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EXPERIENCE-DEPENDENT PLASTICITY OF DENDRITIC SPINES OF LAYER 2/3 PYRAMIDAL NEURONS IN THE MOUSE CORTEX

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Abstract

Previous studies have shown that sensory and motor experiences play an important role in the remodeling of dendritic spines of layer 5 pyramidal neurons in the cortex. In this study, we examined the effects of sensory deprivation and motor learning on dendritic spine remodeling of layer 2/3 pyramidal neurons in the barrel and motor cortices. Similar to layer 5 pyramidal neurons, spines on apical dendrites of layer 2/3 pyramidal neurons are plastic during development and largely stable in adulthood. Sensory deprivation via whisker trimming reduces the elimination rate of existing spines without significant effect on the rate of spine formation in the developing barrel cortex. Furthermore, we show that motor training increases the formation and elimination of dendritic spines in the primary motor cortex. Unlike layer 5 pyramidal neurons, however, there is no significant difference in the rate of spine formation between sibling dendritic branches of layer 2/3 pyramidal neurons. Our studies indicate that sensory and motor learning experiences have important impact on dendritic spine remodeling in layer 2/3 pyramidal neurons. They also suggest that the rules governing experience-dependent spine remodeling are largely similar, but not identical, between layer 2/3 and layer 5 pyramidal neurons.

Keywords

dendritic spines; whisker trimming; motor learning; spine remodeling; two-photon imaging

INTRODUCTION

Changes in synaptic connections are essential for the development and function of the nervous system. In the mammalian cerebral cortex, synapse density increases rapidly before birth and decreases from late postnatal development until puberty (Huttenlocher, 1990;

Markus and Petit, 1987; Rakic et al., 1986). While the number of synapses remain largely stable in adulthood, synaptic connections are continuously modified in response to learning tasks and alterations in sensory experience throughout life (Darian-Smith and Gilbert, 1994; Florence et al., 1998; Grutzendler et al., 2002; Jones and Pons, 1998; Jones et al., 1997; Kleim et al., 1998; Knott et al., 2002; Yang et al., 2014; Yang et al., 2009).

In vivo imaging studies in the mouse cortex have revealed that postsynaptic dendritic spines in apical tuft branches of layer 5 (L5) pyramidal neurons are plastic during development and largely stable throughout adulthood (Grutzendler et al., 2002; Holtmaat et al., 2005; Majewska et al., 2006; Yang et al., 2009; Zuo et al., 2005a). In young adolescent mouse barrel cortex, sensory deprivation via whisker trimming reduces the rate of spine elimination, but not spine formation (Pan et al., 2010; Zuo et al., 2005b). When chessboard trimming is performed to increase the difference in sensory experience coming from adjacent whiskers or mice are placed under enriched environment, an increase in spine formation of L5 pyramidal neurons is observed over a period of days in the barrel cortex (Fox, 2002; Holtmaat et al., 2006; Pan et al., 2010). In addition, motor learning has been shown to promote both spine formation and elimination over days in the primary motor cortex (Xu et al., 2009; Yang et al., 2009). In mice subjected to either rotarod or treadmill training, learning-induced new spines are formed on a subset of apical tuft branches of individual L5 pyramidal neurons (Yang et al., 2014). Together, these findings underscore the important roles of sensory and motor learning experiences in the remodeling of dendritic spines in L5 pyramidal neurons.

Comparing to L5 neurons, much less is known about experience-dependent remodeling of dendritic spines of layer 2/3 (L2/3) pyramidal cells. L2/3 and L5 pyramidal cells have distinct morphological and electrophysiological properties (Anderson et al., 2010; Chagnac-Amitai et al., 1990; Hattox and Nelson, 2007; Helmstaedter et al., 2007). These cells receive different inputs and project to different target regions (Feldmeyer et al., 2006; Hooks et al., 2013; Kiritani et al., 2012; Xu et al., 2012). The patterns of the action potential firing differ among L2/3, L5A and L5B cells (de Kock and Sakmann, 2008; Hattox and Nelson, 2007; Larkman and Mason, 1990). Given the differences in structural and functional properties between L2/3 and L5 pyramidal cells, these neurons may exhibit different degrees of experience-dependent dendritic spine remodeling.

In this work, we used a transcranial two-photon imaging technique to examine the effects of sensory deprivation and motor learning on dendritic spine remodeling of L2/3 pyramidal neurons. Similar to L5 pyramidal cells, we found that sensory deprivation via whisker trimming reduced dendritic spine elimination without affecting the rate of spine formation of L2/3 pyramidal cells in the developing barrel cortex. Furthermore, motor training induced rapid spine formation and elimination in the primary motor cortex. However, unlike L5 pyramidal neurons, the rate of dendritic spine formation was comparable between sibling apical dendritic branches of L2/3 cells in response to motor learning. These findings indicate that experience-dependent dendritic spine remodeling is largely similar but not identical between L2/3 and L5 pyramidal cells.

MATERIALS AND METHODS

Experimental Animals

ICR mice were purchased from Taconic, New York. Kunming mice were purchased from the Guangdong Medical Laboratory Animal Center, China. ICR mice were used exclusively in the study of the barrel cortex, and Kunming mice were used only in the study of the motor cortex. All experiments were done in accordance with the institutional guidelines. Mice aged P30 to 5 months of both sexes were used in the experiments.

Sensory deprivation and motor training

Sensory deprivation via whisker trimming was performed daily by cutting the mystacial vibrissae of the right whisker-pad to skin level with a pair of scissors under a dissecting microscope. For motor training, a custom built treadmill and a rotarod device were used. The devices and training procedures have been described previously (Cichon and Gan, 2015; Marvin et al., 2013; Yang et al., 2014; Yang et al., 2009). Mice subjected to treadmill training were head-fixed under two-photon microscopy with their two forelimbs standing on the treadmill and two hind limbs standing on a fixed board. Mice were forced to run on the linear treadmill at a moderate speed (8 cm/s). The mice were trained for 40 minutes with a short 1-minute break every 5 minutes. Rotarod training was performed in one session (40 trials in total) as described previously (Yang et al., 2014).

In utero electroporation

Plasmids expressing enhanced yellow fluorescent proteins (EYFP) under the CAG promoter were transfected into L2/3 pyramidal neurons in the barrel cortex with an *in utero* electroporation method as described previously (Tsai et al., 2005). In brief, pregnant ICR mice were used, and 1–2 μ l of complementary DNAs (1–5 μ g/ μ l) were injected into the ventricle of embryonic brains at E14–15. A pair of copper alloy oval plates attached to the electroporation generator ECM830 (Harvard Apparatus, MA) transmitted 5 square electric pulses at 50 V for 50 ms at 1-s intervals through the uterine wall.

The DNA plasmids expressing tdTomato were transfected into L2/3 pyramidal neurons of the primary motor cortex by electroporation as described previously (Shimogori and Ogawa, 2008). Pregnant Kunming mice were used in this experiment. 1 μ l of the DNA plasmid solution (1 μ g/ μ l) was mixed with the non-toxic dye fast green (10 $\times$, SIGMA, Tokyo, Japan) and injected into the lateral ventricle of the embryo in uterus via a glass electrode. Five square-wave current electric pulses (34–35 V, at 1-s intervals) were delivered through the electrodes with a pulse generator (CUIY21 Vivo-SQ, BEX). The region of transfection was adjusted by slightly changing the orientation and position of electrodes. After electroporation and surgery, mice were kept warm with a heating pad until recovered from anesthesia.

Imaging YFP-expressing cells and dendrites in fixed brain slices

Mice were anesthetized with pentobarbital (80 mg/kg) and perfused transcardially with 20 ml of 4% paraformaldehyde. Brains were dissected out and post-fixed overnight in 4% paraformaldehyde before slicing. Coronal slices (150–200 μ m thick) were sectioned with a

vibratome (Vibratome 1000, TPI Inc., St. Louis). YFP-labeled cells and dendritic structures were imaged with confocal microscopy (Radiance 2000, Bio-Rad; laser excitation wavelength at 488nm) using either a 20x, N.A. 0.8 or 60X N.A. 1.3 objective. Image stacks at 0.3-2.0 μm steps were acquired to generate three-dimensional data sets of neuronal structures (Fig.1).

Surgical procedure and *in vivo* imaging of dendrites

The procedure of transcranial two-photon imaging was described previously (Grutzendler et al., 2002; Yang et al., 2010). Great precaution was taken not to deflect the skull downwards against the brain surface or to break through the bone during the thinning process, as minor brain trauma or bleeding may potentially cause inflammation and disruption of neuronal structures. Images were acquired with either a 60X objective (N.A. 1.0) on a Bio-Rad multi-photon microscope (Radiance 2001) or a 25X objective (N.A. 1.05) on an Olympus two-photon microscope.

Data quantification

Analysis of spine dynamics was performed similarly as previously described (Grutzendler et al., 2002; Yang et al., 2009). Briefly, the same dendritic segments were identified from 3D image stacks taken from both views with high image quality (signal-to-background-noise ratio > 4-fold). The number and location of dendritic protrusions (protrusion length > 1/3 dendritic shaft diameter) were identified in each view. Filopodia were identified as long thin structures (head diameter/neck diameter < 1.2-fold and length/neck diameter > 3-fold) with fluorescence intensity comparable to those of thin spine necks. The remaining protrusions were classified as spines. Spines or filopodia were considered the same between two views if they were within 0.7 μm from their expected positions based on their spatial relationship to adjacent landmarks.

All the spine turnover data in the barrel cortex (Fig. 2) were acquired by imaging YFP-labeled L2/3 pyramidal neurons. For the quantification of spine turnover in the barrel cortex, the total number of spines was pooled from different dendritic segments of the same animals. More than 150 spines were quantified from each animal.

All the spine turnover data in the motor cortex (Figs. 3 and 4) were acquired by imaging tdTomato-labeled L2/3 pyramidal neurons. For quantifying spine formation on individual dendritic branches in the motor cortex, branches with more than 20 spines were included in order to minimize the variation of spine turnover rate.

Statistics

All data in the barrel cortex were presented as mean $\pm$ S.D. Data on branch-specific spine remodeling in the motor cortex were presented as mean $\pm$ S.E. The student *t*-test was used to determine the difference between groups. $P < 0.05$ was considered statistically significant.

RESULTS

Fluorescent labeling of L2/3 pyramidal neurons

To examine dendritic spine remodeling of L2/3 neurons, we used an *in utero* electroporation method to express yellow fluorescent protein (YFP) or tdTomato in a small subset of L2/3 pyramidal neurons (Fig. 1). When plasmids expressing YFP or tdTomato were electroporated into embryos between embryonic days 14 and 15, we found that fluorescent protein expression was mainly restricted to L2/3 pyramidal neurons in either the barrel or motor cortex. Although the number of fluorescently labeled cells varied among animals (Fig. 1B, C), many labeled L2/3 pyramidal neurons could be observed during early development and throughout adulthood (> 5 months of age).

Higher dendritic spine turnover during development than in adulthood

In vivo imaging studies of L5 pyramidal neurons have shown that dendritic spines become progressively stabilized as development proceeds (Grutzendler et al., 2002; Holtmaat et al., 2005; Yang et al., 2009; Zuo et al., 2005a). To test whether the same is true for L2/3 pyramidal neurons, we imaged apical dendrites of these cells in the barrel cortex over various time intervals using transcranial two-photon microscopy (Fig. 2A-F). In 1-month-old mice, we found that 12.3 ± 2.5 % of new spines were formed and 10.7 ± 2.6 % existing spines were eliminated over 2 days (Fig. 2B; $n = 6$ mice). In adult mice (~5 months old), the formation and elimination rates of spines over 2 days were 4.8 ± 1.2 % and 6.1 ± 1.7 %, respectively ($n = 6$ mice). Similar to dendritic spines on apical dendrites of L5 neurons in the barrel cortex, spines of L2/3 pyramidal neurons are plastic during development and largely stable in adulthood.

In addition to dendritic spines, many dendritic filopodia, long and thin protrusions without bulbous-heads, are found on the developing dendrites. Some of these filopodia do not make synaptic contact with presynaptic axons and are thought to be dendritic spine precursors (Dailey and Smith, 1996; Fiala et al., 1998; Yuste and Bonhoeffer, 2004; Ziv and Smith, 1996). Previous studies of L5 pyramidal cells have shown that dendritic filopodia are abundant in the developing cortex and a small percentage of them are transformed into spines (Grutzendler et al., 2002; Zuo et al., 2005a). Consistent with these studies, we found that 11.7 ± 5.7 % of dendritic protrusions were filopodia, while the rest were spines in mice at 1 month of age ($n = 6$ mice). In adulthood, the percentage of filopodia was 6.3 ± 2.2 % ($n = 6$ mice), significantly lower than that observed in young mice ($P < 0.05$). Furthermore, 88.5 ± 8.3 % and 92.3 ± 8.5 % of filopodia were eliminated within a 2-day interval in 1-month-old and adult mice, respectively. Thus, concomitant with the reduced spine remodeling of L2/3 pyramidal neurons, there is also a reduction in the generation of highly dynamic filopodia, the precursors of dendritic spines, as development proceeds.

Sensory deprivation reduces spine elimination of L2/3 neurons in the barrel cortex

Previous studies have shown that the elimination rate of dendritic spines of L5 pyramidal neurons is substantially higher than the formation rate in the barrel cortex of mice from 1 month to 2 months of age (Grutzendler et al., 2002; Zuo et al., 2005b). As a result, there is ~20% net loss of dendritic spines of L5 neurons during late postnatal period of development.

To determine whether the same holds true for dendritic spines of L2/3 pyramidal neurons, we examined spine elimination and formation of these cells over 1 month in the barrel cortex of 1-month-old mice. We found no significant difference between the rates of spine formation and elimination over 1 month (Fig. 2C; $23.0 \pm 2.6\%$ versus $24.9 \pm 2.9\%$; $n = 7$ mice; $P = 0.19$). Unlike L5 pyramidal neurons, there is no significant net loss of dendritic spines of apical tuft dendrites in L2/3 pyramidal neurons from 1 to 2 months of age.

While the number of spines remained relatively constant in mice from 1 to 2 months of age, there was a net reduction in the number of dendritic filopodia during this period. When the formation and elimination rates of total dendritic protrusions (spines and filopodia) were measured, we found that the formation rate of total protrusions was significantly lower than the elimination rate (Fig. 2D; $26.6 \pm 2.2\%$ versus $31.8 \pm 1.8\%$; $P < 0.005$). As a result, there was ~5% net loss of total dendritic protrusions in the barrel cortex of mice from 1 to 2 months of age. Nevertheless, this net loss of total dendritic protrusions in L2/3 neurons is substantially less than that of L5 pyramidal cells during the same postnatal period of development (Grutzendler et al., 2002; Zuo et al., 2005b).

It has been shown that long-term sensory deprivation via whisker trimming preferentially causes a reduction in the elimination rate of spines of L5 neurons (Zuo et al., 2005b). To examine how sensory deprivation affects the remodeling of L2/3 pyramidal neurons, we examined the elimination and formation of spines in 1-month-old mice after sensory deprivation via whisker trimming for one week. We found that whisker trimming for one week significantly reduced the rate of spine elimination (Fig. 2E; $12.5 \pm 2.7\%$ (trimming, $n = 7$) vs. $18.9 \pm 2.5\%$ (control, $n = 8$); $P < 0.001$) but had no significant effect on the rate of spine formation ($19.0 \pm 2.3\%$ (trimming) vs. $17.1 \pm 2.3\%$ (control); $P = 0.13$). Whisker trimming also reduced the elimination rate of total dendritic protrusions (spines and filopodia) without affecting their formation rate (Fig. 2F). These findings show that the effect of sensory deprivation on dendritic spine remodeling is similar between L2/3 and L5 pyramidal cells.

Motor training increases spine formation and elimination in the motor cortex

Previous studies have shown that motor training on rotarod or treadmill induces spine formation of L5 pyramidal neurons in the primary motor cortex within 8-24 hours (Yang et al., 2014; Yang et al., 2009). To examine the effect of motor training on L2/3 neurons, we first measured the rate of spine formation over 24 hours in 1-month-old mice without motor training. The same mice were then subjected to treadmill training for 40 minutes and the rate of spine formation was measured again over the next 24 hours (Fig. 3A). We found that treadmill training led to a significant increase in the rate of spine formation over 24 hours as compared to the non-training condition (Fig. 3A; $11.1 \pm 1.9\%$ (training) vs. $5.1 \pm 0.6\%$ (non-training), $n = 12$ mice; paired t-test, $P < 0.01$). Thus, similar to layer 5 pyramidal neurons, motor training increases spine formation in layer 2/3 pyramidal neurons within 24 hours.

It has been shown recently that motor training on either a rotarod or treadmill induces spine formation on a subset of apical tuft branches of individual L5 pyramidal neurons over 8-24 hours (Yang et al., 2014). To investigate whether such branch-specific spine formation also

occurs in L2/3 pyramidal neurons, we classified the sibling branch with higher spine formation as a “high-formation branch” (HFB) and the other as a “low-formation branch” (LFB) (Fig. 3B). 24 hours after training, the average rate of spine formation on HFBs in trained mice ($12.5 \pm 1.7\%$; $n = 9$ branches) was 2.2 to 3.8 times that of HFBs ($5.8 \pm 0.9\%$) or LFBs ($3.3 \pm 0.7\%$) in untrained control mice ($n = 11$ branches; unpaired t-test, $P < 0.005$) (Fig. 3C). Spine formation on LFBs in trained mice ($10.0 \pm 1.3\%$) was also significantly higher than that on either HFBs ($P < 0.02$) or LFBs ($P < 0.001$) in untrained controls. The difference in spine formation between HFBs and LFBs in trained mice was not statistically larger for sibling branches than for randomly paired branches ($P > 0.05$) (Fig. 3D). Similarly, when mice were subjected to 40 trial rotarod running, there was also no significant difference in the rate of spine formation between sibling branches of L2/3 neurons over 8 hours (Fig. 3E; $P > 0.05$). These results indicate that, unlike dendritic branch-specific spine formation of L5 pyramidal neurons, motor training does not induce spine formation on a small subset of dendritic branches of an individual L2/3 pyramidal neuron.

In addition to promoting new spine formation, motor training also increases the rate of spine elimination of L5 pyramidal neurons over days (Xu et al., 2009; Yang et al., 2009). 24 hours after treadmill training, we observed a significant increase in the rate of spine elimination of L2/3 pyramidal neurons when compared to the non-training condition (Fig. 4A; $10.0 \pm 1.4\%$ (training) vs. $6.4 \pm 1.0\%$ (no training); $n = 12$ mice; paired t-test, $P < 0.01$). Notably, we found that there was no significant correlation between the degree of spine formation and elimination at the level of individual branches (Fig. 4B, $n = 35$ branches; $P > 0.2$). This finding suggests that dendritic branches with high rates of spine formation do not necessarily exhibit high rates of spine elimination. Consistent with this, when sibling branches were classified into “high-formation branch” (HFB) and “low-formation branch” (LFB), there was no significant difference in spine elimination between HFBs and LFBs (Fig. 4C; $n = 9$ branches; $P = 0.47$). Together, these results suggest that spine formation and elimination induced by motor training do not preferentially occur on the same branches of L2/3 pyramidal neurons.

DISCUSSION

By imaging fluorescently-labeled L2/3 pyramidal neurons over time with transcranial two-photon microscopy, we examined how sensory and motor learning experiences affect spine dynamics of apical tuft dendrites of these neurons in the mouse barrel and motor cortices. We show that similar to L5 pyramidal neurons, sensory deprivation via whisker trimming for 1 week reduces the elimination rate of existing spines without affecting the formation rate of new spines of L2/3 neurons. Furthermore, motor training increases both spine formation and elimination of L2/3 neurons within 24 hours. In contrast to L5 pyramidal neurons, the formation of new spines induced by motor training does not occur selectively on a subset of apical tuft branches of individual L2/3 pyramidal neurons. These findings reveal both similarities and differences in experience-dependent spine remodeling between L2/3 and L5 pyramidal neurons.

Many lines of evidence indicate that a substantial decrease in the number of synapses occurs in the mammalian brain during the late postnatal period (Grutzendler et al., 2002; Huttenlocher, 1990; Markus and Petit, 1987; Rakic et al., 1986; Zuo et al., 2005a). *In vivo* imaging studies of L5 pyramidal cells have shown that there is ~20% net loss of dendritic spines in the barrel cortex of mice from 1 to 2 months of age (Zuo et al., 2005a). In the present study, we observed a small degree of loss of total dendritic protrusions, but not spines, of L2/3 neurons during the same period. This suggests that the loss of spines in L5 pyramidal neurons is a major contributor to the reduced synaptic connections in the superficial layer of the barrel cortex during the late postnatal period. Despite different degrees of spine formation/elimination between L2/3 and L5 neurons in young adolescent mice, long-term sensory deprivation reduces the rate of spine elimination but has no significant effect on spine formation in both cell types. These findings underscore the fundamental role of experience in pruning early formed spines in the developing cortex. Because the effects of sensory deprivation on spine dynamics are similar between these functionally and anatomically diverse cells, the mechanisms underlying experience-dependent spine pruning are likely similar.

Our studies indicate that motor training on a treadmill promotes spine formation and elimination of L2/3 pyramidal neurons with 24 hours. Recent studies have shown that a lever-pressing task promotes spine formation and elimination of L2/3 neurons over days in the motor cortex (Peters et al., 2014). Together with previous findings on spine remodeling of L5 neurons (Xu et al., 2009; Yang et al., 2014; Yang et al., 2009), these studies indicate that motor training induces rapid and wide-spread re-organization of synaptic connections in the motor cortex that is likely important for performance improvement after learning. It has been shown that dendritic spine formation occurs in a branch-specific manner after motor skill learning in L5 pyramidal neurons (Yang et al., 2014). Interestingly, unlike L5 neurons, we did not find evidence supporting branch-specific spine formation in L2/3 pyramidal neurons. These results suggest that mechanisms underlying learning-induced spine formation in L2/3 and L5 neurons likely differ. Furthermore, we did not find a significant correlation between the degree of spine formation and spine elimination at the level of individual branches, suggesting that the increased spine elimination does not simply reflect a compensatory response to the increased spine formation after learning. Recent studies have shown that treadmill training induces branch-specific generation of dendritic Ca^{2+} spikes that are important for synaptic potentiation and depotentiation in L5 pyramidal neurons (Cichon and Gan, 2015). Such branch-specific Ca^{2+} spikes could be involved in promoting branch-specific spine formation and elimination after motor learning. Given the fact that L2/3 and L5 pyramidal cells receive different inputs and have distinct morphological and electrophysiological properties (Anderson et al., 2010; Chagnac-Amitai et al., 1990; Feldmeyer et al., 2006; Helmstaedter et al., 2007; Hooks et al., 2013; Xu et al., 2012), it would be interesting to examine whether L2/3 neurons may lack branch-specific generation of dendritic Ca^{2+} spikes as well as branch-specific spine formation. Future studies are required to address these issues to better understand how experiences shape the development and plasticity of synaptic connections in the cortex.

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Figure 1.

Fluorescent labeling of L2/3 pyramidal cells in the cortex. (A). Schematic showing the use of an *in utero* electroporation method to express YFP or tdTomato in L2/3 pyramidal neurons. (B). Coronal sections from the fixed mouse barrel cortex showing many L2/3 pyramidal neurons were labeled by YFP at various ages (postnatal days 4 to 113). YFP-labeled axonal projections from these L2/3 pyramidal neurons were also seen in L5 (middle and right panels). (C) Higher magnification images of L2/3 pyramidal neurons and their dendritic branches at postnatal days 4 (left), 14 (middle) and 113 (right).

Figure 2.

The effect of sensory deprivation on dendritic spine remodeling. (A) Representative images showing spine formation and elimination in a YFP-labeled dendritic branch over 1 week. Filled arrowhead indicates new spine formed and open arrowheads indicate spines eliminated. Asterisks mark dendritic filopodia. (B) Percentage of spines formed (e.g., number of spines formed/number of pre-existing spines) and eliminated over a period of 2 days in young (1-month-old) and adult (~5 months) animals. Similar to L5 pyramidal neurons, spines of L2/3 pyramidal neurons (labeled by YFP) were more plastic in young mice than in adult mice. (C) In 1-month-old mice, repeated imaging of dendritic branches over a 4-week interval reveals no significant difference between the rate of spine elimination and formation. (D) The elimination rate of total dendritic protrusions (spines and filopodia) was slightly but significantly higher than the formation rate over 1 month in 1-month-old mice. (E, F) Whisker trimming for 1 week reduced the rate of elimination, but not formation, of spines (E) or total protrusions (F) of L2/3 pyramidal neurons in the barrel cortex of 1-month-old mice. Data are presented as mean $\pm$ s.d. $**P < 0.005$, $***P < 0.001$.

Figure 3.

Motor learning induces dendritic spine formation of L2/3 pyramidal neurons in the primary motor cortex. (A) Left, schematic of experimental design to test treadmill training induced spine formation and representative images showing spine formation and elimination in a dendritic segment labeled with tdTomato. Filled arrowhead indicates new spine formed and open arrowheads indicate spines eliminated between 0-24 hours and 24-48 hours. Asterisks mark dendritic filopodia. Right, the rate of dendritic spine formation over 24 hours was significantly higher after 40-minute treadmill training than under non-training condition. (B) An example of two sibling dendritic branches of L2/3 cells labeled with tdTomato. Classification of sibling branches to HFBs and LFBs was based on the spine formation rate relative to each other. (C) Treadmill training significantly increased the rate of spine formation on HFBs or LFBs 24 hours after training as compared to that on HFBs or LFBs in non-trained control. (D-E) The average difference in spine formation between HFBs and LFBs (black circle) was not statistically different from that between randomly paired branches (gray box plot of results from 100 random pairing) after 24 hours treadmill training (D, 9 pairs) or 8 hours rotarod training (E, 12 pairs) ($P > 0.05$). Data are presented as mean $\pm$ sem. * $P < 0.05$, ** $P < 0.005$, *** $P < 0.001$.

Figure 4.

Motor learning induces dendritic spine elimination of L2/3 pyramidal neurons in the primary motor cortex. (A) The rate of dendritic spine elimination over 24 hours was significantly higher after 40-minute treadmill training than under non-training condition. L2/3 pyramidal neurons were labeled by tdTomato as in Fig. 3. $*P < 0.05$. (B) 24 hours after training, there was no significant correlation between the rate of spine formation and elimination at the level of individual branches. (C) There was no significant difference in the rate of spine elimination between HFBs and LFBs ($P = 0.47$). Data are presented as mean $\pm$ sem.