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SUPPLEMENTARY INFORMATION

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# Serotonin-mediated inhibition of ventral hippocampus is required for sustained goal-directed behavior

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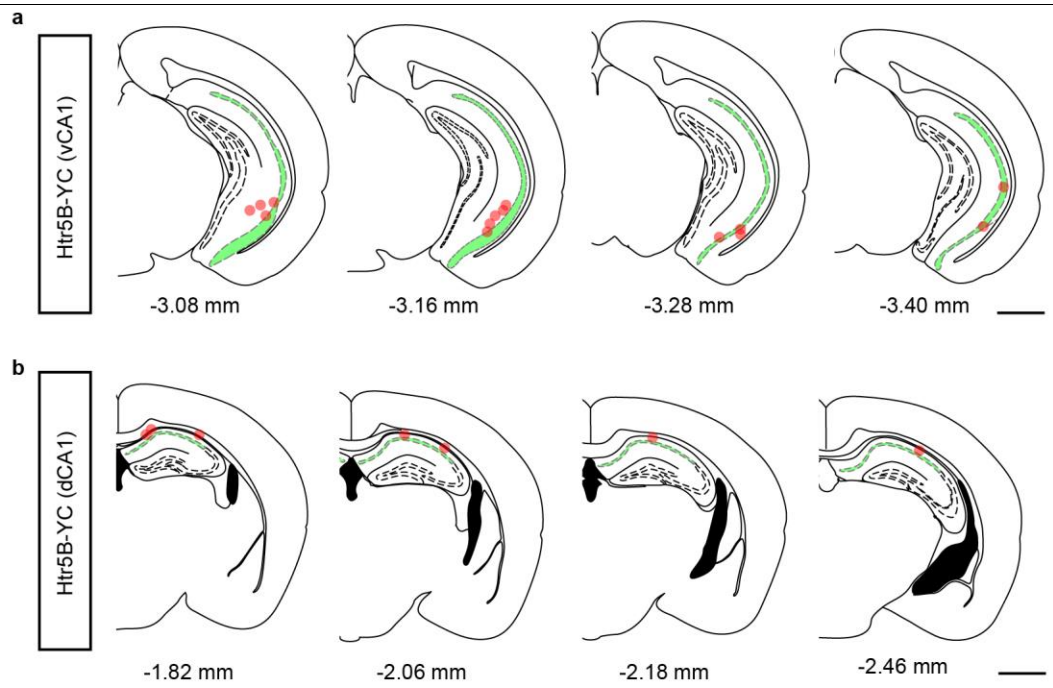
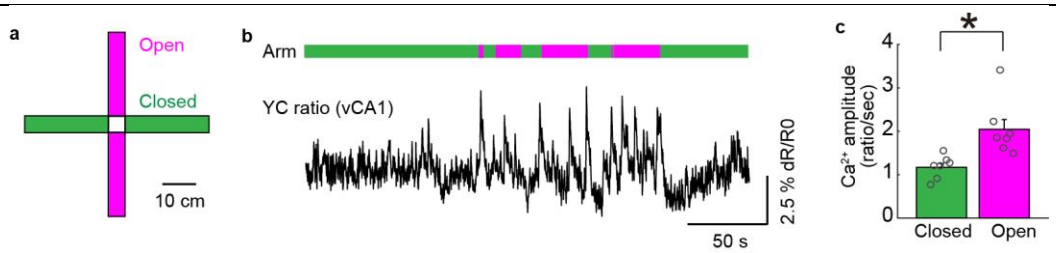



Supplementary Figure 1

**Optical fiber placements for fiber photometry.**

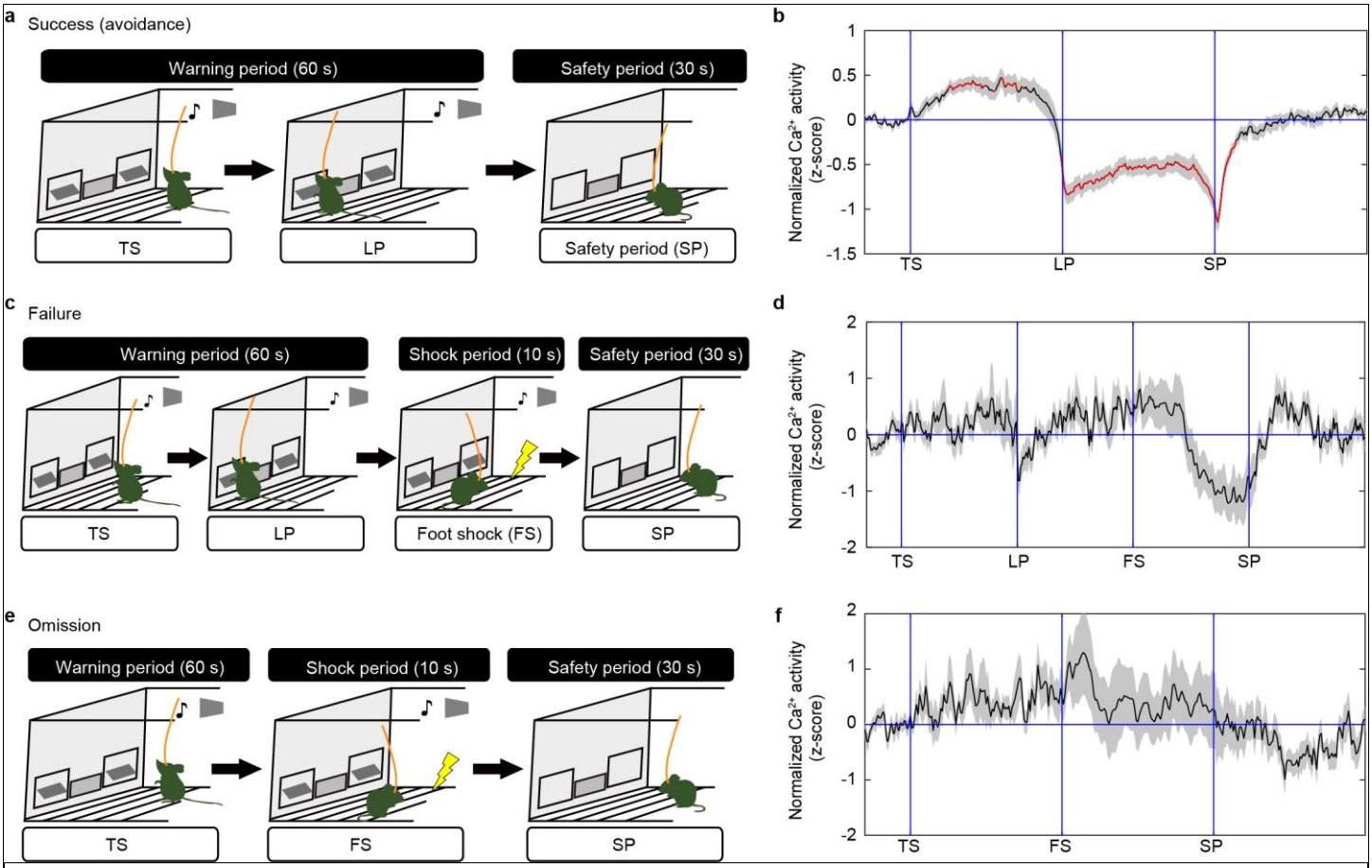
a, b, Histological reconstruction of optical fiber tip placements in the vCA1 (a) and the dCA1 (b). Red dots indicate fiber tips.




**Supplementary Figure 2**

**Compound $Ca^{2+}$ activity of vCA1 neurons increased during exploration of the anxiogenic open-arm compartment in the elevated plus maze (EPM).**

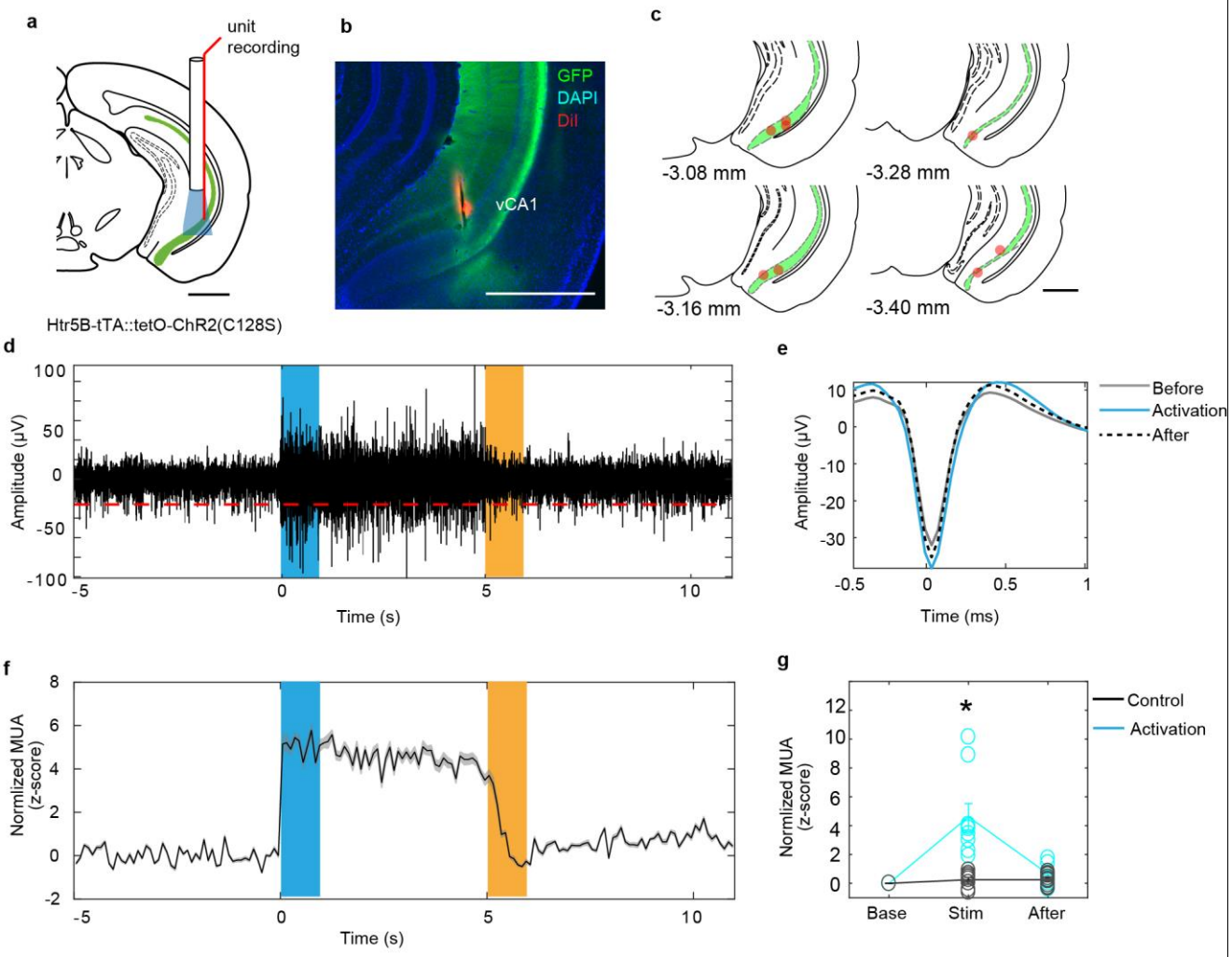
a, An overview of the maze. b, Representative trace of the YC ratio in vCA1 during exploration of the EPM. Upper, Periods when the mouse was located in open and closed arms are represented in magenta and green lines, respectively. c, Mean $Ca^{2+}$ amplitude in vCA1 was significantly higher in open arms compared to closed arms of the EPM (n = 7 mice, 14 sessions, * two-sided paired t test: t(6) = -2.80, p = 0.031). Bars represent the mean and lines represent the s.e.m.




**Supplementary Figure 3**

**Compound $Ca^{2+}$ activity in vCA1 during a lever-press active-avoidance task.**

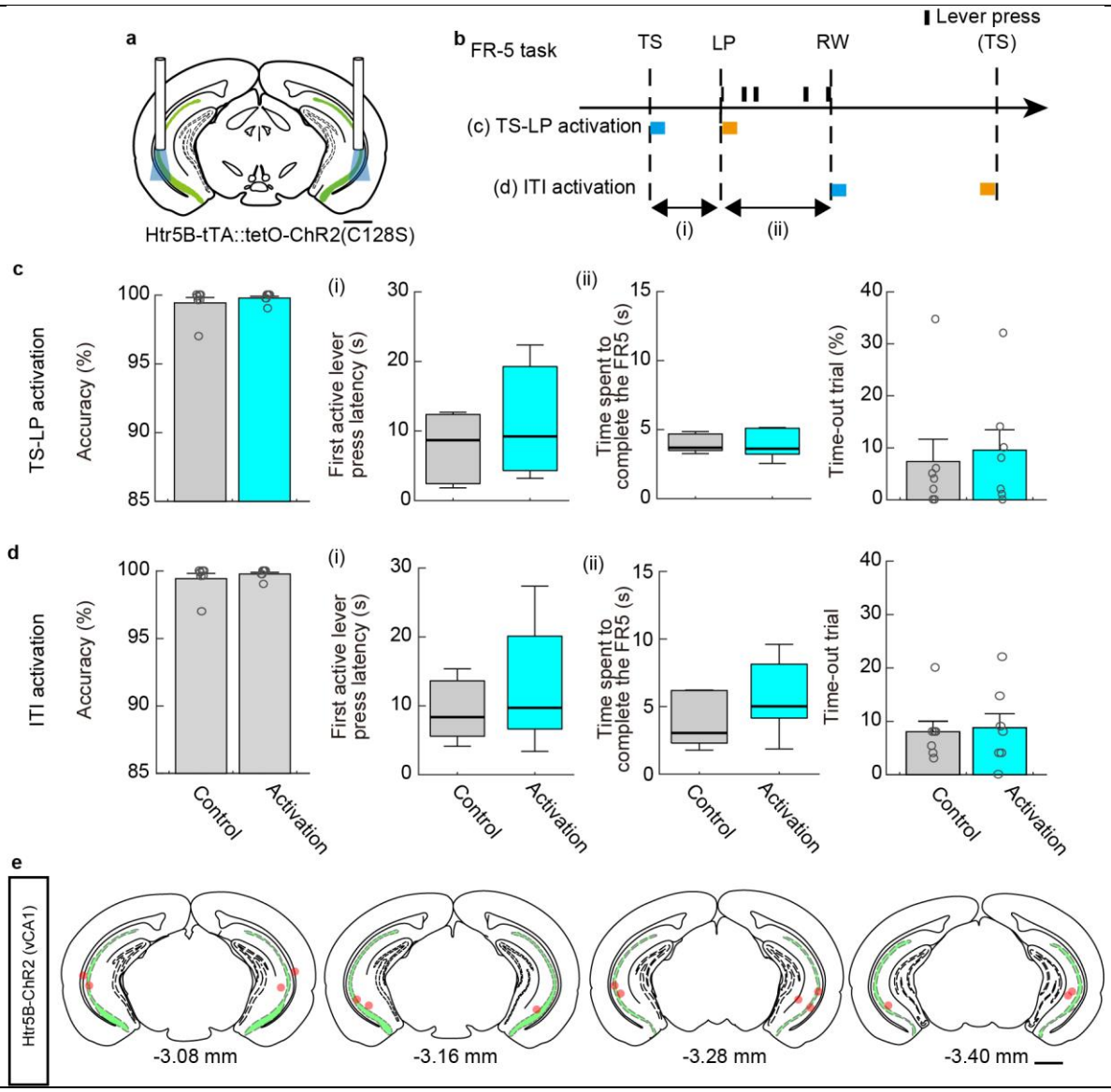
a, c, e, Experimental schedule for the active avoidance task. Trial start; TS, first lever-press; LP, foot shock start; FS, and safety period start; SP, respectively. b, d, f, Trace of averaged $Ca^{2+}$ signals in which the duration between trigger points was normalized (n = 8 mice, 30 sessions). Red, significant increase or decrease (two-sided permutation test: $p$ <0.05). The Shaded areas represent s.e.m.




**Supplementary Figure 4**

**Effect of step-function-type ChR2 on electrophysiological responses in the vCA1 pyramidal cell layer.**

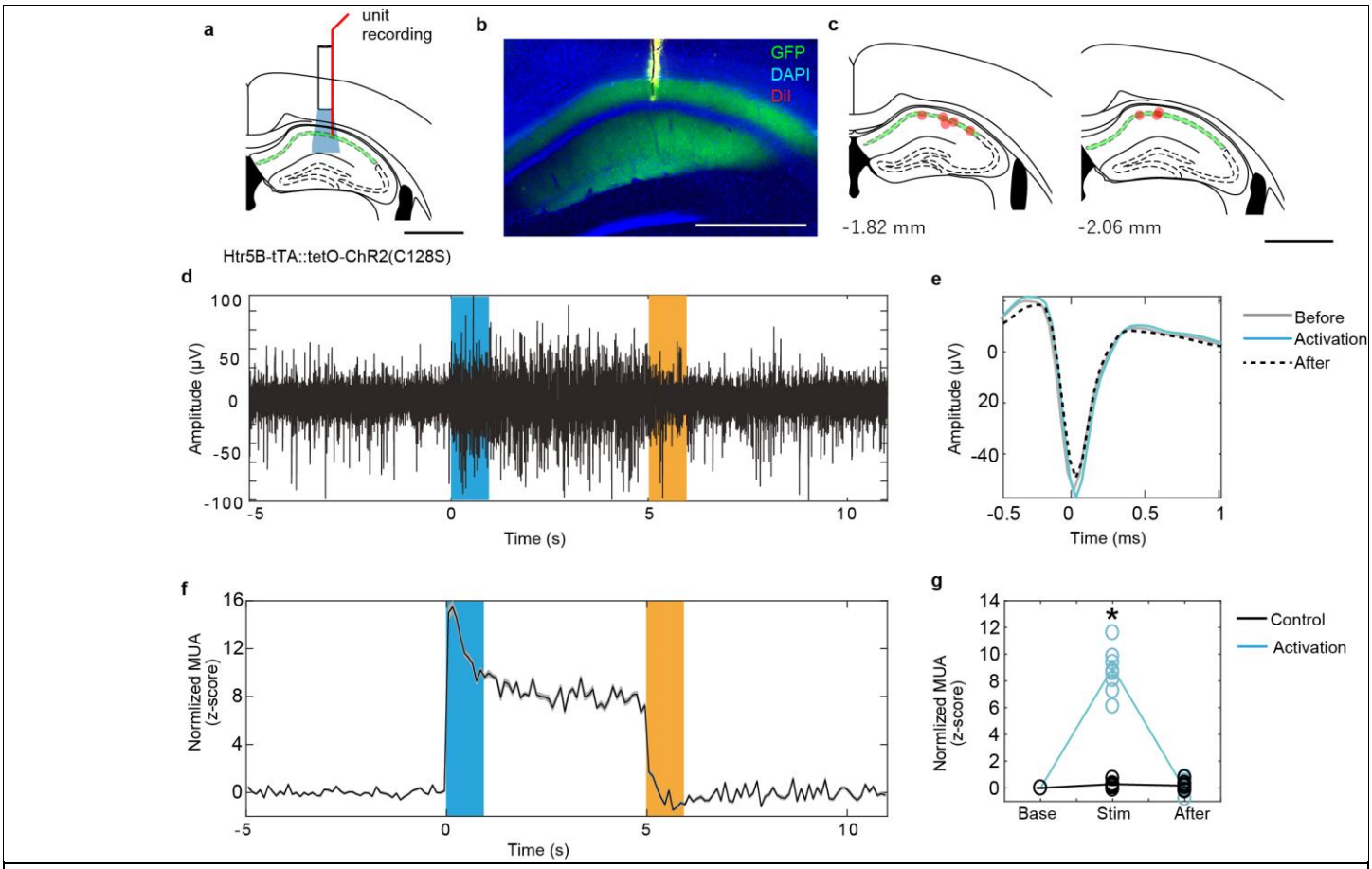
a, Schematic illustration of electrophysiological recording in vCA1 of Htr5B-ChR2(C128S) mice with a custom-made optrode. Scale bar, 1 mm. b, The position of electrode was identified with DiI fluorescence (red). Green shows ChR2(C128S)-EYFP expression in vCA1. Scale bar, 1 mm. c, Electrode placements (red dots) in the vCA1. d, A representative time course of high-pass filtered LFP (> 600 Hz). Vertical blue and yellow lines of indicate 1-s illumination of each light color. ChR2 was activated by blue light and inactivated by yellow light. The red dashed line indicates threshold of MUA detection. e, Representative mean traces of MUA wave forms during a baseline period (-5-0 s, gray line), during the activation period (0-5 s, blue line) and after the activation period (5-10 s, black dash line). f, Time course of normalized MUA counts recorded from the pyramidal cell layer of vCA1 during optogenetic activation (n = 8 traces from 5 mice). The bin size was 100 ms. Shadow areas represent mean ± s.e.m. g, Mean normalized MUA counts during the activation period (0-5 s; control, n = 8 traces from 5 mice; activation, n = 8 traces from 5 mice). Yellow light illumination was used in place of blue light in the control condition. Data are presented as mean ± s.e.m. * $p = 9.14\times10^{-4}$; two-sided paired *t* test compared to the control sessions, $t(7) = 5.11$.




**Supplementary Figure 5**

**No behavioral effects of vCA1 optogenetic activation during TS-LP and ITI periods in the FR-5 task.**

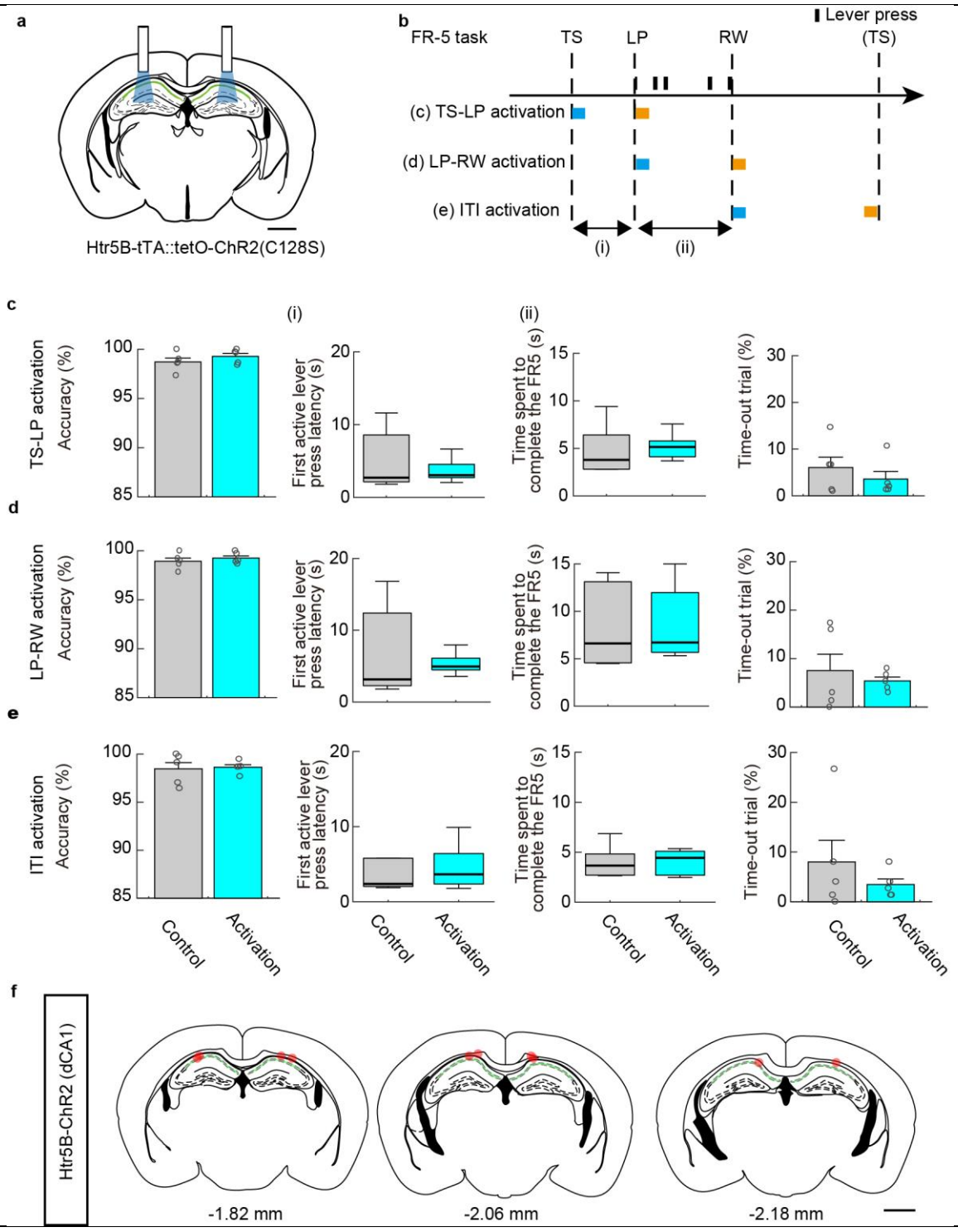
a, Schematic illustration of optogenetic activation in bilateral vCA1 of Htr5B-ChR2(C128S) mice. We used same mice in fig. 2a-2c. b, The timing of illumination in the FR-5 task. Optogenetic activation was performed during every TS-LP or ITI period. Blue and yellow illuminations were 1s in duration. Yellow light illumination was used in place of blue light in the control condition. c, d, The effects of vCA1 activation during TS-LP (c, n = 7 mice, 23 sessions) and ITI (d, n = 7 mice, 20 sessions) periods on behavioral parameters in the FR-5 task. Optogenetic activation during TS-LP and ITI period did not affect behavioral parameters (c, Accuracy: two-sided paired *t* test, t(6) = -1.16, p = 0.29; First active lever press latency: two-sided Wilcoxon signed-rank test, z = -0.34, p = 0.74; Time spent to complete the FR5: two-sided Wilcoxon signed-rank test, z = -0.68, p = 0.49; Time-out trial: two-sided paired *t* test, t(6) = -1.29, p = 0.24; d, Accuracy: two-sided paired *t* test, t(6) = -0.32, p = 0.76; First active lever press latency: two-sided Wilcoxon signed-rank test, z = 0.01, p = 0.99; Time spent to complete the FR5: two-sided Wilcoxon signed-rank test, z = -0.51, p = 0.61; Time-out trial: two-sided paired *t* test, t(6) = -0.34, p = 0.75). Bars represent the mean and lines represent the s.e.m. In box plots, the central mark indicates the median and the bottom and top edges of the box indicate the 25th and 75th percentiles, respectively. Whiskers extend to the most extreme data points not considered outliers. e, Bilateral fiber placements in the vCA1. Red dots indicate fiber tips.




**Supplementary Figure 6**

**Effect of step-function-type ChR2 on electrophysiological responses in the dCA1 pyramidal cell layer.**

a, Schematic illustration of electrophysiological recording in dCA1 of Htr5B-ChR2(C128S) mice with a custom-made optrode. Scale bar, 1 mm. b, The position of electrode was identified with DiI fluorescence (red). Green shows ChR2(C128S)-EYFP expression in dCA1. Scale bar, 1 mm. c, Electrode placements (red dots) in the dCA1. d, A representative time course of high-pass filtered LFP (> 600 Hz). Vertical blue and yellow lines of indicate 1-s illumination of each light color. ChR2 was activated by blue light and inactivated by yellow light. The red dashed line indicates threshold of MUA detection. e, Representative mean traces of MUA wave forms during a baseline period (-5-0 s, gray line), during the activation period (0-5 s, blue line) and after the activation period (5-10 s, black dash line). f, Time course of normalized MUA counts recorded from the pyramidal cell layer of dCA1 during optogenetic activation (n = 8 traces from 5 mice). The bin size was 100 ms. Shadow areas represent mean ± s.e.m. g, Mean normalized MUA counts during the activation period (0-5 s; control, n = 8 sessions from 5 mice; activation, n = 8 traces from 5 mice). Yellow light illumination was used in place of blue light in the control condition. Data are presented as mean ± s.e.m. * $p = 2.15\times10^{-9}$; two-sided paired *t* test compared to the control sessions, $t(7) = 15.79$

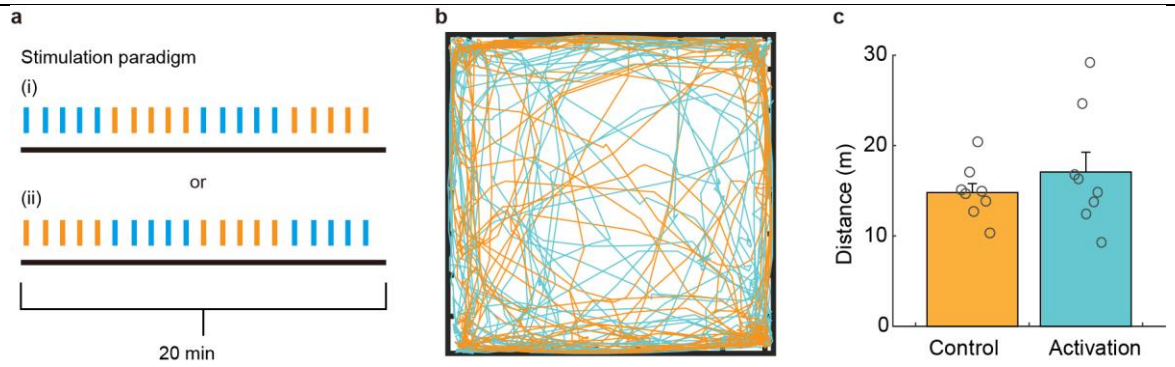



**Supplementary Figure 7**

**No behavioral effects of optogenetic activation of dCA1 in the FR-5 task.**

a, Schematic illustration of optogenetic activation in bilateral dCA1 of Htr5B-ChR2(C128S) mice. Scale bar, 1 mm. b, The timing of illumination in the FR-5 task. Blue and yellow illuminations were 1s in duration. Yellow light illumination was used in place of blue light in the control condition. c-e, The effects of dCA1 activation during TS-LP (c, n = 5 mice, 16 sessions), LP-RW (d, n = 5 mice, 16 sessions), and ITI (e, n = 5 mice, 15 sessions) periods on behavioral parameters in the FR-5 task. Optogenetic activation during TS-LP,

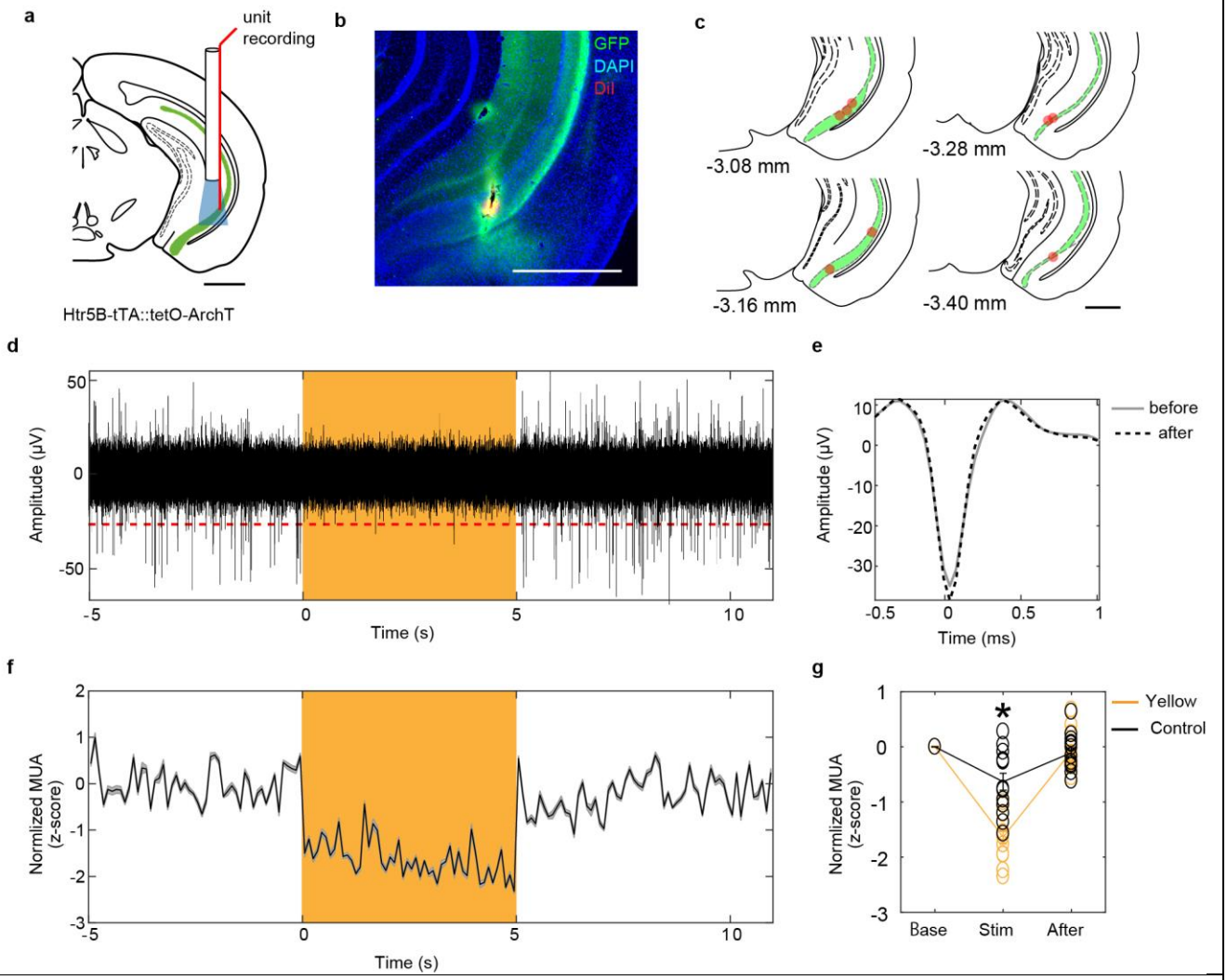
LP-RW and ITI periods did not affect behavioral parameters (c, Accuracy: two-sided paired *t* test, t(4) = -0.99, p = 0.38; First active lever press latency: two-sided Wilcoxon signed-rank test, z = -0.67, p = 0.50; Time spent to complete the FR5: two-sided Wilcoxon signed-rank test, z = -0.14, p = 0.89; Time-out trial: two-sided paired *t* test, t(4) = 1.98, p = 0.12; d, Accuracy: two-sided paired *t* test, t(4) = -0.69, p = 0.53; First active lever press latency: two-sided Wilcoxon signed-rank test, z = -0.41, p = 0.67; Time spent to complete the FR5: two-sided Wilcoxon signed-rank test, z = -0.67, p = 0.50; Time-out trial: two-sided paired *t* test, t(4) = 0.69, p = 0.53; e, Accuracy: two-sided paired *t* test, t(4) = -0.19, p = 0.86; First active lever press latency: two-sided Wilcoxon signed-rank test, z = -0.41, p = 0.69; Time spent to complete the FR5: two-sided Wilcoxon signed-rank test, z = -0.16, p = 0.89; Time-out trial: two-sided paired *t* test, t(4) = 0.98, p = 0.38). Bars represent the mean and lines represent the s.e.m. In box plots, the central mark indicates the median and the bottom and top edges of the box indicate the 25th and 75th percentiles, respectively. Whiskers extend to the most extreme data points not considered outliers. f, Bilateral fiber placements in the dCA1. Red dots indicate fiber tips.




**Supplementary Figure 8**

**No effect of vCA1 activation on locomotor activity.**

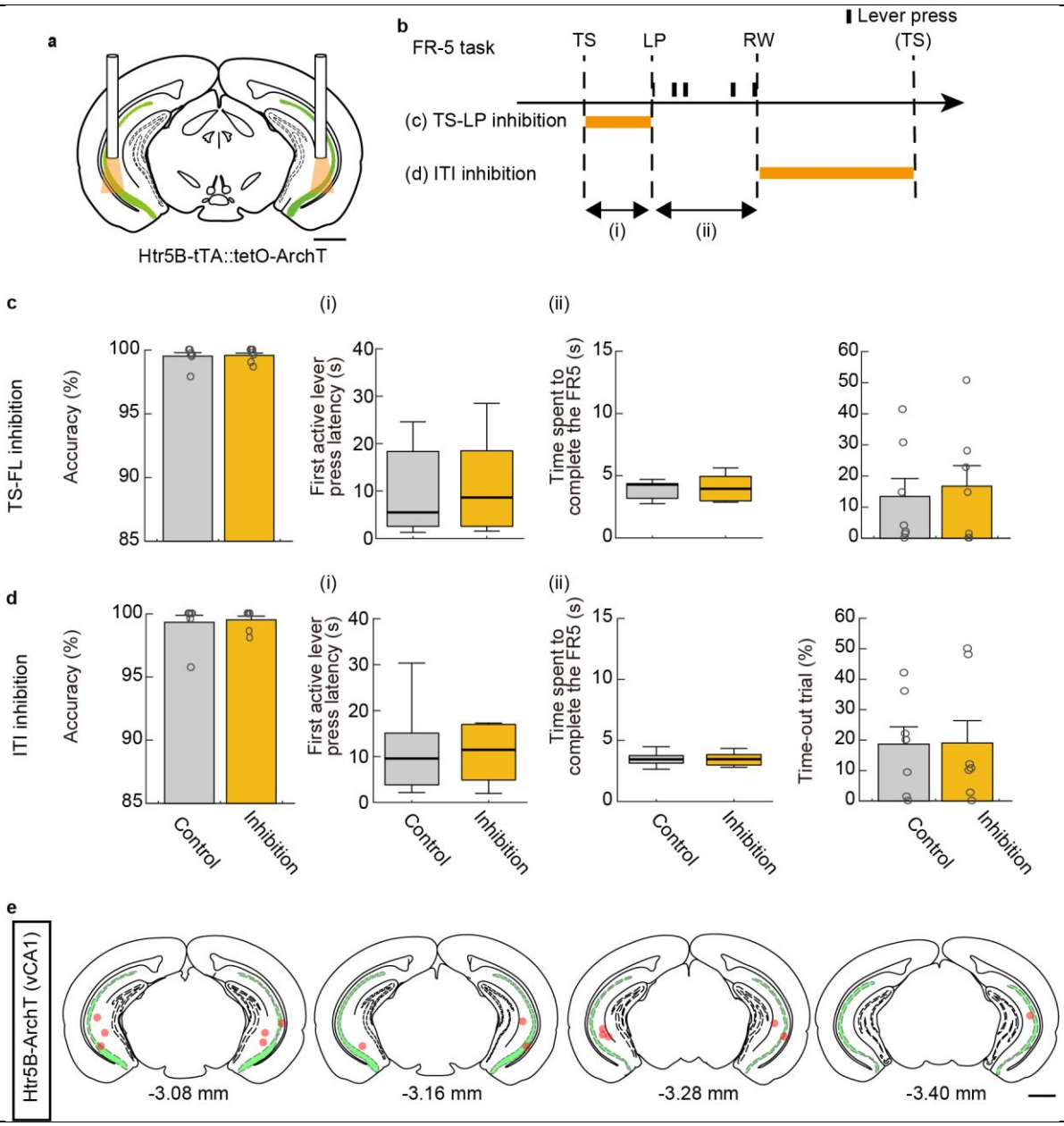
a, Stimulation paradigm. Blue and yellow light illumination trains were delivered (1-s illumination at 1 min interval, 5 pulses) to bilateral vCA1 of Htr5B-ChR2(C128S) mice. The order of the light train blocks was counterbalanced between mice. b, Representative position tracking during the blue and yellow light trains. c, Distance traveled during the ten minutes of blue or yellow light illumination (n = 8 mice, two-sided paired *t* test, t(7) = 1.65, p = 0.14). We used same mice in fig. 2a-2c. Bars represent the mean and lines represent the s.e.m.




**Supplementary Figure 9**

**Effect of ArchT on electrophysiological responses in the vCA1 pyramidal cell layer.**

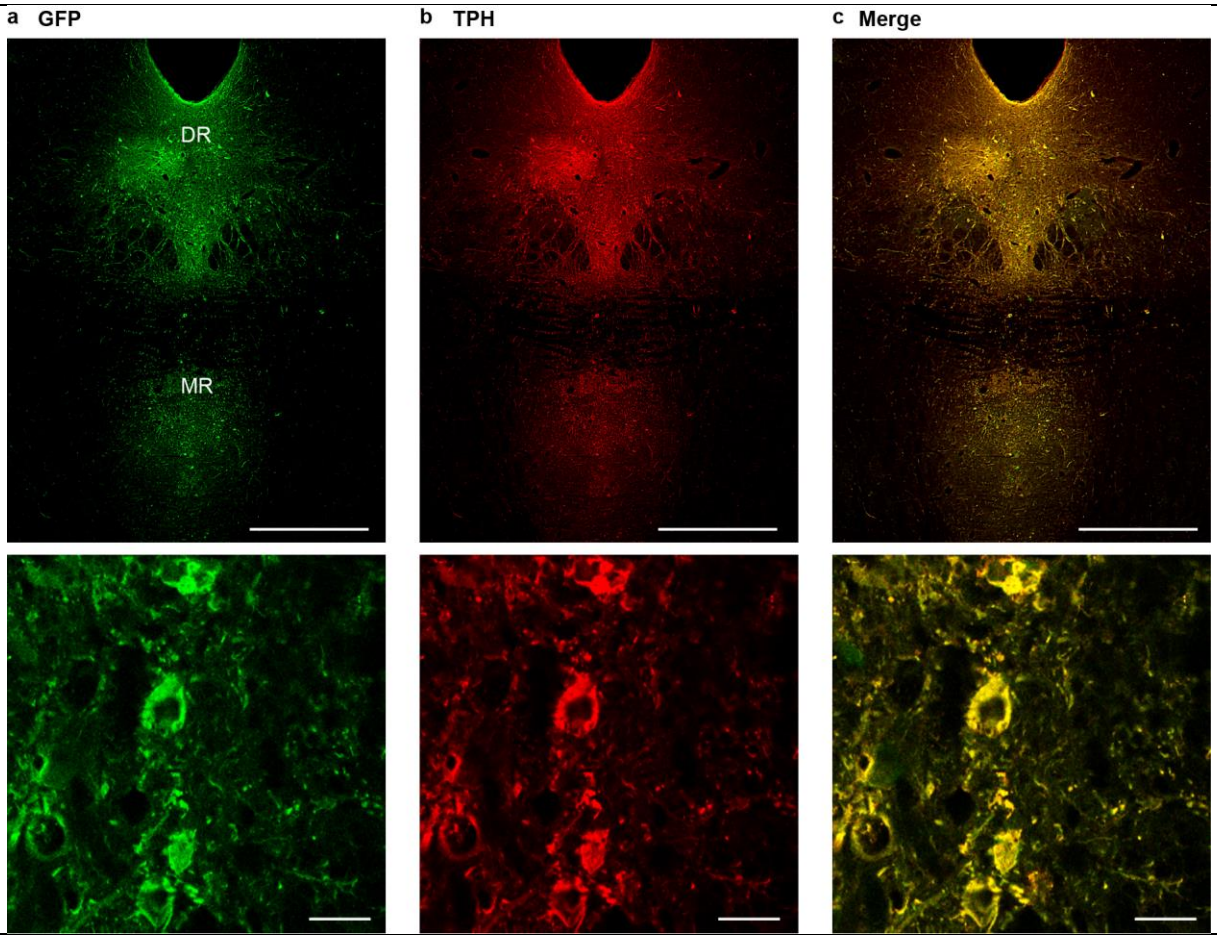
a, A representative time course of high-pass filtered LFP (> 600 Hz). Yellow area indicates the yellow light illumination period. Red dashed line indicates threshold of MUA detection. b, Representative mean traces of MUA wave forms during a baseline period (-5-0 s, gray line) and after the inhibition period (5-10 s, black dashed line). c, Electrode placements (red dots) in the vCA1. d, A representative time course of high-pass filtered LFP (> 600 Hz). Vertical yellow line indicates 5-s illumination. The red dashed line indicates threshold of MUA detection. e, Representative mean traces of MUA wave forms during a baseline period (-5-0 s, gray line) and after the activation period (5-10 s, black dash line).f, Time course of normalized MUA counts recorded from the pyramidal cell layer of vCA1 during optogenetic inhibition (n = 8 traces from 4 mice). The bin size was 100 ms. Shadow areas represent mean ± s.e.m. g, Mean normalized MUA counts during the inhibition period (0-5 s; control, n = 8 traces from 4 mice; inhibition, n = 8 traces from 4 mice). Blue light illumination instead of yellow light in the control condition. Data are presented as mean ± s.e.m. * $p = 4.10\times10^{-5}$; two-sided paired *t* test compared to the control sessions, t(7) = -6.27.




**Supplementary Figure 10**

**No behavioral effects of vCA1 optogenetic inhibition during the TS-LP and ITI periods in the FR-5 task.**

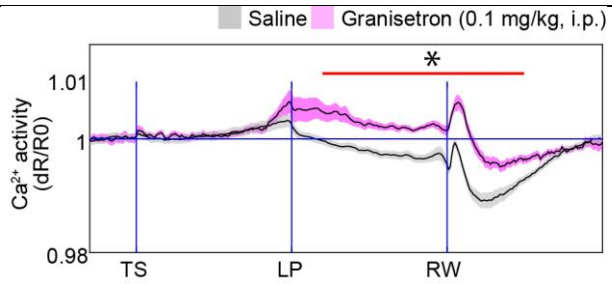
a, Schematic illustration of optogenetic inhibition in bilateral vCA1 of Htr5B-ArchT mice. We used the same mice as in fig. 2d-2f. Scale bar, 1 mm. b, The timing of illumination in the FR-5 task. An optogenetic inhibition was performed during every TS-LP or ITI period. Blue light illumination was used instead of yellow light in the control condition. c, d, The effects of the vCA1 inhibition during TS-LP (c; n = 7 mice, 20 sessions) and ITI (d; n = 7 mice, 16 sessions) periods on behavioral parameters in the FR-5 task. Optogenetic inhibition during TS-LP and ITI periods did not affect behavioral parameters (c, Accuracy: two-sided paired *t* test, t(6) = -0.13, p = 0.89; First active lever press latency: two-sided Wilcoxon signed-rank test, z = -1.01, p = 0.31; Time spent to complete the FR5: two-sided Wilcoxon signed-rank test, z = -1.01, p = 0.31; Time-out trial: two-sided paired *t* test, t(6) = -1.53, p = 0.18; d, Accuracy: two-sided paired *t* test, t(6) = -0.39, p = 0.73; First active lever press latency: two-sided Wilcoxon signed-rank test, z = -1.01, p = 0.31; Time spent to complete the FR5: two-sided Wilcoxon signed-rank test, z = -0.17, p = 0.87; Time-out trial: two-sided paired *t* test, t(6) = -0.12, p = 0.91). Bars represent the mean and lines represent the s.e.m. In box plots, the central mark indicates the median and the bottom and top edges of the box indicate the 25th and 75th percentiles, respectively. Whiskers extend to the most extreme data points not considered outliers. f, Bilateral fiber placements in the vCA1. Red dots indicate fiber tips.




**Supplementary Figure 11**

**YCnano50 is specifically expressed in serotonin neurons in the transgenic mouse brain.**

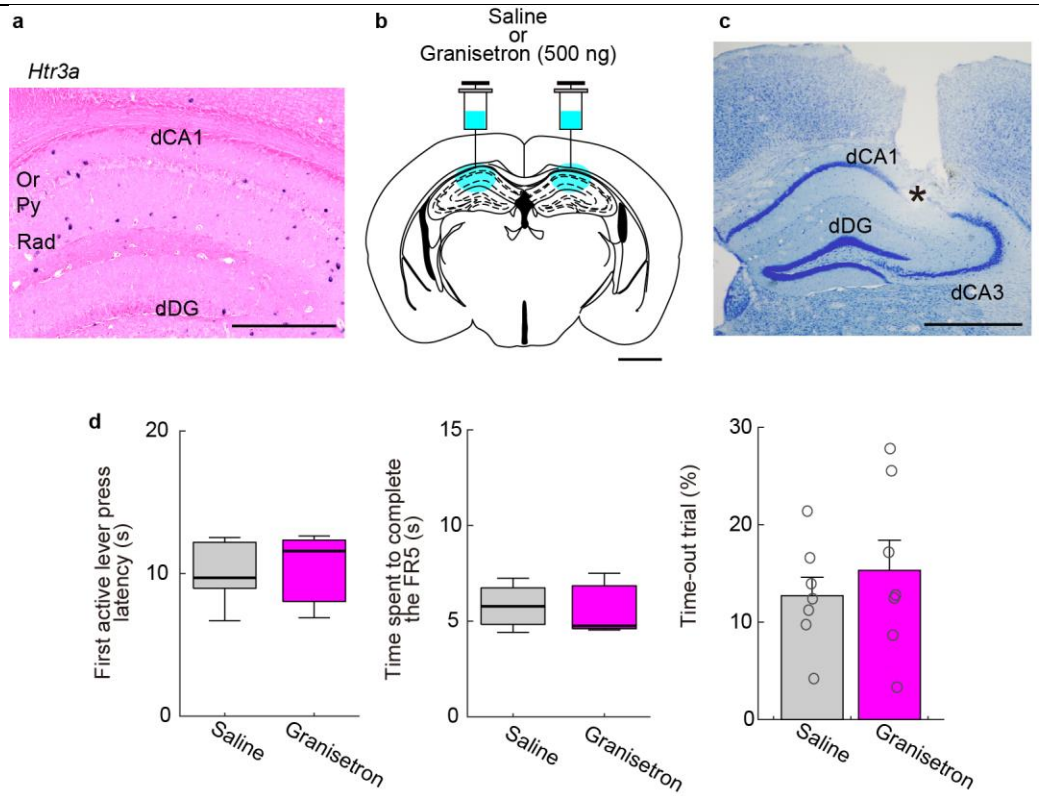
a-c, Upper panels, Representative images of YCnano50 expression in dorsal raphe nucleus (DR) and median raphe nucleus (MR) of Tph2-YC mice. Scale bar, 250 μm. Bottom panels, High-magnification view in the MR. Scale bar, 20 μm. a, Green represents GFP indirect fluorescence of YCnano50. b, Red represents tryptophan hydroxylase (TPH) staining. c, Merged image shows that YCnano50 was selectively expressed in serotonergic neurons; 97.8 ± 0.03 % of TPH+ cells expressed YCnano50 (114 ± 1.4 of 117 ± 1.5 cells, n = 3 mice) and 98.5 ± 0.3% of YCnano50+ cells expressed TPH2 (113 ± 2.9 of 115 ± 2.8 cells, n = 3 mice).




**Supplementary Figure 12**

**Compound $Ca^{2+}$ activity of dCA1 in FR-5 task with systemic granisetron administration.**

The effect of granisetron on $Ca^{2+}$ activity in dCA1 in the FR-5 task (saline, n = 9 mice, 19 sessions; granisetron, n = 9 mice, 16 sessions). *$P$<0.05 (Two-sided permutation test, red line, significant increase or decrease points compared with saline treatment). Shaded areas represent s.e.m.




**Supplementary Figure 13**

**No behavioral effects of local Htr3a antagonist injection into dCA1 in FR-5 task.**

a, *Htr3a* mRNA expression in the dorsal hippocampus (n = 5 mice). Scale bar, 500 μm. Or, stratum oriens; Py, stratum pyramidal cell; Rad, stratum radiatum. b, Schematic illustration of injection sites into bilateral dCA1. Scale bar, 1 mm. c, Representative image of injection site. Asterisk indicates tip of injection canula. Scale bar, 1 mm. d, The effects of local injection of granisetron into dCA1 on behavioral parameters in the FR-5 task (saline, n = 7 mice, 19 sessions; granisetron, n = 7 mice, 20 sessions; two-sided Wilcoxon signed-rank test, First active lever press latency: $z = 0.51$, $p = 0.61$; two-sided Wilcoxon signed-rank test, Time spent to complete the FR5: $z = -0.34$, $p = 0.74$; two-sided paired t test, Time-out trial: $t(6) = -0.95$, $p = 0.38$). Bars represent the mean and lines represent the s.e.m. In box plots, the central mark indicates the median and the bottom and top edges of the box indicate the 25th and 75th percentiles, respectively. Whiskers extend to the most extreme data points not considered outliers.

Supplementary Table 1– Statistical details for main figures

| Figure | Sample size (n) | statistical test | values |
|---|---|---|---|
| 1b | 11 mice | two-sided paired t-test (devalued condition vs non-devalued condition) | t(10) = -4.000, p = 0.003 |
| 1f | 14 mice | two-sided permutation test (1000 permutations for α-level of 0.05) | red lines p < 0.05 |
| 1h | 7 mice | permutation test (1000 permutations for α-level of 0.05) | red lines p < 0.05 |
| 1i | 14 mice | two-sided paired t-test (success vs failure) | t(13) = 5.62 p = 6.30×10-5 |
| 1j | FR-5 task; 14 mice, FR-20 task; 7 mice | two-sided Student's t-test (FR-5 vs FR-20) | t(19) = -5.33, p = 3.80×10-5 |
| 1k | 7 mice | two-sided paired t-test (success vs failure) | t(6) = 3.84, p = 8.51×10-3 |
| 2c (Accuracy) | 7 mice | two-sided paired t-test (blue light activation vs yellow light control) | t(6)=-0.39, p = 0.73 |
| 2c (First active lever press latency) | 7 mice | two-sided Wilcoxon signed-rank test (blue light activation vs yellow light control) | z = -1.35, p = 0.176 |
| 2c (Time spent to complete the FR5) | 7 mice | two-sided Wilcoxon signed-rank test (blue light activation vs yellow light control) | z = -2.37, p = 0.018 |
| 2c (Time-out trial) | 7 mice | two-sided paired t-test (blue light activation vs yellow light control) | t(6)= -3.45, p = 0.14 |
| 2f (Accuracy) | 7 mice | two-sided paired t-test (yellow light inhibition vs blue light control) | t(6) = -1.00, p = 0.37 |
| 2f (First active lever press latency) | 7 mice | two-sided Wilcoxon signed-rank test (yellow light inhibition vs blue light control) | z = -0.51, p = 0.61 |
| 2f (Time spent to complete the FR5) | 7 mice | two-sided Wilcoxon signed-rank test (yellow light inhibition vs blue light control) | z = -0.68, p = 0.50 |
| 2f (Time-out trial) | 7 mice | two-sided paired t-test (yellow light inhibition vs blue light control) | t(6) = -0.62, p = 0.56 |
| 2g (Accuracy) | 7 mice | two-sided paired t-test (yellow light inhibition vs blue light control) | t(6) = 0.48, p = 0.96 |
| 2g (First active lever press latency) | 7 mice | two-sided Wilcoxon signed-rank test (yellow light inhibition vs blue light control) | z = -0.34, p = 0.74 |
| 2g (Time spent to complete the FR10) | 7 mice | two-sided Wilcoxon signed-rank test (yellow light inhibition vs blue light control) | z = -2.37, p = 0.018 |
| 2g (Time-out trial) | 7 mice | two-sided paired t-test (yellow light inhibition vs blue light control) | t(6) = 2.78, p = 0.033 |
| 2h (Accuracy) | 7 mice | two-sided paired t-test (yellow light inhibition vs blue light control) | t(6) = -0.03, p = 0.98 |
| 2h (First active lever press latency) | 7 mice | two-sided Wilcoxon signed-rank test (yellow light inhibition vs blue light control) | z = -0.68, p = 0.50 |
| 2h (Time spent to complete the FR20) | 7 mice | two-sided Wilcoxon signed-rank test (yellow light inhibition vs blue light control) | z = -2.37, p = 0.018 |
| 2h (Time-out trial) | 7 mice | two-sided paired t-test (yellow light inhibition vs blue light control) | t(6) = 5.99, p = 0.001 |
| 3c (Accuracy) | 8 mice | two-sided paired t-test (yellow light inhibition vs blue light control) | t(7)= 0.52, p = 0.62 |

| | | | |
|---|---|---|---|
| 3c (First active lever press latency) | 8 mice | two-sided Wilcoxon signed-rank test (yellow light inhibition vs blue light control) | z = -0.51, p = 0.61 |
| 3c (Break point) | 8 mice | repeated-measures ANOVA with Bonferroni post hoc test | Main effect light $F_{(1,30)}$ = 4.22 p = 0.049, Main effect condition $F_{(1,30)}$ = 100.08 p = $9.57\times10^{-11}$, Interaction effect $F_{(1,30)}$ = 4.44 p = 0.044; post hoc test: Valued condition yellow - control p = $6.44\times10^{-3}$, Devalued condition yellow - control p=0.97 |
| 4e | 21 traces | two-sided paired t-test(blue light activation vs yellow light control) | t(20) = 4.74, p = $1.26\times10^{-4}$ |
| 4j | 21 traces | two-sided paired t-test(blue light activation vs yellow light control) | t(20) = 9.07, p = $1.58\times10^{-8}$ |
| 4m | 6 mice | permutation test (1000 permutations for α-level of 0.05) | red lines p < 0.05 |
| 4p | 8 mice | permutation test (1000 permutations for α-level of 0.05) | red lines p < 0.05 |
| 5f (First active lever press latency) | 7 mice | two-sided Wilcoxon signed-rank test (saline vs WAY100635) | z = -0.70, p = 0.48 |
| 5f (Time spent to complete the FR5) | 7 mice | two-sided Wilcoxon signed-rank test (saline vs WAY100635) | z = -0.42, p = 0.67 |
| 5f (Time-out trial) | 7 mice | two-sided Wilcoxon signed-rank test (saline vs WAY100635) | t(6) = -0.24, p = 0.82 |
| 5g | 7 mice | two-sided permutation test (1000 permutations for α-level of 0.05, saline vs Granisetron) | red lines p < 0.05 |
| 5h (First active lever press latency) | 7 mice | two-sided Wilcoxon signed-rank test (saline vs Granisetron) | z = -1.82, p = 0.69 |
| 5h (Time spent to complete the FR5) | 7 mice | two-sided Wilcoxon signed-rank test (saline vs Granisetron) | z = -2.1, p = 0.036 |
| 5h (Time-out trial) | 7 mice | two-sided Wilcoxon signed-rank test (saline vs Granisetron) | t(6) = -2.66, p = 0.033 |
| 6c (First active lever press latency) | 8 mice | repeated-measures ANOVA with Bonferroni post hoc test (saline vs granisetron) | F4,35 = 0.24, p = 0.913 |
| 6c (Time spent to complete the FR5) | 8 mice | repeated-measures ANOVA with Bonferroni post hoc test (saline vs granisetron) | $F_{(4,35)}$ = 95.01, p = $2.76\times10^{-17}$;0.5 ng, p = 0.069; 5 ng, p = $1.89\times10^{-3}$; 50 ng, p = $3.0\times10^{-5}$, 500 ng, p = $2.66\times10^{-5}$ |
| 6c (Time-out trial) | 8 mice | repeated-measures ANOVA with Bonferroni post hoc test (saline vs granisetron) | $F_{(4,35)}$ = 14.51, p = $7.30\times10^{-7}$;0.5 ng, p = 0.81; 5 ng, p = 0.027; 50 ng, p = $3.71\times10^{-3}$, 500 ng, p = $1.10\times10^{-3}$ |