Cocaine-induced structural plasticity in frontal cortex correlates with conditioned place preference

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Contextual cues associated with previous drug exposure can trigger drug craving and seeking, and form a substantial obstacle in substance use recovery. Using *in vivo* imaging in mice, we found that cocaine administration induced a rapid increase in the formation and accumulation of new dendritic spines, and that measures of new persistent spine gain correlated with cocaine conditioned place preference. Our data suggest that new persistent spine formation in the frontal cortex may be involved in stimulant-related learning driving appetitive behavior.

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The rodent dorsomedial prefrontal cortex (dmPFC) has been shown to support appetitive responses to cocaine-paired stimuli and behavioral sensitization to cocaine[1,2]. Anatomically, the dmPFC is particularly well poised to integrate information from sensory and memory systems and to modulate behavior. Changes in the connectivity of layer 5 neurons in this area could directly affect the output of the frontal cortex to the basal ganglia and other downstream subcortical circuits that are known to be involved in action selection[3,4].

At a structural level, repeated daily exposure to stimulants has been shown to increase dendritic spine density in layer 5 pyramidal neurons of rodent dmPFC after weeks of withdrawal[5–7]. However, little is known about the timing and dynamics of these plastic events and their potential relationship with behavioral changes induced by stimulant exposure. More detailed knowledge of cocaine-induced structural plasticity could enhance our understanding of the mechanisms supporting drug associations that fuel substance use disorders.

To address these questions, we used *in vivo* two-photon imaging via a cranial window in *Thy1* YFP-H transgenic mice[8] to follow structural changes in dendritic spines in the apical dendrites of layer 5 neurons in the dmPFC before and after cocaine exposure. In our first experiment, we imaged neurons in the dmPFC every 48 h for up to 32 d (**Fig. 1**). After a baseline period, intraperitoneal injections of either cocaine (15 mg per kg of body weight) or saline were given daily for 12 d, and afterward imaging continued for a 2-week withdrawal period. In addition, we also measured locomotor behavior in response to drug administration on the days that the mice were not being imaged (**Fig. 1a**). We found that cocaine-treated mice showed significantly greater spine gains than saline-treated control mice ($P < 0.001$), most notable after the first day of treatment (**Fig. 1d**). In contrast, there was no effect of treatment on spine loss ($P > 0.6$; **Supplementary Fig. 1b**). During the withdrawal phase, we observed no significant effect of prior cocaine treatment on the fraction of spines gained or lost ($P > 0.7$; **Fig. 1e** and **Supplementary Fig. 1c**).

Over the course of the treatment and withdrawal periods, we found that spine density diverged between saline- and cocaine-treated groups (**Fig. 1f**). This divergence was explained by the greater accumulation of new spines in cocaine-treated mice (**Fig. 1g**) and enhanced survival of stable spines present before the treatment onset in the cocaine group (**Supplementary Fig. 1e**). Cocaine had no effect on average survival of new spines measured every 48 h after treatment onset (**Supplementary Fig. 1f,g**). Declines in spine density in 2–3-month-old mice, similar to that observed in our saline control mice (**Fig. 1f**), have been observed in other *in vivo* imaging studies in various cortical regions[9–11]. Naive tissue studies suggest that these declines represent late developmental spine pruning and not an imaging-induced decrease in total spine density (**Supplementary Fig. 2a**).

Mice receiving 12 daily cocaine injections showed an expected increase in cocaine-induced locomotion, known as locomotor sensitization (**Fig. 1h**). To determine whether changes in locomotion were related to the effects of cocaine on spines, we performed correlation analyses between spine dynamics and changes in locomotion (**Fig. 1i,j**). When the measurements of spine dynamics and locomotion change of all the mice were pooled and standardized (z normalized), no correlations were found with net spine change (**Fig. 1j**) or other measures (**Supplementary Figs. 3** and **4**). Furthermore, a cohort of mice allowed to run on a running wheel (**Supplementary Figs. 5** and **6**) showed no correlation between spine gains and locomotion (**Supplementary Fig. 5e**), and running wheel experience did not occlude the effect of cocaine on spine gain (**Supplementary Fig. 6c**).

Using a new cohort of mice, we measured spine gains after acute cocaine treatment with greater temporal resolution (**Fig. 2a**). We found that a single cocaine injection enhanced spine gains ($P < 0.01$; **Fig. 2b**) and spine density ($P < 0.01$; **Fig. 2c**) as early as 2 h after drug administration. We found no effect of cocaine on spine loss (**Fig. 2d**). We replicated previous observations of gains and loss made 24 h after treatment (**Supplementary Fig. 7b,c**). The day after treatment, new spines, those observed just 2 h after cocaine injection, comprised a >3-fold larger fraction of the total spines (20–24 h later) in cocaine-treated mice than in saline-treated mice ($P < 0.01$; **Fig. 2e**).

Increases in spine gain immediately after cocaine exposure (**Fig. 2b**) and accumulation of these new spines (**Fig. 1g**) could represent a mechanism by which cocaine facilitates new associations made between drug experience and predictive contextual cues.

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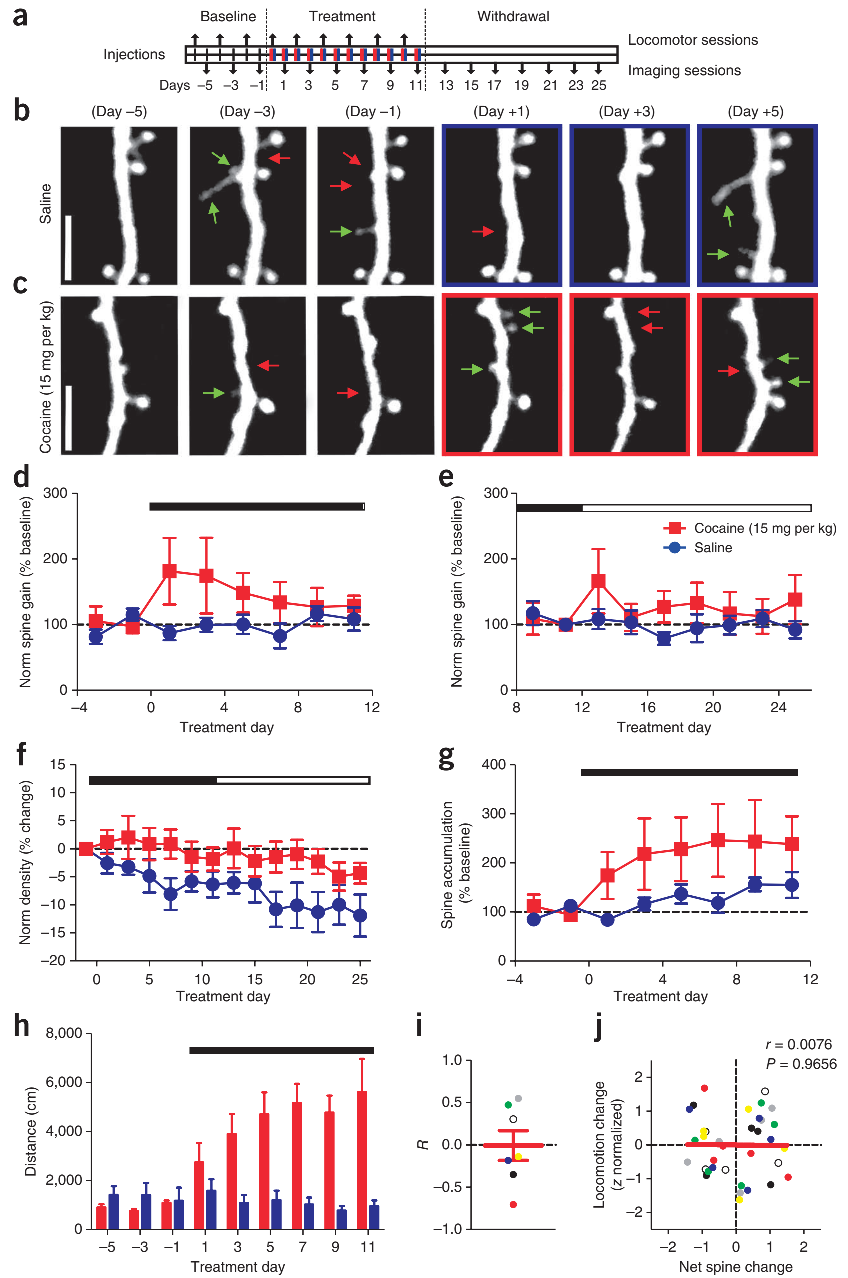

Figure 1 Cocaine exposure increases spine gains in the frontal cortex. (**a**) Schematic of the protocol schedule (see Online Methods). (**b**,**c**) Repeated imaging of dendrites from *Thy1* YFP-H mice. Light green arrows indicate spines gained, red arrows indicate spines lost. Scale bars represent 5 µm. (**d**) Fraction of spines gained between imaging sessions normalized to the gains measured during the baseline period (group: $F_{1,16} = 3.72$, $P = 0.001$; time: $F_{6,96} = 1.22$, $P > 0.86$; interaction: $F_{6,96} = 2.94$, $P < 0.0001$, two-way mixed ANOVA; cocaine, $n = 7$ mice; saline, $n = 11$ mice). Solid black line represents the treatment phase. (**e**) Normalized spine gain during the withdrawal period (group: $F_{1,16} = 0.78$, $P > 0.72$; time: $F_{7,112} = 1.15$, $P > 0.8$; interaction: $F_{7,112} = 1.16$, $P > 0.8$, two-way mixed ANOVA; cocaine, $n = 10$ mice; saline, $n = 8$ mice). White line represents the withdrawal phase. (**f**) Normalized spine density (group: $F_{1,10} = 1.84$, $P > 0.6$; time: $F_{13,130} = 6.11$, $P < 0.0001$; interaction: $F_{13,130} = 1.64$, $P < 0.001$, two-way mixed ANOVA; cocaine, $n = 6$ mice; saline, $n = 6$ mice). (**g**) Summary plot showing spine accumulation (see Online Methods) during the treatment phase (group: $F_{1,16} = 3.29$, $P < 0.002$; time: $F_{6,96} = 6.09$, $P < 0.0001$; interaction: $F_{6,96} = 2.92$, $P < 0.0001$, two-way mixed ANOVA; cocaine, $n = 7$ mice; saline, $n = 11$ mice). (**h**) Average plot of the locomotor activity measured for 120 min after intraperitoneal injection (group: $F_{1,12} = 14.28$, $P < 0.0001$; time: $F_{6,72} = 3.72$, $P < 0.0001$; Interaction: $F_{6,72} = 5.17$, $P < 0.0001$, two-way mixed ANOVA; cocaine $n = 7$ mice; saline, $n = 7$ mice). (**i**,**j**) Summary plot of the correlation between the net change in spine number between consecutive imaging sessions and the changes in distance traveled between consecutive treatment sessions (**Supplementary Fig. 3a–c**). Each dot in **i** represents correlations obtained from individual mice during treatment. Each dot in **j** represents a *z*-normalized plot of net spine change and the subsequent locomotor change on a given day of the treatment. Each individual color represents a mouse. Bars are means and error bars are s.e.m.

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To test the relationship between cocaine exposure and associative learning, we measured spine dynamics in a cocaine conditioned place preference (CPP) procedure (**Fig. 3**). Mice underwent two sessions of CPP training with either saline or cocaine (30 mg per kg) paired with a conditioning chamber (**Fig. 3a**). Preference for the cocaine versus saline paired context was measured 24 h after cocaine pairing. We found that mice gained more new spines on the day that they received cocaine than on the day that they received saline ($P = 0.002$; **Fig. 3b**). New spines observed after cocaine-conditioning had shorter length-to-width ratios than new spines gained after saline conditioning ($P = 0.006$; **Supplementary Fig. 8d**), suggesting they were more likely to persist and potentially form synapses[12–16]. When we isolated the population of new spines that persisted 96 h or more (suggesting they formed synapses[16]), we found that new persistent spines gained after cocaine conditioning represented a significantly greater fraction of total spines than those gained after saline conditioning ($P = 0.007$; **Fig. 3d**).

The percentage of new spines that persisted 96 h or more that were gained on cocaine-pairing day strongly correlated ($r = 0.76$, $P = 0.017$) with the magnitude of the change in preference for the cocaine-paired side (**Fig. 3e**), suggesting a relationship between the two variables. There was no correlation between CPP preference measures and spine loss or density (**Supplementary Fig. 8f,h**). Control experiments in which only saline was given on both conditioning days revealed no correlation between new 96 h persistent spine gains and CPP preference measures ($r = -0.22$, $P > 0.54$; **Supplementary Fig. 9c**).

The correlation that we observed between new persistent spine gains and CPP preference score (**Fig. 3e**) suggests that new persistent spine gain may support learning about drug context cues or appetitive expression of these associations. Associative or reconsolidation processes involved in CPP might also be involved in stabilizing new

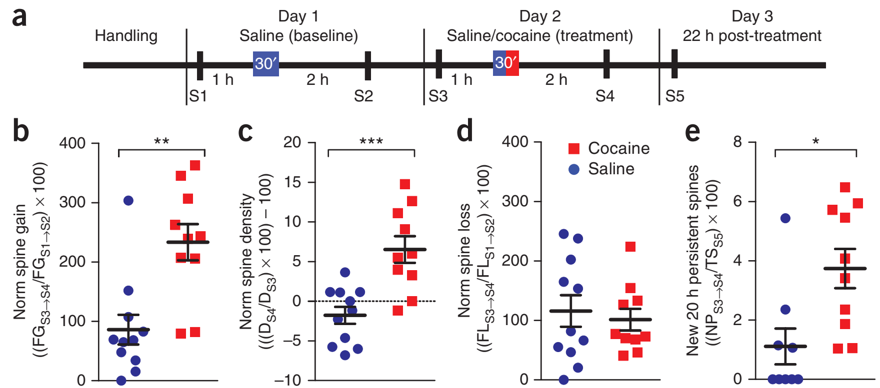

Figure 2 Cocaine increases spine gains in the frontal cortex within 2 h of injection. (**a**) Schematic of the imaging and treatment schedule. S1 to S5 refers to the imaging session number. (**b**) Fraction of spines gained (FG) 2 h after injection ($FG_{S3→S4}$) normalized to the baseline ($FG_{S1→S2}$) ($U = 13$, $P < 0.004$, Mann-Whitney U test; cocaine, $n = 10$ mice; saline, $n = 11$ mice). (**c**) Normalized spine density (D) observed 2 h after cocaine treatment ($t = 4.25$, $P < 0.001$, unpaired Student's t test; cocaine, $n = 10$ mice; saline, $n = 11$ mice). (**d**) Fraction of spines lost (FL) 2 h after injection ($U = 52$, $P > 0.8$, Mann-Whitney U test; cocaine, $n = 10$ mice; saline, $n = 11$ mice). (**e**) New spines that were first observed 2 h after cocaine injection ($NP_{S3→S4}$) and persisted 20 h later (S5), made up a greater percentage of the total number of spines (TS_{S5}) ($U = 10$, $P = 0.011$, Mann-Whitney U test; cocaine, $n = 10$ mice; saline, $n = 9$ mice). Each symbol represents one mouse. Bars are means and error bars are s.e.m. $*P < 0.05$, $**P < 0.01$ and $***P < 0.001$.

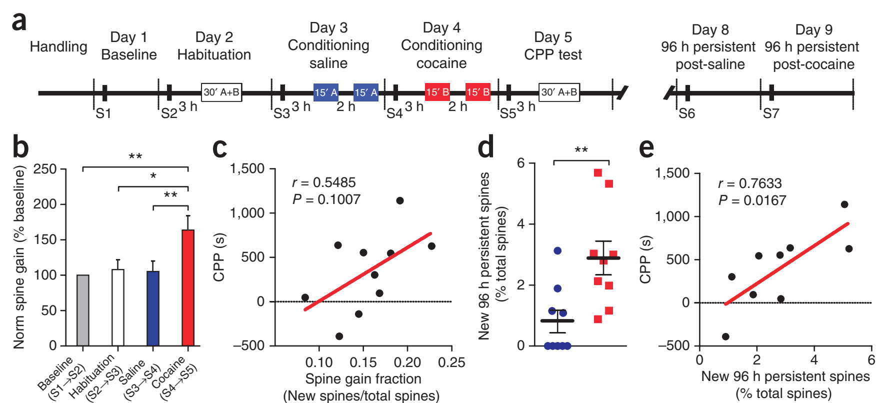

Figure 3 New persistent spine gains after cocaine CPP training correlate with the magnitude of gain in preference for the cocaine-paired context. (**a**) Schematic of the imaging and CPP protocol (see Online Methods). S1 to S7 refers to the imaging session. (**b**) Fraction of spines gained between consecutive imaging sessions. A one-way repeated-measures ANOVA revealed significant differences between sessions ($F_{3,27} = 6.84$, $P = 0.001$, $n = 10$ mice). A Bonferroni *post hoc* analysis revealed that more spines were gained during the cocaine treatment (S4→S5) than during previous sessions (cocaine versus baseline, $P < 0.01$; cocaine versus habituation, $P < 0.05$; cocaine versus saline, $P = 0.01$; $n = 10$ mice). (**c**) Correlation between the fraction of spines gained after cocaine treatment (S4→S5) and preference for the cocaine-paired chamber. (**d**) New persistent spines (present for >96 h) gained during cocaine conditioning (S4→S5) accounted for a greater percentage of total spines (at S7) than after saline (at S6) ($t = 3.56$, $P = 0.007$, $n = 9$ mice, paired Student's t test). (**e**) New persistent spines first gained during cocaine conditioning (S4→S5) showed a significant positive correlation with the change in magnitude of preference for the cocaine-paired chamber between habituation and test day. Bars are means and error bars are s.e.m. $*P < 0.05$, $**P < 0.01$.

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spines formed after cocaine exposure. Future studies investigating mechanisms underlying cocaine-induced spine formation and persistence should shed further light on their role in learning and substance use and abuse, and potentially aid the development of therapeutic interventions for addiction.

METHODS

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

Note: Any Supplementary Information and Source Data files are available in the online version of the paper.

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AUTHOR CONTRIBUTIONS

F.J.M.-C., J.A. and D.P. performed the experiments. L.W. and F.J.M.-C. designed the experiments, analyzed the data and wrote the paper.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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ONLINE METHODS

Mice. Male C57BL/6J transgenic mice (*n* = 77) expressing YFP (line H, 00378, Jackson Laboratory)[8] were housed on a 12/12-h reverse light-dark cycle (lights off at 10:00 a.m.). Mice were weaned at postnatal day 21 (P21) and housed with siblings (2–5 mice per cage), nesting material and a round plastic hut. In experiments where cocaine or saline treatment was required, littermates were evenly distributed to each group (except within-subject design experiments shown in **Supplementary Fig. 9a**,**d**, where the experiments were performed several months apart). At the first imaging session, mice were P58–112. All procedures were approved by the Ernest Gallo Clinic and Research Center Animal Care and Use Committee.

Surgery. Under isoflurane anesthesia, we made a ~3-mm diameter craniotomy over the dorsomedial frontal cortex of both hemispheres. Surgical procedures used were described previously[17]. Mice were left to recover a minimum of 7 d for the repeated-treatment and withdrawal (**Fig. 1** and **Supplementary Figs. 1**–**4**), running wheel (**Supplementary Figs. 5** and **6**), and CPP experiments (**Fig. 3** and **Supplementary Figs. 8** and **9**). For the acute-treatment experiments (**Fig. 2** and **Supplementary Fig. 7**), all the mice were imaged 1–3 d after surgery except for one mouse belonging to the saline group that was imaged after >7 d of recovery. We found no significant differences in the fraction of spines gained or lost between these two recovery time groups (**Supplementary Fig. 10**). Similar results were described previously[17].

Repeated cocaine and withdrawal experiment (Fig. 1 and **Supplementary Figs. 1**–**4).** During the last days of recovery from surgery, mice were handled to habituate them to restraint for intraperitoneal injections and they were habituated to locomotor chambers for at least 2 d for 3 h (**Fig. 1a**). After this habituation period, the mice entered the baseline phase in which we performed all the same procedures as in the treatment phase, but mice received only sham intraperitoneal injections. To measure cocaine sensitization, we performed locomotor test sessions every second day during the baseline and treatment phase on days when mice were not imaged. For locomotor testing, mice were introduced into a clear chamber (7.5 × 7.5 × 7.5 inch) with an infrared monitoring system (MED Associates) for 60 min (pre-test period), then removed to receive the intraperitoneal injection (sham for the baseline, saline or cocaine (15 mg per kg, Sigma) for the treatment phase). After injection, mice were immediately placed back in the chamber for 2 h (test period). Locomotor data on four saline-treated mice were lost during a computer upgrade and they could not be included for analysis. Mice were imaged on alternate days in the morning under isoflurane anesthesia. The time at which each mouse was imaged was held constant to eliminate any potential effects of sleep-wake cycle on spine dynamics. Mice were scheduled for treatment injections 3 h after the imaging session was concluded. Throughout the treatment phase, mice received 12 daily injections of either saline or cocaine (15 mg per kg). During the withdrawal phase, mice were imaged every other day for 2 weeks (seven sessions) and were returned to their home cage after recovery from anesthesia. In 12 mice (six per group), we were able to image the same dendrites through the entire baseline, treatment and withdrawal protocol without bone growth obscuring the window. A subset of mice in both groups were imaged only for the treatment or withdrawal portion of the protocol. Values for measures of spine density and spine dynamics were normalized to baseline for analysis of treatment and to the final session of treatment for withdrawal specific analysis.

Running wheel experiment (Supplementary Figs. 5 and **6).** Mice were handled and habituated to sham injection procedures and running wheel for five sessions in which the wheel was unlocked for ~30 min (mouse was allowed to run in the wheel) and ~30 min where the wheel was locked (the wheel was blocked so the mouse could not run). Imaging was then started and mice were imaged during an additional habituation session in which they were in the wheel for 20 min with the wheel unlocked for 5 of the 20 min (day 1: 5 min) (**Supplementary Fig. 5a**). The next day, the wheel was unlocked for 15 of the 20 min to allow more running (day 2: 15 min). On day 3, the wheel was unlocked for only 5 min of the 20 min session (day 3: 5 min). The rationale behind this unlocked-locked running wheel protocol was to standardize the amount of time in the chamber (20 min) and to regulate the amount of running within that time. Mice were imaged daily in the morning and were allowed at least 3h of recovery from anesthesia before running wheel exposure. Between days 1 (5 min running) and 2 (15 min running), mice ran a proportional distance similar to that observed between the last session of the baseline and first day of treatment in the repeat cocaine treatment experiment (**Supplementary Fig. 5c**). This allowed us to test the effect of increased locomotion on dorsomedial frontal cortex spine dynamics in the absence of cocaine.

Mice running in the running wheel for 15 min traveled longer distances than those measured in the open field over the same time frame (running 5 min = 3,792 ± 453 cm versus pre-treatment locomotion = 1,026 ± 107 cm; running 15 min = 10,824 ± 1,972 cm versus treatment day 1 locomotion = 2,973 ± 1,013 cm). Given the large amount of locomotion on the running wheel in even 5′, it was possible there was a saturation effect on the first measure of the fraction of spines gained. Under this saturation scenario, any further manipulation would not have any effect on spine gain as a result of occlusion. To test this possibility, we included an extra running wheel session (day 4) in which mice were allowed to run for 5 min on the running wheel after a cocaine (15 mg per kg) intraperitoneal injection (**Supplementary Fig. 6**). The results indicated that there was room for further increases in spine gains illustrated by the significant increase in the fraction of spines gained after the cocaine +5 min running protocol when compared to the value obtained after the 15 min running-session (without drug) ($P = 0.03$; **Supplementary Fig. 6c**).

Acute treatment experiment (Fig. 2 and **Supplementary Fig. 7).** Mice were handled and habituated to sham injection procedures before cranial window implant surgery and recovered 1–3 d afterwards. Usually, saline and cocaine groups were formed by littermates for whom the surgery-recovery times were identical. Mice were then imaged twice daily (starting in the morning) with a 3-h interval between imaging sessions. Mice were scheduled for treatment injections when 1 h had passed since the conclusion of isoflurane anesthesia and were imaged again in the afternoon starting 2 h after injection of either saline or cocaine (15 mg per kg; **Fig. 2a**).

Cocaine CPP experiment (Fig. 3 and **Supplementary Figs. 8** and **9).** Mice were handled and habituated to sham injection procedures after cranial window implant surgery. All mice were imaged daily in the morning and allowed to recover for at least 3 h from anesthesia before any behavioral manipulation was performed (**Fig. 3a**). CPP conditioning was performed in the afternoon. The CPP chamber (MED associates) consisted of two compartments separated by a black (infrared transparent) wall. On the habituation and test sessions (days 2 and 5 of the schedule; **Fig. 3a**), the wall had an opening that allowed the mice to freely explore both chambers. Each chamber contained a set of three different contextual cues (**Supplementary Fig. 8b**): a visual cue (horizontal versus vertical lines) on the chamber walls, texture cues (square versus random textures) on the chamber floor and odor cues (vanilla versus cinnamon extract, 200 μl on filter paper positioned on opposite corners of the chambers). On experimental days, when two conditioning sessions were performed, 100 μl of odor was added before the second session to prevent the loss of odorant intensity. The combination of sensory cues for each chamber was counterbalanced between mice.

After a habituation session in which the mice were allowed to explore both chambers for 30 min, an initial measurement of baseline preference was taken (CPP_{hab}, defined as the time difference between the time spent in the two chambers). The next day (**Fig. 3a**), mice underwent saline paired conditioning (two 15-min sessions separated by 2 h) in the chamber for which they showed preference on the habituation day (designated chamber A). The following day, the mice were given cocaine (30 mg per kg, intraperitoneal; **Fig. 3** and **Supplementary Figs. 8** and **9d**–**f**) or saline (**Supplementary Fig. 9a**–**c**) in the opposite, non-preferred chamber (designated chamber B) for two 15-min conditioning sessions separated by 2 h. During the conditioning days, the mice were restricted to one side of the chamber. On the CPP test day, the mice were introduced into the CPP chamber with the open gate configuration (**Fig. 3a**) for 30 min and preference for chamber A versus B was again measured (CPP_{test}). CPP was defined as the extent of the preference shift after cocaine, $CPP = CPP_{hab} - CPP_{test}$ (**Supplementary Fig. 9b**,**e**).

To measure the persistence of the new spines gained after the saline- and cocaine-conditioning sessions, we performed two more imaging sessions 96 h after the sessions 4 and 5 (**Fig. 3a**). In two mice from the saline-treated group (**Supplementary Fig. 9a**–**c**), some regions of interest were obscured by bone growth between the CPP test day and the 96-h persistent imaging sessions (**Supplementary Fig. 9a**). On day 9, we imaged visible regions of interest

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in vivo and then perfused with mice with fixative. For these two special cases, some images were then obtained in a whole-mount preparation. Previous studies have found that majority of spines observed after fixation correspond to spines observed *in vivo* in a session just before fixation[16].

Imaging procedure and analysis. Our procedures for *in vivo* imaging have been described[17]. Briefly, we imaged the apical dendrites of YFP expressing pyramidal neurons using a Mai Tai HP laser (920 nm, Spectra Physics), Ultima IV *in vivo* laser-scanning microscope (Prairie Technologies) and a 40× 0.8 NA objective (Olympus). 40-µm segments of third order (and higher) dendrites were imaged with high resolution (0.085–0.17 µm per pixel). Branches were located within 100 µm from the surface (layer I). In about 50% of the dendrites imaged, we could follow the dendritic arbor until its main bifurcation. In these neurons, we found that the dura-initial apical bifurcation was 314.8 ± 17.63 in cocaine-treated mice ($n = 21$ neurons) and 277.9 ± 23.16 ($n = 17$ neurons) in saline-treated mice ($P = 0.45$; **Supplementary Fig. 2b**).

To control for the effect of surgery and anesthesia, we repeated the same schedule of handling and injection in a subset of mice that did not receive a craniotomy or repeated anesthesia. We killed these mice 2 weeks after the last injection of saline and compared their dendritic spine density with that of the mice subject to *in vivo* imaging. There were no significant differences (saline *in vivo*, 0.401 ± 0.02 spines per µm, $n = 6$ mice; saline fixed, 0.373 ± 0.02 spines per um, $n = 6$ mice; $U = 12$, $P > 0.39$, Mann-Whitney U test; **Supplementary Fig. 2a**).

We used Matlab (MathWorks) and custom SpineAnalysis image software to manually score the spines using guidelines for scoring from a previous study[17]. On average, we analyzed 101.6 ± 1.8 spines per mouse ($n = 77$ mice) measured on the first imaging session. There were no differences in sampling between the cocaine and saline groups used in this study ($P > 0.69$). Mice for which we could not analyze a minimum of 80 spines at day 1 of the experiment were excluded from analysis. All images were scored by an observer blind to the mouse's treatment.

Spine gain fraction, as defined in **Figure 1d**, was obtained from $FG_{ab} = (NG_{ab} | TS_a)$, where FG_{ab} represents the number of new spines gained (NG_{ab}) between two consecutive sessions a and b divided by the total number of spines (TS_a) present on session a. This value is then normalized to the averaged fraction gain between baseline sessions

$$\text{norm}FG_{ab} = (FG_{ab} | \overline{FG_{\text{base}}}) \times 100$$

where $\overline{FG_{\text{base}}}$ is defined as the average of the fraction of spines gained ($FG_{(i-1)\to i}$) between consecutive baseline sessions $i - 1$ and i, where j represents the total number of baseline gain sessions

$$\overline{FG_{\text{base}}} = \frac{\sum_{i=1}^{j} FG_{(i-1)\to i}}{j}$$

Identical calculations were made for spine loss as for gain using loss data.

To understand how the accumulation of new spines (SA) after cocaine treatment could alter spine density, we created a measure of normalized spine accumulation (**Fig. 1g**). We defined normalized spine accumulation ($\text{norm}SA_{p\to n}$) between the last pre-treatment session (p) and a treatment session (n) as

$$\text{norm}SA_{p\to n} = \left(\frac{SA_{p\to n}}{FG_{\text{base}}}\right) \times 100$$

where $SA_{p\to n}$ is the number of spines present in session n that were not present on the last session of the pretreatment baseline p, and $\overline{FG_{\text{base}}}$ is defined as the average of the fraction of spines gained ($FG_{(i-1)\to i}$) between consecutive baseline sessions $i - 1$ and i, where j represents the total number of baseline gain sessions. In the theoretical case in which a mouse would always gain the same amount of spines between any given two consecutive sessions, and all those spines gained would be transient (not present on the next imaging session), the value for $SA_{p\to n}$ would be the same than the value of the baseline ($\overline{FG_{\text{base}}}$) and the percentage would be 100% (where 100% means no accumulation).

For the morphological analysis of new spines (**Supplementary Fig. 8c**,**d**), we measured the length of the dendritic spine from the base of the dendrite to the furthest tip of the spine and divided this value by the maximal head width to obtain the length-width ratio.

Statistics. Two-way mixed (within-between) ANOVAs for groups of different sizes were performed with Matlab (http://phy.ucsf.edu/~loren/NS248/Matlab/BetweenWithinAnova/). Comparisons of two groups at a single time point were performed after testing for normality by using the D'Agostino-Pearson omnibus test (Graphpad Prism 5). Whenever the distribution was considered normal, paired or unpaired Student's t tests were performed. For unpaired t tests, homoscedasticity (equal variances) was tested with an F test (GraphPad Prism 5). Nonparametric tests were used to compare non-normal distributions and experiments with small samples. Wilcoxon rank test was used for paired groups and Mann-Whitney test for unpaired groups (GraphPad Prism 5). One-way repeated-measures ANOVA was used to analyze the effects of conditioning session on spine dynamics in the CPP-experiments (GraphPad Prism 5). Whenever the distribution of these values was not normal, a Friedman's test for repeated measures was used (GraphPad Prism 5). To analyze the potential differences between cumulative histogram distributions, a two-sample Kolmogorov-Smirnov test was used (Matlab). Pearson's r values were used to measure correlations between parameters (GraphPad Prism 5). No statistical methods were used to predetermine sample sizes, but our sample sizes are similar to those reported in previous publications[9–11,16].

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