Visual recognition memory, manifested as long-term habituation, requires synaptic plasticity in V1

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Familiarity with stimuli that bring neither reward nor punishment, manifested through behavioral habituation, enables organisms to detect novelty and devote cognition to important elements of the environment. Here we describe in mice a form of long-term behavioral habituation to visual grating stimuli that is selective for stimulus orientation. Orientation-selective habituation (OSH) can be observed both in exploratory behavior in an open arena and in a stereotyped motor response to visual stimuli in head-restrained mice. We found that the latter behavioral response, termed ‘vidget’, requires V1. Parallel electrophysiological recordings in V1 revealed that plasticity, in the form of stimulus-selective response potentiation (SRP), occurred in layer 4 of V1 as OSH developed. Local manipulations of V1 that prevented and reversed electrophysiological modifications likewise prevented and reversed memory demonstrated behaviorally. These findings suggest that a form of long-term visual recognition memory is stored via synaptic plasticity in primary sensory cortex.

The cerebral cortex stores memory1, but precisely how and where specific types of information are retained in the neocortex remains poorly understood. The minimal criteria necessary to conclude that experience-dependent modification of a particular cortical area is an essential substrate of learning and memory would include the following evidence: that cortical electrophysiological responses are persistently modified by experiences that are encoded as memory; that such modifications coincide with changes in behavior that depend upon this cortical area; and that local manipulations of the cortex that prevent or reverse electrophysiological modifications likewise prevent or reverse memory demonstrated behaviorally.

Previous studies in our laboratory have documented a robust and long-lasting potentiation of electrophysiological responses in the primary visual cortex (V1) following the controlled exposure of head-fixed awake mice to high-contrast visual grating stimuli2. The underlying synaptic mechanism of this response modification has been localized to V1 (ref. 3). Because the effect is highly selective for the features of the stimulus experienced (for example, grating orientation), the phenomenon has been termed ‘stimulus-selective response potentiation’ (SRP). The current study was designed to determine the behavioral importance of SRP in V1.

Here we show that V1 activity is required for the expression of a quantifiable behavioral response to novel visual stimuli. We found that in both head-fixed and freely behaving mice, behavioral responses to grating stimuli habituated in a stimulus-selective manner as SRP developed across days, and that local manipulations of V1 that prevented and reversed SRP did the same to stimulus-selective behavioral habituation. Together our results support the conclusion that experience-dependent plasticity in V1 is a substrate for visual recognition memory, manifested behaviorally as long-term habituation to familiar stimuli.

RESULTS

The vidget: visually driven behavior in head-fixed mice

We developed an assay for visual detection based on our observation that head-fixed mice spontaneously fidget in response to visual stimuli. We called this response, induced with full-field, phase-reversing (2 Hz) sinusoidal grating stimuli, a ‘vidget’ (visually induced fidget) and measured it via a piezoelectric sensor located beneath the forepaws of restrained mice (Fig. 1a and Supplementary Video 1). These vidgets were quantified as the average stimulus-locked voltage signal, rectified and normalized to a pre-stimulus baseline (Fig. 1b). We found that vidget onset latency, determined by the first time point greater than one s.d. above the pre-stimulus baseline in 75 stimulus onsets from 15 mice, was ~150 ms (Fig. 1c). The response to individual stimuli was variable, with approximately 30% of trials failing to induce movement (Supplementary Fig. 1). Nevertheless, after averaging responses to the five stimulus onsets delivered to each mouse, all 15 mice had quantifiable responses above baseline (Fig. 1d). Unless stated otherwise, all subsequent behavioral data are reported as per-subject averages, with complete distributions shown in Supplementary Figures 1–10.

We next determined if this behavior could be used to assess visual contrast sensitivity and acuity by simultaneously recording vidgets and visually evoked potentials (VEPs) in binocular layer 4 of V1, a surrogate measure of visual detection1–4. We presented 100-s blocks of grating stimuli at various contrasts and spatial frequencies to 15 mice into which electrodes had been implanted. High-contrast stimuli evoked vidgets significantly larger than those evoked by low contrast stimuli (Fig. 1e) and low spatial frequencies elicited larger vidgets than those elicited by high spatial frequencies (Fig. 1f). Simultaneously recorded VEPs had a similar contrast sensitivity (Fig. 1g) and spatial acuity (Fig. 1h). Thus, the vidget serves as a behavioral metric of visual detection aligned with V1 electrophysiology.

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all subsequent experiments, we used five blocks of 100% contrast, 0.05 cycles per degree, and full-field grating stimuli, separated by 30 s of gray stimulus, for each session because these stimulus parameters yield large and reliable vidgets and VEPs.

The vidget requires activity within V1

Many reflexive behavioral responses to visual stimuli occur without V1 (ref. 5). We therefore locally suppressed cortical activity by micro-infusing the GABA_A receptor agonist muscimol (4 nmol in 1 µl over 10 min in each hemisphere; Supplementary Fig. 2a–c) to investigate whether the vidget requires V1. To confirm inactivation, we recorded VEPs in eight mice before and 30 min after infusion of muscimol. We then waited 2 d for full recovery from muscimol before infusing vehicle and recording VEPs again. Muscimol significantly reduced the VEP magnitude (28.18 ± 7.46 µV) compared with its magnitude pre-infusion (78.58 ± 14.16 µV) or after infusion of vehicle (74.13 ± 21.55 µV) (Supplementary Fig. 2d).

Muscimol also significantly reduced vidget magnitude (1.82 ± 0.39 arbitrary units (a.u.)) compared with its magnitude pre-infusion (4.79 ± 0.73 a.u.) or after treatment with vehicle (5.01 ± 0.91 a.u.) (Supplementary Fig. 2e,f). Onset-by-onset analysis also revealed a significantly greater impact of muscimol treatment than the control conditions (Supplementary Fig. 2g).

Pharmacological blockade of activity using muscimol lasts for an extended period and may spread beyond V1. In order to overcome these potential issues, we transiently inactivated V1 by expressing channelrhodopsin-2 (ChR2) in putative fast-spiking interneurons via local delivery of an adeno-associated virus (AAV) vector (AAV5-EF1α-DIO-hChR2(H134R)-eYFP) into V1 of mice expressing Cre recombinase only in parvalbumin-positive (PV^+^) cells (B6;129P2-Pvlapf^cre^/J mice; called 'PV-Cre mice' here) (Fig. 2a–e and Supplementary Fig. 3a,b). Ten PV-Cre mice and eight wild-type littermate control mice were infected bilaterally within lateral (binocular) V1 (see Online Methods and Supplementary Fig. 10 for histological confirmation). VEP electrodes and optical fibers were also implanted. One month later, mice were presented with a sinusoidal grating stimulus of a single orientation (10-s blocks of stimuli). Using a laser, we delivered blue light (473 nm) into both hemispheres throughout half of the stimulus presentations, commencing 0.5 s before the onset of the visual stimulus and terminating 0.5 s after stimulus offset. The VEP magnitude was significantly suppressed during laser stimulation in the PV-Cre mice (207.90 ± 27.76 µV) compared with its magnitude when the laser was off (292.28 ± 36.89 µV).
Figure 2 The vidget requires activity in V1. Optical fibers were implanted below the cortical surface targeting V1 and VEP electrodes positioned in layer 4. Light could then be delivered to the recording site in vivo while the animal viewed a visual stimulus and optogenetic strategies used to alter activity locally. Through the selective expression of ChR2 in PV+ cells via Cre recombinase technology, blue light (473 nm) could be used to suppress activity in V1 through PV+ inhibition. (a) An example coronal section through a mouse brain (stained with the DNA dye DAPI) showing cells labeled with enhanced yellow fluorescent protein (eYFP) (green) that express ChR2 bilaterally restricted to lateral (binocular) V1. (b) Example histology showing eYFP labeling in select cortical cells expressing ChR2 (green). (c) Immunohistochemistry for parvalbumin reveals all the PV+ putative fast-spiking interneurons (red) in the image in b. (d) DAPI (blue) labels the nuclei of all cells in the same region as in b and c. (e) eYFP cells are also PV+. Other DAPI+ cells (blue) are not co-labeled in green, demonstrating that they do not express ChR2. Scale bars, 500 µm (a) and 10 µm (e). (f) VEPs evoked by full-field sinusoidal gratings were significantly suppressed in PV-Cre mice (n = 10) when blue light (473 nm) was applied with a laser (two-way repeated-measures ANOVA (genotype × treatment interaction): F₁,₅₅ = 13.395; P = 0.001), measuring 207.90 ± 27.76 µV while the laser was on (+) (light blue) and 292.28 ± 36.89 µV when it was off (−) (black) (n = 20 hemispheres; Student-Newman-Keuls post-hoc test, q₁₅ = 6.906, P < 0.001). This suppression was not observed in wild-type (WT) littermates (n = 8) infected with the same AAV5 virus, demonstrating that the laser had no effect without ChR2; in these animals, VEPs measured 303.19 ± 32.68 µV when the laser was on (blue) and 290.16 ± 33.49 µV when it was off (gray) (n = 16 hemispheres; Student-Newman-Keuls post-hoc test, q₁₅ = 0.866, P = 0.546). Averaged VEPs are presented at top. Dashed line represents noise levels. (g) VEPs were significantly suppressed by blue light (473 nm) in the same PV-Cre mice as in f (two-way repeated-measures ANOVA (genotype × treatment interaction): F₁,₁₅ = 6.639; P = 0.020). The vidget was suppressed in the PV-Cre mice (light blue, 1.26 ± 0.24 a.u.) relative to vidgets in the absence of laser stimulation (black; 2.89 ± 0.39 a.u.; n = 10 mice; Student-Newman-Keuls post-hoc test, q₁₅ = 3.919, P = 0.014) and in control littermates during laser stimulation (blue; 2.90 ± 0.63 a.u.; n = 8 mice; Student-Newman-Keuls post-hoc test, q₁₅ = 0.866, P = 0.546). Averaged vidget responses are presented at top. Dashed line represents pre-stimulus baseline. (h) Cumulative distribution of averaged vidget per PV-Cre mouse pre-laser stimulation (black) and during laser stimulation (light blue). (i) Cumulative distribution of averaged vidget per infected wild-type littermate (n = 8) pre-laser stimulation (gray) and during laser stimulation (blue). Dashed line represents pre-stimulus baseline. Error bars indicate s.e.m. *P < 0.05; n.s., non-significant comparisons.

(Fig. 2f). We did not observe this suppression in their wild-type littermates (VEP with laser on, 303.19 ± 32.68 µV; VEP with laser off, 290.16 ± 33.49 µV) (Fig. 2f), demonstrating that suppression was due to ChR2-mediated activation of PV+ inhibitory cells. The reduction in V1 activity also significantly affected the vidget. Laser stimulation suppressed the vidget in the PV-Cre mice (1.26 ± 0.24 a.u.) relative to the vidget in both the absence of laser stimulation (2.89 ± 0.39 a.u.) and the littermate control mice during laser stimulation (2.90 ± 0.63 a.u.) (Fig. 2g–i). The impact of laser stimulation on wild-type control mice was not significant (2.26 ± 0.57 a.u.) (Fig. 2g–i). Analysis conducted per behavioral onset is presented in Supplementary Figure 3c,d. Overall, these results demonstrate that the vidget is driven by activity in V1.

Stimulus-selective response potentiation

We next sought to determine if the vidget was modified as SRP developed in V1. SRP is a long-lasting potentiation of VEPs as a consequence of brief daily exposure to oriented grating stimuli and bears all the hallmarks of Hebbian synaptic plasticity and visual perceptual learning. Following SRP induction, VEPs evoked by familiar grating orientations are significantly larger than those evoked by novel orientations.

Previous recordings of SRP have been limited to VEPs in layer 4 of V1. In addition to determining the behavioral correlates of SRP, we wished to better understand the modification of translaminar patterns of V1 activity. We therefore implanted laminar probes (16 recording sites separated by 50 µm spanning the depth of V1) (Fig. 3a). After recovery and acclimation to head fixation, mice viewed a sinusoidal grating stimulus of a fixed orientation (X°) repeatedly over 6 d. On day 7, we pseudo-randomly interleaved blocks of the familiar stimulus (X°) with a stimulus of novel orientation (X + 90°) while acquiring VEPs. We then performed current-source-density analysis to determine the laminar flow of current sinks and sources through V1 (ref. 7). In response to each stimulus phase reversal, current sinks appeared with different latencies at different cortical depths (Fig. 3b), reflecting the spread of synaptic activity across the canonical cortical circuit (activation of thalamo-recipient layers 4 and 6, followed by layers 2 and 3, and then layer 5). A comparison of current sinks in response to familiar stimuli (X°) and novel stimuli (X + 90°) revealed that the layer 4 sink was greater in magnitude for familiar stimuli than for novel stimuli, while the deep layer 6 sink remained unchanged as a result of stimulus familiarity (Fig. 3b). Thus, while SRP is distributed within the cortical circuit, it is not uniform throughout V1. We therefore restricted our recordings to layer 4 for the remainder of this study, as this is a major site of SRP.

Although SRP has previously been reported as a synaptic phenomenon, we wished to determine its effect on the firing of single units within layer 4, because changes in neural firing would be necessary to support changes in behavior. To do this, we implanted bundles of eight recording electrodes targeting layer 4 of binocular V1 in ten mice. After recovery,
Figure 3 SRP is distributed but not uniform and affects neural spiking. (a) Example of the implantation of a laminar probe in mouse V1 with 16 recording sites separated by increments of 50 μm. Scale bar, 500 μm. (b) Current source density calculated from the local field potential data, presented for a familiar stimulus (left), a novel stimulus (middle) and a scaled subtraction of novel from familiar (right). Dark colors indicate current sinks and light colors indicate current sources. (c) Example raster plots showing multiunit firing from a single animal across the course of a familiarity test session in which familiar stimuli and novel stimuli were pseudo-randomly interleaved and separated by viewing of a gray screen. At left is the unit response to 30-s bouts of static gray stimuli interleaved between grating stimuli. This is presented arbitrarily time-locked for comparison with the phase reversing stimuli in the adjacent two panels, which show the event-related response to each of 500 phase reversals across five blocks of a 2-Hz phase-reversing familiar stimulus (middle) or novel stimulus (right). (d) Summary plot of peak firing rates for multi-unit recordings for ten mice. Peak firing after phase reversal is significantly elevated for the familiar stimulus (blue; 13.39 ± 2.51 Hz, n = 10; one-way repeated-measures ANOVA; F2,18 = 20.01, P < 0.001) relative to peak firing for the novel stimulus (red; 5.81 ± 1.61 Hz; Student-Newman-Keuls post-hoc test, q9 = 5.51, P = 0.001) and gray screen (gray, 1.20 ± 0.21 Hz; Student-Newman-Keuls post-hoc test, q9 = 8.86, P < 0.001). The ratio of peak firing rate in response to the familiar stimulus to that in response to the novel stimulus (Fam/nov (black)) is 4.38 ± 1.71, indicating that on average, the familiar stimulus evokes a peak firing rate around four times greater than that evoked by the novel stimulus. Error bars are s.e.m. *P < 0.05.

OSS in the freely moving mouse

Animals preferentially explore novel objects3 and thereby demonstrate memory of familiar objects. To investigate the possibility that a similar preference can be observed for a novel orientation, we developed an assay to measure the emergence of OSS in freely moving mice (Fig. 5a,b and Supplementary Video 2). Mice (n = 18) explored an open field arena with two video monitors positioned on opposite ends. The monitors showed a uniform gray stimulus during habituation sessions of ~30 min on each of 2 d. Over the next 8 d, mice were presented with five blocks of oriented, phase-reversing grating stimuli (X°) on each day on each monitor in a pseudo-random (counterbalanced) sequence. Exploration was quantified as time spent actively moving (velocity > 5 cm/s) within the zone next to the stimulus. Exploration on day 1 was significantly influenced by the visual stimulus, and mice spent more time exploring the area proximal to the stimulus presentation, whether on the left (59.5% ± 0.97%) or right (64.1% ± 0.73%) (Fig. 5c). Exploration bias was measured using a preference index (see Online Methods). We observed that preference for the previously viewed stimulus decreased significantly over 8 d as familiarity developed (Fig. 5d).

On day 9, blocks of novel stimuli (X + 90°) and familiar stimuli (X°) were shown on each monitor to determine if OSS had occurred. Mice exhibited preference for the stimulus of novel orientation whether it was presented on the left side (65.8% ± 0.69%) or the right side (59.4% ± 0.65%) of the arena (data not shown). No significant preference was observed for the familiar stimulus on the left (50.9% ± 0.77%) or the right (53.1% ± 0.60%) (data not shown). Overall, there was greater preference for the novel stimulus (0.28 ± 0.09) than for the familiar stimulus (0.03 ± 0.17) (Fig. 5e,f). Thus, OSS occurs in freely moving mice.

We next head fixed mice (n = 18) that had undergone free exploration of stimuli to determine if SRP of the VEP and OSS of the vidget had been induced by experience in the arena. The animals were presented with interleaved blocks of a stimulus that had been viewed over the previous 9 d (X°) and a stimulus they had just viewed for the first time, but only in the arena (X + 90°). SRP was clearly induced in V1 by the
SRP is eye specific, consistent with extensive evidence that it is induced by synaptic modifications within V1 (refs. 2, 3). To determine if OSH is also eye specific, we restricted the presentation of one eye to V1 on a group of mice on day 1 and subsequently measured VEPs to evaluate the induction of SRP and OSH in both restrained mice and freely behaving mice. This indicates that V1-dependent behavioral modifications occur as stimuli become familiar, regardless of context.

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stimulus (X°) to the left eye and another stimulus (X + 90°) to the right eye over 8 d in 14 mice. On day 9, we interleaved blocks of each of these stimuli, along with blocks of a third, completely novel stimulus (X + 45°; ‘true novel’) shown to each eye independently (Fig. 6a,b). Vidgets driven by the familiar stimulus were significantly lower in magnitude (1.54 ± 0.26 a.u.) than vidgets driven by the stimulus novel to the eye (blue) elicited by the stimulus novel to the contralateral eye (Novel to eye; orange) (2.58 ± 0.36 a.u.; Student-Newman-Keuls post-hoc test, q20 = 6.55, P < 0.05) or the ‘true novel’ stimulus (red) (3.10 ± 0.50 a.u.; Student-Newman-Keuls post-hoc test, q20 = 4.73, P < 0.05). There was no significant difference between the averaged magnitude of vidgets elicited by the stimulus novel only to the eye (orange) and those elicited by the ‘true novel’ stimulus (red) (Student-Newman-Keuls post-hoc test, q20 = 0.13, n.s.). (d) The cumulative distribution of average vidget magnitude elicited through each eye by the familiar stimulus (blue), the stimulus novel only to the eye (orange circles) and the ‘true novel’ stimulus (red circles) on day 9 shows that OSH is reliably eye specific. (e) The stimulus familiar to the eye (blue) elicited VEPs of significantly greater magnitude (124.21 ± 15.29 µV; n = 28 hemispheres; Friedman one-way repeated-measures ANOVA on ranks, χ2 = 14.72, P < 0.001) than did either the ‘true novel’ stimulus (red) (70.70 ± 9.71 µV; Student-Newman-Keuls post-hoc test, q20 = 4.68, P < 0.05) or the stimulus novel only to the eye (orange) (77.02 ± 10.89 µV; Student-Newman-Keuls post-hoc test, q20 = 8.65, P < 0.05). There was also no significant difference between VEPs driven by the stimulus novel to the eye (orange) and those driven by the ‘true novel’ stimulus (red) (Student-Newman-Keuls post-hoc test, q20 = 0.52, n.s.). Error bars are s.e.m. *P < 0.05.

OSH and SRP require NMDA receptors in V1

SRP induction requires activation of NMDA receptors (NMDARs)2. To determine whether OSH shares this mechanism, we locally knocked down the mandatory GRIN1 subunit in 1-month old mice expressing loxP-flanked Grin1 alleles (Grin1fl/fl mice: B6.129S4–Grin1tm1Stl/J)10,11 by expressing Cre recombinase bilaterally in V1 via an AAV8 viral vector (AAV8-hSyn-GFP-Cre). Infected cells were labeled with green fluorescent protein (GFP) to track the spread of infection. Control mice were Grin1fl/fl litters that received local injection to express GFP alone under control of the same promoter via a viral vector of the same serotype (AAV8-hSyn-GFP) (Fig. 7a,b). Three weeks after infection, head-fixed mice were shown five blocks of 100 phase reversals of the grating stimulus per day over six consecutive days, while VEPs and vidgets were recorded. On day 7, the now familiar stimulus (X°) and a novel stimulus (X + 90°) were pseudo-randomly interleaved to assess both SRP and OSH. In comparison to the potentiation of VEPs in control littermate mice, the potentiation of VEPs over days was significantly impaired as a result of the deletion of GRIN1 in V1 (Fig. 7c). Additionally, expression of SRP on day 7 was selectively disrupted by loss of NMDARs in V1, as VEPs evoked by familiar stimuli (113.73 ± 14.57% day 1 baseline) and novel stimuli (100.84 ± 12.95% baseline) were of similar magnitude after local expression of Cre in Grin1fl/fl mice (Fig. 7d). In littermate control mice, by contrast, the familiar stimulus evoked VEPs of significantly greater magnitude (188.83 ± 19.40% baseline) than those evoked by the novel stimulus (115.01 ± 14.58% baseline) (Fig. 7d), demonstrating SRP. OSH was also selectively disrupted only in Grin1fl/fl mice in which Cre had been expressed. Vidgets of similar magnitude were evoked by familiar stimuli (2.64 ± 0.48 a.u.) and novel stimuli (3.21 ± 0.61 a.u.) in these mice, whereas in their control littermates, the familiar stimulus evoked vidgets of significantly lower magnitude (2.03 ± 0.65 a.u.) than those evoked by the novel stimulus (6.92 ± 1.15 a.u.) (Fig. 7c,d), demonstrating OSH. We observed this same selective deficit in OSH in the Cre-expressing Grin1fl/fl mice for comparisons made across all stimulus onsets (Supplementary Fig. 7a,b). These data support the conclusion that NMDARs within V1 are required for both SRP and OSH.
Acute blockade of NMDARs prevents acquisition of OSH

Our strategy of local knockdown of Grin1 resulted in a chronic loss of NMDAR function in V1, which could potentially impair memory recall as well as learning. To address this concern, we conducted an experiment in which we bilaterally infused the NMDAR antagonist AP5 (5 nmol in 1 µl, delivered over 10 min in each hemisphere) into V1 of 18 mice before stimulus delivery. A crossover experimental design was employed in which mice were divided into two groups of nine mice per group. One group received infusions of vehicle and the other received infusions of AP5 30 min before viewing a stimulus of a designated orientation (X°). After a day of rest to allow complete drug washout, we assessed whether OSH was present by showing interleaved blocks of the original stimulus (X°) and a novel stimulus (X + 25°). After a further day of rest, each group then received the opposite drug treatment before viewing another novel stimulus (X + 25°). On day 7, allowing another rest day for drug washout, we assessed OSH by presenting interleaved blocks of the second stimulus (X + 25°) and a final stimulus of novel orientation (X + 115°) (Fig. 8a).

Following treatment with vehicle, vidgets in response to novel stimuli were significantly greater in magnitude (5.59 ± 0.56 a.u.) than those in response to familiar stimuli (2.72 ± 0.43 a.u.) (Fig. 8b), demonstrating OSH. In contrast, the same mice did not discriminate the previously viewed stimulus (4.16 ± 0.68 a.u.) from the novel stimulus (4.97 ± 0.52 a.u.) (Fig. 8c) following treatment with AP5. Significant OSH was apparent in a comparison across stimulus onsets, although discrimination of familiar and novel stimuli was restricted to post-vehicle sessions (Supplementary Fig. 8c,d). Thus, blockade of NMDAR within V1 prevented OSH.

It was possible that the blockade of NMDARs impeded OSH by reducing activity in V1 and preventing flow of information to the site of storage elsewhere. With this question in mind, we compared the magnitude of VEPs evoked in the presence of AP5 (67.93 ± 10.81 µV) and those evoked in the presence of vehicle (59.82 ± 7.06 µV) and found no difference between treatments (Supplementary Fig. 8a). We also confirmed that SRP did not occur during treatment with AP5 (Supplementary Fig. 8b). In discrimination sessions during treatment with AP5, VEPs driven by the previously viewed stimulus (57.71 ± 8.53 µV) did not differ significantly from those evoked by the novel stimulus (57.43 ± 7.31 µV) (Supplementary Fig. 8b). In contrast, familiar stimuli evoked VEPs of significantly greater magnitude (76.17 ± 10.02 µV) than those evoked by novel stimuli (58.95 ± 7.61 µV) during treatment with vehicle (Supplementary Fig. 8b). Familiar stimuli experienced previously during treatment with vehicle also evoked VEPs of significantly greater magnitude than the VEPs evoked by stimuli experienced during treatment with AP5 (Supplementary Fig. 8b). Thus, blockade of NMDARs within V1 prevents both SRP and OSH.

Figure 7 NMDARs in V1 are required for the induction of OSH. (a) NMDARs were ablated bilaterally in V1 of Grin1KO mice by local expression of Cre recombinase through infection with an AAV8 virus. A GFP tag revealed the local spread of infection in V1 targeting the lateral binocular portion (V1b) and not the medial monocular region (V1m). (b) Grin1KO littermates locally expressing only GFP through infection with a matched AAV8 viral vector served as controls. Scale bars represent 500 µm (a) and 100 µm (b). (c) SRP induction was significantly deficient in Cre-expressing Grin1KO mice (n = 11; light green circles) relative to that in Grin1KO littermates expressing GFP only (dark green circles) (two-way repeated-measures ANOVA (treatment × day interaction): F1,17 = 5.523; P < 0.001). (d) Selective failure of SRP expression (ANOVA (treatment × stimulus interaction): F1,17 = 25.634; P < 0.001) is reflected by the equal magnitude of VEPs driven by novel stimuli (red outlines; 100.84% ± 12.95% baseline) and those driven by familiar stimuli (blue outlines; 113.73% ± 14.57% baseline) in Cre-expressing Grin1KO mice (V1 GRIN1 KO; light green bars; n = 22 hemispheres; Student-Newman-Keuls post-hoc test, q21 = 2.142, P = 0.137) during the final session of the familiarity test. Normal selectivity between novel stimuli (red outlines; 115.01 ± 14.58% baseline) and familiar stimuli (blue outlines; 188.83 ± 19.40% baseline) was observed in Grin1KO mice expressing GFP only (V1 GRIN1 KO; dark green bars; n = 22 hemispheres; Student-Newman-Keuls post-hoc test, q21 = 12.268, P < 0.001) during interleaved test sessions. Stimulus is described as ‘familiar’ because it is not actually familiar to those animals in which learning was blocked. Average VEPs are shown at top in c, d. (e) OSH was similarly selectively blocked by knockdown of NMDAR function in V1 (two-way repeated-measures ANOVA (treatment × stimulus interaction): F1,42 = 8.615; P = 0.008). The cumulative distributions of average vidget magnitude per mouse driven by familiar stimuli (blue outlines; 2.03 ± 0.65 a.u.) and novel stimuli (red outlines; 6.92 ± 1.15 a.u.) in Grin1KO mice expressing GFP only (V1 GRIN1 KO; dark green bars; n = 22 hemispheres; Student-Newman-Keuls post-hoc test, q21 = 6.643, P < 0.001), reflecting OSH. (f) Vidgets of similar magnitude were evoked by familiar stimuli (blue outlines; 2.64 ± 0.48 a.u.) and novel stimuli (red outlines; 3.21 ± 0.61 a.u.) (n = 11 mice; Student-Newman-Keuls post-hoc test, q10 = 0.773, P = 0.591) after deletion of GRIN1 in V1 (light green), demonstrating blockade of OSH. Average vidgets are presented at top of insets in e, f. Dashed lines represent pre-stimulus baseline for e, f. Error bars are s.e.m. *P < 0.05, **P < 0.01, ***P < 0.001.
The inhibitor ZIP in V1 ‘erases’ OSH

In order to determine if OSH requires memory storage in V1, we applied ZIP (‘PKMζ inhibitor peptide’) after OSH had been saturated. This peptide has been shown to reverse long-term synaptic potentiation (LTP)\(^1\)\(^2\), cortical memory\(^4\) and SRP\(^3\). Mice (n = 36) underwent a typical OSH induction protocol over 8 d (Fig. 8d). On day 9, blocks of the familiar stimulus (X\(^\ddagger\)) and a novel stimulus (X + 45\(^\circ\)) were interleaved. On day 10, half of the mice (n = 18) were given bilateral infusions of ZIP (10 nmol ZIP in 1 \(\mu\)l delivered over 10 min), while the other half (n = 18) were given bilateral infusions of vehicle through infusion cannulae implanted bilaterally in binocular V1. This experiment revealed that ZIP was susceptible to ‘erasure’ by ZIP (three-way ANOVA (interaction of treatment \(\times\) session \(\times\) stimulus): \(F_{1,34} = 4.27, P = 0.041\)). Prior to the infusions, OSH occurred (n = 18; two-way repeated-measures ANOVA (effect of stimulus): \(F_{1,17} = 11.80, P = 0.002\)) and did not differ across treatment (treatment \(\times\) stimulus: \(F_{1,34} = 0.52, P = 0.48\)). Prior to infusions of ZIP on day 9 (black bars), vidgets of significantly greater magnitude were observed in response to the novel stimuli (red outlines; 4.47 \(\pm\) 0.81 a.u.) than in response to the familiar stimuli (blue outlines; 2.32 \(\pm\) 0.40 a.u.) (Student-Newman-Keuls post-hoc test, \(q_{1,17} = 4.00, P = 0.006\)). On day 11 after the infusion, a difference between the groups emerged (two-way repeated-measures ANOVA (treatment \(\times\) stimulus): \(F_{1,34} = 4.63, P = 0.039\)). On day 11, discrimination between novel stimuli and familiar stimuli was still present in the control group treated with vehicle. Cumulative distributions of average vidget per mouse overlapped for familiar stimuli (blue outlines; 3.17 \(\pm\) 0.76 a.u.) and novel stimuli (red outlines; 3.01 \(\pm\) 0.53 a.u.), and any difference was not statistically significant (inset; Student-Newman-Keuls post-hoc test, \(q_{1,17} = 0.31, P = 0.830\)). Dashed lines represent pre-stimulus baseline. Error bars are s.e.m. \(*P < 0.05.

Figure 8 Local blockade of learning and erasure of memory in V1 (a) Either the selective NMDAR antagonist AP5 (5 nmol in 1 \(\mu\)l over 10 min) or vehicle was infused bilaterally 30 min before stimulus onset into binocular V1 of mice with bilateral implantation of injection cannulae and VEP recording electrodes. Mice were separated into two groups of nine for a crossover experimental design. (b) OSH occurred selectively during treatment with vehicle but not during treatment with AP5 (two-way repeated-measures ANOVA; n = 18; \(F_{1,17} = 7.79, P = 0.013\)). The cumulative distributions of average vidget per mouse driven by familiar stimuli (F (blue); 2.72 \(\pm\) 0.43 a.u.) and novel stimuli (N (red); 5.59 \(\pm\) 0.56 a.u.) after vehicle treatment revealed a consistent suppressive effect of familiarity (Student-Newman-Keuls post-hoc test, \(q_{1,17} = 4.82, P = 0.002\)). (c) After treatment with AP5, cumulative distributions of average vidget per mouse driven by the previously viewed familiar stimulus (blue; 4.16 \(\pm\) 0.68 a.u.) and the novel stimulus (red; 4.97 \(\pm\) 0.52 a.u.) were largely overlapping (n = 18) and no significant difference was observed (inset; Student-Newman-Keuls post-hoc test, \(q_{1,17} = 0.48\)). Prior to infusions of ZIP on day 9 (black bars), vidgets of significantly greater magnitude were observed in response to the novel stimuli (red outlines; 4.47 \(\pm\) 0.81 a.u.) than in response to the familiar stimuli (blue outlines; 2.32 \(\pm\) 0.40 a.u.) (Student-Newman-Keuls post-hoc test, \(q_{1,17} = 4.16, P = 0.006\)), reflective of OSH. (f) On day 11 after the infusions, a difference between the groups emerged (two-way repeated-measures ANOVA (treatment \(\times\) stimulus): \(F_{1,34} = 4.63, P = 0.039\)). On day 11, discrimination between novel stimuli and familiar stimuli was still present in the control group treated with vehicle. Cumulative distributions of average vidget per mouse overlapped for familiar stimuli (blue outlines; 3.17 \(\pm\) 0.76 a.u.) and novel stimuli (red outlines; 3.01 \(\pm\) 0.53 a.u.), and any difference was not statistically significant (inset; Student-Newman-Keuls post-hoc test, \(q_{1,17} = 0.31, P = 0.830\)). Dashed lines represent pre-stimulus baseline. Error bars are s.e.m. \(*P < 0.05.

DISCUSSION

We have characterized a spontaneous, V1-dependent behavior in the head-fixed mouse, termed the ‘vidget’, which accurately reports an
Measuring V1-dependent mouse vision with the vidget

The mouse has gained popularity as a species with which to study the neurobiology of vision and visual cortical plasticity, but it has been challenging to assay V1-dependent vision with behavior. Previous attempts have involved operant conditioning or optokinetic reflexes. However, operant conditioning is necessarily limited to specific stimulus sets and requires extensive training; and optokinetic reflexes do not require the participation of V1 (ref. 18). The presentation-selective behavioral habituation in the open field transferred we have described a form of long-term habituation in the mouse that manifested through behavioral habituation, enables organisms to familiarize with stimuli that bring neither reward nor punishment, thus providing a means to measure plasticity in V1 as long as different stimuli are used. This transfer of habituation to novel stimuli the animals find interesting and worthy of exploration.

Because the vidget is a response to novel stimuli that does not require pre-training, it has the potential to be used in longitudinal studies of mouse vision—for example, after periods of monocular deprivation. The obvious complication is OSH. However, like VEPs, vidgets of comparable magnitude can be elicited in naive mice with few stimuli the animals find aversive, raising the possibility that mice actually detect low-contrast stimuli but fail to respond because they find them less aversive than high-contrast stimuli. Arguing against this interpretation were the simultaneous recordings of V1 VEPs, which disappeared into the noise at the same contrasts as the vidgets. Furthermore, the reactions of the mice in the open field to presentation of the same high-contrast full-field stimuli are more compatible with the view that the vidget reflects an orienting response to novel stimuli the animals find interesting and worthy of exploration.

Long-term behavioral habituation occurs via synaptic plasticity in V1

Familiarity with stimuli that bring neither reward nor punishment, manifested through behavioral habituation, enables organisms to devote cognition to important elements of the environment. Here we have described a form of long-term habituation in the mouse that enables the detection of novel visual stimuli. We found that freely behaving mice actively explored novel visual gratings and that behavioral habituation occurred as these stimuli became familiar. The orientation-selective behavioral habituation in the open field transferred to the vidget responses in the head-fixed mouse. Using the head-fixed mouse preparation, we have been able to identify the locus and some mechanistic requirements of OSH.

Behavioral expression of the vidget requires V1, and several converging lines of evidence suggest that a mechanism that contributes to OSH also resides within V1. First, vidget habituation was eye specific as well as orientation selective. Although these properties do not rule out the possibility of involvement of other cortical areas, they are consistent with a V1 locus. Second, local genetic knockdown of NMDARs in V1 or microinfusion of AP5 prevented the induction of OSH. These results show that activation of V1 neurons is required for OSH and suggest a critical involvement of NMDARs in the synaptic mechanism. We cannot exclude the possibility that the infusion of AP5 during learning interrupted the flow of information to other cortical areas where the information is stored, but we note that this treatment did not suppress the V1 VEPs. Third, OSH was disrupted by local infusion of ZIP into V1. Although the data do not allow us to conclude that ZIP-treated mice respond to familiar stimuli as if they were novel, the results clearly indicate that vidget responses fail to discriminate familiar stimuli versus novel stimuli after infusion of ZIP confined to V1.

ZIP was developed to selectively inhibit the kinase PKMζ, which has been linked to the mechanism for stable LTP at many synapses. Although it has been questioned whether PKMζ is the relevant target of ZIP, there is broad consensus that this peptide can reverse established LTP and memory in a variety of neural systems, including the neocortex. In mouse V1, previous work from our laboratory has shown that ZIP reverses SRP. SRP is also prevented by local microinfusion of AP5 into V1 and is produced by mechanisms shared with LTP. Together, the findings that vidget habituation and SRP were both induced by the same stimuli over the same time course, had similar properties of orientation and eye specificity and were similarly sensitive to local infusion of ZIP or AP5 into V1 strongly suggest these phenomena are closely related. We hypothesize that SRP is an electrophysiological consequence of synaptic modifications that contribute to OSH. The finding that selective visual experience that produced OSH in the open field also induced VEP potentiation supports this hypothesis.

Given that habituation features a decrement in behavioral response, it is intuitive to imagine synaptic depression as an underlying mechanism. Indeed, there is evidence for synaptic depression in some neural pathways displaying habituation. However, a viable alternative is that the synaptic potentiation observed in SRP enforces a selective suppression of a separate response pathway. Given that inactivation of V1 prevented performance of the vidget, a logical extension of this hypothesis is that parallel pathways pass through V1: a ‘response’ pathway that directly mediates the vidget and does not undergo long-term modification, and a ‘learning’ pathway that is selectively strengthened through Hebbian plasticity and subsequently suppresses the output of the ‘response’ pathway. Although speculative, this proposal is anatomically plausible and shares features in common with influential theories of habituation.

We note that deficits in habituation are well documented in schizophrenia. These may contribute to the disrupted attention that characterizes the cognitive symptoms of this disorder. Deficits in a physiological phenomenon similar to SRP have also been observed in individuals with schizophrenia. Assays of SRP and OSH in mutant mice engineered to carry genetic disruptions linked to schizophrenia therefore have the potential to uncover aspects of cortical pathophysiology that could suggest new treatment strategies.

V1 as a cortical substrate for visual recognition memory

Although much experimental work has now revealed that primary sensory cortices retain the capacity for change in response to injury.
or sensory deprivation, it has previously been unclear to what degree they encode memory resulting from everyday experience. We have shown here that plasticity in V1 contributes to memory. The observation that OSH transferred from free exploration of an arena to an apparatus in which the animal was restrained suggests that this plasticity supports context-independent recognition based on familiarity. The perirhinal cortex, a higher-order visual region, is a major focus of work on familiarity because this region is necessary for preferential exploration of novel objects. Interestingly, exploration of a novel object requires mechanisms of synaptic long-term depression in the perirhinal cortex, and the neuronal response to the object is observed, a property called ‘invariance’. OSH is not invariant because very simple stimuli are discriminated simply on the basis of shifted orientation. Our findings suggest the possibility that low-level plasticity in V1 may serve as a building block for more complex representations that contribute to invariant visual recognition memory.

METHODS

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

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AUTHOR CONTRIBUTIONS

S.F.C. designed all experiments, conducted and analyzed all data from experiments described in Figures 1A-4E, 7A and all Supplemental Figures, collected data shown in Figure 3, and wrote the manuscript. R.W.K. designed, conducted, analyzed and summarized all experiments shown in Figure 5, and participated in analysis of Figure 3. E.S.K. conducted experiments described in Figures 2 and 7. J.P.G. designed and conducted experiments in Figure 3 and developed the stimulus-generation and recording system for acquisition of data and participated in data analysis. M.F.B. designed experiments and wrote the manuscript.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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**ONLINE METHODS**

**Animals.** All procedures were approved by the Committee on Animal Care at the Massachusetts Institute of Technology and were in accordance with the guidelines of the US National Institutes of Health. Mice were male C57BL/6 mice (Charles River Laboratory International) aged postnatal days 30–45. For optogenetic experiments, mice expressing Cre recombinase directed by the promoter of the gene encoding parvalbumin were used (B6;129P2-Grin1tm1(cre)Arbr; Jackson Laboratory). For local NMDAR-knockdown experiments, Grin1fl/fl mutant mice were used (B6;129S4-Grin1tm2Sdl; Jackson Laboratory). In all cases, mice were housed in groups of two to five with food and water available ad libitum and were maintained on a 12-h light-dark cycle. All mice participated only in the individual experiment described and did not undergo any prior or subsequent experimental treatment or procedure.

**Electrode and cannula implantation.** Mice were anesthetized for surgery by intraperitoneal (i.p.) injection of 50 mg ketamine per kg body weight and 10 mg xylazine per kg body weight. 1% lidocaine hydrochloride anesthetic was injected under the scalp of the mouse before incision. 0.1 mg Buprenex per kg body weight was delivered subcutaneously for analgesia. The skull was cleaned with iodine and 70% ethanol. A steel headpost was affixed to the skull anterior to bregma using cyanoacrylate glue. Burr holes (~0.5 mm) were then drilled in the skull over binocular V1 (3.2 mm lateral of lambda). Tungsten electrodes (FHC) 75 µm in diameter at the widest point were implanted in each hemisphere, 450 µm below the cortical surface. Silver wire (A-M Systems) reference electrodes were placed over prefrontal cortex. For layer-specific analysis, linear silicon probes (16 recording sites with 50-µm spacing; NeuroNexus) were implanted with the most superficial recording site just below the cortical surface. For unit recordings, custom-made bundles (tungsten H-Formvar wire with an outer diameter of 20 µm; California Fine Wire Company) were implanted 450 µm below the cortical surface. For local drug infusions, mice were also given bilateral implantation of 26 GA guide cannulae (Plastics One) positioned lateral (3.5 mm lateral to lambda) to the recording site at a 45° angle to the recording electrode, 0.1 mm below the surface. All implants were secured in place using cyanoacrylate glue. Finally, dental cement was applied to form a stable, protective head cap. Dummy cannulae were inserted into guides. Mice were monitored postoperatively for signs of discomfort and were allowed 24 h for recovery.

**Stimulus presentation.** Visual stimuli were generated using custom software written in Matlab (MathWorks) using the PsychToolbox extension (http://psychtoolbox.org) to control stimulus drawing and timing. The display was positioned 20 cm in front of the mouse and centered, thereby occupying 92° × 66° of the visual field. Mean luminance was 27 cd/m². Visual stimuli consisted presented full fields of gray. Phase-reversing stimulus presentation began the next day. Each day consisted of two free-exploration sessions separated by ~1 h, in which the mouse was returned to its home cage. Each session began with 5 min of full-field gray on both monitors, followed by presentation of the visual stimulus on one side of the arena (the actual location of the visual stimulus was counterbalanced from day to day). The stimulus consisted of a 100% contrast, sinusoidal grating that phase-reversed at a frequency of 2 Hz. The stimulus had a spatial frequency of 0.05 cycles per degree, as calibrated from the center of the testing area. Visual stimuli were presented as five blocks of 100 phase-reversals per block with 30 s of gray-screen stimulus during the interblock interval. For the second training session, the stimulus was presented on the opposite side of the arena, following the same protocol. This training paradigm was implemented for 8 d with a single orientated stimulus. Day 9 consisted of four training sessions, two using the new familiar orientation and two using an orthogonal novel orientation. Once again, the order of stimulus presentation was counterbalanced from mouse to mouse. Subsequently, the mouse was head-fixed for recordings of VEP and vidget.

To measure each mouse’s preference for the visual stimulus, we split the testing area into halves (zones) and quantified the amount of time the mouse spent within the zone near the visual stimulus versus time spent on the side near the gray screen. To limit our analyses only to periods of active exploration, we quantified the mouse’s location for periods during which running velocity exceeded 5 cm/s. Side preferences were then quantified as the percentage of total exploration time spent within each zone. Preference for the stimulus zone was expressed as the ratio of time spent exploring within the zone closest to the stimulus minus the time spent within the opposite zone over total exploration time (stimulus zone – opposite zone)/overall exploration).

**Electrophysiological data acquisition and analysis.** All data were amplified and digitized using the Recorder-64 system (Plexon Inc.). Two recording channels were dedicated to recording EEG and VEPs from V1 in each implanted hemisphere and a third recording channel was reserved for the Piezo-electrical input carrying the behavioral information for the majority of experiments. Fields were recorded with 1-kHz sampling and a 500-Hz low-pass filter. All data were extracted from the binary storage files and analyzed using custom software written in C++ and Matlab. VEPs were averaged across all phase reversals within a block and trough–peak difference measured during a 200-ms period from phase
reversal. For experiments described in Figure 3, 16 separate channels were used for laminar probe local field potential (LFP) recordings, each dedicated to an individual recording site. Current source density analysis measured sink and source magnitudes across all cortical layers by calculating the second spatial derivative of the averaged VEP responses to familiar and novel stimuli. For spike recordings, eight separate channels were used, each dedicated to a single wire within the electrode bundle. Spiking activity was digitized with 25-kHz sampling, and multi-unit spikes were isolated using Offline Sorter (Plexon Inc.).

Viral infection. All viruses used to locally infect V1 were adenovirus-associated viruses (AAVs). For optogenetic experiments, we infected V1 of 1–1-month-old mice expressing Cre recombinase directly by the promoter of the gene encoding parvalbumin (PV-Cre, B6;129S4-Crt61tm1(cre); Jackson Laboratory) or their wild-type littermates with AAV5-EF1α-dIO-hChR2(H134R)-eYFP (University of North Carolina viral core; generated by K. Deisseroth's laboratory). Using a glass pipette and nanoinjection system (Drummond Scientific), we delivered 81 nl of virus at each of three cortical depths: 600 µm, 450 µm, and 300 µm (distance below surface). At each depth, six injections of 13.5 nl were delivered, each separated by 15 s, and 5 min was allowed between repositioning for depth. For local knockdown of GRIN1, 1–1-month-old mice Grin1fl/fl mutant mice (B6.12994-Grin1tm5205J; Jackson Laboratory) were infected locally in V1 with either AAV8-Hs35-GFP-Cre (knockdown; UNC viral core) or AAV8-Hs35-GFP (control; UNC viral core; generated by B. Roth's laboratory). Again, injections were made at multiple depths. In this case, ten injections of 13.5 nl were made for a total of 135 nl at four cortical depths: 600 µm, 450 µm, 300 µm and 150 µm (distance below surface). As before, each injection was separated by 15 s, and 5 min was allowed between repositioning for depth. Mice were allowed 4 weeks recovery for viral expression to peak before experiments were initiated.

Optogenetics. After viral infection, mice were also bilaterally implanted with VEP recording electrodes positioned in layer 4. Ready-made optic fibers (200 µm in girth) mounted in stainless steel ferrules (1.25 mm in diameter; 2 mm fiber projection; Thor Labs) were then implanted positioned lateral (3.5 mm lateral to lambda) to the recording site and at a 45° angle to the recording electrode, 0.1 mm below surface in each hemisphere. 1 month later, after the peak of viral expression, mice were habituated to the head-fixation apparatus over 2 d before optogenetic experiments were conducted. We delivered continuous pulses of blue light (473 nm; 150 mW) 1 s in duration into V1 using a laser (Optogone). These light pulses were delivered simultaneously to 50% of the 10-s visual stimulus presentations, commencing 30 ms before visual stimulus onset and ending 30 ms after offset. Animals were sacrificed and perfused within a week after this experiment for histological analysis.

Drug infusion. Infusions of ZIP and AP5 were conducted by researchers ‘blinded’ to treatment conditions. Prior to infusion experiments, during which mice became accustomed to the recording apparatus, ‘dummy’ cannulae were removed and repositioned in order to prevent blockage. On the day of infusion, syringes and guide tubing, attached to 33 GA injection cannulae, were filled with distilled water, which was separated from the injected solution with an air bubble. The ‘dummies’ were again removed, and injection cannulae were inserted through guides and were allowed to sit in place for 5 min before injection. If blockade prevented smooth infusion, the animal was excluded from the study (ten animals were excluded for this reason before inclusion in any data set). Using a KD scientific infusion pump, slight positive pressure was maintained on the syringe while the injection cannulae was inserted in order to prevent blockage. The vehicle solution was aCSF (124 mM NaCl, 5 mM KCl, 1.25 mM Na2PO4, 26 mM NaHCO3, 1 mM MgCl2, and 2 mM CaCl2) stored and defrosted on the day of injection. Muscimol (4 mM, Sigma), AP5 (5 mM; Tocris) and ZIP (myr-SYRRGARRWKLR-OH; 10 mM; Sigma) were infused at a rate of 6 µl/hour over 10 min to inject 1 µl. The resulting local quantities are described in the manuscript. Hemispheres were injected in sequence.

Histology. Mice were deeply anaesthetized with Fatal-Plus (pentobarbital) and were perfused with saline followed by 4% paraformaldehyde in 0.1 M phosphate buffer. The brain was removed and post-fixed for 24 h at room temperature. After fixation, the brain was sectioned into coronal slices 60-µm in thickness using a vibratome. For assessment of the spread of viral infusions, Hoechst and FluoroMyelin Red stains were performed. Slices were incubated with a permeabilization solution (0.2% Triton X-100 in PBS) for 30 min at room temperature and then with a staining solution (Hoechst 33342 at a dilution of 1:10,000 (Life Technologies) and FluoroMyelin Red at a dilution of 1:100 in PBS (Life Technologies)) for 30 min. Slices were washed three times with PBS and mounted. Fluorescence images were obtained with a confocal fluorescence microscope (Olympus). The margins of V1 were determined using the established techniques of observing the pronounced increase in thickness of layer 4 (refs. 44–46) and of increased myelination demarcating primary sensory areas47. A description of this approach is provided in Supplementary Figure 10.

Immunohistochemistry. Mice were deeply anaesthetized with Fatal-Plus (pentobarbital) and were perfused with saline followed by 4% paraformaldehyde in 0.1 M phosphate buffer. The brain was removed and post-fixed for 24 h at room temperature. After fixation, the brain was sectioned into coronal slices 60-µm in thickness using a vibratome. Slices were incubated with blocking solution (10% FBS in PBS with 0.2% Triton X-100) for 1 h at room temperature and then with mouse primary antibody to parvalbumin (MAB1572; 1:1,000 dilution in blocking solution; Millipore) overnight at 4 °C. Slices were then washed three times with PBS and incubated with the secondary antibody (Alexa Fluor 594–conjugated antibody to mouse immunoglobulin G; 1:500 dilution; Invitrogen) for 1 h at room temperature. Slices were washed three times with PBS and were mounted with Vectashield –containing DAPI (49,6-diamidino-2-phenylindole) (Vector Laboratories). Fluorescence images were obtained with a confocal fluorescence microscope (Olympus).

Statistical analyses. In the Results section, all data are presented as a mean ± s.e.m. Sigmaplot and SPSS were used for statistical analysis. For all experiments, normality of distribution and homogeneity of variation was tested. A parametric ANOVA (for multiple groups) or two-tailed t-test (for two groups) was performed when data passed these tests. Otherwise, a non-parametric ANOVA or t-test on ranks was used. If the ANOVA yielded significance, a Student-Newman-Keuls post-hoc test with adjustment for multiple comparisons was applied for individual comparisons. A repeated-measures ANOVA or paired t-test was applied for all within-subject comparisons. For other comparisons, an unpaired ANOVA or t-test was used. Individual tests used are described in the Results section. P<0.05 is used as a threshold for significance throughout, but exact P values are given for all comparisons for which the P value was above 0.001, except for post-hoc tests conducted after non-parametric tests on ranks. No explicit statistical methods were used to predetermine sample sizes, but our sample sizes throughout are similar to or greater than those generally employed to assess mouse behavior.

A Supplementary Methods Checklist is available.

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Erratum: Visual recognition memory, manifested as long-term habituation, requires synaptic plasticity in V1

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In the version of this article initially published, there were quotation marks around “encoded” in the first paragraph and around “encode” in the last paragraph of the main text; these have been deleted. The second sentence of the third paragraph read “habituated in a stimulus-selective manner in V1 as SRP developed”; “in V1” has been deleted. The Figure 7d legend began “Failure of SRP induction”; the correct text is “Selective failure of SRP expression.” The sixth paragraph of the Discussion began “Behavioral manifestation of the vidget required V1”; the correct text is “Behavioral expression of the vidget requires V1.” The eighth paragraph of the Discussion included “first, a ‘response’ pathway that directly mediates the vidget and does not undergo long-term modification, and second, a ‘learning’ pathway”; “first,” and “second,” have been deleted. The errors have been corrected in the HTML and PDF versions of the article.