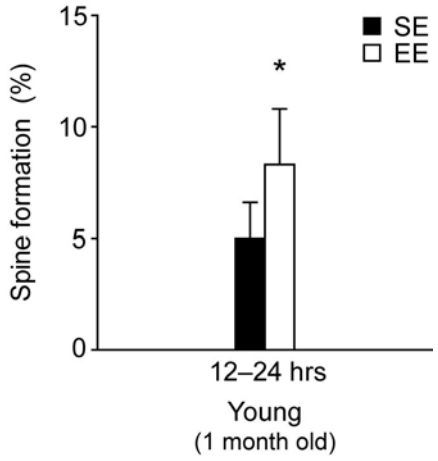
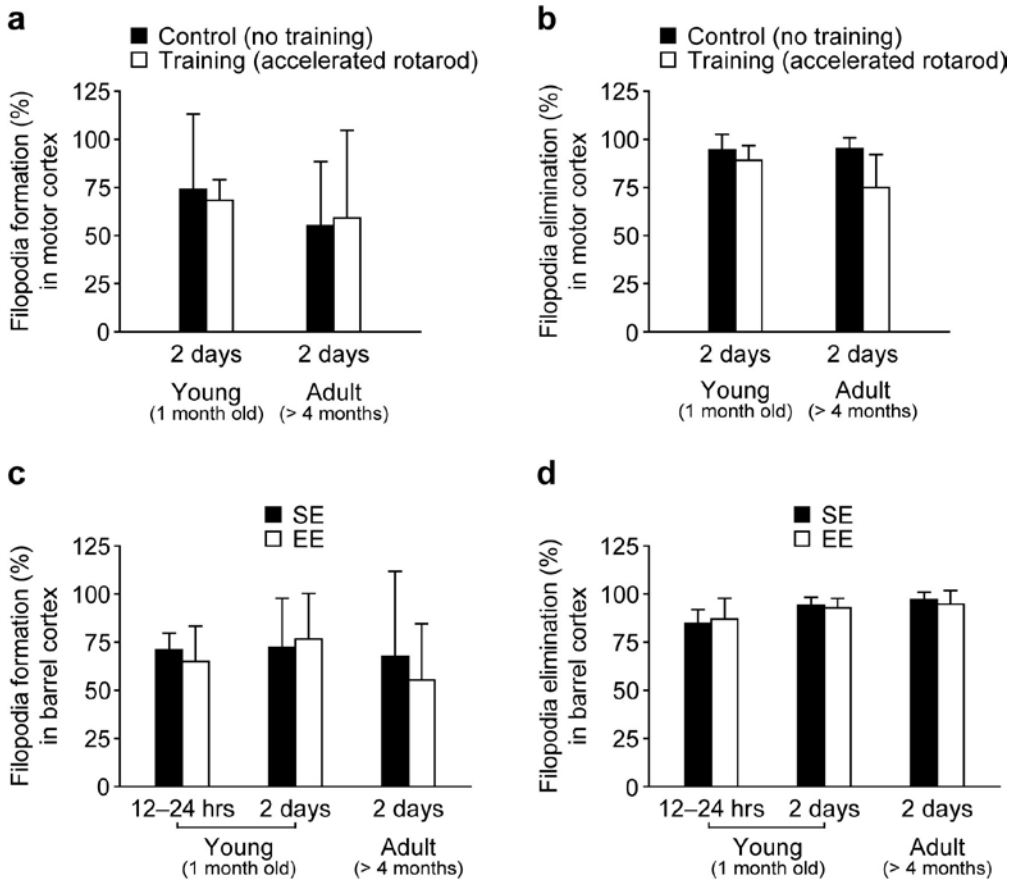
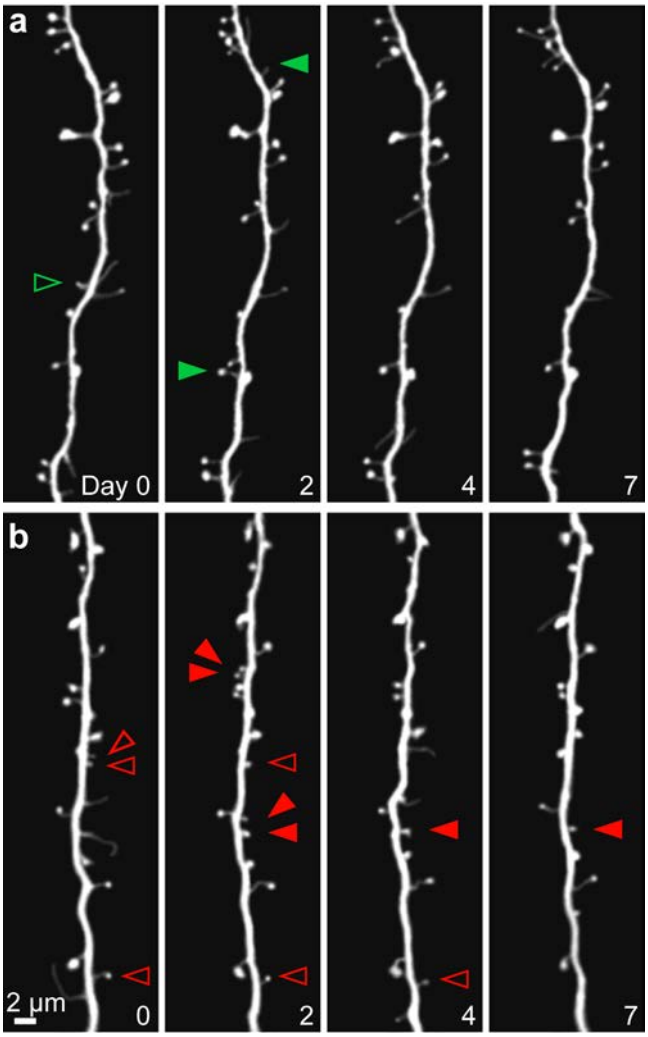
## Supplementary Figures and Legends



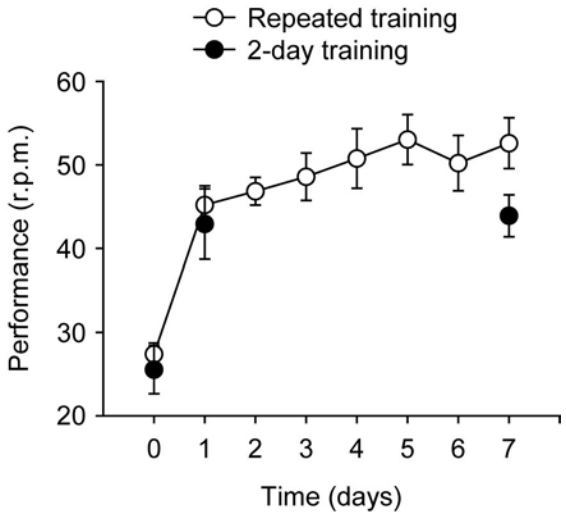

**Supplementary Figure 1 | Percentage of spines formed over 12–24 hours in the barrel cortex of mice housed under standard environment (SE) and enriched environment (EE).** A small but significant increase in spine formation was found as early as 12–24 hours after EE in young animals ($P < 0.05$). Data are presented as mean ± s.d. See Supplementary Table for the number of animals in each group.



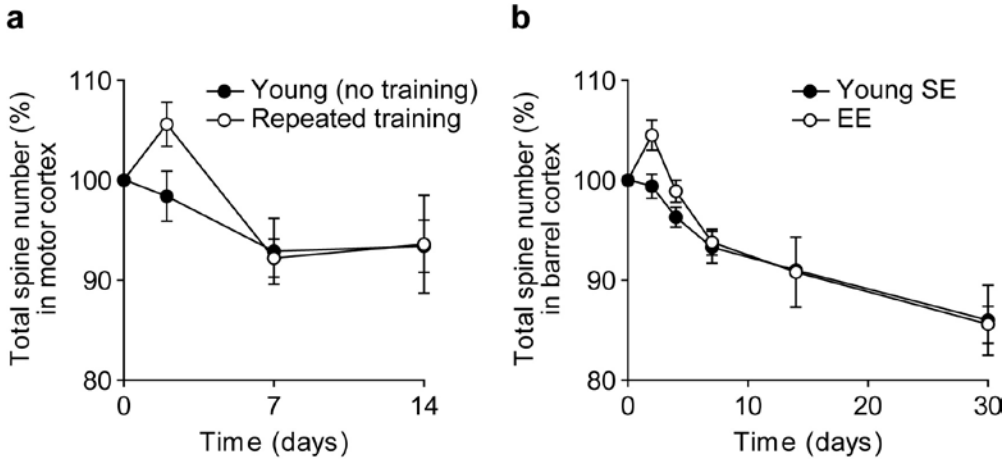

**Supplementary Figure 2 | Filopodial dynamics over 2 days were not significantly altered after motor training or by sensory enrichment. a**, **b**, Percentage of filopodia formed (**a**) and eliminated (**b**) within 2 days in control and motor training animals. Accelerated rotarod training over 2 days had no significant effect on filopodia formation and elimination in both young and adult animals ($P > 0.1$). **c**, **d**, Percentage of filopodia formed (**c**) and eliminated (**d**) over 1–2 days in barrel cortex of animals housed in SE and EE. Sensory enrichment over 1–2 days had no significant effect on filopodia formation and elimination in both young and adult animals ($P > 0.2$). 7–11 animals were used for each group. Data are presented as mean ± s.d.



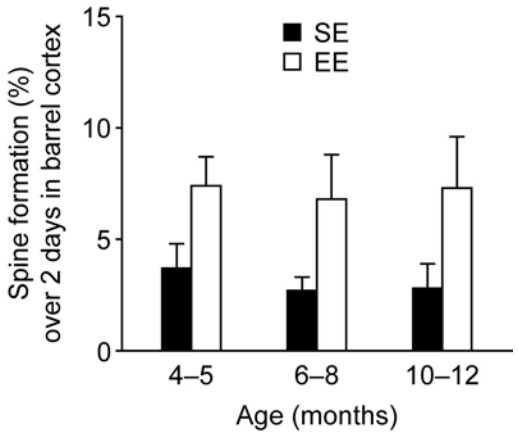

**Supplementary Figure 3 | A small fraction of newly-formed spines persists. a**, **b**, Repeated imaging of dendritic branches in the barrel cortex under SE (**a**) and EE (**b**). Filled arrowheads indicate new spines that were formed during the first 2 days, and open arrowheads indicate spines existing on day 0 and eliminated over next 7 days. Scale bar, 2 µm.



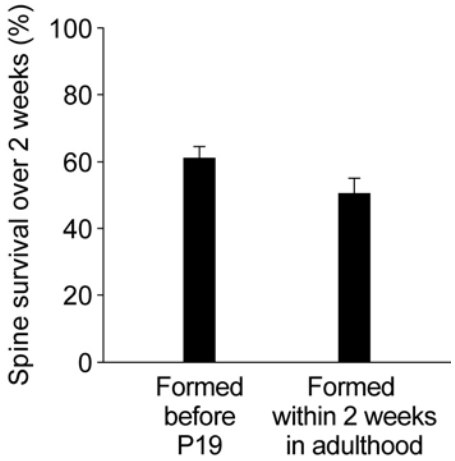

**Supplementary Figure 4 | Plot of rotarod performance of young animals on each training day.** On day 7, animals that had received repeated training for 7 days (open circles; 10 animals) performed better than animals that were trained for only 2 days (filled circles; 5 animals). $P < 0.05$. Data are presented as mean ± s.e.m.




**Supplementary Figure 5 | Dynamic regulation of spine number by motor training and sensory enrichment in 1 month old animals**. In addition to rapidly promoting new spine formation over days, motor learning and novel sensory experience increased elimination of existing spines over weeks. As a result, the total number of spines increased during the first week of motor training (**a**) or EE (**b**) and decreased afterwards, in consistent with previous publications[34, 35]. Data are presented as mean ± s.d. See Supplementary Table for the number of animals in each group.




**Supplementary Figure 6 | The rate of spine formation is relatively constant throughout adulthood**. Percentages of spine formation over 2 days in mice of various ages (4–5 months, 6–8 months, 10–12 months) were comparable under SE. Likewise, percentages of spine formation over 2 days in mice of various ages (4–5 months, 6–8 months, 10–12 months) were comparable under EE. Data are presented as mean ± s.d.




**Supplementary Figure 7 | A fraction of spines formed during early development (before P19) survived later in life.** Over two weeks, the survival fraction of early-formed new spines (before P19) was comparable to that of new spines formed within two weeks later in adulthood, suggesting that maintenance of a fraction of new spines via a prolonged process is a fundamental attribute of neural circuits throughout development and in adulthood. Data are presented as mean ± s.d.

## Supplementary Table

**a. The percentage of spines eliminated and formed (number of spines eliminated or formed/number of pre-existing spines) over various intervals under different experimental conditions. Data are presented as mean ± s.d.**

**Motor cortex**

| Time interval | Condition | Animal # | Spine # in 1st view | Formation (%) | Elimination (%) |
|---|---|---|---|---|---|
| **Young (1 month old)** | | | | | |
| 2 days | Rotarod training | 10 | 1415 | 14.7 ± 1.9 | 9.2 ± 1.7 |
| 2 days | No training control | 7 | 966 | 7.3 ± 1.3 | 8.9 ± 1.8 |
| 2 days | Non-accelerated rotarod control | 4 | 513 | 8.0 ± 1.5 | 9.4 ± 1.1 |
| 7 days | Repeated training | 4 | 643 | 10.7 ± 2.1 | 18.5 ± 2.0 |
| 7 days | No training control | 4 | 503 | 6.6 ± 2.7 | 13.8 ± 1.3 |
| 7 days | 2 day training | 4 | 619 | 10.7 ± 2.2 | 13.3 ± 0.9 |
| 2 weeks | Repeated training | 4 | 616 | 14.0 ± 5.5 | 20.3 ± 0.6 |
| 2 weeks | No training control | 4 | 576 | 9.8 ± 2.0 | 16.4 ± 1.3 |
| **Adult ( > 4 months)** | | | | | |
| 2 days | Rotarod training | 4 | 462 | 8.1 ± 0.7 | 3.5 ± 0.8 |
| 2 days | No training control | 4 | 480 | 3.1 ± 0.6 | 2.9 ± 0.9 |
| 2 days | Pre-trained at P30, trained with the same regime at 4 months of age | 4 | 559 | 4.2 ± 1.4 | 3.8 ± 1.8 |
| 2 days | Pre-trained at P30, trained with new regime at 4 months of age | 4 | 455 | 8.1 ± 1.3 | 3.9 ± 0.5 |

**Barrel cortex**

| Time interval | Condition | Animal # | Spine # in 1st view | Formation (%) | Elimination (%) |
|---|---|---|---|---|---|
| **P19** | | | | | |
| 2 days | SE | 6 | 904 | 14.4 ± 4.3 | 19.6 ± 3.8 |
| 11 days | SE | 8 | 1262 | 14.2 ± 5.9 | 33.0 ± 4.6 |
| **Young (1 month old)** | | | | | |
| 12-24 hrs | SE | 7 | 979 | 5.0 ± 1.6 | 4.3 ± 1.6 |
| 12-24 hrs | EE | 8 | 1149 | 8.3 ± 2.5 | 5.8 ± 1.6 |
| 2 days | SE | 10 | 1904 | 7.2 ± 0.9 | 7.8 ± 0.9 |
| 2 days | EE | 11 | 1994 | 12.9 ± 1.3 | 8.4 ± 1.1 |
| 2 days | SE plus whisker trimming | 4 | 781 | 6.2 ± 0.5 | 3.6 ± 1.0 |
| 2 days | EE plus whisker trimming | 4 | 697 | 5.8 ± 0.5 | 3.8 ± 0.6 |
| 7 days | SE | 5 | 1033 | 6.9 ± 1.1 | 13.6 ± 0.8 |
| 7 days | EE | 4 | 760 | 11.1 ± 1.8 | 17.3 ± 0.5 |
| 7 days | EE (2 days)–SE (5 days) | 4 | 642 | 6.5 ± 2.1 | 14.6 ± 1.4 |
| 7 days | EE(2 days)–EE plus trimming (5 days) | 4 | 737 | 8.1 ± 1.7 | 12.0 ± 0.9 |
| 2 weeks | SE | 6 | 816 | 7.3 ± 1.1 | 16.3 ± 1.2 |
| 2 weeks | EE | 7 | 1252 | 12.1 ± 1.9 | 21.3 ± 2.7 |
| 1 month | SE | 5 | 894 | 8.0 ± 1.3 | 22.0 ± 2.5 |
| 1 month | EE | 4 | 827 | 13.0 ± 1.0 | 27.5 ± 1.8 |
| 3 months | SE | 4 | 579 | 10.2 ± 1.1 | 32.2 ± 1.5 |

| Adult ( > 4 months) | | | | | |
|---|---|---|---|---|---|
| 2 days | SE | 5 | 766 | 2.7 ± 0.6 | 2.7 ± 1.1 |
| 2 days | EE | 5 | 833 | 7.2 ± 1.3 | 3.1 ± 1.6 |
| 4 days | SE | 4 | 606 | 3.3 ± 1.0 | 3.3 ± 0.5 |
| 4 days | EE | 4 | 594 | 8.9 ± 2.8 | 4.0 ± 0.5 |
| 7 days | SE | 4 | 616 | 4.1 ± 1.0 | 4.4 ± 0.8 |
| 7 days | EE | 5 | 710 | 10.3 ± 1.4 | 5.0 ± 0.5 |
| 2 weeks | SE | 4 | 639 | 4.6 ± 0.4 | 5.1 ± 0.4 |
| 2 weeks | EE | 5 | 679 | 10.9 ± 1.7 | 5.7 ± 1.6 |
| 1 month | SE | 5 | 870 | 5.9 ± 0.9 | 7.0 ± 0.4 |
| 1 month | EE | 5 | 626 | 11.9 ± 2.5 | 8.0 ± 0.9 |
| 2 months | SE | 4 | 705 | 7.9 ± 0.9 | 9.9 ± 0.9 |
| 2 months | EE | 4 | 640 | 13.7 ± 1.7 | 12.4 ± 1.0 |
| 5 months | SE | 5 | 814 | 11.4 ± 0.8 | 13.9 ± 1.2 |
| 5 months | EE | 2 | 211 | 18.0 ± 2.3 | 17.1 ± 1.0 |
| 10 months | SE | 2 | 204 | 14.7 ± 1.5 | 18.6 ± 1.6 |
| 14 months | SE | 3 | 400 | 16.9 ± 2.0 | 22.1 ± 1.4 |

**b. Survival fraction of newly formed spines over different time intervals (Fig. 2). Data are presented as mean ± s.d.**

| Conditions under which new spines were formed (2 days) | Conditions under which the survival fraction of new spines was measured | Time interval | Animal # | New spine # | Survival fraction (%) |
|---|---|---|---|---|---|
| **Motor cortex** | | | | | |
| No training | No training | 2 days | 4 | 35 | 44.0 ± 6.3 |
| No training | No training | 5 days | 4 | 35 | 28.0 ± 4.3 |
| No training | No training | 12 days | 4 | 37 | 21.6 ± 4.4 |
| Training | Additional training for 2 days | 2 days | 5 | 124 | 43.6 ± 5.0 |
| Training | Additional training for 5 days | 5 days | 4 | 99 | 30.3 ± 4.8 |
| Training | Additional training for 2 days | 5 days | 1 | 9 | 22.2 |
| Training | Additional training for 12 days | 12 days | 4 | 65 | 21.5 ± 5.2 |
| Training | No additional training | 2 days | 4 | 84 | 27.4 ± 1.8 |
| Training | No additional training | 5 days | 4 | 84 | 13.1 ± 3.1 |
| Training | No additional training | 12 days | 4 | 44 | 9.1 ± 2.5 |
| **Barrel cortex** | | | | | |
| SE | SE | 2 days | 5 | 84 | 36.9 ± 6.3 |
| SE | SE | 5 days | 6 | 90 | 22.2 ± 6.0 |
| SE | SE | 12 days | 4 | 68 | 14.7 ± 4.1 |
| EE | EE | 2 days | 5 | 110 | 37.3 ± 5.7 |
| EE | EE | 5 days | 4 | 100 | 21.0 ± 3.6 |
| EE | EE | 12 days | 4 | 74 | 16.2 ± 4.0 |
| EE | SE | 2 days | 4 | 83 | 20.5 ± 3.8 |
| EE | SE | 5 days | 4 | 64 | 12.5 ± 3.4 |
| EE | SE | 12 days | 5 | 98 | 7.1 ± 2.7 |
| EE | EE plus whisker trimming | 2 days | 4 | 91 | 18.7 ± 3.6 |
| EE | EE plus whisker trimming | 5 days | 4 | 91 | 12.1 ± 5.0 |

## Supplementary Information 1

### Estimating the survival of new spines over time

Assuming (1) the formation rate of spines between $t$ and $t + \Delta t$ is $F(t)$ and (2) the survival fraction of $F(t)\Delta t$ at time $T$ is $S(T)$:

$$S(T) = a \cdot e^{-(T-t)/\tau_1} + b \cdot e^{-(T-t)/\tau_2} + c \cdot e^{-(T-t)/\tau_3}, \quad a + b + c = 1$$

Accumulation of new spines from $T_0$ to $T$ can be expressed as

$$N_{accumulation}(T) = \int_{T_0}^{T} F(t) \cdot (a \cdot e^{-(T-t)/\tau_1} + b \cdot e^{-(T-t)/\tau_2} + c \cdot e^{-(T-t)/\tau_3}) dt$$

If one assumes that $F(t)$ is a constant ($F_a$) throughout adulthood,

$$\begin{aligned} N_{accumulation}(T) &= F_a \cdot a \cdot \tau_1 (1 - e^{-(T-T_0)/\tau_1}) + F_a \cdot b \cdot \tau_2 (1 - e^{-(T-T_0)/\tau_2}) + F_a \cdot c \cdot \tau_3 (1 - e^{-(T-T_0)/\tau_3}) \\ &= F_a \cdot a \cdot \tau_1 + F_a \cdot b \cdot \tau_2 + F_a \cdot c \cdot \tau_3 - F_a \cdot a \cdot \tau_1 \cdot e^{-(T-T_0)/\tau_1} \\ &\quad - F_a \cdot b \cdot \tau_2 \cdot e^{-(T-T_0)/\tau_2} - F_a \cdot c \cdot \tau_3 \cdot e^{-(T-T_0)/\tau_3} \end{aligned}$$

When we applied three exponential curve fitting to experimental measurement of new spine accumulation over time intervals ranging from 2 days to 18.5 months under SE, we found that

$$N_{accumulation}(t) = 0.5770 - 0.0320 \cdot e^{-t/1.3541 days} - 0.0585 \cdot e^{-t/66.6667 days} - 0.4865 \cdot e^{-t/2399 days};$$

$$r^2 = 0.9999$$

By matching this curve fitting result with the mathematically derived function, we obtained the following:

$$\tau_1 = 1.3541 \text{ days}; \tau_2 = 66.6667 \text{ days}; \tau_3 = 2399 \text{ days};$$

$$F_a \cdot a \cdot \tau_1 = 0.032; F_a \cdot b \cdot \tau_2 = 0.0585; F_a \cdot c \cdot \tau_3 = 0.4865.$$

Based on $a + b + c = 1$, we found that:

$$F_a = 2.47\%; a = 95.63\%; b = 3.55\%; c = 0.82\%.$$

Furthermore, when we applied three exponential curve fitting to experimental measurement of new spine accumulation over time intervals ranging from 2 days to 5 months under EE, we found that:

$$N_{accumulation}(t) = 1.1698 - 0.0953 \cdot e^{-t/1.6018 days} - 0.0138 \cdot e^{-t/25 days} - 1.0605 \cdot e^{-t/2189 days};$$

$$r^2 = 0.9991$$

Through the same calculation mentioned above, we found that:

$$\tau_1 = 1.6018 \text{ days}; \tau_2 = 25 \text{ days}; \tau_3 = 2189 \text{ days};$$

$$F_a = 6.05\%; a = 98.29\%; b = 0.91\%; c = 0.80\%.$$

In summary, based on the exponential curve fittings in Fig. 4a, we estimated $\tau_1 \approx 1.5$ days, $\tau_2 \approx$ 1–2 months and $\tau_3 \approx$ 73–80 months. Importantly, ~0.8% ($c$) of daily-formed new spines have an average lifetime of 73–80 months under SE and EE, suggesting a small fraction of daily-formed spines could last through the animal's life span (~36 months).

It is worth to mention that when $T - T_0 >> \tau_2$,

$$N_{accumulation}(T) \approx F_a \cdot a \cdot \tau_1 + F_a \cdot b \cdot \tau_2 + F_a \cdot c \cdot \tau_3 - F_a \cdot c \cdot \tau_3 \cdot e^{-(T-T_0)/\tau_3}$$

This suggests that after the first 2–3 months, accumulation of new spines will increase according to the exponential function with the time constant $\tau_3$.

Finally, we would like to point out that the fitting curves in Fig. 4a (also in Fig. 4b) look like they are going right through the data points. This is in part due to the small scale of the figure and also in part because there are 7 degrees of freedom for Fig. 4a (6 degrees of freedom for Fig. 4b). At higher magnification, however, one can see that the fitting curves do not go right through all the data points.

## Supplementary Information 2

### Estimating the number of spines formed after learning and remained at the end of life

Based on the exponential fittings in Fig. 4a, we calculated that ~0.8% of daily formed spines in the barrel cortex have a life time of ~73–80 months under standard housing or enriched environment (see Supplementary Information 1). Because the degree of spine formation (Fig. 1f–i) and the survival of new spines (Fig. 2b, c) are comparable between motor and barrel cortices, we expect a similar degree of daily-generated new spines are also long-lasting in the motor cortex.

We found ~5–7% increase in spine formation over 2 days under motor learning or enriched environment conditions (Fig. 1). Furthermore, new experience leads to ~3–4% increase in new spine formation over 1 day in the barrel cortex (Supplementary Fig. 1). After 1-day rotarod training, a similar degree of increase (~4%) in new spine formation has also been found in the motor cortex (data not shown). Assuming ~0.8% of daily formed spines in the barrel or motor cortex have a life time of ~73–80 months, the number of new spines that are formed over 2 days and persist until the end of life would be $2 \times 4\% \times 0.8\% \times e^{-36/80} \approx 0.04\%$ of the total spines at the time when the animals are exposed to novel experience (assuming the mouse lifespan ~36 months).

Assuming the motor cortex occupies 1/10 of the entire cortical region[36] and the half of it is involved in the rotarod learning[37-40], we estimate that $\sim 8 \times 10^{10} \times (1/10) \times (1/2) \times 5\sim 7\% \approx 2 \times 10^{8}$ new spines are formed 2 days after motor training. Furthermore, $\sim 8 \times 10^{10} \times (1/10) \times (1/2) \times 0.04\% \approx 2 \times 10^{6}$ spines can persist until the end of life. The number of synapses formed and subsequently maintained after motor learning are likely several-fold larger than what we estimated here because other brain regions such as cerebellum are also important for the process. Nevertheless, $\sim 2 \times 10^{6}$ stable spines are more than enough to have significant impact on the animals' behaviour because sensory representation could be achieved with few hundred synapses and activation of 1–100 neurons could impact animals' behaviors[41-43]. These estimates suggest that after a protracted process, a fraction of new spines induced by novel experience persist and are sufficiently many to modify neural network functions and directly contribute to the lifelong storage of information.

## Supplementary Information 3

### Estimating the survival of existing spines over time

Assuming that (1) the formation rate of adult spines between $t$ and $t + \Delta t$ is $F(t)$ and (2) the survival fraction of $F(t)\,\Delta t$ at time $T$ is $S(T)$:

$$S(T) = a \cdot e^{-(T-t)/\tau_1} + b \cdot e^{-(T-t)/\tau_2} + c \cdot e^{-(T-t)/\tau_3}, \quad a + b + c = 1$$

The survival of adult spines formed from $T_a$ to $T_0$ and persisting at time $T$ can be expressed as

$$S_a(T) = \int_{T_a}^{T} F(t) \cdot (a \cdot e^{-(T-t)/\tau_1} + b \cdot e^{-(T-t)/\tau_2} + c \cdot e^{-(T-t)/\tau_3}) dt - \int_{T_0}^{T} F(t) \cdot (a \cdot e^{-(T-t)/\tau_1} + b \cdot e^{-(T-t)/\tau_2} + c \cdot e^{-(T-t)/\tau_3}) dt$$

If one assumes that $F(t)$ is a constant $F_a$ throughout adulthood,

$$S_a(T) = F_a \cdot a \cdot \tau_1 (e^{-(T-T_a)/\tau_1} - e^{-(T-T_0)/\tau_1}) + F_a \cdot b \cdot \tau_2 (e^{-(T-T_a)/\tau_2} - e^{-(T-T_0)/\tau_2}) + F_a \cdot c \cdot \tau_3 (e^{-(T-T_a)/\tau_3} - e^{-(T-T_0)/\tau_3})$$

According to the exponential curve fitting in Fig. 4a, $\tau_1 \approx 1.5$ days, $\tau_2 \approx 1$–2 months and $\tau_3 \approx 73$–80 months. When $T–T_0 >> \tau_2 >> \tau_1$, the survival of adult spines formed from $T_a$ to $T_0$ and persisting at time $T$

$$S_a(T) \approx F_a \cdot c \cdot \tau_3 (e^{-(T-T_a)/\tau_3} - e^{-(T-T_0)/\tau_3}) \approx A \cdot e^{-T/\tau_3}$$

We have found that spines formed from P19 to P21 and spines formed later in life are maintained by a similar protracted process (Fig. 5e). Assuming that spines formed early in life and surviving into adulthood follow a similar equation as shown above, the survival of all the existing spines in adulthood could be expressed as

$$S_{all}(T) \approx A \cdot e^{-T/\tau_3} + B \cdot e^{-T/\tau_3'} \text{ (when } T–T_0 >> \tau_2 >> \tau_1)$$

$\tau_3$ is the decay time constant for spines formed in adult life and $\tau_3$' is the decay time constant for spines formed during development.

When we applied the exponential curve fitting to our measurements of the survival of all the existing spines in adulthood under SE or EE in Fig. 4b, we found that:

Under SE:

$$S(t) = 0.0293 \cdot e^{-t/1.4073 days} + 0.0621 \cdot e^{-t/40.9836 days} + 0.9085 \cdot e^{-t/2703 days};\ r^2 = 0.9997$$

Under EE:

$$S(t) = 0.0306 \cdot e^{-t/0.9716 days} + 0.0829 \cdot e^{-t/40.9836 days} + 0.8866 \cdot e^{-t/2137 days};\ r^2 = 0.9960$$

The above exponential curve fittings suggest that a small fraction of existing spines in adulthood have average lifetime of ~1–1.4 days ($\tau_1$) and ~1.5 months ($\tau_2$). Importantly, ~89–91% of existing spines in adulthood have an average lifetime of 71–90 months ($\tau_3$ or $\tau_3$') under EE and SE, suggesting a large fraction of adult spines could last through the animal's life span.

## Supplementary Information 4

### Estimating the time course over which a fraction of new spines are maintained based on the developmental profile of spine number

Assuming (1) the formation rate of spines between $t$ and $t + \Delta t$ is $F(t)$:

$$F(t) = f(t) \qquad \text{when } t < \text{the peak of spinogenesis } T_p$$

$$F_p \cdot e^{-(t-T_p)/\tau_f} \qquad \text{when the peak of spinogenesis} < t < \text{adulthood}$$

$$F_a \qquad \text{when } t > \text{adulthood}$$

(2) the survival fraction of $F(t)\Delta t$ at time $T$ is

$$S(T) = a \cdot e^{-(T-t)/\tau_1} + b \cdot e^{-(T-t)/\tau_2} + c \cdot e^{-(T-t)/\tau_3}$$

The total spine number at time $T$ (during late postnatal development) can be expressed as:

$$\begin{aligned} N(T) &= \int_0^T F(t) \cdot S(T-t)dt \\ &= \int_0^{T_p} f(t) \cdot (a \cdot e^{-(T-t)/\tau_1} + b \cdot e^{-(T-t)/\tau_2} + c \cdot e^{-(T-t)/\tau_3})dt \\ &\quad + \int_{T_p}^T (F_p \cdot e^{-(t-T_p)/\tau_f}) \cdot (a \cdot e^{-(T-t)/\tau_1} + b \cdot e^{-(T-t)/\tau_2} + c \cdot e^{-(T-t)/\tau_3})dt \end{aligned}$$

Based on the exponential curve fittings in Fig. 4a, b and 5d,

$$\tau_1 \text{ (~1 day)} << \tau_f \text{ (~7.5 days)} << \tau_2 \text{ (~1–2 months)} << \tau_3 \text{ (~73–80 months)}$$

When $\tau_f << T - T_p < \tau_2$,

$$\begin{aligned} N(T) &\approx \int_0^{T_p} f(t) \cdot (b \cdot e^{-(T-t)/\tau_2} + c)dt + \int_{T_p}^T (F_p \cdot e^{-(t-T_p)/\tau_f}) \cdot (b \cdot e^{-(T-t)/\tau_2} + c)dt \\ &\approx \int_0^{T_p} f(t) \cdot c dt + (\int_0^{T_p} f(t) \cdot b \cdot e^{t/\tau_2} dt) \cdot e^{-T/\tau_2} + \tau_f \cdot F_p \cdot c \cdot (1 - e^{-(T-T_p)/\tau_f}) \\ &\quad + F_p \cdot b \cdot \tau_2 \cdot \tau_f / (\tau_2 - \tau_f) \cdot (e^{-(T-T_p)/\tau_2} - e^{-(T-T_p)/\tau_f}) \\ &\approx A + B \cdot e^{-T/\tau_2} \end{aligned}$$

This suggests that during late postnatal development, the net spine loss occurs according to an exponential function with a time constant $\tau_2$, which reflects the time course over which newly-formed spines are progressively integrated into the existing circuitry (Fig. 5 a–c).

In both barrel and motor cortices, we found that the developmental decline in spine formation occurred faster than the prolonged process of maintaining newly-formed spines (Fig. 5d–e). Consequently, the time course of the developmental spine loss

mainly reflected that of the process of new spine maintenance (Fig. 5a). Indeed, we found that the spine number decreased from P37 to 4 months of age with a time constant of ~1.5 months, which is comparable to the time course over which a fraction of newly-formed spines were stably maintained on layer V apical dendrites (Fig. 2b, c, 4a). Furthermore, based on the developmental profiles of spine number in the basal dendrites of layer V and VI pyramidal cells in the mouse barrel cortex (Fig. 5b, c), we estimated that the process over which a fraction of newly-formed spines are maintained on these basal dendrites lasted ~0.6–0.8 months.

## Supplementary Information 5

### Estimating the lifetime of stably-maintained spines based on age-dependent decline in spine number

Assuming that (1) the formation rate of spines between $t$ and $t + \Delta t$ is $F(t)$:

$$F(t) = f(t) \qquad \text{when } t < \text{adulthood } T_a$$

$$F_a \qquad \text{when } t > \text{adulthood } T_a$$

(2) the survival fraction of $F(t)\Delta t$ at time $T$ is

$$S(T) = a \cdot e^{-(T-t)/\tau_1} + b \cdot e^{-(T-t)/\tau_2} + c \cdot e^{-(T-t)/\tau_3}$$

The total spine number at time $T$ (in adulthood) can be expressed as:

$$\begin{aligned} N(T) &= \int_0^T F(t) \cdot S(T-t)dt \\ &= \int_0^{T_a} f(t) \cdot (a \cdot e^{-(T-t)/\tau_1} + b \cdot e^{-(T-t)/\tau_2} + c \cdot e^{-(T-t)/\tau_3})dt \\ &\quad + \int_{T_a}^T F_a \cdot (a \cdot e^{-(T-t)/\tau_1} + b \cdot e^{-(T-t)/\tau_2} + c \cdot e^{-(T-t)/\tau_3})dt \end{aligned}$$

Based on the exponential curve fittings in Fig. 4a, b,

$$\tau_1 \text{ (~1 day)} << \tau_2 \text{ (~1–2 months)} << \tau_3 \text{ (~73–80 months).}$$

When $T - T_a >> \tau_2$, then

$$N(T) \approx \int_0^{T_a} f(t) \cdot c \cdot e^{-(T-t)/\tau_3} dt + F_a \cdot a \cdot \tau_1 + F_a \cdot b \cdot \tau_2 + F_a \cdot c \cdot \tau_3 - F_a \cdot c \cdot \tau_3 \cdot e^{-(T-T_a)/\tau_3}$$

$$\approx F_a \cdot a \cdot \tau_1 + F_a \cdot b \cdot \tau_2 + F_a \cdot c \cdot \tau_3 + (\int_0^{T_a} f(t) \cdot c \cdot e^{t/\tau_3} dt - F_a \cdot c \cdot \tau_3 \cdot e^{T_a/\tau_3}) \cdot e^{-T/\tau_3}$$

$$\approx A + B \cdot e^{-T/\tau_3}$$

This suggests that a few months into adulthood, the total number of spines decreases according to an exponential function with a time constant $\tau_3$, which reflects the average lifetime of stably-maintained spines in the circuits (Figs. 4a, b and 5a–c).

## Supplementary Discussion

Recent studies indicate that adult spines can persist throughout a lifetime, but it is unclear how adult spine stability relates to learning-induced synaptic reorganization and participates in memory storage. Here we have provided three lines of evidence indicating that despite ongoing and critical circuit plasticity, there are two populations of stable spines in synaptic circuits that are important for maintaining long-lasting memories.

First, although new spines are induced by learning and novel sensory experience, a small fraction of them are transformed, via a prolonged process, into spines that are stably maintained over the animal's lifespan. These new stable spines are specifically induced by novel experience and their maintenance is facilitated by persistent experience. Their number strongly correlates with the animal's behavioural performance after learning and is sufficiently large to impact behaviour throughout the animal's life (Figs. 2, 4 and Supplementary Information 2). Therefore, new stable spines could represent unique and permanent structural marks for lifelong information storage in the cortex. Second, a large pool of spines exists during early postnatal development due to a rapid burst in spinogenesis. These early-formed spines that survive into adulthood constitute the major population of spines in mature circuits and are largely maintained later in life (Fig. 4). Importantly, early-formed spines undergo experience-dependent pruning, the degree of which also strongly correlates with the animal's performance after learning. Therefore, early-formed spines surviving the process of pruning carry new information and likely contribute to memory storage throughout life. Third, based on age-dependent changes in spine number, we found that stably maintaining new spines and spines formed early in life are likely a general phenomenon, occurring not only in the apical dendrites of layer V pyramidal neurons but also in other cell types and cortical layers in the mouse cortex. Together, these findings suggest that new stable

spines induced by novel experience and existing spines that participate in a sensory or behavioral event, represent an integrated and stable physical entity for lifelong memory storage, despite ongoing plasticity in synaptic networks (Fig. 5f).

It is important to point out that because of technical limitations, the present study is restricted to an analysis of spine dynamics on apical dendrites of layer V pyramidal cells in the motor or barrel cortex. Even though our studies suggest that similar rules governing spine development and maintenance may apply to different pyramidal cells, future studies are required to directly examine spine dynamics of other neuronal cell types, cortical layers and regions. Furthermore, other than synapse formation and elimination, alterations in synaptic efficacy and neuronal excitability represent essential aspects of brain plasticity that require further investigation in order to better understand the brain's extraordinary ability to learn and to remember.

Finally, it is important to mention that previous studies have examined the effect of sensory deprivation and peripheral injury on spine turnover in the mouse barrel and visual cortices. One study has shown that sensory deprivation via whisker trimming for weeks predominantly prevents spine loss in the barrel cortex of young adolescent mice but has no significant effect on adult spine turnover[9]. In contrast, other studies have shown that sensory deprivation over a few days (chessboard trimming or monocular deprivation) leads to a substantial formation or elimination of persistent spines (~8–15%) in adult mice[15, 16]. These latter studies are also inconsistent with our present findings that novel experience over days causes a small degree (<1%) of remodelling of persistent spines. The reason for the discrepancies over the degree of experience-dependent spine remodelling remains unknown. It is clear, however, that studies showing substantial spine turnover after sensory deprivation or peripheral injury used open-skull windows for chronic imaging of spines whereas studies showing orders of magnitude lower remodelling of persistent spines used thinned-skull windows for spine imaging. Because open-skull preparations involve skull removal (~5 mm in diameter) and implantation of a glass window, such preparations often show significant activation of microglia and astrocytes, initial dendritic spine loss after surgery and a dramatic increase in spine turnover in the cortex[44-46]. Furthermore, potent immunomodulators such as dexamethasone are frequently used following open-skull preparations to reduce inflammation and achieve optimal imaging properties[47-53]. It is therefore possible that data on spine dynamics and its modification by experience using the open-skull window soon after surgery may not apply to normal physiological conditions. Future studies comparing experience-dependent spine remodelling using thinned-skull and open-skull windows are necessary to resolve the discrepancies between different studies.

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