analyzed the fate of newly formed spines during and after motor training in control and MPTP-injected mice. We found that, in control mice, newly formed, learning-induced spines were significantly more stable (Fig. 8h), which is consistent with previous findings. At the initial training phase, new spines were more stable in trained MPTP-injected mice than in naive MPTP-injected mice. However, most of the new spines in the trained MPTP-injected mice were then eliminated, and the spine survival rate became not significantly different from that in MPTP-injected naive mice. Taken together, these data indicate that dendritic spines on layer V pyramidal neurons in the motor cortex undergo rapid structural remodeling following dopamine depletion. In addition, selective stabilization of newly formed, learning-induced spines was impaired, as most of these spines were unable to become stable synapses following dopamine depletion. Learning-induced rewiring was unable to undergo stabilization, presumably because of impairments in functional plasticity, such as LTP. The impairment in stabilization of neural circuits provides an additional synaptic mechanism for the motor deficits seen in PD.

**DISCUSSION**

PD results from the degeneration of midbrain dopaminergic neurons, and most research has focused on synaptic adaptations in the striatum, a region that receives the highest density of dopaminergic innervation. Cortical areas, by contrast, have received very little attention, even though the motor cortex, which is the command center that governs precise fine motor control, also receives rich projections from midbrain dopamine neurons. In this study, we investigated dendritic spine mobility and functional plasticity in the motor cortex in PD. We found that both spine elimination and formation in M1 were elevated in PD mouse models. Activity of M1 can be either directly elicited by activating mesocortical dopaminergic projections or indirectly modulated by nigrostriatal projections through the basal ganglia–thalamus–cortex closed-loop feedback system. Our data indicate that structural and functional plasticity in M1 is mainly
influenced by the activity of mesocortical dopaminergic direct projections. The rewiring in the motor cortex was differentially regulated by D1 and D2 dopamine receptors in M1: D1 receptor signaling was directly linked to spine elimination, while D2 dopamine receptor signaling was directly linked to spine formation. Therefore, spine formation and elimination, two distinct structural plasticity mechanisms, are dissociable at the dopamine receptor level. In addition, we found a new relationship among LTP, LTD, spine elimination and de novo spine formation. Furthermore, by combining motor skill training and in vivo imaging, we demonstrated that dopamine depletion impaired performance of newly learned motor skills and compromised learning-induced spine dynamics and selective stabilization in the motor cortex. The motor cortex has critical functions in movement control, and given that these functions are regulated by the mesocortical dopaminergic system and disrupted by dopamine depletion, our study suggests that abnormal spine turnover in the motor cortex may contribute to motor deficits observed in PD.

D1 and D2 signaling in functional and structural plasticity
Dopamine exerts its function by activating G protein–coupled receptors. Two main classes of dopamine receptors are expressed in M1 (refs. 3,4), and we found here that D1 and D2 receptors had distinct roles in regulating dendritic spine elimination and formation. In particular, D1 receptor signaling was selectively linked to spine elimination, while D2 dopamine receptor signaling was selectively linked to spine formation. Blocking D2 receptor activity and depleting dopamine in mouse models of PD promoted spine formation. How activation of D1 and D2 receptors leads to downstream intracellular signaling cascades that regulate spine dynamics is not well understood. Activation of D1 receptors leads to an increase in cytosolic cAMP and activation of PKA, and, conversely, activation of D2 receptors leads to inhibition of cAMP and decrease of PKA activity. A recent study showed that activity induced spinogenesis can be bidirectionally regulated by PKA13. It is therefore possible that PKA activity is involved in regulating spine dynamics in M1 following dopamine depletion.

Dopamine neurons fire action potentials spontaneously, which provide tonic dopamine release in targeted areas. Moreover, dopamine neurons can switch from low-frequency tonic activity to phasic bursts of action potentials. Such firing patterns could encode reward prediction errors and incentive salience38, which are critical for motor skill learning. D2 receptors have higher binding affinity to dopamine and can be preferentially activated during tonic dopaminergic neuron activity. Therefore, tonic activation of D2 receptor may serve as a checkpoint for preventing ectopic growth of dendritic spines that are irrelevant to experience. During reward-based associative learning, phasic dopamine neuron firing will enhance dopamine release and activate low-affinity D1 receptors. Activation of D1 receptors may enhance synaptic connections by regulating the function and trafficking of AMPA and NMDA receptors39. Consistent with this notion, we have found that the D1 receptor is critically involved in LTP induction, which may be required for stabilizing dendritic spines and encoding motor memory during skill learning9. Loss of D1 receptor activation may compromise the consolidation of motor memory and may destabilize pre-existing synapses. Therefore, the changes in spine dynamics in mouse models of PD can be explained by the simultaneous loss of D1 and D2 receptor activation following dopamine depletion: an increase in spine elimination by D1 antagonism mimicked the changes observed in spine elimination in MPTP-treated mice, and an increase in spine formation by D2 antagonism resembled the increase in spine formation rates in MPTP-treated mice. It is worth noting that D1 receptor activation also contributes to LTP at corticostratial synapses. Abnormal information storage in these synapses is linked to the development of l-DOPA-induced dyskinesia40.

Relationship between functional and structural plasticity
One existing model for synaptic plasticity is that long-term potentiation (LTP) stimulates spine formation while long-term depression (LTD) promotes spine elimination, and many lines of evidence support this theory12–16. However, these previous studies may have overlooked the issue of age, as synapses can undergo bidirectional plasticity through early development stages. In addition, LTP promotes the transition of nascent spines to persistent, stable spines30. Notably, we found that spine formation was enhanced in our PD models, even though LTP was blocked by dopamine depletion. We also found that LTD had a minimal presence in the adult cortex, even though dendritic spines continued pruning. Together this suggests a dissociation between LTD and spine elimination. Therefore, our findings suggest an alternative interpretation for dopamine regulation of structural and functional plasticity in the adult motor cortex (Supplementary Fig. 6). This explains our results, which demonstrate that dopamine signaling through D1 receptor activation is required for LTP. Functionally, chronic dopamine depletion or blockade of the D1 receptor prevented LTP induction, while structurally, it promoted spine elimination. In parallel, chronic dopamine depletion or D2 receptor blockage promoted spine formation, without involving functional synaptic potentiation. In PD models, owing to the concurrent loss of both D1 and D2 receptor activation, both synapse elimination and formation in M1 were dramatically enhanced, decreasing synaptic stability. In addition, performing a newly learned motor skill failed to induce further enhancement of spine turnover in MPTP-injected mice.

Involvement of the motor cortex in PD
Patients with PD have deficits in skill learning34, and, although this disease is mostly characterized by degradation of the nigrostriatal dopaminergic system, extrastriatal dopaminergic neurons and other monoamine systems also degenerate, particularly as the disease progresses. PD patients show reduced [18F]DOPA uptake in the motor cortex, which has been used as a marker for the degradation of cortical dopaminergic terminals42. Degeneration of mesocortical projections to M1 may contribute to the synaptic adaptations and skill learning deficits in human PD.

The primary motor cortex not only provides major glutamatergic afferents to the input nucleus of the basal ganglia, but it is also a major target of the basal ganglia output; therefore, this region is likely to transforms patterns of pathological activity into the motor symptoms in PD. There have been few studies investigating the synaptic adaptations and neuronal activities in primary motor cortical neurons in PD.43–45. We found dramatic changes in synaptic dynamics in M1 following dopamine depletion. The consequence of structural remodeling is a net loss of stable spines in layer V pyramidal neurons in PD. Our study further showed that training on a new skill failed to enhance spine formation and elimination, and failed to stabilize new, learning-induced spines in MPTP-treated mice. Taken together, the abnormally enhanced dynamics of neural circuitry and the destabilization of both pre-existing and newly formed, learning-induced spines in response to dopamine depletion may jointly contribute to motor deficits in PD.

Recent studies suggest that neuronal activities in M1 layer V pyramidal neurons undergo drastic changes at both the single-cell and the population level in PD model mice. Therapeutic deep brain stimulation targeting the subthalamic nucleus may directly interfere...
with pathological cortical oscillations in PD models. In addition, a recent study showed that manipulation of subthalamic nucleus neuronal activity by optogenetic stimulation is not sufficient to produce therapeutic effects in PD model mice. In contrast, stimulation of the layer V neurons in M1 or in their axons is beneficial for restoring motor ability. Taken together, the motor cortex is emerging as important in a picture that has implications for both pathology and treatment strategies for PD: the direct modification of abnormal synaptic plasticity and neuronal activities in the motor cortex could have therapeutic effects in patients with PD.

**METHODS**

Methods and any associated references are available in the online version of the paper.

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**AUTHOR CONTRIBUTIONS**

T.X. and I.B.D. designed the experiments, performed pilot experiments and supervised the project. L.G., H.X., Y.C., Y.S. and T.X. performed in vivo imaging experiments. J.-I.K. and I.B.D. performed the electrophysiology experiments. Y.-W.W. performed two-photon uncaging experiments. T.X., R.R.L. and I.B.D. wrote the manuscripts with contributions from all authors.

**COMPETING FINANCIAL INTERESTS**

The authors declare no competing financial interests.

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ONLINE METHODS

Animals. All procedures were approved by Stanford University’s Administrative Panel on Laboratory Animal Care. Adult (1–3 months old) mice (male or female) were used for in vivo imaging and electrophysiology, and postnatal day 15–18 mice (male or female) were used for de novo spinogenesis experiments. Transgenic mice expressing YFP in a small subset of cortical neurons (Thy1-YFP-H line, Jackson Laboratory, JAX 003782)7 were purchased from Jackson Laboratory and were housed and bred in the animal facilities at Stanford University and Wuhan National Laboratory for Optoelectronics with a 12 h light/dark cycle. Animals were randomly assigned to either control saline or drug treatments. All animal experiments were carried out in compliance with protocols approved by Stanford University’s Administrative Panel on Laboratory Animal Care, the Hubei Provincial Animal Care and Use Committee and the experimental guidelines of the Animal Experimentation Ethics Committee of Huazhong University of Science and Technology, China.

Thinned-skull surgical procedure. The procedure for transcranial two-photon imaging was modified from Yang et al.48. Mice were anesthetized via intraperitoneal injection with a cocktail of 17 mg/ml ketamine and 1.7 mg/ml xylazine or with a cocktail of 2% chloral hydrate, 8% urethane and 1.7 mg/ml xylazine in 0.9% NaCl. After resecting the scalp to expose the skull and removing the periosteum, the skull above the cortical area of interest was manually thinned using either a high-speed microdrill or a microsurgical knife under a dissection microscope. The skull was kept cool by periodically dropping sterile saline solution on the surface to avoid friction-induced overheating, which may damage the underlying cortex. The thinned region for imaging was ~200 µm in diameter and ~20 µm in thickness.

Surgical procedure for implanting chronic cranial windows. Mice age 45 d were anesthetized with an intraperitoneal injection containing 2% chloral hydrate, 8% urethane and 1.7 mg/ml xylazine in 0.9% NaCl. The head of the animal was stabilized in a stereotaxic frame. The skin and periosteum were removed to expose the skull from olfactory bulb to cerebellum. A high-speed drill was used to drill a circular groove in the skull above primary motor cortex. Drilling was intermittent for heat dissipation and a cool sterile solution was periodically added to the skull to avoid damage to the underlying cortex from friction-induced heat. When the island of cranial bone moved in response to a light touch by the drill head, it was removed and replaced with a circular cover-glass window. Animals with compromised dura were excluded. A thin layer of cyanoacrylate was applied to the surface of the skull and the edge of cover glass to seal off the exterior, and this was followed by dental resin to cover both exposed skull and wound edge. Finally, a titanium bar with threaded holes was attached to skull posterior to the cranial window for stabilizing the head during subsequent imaging sessions. Animals recovered from surgery for at least 2 weeks before imaging in vivo.

In vivo repeated imaging of dendritic spines. For achieving stable, high-resolution imaging, the skull was glued to a stainless steel plate with an opening over the thinned skull, and the plate was fixed to a metal base. For chronic cranial window imaging, the skull was fixed to the metal base through the titanium bar. Repeated imaging of apical dendritic stretches of 5 µm diameter neurons 10–100 µm below the cortical surface were performed through the thinned area or chronic cranial window using a custom-built two-photon microscope or an Olympus microscope (FV1200) with a Mai Tai Ti:sapphire laser (Spectra-Physics) at 925 nm with a low laser power (output optical power <40 mW) to avoid phototoxicity. Stacks of image planes were obtained with a step size of 0.70 µm using an Olympus water-immersion objective. To relocate and align the same imaged area located according to the stereotactic coordinates and the description from ref. 20. The location of motor cortex for imaging was 1.2 mm lateral from the midline and 1.3 mm anterior to the bregma. That of barrel cortex was 3.4 mm lateral from the midline and 1.1 mm posterior to the bregma. Identifications of spines and filopodia. Individual dendritic protrusions (length greater than one-third of the dendritic shaft diameter) were tracked manually along dendrites. Conservative criteria were used to define filopodia as long thin protrusions with length/neck diameter ratio >3 and head diameter/neck diameter ratio <0.2. Other protrusions were classified as spines. Spine changes, such as elimination and formation, were determined by comparing images collected at two time points. A stable spine was considered as such if it was present in both images. Spines were considered the same on the basis of their spatial relationship to adjacent landmarks and/or their position relative to adjacent spines. An eliminated spine was the one that appeared in the initial image but not the second image. A newly formed spine was the one that was absent in the initial image and then appeared in the second image.

Data quantification and image presentation. All image analysis of spine dynamics was measured manually on the raw image stacks using ImageJ software (NIH ImageJ), blind to the experimental conditions. The same dendritic segments from three-dimensional image stacks with high image quality were used for analysis to ensure that tissue rotation or movements between imaging sessions did not affect identification of the protrusions. Spine heads that contained saturated pixels were excluded. The total number of spines was pooled from the dendritic segments of different mice. The elimination and formation rates of spine were, respectively, the percentages of spines that disappeared or appeared between two imaging sessions, relative to the total spine number of the previous image. Percentages of stable, eliminated and newly formed spines were all normalized to the initial image. Percentage change in total spine number between sessions was relative to the total number of spines in the first image and was calculated as formation rate minus elimination rate. Data on spine dynamics throughout our study are presented as mean ± s.e.m. 2D projections of 3D image stacks containing in-focus dendritic segments with high image quality were selected for making all figures. The projected images were thresholded, contrasted, and median filtered for the final figures.

6-OHDA and drug injections. 6-OHDA or saline (as a control) microinjections were performed after the first imaging session. In brief, 6-OHDA was dissolved with 0.2 mg/ml ascorbic acid at a concentration of 4 µg/ml in saline. Anesthetized animals were positioned in a stereotactic apparatus (STOELTING). A glass electrode filled with 6-OHDA and aimed at M1 or caudate putamen (CPU) was used for injection. The intended stereotactic coordinates were as follows: AP: −1.0 mm; ML: +1.2 mm (with an angle of 60° or 30° from the posterior to the anterior for M1 or CPU, respectively); DV: +0.7 mm or 2.0 mm for M1 or CPU, separately. Animals were randomly assigned into two groups: one received 100 nl 6-OHDA into the M1 forelimb representation area, the other received 500 nl 6-OHDA into the CPU of either hemisphere. The injection was conducted at a rate of 1 µl per 30 min, and the glass electrode was left in place for another 10 min before it was slowly withdrawn over 10 min. The second imaging session was conducted 8 d after injection. Spine dynamics in the motor cortex of 6-OHDA-lesioned and saline-injected animals were determined by comparing images from two time points and then normalizing to the initial images. After the final imaging session, the mice were anesthetized and then transcardially perfused with 4% paraformaldehyde (PFA) in phosphate-buffered saline. The cortical and striatal denervation of dopaminergic fibers were determined by tyrosine hydroxylase (TH) staining. SCH23390 (0.25 mg/kg i.p., twice daily), haloperidol (3 mg/kg i.p., once daily) and raclopride (0.4 mg/kg, i.p., twice daily) were used to block D1 and D2 receptors in vivo. Because sulpiride does not pass through the blood-brain barrier, it was used only in acute brain slices.

Tyrosine hydroxylase (TH) staining and quantification. Mice were deeply anesthetized and perfused with 4% paraformaldehyde (PFA) transcardially. The brains were removed and postfixed in 4% PFA for 5 h. After cryoprotection in 30% sucrose at 4 °C for 2–3 d, brains were sectioned (30 µm) encompassing the entire substantia nigra pars compacta on a cryostat microtome. For studies of cortical and striatal TH-positive fiber, the brain was sectioned (50 µm) on a vibration microtome. After blocking, sections were incubated in rabbit polyclonal anti-TH primary antibody (1:700; Sigma, cat. no. T8700) for 24 h at 4 °C. After washing three times in PBS, sections were incubated in Cy3-conjugated goat anti–rabbit IgG (1:100, Jackson ImmunoResearch Laboratories, cat. no. 111–165–003) secondary antibody for 2 h at room temperature, followed by washes in PBS.

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Numbers of TH-positive neurons were bilaterally counted manually at five-section intervals throughout the entire extent of ventral tegmental area and substantia nigra pars compacta (SNC/VTA) by bright-field microscopy (Nikon Ni-E upright, 10×, NA 0.45, dry, working distance 4 mm) using ImageJ (NIH) software. To quantify changes in the number of TH-positive neurons in the SNC/VTA regions, the number of TH-positive neurons in control mice was normalized to 100%, and the number of TH-positive neurons in MPTP-treated mice was expressed as a percentage of the control (mean ± s.e.m.). TH-positive fibers in the cortex and striatum were imaged (Nikon A1R MP+ upright, 16×, NA 0.8, water, working distance 3 mm) and quantified by measuring the optical density of TH immunofluorescence using MATLAB, and the value was reported as the percentage optical density (mean ± s.e.m.).

Whole-cell patch clamp recording in mouse primary motor cortex (M1). Thy1-YFP-H mice 3–5 weeks old (male and female) were anesthetized with isoflurane and decapitated, and brains were briefly exposed to chilled artificial cerebrospinal fluid (ACSF) containing 125 mM NaCl, 2.5 mM KCl, 1.25 mM NaH2PO4, 25 mM NaHCO3, 15 mM glucose, 2 mM CaCl2 and 1 mM MgCl2 oxygenated with 95% O2 and 5% CO2 (300–305 mosM, pH 7.4). Coronal slices (300-350 µm) containing M1 were prepared using a tissue vibratome (VT1200S, Leica), and slices were first maintained in ACSF for 30 min at 34 °C and then subsequently recovered at room temperature for 30 min. After recovery, slices were transferred to a submersed recording chamber perfused with ACSF at a rate of 2–3 ml/min and maintained at 30–31 °C. Picrotoxin (50 µM) was present to block GABA_A receptor–mediated currents. Motor cortex M1 and barrel cortex layer V pyramidal neurons were identified by YFP signal (BX51, Olympus), and EPSCs were induced by stimulating the superficial layer of M1 and barrel cortex via a concentric bipolar stimulating electrode (FHC) in the area. Tissues surrounding the recording area were cut to prevent polysynaptic responses. Whole-cell voltage clamp recordings were made with glass pipettes (3–4 MΩ) filled with an internal solution containing 126 mM CsMeSO3, 10 mM HEPES, 1 mM EGTA, 2 mM QX-314 chloride, 0.1 mM CaCl2, 4 mM Mg-ATP, 0.3 mM Na2-GTP, 8 mM disodium phosphocreatine (280–290 mosM, pH 7.3 with CsOH), and cells were voltage clamped at −70 mV. EPSCs were evoked at 0.05 Hz and three successive EPSCs were averaged, expressed relative to the normalized baseline. Access resistance was 15–25 MΩ and only cells with a change in access resistance <20% were included in the analysis. Whole-cell patch clamp recordings were performed using Multiclamp 700B (Molecular Devices), monitored with WinWCP (Strathclyde Electrophysiology Software) and analyzed offline using Clampfit 10.0 (Molecular Devices). Signals were filtered at 2 kHz and digitized at 10 kHz (NI PCIe-6259, National Instruments). For pairing LTP, conditioning stimulation was delivered within 13 min of achieving whole-cell configuration. After establishing a stable baseline (5 min), presynaptic stimulation (2 Hz, 360 pulses) was paired with postsynaptic depolarization at +10 mV. For pairing LTD, conditioning stimulation was delivered within 30 min of achieving whole-cell configuration. After establishing a stable baseline (10 min), low frequency stimulation (1 Hz, 200 pulses) was paired with postsynaptic depolarization at −30 mV.

For reserpine experiments, each Thy1-YFP-H mouse was injected once daily with reserpine (3–5 mg/kg i.p., dissolved in filtered 0.9% NaCl) for 5 d. Brain slices were prepared 2 h after the last injection. For 6-OHDA experiments, each Thy1-YFP-H mouse was stereotaxically microinjected with 6-OHDA (2.5 mg/ml, 100 nl/min speed, 1 µl, dissolved in filtered 0.9% NaCl) targeting the medial forebrain bundle in the right hemisphere (AP: −0.9 mm, ML: −1.1 mm, DV: −4.8 mm). Brain slices were prepared for whole-cell recording 5–7 d after 6-OHDA injection.

De novo spine formation. De novo spine formation methods were described previously by Kwon et al. Because dopamine is critically involved in initial synaptogenesis during the second postnatal week (postnatal days 7–14) in vivo, we performed our analysis in acute cortical brain slices from young animals at postnatal days 15–18. Thi1-YFP-H mice were deeply anesthetized with isoflurane and decapitated. The brain was quickly removed and placed into chilled artificial cerebrospinal fluid (ACSF) and 300 µm coronal slices were prepared as described above. Slices were recorded within 4 h after recovery and all recordings were performed at 33–34 °C. Two-photon imaging and uncaging of 4-methoxy-7-nitroindolinyl (MNI)-caged glutamate was performed with a custom-built microscope equipped with two Ti:sapphire lasers (Mai Tai, Spectra-Physics) as described previously. Laser wavelengths were tuned to 925 nm and 720 nm for imaging of eYFP and glutamate uncaging, respectively. Slices were superfused with Mg2+-free ACSF containing 5 mM MN1-caged glutamate. Two-photon imaging and glutamate uncaging were performed on apical oblique dendrites of layer V pyramidal neurons in primary motor cortex (M1). Uncaging laser power was tuned to ∼50 mW at the back aperture of the objective (60×, NA 1.1, Olympus) to trigger glutamate uncaging. Uncaging position was chosen on the basis of criteria described previously. Briefly, the surface of the dendrite was required to be smooth in the immediate vicinity of the uncaging spot, and there was required to be at least one spine presented within 5 µm of the uncaging spot to ensure the capability of the dendrite for spinogenesis. De novo spine formation was induced by 40 pulses (0.5 ms) of uncaging laser at 5 Hz at a spot approximately 0.5 µm away from the border of the selected dendrites. To test the efficacy of glutamate uncaging, whole-cell patch-clamp recording and two-photon Ca2+ imaging were simultaneously performed. For voltage-clamp recording, layer V pyramidal neurons were filled with an internal solution containing 130 mM CsCH2SO3, 8 mM NaCl, 10 mM HEPES, 10 mM disodium phosphate, 0.4 mM Na2-GTP, 4 mM Mg-ATP, 3 mM sodium ascorbate and 1.7 mM QX-314 (pH 7.2). For two-photon Ca2+ imaging, Alexa Fluor 594 (25 µM) and Fluorescein 5F (300 µM) were added to the internal solution. Cells were filled with the dyes for at least 20 min before the start of recording. Imaging and uncaging lasers were tuned to 830 and 720 nm, respectively. Glutamate uncaging was performed in the same conditions as for de novo spine formation, but at the spine head to induce Ca2+ influx to test the efficacy of glutamate uncaging. Uncaging-induced EPSCs and Ca2+ transients were recorded at −70 mV.

Motor skill training: single-seed reaching task. The mouse single-seed reaching task protocol has been described previously. Before training, animals were food-restricted to maintain about 90% of their free-feeding weight. The training chamber was constructed as a transparent Plexiglas box. A vertical slit was made on the front wall of the box for the mouse to reach for millet seeds. The training included a shaping phase and a training phase. In the shaping phase, mice were familiarized with the training chamber and task requirements and developed a preferred limb. A small pile of millet seeds were placed in front of the slit and mice could use both paws to reach for them. Shaping was finished when 20 reach attempts were achieved within 20 min and 70% limb preference was established. Both control and MPTP-treated mice finished shaping in 2–5 d. Surgery for an implanting chronic cranial window above motor cortex was carried out after shaping. The side of implanting was selected according to limb preference demonstrated during shaping: if the preferred limb was left the window was implanted above the right motor cortex, and vice versa. The training phase started at least 2 weeks after surgery. Every training day consisted of one session of 30 trials with the preferred limb or 20 min. Seeds were presented one by one in front of the slit. A successful reach was one in which the mouse used the preferred limb, retrieved the seed and put it into its mouth smoothly. Otherwise it was considered an unsuccessful or failed reach. Success rates were the percentages of successful reaches over total reach attempts. All shaping and training sessions were carried out at the same time of day. The control mice were littersmates that also underwent the food restriction and were housed in standard mouse cages with up to four mice per cage. Mice that experienced complications after chronic window implantation surgery were excluded from the study.

Statistical analysis. No statistical methods were used to predetermine sample sizes, but our sample sizes are similar to those reported in previous publications. For in vivo experiments, slice physiology and uncaging experiments, n is reported as the number of animals, the number of cells (one cell from one slice) and the number of trials, respectively. Statistical analysis for long-term plasticity (LTP and LTD) among two or more groups was performed by comparing the average amplitude of responses over a 5-min period (25–30 min after induction protocol). Statistical significance of data was determined using non-parametric Mann–Whitney rank sum test and Kruskal–Wallis one-way ANOVA, followed by the appropriate post hoc test. For comparing the probability of de novo spine formation across conditions, Fisher’s exact test was used. For comparing food reaching behavior performance, repeated-measures two-way ANOVA was used. A Supplementary Methods Checklist is available.

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