Spatial learning induces activity-dependent circuit plasticity (or changes in functional connectivity), thereby strengthening vHPC–mPFC connectivity and supporting improved performance. However, strong pre-existing vHPC–mPFC connections impair adaptation to new tasks, whereas disruption of pre-existing theta-frequency connectivity enhances performance. Synaptic depression facilitates the learning of new rules, whereas induction of long-term potentiation (LTP) blocks subsequent learning. Furthermore, LTP-expressing synapses cannot be further potentiated, but low-frequency theta stimulation de-potentiates existing LTP and allows subsequent induction of LTP. We hypothesized that weakening established circuit connectivity might increase learning capacity by permitting subsequent learning-associated plasticity. To test this hypothesis, we investigated how novelty, which induces synaptic depression, affects vHPC–mPFC circuitry during adaptive spatial learning.

Novelty facilitates learning

Cognitive challenges often require old routines to be updated. We tested this capacity to modify an existing strategy by training mice to freely choose either arm of a T-shaped maze for rewards. Over three days, mice developed the efficient strategy of choosing one arm consistently. Then, in the same maze, the mice underwent ‘flexible choice’ training, which required them to flexibly choose a goal in a delayed-non-match-to-sample task. To successfully perform this new task, the mice had to overcome their established arm bias. Learning across training was assessed by a logistic regression model learning curve. Under control conditions, mice did not fully overcome their bias, but their performance did gradually improve over 40 training trials.

To investigate how novelty affects learning, we exposed additional mice to a novel arena 1 h before flexible choice training. To control for non-novelty-related aspects of arena exploration, another group was exposed to a familiar arena that they had previously explored for three consecutive days. Both groups initially retained their arm bias during flexible choice training. However, mice that had been exposed to the novel arena progressively overcame this bias, improving their performance more rapidly than mice exposed to a familiar arena. On average, the novel-exposed group learned the rule midway through the task, whereas the control group did not fully overcome their bias. To further investigate the role of vHPC–mPFC connectivity in the development of a new arm bias, we exposed mice to a novel arena that they had previously explored for three consecutive days. Both groups initially retained their arm bias during flexible choice training. However, mice that had been exposed to the novel arena progressively overcame this bias, improving their performance more rapidly than mice exposed to a familiar arena (familiar-exposed) on trials in which their arm bias conflicted with the new task. We estimated the trial at which mice learned the new rule using the learning curve inflection point. On average, the novel-exposed group learned the rule midway through the task, whereas the control group did not fully overcome their bias.

The ability to rapidly adapt to novel situations is essential for survival, and this flexibility is impaired in many neuropsychiatric disorders. Thus, understanding whether and how novelty prepares, or primes, brain circuitry to facilitate cognitive flexibility has important translational relevance. Exposure to novelty recruits the hippocampus and medial prefrontal cortex (mPFC) and may prime hippocampal–prefrontal circuitry for subsequent learning-associated plasticity. Here we show that novelty resets the neural circuits that link the ventral hippocampus (vHPC) and the mPFC, facilitating the ability to overcome an established strategy. Exposing mice to novelty disrupted a previously encoded strategy by reorganizing vHPC activity to local theta (4–12 Hz) oscillations and weakening existing vHPC–mPFC connectivity. As mice subsequently adapted to a new task, vHPC neurons developed new task-associated activity, vHPC–mPFC connectivity was strengthened, and mPFC neurons updated to encode the new rules. Without novelty, however, mice adhered to their established strategy. Blocking dopamine D1 receptors (D1Rs) or inhibiting novelty-tagged cells that express D1Rs in the vHPC prevented these behavioural and physiological effects of novelty. Furthermore, activation of D1Rs mimicked the effects of novelty. These results suggest that novelty promotes adaptive learning by D1R-mediated resetting of vHPC–mPFC circuitry, thereby enabling subsequent learning-associated circuit plasticity.

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Novel experience enhances learning. a, Experimental design. b, Mice (n = 10) established an arm bias after free choice sessions (t-test, t_{18} = 8, P < 0.0001). c, Mice performed more trials on their non-biased side during flexible choice training (F_{19,168} = 25.4, P < 0.0001). d, Mice performed below chance level in the flexible choice task measured with a logistic regression model learning curve. e, Arm type–dependent performance during flexible choice training. Novel-exposed, n = 17; familiar-exposed, n = 20 mice; trial × group: biased goal, F_{19,168} = 0.8, P = 0.7; non-biased goal, F_{19,168} = 3.5, P < 0.0001. f, Learning curve (logistic regression model; F_{19,168} = 2.4).

We re-tested a subset of mice in the free choice task after flexible choice training, and found that novel-exposed mice reverted to consistently choosing one arm in the free choice task, indicating that they adaptively used task-specific strategies (Fig. 1f). Next, we tested whether novelty facilitated learning. Exposure to a novel mouse also enhanced learning (Extended Data Fig. 1), demonstrating that this effect generalizes across different novel stimuli. In an additional cohort, we induced arousal from sleep or rest 1 h before flexible choice training. Although, like novelty-exposed animals, these mice remained active until the onset of flexible choice training, their task performance was not facilitated (Extended Data Fig. 1). These convergent data demonstrate that novelty facilitates new learning.

Novelty resets vHPC–mPFC circuitry

If novelty primes neural circuitry for subsequent learning, it should elicit neural signals that persist for at least 1 h (the time between exposure to novelty and flexible choice training). We therefore looked for persistent novelty-induced neural signals (Extended Data Fig. 2a). We hypothesized that local field potential (LFP) power, which reflects collective local activity, might show this priming signature. The novel-exposed group exhibited higher theta power in the vHPC during and 1 h after arena exposure than did the familiar-exposed group (Fig. 2a, Extended Data Fig. 2b). In addition, in mice that explored a novel maze for two consecutive days, vHPC theta power decreased as the environment became familiar (Extended Data Fig. 2e, f). Novelty also increased the entrainment of single units in the vHPC to local theta oscillations, and theta phase-locked cells displayed higher firing rates than non-phase-locked cells (Fig. 2b). Moreover, vHPC units in the novel-exposed group preferentially fired at the local theta trough (Fig. 2c). These findings indicate that novelty induces persistent theta rhythms that reorganize vHPC activity.

Because adapting to new spatial rules requires communication between the vHPC and mPFC and increased neuronal activity at the theta trough (Fig. 2c) causes synaptic depression, we checked whether novelty altered vHPC–mPFC functional connectivity. We examined the phase-locking of mPFC single units to vHPC theta oscillations, as phase-locking reflects synaptic connectivity. Both during arena exposure and 1 h later, the novel-exposed group displayed lower phase-locking than the familiar-exposed group (Fig. 3a, Extended Data Fig. 3a). We also examined single-unit spike firing in the mPFC in response to multiunit activity (MUA) in the vHPC (Extended Data Fig. 3b). The novel-exposed group exhibited lower vHPC-evoked mPFC unit activity than the familiar-exposed group, during and 1 h after arena exposure (Extended Data Fig. 3c). To test whether this persistent novelty-induced weakening facilitated subsequent learning-dependent strengthening of connectivity, we compared vHPC–mPFC connectivity before and after flexible choice trial 21, the average learning point of the novel-exposed group. As in pre-training (Fig. 3a), phase-locking of mPFC single units to vHPC theta activity was lower in the novel-exposed group than the familiar group during early training (trials 1–20; P = 0.02). However, both phase-locking of mPFC single units to vHPC theta activity and mPFC spike firing in response to MUA in the vHPC increased in late training (trials 21–40) only in the novel-exposed group (Fig. 3b, Extended Data Fig. 3d). In fact, phase-locking in the novel-exposed group reached the same levels as in the familiar-exposed group (P = 0.1). Novelty particularly affected vHPC–mPFC circuitry, as dorsal hippocampal–prefrontal (dHPC–mPFC) circuitry displayed neither prolonged novelty signals (Extended Data Figs. 2c, d, g, h, 3e) nor learning-dependent changes in functional connectivity (Extended Data Fig. 3f). These results indicate that exposure to novelty weakens existing vHPC–mPFC synapses, thereby facilitating subsequent learning-associated plasticity.

To test this hypothesis directly, we induced plasticity in vivo by optogenetically stimulating vHPC terminals in the mPFC (Fig. 3c). Optical stimulation (Methods, Fig. 3e) potentiated vHPC–mPFC synapses. Exposing mice to the novel arena, but not the familiar arena, de-potentiated synapses back to baseline strength (Fig. 3d). Subsequent stimulation delivered 1 h after exposure to the arena re-potentiated vHPC–mPFC synaptic activity in the novel-exposed group, but not in the familiar-exposed group (Fig. 3d). These findings suggest that novelty facilitates new learning by weakening existing vHPC–mPFC connectivity and thereby opening a window for learning-associated circuit plasticity. Indeed, closing this window by stimulation immediately after novelty blocked subsequent novelty-enhanced learning (Fig. 3e).
Novelty facilitates strategy updating

We assessed encoded task-specific information by training machine-learning classifier models on vHPC unit activity in the free choice condition. We collected unit firing patterns during the centre arm run (before turning to the chosen arm) to test whether they predicted arm choice (Methods, Extended Data Fig. 4). Each mouse had a specific arm bias (Methods), and the models successfully classified (across all mice) biased versus non-biased arm trials (Extended Data Fig. 4a). This suggests that vHPC units encode free choice strategy.

To determine whether novelty-induced reorganization of activity (Fig. 2b, c) alters this encoding, we tested vHPC unit activity from flexible choice trials using models trained on free choice data (Extended Data Fig. 4b). If vHPC unit activity continues to encode the free choice strategy, the models should predict biased arm choice during early flexible choice trials, when both groups maintained their arm bias (Fig. 1e).

While the model did so in the familiar-exposed group, it performed at chance levels in the novel-exposed group (Extended Data Fig. 4c). Thus, novelty disrupts vHPC unit activity underlying the established free choice strategy.

Because direct projections from the vHPC to the mPFC provide task-relevant information (Extended Data Fig. 4d), we postulated that novelty-induced changes in vHPC encoding and vHPC–mPFC circuit plasticity might...
facilitate the updating of information in mPFC neurons. During the centre arm run in flexible choice training, mPFC neurons may encode the future arm choice based on a free choice (biased versus non-biased arm) or flexible choice (rewarding versus non-rewarding arm) strategy. We used mutual information, or the amount of information shared by mPFC spikes and arm choice, to quantify learning-dependent changes in arm choice encoding for each strategy. Training reduced only free choice-based encoding (arm bias mutual information) in the novel-exposed group (Methods, Fig. 3F). Conversely, flexible choice-based encoding (rewarding arm mutual information) increased in all groups, with the novel-exposed group showing the largest increase (Fig. 3F). Thus, mPFC neurons in the novel-exposed group update with task-relevant information during training. Indeed, proportionally more mPFC neurons switched strategy encoding in the novel-exposed group than the familiar-exposed group (Methods, Fig. 3G). Notably, it was these ‘switching’ neurons that increased their phase-locking to vHPC theta activity in late training (Fig. 3G). Thus, exposure to novelty enhances information updating in the mPFC by increasing vHPC–mPFC functional connectivity.

D1Rs mediate the effects of novelty

Consistent with our findings that novelty persistently activates the vHPC, but not dHPC or mPFC, dopaminergic inputs from the ventral tegmental area (VTA) to the vHPC convey information about novelty. Indeed, the VTA projects densely to area CA1 of vHPC, but minimally to dHPC CA1 (Extended Data Fig. 5). Activation of D1Rs induced depression of vHPC–mPFC synapses and enhanced learning (Extended Data Fig. 6). We tested whether infusion of the D1R-like antagonist SCH23390 (SCH), which blocks novelty-induced synaptic depression26,27, into the vHPC abolished the priming effect of novelty (Fig. 4A). Treatment with SCH, but not vehicle, before exposure to novelty impaired flexible choice task learning (Fig. 4B). Moreover, SCH reversed all of the physiological effects of novelty. Compared with the vehicle group, the SCH-treated group showed lower vHPC theta power (Fig. 4C, Extended Data Fig. 7A), higher phase-locking of mPFC units to vHPC theta activity during exposure to novelty (Fig. 4D, Extended Data Fig. 7B). The vehicle-treated group, but not the SCH-treated group, showed increased vHPC–mPFC connectivity with training, measured by phase-locking or evoked firing (Extended Data Fig. 7C, D). Furthermore, SCH prevented the training-induced reduction in arm bias mutual information seen in the vehicle-treated group (Fig. 4E). Rewarding arm mutual information decreased in the SCH-treated group with training, but increased in the vehicle-treated group (Fig. 4E). Thus, vHPC D1Rs mediate novelty-enhanced learning. SCH did not affect dHPC theta power or dHPC–mPFC connectivity (Extended Data Fig. 7E, G, H), consistent with the absence of novelty-induced changes in these parameters. SCH did impair mPFC theta power during exposure to novelty, but not 1 h later (Extended Data Fig. 7F).

Novelty or D1R activation increases cell excitability, and vHPC cells phase-locked to novelty-induced theta activity are more active than non-phase-locked neurons (Fig. 2B), suggesting that D1R-expressing vHPC neurons might mediate the effects of novelty. We used the Cal-Light technique—which induces reporter expression in virally infected cells that are active during blue light illumination—to label novelty-responsive vHPC cells with high spatiotemporal resolution. In Cal-Light–injected mice exposed to blue light alone, the novel arena alone, or both, only the last group showed robust expression of the reporter enhanced green fluorescent protein (eGFP) in the vHPC (Extended Data Fig. 8A–C).

We next expressed the inhibitory halorhodopsin (eNpHR) instead of eGFP. Mice exposed to blue light in the novel arena robustly expressed eNpHR in activated vHPC cells, and these novelty-tagged cells co-expressed D1Rs and projected to the mPFC (Fig. 4F, Extended Data Fig. 8E, F). Green light inhibited the spiking of these cells in vitro (Extended Data Fig. 8D). To specifically interfere with novelty-induced priming without affecting cell activity during novelty exposure or flexible choice training, we inhibited eNpHR-expressing novelty-tagged vHPC cells with green light for 10 min immediately after exposure.
to novelty. This inhibition disrupted task acquisition relative to eGFP-expressing mice (Fig. 4g). As a control, we labelled vHPC cells while mice consumed reward in the start box during a free choice session on the day before novelty exposure. Comparing eNpHR expression from this non-novelty labelling with novelty-specific labelling (Extended Data Fig. 8a–c) revealed that the non-novelty condition similarly labelled vHPC cells, but with greater eNpHR expression (green:red ratio; novelty-specific, 0.69 ± 0.01 (975 cells); novelty-non-specific, 0.8 ± 0.01 (1,008 cells); Kolmogorov–Smirnov test, P < 0.0001). Nonetheless, inhibition of these non-novelty-associated cells after exposure to novelty did not disrupt novelty-enhanced learning (Fig. 4h). Furthermore, inhibition of ‘familiar tagged’ vHPC cells did not affect learning (Extended Data Fig. 8g), confirming the critical and specific role of novelty-responsive vHPC cells in novelty-enhanced learning.

Discussion

Our study provides a model in which novel experience restructures vHPC–mPFC circuitry to facilitate learning. Novelty reorganizes vHPC neuronal activity to a local theta trough when membrane potential drops30, disrupting established encoding of the free choice strategy and weakening vHPC–mPFC connectivity. These changes prime vHPC–mPFC circuitry, enabling subsequent learning-associated plasticity. During novel learning, the vHPC develops new task-driven activity and vHPC–mPFC connectivity improves. Then, vHPC neurons convey new task-specific information to mPFC neurons, which encode the new task strategy alongside the old one (Extended Data Fig. 9). The DIR-dependent activation of vHPC neurons triggers these effects of novelty. Future studies will determine how DIR activation reorganizes vHPC neuronal activity to the theta trough. We have provided detailed mechanisms underlying the general learning principle that disruption of existing representations facilitates subsequent learning-associated plasticity.

Our findings expand the role of dopaminergic signalling along the hippocampal dorsoventral axis. Although dopaminergic inputs from the locus coeruleus to the dHPC mediate novelty-induced memory enhancement32, we found no evidence of novelty-triggered priming signals in the dHPC. Whereas other studies introduced novelty after training to study memory retention33, we show that introducing novelty before training encodes encoding. We speculate that the VTA–vHPC circuit underlies novelty-enhanced learning, whereas the locus coeruleus–dHPC connection enhances subsequent memory retention. Indeed, theta oscillations in the dHPC and vHPC represent distinct entities33. However, the dHPC may contribute to novelty-enhanced learning, as knocking out DIRs impairs spatial novelty detection in the dHPC33, perhaps mediated by inputs from the vHPC33 and VTA (Extended Data Fig. 5e,f). Because many neuropsychiatric disorders are associated with rigid vHPC–mPFC circuit plasticity33, our study provides new mechanistic insights for potential therapeutic interventions.

Online content

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41586-021-03272-1.
Methods

Subjects
Male and female C57BL/6j mice (Jackson Labs) were used for all experiments. Mice were maintained on a 12-h light/12-h dark cycle with lights on at 07:00. Food and water were available ad libitum until mice were food-restricted for the behavioural experiments. Behavioural experiments were conducted between 09:00 and 19:00. After chronic implantation of drive/optic fibres, mice were singly housed in cages divided into two compartments by a perforated plastic divider. Mice were randomly assigned to each experimental group. For the experiments in Extended Data Fig. 2e–h, five male and four female mice were used. No sex differences were observed (P > 0.8). Power analysis (alpha: 5%, power: 80%) showed that a sample size of 4 is sufficient to see the novelty effect on behaviour. Experimenters were blinded to the groups when treatments were the same. All procedures were carried out following the NIH Guidelines and approved by Columbia University and the New York State Psychiatric Institute Institutional Animal Care and Use Committees (IACUC).

Surgical procedures
Three-month-old mice were anaesthetized with 2% isoflurane in a flow box until sedated and placed in a stereotaxic apparatus. For the duration of the surgery, isoflurane was maintained at 0.8%, and mice were kept warm on a heating pad. Carprofen (0.15 ml) and dexamethasone (0.05 ml) were injected subcutaneously before the surgery. Mice were allowed to recover for 4 weeks before behavioural experiments were conducted. Because of a discrepancy across mouse brain atlases36, we used custom coordinates and confirmed the placements of our electrodes, cannula, and optic fibres (see ‘Histology’).

Drive implant. For 44 mice (9 for novel, 11 for familiar, 10 for control, 7 for D1 antagonist, and 7 for vehicle group), a tungsten wire field electrode (76 μm diameter) was implanted into the dHPC (targeting CA1 pyramidal layer; 1.9 mm posterior to, 1.3 mm lateral to, 1.26 mm below bregma) and vHPC (targeting ventral CA1/subiculum; 3.2 mm posterior to, 3.3 mm lateral to, 4.59 mm below bregma), each to record LFPs. For combined LFP and single-unit recordings, a bundle of 13 tungsten wire stereotrodes (13 μm diameter) was implanted into the mPFC (targeting prelimbic/infrahilar cortex, layer 2/3; 1.85 mm anterior to, 0.3 mm lateral to, 2.1 mm below bregma). For 17 mice (8 for novel, and 9 for familiar groups), the stereotrode bundle was implanted into the vHPC, and the field electrodes were implanted into the dHPC and mPFC. Reference and ground screws were placed into the skull overlaying the frontal cortex and cerebellum, respectively. Wires were then connected to a 36-channel electrode interface board (Neuralynx), which was fixed to the skull with dental cement.

Cannula implant. Guide cannulae (26 gauge; Plastics One, Roanoke, VA 24018) were implanted bilaterally into the vHPC (angled by 10°, 3.2 mm posterior to, 3.88 mm lateral to, 3.3 mm below bregma) and held by dental cement. The dummy cannulae (Plastics One) were inserted into the guide cannulae until the day of drug infusion. On the day of infusion, 33-gauge internal cannulae with a 0.5-mm projection were inserted into the guide cannulae. To record LFPs in the vHPC, a tungsten wire field electrode was attached to a guide cannula with the tip of the electrode 0.7 mm below the cannula. Another field electrode was implanted into the dHPC and a stereotrode bundle was implanted into the mPFC as described above for this cohort.

Histology
Electrode placements were verified after behavioural experiments by visual examination of electrolytic lesions. Mice were anaesthetized with a ketamine–xylazine mix, and lesions were induced by passing 50 μA current through an electrode for 20 s. Mice were transcardially perfused with PBS, followed by 4% paraformaldehyde in PBS. Brains were fixed in 4% paraformaldehyde at 4 °C overnight and cryoprotected in 30% phosphate-buffered sucrose for 3 days at 4 °C. Brains were sectioned (40 μm) using a cryostat and mounted with DAPI Fluoromount-G mounting medium (Southern Biotech, 0100-20). Only recordings from verified recording sites were used for analyses.

Drugs
SCH23390 (Tocris, 0925), a standard selective D1R-like antagonist, was prepared as a 100 mM stock solution in saline and delivered at 1 mM final concentration in saline for cannulation experiments. The potent full efficacy D1 agonist dibydroxetidine (Tocris, 0884) was prepared as a 10 mM stock solution in deionized water.

Behaviour
Our delayed-non-match-to-sample flexible choice task is fundamentally distinct from the conventional delayed-non-match-to-sample working memory task24,37. Conventional T-maze working memory task protocols include ‘shaping’ sessions in which mice are guided to spontaneously alternate goal arms before training. Then the mice undergo days of training sessions until they learn the delayed non-match-to-sample rule to reach more than 70% correct criterion performance levels. Therefore, spatially unbiased working memory representation is assessed from subjects who already know about the task rule during working memory testing24,37. The present study, however, omitted both shaping and repeated training sessions. As a result, after free choice sessions, mice developed a strong arm bias, which they had to overcome in order to successfully perform in the delayed-non-match-to-sample flexible choice task. Both goal arms were in the same conditions (colour, scent, brightness, reward amount, and so on). Also, the orientation of the experimental area was kept consistent. Moreover, the arm bias of each mouse was not consistent across all groups, indicating that the bias was not guided by external cues. Specifically, among total 131 mice, 50 mice switched their biased arm side over the course of three free choice sessions. On the last day of free choice, 74 mice were biased towards the left arm and 57 mice were biased towards the right arm. This indicates that mice were not exclusively biased to one particular side and their arm choice was not guided by external cues.

Mice underwent behavioural procedures 4 weeks after surgeries. Food restriction was started 3 days before the beginning of free choice sessions to maintain 85% of the pre-restriction weight of mice. All mice were gently handled for 3 min for 3 days before starting free choice sessions, to acclimate mice to experimenters. The circular arena was 50 cm in diameter and 25 cm in height. For behavioural scoring of exploration, the centre of the circular arena was defined as the innermost half of the total area. Each arm of the custom-built automated T-maze was 10 cm wide and 15 cm high, and the length of the arms was 35 cm for the centre arm and 32 cm for the goal arms.

Because novelty elicits a complex mixture of behavioural states, including anxiety, we designed experiments to minimize the impact of non-specific anxiety or arena exploration. Previous reports demonstrated that exposing mice to a brightly lit open arena induces anxiety-related behaviour and increases synchrony between the vHPC and mPFC, effects not seen when the experiments were performed in the dark38. Moreover, delivering bright light to a novel arena abolishes novelty-induced synaptic depression38. Hence, all experiments were performed in the dark to reduce the possibility of non-specific anxiety-related effects. Indeed, we did not observe anxiety-related behaviour (reduced path length and time spent in the centre of the arena)38 in the novel arena (Extended Data Fig. 10).

On days 1–3, all groups of mice had free choice sessions in the T-maze for 30 min each day. The familiar-exposed group also explored in the circular arena for 30 min, 1 h before free choice sessions. During free choice sessions, mice were allowed to freely visit all of the arms to get rewards (condensed milk diluted 1:3 in deionized water). After getting
a reward from one of the two goal arms, mice had to return to and consume a reward in the start box before entering another goal arm in order to initiate another round of rewards. By the last day of free choice sessions, mice across groups displayed similar numbers of arm visits (control: 13.8 ± 0.6, familiar-exposed: 14.7 ± 0.7, novel-exposed: 13.8 ± 0.5, F2,44 = 0.7, P = 0.5) and had developed a strong arm bias, choosing that arm approximately 90% of the time (control: 86.6 ± 4.5, familiar-exposed: 87.1 ± 3.2, novel-exposed: 86.6 ± 3.8, F2,44 = 0.006, P > 0.9) (Fig. 1b). On day 4, the novel and familiar groups underwent 10 min of exposure to the circular arena followed 1 h later by 40 trials of training in a delayed-non-match-to-sample flexible choice training. The 1-h interval was chosen because novelty effects typically last about 2 h.13,14 Mice in the control group went directly from their home cages to flexible choice training without exposure to the circular arena. The delayed non-match-to-sample task consisted of sample, delay, and choice phases. In the sample phase, mice were guided to receive the reward in one of the goal arms. Then they returned to the start box and stayed there for a delay of 60 s. In the choice phase, mice had to choose the arm opposite to the arm they visited during the sample phase in order to get the reward (Fig. 1c). After finishing flexible choice training, mice were returned to their home cage. Once the maze had been cleaned, a subset of mice (for novel-exposed and 9 for familiar-exposed) underwent another 30-min free choice session to test whether flexible choice training affected previously established free choice behaviour.

**Social novelty experiments.** Three-month-old male mice interacted with a novel one-month-old male juvenile mouse in their home cage for 5 min, 1 h before flexible choice training.

**General arousal experiments.** Mice were awakened by opening the cage lid and disturbing nesting materials once. Ten minutes later, mice were returned to the cage rack for 1 h until flexible choice training started. As observed after the arena exposure or interaction with a novel mouse, this waking procedure made the mice active in the home cage until the onset of training. Mice were habituated to the waking procedures for 3 days before the training day to minimize potential novelty of the procedures. This daily acclimation handling procedure awakened mice without affecting neuronal physiology, behaviour, or blood corticosterone levels40.

**Optical stimulation of vHPC terminals in the mPFC**

To measure vHPC–mPFC synaptic transmission, AAV5-CaMKIIα-hChR2(H134R)-eYFP (UNC Viral Vector Core) was injected into the vHPC. The coordinates of the injection sites were three mediolateral rows at posterior 3.1, 3.2 and 3.3 mm, with sites at lateral/ventral: 3.4/89 and 4.59, 3.55/4.69 and 4.39 mm, with respect to bregma. A glass micro-micropette (20–40 μm diameter) was used to inject 100 nl per site at a rate of 100 nl/min. The time interval between injections was 5 min. To optically stimulate vHPC terminals in the mPFC and record synaptic responses, a tungsten wire electrode (13 μm diameter) was attached to an optical fibre (Thorlabs, 200 μm diameter, 0.22 NA) with the tip of the electrode 0.5 mm below the fibre. The coordinates for the optrode implant into the mPFC were the same as those described in ‘Drive implant’.

Six weeks after the viral infection, mice were food-restricted to maintain 85% of their pre-restriction weight and habituated to the recording procedures in their home cage for six days. Food was provided at 19:00 so that optically evoked fEPSP recordings were not interfered with by noise from chewing food. Neurolynx was used to amplify, band-pass filter (1–1,000 Hz) and digitize fEPSPs collected at 32 kHz, as described in ‘Neural data acquisition’. Blue light test pulses (473 nm, 500-μs pulses at 0.033 Hz, OEM Laser) were given to evoke 40% of maximum fEPSP amplitude. A pulse stimulus (A.M.P.I. Master8) was used to control the laser.

To assess the effect of novelty exposure on vHPC–mPFC synaptic transmission (Fig. 3c, d), a stable baseline was established for 30 min in home cage. Then, LTP was induced by 10 trains of 20-Hz burst stimulation, each containing 300 500-μs pulses at 20 Hz, with 45-s intertrain intervals41 (Fig. 3e). One hour after the first stimulation, mice were exposed to either the novel or familiar arena for 10 min and then returned to home cage. The second 20 Hz burst stimulation was delivered 1 h later, and fEPSP recording continued for 2 h afterwards. The initial slope of the evoked fEPSPs was averaged across 5 min.

To block the effect of novelty by inducing LTP (Fig. 3e), separate groups of mice were injected with AAV5-CaMKIIα-hChR2(H134R)-eYFP or AAV5-CaMKIIα-eYFP (UNC Viral Vector Core) into the vHPC. Optical fibres were implanted bilaterally into the mPFC (1.8 mm anterior to, 0.3 mm lateral to, 1.6 mm below bregma). Six weeks after the viral injection, mice underwent behavioural procedures as described in ‘Behaviour’. The 10 trains of 20 Hz burst stimulation were delivered immediately after novelty exposure to reverse novelty-induced vHPC–mPFC synaptic depression.

**DI antagonist experiment**

SCH23390 (100 nl, 1 mM) or vehicle (saline, 100 nl) was loaded into a 10-μl Hamilton syringe and delivered bilaterally to the vHPC at 50 nl/min using a Harvard Apparatus Pump II Dual Syringe micropump. Injection cannulae remained in place for 5 min to allow the injected fluid to diffuse. Twenty minutes later, mice were exposed to the novel circular arena (Fig. 4a). Compared with vehicle treatment, treatment with SCH 20 min before arena exposure did not affect behaviour in the novel arena (speed: SCH, 11.5 ± 1.3 cm/s; vehicle, 11.7 ± 0.5 cm/s; t12 = 0.1, P = 0.9; path length: SCH, 69.7 ± 7.6 m; vehicle, 70.8 ± 2.9 m; t12 = 0.1, P = 0.9; per cent centre time: SCH, 14.7 ± 2.4%; vehicle, 14.3 ± 3.4%; t12 = 0.1, P = 0.9). Notably, injecting 3.1 mM of SCH into the dHPC blocks novelty-enhanced memory consolidation31, but injecting such a high concentration into the vHPC caused severe sedation or bradykinesia for several hours. Mice were also habituated to the cannulation procedures for 5 days before the day of infusion to minimize the potential novelty of the procedures.

**DI agonist experiment**

To measure the effect of D1R activation on vHPC–mPFC synaptic transmission, experiments were prepared as described in ‘Optical stimulation of vHPC terminals in the mPFC’. A tungsten wire electrode (76 μm diameter) was implanted into the mPFC. Saline (0.3 ml) was injected subcutaneously after establishing a stable baseline for 20 min in the home cage. One hour later, the DI agonist dihydrexidine was subcutaneously injected at 10 μg/g (in 0.3 ml saline), and recordings continued for another 90 min. The initial slope of the evoked fEPSPs was averaged across 5 min.

To test whether D1R activation mimics the effects of novelty on learning, a separate cohort of mice underwent free choice sessions as described in ‘Behaviour’. On the day of flexible choice training, mice were subcutaneously injected with dihydrexidine (1 μg/g in 0.3 ml saline) or saline (0.3 ml) 70 min before the onset of training. This time window was chosen because flexible choice training followed 70 min after the onset of novelty exposure (see ‘Behaviour’). Compared with vehicle treatment, dihydrexidine did not affect behaviour in the familiar arena (n = 5 for each group; speed: dihydrexidine, 9.6 ± 0.4 cm/s; vehicle, 10 ± 0.5 cm/s; t12 = 0.6, P = 0.6; path length: dihydrexidine, 58 ± 2.4 m; vehicle, 60.3 ± 3.1 m; t12 = 0.6, P = 0.6; per cent centre time: dihydrexidine, 22.6 ± 3%; vehicle, 19.5 ± 7.5%; t12 = 0.4, P = 0.7; per cent centre path length: dihydrexidine, 30.7 ± 3.3%; vehicle, 22.6 ± 7%; t12 = 1, P = 0.3).

**Optical inhibition of tagged vHPC neurons**

The Cal-Light viruses AAV1-syn-NSLOV2-TEVseq-TTA, AAV1-syn-M13-TEV-C-P2A-tTomato, and AAV1-TRE-eGFP (or AAV1-TRE-ePnP-3HR-eYFP) were purchased from Vigenec Biosciences Inc. (Rockville, MD 20850) and pre-mixed in 1:1:2 ratio29. With the Cal-Light system, elevated calcium levels in active cells allow binding of calmodulin.
to M13, thereby activating TEV protease. In the presence of blue light, TEV protease recognizes the TEV sequence and cleaves tetracycline transactivator (tTA), which induces the expression of a reporter. Thus the reporter is expressed in those cells that are active during the period of illumination with blue light. The cocktail of Cal-Light viruses was injected into 12 sites per hemisphere in the vHPC. The viral injection procedures are described in 'Optical stimulation of vHPC terminals in the mPFC'. Once the viral injection was done, optical fibres (Thorlabs, 200 μm diameter, 0.22 NA) were implanted bilaterally into the vHPC 500 μm above the CA1/subiculum area (angled by 10°, 3.2 mm posterior to, 3.88 mm lateral to, 3.5 mm below bregma) and held by dental cement.

Four weeks later, blue light (473 nm, 10 mW at the optic fibre tip) was bilaterally delivered (2 s on/28 s off) to tag vHPC neurons. Among novelty-tagged neurons, 92.1% of cells co-expressed D1Rs. Green light (532 nm, 10 mW at the optic fibre tip) was bilaterally delivered (2 s on/28 s off) to inhibit tagged vHPC neurons during the first 10 min of the 1-h home cage interval. Notably, the induced gene expression does not terminate immediately when we stop delivering blue light. tTA, which becomes available as a consequence of blue light delivery and elevated calcium levels, continues to induce gene expression until it is depleted. Because we delivered blue light during 10-min novelty exposure and green light for 10 min afterwards for behaviour (Fig. 4g), we silenced cells that started expressing eNpHR for 10-20 min. This timeframe also matched that of immunostaining shown in Extended Data Fig. 8a (also see 'Optical labelling of active cells for immunostaining'). Mice were habituated to the optical procedures for 5 days before the experiment to minimize potential novelty of the procedures.

Learning curve
A logistic regression model and a state–space model were independently used to examine mouse behaviour during flexible choice training. To fit the behaviour to the logistic regression model, the MATLAB (MathWorks) function glmft was used to calculate logistic regression coefficient estimates. These estimates were then used as inputs for the function glmvst to obtain predicted performance levels across trials. Because mice exhibited arm bias (Fig. 1c, e), performance could be falsely counted as correct when the goal arm was the biased arm. To account for this potential error, logistic regression coefficients were weighted according to the probability (P) of visiting the biased arm as measured during the last day of free choice sessions. When P > 0.7, correct observations for the biased arm were weighted by 1 − P. When there was no strong bias (P < 0.7), all correct observations were weighted by 1 − P. This weighting procedure was critical because mice kept their arm bias throughout training (Fig. 1c, e). To estimate at which trial mice learned the rule governing successful flexible choice performance, the inflection point of the learning curve was calculated. At the inflection point, the curvature of the learning curve changes its direction, which reflects that learning has occurred. The inflection points of five mice (four mice in the familiar-exposed group and one mouse in the novel-exposed group, Fig. 1f) were undetermined because the overall slopes of their learning curves were negative, indicating that learning had not occurred. The learning trial of one mouse in the ChR group in Fig. 3e was undetermined for the same reason. The state–space model defines the probability of a correct response as a function of the unobservable learning state process. The probability of a correct response for each trial was calculated using a MATLAB script. This model does not explicitly account for the arm bias.

Neural data acquisition
For the cohorts of mice from which mPFC single-unit data were obtained, recordings were conducted during the last day of free choice sessions, in addition to arena exploration and flexible choice training. Neurophysiological recordings were not performed for the social novelty, arousal (Extended Data Fig. 1) or Cal-Light experiments (Fig. 4g, h, Extended Data Fig. 8g), for behavioural experiments following optical stimulation (Fig. 3e) or D1R agonist treatment (Extended Data Fig. 6b), or during the free choice session immediately after flexible choice training (Fig. 1h). A Digital Lynx system (Neuralynx, Bozeman, MT) was used to amplify, band-pass filter (1–1,000 Hz for LFPs, and 600–6,000 Hz for spikes) and digitize the electrode recordings. LFPs and spikes were collected at 2 kHz and 32 kHz, respectively. Klustakwik (Ken Harris) was used to initially cluster single units on the basis of the first two principal components (peak and energy) from each channel. Clusters were then manually accepted, merged, or eliminated on the basis of visual inspection of waveform appearance, inter-spike interval distribution, isolation distance, and L-ratio. Only single units that had more than 50 spikes over the whole recording session were used for data analyses. The total number of mPFC single units used for analyses for each recording session was as follows. Circular arena: 113 (familiar), 110 (novel), 31(SCH), 69 (vehicle); 1h after arena exposure: 8 (familiar), 19 (control), 29 (novel), 2(SCH), 4 (vehicle); flexible choice training: 43 (familiar), 62 (control), 66 (novel), 18 (SCH), 36 (vehicle). Because neither the familiar-exposed nor control group was exposed to the novel arena and both groups performed at similar levels during flexible choice training, single units from these groups were combined. For the SCH and vehicle groups, single-unit data were not analysed 1 h after arena exposure as there were too few units. The total number of vHPC single units used for analyses for each recording session was as follows. Circular arena: 26 (familiar), 29 (novel); free choice/flexible choice session: 22 (familiar), 30 (novel). Ventral HPC single-unit data were not analysed 1 h after arena exposure as there were too few units. We also attempted to classify mPFC and vHPC cells into putative cell types (for example. excitatory vs. inhibitory) using waveform features, but they did not separate into clear groups.

To account for the influence of animal movement, recordings collected during circular arena exposure were analysed when mice were moving (6–30 cm/s); these ranges were chosen on the basis of the observed bimodal distribution of speeds during the exposure. Mice moved at a comparable speed in the novel and familiar arenas, as previously reported (novel, 13.8 ± 0.5 cm/s; familiar, 13 ± 0.5 cm/s; t5 = 1.1, P = 0.3). To assess neuronal activity 1 h after novelty exposure, we analysed recordings collected from mice running in the centre arm for the first time in flexible choice training, which started 1 h after arena exposure. This first centre-arm run is conceptually similar to the free choice session as it occurs before the first task-specific cue (encounter with the arm door that guided mice to the sample goal arm).

Neural data analysis
For the free choice and flexible choice training data, analyses were conducted when mice were running in the centre arm, before turning to the chosen arm, to avoid any signals related to reward consumption. All data analyses were conducted using custom-written scripts in MATLAB. To account for impedance differences across electrodes, raw LFP data were normalized to the root mean square of the voltage signal over the whole recording session. To calculate power, the wavelet method (the MATLAB wavelet toolbox) was used. To analyse phase-locking, the phase of the LFP was calculated using a Hilbert transform, and spikes were assigned to corresponding phases. To avoid bias from variable spike numbers, we calculated pairwise phase consistency (PPC), which computes the mean of the cosine of the absolute angular distance for all given pairs of spike phases.

Mutual information. To determine whether mPFC neurons encode arm choice based on a free choice or flexible choice strategy, we calculated the amount of information shared by mPFC spikes and arm choice...
(biased vs. non-biased arm for arm bias mutual information, or reward vs. non-reward arm for rewarding arm mutual information)48. Spike data (variable X) were assigned into 100-ms bins. The arm choice for each trial (variable Y) was transformed into binary vectors. The amount of information (entropy) for variable X (or Y) was calculated using the formula below: \( H(X) = -\sum_{i=1}^{n} p(x_i) \log p(x_i) \)

Joint entropy, which is the total entropy of a pair of variables X and Y, was calculated as below. Variable Y has corresponding counting indices j and m.

\[ H(X, Y) = -\sum_{j=1}^{m} \sum_{i=1}^{n} p(x_i, y_j) \log p(x_i, y_j) \]

Finally, mutual information (MI) between spikes (X) and arm choice (Y) was calculated by the following formula.

\[ MI(X, Y) = H(X) + H(Y) - H(X, Y) \]

Because mutual information is sensitive to arbitrary influences of the number of spikes (X) and recorded neurons were different between the novel-exposed and familiar-exposed groups, comparing mutual information values between the two groups is not desirable. In each group, mPFC neurons did not show firing rate differences between the early and late phase of flexible choice training (familiar, \( P = 0.57 \); novel, \( P = 0.91 \)). Mutual information represents the total amount of information and does not contain information about directionality. Rewarding arm (or arm bias) mutual information is total information about both reward and non-reward (or biased and non-biased) arm choice. For example, higher rewarding arm mutual information than arm bias mutual information does not mean that mPFC neurons encode more information about reward arm than biased arm, per se. Also, switching indices between biased- and non-biased arm (or reward- and non-reward arm) does not change the entropy for arm choice (\( H(X) \)).

For each group, we calculated rewarding arm and arm bias mutual information for each mPFC neuron before and after the learning point and presented differences for each mutual information to demonstrate learning-dependent changes in each mutual information for each mPFC neuron.

To define cells that switched information content from arm bias mutual information to rewarding arm mutual information (‘switcher’ cells), bootstrapping was used (\( \alpha = 0.05 \)). All cells from all groups were pooled, and mutual information values for arm bias or rewarding arm were separately treated for bootstrapping. Specifically, eighty mutual information values from each category were randomly picked from the pooled data and averaged. This procedure was repeated 10,000 times to make a distribution of the sampled means. A significant increase was defined when mutual information values were bigger than the upper 2.5% cut-off value of the sampled mean distribution. A significant decrease was defined when mutual information values were smaller than the lower 2.5% cut-off value of the sampled mean distribution. Then, switcher cells were defined when they showed significant decreases in arm bias mutual information as well as significant increases in rewarding arm mutual information. The rest of the cells were defined as non-switchers.

**Machine learning.** The MATLAB machine learning toolbox was used. Ventral hippocampal spike data were assigned into 100-ms bins. The arm choice for each trial was transformed into binary vectors. To train machine learning classifiers models, spike and arm choice data from all groups of mice were obtained from the last day of free choice sessions. Among all classifier models in the toolbox, the weighted k-nearest neighbours (KNN) model gave the best classification results (>95% prediction accuracy, and the area under the receiver operating characteristic curve was 0.99, Extended Data Fig. 4). To avoid over-representation of trials in which mice chose their biased arm, equal numbers of trials of either biased- or non-biased arm choice were randomly selected to train the model. This procedure was repeated ten times to generate ten independent models. Then, vHPC spike data obtained from flexible choice training were fed into the trained models to predict flexible choice behaviour. If spike firing patterns from free choice were well maintained through flexible choice training, the model should predict flexible choice behaviour well. For control analyses, vHPC spike data obtained from flexible choice training were randomly shuffled and fed into the trained models.

**Multi-unit activity.** The amplitude of LFP activity in high gamma or epsilon band (>100 Hz) represents MUA and shows modulation by theta oscillations15–18. To confirm this, we obtained sample LFP and MUA recordings from the vHPC when mice were exploring in a familiar box (a kind gift from Dr. Nancy Padilla-Coreano at Salk Institute). LFP data were bandpass-filtered at 200–300 Hz using a Butterworth filter, and then the square of the filtered data was z-scored. Peaks of the z-scored data above 2 s.d. were considered MUA spikes. We found that these peaks correlated well with actual MUA spikes (\( r > 0.9, P < 0.0001 \)) and that the amplitude of the z-scored data also correlated well with the actual MUA firing rate (\( r = 0.7, P < 0.0001 \)). Thus, epsilon power represents MUA activity in the vHPC well.

**MUA-evoked mPFC spike firing.** Because vHPC spike activity leads mPFC spike firing by 5–25 ms24, mPFC single-unit firing rate was calculated after each MUA spike event within this window and averaged. MUA spikes over 50 were used for analyses.

**Ex vivo electrophysiology**

Mice were injected with the Cal-Light viruses into the vHPC. Four weeks later, blue light was delivered during novelty exposure to tag novelty-responsive vHPC neurons as described in ‘Optical inhibition of tagged vHPC neurons’. Then, the mice were cervically dislocated, and brains were quickly removed and chilled in ice-cold oxygenated artificial cerebrospinal fluid (ACSF) consisting of 126 mM NaCl, 2.5 mM KCl, 2 mM MgCl₂, 1.25 mM NaH₂PO₄, 2 mM CaCl₂, 26.2 mM NaHCO₃, and 10 mM d-glucose, pH 7.45, 300–310 mOsm. Several 300-μm coronal sections spanning the rostral-caudal axis of the vHPC were made in ice-cold ACSF using a vibratome. Slices were immediately transferred to oxygenated ACSF at 32 °C for 30 min and then kept at room temperature. Electrodes were pulled from 1.5 mm borosilicate glass pipettes at a typical resistance of 3–6 MΩ and filled with internal solution consisting of 130 mM K-glucuronate, 5 mM NaCl, 10 mM HEPES, 0.5 mM EGTA, 2 mM MgATP, 0.3 mM NaGTP, pH 7.3, 280 mOsm. Recordings were made using a MultiClamp 700B amplifier, a Digidata 1440A acquisition system, Clampex 10, and pClamp 10 (all from Molecular Devices). To select eNpHR-eYFP-positive neurons labelled by Cal-Light, neurons expressing both tdTomato (expression marker for Cal-Light viral transfection) and eYFP were identified (Extended Data Fig. 8d). In whole-cell current-clamp mode, membrane potential was maintained at ~−65 mV. Step currents (500 ms) were injected from 0 pA to 200 pA, and the number of spikes was quantified with and without 350 nm LED (pE-4000, CoolLED). Considering all experimental procedures including the incubation period, recordings were made at least 1 h after the termination of Cal-Light labelling.

**Optical labelling of active cells for immunostaining**

Mice were allowed to recover for 4 weeks after the injection of the Cal-Light viruses. Mice were habituated to optical procedures for 3 days to avoid potential novelty effects. To label vHPC cells that were active
Further information on research design is available in the Nature Research Reporting Summary linked to this paper.
Extended Data Fig. 1 | Spatial and social novelty, but not general arousal, enhance learning. After 3 days of free choice sessions, mice were exposed to the novel or familiar arena (Fig. If), a novel juvenile male mouse (n = 7), or arousal handling (n = 7) 1 h before flexible choice training. Mice exposed to the novel juvenile mouse performed similarly to mice exposed to the novel arena (two-way RM ANOVA, F(1,22) = 0.03, P = 0.9). Conversely, mice that underwent arousal handling performed similarly to mice exposed to the familiar arena (two-way RM ANOVA, F(1,25) = 0.4, P = 0.5). The average inflection points (learning trial) were 21 (spatial novelty), 19 (social novelty), 38 (familiar), and 39 (arousal) (Kruskal–Wallis test, P = 0.002; familiar versus arousal, P > 0.9; spatial novelty versus familiar, P = 0.03; social novelty versus familiar, P = 0.03). Inset, learning trial of each mouse. n.s., not significant. *P < 0.05, ***P < 0.0005. Data represented as mean ± s.e.m.
Extended Data Fig. 2 | Novelty induces prolonged increases in theta power in the vHPC, but not the dHPC or mPFC.  

a, LFP power was measured during and 1 h after arena exposure, at the onset of flexible choice training.  

b, The novel-exposed group displayed higher vHPC theta power 1 h after arena exposure than the other groups (Kruskal–Wallis test, $P = 0.0007$; novel vs. familiar, $P = 0.001$; novel versus control, $P = 0.008$).  

c, Theta power in the dHPC was comparable across all groups during (Mann–Whitney test, $P = 0.1$) and 1 h after arena exposure (Kruskal–Wallis test, $P = 0.4$).  

b, Novelty exposure increased mPFC theta power (Mann–Whitney test, $P = 0.002$), but this increase was not seen at the onset of flexible choice training (Kruskal–Wallis test, $P = 0.4$).  

e, A separate cohort of mice explored a T-shaped arena for two consecutive days.  

f, Theta power in the vHPC decreased on day 2 compared with day 1 (Wilcoxon signed-rank test, $P = 0.04$).  

g, h, Theta power in the dHPC or mPFC was comparable between day 1 and day 2 (Wilcoxon signed-rank test; 

g, $P = 0.8$; h, $P = 0.3$).  

Insets, average theta power of each mouse.  

n.s., not significant.  

* $P < 0.05$, ** $P < 0.005$.  

Data represented as mean ± s.e.m.
Extended Data Fig. 3 | Novelty-induced connectivity weakening permits subsequent learning-associated connectivity strengthening in the vHPC–mPFC, but not dHPC–mPFC, circuit. 

**a.** Left, rose plots illustrating the phase-locking of example mPFC single units to vHPC theta oscillations. The novel-exposed group showed lower phase-locking than the familiar-exposed group during arena exposure (novel, 110; familiar, 113 cells; Mann–Whitney test, \( P = 0.04 \)). 

**b.** Measuring vHPC MUA-evoked mPFC spike firing. 

**c.** The novel-exposed group exhibited lower evoked firing during (novel, 110; familiar, 113 cells; \( P = 0.02 \)) and 1 h after arena exposure (novel, 12; familiar, 24 cells; Mann–Whitney test, \( P = 0.01 \)). 

**d.** In the late phase of flexible choice training, evoked firing increased in the novel-exposed group (66 cells; \( P = 0.03 \)), but decreased in the familiar-exposed group (97 cells; \( P = 0.01 \)). Wilcoxon signed-rank test. 

**e.** Rose plots illustrate the phase-locking of example mPFC single units to dHPC theta oscillations. The novel- and familiar-exposed groups showed comparable phase-locking levels during (novel, 110; familiar, 107 cells; \( P = 0.3 \)) and 1 h after arena exposure (novel, 29; familiar, 25 cells; \( P = 0.07 \)). Mann–Whitney test. 

**f.** Both the novel- and familiar-exposed groups exhibited increased phase-locking in the late phase of flexible choice training (Wilcoxon signed-rank test; novel, 66 cells, \( P = 0.0002 \); familiar, 103 cells, \( P = 0.04 \)). Cumulative distribution shows all mPFC single unit values. n.s., not significant. \( ^* P < 0.05 \), \( ^{**} P < 0.005 \), \( ^{***} P < 0.0005 \). Data represented as mean ± s.e.m. for a–d, and median with 95% confidence interval for e, f.
Extended Data Fig. 4 | Novelty disrupts vHPC encoding of free choice strategy and permits encoding of flexible choice strategy. a, Machine learning classifier models trained with free choice data (vHPC unit activity and arm bias) successfully classified differences in vHPC unit activity patterns between biased and non-biased arm visits (10 models; 95.8% ± 0.3). b, Machine learning classifier models trained with free choice arm bias data (a) were used to decode flexible choice vHPC spiking data. c, For the first half of the flexible choice training, the models predicted biased arm choice of the familiar-exposed group, but not the novel-exposed group (10 models; two-way RM ANOVA, F(1,18) = 25.1, P < 0.0001). d, Once the novel-exposed group had learned the flexible choice task rule in later trials, the models predicted getting the reward for the novel-exposed group but not the familiar-exposed group (10 models; two-way RM ANOVA, F(1,18) = 5.7, P = 0.02). Insets, model predictions with shuffled flexible choice vHPC spiking data. *P < 0.05, **P < 0.005, ***P < 0.0005. Data represented as mean ± s.e.m.
Extended Data Fig. 5 | VTA inputs to the HPC. Top, AAV-mCherry was injected into the VTA to visualize VTA-to-HPC projections. Bottom, maximum-intensity projection images. a–c, VTA terminals in vHPC CA1 (a), CA3 (b), and dentate gyrus (DG; c). d–f, VTA terminals in dHPC CA1 (d), CA3 (e), and DG (f). g, The expression of mCherry in the VTA. h, VTA dopaminergic neurons expressing tyrosine hydroxylase (TH). i, Merged image of g and h. Blue, DAPI. LMol, lacunosum moleculare layer; Or, oriens; Py, pyramidal; Rad, radiatum. Scale bars, 50 μm.
Extended Data Fig. 6 | D1R activation mimics the effect of novelty on vHPC–mPFC synaptic transmission and learning. a, Optical test pulses were delivered as in Fig. 3c–e. Left, systemic administration of the D1R agonist dihydrexidine induced vHPC–mPFC synaptic depression compared with the vehicle condition (n = 5 mice; one-way RM ANOVA, F(1.6,6.3) = 52.2, P = 0.0002; baseline versus vehicle, P = 0.7; baseline versus dihydrexidine, P = 0.003; vehicle versus dihydrexidine, P = 0.0009). Top right, example average fEPSP traces. Bottom right, average fEPSPs. b, Dihydrexidine treatment enhanced learning relative to vehicle treatment (n = 5 (vehicle), n = 6 (dihydrexidine); two-way RM ANOVA, F(1,9) = 8.7, P = 0.02). The average inflection points (learning trial) were 15 (dihydrexidine) and 46 (vehicle) (Mann–Whitney test, P = 0.009). Inset, learning trials of each mouse. The learning trial of one mouse in the vehicle group was undetermined because the overall slope of its learning curve was negative, indicating that learning had not occurred. *P < 0.05, **P < 0.005, ***P < 0.0005. Data represented as mean ± s.e.m.
Extended Data Fig. 7 | Blocking D1Rs in the vHPC abolishes the effects of novelty on hippocampal–prefrontal circuitry. a, SCH infusion impaired novelty-induced vHPC theta power 1 h after novelty exposure (n = 7 mice each; Mann–Whitney test, P = 0.02). b, During novelty exposure, the SCH group exhibited higher vHPC MUA-evoked mPFC spike firing (SCH, n = 31; vehicle, n = 69 cells; Mann–Whitney test, P = 0.04). c, In late training, mPFC unit phase-locking to vHPC theta activity was not significantly changed in the SCH group (n = 36 cells; P = 0.1) but was increased in the vehicle group (n = 36 cells; P = 0.004). Wilcoxon signed-rank test. d, In late training, evoked mPFC spike firing was not significantly changed in the SCH group (n = 36 cells; P = 0.2) but was increased in the vehicle group (n = 36 cells; P = 0.03). Wilcoxon signed-rank test. e, Mice infused with either vehicle or SCH into the vHPC displayed similar dHPC theta power during and 1 h after novel arena exploration (Mann–Whitney test, P = 0.3 and P = 0.1, respectively; n = 7 mice each). f, SCH infusion impaired novelty-induced mPFC theta power during novel arena exposure (P = 0.04), but did not have an effect 1 h later (P = 0.2). Mann–Whitney test, n = 7 mice each. g, Phase-locking of mPFC single units to dHPC theta oscillations. h, Phase-locking remained stable during training in both groups (SCH, n = 31, P = 0.6; vehicle, n = 69 cells, P = 0.8; Wilcoxon signed-rank test). Cumulative distribution shows all mPFC unit values. Insets (a, e, f), individual average theta power. n.s., not significant. *P < 0.05, **P < 0.005. Data represented as mean ± s.e.m. (a–f) or median with 95% confidence interval (g, h).
Extended Data Fig. 8 | The Cal-Light technique to tag and inhibit novelty-responsive vHPC cells. **a**, Labelling active cells in vHPC CA1 area using the Cal-Light system. Scale bars, 50 μm. **b**, Cumulative distribution of the green:red ratio of each cell (light + familiar, 1,014 cells/2 mice; no light + novel, 920 cells/2 mice; light + novel, 975 cells/2 mice). As eGFP expression is induced in the virus-infected cells that express the red fluorophore tdTomato, the green:red ratio for each cell was measured. Relative to the other conditions, vHPC cells in the light + novel condition displayed a higher green:red ratio (one-way ANOVA, \( F_{(2,2906)} = 171.9, P < 0.0001 \); light + novel versus no light + novel, \( P < 0.0001 \); light + novel versus light + familiar, \( P < 0.0001 \)). **d**, Green light inhibited spiking of eNpHR-expressing novelty-tagged cells (two-way RM ANOVA, \( F_{(1,12)} = 10.2, P = 0.008 \), \( n = 7 \)). Inset, vHPC cells expressing eNpHR–eGFP reporter. **e**, The mPFC projections of vHPC cells infected with the Cal-Light viruses. Maximum-intensity projection images. Scale bars, 10 μm. **f**, The projections of vHPC cells expressing D1Rs to the mPFC. Left, Cre-dependent eYFP expression in vHPC cells of Drd1<sup>Cre</sup> mice. Middle, co-localization of eYFP (green) and D1Rs (red) in the vHPC. Right, vHPC terminals (green) in the mPFC. Blue, DAPI. Scale bars, 20 μm (middle), 500 μm (right). **g**, Inhibiting familiar-responsive vHPC cells did not affect flexible choice training performance (\( n = 5 \) for each group, two-way RM ANOVA, \( F_{(1,8)} = 0.2, P = 0.7 \)). Inset, learning trials of each mouse. The average learning trials were 40 (eGFP) and 36 (eNpHR) (Mann–Whitney test, \( P = 0.8 \)). The learning trials of two mice in the eGFP group were undetermined because the overall slopes of their learning curves were negative, indicating that learning had not occurred. n.s., not significant. *\( P < 0.05 \), ***\( P < 0.0005 \). Data represented as mean ± s.e.m.
A model illustrating the effects of novelty on vHPC–mPFC circuitry and information encoding. The vHPC–mPFC circuit encodes a strategy to get the reward after free choice sessions. This circuit encoding of the free choice strategy remains stable under familiar conditions and conflicts with learning on flexible choice training. By contrast, exposure to novelty disrupts vHPC activity patterns encoding the free choice strategy and weakens existing vHPC–mPFC connectivity, reducing adherence to the free choice strategy. During flexible choice training, the vHPC develops new task-driven activity patterns and vHPC–mPFC functional connectivity undergoes learning-dependent strengthening. The vHPC then transmits newly encoded task-specific information to the mPFC, updating mPFC encoding with new task-relevant information. Hence, exposure to novelty enhances new learning by resetting the vHPC–mPFC circuit.
Extended Data Fig. 10 | The novel arena is not anxiogenic. To avoid anxiogenic effects of the novel arena exposure, experiments were performed in the dark. Top, example behaviour trajectories in the novel and familiar arenas. a, Total path length was comparable between the novel and familiar groups ($t$-test, $t_{35} = 1.1, P = 0.3$). b, c, Percentage path length (b; $t$-test, $t_{35} = 0.3, P = 0.7$) or time spent in the centre (c; $t$-test, $t_{35} = 0.6, P = 0.6$) was similar between the two groups. Novel, $n = 17$, familiar, $n = 20$ mice. Data represented as mean ± s.e.m.
Reporting Summary

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- The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
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- Only common tests should be described solely by name; describe more complex techniques in the Methods section.
- A description of all covariates tested
- A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
- A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
- For null hypothesis testing, the test statistic (e.g. F, t, r) with confidence intervals, effect sizes, degrees of freedom and P value noted
- Give P values as exact values whenever suitable.
- For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- Estimates of effect sizes (e.g. Cohen’s d, Pearson’s r), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection Electrophysiological data were collected using Neuralynx (Digital Lynx SX with Cheetah 5.6.3.).

Data analysis Data analysis was performed with custom Matlab (R2018a) scripts that are available upon request. Graphpad Prism 8.3, and ImageJ 1.52p were used.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

All data are available from the corresponding authors upon reasonable request.
Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☑ Life sciences   ☐ Behavioural & social sciences   ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size

| Power analysis (alpha: 5%, power: 80%) showed sample size of 4 is sufficient to see the novelty effect on behavior. Regardless, we used animal numbers often used in the literature and they were sufficient to discover statistically significant findings. |

Data exclusions

| Single units having less than 50 spikes were excluded. This was established in our previous papers to avoid spurious findings resulting from few spikes. Fig. 3abfg, 4de / Ext. Fig. 3aef, 7bgh. |

Replication

| Behavioural and physiological findings were replicated at least twice across different sets of experiments presented in the current study. All replications were successful. |

Randomization

| All mice were randomly assigned to experimental groups. |

Blinding

| Animals were randomly assigned to experimental groups, and analyses were conducted blind to experimental condition. |

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

| n/a | Involved in the study |
|-----|------------------------|
| ☐ ☑ Antibodies |
| ☑ ☑ Eukaryotic cell lines |
| ☑ ☑ Palaeontology and archaeology |
| ☑ ☑ Animals and other organisms |
| ☑ ☑ Human research participants |
| ☑ ☑ Clinical data |
| ☐ ☑ Dual use research of concern |

Methods

| n/a | Involved in the study |
|-----|------------------------|
| ☑ ☑ ChIP-seq |
| ☑ ☑ Flow cytometry |
| ☑ ☑ MRI-based neuroimaging |

Antibodies

| Antibodies used |
|-----------------|
| rabbit DsRed (Takara, #632496), chicken GFP (Abeam, ab13970), chicken cy2 (Jackson Immunoresearch, AB_2340370), rabbit cy3 (Jackson Immunoresearch, AB_2307443), rat D1-receptor (Frontier, afl000), rat cy3 (Jackson Immunoresearch, AB_2340667), sheep TH (Abcam, ab113), goat DyLight 405 (Jackson Immunoresearch, AB_2340426), and rat Dylight 405 (Jackson Immunoresearch, AB_2340681). |

Validation

| All reported antibodies are previously validated and have been used in the literature. They all have been extensively and commonly used for immuno staining in mice as in the present study. |

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals

| Male and female 3- to 4-month-old C57BL/6J mice (Jackson Labs) were used for all experiments. Mouse colony was maintained at 25 degrees and 30% humidity. |

Wild animals

| This study did not involve wild animals. |

Field-collected samples

| This study did not involve samples collected from the field. |

Ethics oversight

| All procedures were carried out in accordance with the NIH Guidelines and approved by Columbia University and the New York State Psychiatric Institute Institutional Animal Care and Use Committees. |
Note that full information on the approval of the study protocol must also be provided in the manuscript.