

1 **EphB2 mediates social isolation induced memory forgetting**

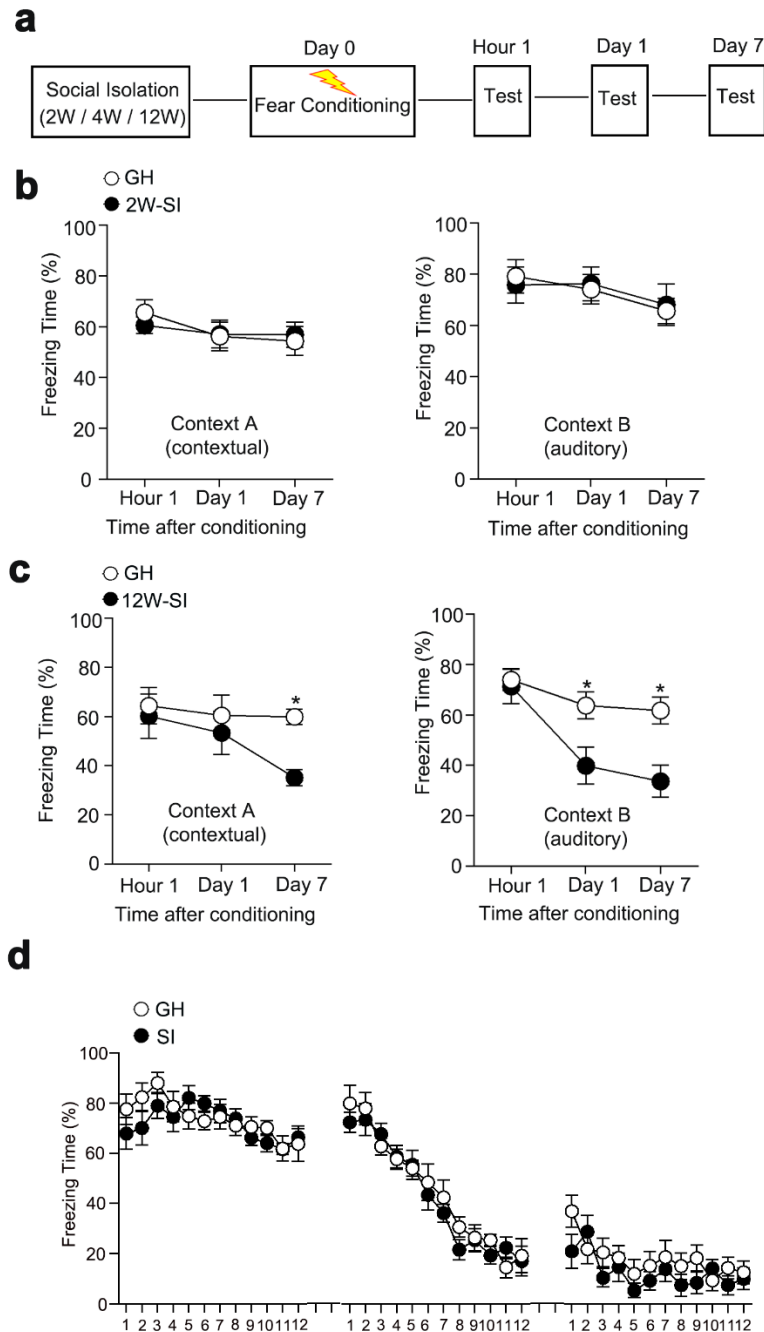
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5 **Supplementary Figures and Tabela**

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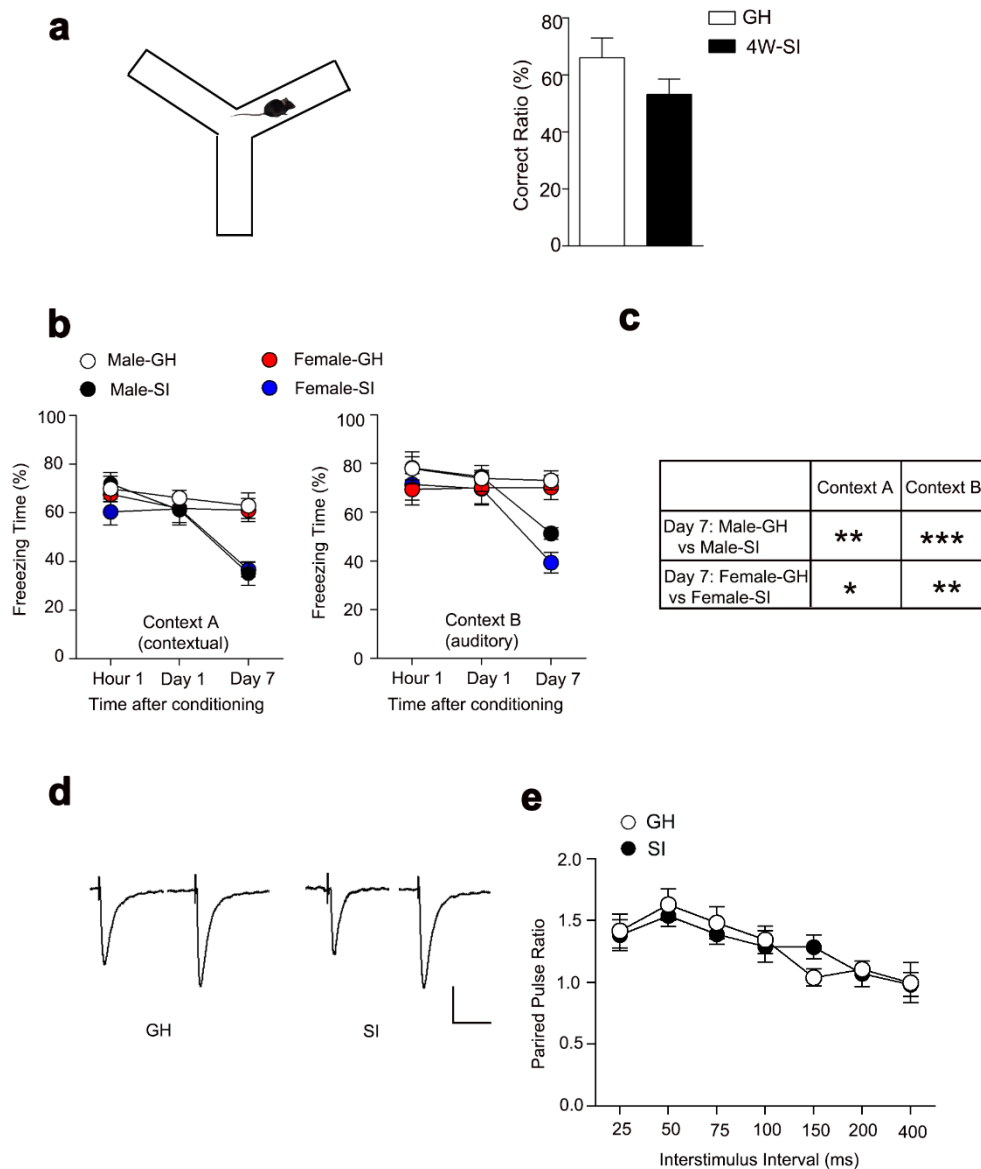
8 **Supplementary Fig. 1 SI induces the forgetting of long-term fear memory.**

9 **a** Experimental paradigm of memory process in fear conditioning. **b** No difference was

10 seen in short-term memory or long-term memory between GH mice and SI (2W) mice

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11 in fear conditioning. GH,  $n = 12$  mice; SI,  $n = 12$  mice, two-way *ANOVA*, context A,  
12 effect of time,  $F_{(2, 66)} = 1.277$ ,  $p > 0.05$ ; effect of group,  $F_{(1, 66)} = 0.01826$ ,  $p > 0.05$ ;  
13 group  $\times$  time,  $F_{(2, 66)} = 0.3063$ ,  $p > 0.05$ ; context B, effect of time,  $F_{(2, 66)} = 1.428$ ,  $p >$   
14  $0.05$ ; effect of group,  $F_{(1, 66)} = 0.01826$ ,  $p > 0.05$ ; group  $\times$  time,  $F_{(2, 66)} = 0.1231$ ,  $p >$   
15  $0.05$ , Tukey post hoc analysis was performed. **c** Long-term memory was impaired in SI  
16 (12W) mice compared with GH mice. GH,  $n = 11$  mice; SI,  $n = 7$  mice, two-way *ANOVA*,  
17 context A, effect of time,  $F_{(2, 49)} = 2.134$ ,  $p > 0.05$ ; effect of group,  $F_{(1, 49)} = 4.163$ ,  $p <$   
18  $0.05$ ; group  $\times$  time,  $F_{(2, 49)} = 1.184$ ,  $p > 0.05$ , Tukey post hoc analysis was performed;  
19 context B, effect of time,  $F_{(2, 49)} = 10.2$ ,  $p < 0.001$ ; effect of group,  $F_{(1, 49)} = 14.19$ ,  $p <$   
20  $0.001$ ; group  $\times$  time,  $F_{(2, 49)} = 2.668$ ,  $p > 0.05$ ; Tukey post hoc analysis was performed.  
21 **d** No difference was seen in fear extinction between GH and SI mice. GH = 7 mice, SI  
22 = 7 mice, two-way *ANOVA*, effect of time,  $F_{(35, 432)} = 59.46$ ,  $p < 0.0001$ ; effect of group,  
23  $F_{(1, 432)} = 6.980$ ,  $p < 0.01$ ; group  $\times$  time,  $F_{(35, 432)} = 0.7259$ ,  $p > 0.05$ , Tukey post hoc  
24 analysis was performed. All data are presented as mean  $\pm$  SEM.  $*p < 0.05$ .

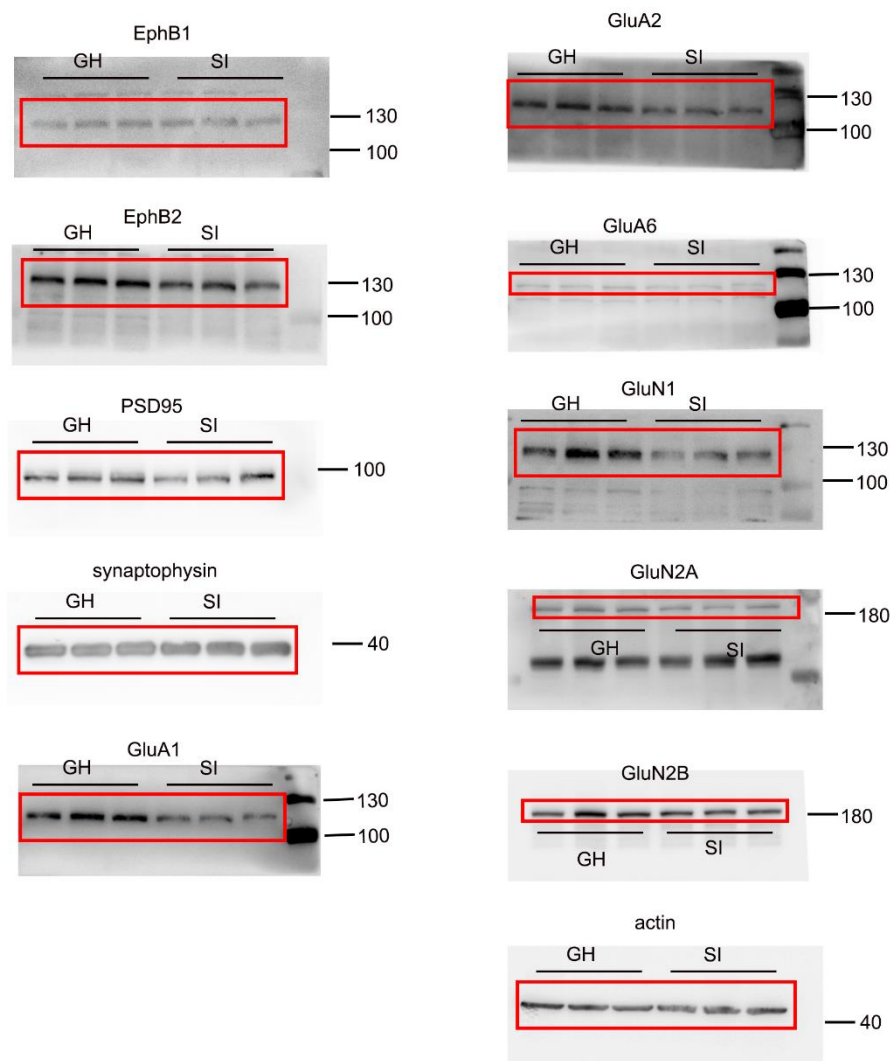


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26 **Supplementary Fig. 2 SI do not affect the fear extinction and presynaptic**27 **function. a** No difference was seen in short-term memory or spatial memory between28 group housed mice and 4-week isolated mice in Y-maze. GH,  $n = 9$  mice; SI,  $n = 7$ 29 mice, unpaired Student's  $t$  test,  $t_{(14)} = 1.406$ ,  $p > 0.05$ . **b** No difference was seen30 between males and females on social isolation induced fear memory forgetting. **c** The31 list showed the statistical results. **d** Representative PPR trace at 100 ms interval .32 Calibration: 100 pA, 25 ms. **e** Quantification of PPR from GH and SI mice. GH,  $n =$

33 16 neurons from 4 mice; SI,  $n = 15$  neurons from 4 mice; two-way ANOVA; effect of  
34 interval time,  $F_{(6, 203)} = 7.292$ ,  $p < 0.0001$ ; effect of group,  $F_{(1, 203)} = 0.0322$ ,  $p > 0.05$ ;  
35 group  $\times$  interval time,  $F_{(6, 203)} = 0.5651$ ,  $p > 0.05$ , Tukey post hoc analysis was  
36 performed. All data are presented as mean  $\pm$ SEM. \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p <$   
37 0.01.

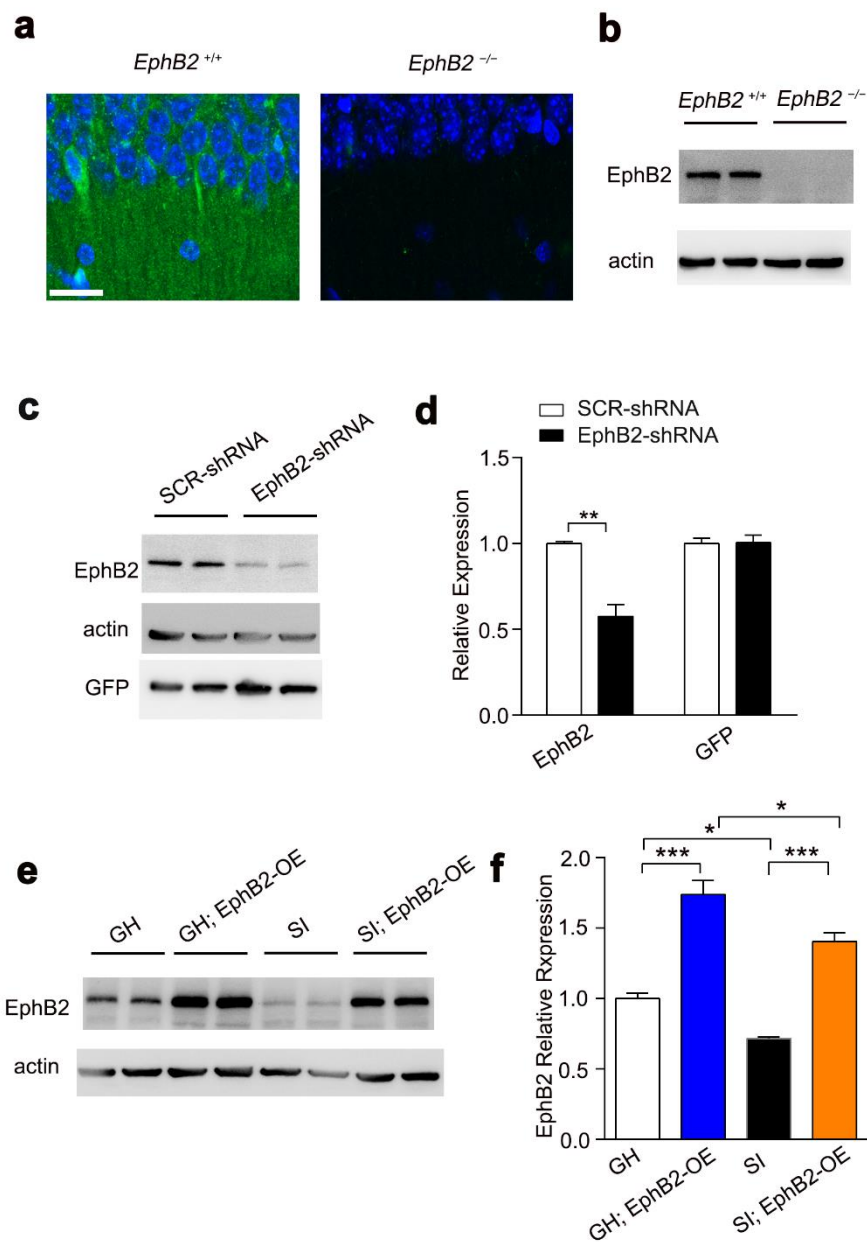
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40 **Supplementary Fig. 3 Whole western blotting related to Fig. 2a.**

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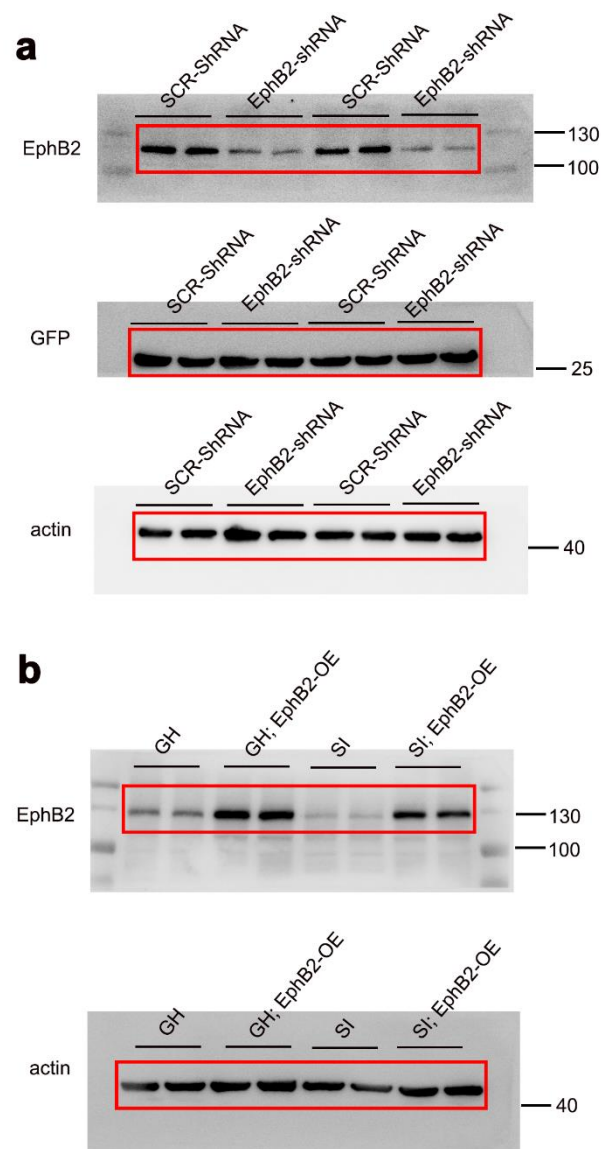
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43 **Supplementary Fig. 4 Verification of EphB2 antibody and virus. a** Representative  
 44 confocal image of EphB2 staining in *EphB2*<sup>+/+</sup> and *EphB2*<sup>-/-</sup> mice. Scale bar: 25  $\mu$ m.  
 45 **b** Western blotting showed the EphB2 and actin from *EphB2*<sup>+/+</sup> and *EphB2*<sup>-/-</sup> mice. **c**  
 46 The expression of EphB2 in hippocampus CA1 region from SCR-shRNA and EphB2-  
 47 shRNA mice were detected by western blotting. **d** The quantification of EphB2, GFP

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48 proteins in hippocampus.  $n = 3$  mice for each group, paired Student's  $t$  test, EphB2,  $t_{(2)}$   
49  $= 5.991$ ,  $p < 0.01$ ; GFP,  $t_{(2)} = 0.0706$ ,  $p > 0.05$ . **e** The expression of EphB2 in  
50 hippocampus from GH, GH with AAV-EphB2 (EphB2-OE), SI, SI with AAV-EphB2  
51 (EphB2-OE) mice was detected by western blotting. **f** The quantification of EphB2  
52 proteins in hippocampus.  $n = 3$  mice for each group, two-way ANOVA; effect of group,  
53  $F_{(1,8)} = 24.79$ ,  $p < 0.01$ ; effect of treatment,  $F_{(1,8)} = 132.1$ ,  $p < 0.0001$ ; group  $\times$  treatment,  
54  $F_{(1,8)} = 0.1529$ ,  $p > 0.05$ ; Tukey post hoc analysis was performed. All data are presented  
55 as mean  $\pm$  SEM. \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.01$ .





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57 **Supplementary Fig. 5 Whole western blotting related to Supplementary Fig. 3c (a)**

58 **and 3e (b).**

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60 **Supplementary Table 1. ANOVA results related to Fig. 3b**61 **Context A**

		<i>F</i>	<i>P</i>
Hour 1	group	$F_{(1,27)} = 0.04483$	$p > 0.05$
	treatment	$F_{(1,27)} = 0.7798$	$p > 0.05$
	interaction	$F_{(1,27)} = 0.1030$	$p > 0.05$
Day 1	group	$F_{(1,27)} = 0.7583$	$p > 0.05$
	treatment	$F_{(1,27)} = 0.2864$	$p > 0.05$
	interaction	$F_{(1,27)} = 0.6971$	$p > 0.05$
Day 7	group	$F_{(1,27)} = 11.92$	$p < 0.01$
	treatment	$F_{(1,27)} = 6.012$	$p < 0.05$
	interaction	$F_{(1,27)} = 1.054$	$p > 0.05$

62 **Context B**

		<i>F</i>	<i>P</i>
Hour 1	group	$F_{(1,27)} = 0.08326$	$p > 0.05$
	treatment	$F_{(1,27)} = 0.2988$	$p > 0.05$
	interaction	$F_{(1,27)} = 0.08326$	$p > 0.05$
Day 1	group	$F_{(1,27)} = 1.352$	$p > 0.05$
	treatment	$F_{(1,27)} = 0.4632$	$p > 0.05$
	interaction	$F_{(1,27)} = 0.02596$	$p > 0.05$
Day 7	group	$F_{(1,27)} = 9.527$	$p < 0.01$
	treatment	$F_{(1,27)} = 10.05$	$p < 0.01$
	interaction	$F_{(1,27)} = 2.749$	$p > 0.05$

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65 **Supplementary Table 2. ANOVA results related to Fig. 3d**

		<i>F</i>	<i>P</i>
Spine density	group	$F_{(1,71)} = 1.56$	$p > 0.05$
	treatment	$F_{(1,71)} = 0.0005791$	$p > 0.05$
	interaction	$F_{(1,71)} = 0.7928$	$p > 0.05$
Mushroom	group	$F_{(1,71)} = 1.55$	$p > 0.05$
	treatment	$F_{(1,71)} = 10.62$	$p < 0.01$
	interaction	$F_{(1,71)} = 0.00514$	$p > 0.05$
Stubby	group	$F_{(1,71)} = 0.1221$	$p > 0.05$
	treatment	$F_{(1,71)} = 0.7599$	$p > 0.05$
	interaction	$F_{(1,71)} = 4.668$	$p < 0.05$
Thin	group	$F_{(1,71)} = 14.33$	$p < 0.001$
	treatment	$F_{(1,71)} = 12$	$p < 0.001$
	interaction	$F_{(1,71)} = 1.377$	$p > 0.05$

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68 **Supplementary Table 3. ANOVA results related to Fig. 3f**

		<i>F</i>	<i>P</i>
Amplitude	group	$F_{(1,59)} = 14.25$	$p < 0.001$
	treatment	$F_{(1,59)} = 14.36$	$p < 0.001$
	interaction	$F_{(1,59)} = 3.168$	$p > 0.05$
Frequency	group	$F_{(1,59)} = 1.071$	$p > 0.05$
	treatment	$F_{(1,59)} = 0.09728$	$p > 0.05$
	treatment	$F_{(1,59)} = 1.28$	$p > 0.05$

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70 **Supplementary Table 4. ANOVA results related to Fig. 3h**

		<i>F</i>	<i>P</i>
fEPSP slope	group	$F_{(3,319)} = 34.55$	$p < 0.001$
	treatment	$F_{(10,319)} = 0.01840$	$p > 0.05$
	interaction	$F_{(30,319)} = 0.02592$	$p > 0.05$

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73 **Supplementary Table 5. ANOVA results related to Fig. 4a**74 **Context A**

		<i>F</i>	<i>P</i>
Hour 1	group	$F_{(1,29)} = 0.034$	$p > 0.05$
	treatment	$F_{(1,29)} = 0.04654$	$p > 0.05$
	interaction	$F_{(1,29)} = 0.0001$	$p > 0.05$
Day 1	group	$F_{(1,29)} = 1.07$	$p > 0.05$
	treatment	$F_{(1,29)} = 1.249$	$p > 0.05$
	interaction	$F_{(1,29)} = 0.001656$	$p > 0.05$
Day 7	group	$F_{(1,29)} = 5.374$	$p < 0.05$
	treatment	$F_{(1,29)} = 6.836$	$p < 0.05$
	interaction	$F_{(1,29)} = 1.892$	$p > 0.05$

75 **Context B**

		<i>F</i>	<i>P</i>
Hour 1	group	$F_{(1,29)} = 0.08513$	$p > 0.05$
	treatment	$F_{(1,29)} = 0.01505$	$p > 0.05$
	interaction	$F_{(1,29)} = 0.3495$	$p > 0.05$
Day 1	group	$F_{(1,29)} = 0.3818$	$p > 0.05$
	treatment	$F_{(1,29)} = 0.1331$	$p > 0.05$
	interaction	$F_{(1,29)} = 0.06949$	$p > 0.05$
Day 7	group	$F_{(1,29)} = 10.73$	$p < 0.01$
	treatment	$F_{(1,29)} = 11.77$	$p < 0.01$
	interaction	$F_{(1,29)} = 5.916$	$p < 0.05$

77 **Supplementary Table 6. ANOVA results related to Fig. 4c**

		<i>F</i>	<i>P</i>
Spine density	group	$F_{(1,63)} = 5.565$	$p < 0.05$
	treatment	$F_{(1,63)} = 0.3292$	$p > 0.05$
	interaction	$F_{(1,63)} = 0.1663$	$p > 0.05$
Mushroom	group	$F_{(1,63)} = 0.3757$	$p > 0.05$
	treatment	$F_{(1,63)} = 0.05802$	$p > 0.05$
	interaction	$F_{(1,63)} = 4.972$	$p < 0.05$
Stubby	group	$F_{(1,63)} = 2.05$	$p > 0.05$
	treatment	$F_{(1,63)} = 1.447$	$p > 0.05$
	interaction	$F_{(1,63)} = 0.005215$	$p > 0.05$
Thin	group	$F_{(1,63)} = 5.578$	$p < 0.05$
	treatment	$F_{(1,63)} = 3.164$	$p > 0.05$
	interaction	$F_{(1,63)} = 8.956$	$p < 0.01$

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81 **Supplementary Table 7. ANOVA results related to Fig. 4e**

		<i>F</i>	<i>P</i>
Amplitude	group	$F_{(1,66)} = 4.876$	$p < 0.05$
	treatment	$F_{(1,66)} = 8.257$	$p < 0.01$
	interaction	$F_{(1,66)} = 6.486$	$p < 0.05$
Frequency	group	$F_{(1,66)} = 0.4383$	$p > 0.05$
	treatment	$F_{(1,66)} = 0.6536$	$p > 0.05$
	treatment	$F_{(1,66)} = 2.266$	$p > 0.05$

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83 **Supplementary Table 8. ANOVA results related to Fig. 4g**

		<i>F</i>	<i>P</i>
fEPSP slope	group	$F_{(3,330)} = 77.8$	$p < 0.0001$
	treatment	$F_{(10,330)} = 0.2552$	$p > 0.05$
	interaction	$F_{(30,330)} = 0.2882$	$p > 0.05$

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