

Long-term sensory deprivation prevents dendritic spine loss in primary somatosensory cortex

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A substantial decrease in the number of synapses occurs in the mammalian brain from the late postnatal period until the end of life^{1–5}. Although experience plays an important role in modifying synaptic connectivity^{6–17}, its effect on this nearly lifelong synapse loss remains unknown. Here we used transcranial two-photon microscopy to visualize postsynaptic dendritic spines in layer I of the barrel cortex in transgenic mice expressing yellow fluorescent protein. We show that in young adolescent mice, long-term sensory deprivation through whisker trimming prevents net spine loss by preferentially reducing the rate of ongoing spine elimination, not by increasing the rate of spine formation. This effect of deprivation diminishes as animals mature but still persists in adulthood. Restoring sensory experience after adolescent deprivation accelerates spine elimination. Similar to sensory manipulation, the rate of spine elimination decreases after chronic blockade of NMDA (*N*-methyl-D-aspartate) receptors with the antagonist MK801, and accelerates after drug withdrawal. These studies of spine dynamics in the primary somatosensory cortex suggest that experience plays an important role in the net loss of synapses over most of an animal's lifespan, particularly during adolescence.

In the cerebral cortex of humans and other mammals, rapid synaptogenesis during early postnatal life is followed by a substantial (~50%) loss of synapses that extends through adolescence^{1–5}. In adulthood, the number of synapses continues to decline, but at a much slower rate^{1–5}. The mechanisms that underlie this extensive loss of synapses occurring throughout life are unknown. It is well established that experience profoundly influences synaptic connectivity and behaviour^{6–11}. Although experience or activity-dependent synaptic plasticity varies with experimental systems and time scales of manipulation^{6–16}, it is generally believed that experience leads to an increase rather than a decrease in the number of synapses^{8,9,17}. The influence of experience on the extensive loss of synapses that occurs from young childhood until the end of life, however, remains unknown.

To address this question, we examined the long-term effect of sensory experience on the rates of dendritic spine formation and elimination in mouse barrel cortex, a cortical region to which sensory input can be easily manipulated. Dendritic spines are the postsynaptic sites of the vast majority of excitatory axo-dendritic synapses in the brain, and their dynamism therefore serves as a good indicator of synaptic plasticity¹⁸. We used transcranial two-photon microscopy and transgenic mice that express yellow fluorescent protein (YFP) predominantly in layer V pyramidal neurons^{4,19} to repeatedly image individual dendritic branches and spines in layer I of the barrel cortex, after various periods of sensory deprivation and recovery. At the end of repeated imaging, cytochrome oxidase staining was used to confirm that the imaged area was located within barrel cortex (Fig. 1a, b).

In control mice at one month of age, we found that the percentage of spines eliminated over a two-week interval was significantly higher than the percentage of spines formed ($16.9 \pm 2.5\%$ (mean \pm s.d.) elimination versus $6.3 \pm 2.3\%$ formation; 1,366 spines; $n = 5$ animals; $P < 0.01$) (Fig. 1c, d, g). Similar results have been found in other cortical regions at the same age^{4,5}, suggesting that a reduction in total spine number occurs in different cortices during this period of postnatal life. To determine the effect of sensory experience on spine turnover, we performed daily trimming of all the whiskers on one side of the facial pad from weeks 4–6, and examined the percentages of spines formed or eliminated in the barrel cortex contralateral to the trimmed side. Trimming whiskers for two weeks significantly reduced the percentage of spines eliminated compared with that in control littermates (trimmed: $9.5 \pm 1.5\%$ elimination; 1,581 spines, $n = 5$; $P < 0.01$) (Fig. 1c–g). In contrast, there was no significant difference in the percentage of spines formed between whisker-trimmed and control mice (trimmed: $5.4 \pm 1.0\%$; $P > 0.8$) (Fig. 1g). Furthermore, extending the period of whisker trimming for an additional two weeks continued to have a significant effect on spine elimination (trimmed: $7.1 \pm 2.3\%$; 550 spines; $n = 4$; control (weeks 6–8): $10.7 \pm 0.8\%$; 753 spines; $n = 5$; $P < 0.05$) but not spine formation (trimmed $4.3 \pm 1.5\%$ versus control $5.4 \pm 0.4\%$; $P > 0.4$). Thus, during the second postnatal month, when extensive spine loss occurs, sensory deprivation preferentially reduces the rate of spine elimination but not formation.

Dendrites in the developing cortex contain not only spines but also filopodia, which are long and thin protrusions without a bulbous head^{4,5,18,20} (Fig. 1c–f, i). As animals mature, filopodia become less abundant^{4,5} (Fig. 1i). Unlike spines that persist over weeks to months, filopodia undergo rapid turnover (within hours) and might serve as precursors of spines^{4,5,18,20}. In barrel cortex from one-month-old mice, we found that $9.9 \pm 3.5\%$ of dendritic protrusions were filopodia (5,388 protrusions; 18 animals) (Fig. 1i). Whisker trimming for two weeks had no significant effect on filopodia elimination ($P > 0.4$) or formation ($P > 0.8$) (Fig. 1h), even though spine elimination was significantly reduced during the same period (Fig. 1g). These results indicate that filopodia and spines have very different susceptibilities to manipulation of experience.

As mice mature into adulthood (>4 months of age), the rate of spine elimination over a two-week period is significantly reduced ($5.1 \pm 1.2\%$; 707 spines; $n = 4$) and becomes comparable to that of spine formation ($4.4 \pm 1.3\%$; $P > 0.4$). In contrast to young adolescent animals (1–2 months of age), whisker trimming for two weeks in adulthood had no significant effect on spine elimination (trimmed $4.9 \pm 1.1\%$; 547 spines; $n = 4$; $P > 0.6$) (Fig. 2i), suggesting that the effect of sensory deprivation on spine elimination decreases as animals reach adulthood. Furthermore, no significant difference in spine formation was found between control and deprived adult mice

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over two weeks ($P > 0.6$) (Fig. 2i). However, when the duration of sensory deprivation was extended from two weeks to two months, we did find a significant reduction in spine elimination (trimmed: $7.1 \pm 1.4\%$; 672 spines; $n = 5$; control: $10.2 \pm 1.6\%$; 907 spines; $n = 6$; $P < 0.01$) (Fig. 2a–h, j). No significant difference in spine formation was found between the control and trimmed groups even after two months of whisker trimming ($P > 0.7$). Thus, sensory deprivation continues to affect spine elimination in adulthood, albeit to a lesser degree than in young adolescence. The same conclusion can be generalized to all dendritic protrusions ($P < 0.05$ for elimination and $P > 0.3$ for formation), as filopodia represent only $4.2 \pm 3.3\%$ of total protrusions in adulthood (Fig. 1i; 2,916 protrusions, $n = 18$) and their number is not significantly affected by long-term deprivation.

Because more spines were eliminated than formed over weeks to months in the maturing barrel cortex, spine density in one-month-old barrel cortex (0.47 ± 0.04 spines μm^{-1} ; 933 spines; $n = 6$) is $\sim 27\%$ higher than that at six months of age (0.37 ± 0.03 spines μm^{-1} ; 845 spines; $n = 6$; $P < 0.005$). As whisker trimming preferentially reduces spine elimination in young adolescence and adulthood, we predicted that a higher spine density should be found in barrel cortex that has been subjected to long-term sensory deprivation. To test this directly, we trimmed whiskers unilaterally on a daily basis from 1–6 months of age and compared spine density of layer V pyramidal cells in layer I of barrel cortex both ipsilateral and contralateral to the whisker-trimming side. We found that spine density on the side ipsilateral to deprivation (0.36 ± 0.04 spines μm^{-1} ; 886 spines; $n = 6$) is not significantly different from age-matched, non-deprived control mice ($P > 0.4$), but that spine density is $\sim 22\%$ higher on the side contralateral to

deprivation (Fig. 2k; 0.44 ± 0.03 spines μm^{-1} ; 754 spines; $P < 0.005$). These results indicate that long-term sensory deprivation from young adolescence to adulthood leads to a significant reduction in spine loss.

To determine whether the effect of deprivation on spine loss is reversible upon sensory restoration, we first trimmed whiskers in one-month-old mice for two weeks and then allowed the whiskers to re-grow for the next two weeks. We found that during the two-week recovery period (weeks 6–8), the percentage of spine elimination exceeded that of age-matched controls, with no significant change in spine formation (Fig. 3a) (elimination: $14.9 \pm 1.7\%$ versus control $10.7 \pm 0.8\%$, $P < 0.05$; formation: $6.6 \pm 2.0\%$ versus control $5.4 \pm 0.4\%$, $P > 0.3$). Similar results were also found in mice whose whiskers were trimmed for four weeks and then allowed to re-grow in the next two weeks (Fig. 3b) (elimination: $13.5 \pm 1.6\%$ versus control $6.7 \pm 1.1\%$, $P < 0.05$; formation: $5.9 \pm 1.8\%$ versus control $5.4 \pm 1.5\%$, $P > 0.8$). In both cases, the reduction in total spine loss due to previous adolescent deprivation was largely ($\sim 80\%$) compensated for during the subsequent two-week recovery (Fig. 3c; see Methods for calculation of total spine number).

To further examine whether sensory restoration can accelerate spine elimination after animals reach adulthood, we trimmed all the whiskers on one side of the facial pad in one-month-old mice on a daily basis for five months. We found that the percentages of spine elimination and formation over the last two weeks of whisker trimming ($5.9 \pm 3.1\%$ elimination and $5.4 \pm 0.8\%$ formation; 297 spines; $n = 3$) were not significantly different from those in adult controls ($P > 0.8$ for both elimination and formation), suggesting that lack of sensory experience from young adolescence to adulthood

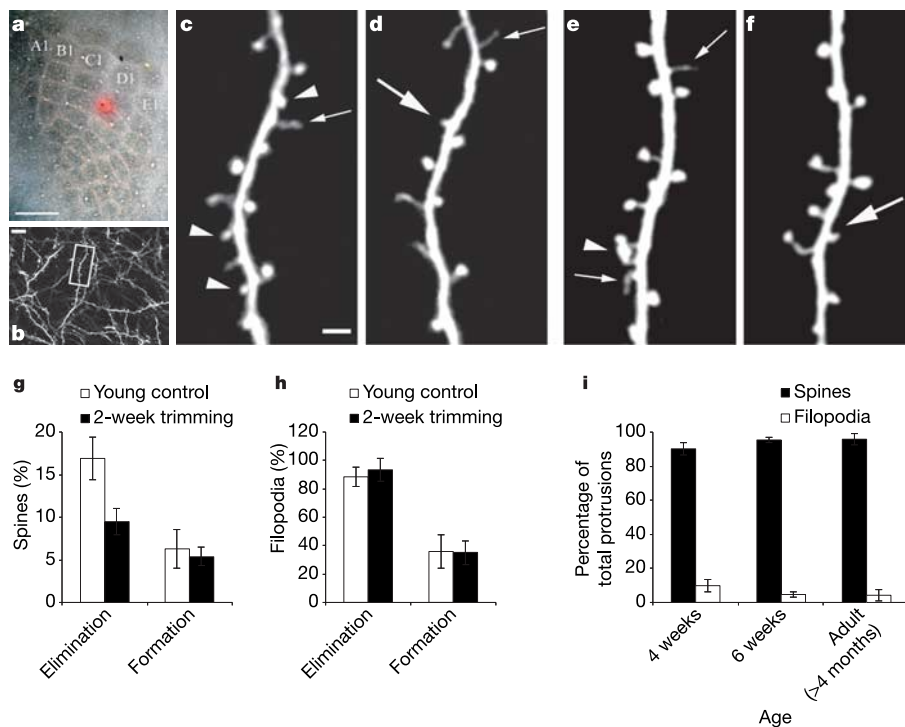


Figure 1 | Long-term whisker trimming reduces spine elimination in barrel cortex of young adolescent mice at one month of age. **a**, A cytochrome oxidase stained tangential section indicates that the imaged region (red; labelled with DiI) is within the barrel cortex. Labels A1–E1 refer to barrels corresponding to the first rows of the mystacial pad. **b**, Low-magnification image of apical dendrites of layer V pyramidal neurons. A higher-magnification view of a dendritic segment (box in **b**) is shown in **c**. **c–f**, Repeated imaging of two dendritic branches from 4–6 weeks of age reveals spine elimination (arrowheads) and formation (large arrows) as well

as filopodium turnover (small arrows) in control (**c, d**) and whisker-trimmed mice (**e, f**). **g**, Percentage of spines eliminated or formed (number of spines eliminated or formed divided by the number of pre-existing spines) over two weeks for control and whisker-trimmed animals. Whisker trimming preferentially reduces spine elimination ($P < 0.01$). **h**, Whisker trimming for two weeks has no significant effect on filopodium turnover ($P > 0.4$). **i**, Percentages of spines and filopodia as a proportion of total protrusions at different ages. Data are presented as mean \pm s.d. Scale bars, 500 μm (**a**), 10 μm (**b**), 2 μm (**c–f**).

does not prevent spines from reaching adult stability. Notably, when whiskers were allowed to re-grow and sensory experience was restored over two months, we found that spine elimination and formation are not significantly different between control and sensory-restored mice (Fig. 3d; $P > 0.8$ for both elimination and formation). Thus, although the effect of sensory deprivation on spine elimination can be largely reversed in young adolescence, it cannot be reversed

once spines are stabilized in adulthood.

Because NMDA receptor activation is essential for synaptic plasticity in a variety of systems^{21–24}, we tested the effect of chronic blockade of these receptors by applying the antagonist MK801 (0.1 or 0.25 $\mu\text{g g}^{-1}$ body weight, twice per day) for two weeks in one-month-old mice. This blockade resulted in a significant reduction in the rate of spine elimination (Fig. 4a; $P < 0.05$ for both dosages). Similar to the effects of sensory deprivation, the rate of spine formation was not significantly altered (Fig. 4a; $P > 0.5$ for both dosages). Furthermore, two weeks after the withdrawal of MK801 (0.25 $\mu\text{g g}^{-1}$ body weight), spine elimination ($15.1 \pm 2.3\%$; 682 spines; $n = 5$) was significantly increased compared with age-matched controls, and spine formation was unaffected (Fig. 4b; $P < 0.01$ for elimination and $P > 0.1$ for formation). Finally, no significant effect on NMDA receptor formation or elimination was observed upon NMDA receptor blockade and subsequent removal over two weeks ($P > 0.2$, for all conditions). Together, these results suggest that NMDA receptor-dependent mechanisms are involved in the extensive spine elimination during adolescence.

Our results indicate that in young adolescence, when the peak of synaptogenesis is over and synapse elimination dominates^{1–5}, long-term sensory deprivation reduces the rate of spine elimination but has no significant effect on spine formation. Furthermore, as spines become stabilized in adulthood, the effect of sensory deprivation on spine elimination is substantially reduced but still exists. As a result, sensory deprivation from 1–6 months of age increases spine density by ~22% after preventing spines from undergoing naturally occurring elimination (Fig. 2k). These observations underscore the importance of sensory experience in regulating the extensive loss of spines from the late postnatal period and throughout adulthood.

Owing to technical limitations, we only imaged dendritic spines of layer V pyramidal cells in layer I of the barrel cortex. Whether our results can be generalized to other neuronal types, cortical layers or

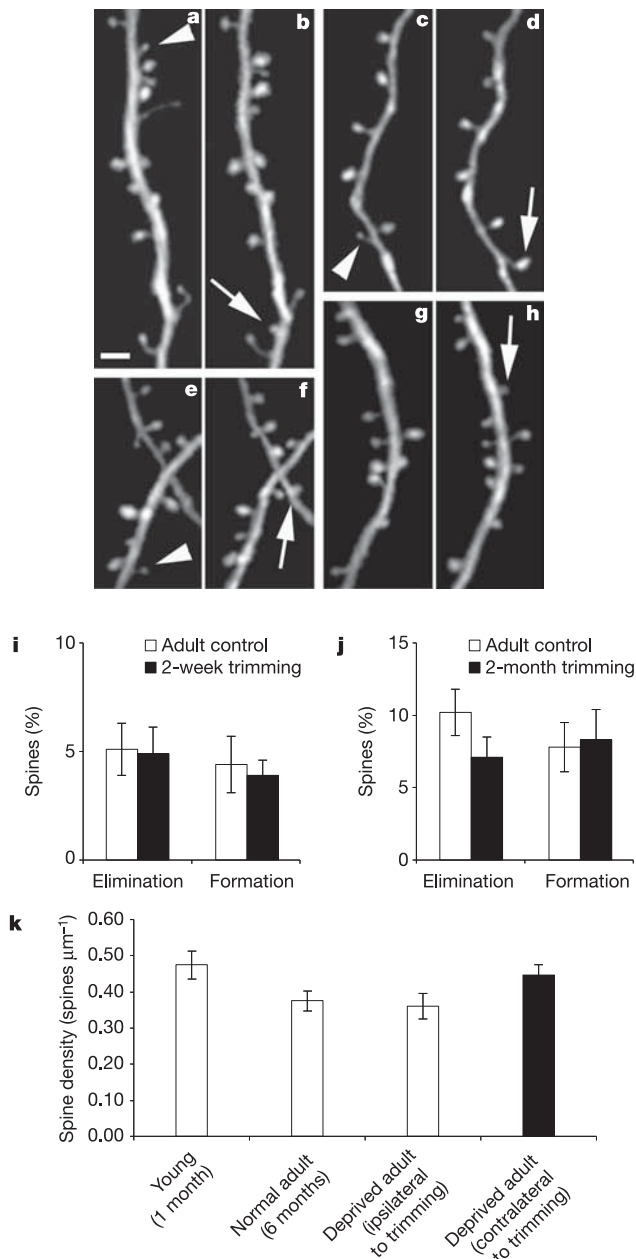


Figure 2 | The effect of long-term deprivation on spine elimination decreases but still exists in adulthood. **a–h**, Repeated imaging of dendritic branches from two normal (**a–d**) and two whisker-trimmed (**e–h**) adult mice shows elimination (arrowheads) and formation (arrows) of few spines over two months. Scale bar for **a–h**, 2 μm . **i, j**, Percentage of spines eliminated and formed over two weeks (**i**) or two months (**j**) in control and deprived adults. Whisker trimming significantly reduces spine elimination over two months ($P < 0.01$) but not over two weeks ($P > 0.6$). **k**, Spine density in one-month-old barrel cortex is ~27% higher than that in six-month-old adult barrel cortex. Unilateral whisker trimming from 1–6 months reduces spine loss on the side contralateral to deprivation compared with the ipsilateral side and non-deprived controls ($P < 0.005$). Data are presented as mean \pm s.d.

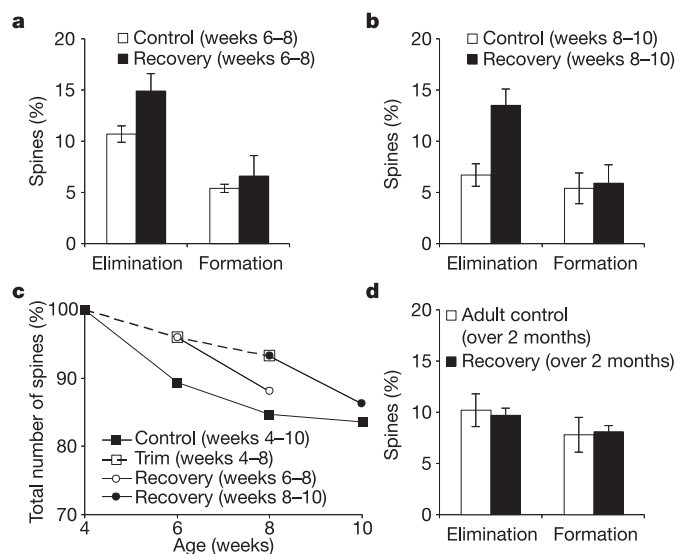


Figure 3 | Restoring sensory experience after previous deprivation accelerates spine elimination in adolescence but not in adulthood. **a**, The percentage of spine elimination, but not formation, is higher over a two-week recovery period (weeks 6–8) after a previous two-week deprivation (weeks 4–6) compared with age-matched controls ($P < 0.05$). **b**, Similar results were observed over a two-week recovery period after a previous four-week deprivation ($P < 0.05$). **c**, Percentage of total spine number from weeks 4–10 in control, trimmed and recovered animals. Whisker trimming reduces total spine loss, and sensory restoration accelerates it. **d**, Spine elimination and formation over a two-month interval are not significantly different between the control and recovered adult mice following previous deprivation from 1–6 months of age ($P > 0.8$). Data are presented as mean \pm s.d.

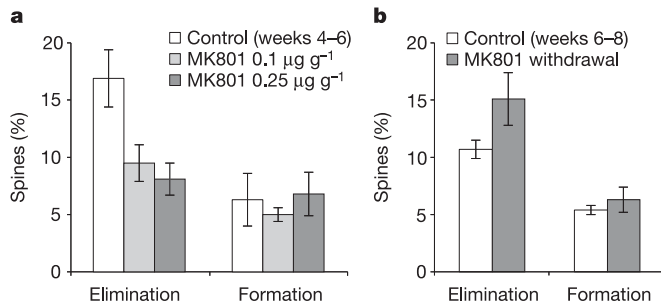


Figure 4 | Spine elimination involves NMDA receptor activation. **a**, Spine elimination, but not formation, over two weeks is significantly reduced in mice treated with MK801 (0.1 µg g⁻¹ or 0.25 µg g⁻¹ body weight, twice a day) compared with control mice ($P < 0.05$ for both doses). **b**, Spine elimination, but not formation, over two weeks is significantly increased upon MK801 withdrawal after a two-week blockade (0.25 µg g⁻¹) compared with age-matched controls ($P < 0.01$). Data are presented as mean \pm s.d.

regions remains to be investigated. Activity-dependent competition and homeostatic regulation are fundamental mechanisms underlying synaptic plasticity, and both involve NMDA receptor activation^{7,8,11,13,21,23-25}. A combination of these mechanisms may be responsible for the observed effect of sensory deprivation on spine dynamics. Because of the complexity of cortical connectivity, it is possible that the effect of sensory experience on spine loss via these two mechanisms varies with cortical layers and regions. Nevertheless, as substantial synapse loss (30–50%) occurs in different cortical regions and layers in various species, including humans¹⁻⁵, our results raise the possibility that experience-dependent net loss of synapses is fundamental to the development and plasticity of the brain.

Previous studies have shown that synapse number in sensory cortices is reduced as a consequence of sensory deprivation during early postnatal life, and is increased in an enriched environment^{12,14,15,17,26}. These and other studies have led to the prevailing view that experience and/or neuronal activity leads to a gain rather than a loss of synapses^{8,9,17}. However, this view is not easily compatible with our findings that sensory deprivation prevents net synapse loss in the maturing mammalian brain¹⁻⁵. It is important to note that experience or activity-dependent modification of synapse number and strength often varies with experimental systems, synapse types and time scales of manipulation^{6,9-12,14-16,27,28} (see Supplementary Information). For example, sensory deprivation during early postnatal life results in a decrease in the number of excitatory synapses, whereas prolonged deprivation until adolescence leads to no significant changes^{14,15,26}. Our findings indicate that the effect of sensory experience on synapse number depends on the rates of synapse formation and elimination at different stages of the animal's life (Figs 1 and 2). The significant impact of adolescent deprivation on spine elimination underscores the fundamental role of childhood experience on sculpting synaptic connections. Furthermore, our observation that the effect of sensory deprivation on spine loss can only be partially reversed during adolescence (Fig. 3c) suggests that childhood experience has a long-lasting and perhaps permanent effect on later life. Experience-based pruning of synaptic connections might continue to serve as an important mechanism in learning and memory throughout adulthood^{11,29}. As sensory deprivation over periods of weeks in adulthood does not cause significant modification in spine turnover, changes in synaptic strength, but not number, might be more essential for rapid plasticity and short-term storage of information^{16,22,30}. It is worth noting that our results demonstrate long-term effects of sensory deprivation on spine dynamics in animals under laboratory housing conditions. The timescale for modifying synaptic strength and number and the degree of such modifications in complex environments and learning tasks will require future studies.

METHODS

Experimental animals. Mice expressing YFP in layer V pyramidal neurons (H-line) were purchased from the Jackson Laboratory. Mice were group-housed and bred in the Skirball animal facilities, and all experiments were done in accordance with animal protocols. 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. Control mice were handled under identical conditions for the same duration. The NMDA receptor antagonist MK801 (15 µg ml⁻¹ in saline; 0.1 or 0.25 µg g⁻¹ body weight) was injected twice a day for two weeks into the peritoneum of mice, starting at four weeks of age.

Surgical procedure for *in vivo* transcranial imaging and data quantification.

The procedures of transcranial two-photon imaging and data quantification have been described previously⁴. The percentage of spines eliminated or formed is defined as the number of spines eliminated or formed, divided by the pre-existing number of spines. The number of spines analysed and the percentage of spine elimination or formation under various experimental conditions are summarized in Supplementary Table S1. We calculated changes in total spine number with age as follows. The total spine number in one-month-old mice was assumed to be 100%. Percentage change in the total spine number over a given interval (for example, weeks 6–8) is relative to the animal age at the first view (for example, week 6) and calculated as the percentage of formation minus the percentage of elimination measured over that interval. Data throughout the text is presented as mean \pm standard deviation (s.d.). P -values were calculated using the non-parametric Mann-Whitney U -test. Use of the Student's t -test also confirmed all the conclusions.

Determining the location of the imaged region using cytochrome oxidase staining.

At the end of repeated imaging sessions, the imaged region was identified under a dissecting microscope on the basis of CCD images of blood vasculature, and was subsequently labelled with a small lipophilic dye (DiI) crystal using a small needle tip (~100 µm in diameter). The mice were then perfused with 4% paraformaldehyde. The brains were removed, post-fixed, and tangentially sectioned into 150 µm slices using a vibratome. Sections were stained for cytochrome oxidase using a standard method.

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- Rakic, P., Bourgeois, J. P., Eckenhoff, M. F., Zecevic, N. & Goldman-Rakic, P. S. Concurrent overproduction of synapses in diverse regions of the primate cerebral cortex. *Science* **232**, 232–235 (1986).
- Markus, E. J. & Petit, T. L. Neocortical synaptogenesis, aging, and behaviour: lifespan development in the motor-sensory system of the rat. *Exp. Neurol.* **96**, 262–278 (1987).
- Huttenlocher, P. R. & Dabholkar, A. S. Regional differences in synaptogenesis in human cerebral cortex. *J. Comp. Neurol.* **387**, 167–178 (1997).
- Grutzendler, J., Kasthuri, N. & Gan, W. B. Long-term dendritic spine stability in the adult cortex. *Nature* **420**, 812–816 (2002).
- Zuo, Y., Chang, P., Lin, A. & Gan, W. B. Development of long-term dendritic spine stability in diverse regions of cerebral cortex. *Neuron* **46**, 181–189 (2005).
- Shatz, C. J. & Stryker, M. P. Ocular dominance in layer IV of the cat's visual cortex and the effects of monocular deprivation. *J. Physiol. (Lond.)* **281**, 267–283 (1978).
- Katz, L. C. & Shatz, C. J. Synaptic activity and the construction of cortical circuits. *Science* **274**, 1133–1138 (1996).
- Yuste, R. & Bonhoeffer, T. Morphological changes in dendritic spines associated with long-term synaptic plasticity. *Annu. Rev. Neurosci.* **24**, 1071–1089 (2001).
- Bailey, C. H. & Kandel, E. R. Structural changes accompanying memory storage. *Annu. Rev. Physiol.* **55**, 397–426 (1993).
- Buonomano, D. V. & Merzenich, M. M. Cortical plasticity: from synapses to maps. *Annu. Rev. Neurosci.* **21**, 149–186 (1998).
- Lichtman, J. W. & Colman, H. Synapse elimination and indelible memory. *Neuron* **25**, 269–278 (2000).
- Valverde, F. Rate and extent of recovery from dark rearing in the visual cortex of the mouse. *Brain Res.* **33**, 1–11 (1971).
- Ballice-Gordon, R. J. & Lichtman, J. W. Long-term synapse loss induced by focal blockade of postsynaptic receptors. *Nature* **372**, 519–524 (1994).
- Winkelman, E., Brauer, K. & Klutz, K. Spine density of lamina V pyramidal cells in the visual cortex of laboratory rats after lengthy dark exposure [in German with English abstract]. *J. Hirnforsch.* **18**, 21–28 (1977).
- Micheva, K. D. & Beaulieu, C. An anatomical substrate for experience-dependent plasticity of the rat barrel field cortex. *Proc. Natl Acad. Sci. USA* **92**, 11834–11838 (1995).
- Matsuzaki, M., Honkura, N., Ellis-Davies, G. C. & Kasai, H. M. Structural basis of long-term potentiation in single dendritic spines. *Nature* **429**, 761–766 (2004).
- Grossman, A. W., Churchill, J. D., Bates, K. E., Kleim, J. A. & Greenough, W. T. A brain adaptation view of plasticity: is synaptic plasticity an overly limited concept? *Prog. Brain Res.* **138**, 91–108 (2002).

18. Yuste, R. & Bonhoeffer, T. Genesis of dendritic spines: insights from ultrastructural and imaging studies. *Nature Rev. Neurosci.* **5**, 24–34 (2004).
19. Denk, W., Strickler, J. H. & Webb, W. W. Two-photon laser scanning fluorescence microscopy. *Science* **248**, 73–76 (1990).
20. Ziv, N. E. & Smith, S. J. Evidence for a role of dendritic filopodia in synaptogenesis and spine formation. *Neuron* **17**, 91–102 (1996).
21. Bock, J. & Braun, K. Blockade of *N*-methyl-D-aspartate receptor activation suppresses learning-induced synaptic elimination. *Proc. Natl Acad. Sci. USA* **96**, 2485–2490 (1999).
22. Nicoll, R. A. & Malenka, R. C. Expression mechanisms underlying NMDA receptor-dependent long-term potentiation. *Ann. NY Acad. Sci.* **868**, 515–525 (1999).
23. Sawtell, N. B. *et al.* NMDA receptor-dependent ocular dominance plasticity in adult visual cortex. *Neuron* **38**, 977–985 (2003).
24. Sin, W. C., Haas, K., Ruthazer, E. S. & Cline, H. T. Dendrite growth increased by visual activity requires NMDA receptor and Rho GTPases. *Nature* **419**, 475–480 (2002).
25. Turrigiano, G. G. & Nelson, S. B. Homeostatic plasticity in the developing nervous system. *Nature Rev. Neurosci.* **5**, 97–107 (2004).
26. Sadaka, Y., Weinfeld, E., Lev, D. L. & White, E. L. Changes in mouse barrel synapses consequent to sensory deprivation from birth. *J. Comp. Neurol.* **457**, 75–86 (2003).
27. Trachtenberg, J. T. *et al.* Long-term *in vivo* imaging of experience-dependent synaptic plasticity in adult cortex. *Nature* **420**, 788–794 (2002).
28. Knott, G. W., Quairiaux, C., Genoud, C. & Welker, E. Formation of dendritic spines with GABAergic synapses induced by whisker stimulation in adult mice. *Neuron* **34**, 265–273 (2002).
29. Changeux, J. P. & Danchin, A. Selective stabilisation of developing synapses as a mechanism for the specification of neuronal networks. *Nature* **264**, 705–712 (1976).
30. Shi, S. H. *et al.* Rapid spine delivery and redistribution of AMPA receptors after synaptic NMDA receptor activation. *Science* **284**, 1811–1816 (1999).

Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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