Supplementary Figure 1

Vidgets occur in response to most, but not all, novel stimulus onsets.

Cumulative distribution of vidgets evoked by each stimulus onset revealing that a movement above baseline (dashed line) occurs on approximately 70% of stimulus onsets. These data (n = 75) comprise the averages presented from 15 animals in Figure 1. Data are plotted on a log 10 scale for clarity.
Inactivation of V1 with muscimol prevents vidgets.

a) In mice, recording electrodes were implanted within layer 4 of V1 and indwelling cannulae angled to deliver drug (4 nMol muscimol in 1 µL over 10 minutes) to the recording site. b) Dorsal reconstruction of electrode implantation/cannulation sites bilaterally in visual cortex of a brain removed from an example animal. c) Example histology slide of light cresyl violet stain showing recording electrode track and guide cannula position. d) Averaged VEPs driven by novel sinusoidal grating stimuli in 8 mice 30 minutes after muscimol treatment (purple, 28.18 ± 7.46 µV) were significantly reduced relative to pre-injection (gray, 78.58 ± 14.16 µV; n = 16 hemispheres; 1-way repeated measures ANOVA, F(2,30) = 11.21, p < 0.001; Student-Newman-Keuls post-hoc test, q(15) = 6.05, p < 0.001) or vehicle infusion (open bar, 74.13 ± 21.55 µV; Student-Newman-Keuls post-hoc test, q(15) = 5.51, p < 0.001). Dashed line represents noise levels. Averaged VEPs are presented at top of panel. All error bars are SEMs. e) Averaged vidgets are suppressed after muscimol (purple, 1.82 ± 0.39 a.u.) compared to pre-infusion (gray, 4.79 ± 0.73 a.u.; n = 8; one-way repeated measures ANOVA, F(2,14) = 5.59, p = 0.016; Student-Newman-Keuls post-hoc test, q(7) = 3.94, p = 0.015) or vehicle (open bar, 5.01 ± 0.91 a.u.; Student-Newman-Keuls post-hoc test, q(7) = 4.24, p = 0.025). Averaged vidgets are presented at top of panel. f) Cumulative distribution of averaged vidget per mouse pre-infusion.
(gray), post-muscimol (purple) or vehicle (open circles, n = 8). g) Cumulative distributions of each vidget per stimulus onset recorded from 8 animals prior to infusion (gray circles), during muscimol inactivation of V1 (purple circles) and vehicle treatment (open circles). Onset-by-onset analysis also reveals significant impact of treatment (n = 40; Kruskal-Wallis 1-way ANOVA on ranks, $H_{(2)} = 8.48, p = 0.014$). Data are plotted on a log 10 scale for clarity. Dashed lines represent pre-stimulus baseline for behavioral panels. Throughout figure error bars are S.E.M.s and asterisks denote significance of $p < 0.05$. 

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Supplementary Figure 3

Optogenetic activation of inhibition within V1 suppresses the vidget.

a) Optical fibres were implanted below the cortical surface targeting V1 and VEP electrodes implanted in layer 4. Light could then be delivered to the recording site *in vivo* while the animal views a visual stimulus and optogenetic strategies used to alter activity locally. b) By selectively expressing channelrhodopsin2 (ChR2) in parvalbumin expressing (PV+) cells using Cre recombinase technology, blue light (473 nm) could be used to suppress activity in V1 through PV+ inhibition. c) Cumulative distribution of vidgets evoked by each stimulus onset during optogenetic inhibition of V1. ChR2-mediated activation of PV+ inhibitory cells locally within V1 during blue light (473 nm) application suppressed vidget responses (light blue, n = 50) compared to interleaved blocks during which light was not applied (black, n = 50). d) Wild-type littermate controls subjected to the same AAV5 viral treatment in V1 showed equivalent responses whether the laser was on (blue, n = 40) or off (gray, n = 40), demonstrating that blue light itself has no impact on visually-driven responses (Kruskal-Wallis 1-way ANOVA on ranks, $H_{(3)} = 9.76, p = 0.021$). Data are plotted on a log 10 scale for clarity. Dashed lines represent pre-stimulus baseline throughout. This figure re-presents by stimulus onset data shown in Figure 2.
Supplementary Figure 4

Orientation selective habituation (OSH) occurs reliably across animals and stimulus onsets.

a) The cumulative distributions of average vidget magnitudes recorded in 19 animals on day-1 (gray circles), day-2 (gray squares) and day-8 (gray diamonds), reveals the significant habituation over 24 hours and further saturation after 8 days. b) The cumulative distributions of individual vidget magnitudes recorded in 19 animals for each stimulus onset on day-1 (gray circles, n = 95), day-2 (gray squares, n = 95) and day-8 (gray diamonds, n = 95), reveals the significant habituation over 24 hours and further saturation after 8 days (n = 95 onsets; Kruskal-Wallis 1-way ANOVA on ranks, $H_{(7)} = 29.31$, $p < 0.001$). c) The cumulative distributions of average vidget magnitudes recorded in 19
animals on day-1 (gray circles) and day-9 for familiar (blue circles) and novel (red circles) stimuli, revealing the orientation-selectivity of habituation. d) The cumulative distributions of individual vidget magnitudes recorded in 19 animals for each stimulus onset on day-1 (gray circles, n = 95) and day-9 for familiar (blue circles) and novel (red circles) stimuli, revealing the orientation-selectivity of habituation (n = 95; Mann Whitney rank sum test, $U_{(188)} = 3431.00$, $p = 0.004$). Data are plotted on a log 10 scale for clarity in panels c and d. Dashed lines represent pre-stimulus baseline throughout. This figure re-presents the same dataset as presented in Figure 4.
Supplementary Figure 5

Animals exhibit OSH under head fixation after previously experiencing stimulus only under free exploration.

a) Cumulative distributions of average vidget in 12 animals during head-fixed familiarity tests after freely moving stimulus exploration. The separation of the distributions of behavioral responses evoked by familiar (blue) and novel stimuli (red) is reflective of OSH. b) Cumulative distributions of all vidgets in response to each stimulus onset recorded from 12 animals during head-fixed familiarity tests after freely moving stimulus exploration. The separation of the distributions of behavioral responses evoked by familiar (blue, n = 60) and novel stimuli (red, n = 60) is significantly reflective of OSH (Mann Whitney rank sum test, U_{118} = 3173.00, p = 0.017). Data are plotted on a log 10 scale for clarity. Dashed lines represent pre-stimulus baseline throughout. This figure re-presents the same dataset as presented in Figure 5.
Supplementary Figure 6

OSH is eye specific (per stimulus onset).

The cumulative distribution of the amplitude of individual vidgets recorded from 14 animals through each eye for every onset of the stimulus familiar to that eye (blue, n = 140), novel only to that eye (orange, n = 140) or the 'true novel' stimulus (red, n = 140), revealing the significant eye-specificity of the effect (Kruskal-Wallis 1-way ANOVA on ranks, $H_{(2)} = 11.38$, $p = 0.003$). Data are plotted on a log 10 scale for clarity. Dashed lines represent pre-stimulus baseline. This figure re-presents the same dataset as presented in figure 6.
Supplementary Figure 7

Local knockdown of NMDAR in V1 prevents OSH.

a) Cumulative distributions of each vidget response evoked by familiar (blue outlines) and novel stimulus onsets (red outlines) in 11 GRIN1
fl/fl mice expressing GFP (as a result of AAV8 infection) in V1 are separated, demonstrating normal OSH. b) Cumulative distributions of each vidget response evoked by familiar (blue outlines) and novel stimulus onsets (red outlines) in 11 GRIN1
fl/fl mice lacking GRIN1 in V1 as a result of local Cre recombinase expression (as a result of AAV8 infection) reveal deficient OSH (n = 55; Kruskal-Wallis one-way ANOVA on ranks, H[3] = 16.97, p < 0.001). Data are plotted on a log 10 scale for clarity. Dashed lines represent pre-stimulus baseline. This figure represents the same dataset as presented in figure 7.
Supplementary Figure 8

Local blockade of NMDAR in V1 prevents OSH.

a) VEPs recorded during and after AP5 or vehicle infusions from all hemispheres (n = 36) reveal that AP5 (closed gray bar, 67.93 ± 10.81 µV) did not impact activity in V1 to any greater degree than vehicle (open gray bar, 59.82 ± 7.06 µV; n = 36 hemispheres; Wilcoxon signed rank test, Z-stat(35) = 0.53, p = 0.599). b) VEPs driven by the same ‘familiar’ stimulus on day-3 as previously viewed on day-1 (blue bars) were significantly greater in magnitude relative to the novel stimulus (red bars) only after vehicle treatment and not after AP5 treatment, indicating a selective blockade of SRP by AP5 (n = 36; 2-way repeated measures ANOVA, interaction of treatment x stimulus, F(1,35) = 21.08, p < 0.001). VEPs driven by the stimulus previously viewed during AP5 application (57.71 ± 8.53 µV) did not differ significantly from those evoked by a novel stimulus (57.43 ± 7.31 µV; Student-Newman-Keuls post-hoc test, q(35) = 0.09, p = 0.950). In contrast, VEPs evoked by the familiar stimulus were significantly greater in magnitude (76.17 ± 10.02 µV) than those evoked by the novel (58.95 ± 7.61 µV; Student-Newman-Keuls post-hoc test, q(35) = 6.98, p < 0.001) under vehicle treatment. Familiar stimuli experienced previously under vehicle also evoked VEPs of significantly greater magnitude than those experienced under AP5 (Student-Newman-Keuls post-hoc test, q(35) = 3.37, p = 0.022). Average VEPs (n = 36) are shown at top of panel. Scale bar is 50 µV vertically and 100 ms horizontally. Error bars are S.E.Ms. Asterisk denotes significance of p < 0.05. Non-significant comparisons are denoted with n.s. Dashed line represents noise. Dataset is acquired from the same animals as the behavioral data presented in figure 8. c) The cumulative distributions of all vidgets recorded from 18 animals during familiarity tests for each familiar (blue, n = 90) or novel stimulus onset (red, n = 90) after vehicle treatment during learning show separation reflective of learning (n = 90 onsets; 2 Way ANOVA, F(1,356) = 3.00, p = 0.002; Student-Newman-Keuls post-hoc test, q(178) =
4.82, p < 0.001). d) In contrast, vidgets evoked by the previously viewed ‘familiar’ (blue, n = 90) or novel stimulus onsets (red, n = 90) after AP5 treatment during learning produce overlapping distributions (Student-Newman-Keuls post-hoc test, q(178) = 1.36, p = 0.336), indicating blockade of learning. Data are plotted on a log 10 scale for clarity. Dashed lines represent pre-stimulus baseline throughout. These figures re-present the same dataset as presented in Figure 8.
Supplementary Figure 9

Local application of ZIP in V1 'erases' OSH.

a) SRP is significantly erased by ZIP in V1 (n = 36 hemispheres; 2-way repeated measures ANOVA, treatment x stimulus: F(3,210) = 2.90, p = 0.036). Vehicle infusions into V1 did not impact SRP (open bars) as potentiation of VEP magnitude through experience (blue outline; 146.61 ± 18.19 µV) was retained after infusion of vehicle (black outline; 140.08 ± 18.39 µV; Student-Newman-Keuls post-hoc test, q(35) = 0.37, p < 0.001), and remained significantly greater than VEP magnitude to novel (red outline; 84.28 ± 8.80 µV; Student-Newman-Keuls post-hoc test, q(35) = 7.01, p < 0.001). By contrast, ZIP had an erasing effect (black bars). VEPs that had been potentiated through visual experience (blue outline; 144.94 ± 17.66 µV) were significantly reduced in magnitude as a result of ZIP treatment (black outline; 98.00 ± 15.32 µV; Student-Newman-Keuls post-hoc test, q(35) = 6.69, p < 0.001), such that they were no longer significantly different in magnitude from those driven by the novel stimulus (red outlines; 78.94 ± 8.81 µV; Student-Newman-Keuls post-hoc test, q(35) = 2.60, p = 0.066).

Average VEPs (n = 36 per group) are shown at top of panel. Scale bar is 50 µV vertically and 100 ms horizontally. Error bars are S.E.Ms. Asterisk denotes significance of p < 0.05. Non-significant comparisons are denoted with n.s. Dashed line represents noise.

b) The cumulative distributions of all vidgets recorded from 18 animals during familiarity tests for each familiar (blue outlines, n = 90) or novel stimulus onset (red outlines, n = 90) reveals a suppressive familiarity effect in the vehicle group only (white fill, n = 90). When analyzed per stimulus onset, failure to discriminate familiar and novel stimuli was observed only after application of ZIP (3-way ANOVA, interaction of treatment x session x stimulus: F(1,712) = 5.00, p = 0.025). Vidgets evoked a day after ZIP treatment are presented alone for clarity. Data are plotted on a log 10 scale for clarity. Dashed lines represent pre-stimulus baseline throughout.

c) Distributions are overlapping for familiar and novel stimuli if previously presented in the presence of ZIP. When analyzed per stimulus onset, failure to discriminate familiar and novel stimuli was observed only after application of ZIP (3-way ANOVA, interaction of treatment x session x stimulus: F(1,712) = 5.00, p = 0.025). Vidgets evoked a day after ZIP treatment are presented alone for clarity. Data are plotted on a log 10 scale for clarity. Dashed lines represent pre-stimulus baseline throughout. These figures re-present the same dataset as presented in figure 8.
Supplementary Figure 10

Evaluation of viral infections in V1.

a) Hoechst stain revealing increased thickness of layer 4 throughout V1. Arrows indicate identified margins of V1 using this established method (see reference 44, 45 and 46) b) Imaging of fluorescent protein (FP) signal to reveal spread of example infection in same section as (a). c) A complementary approach to identification of the margins of V1 (see reference 47) using a myelin stain in the same section. d) Blue Hoechst, Green FP and Red myelin labels are merged in the same section for clarity. Scale bar represents 500 µm. Using this approach we determined the spread of viral infections to be sure that they remained within the confines of V1. e) Three example infections of PV+ cells with ChR2-eYFP (green) in V1 of three separate animals are shown. The Hoechst stain (blue) is shown for structural reference. Note that these are three different animals from that already shown in figure 2. f)
Three example infections of Cre-GFP (green) in V1 of three separate animals are shown. The Hoechst stain (blue) is shown for structural reference. Note that these are three different animals from that already shown in figure 7. Also note that our viral infections were delivered using stereotaxic coordinates (3.1 mm lateral to lambda and, respectively, 3 (in e) or 4 infection depths (in f) of 600, 450, 300 and 150 µm – see methods for further clarification) that targeted the binocular region of V1, which is the lateral portion of V1.