# Selective activation of parvalbumin interneurons prevents stress-induced synapse loss and perceptual defects

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## Supplementary Information

### Stress does not affect whisker length, baseline behavior and short-term memory in the whisker-dependent texture discrimination task

As intact whiskers are important for the mouse to perform the texture discrimination task (Supplementary Figure 1a), we asked if the failure of RS mice in the task was due to alterations of whisker length. We measured the length of the longest whiskers of both control and RS mice, and found no difference in their lengths (Supplementary Figure 2b).

Earlier studies have shown that stress increases anxiety in mice[1]. We thus asked if the defective texture discrimination was due to altered exploratory behavior. We measured the total traveled distance (Supplementary Figure 2c) as well as the time spent in the center vs. the periphery of the arena (Supplementary Figure 2e) during the free exploration period. We observed no significant difference between control and 7 day RS mice in either the total traveled distance or the percentage of time spent in the center (Supplementary Figure 2d,f). To rule out the possibility that the stressed mouse does not explore the textured columns sufficiently during the encoding phase, or possesses intrinsic bias to one side of the arena, we measured the total number of approaches and the relative time spent on the left versus the right side of the arena. Again we found comparable results between control and RS mice (Supplementary Figure 2h,i).

In addition, when the textured columns were replaced by two objects of different shapes and colors (Supplementary Figure 3a), or when the textural difference was amplified (50 vs. 1500 grits, Supplementary Figure 3c), the performance of 7d RS mice was not significantly different from that of control mice (Supplementary Figure 3b,d). This suggests that the defective performance of RS mice in the original task was not due to short-term memory loss, but rather the incapability to distinguish the more subtle textural differences.

**Stress reduces PV expression of PV+ INs**

A previous study reported that the expression level of PV in the hippocampus changes with altered experience[2]. Therefore we analyzed PV expression level under stress and EE conditions. We found that stress significantly decreased the PV intensity, while EE did not change it (Supplementary Figure 7d). Such data are consistent with the changes in PV activation as revealed by c-Fos immunohistochemistry (Figures 2j, 5b). Thus stress affects the expression level of PV in addition to the activation of PV+ INs.

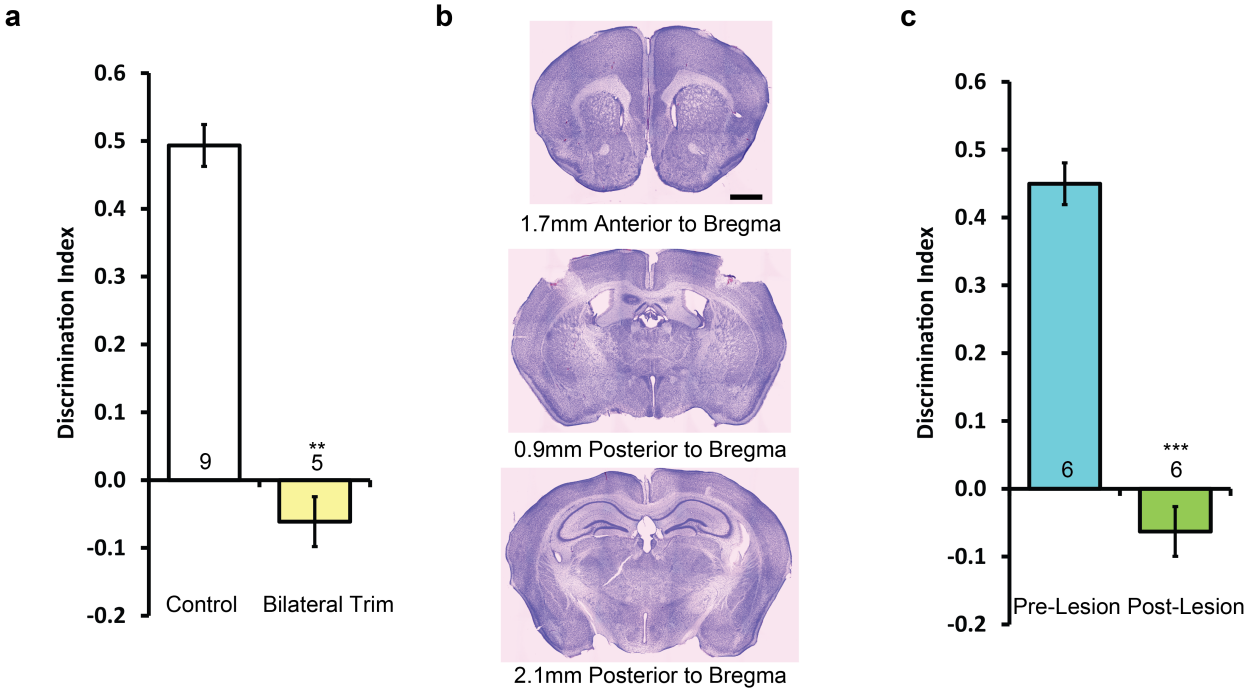
**Supplementary Methods**

**Whisker trimming and barrel cortex lesion:** Whisker trimming was performed by cutting the mystacial vibrissae on both sides to the skin level, as previously described[3, 4]. Bilateral barrel cortex lesion was performed as previously described[5]. The extent of lesion was confirmed for each mouse by *post hoc* histology with Nissl staining (Supplementary Figure 1b).

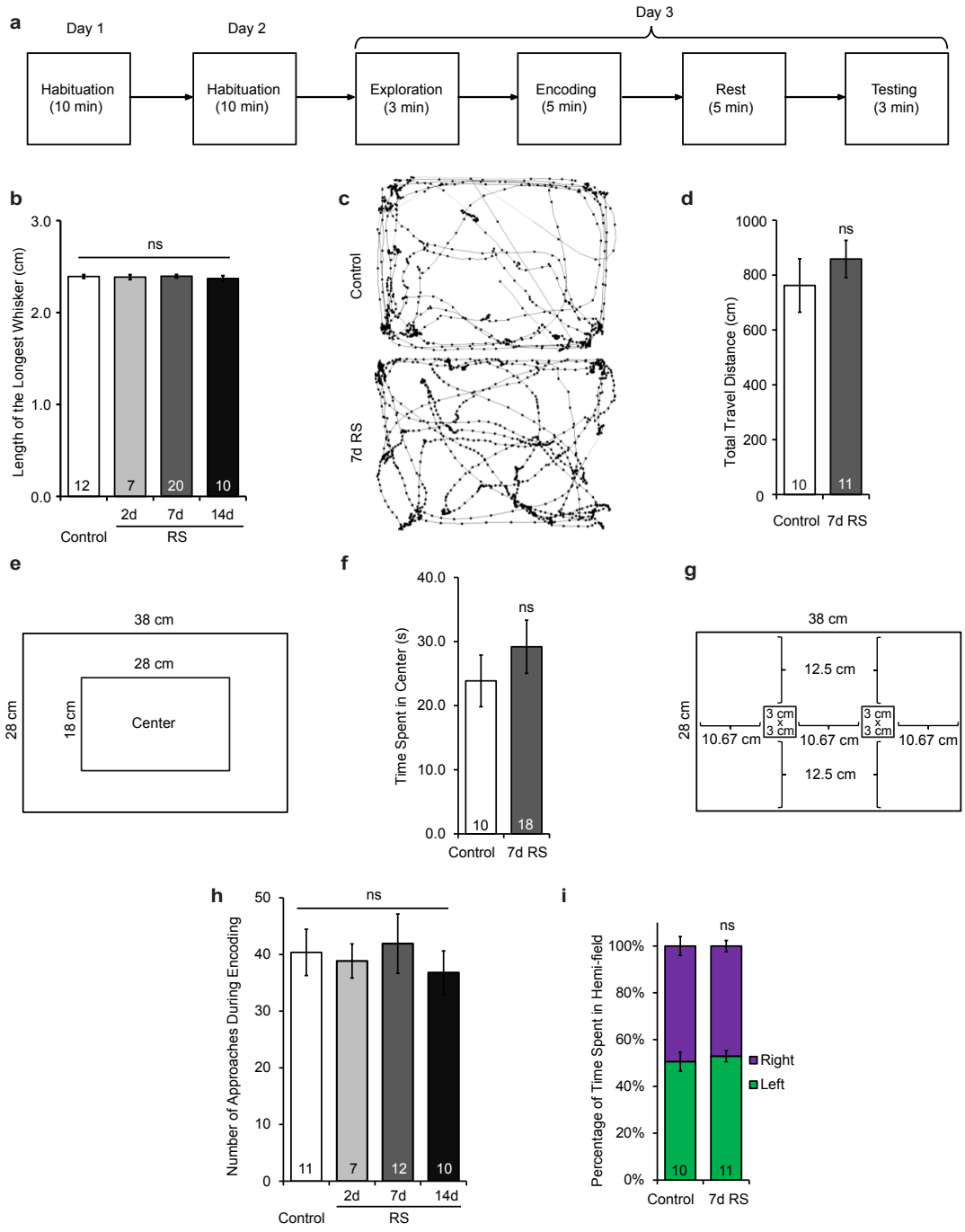
**Nissl staining:** Nissl staining was performed as previously described[6].

**Systemic drug administration:** MK801 (0.25 mg/kg body weight)[3] or diazepam (2.5 mg/kg body weight)[7] was injected intraperitoneally approximately 30 min before RS. Because the half-life of diazepam in mouse (< 90 min)[8] is shorter than the duration of the RS session (2 h), the mouse was given a booster shot (1.25 mg/kg body weight) at 1 h into the RS session through the perforated hole in the restraint tube without being removed from the tube.

## Supplementary Figures

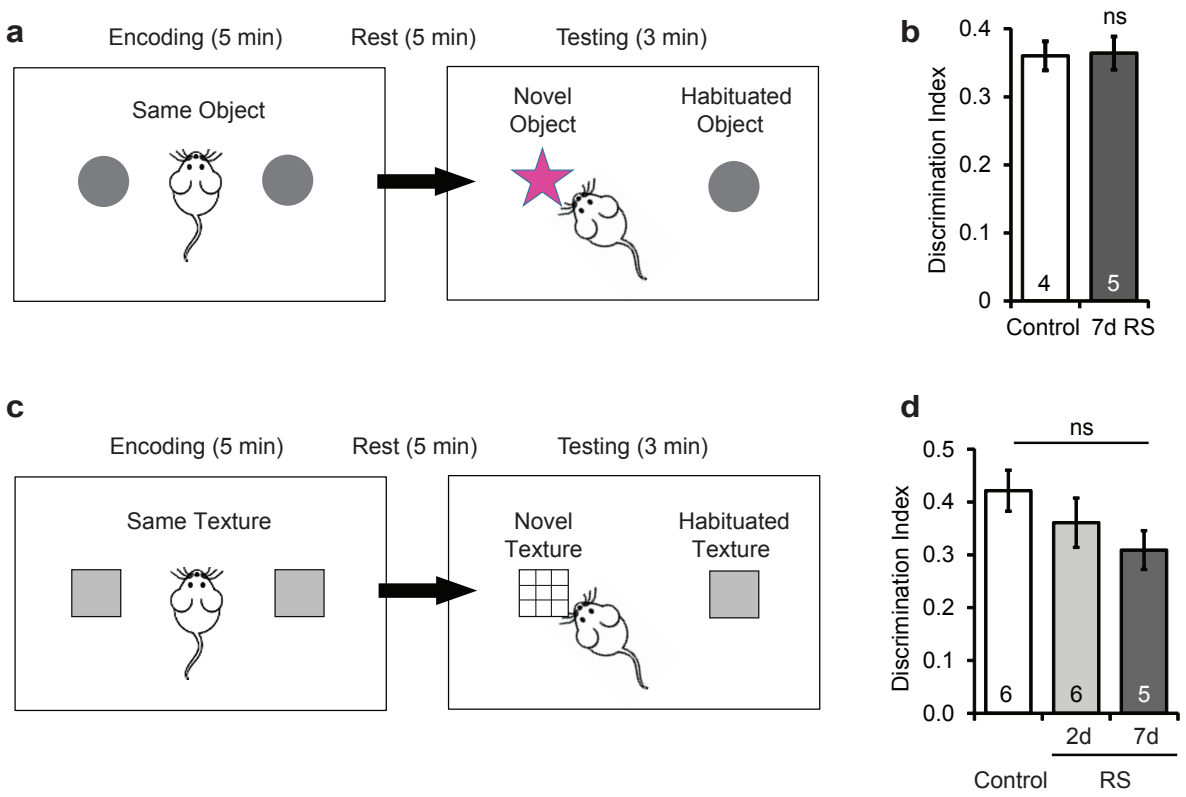



**Supplementary Figure 1: The whisker-dependent texture discrimination task requires both intact whiskers and intact barrel cortex. a,** Mice subjected to bilateral whisker trimming failed to discriminate between novel and habituated textures. **b,** Nissl staining confirmed restricted lesion in the mouse barrel cortex. Scale bar: 1 mm. **c,** Mice could not discriminate between the two textures after bilateral barrel cortex lesion. Hereinafter unless otherwise indicated, comparisons are made to the control group, data are presented as mean ± S.E.M., and asterisks represent the following *P*-values: *$p<0.05$, **$p<0.01$, ***$p<0.001$.

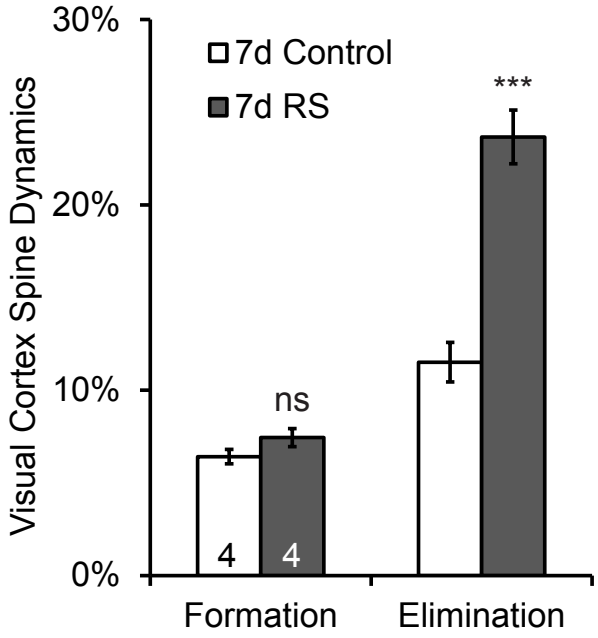



**Supplementary Figure 2: Stress does not affect whisker length or exploration/encoding behavior in the texture discrimination task. a,** Experiment setup. **b,** 2 to 14 days of RS did not alter whisker length. **c,** Representative movement maps of an unstressed control (upper panel) and a stressed mouse (lower panel) during the 3 min exploration phase on the testing day. **d,** Control and RS mice traveled over comparable distances during the exploration phase. **e,**

Demarcation of the center vs. periphery in the arena for analyzing the exploratory behavior. **f**, Control and RS mice spent similar amount of time in the center during the exploration phase. **g,** Layout of the arena with textured columns during encoding and testing phases. **h,** Control mice and mice subjected to RS for 2 to 14 days made comparable numbers of approaches during the encoding phase. **i,** Control and RS mice spent similar amount of time in both hemi-fields of the arena during the encoding phase.

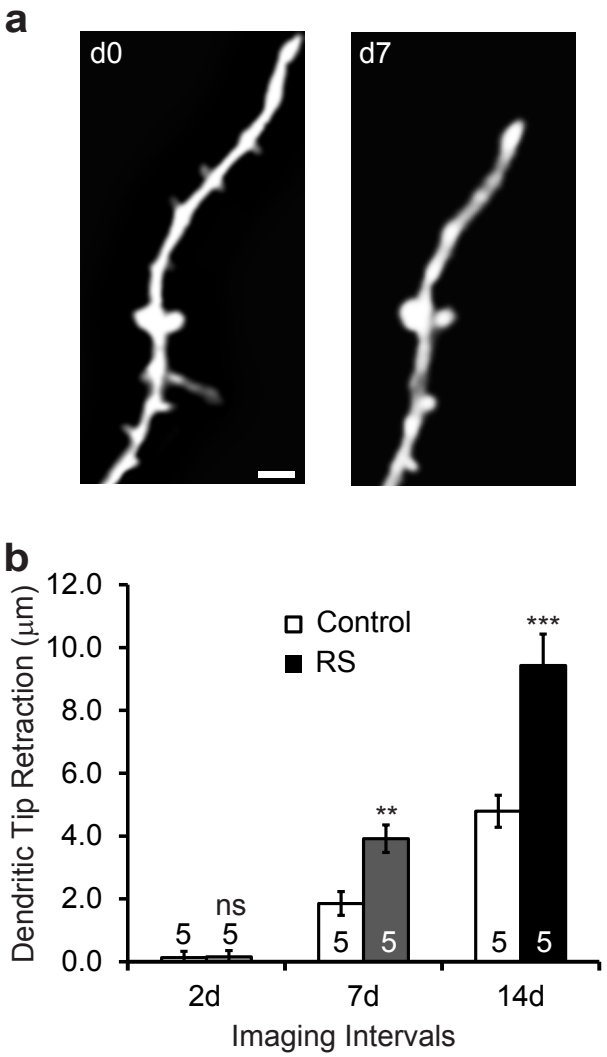



**Supplementary Figure 3: 7-day RS does not impair short-term memory. a,b,** 7-day RS mice discriminated between the novel and the habituated objects with different shapes and colors as effectively as control mice. **c,d,** 2-day and 7-day RS mice effectively discriminated between textures with amplified textural differences (50 vs. 1500 grits).

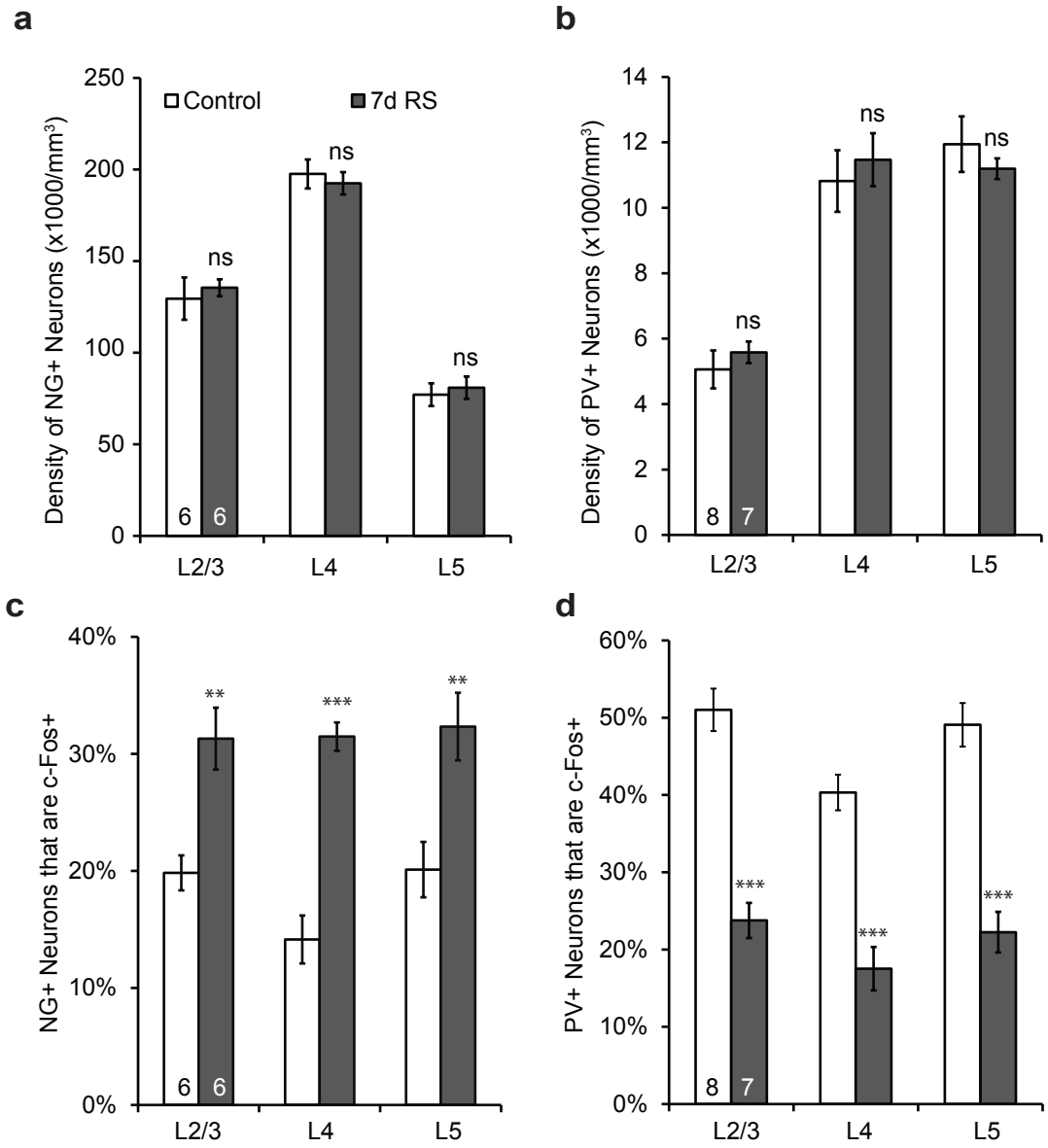



74 **Supplementary Figure 4: Spine elimination in the visual cortex is increased in 7-day RS mice**

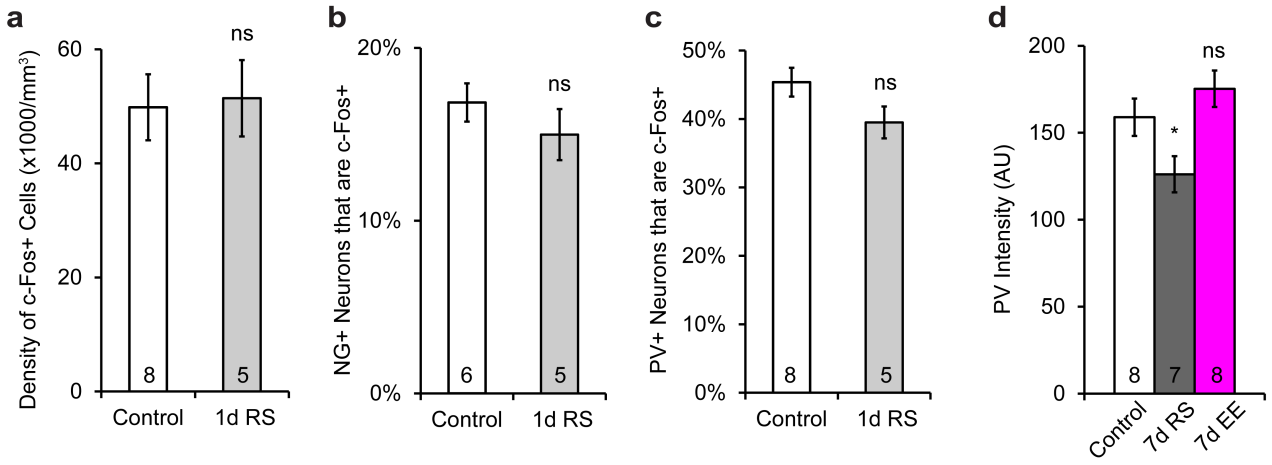
75 **compared to controls, while spine formation is unperturbed.**



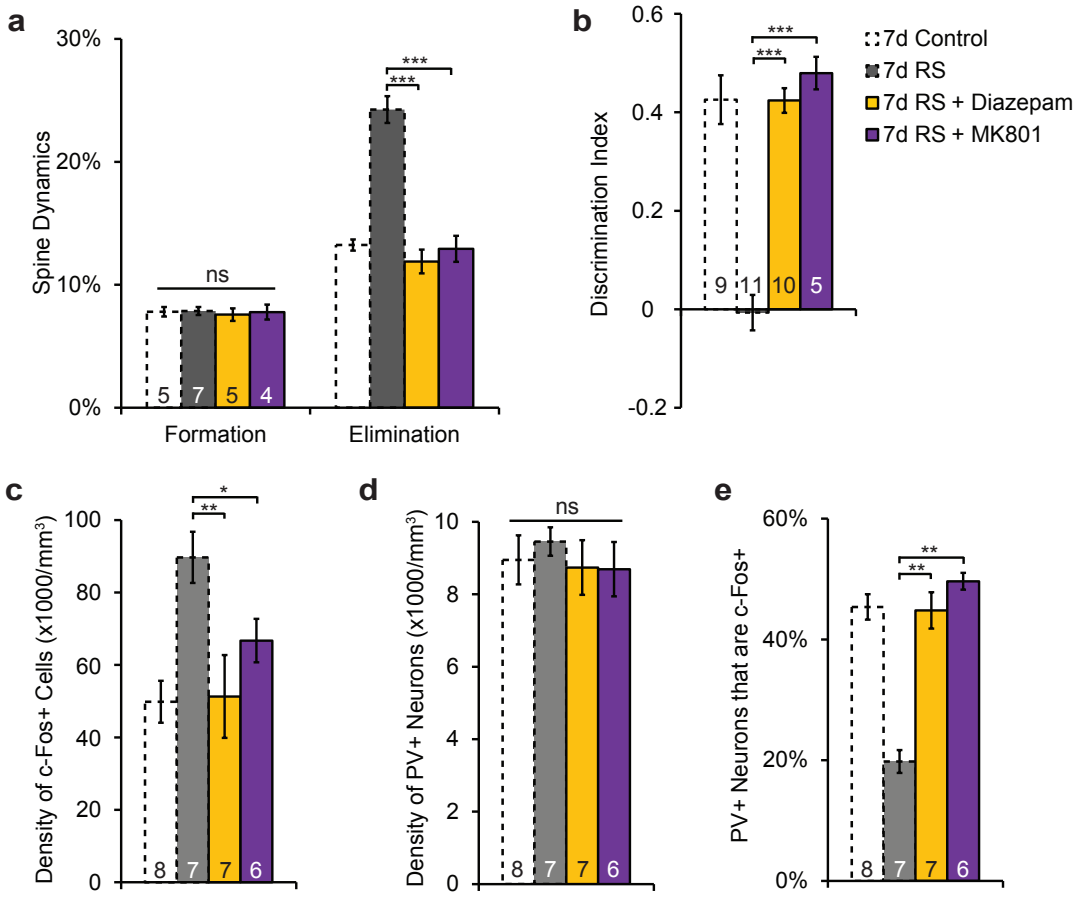

**Supplementary Figure 5: Stressed mice show progressive retraction of dendritic tips. a,** An example of dendritic tip retraction in 7-day RS mice. Scale bar: 2 μm. **b,** Quantification of tip retraction over 2, 7, and 14 days in control and RS mice. *P*-values represent the comparison to control mice over the same time interval.



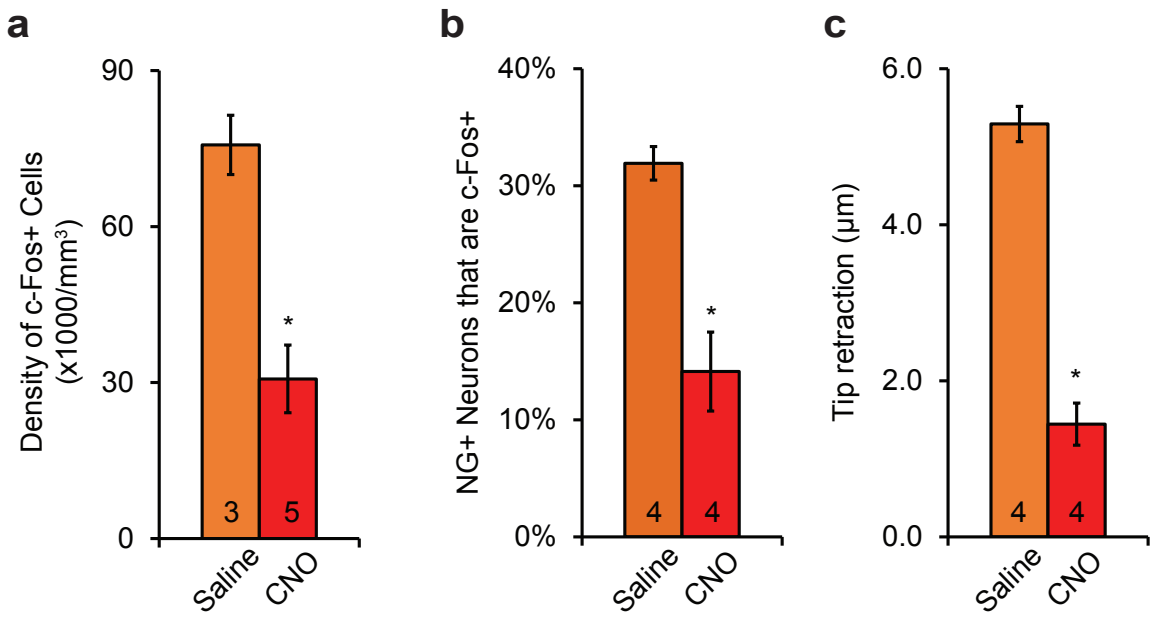

**Supplementary Figure 6: Stress perturbs the excitation-inhibition balance across cortical layers. a, b,** Stress did not affect the density of NG+ neurons (**a**) or PV+ INs (**b**) in L2/3, 4, and 5 of the mouse barrel cortex. **c,d,** After 7-day RS, the percentage of NG+ neurons that were c-Fos+ (**c**) was significantly increased, while the percentage of PV+ INs that were c-Fos+ (**d**) was significantly reduced, in L2/3, 4, and 5 of the mouse barrel cortex.



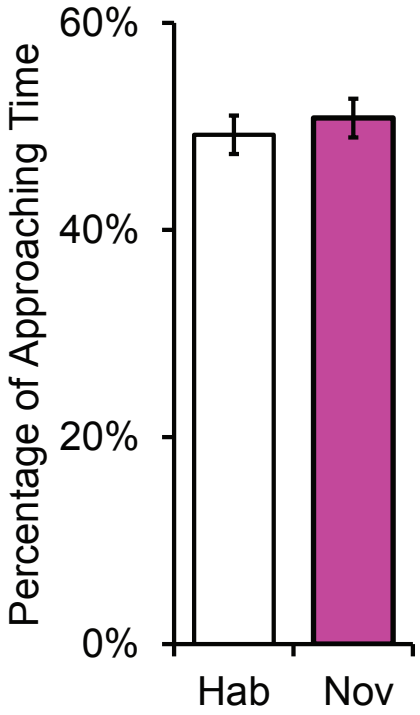

**Supplementary Figure 7: A single RS session does not affect global c-Fos activation (a), or activity of NG+ neurons (b) and PV+ INs (c) in the mouse barrel cortex. PV intensity in PV+ INs is decreased by RS, but not affected by EE (d).**




**Supplementary Figure 8: MK801 and diazepam treatment alleviates stress-induced spine loss and texture discrimination defects. a,b,** Systemic administration of MK801 or diazepam before RS prevented both stress-induced acceleration of spine elimination (**a**) and texture discrimination defects (**b**). **c-e,** MK801 and diazepam treatment both alleviated stress-induced increase in global neuronal activity (**c**) and decrease in PV+ IN activity (**e**), but neither affected PV+ IN density (**d**).

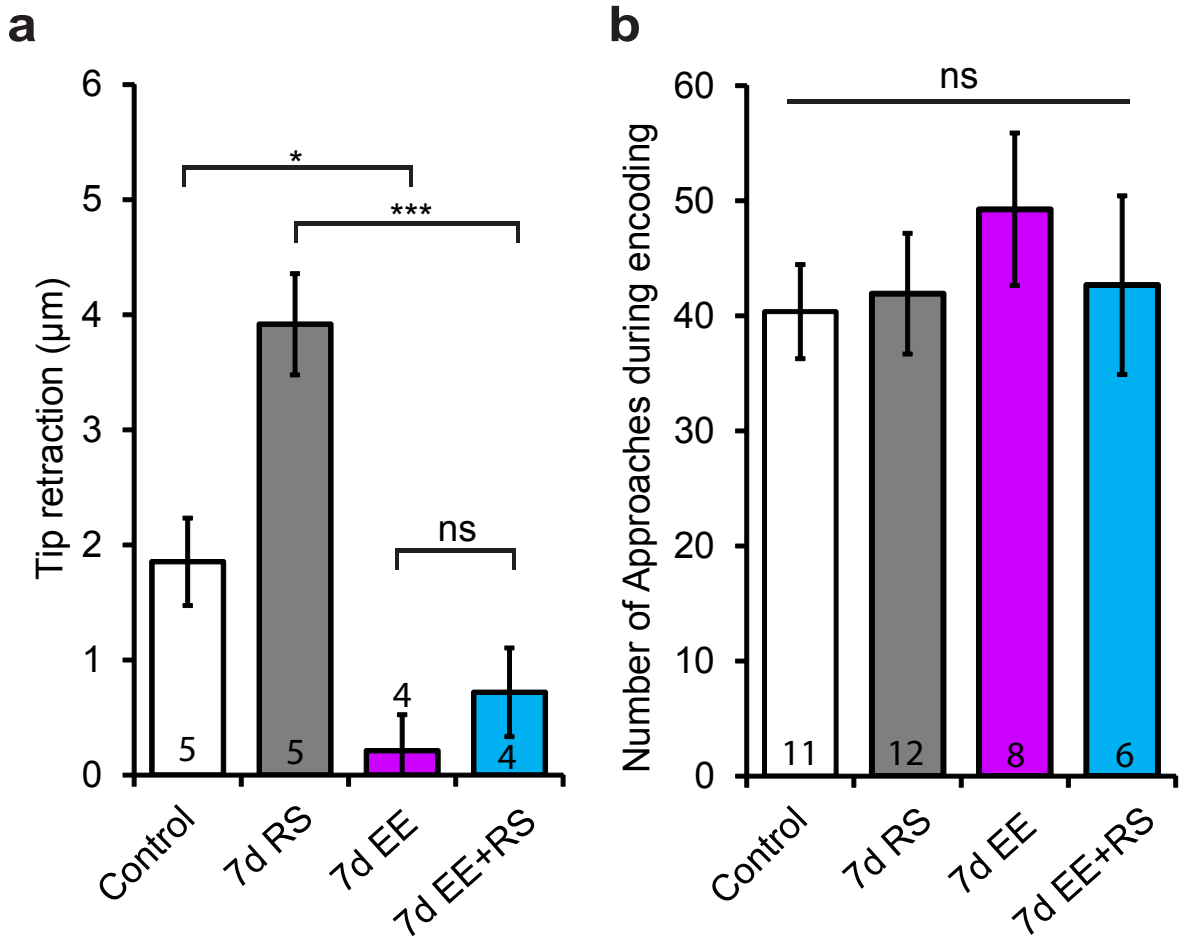



**Supplementary Figure 9: Activation of PV+ INs during stress prevents global neuronal activation and dendritic tip retraction.** Among DREADD virus-infected RS mice, those treated with CNO had significantly lower density of c-Fos+ cells (**a**), fewer NG+ neurons that were activated (**b**), and less dendritic tip retraction (**c**), compared to those treated with saline.



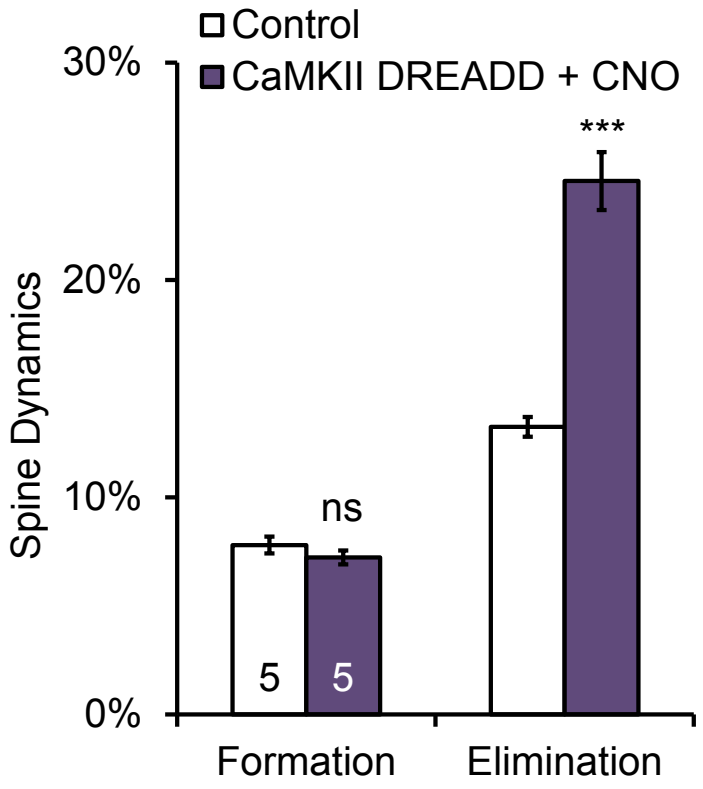

**Supplementary Figure 10: 7-day RS mice with PV+ INs activated outside barrel cortex fail to discriminate between novel and habituated textures.**

# Supplementary Figure 11

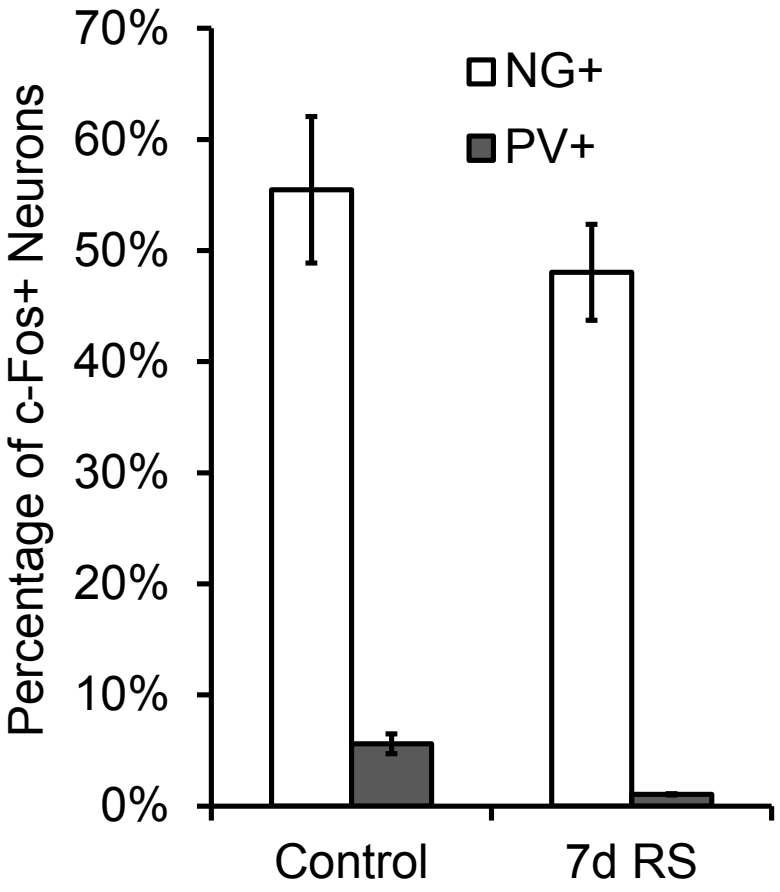



100 **Supplementary Figure 11: Dendritic tip retraction (a) and behavior during the encoding**

101 **phase (b) under EE conditions**.




**Supplementary Figure 12: DREADD activation of excitatory neurons in the barrel cortex increases spine elimination, but does not affect spine formation.**




104 **Supplementary Figure 13: Composition of c-Fos+ neurons under control and 7-day RS**

105 **conditions.**

**Supplementary Table 1.** Performance in the whisker-dependent texture discrimination task during the testing phase under various experimental conditions. RS: Restraint Stress; UMS: Unpredictable Mild Stress; EE: Environmental Enrichment. Data are presented as mean ± s.e.m.

| Experimental conditions | Percentage of time exploring novel texture | # Mice (male, female) |
|---|---|---|
| Control | 71.3 ± 2.5 | 9 (5,4) |
| 2d RS | 60.6 ± 2.0 | 7 (4,3) |
| 7d RS | 49.1 ± 1.8 | 11 (5,6) |
| 14d RS | 49.3 ± 1.1 | 6 (2,4) |
| 14 UMS | 52.1 ± 2.7 | 7 (3,4) |
| 7d RS + MK801 | 73.4 ± 2.0 | 5 (3,2) |
| 7d RS + DZ | 71.2 ± 1.3 | 10 (5,5) |
| 7d EE | 74.7 ± 4.2 | 6 (3,3) |
| 7d EE + RS | 72.5 ± 3.4 | 8 (4,4) |
| DREADD virus + Saline, 7d RS | 50.6 ± 1.0 | 6 (3,3) |
| DREADD virus + CNO, 7d RS | 75.8 ± 2.4 | 7 (4,3) |
| DIO-mCherry virus (off target) + CNO, 7d RS | 50.8 ± 1.9 | 5 (4,1) |
| DIO-mCherry virus + CNO, 7d RS | 48.9 ± 3.1 | 4 (3,1) |

**Supplementary Table 2.** Percentages of spines eliminated and formed under various experimental conditions. RS: Restraint Stress; UMS: Unpredictable Mild Stress; EE: Environmental Enrichment. Data are presented as mean ± s.e.m.

| Imaging interval (Days) | Experimental conditions | Spine formation (%) | Spine elimination (%) | Total analyzed spine number on day 1 | # Mice (Male, Female) |
|---|---|---|---|---|---|
| 2 | Control | 5.7 ± 0.2 | 6.0 ± 0.1 | 835 | 5 (3,2) |
| | RS day 0-2 | 5.7 ± 0.6 | 14.5 ± 0.6 | 773 | 5 (2,3) |
| | 2 days Recovery following 7d RS | 5.8 ± 0.4 | 9.1 ± 0.8 | 812 | 5 (2,3) |
| | RS day 7-9 | 6.4 ± 0.3 | 18.4 ± 0.6 | 594 | 4 (2,2) |
| 7 | Control | 7.8 ± 0.4 | 13.2 ± 0.5 | 778 | 5 (2,3) |
| | RS | 7.9 ± 0.3 | 24.3 ± 1.1 | 1207 | 7 (5,2) |
| | MK801 + RS | 7.8 ± 0.6 | 12.9 ± 1.1 | 604 | 4 (3,1) |
| | DZ + RS | 7.6 ± 0.5 | 11.9 ± 1.0 | 737 | 5 (2,3) |
| | DREADD virus + Saline, RS | 7.2 ± 1.0 | 25.2 ± 1.2 | 557 | 4 (1,3) |
| | DREADD virus + CNO, RS | 7.2 ± 0.2 | 12.0 ± 0.8 | 599 | 4 (0,4) |
| | DIO-mCherry virus + CNO, RS | 7.1 ± 0.4 | 25.3 ± 0.3 | 450 | 3 (2,1) |
| | CaMKII DREADD + CNO | 7.2 ± 0.7 | 24.6 ± 3.0 | 814 | 5 (3,2) |
| | EE | 16.5 ± 2.0 | 17.6 ± 0.9 | 587 | 4 (2,2) |
| | EE +RS | 16.8 ± 1.7 | 17.8 ± 0.6 | 622 | 4 (2,2) |
| | Visual Cortex Control | 6.5 ± 0.4 | 11.5 ± 1.1 | 609 | 4 (2,2) |
| | Visual Cortex RS | 7.4 ± 1.0 | 23.7 ± 2.9 | 603 | 4 (2,2) |
| 14 | Control | 7.1 ± 1.0 | 16.8 ± 1.1 | 1515 | 6 (4,2) |
| | RS | 7.2 ± 0.6 | 32.9 ± 0.8 | 664 | 4 (1,3) |
| | UMS | 7.7 ± 0.3 | 24.5 ± 1.3 | 777 | 5 (3,2) |

**Supplementary Table 3.** Dendritic tip retraction under various experimental conditions. RS: Restraint Stress; UMS: Unpredictable Mild Stress; EE: Environmental Enrichment. Data are presented as mean ± s.e.m.

| Imaging interval (Days) | Experimental conditions | Dendritic retraction length (μm) | # Dendritic tips | # Mice (Male, Female) |
|---|---|---|---|---|
| 2 | Control | 0.13 ± 0.20 | 56 | 5 (3,2) |
| | RS day 0-2 | 0.15 ± 0.20 | 56 | 5 (2,3) |
| | RS day 7-9 | 2.21 ± 0.37 | 45 | 4 (2,2) |
| | Recovery following 7d RS | 1.61 ± 0.44 | 70 | 5 (2,3) |
| 7 | Control | 1.85 ± 0.38 | 61 | 5 (2,3) |
| | RS | 3.92 ± 0.44 | 66 | 7 (5,2) |
| | DREADD virus + Saline, RS | 5.29 ± 0.23 | 34 | 4 (1,3) |
| | DREADD virus + CNO, RS | 1.44 ± 0.27 | 52 | 4 (0,4) |
| | EE | 0.21 ± 0.28 | 47 | 4 (2,2) |
| | EE + RS | 0.72 ± 0.34 | 48 | 4 (2,2) |
| 14 | Control | 4.79 ± 1.13 | 26 | 6 (4,2) |
| | RS | 9.43 ± 1.99 | 53 | 4 (1,3) |

**Supplementary Table 4.** Number of mice and sex used for the immunohistochemistry experiments.

| Conditions | # Mice (Male, Female) |
|---|---|
| Control | 8 (5,3) |
| 1d RS | 5 (2,3) |
| 7d RS | 8 (5,3) |
| 7d RS + MK801 | 6 (3,3) |
| 7d RS + DZ | 7 (5,2) |
| 7d EE | 7 (4,3) |
| 7d EE + RS | 7 (3,4) |
| DREADD virus + Saline, 7d RS | 4 (2,2) |
| DREADD virus + CNO, 7d RS | 5 (3,2) |

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