

Supplementary Materials for
**Modulation of prefrontal cortex excitation/inhibition balance rescues
social behavior in *CNTNAP2*-deficient mice**

Aslihan Selimbeyoglu, Christina K. Kim, Masatoshi Inoue, Soo Yeun Lee,
Alice S. O. Hong, Isaac Kauvar, Charu Ramakrishnan, Lief E. Fenno,
Thomas J. Davidson, Matthew Wright, Karl Deisseroth*

*Corresponding author. Email: deissero@stanford.edu

Published 2 August 2017, *Sci. Transl. Med.* **9**, eaah6733 (2017)
DOI: 10.1126/scitranslmed.aah6733

The PDF file includes:

Fig. S1. Bilateral expression of SwiChR++ in mPFC.
Fig. S2. CNTNAP2 KO and WT mice interact with objects similarly both in the presence and absence of SSFO recruitment.
Fig. S3. Both SSFO and SwiChR++ recruitments rescue CNTNAP2 KO hyperactivity in the open-field test.
Fig. S4. Modulation of outgoing corticostriatal activity traffic by SSFO recruitment in mPFC PV neurons.
Fig. S5. Temporal details of interactions with familiar mouse and novel object.
Fig. S6. Modulation of PV neuron activity with locomotion.
Table S1. Raw data of Fig. 1E.
Table S2. Raw data of Fig. 1H.
Table S3. Raw data of Fig. 1I.
Table S4. Raw data of Fig. 1K.
Table S5. Raw data of Fig. 2B.
Table S6. Raw data of Fig. 2C.
Table S7. Raw data of Fig. 2D.
Table S8. Raw data of Fig. 2E.
Table S9. Raw data of Fig. 2G.
Table S10. Raw data of Fig. 2H.
Table S11. Raw data of Fig. 2I.
Table S12. Raw data of Fig. 2J.
Table S13. Raw data of Fig. 3G.
Table S14. Raw data of Fig. 3H.

Legends for movies S1 and S2

Other Supplementary Material for this manuscript includes the following:

(available at

www.sciencetranslationalmedicine.org/cgi/content/full/9/401/eaah6733/DC1)

Movie S1 (.mov format). *CNTNAP2* KO mPFC PV neuron activity during social behavior.

Movie S2 (.mov format). WT mPFC PV neuron activity during social behavior.

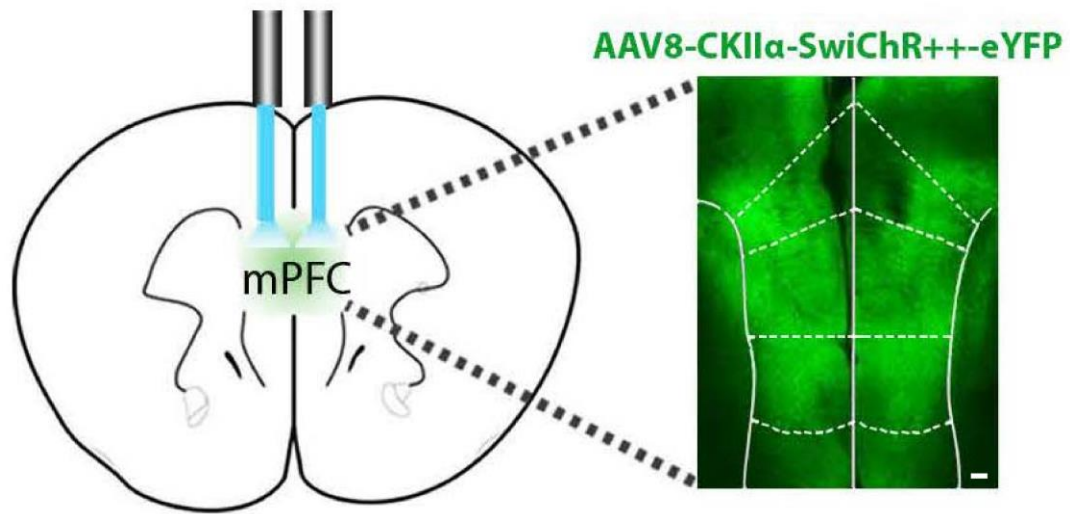


Fig. S1. Bilateral expression of SwiChR $^{++}$ in mPFC. AAV8-CKII α -SwiChR $^{++}$ -eYFP (green) in mPFC.in PYR neurons and fiber optic implants above mPFC. Scale bar represents 100 μ m.

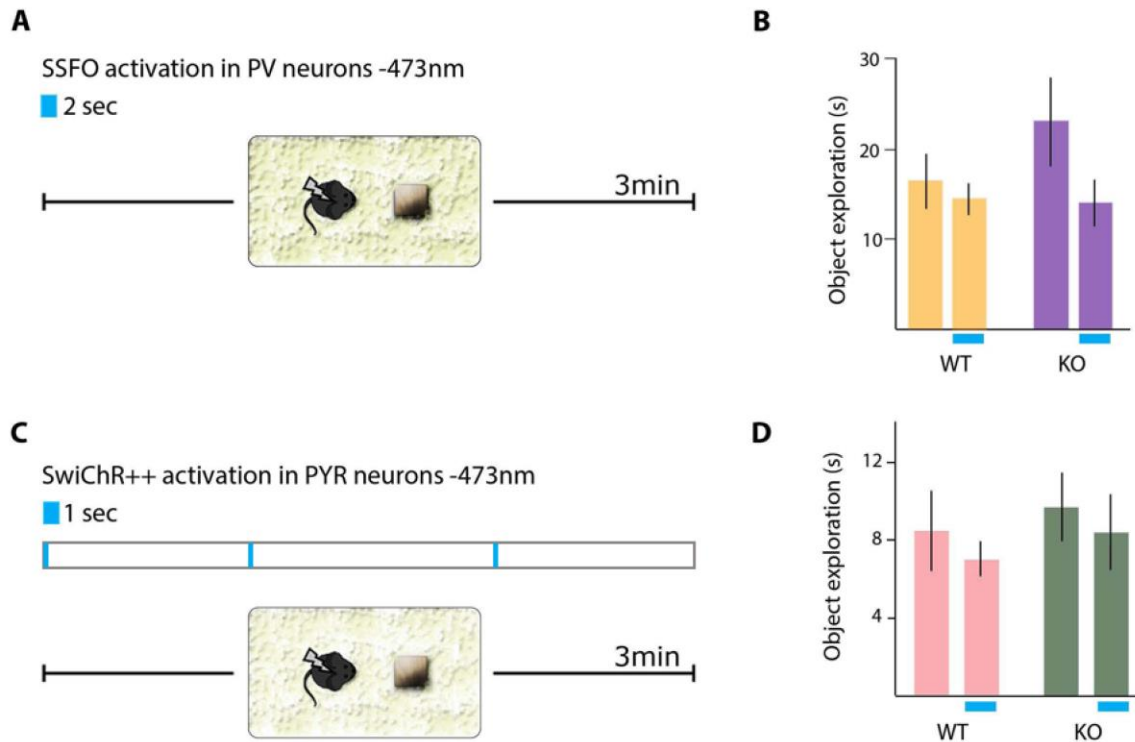


Fig. S2. CNTNAP2 KO and WT mice interact with objects similarly both in the presence and absence of SSFO recruitment. (A) 3 min object exploration upon 2 sec bilateral SSFO activation in mPFC. CNTNAP2 KO and WT mPFC were infected with AAV5-EF1 α ::DIO-SSFO-YFP. (B) Duration of social exploration over 3 min exploration time (Two-way ANOVA stimulation/genotype interaction $F_{1,32} = 1.348$, $p = 0.2542$; genotype effect $F_{1,32} = 1.035$, $p = 0.3166$; stimulation effect $F_{1,32} = 3.298$, $p = 0.0787$, followed by Dunnett's pairwise comparisons, no significant results. WT_{baseline}, $n = 10$; WT_{stimulation}, $n = 10$; KO_{baseline}, $n = 8$; KO_{stimulation}, $n = 8$ pairs). (C) 3 min object exploration during bilateral mPFC SwiChR++ activation with 1 sec light pulse/min. CNTNAP2 KO and WT mPFC were infected with AAV8-CKII α -SwiChR++-eYFP. (D) Duration of social exploration over 3 min exploration time (Two-way ANOVA stimulation/genotype interaction $F_{1,27} = 0.0025$, $p = 0.9599$; genotype effect $F_{1,27} = 0.5337$, $p = 0.4714$; stimulation effect $F_{1,27} = 0.81$, $p = 0.4525$, followed by Dunnett's pairwise comparisons, no significant results. WT_{baseline}, $n = 8$; WT_{stimulation}, $n = 7$; KO_{baseline}, $n = 8$; KO_{stimulation}, $n = 8$ pairs).

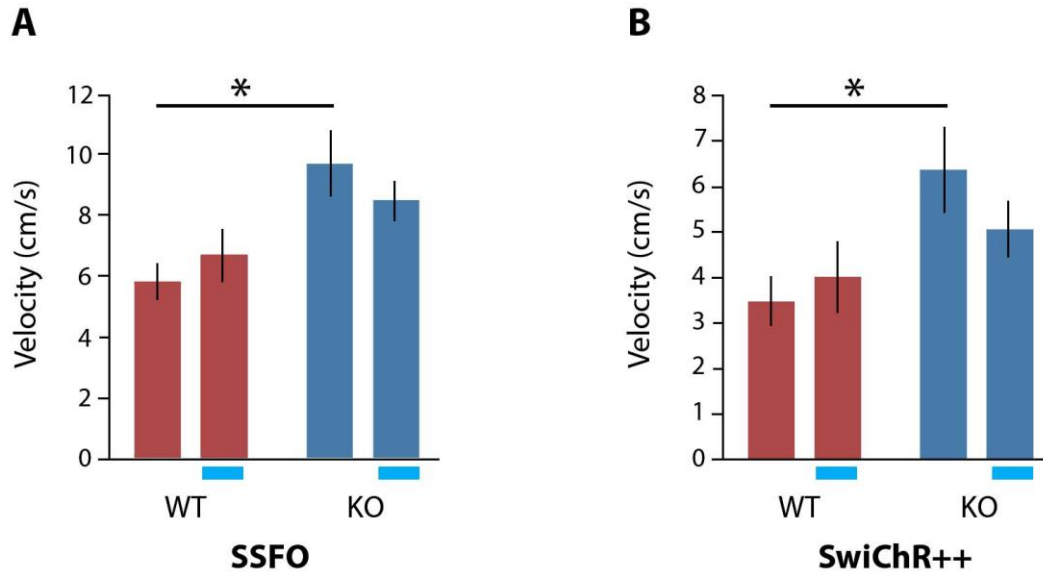


Fig. S3. Both SSFO and SwiChR++ recruitments rescue CNTNAP2 KO hyperactivity in the open-field test. (A) Average movement speed in the open-field box with and without 2 sec SSFO stimulation onto bilateral WT and KO mPFC that express AAV5-EF1 α ::DIO-SSFO-YFP. Two-way ANOVA stimulation/genotype interaction $F_{1,34} = 1.904$, $p = 0.1794$; genotype effect $F_{1,34} = 12.45$, $p = 0.0016$; stimulation effect $F_{1,34} = 0.0311$, $p = 0.8613$, followed by Dunnett's pairwise comparisons WT_{baseline} vs. KO_{baseline} $p < 0.001$, $WT_{\text{stimulation}}$ vs. $KO_{\text{stimulation}}$ $p > 0.05$. WT_{baseline} , $n = 8$; $WT_{\text{stimulation}}$, $n = 8$; KO_{baseline} , $n = 7$; $KO_{\text{stimulation}}$, $n = 7$ pairs. (B) Average movement speed of WT and KO mice in the open-field box in the presence and absence of bilateral SwiChR++ activation with 1 sec pulse/min. mPFC was targeted with AAV8-CKII α -SwiChR++-eYFP. Two-way ANOVA stimulation/genotype interaction $F_{1,30} = 0.148$, $p = 0.2328$; genotype effect $F_{1,30} = 6.77$, $p = 0.0143$; stimulation effect $F_{1,30} = 0.27$, $p = 0.6060$, followed by Dunnett's pairwise comparisons WT_{baseline} vs. KO_{baseline} $p < 0.05$, $WT_{\text{stimulation}}$ vs. $KO_{\text{stimulation}}$ $p > 0.05$. WT_{baseline} , $n = 8$; $WT_{\text{stimulation}}$, $n = 7$; KO_{baseline} , $n = 7$; $KO_{\text{stimulation}}$, $n = 8$ pairs.

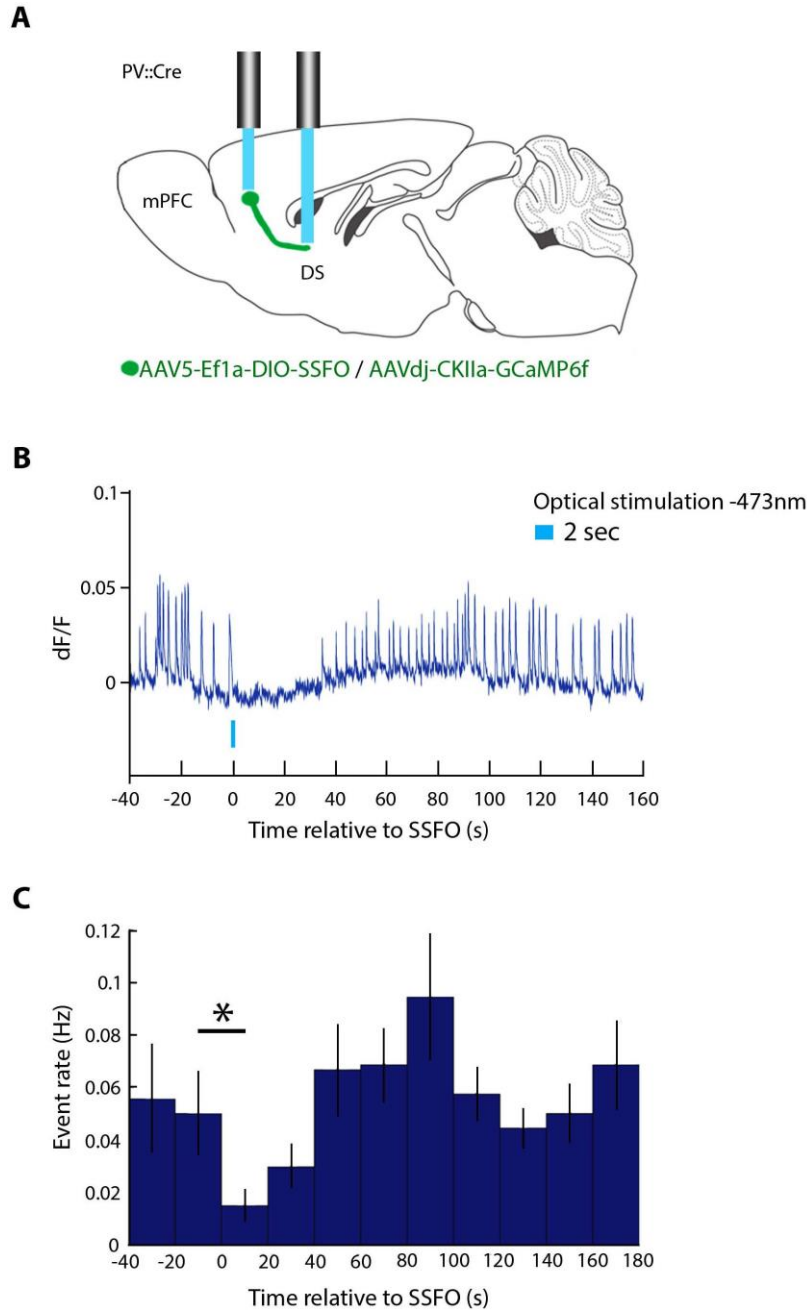


Fig. S4. Modulation of outgoing corticostriatal activity traffic by SSFO recruitment in mPFC PV neurons. (A) SSFO was expressed in mPFC PV+ inhibitory neurons, and GCaMP6f was expressed in CaMKII α + pyramidal neurons. Optical fibers were placed as indicated in mPFC and dorsal striatum. (B) Sample fiber photometry recording in dorsal striatum before and after mPFC PV SSFO activation. (C) Mean Ca²⁺ transient rates in mPFC axons projecting to dorsal striatum; note onset time of SSFO activation (time = 0). Wilcoxon rank-sum test comparing mean Ca²⁺ transient rates across trials 20s before and after SSFO activation (n = 9 trials from 3 mice, p = 0.038). Data presented as mean \pm SEM.

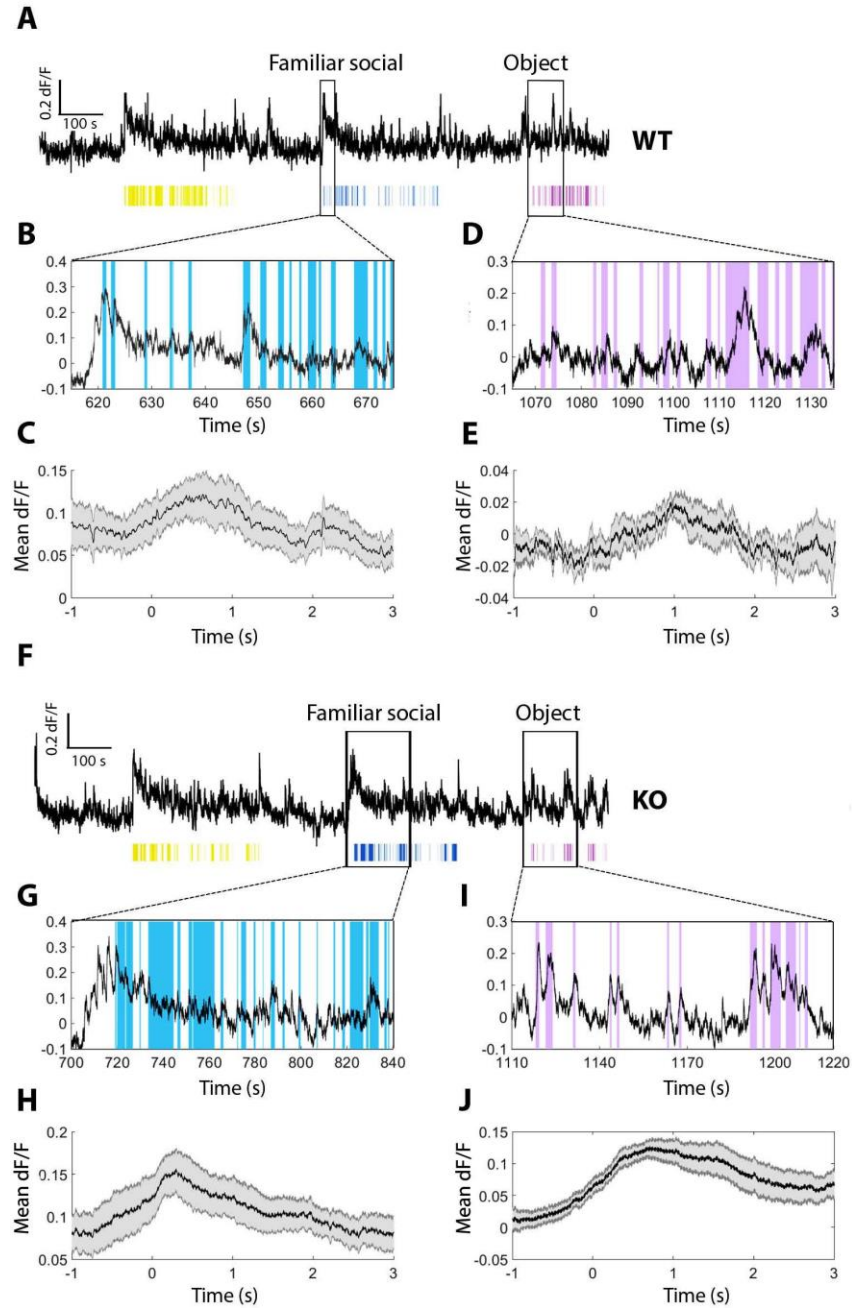


Fig. S5. Temporal details of interactions with familiar mouse and novel object. (A) Sample photometry trace of a WT animal. (B) Zoomed image of the beginning of familiar mouse social exploration. (C) Mean df/F aligned to the first 10 familiar mouse interaction bouts. (D) Zoomed image of the beginning of novel object exploration. (E) Mean df/F aligned to the first 10 novel object interaction bouts. (F) Sample photometry trace of a KO animal. (G) Zoomed image of the beginning of familiar mouse social exploration. (H) Mean df/F aligned to the first 10 familiar mouse interaction bouts. (I) Zoomed image of the beginning of novel object exploration. (J) Mean df/F aligned to the first 10 novel object interaction bouts. Colored vertical shaded areas correspond to interaction bouts.

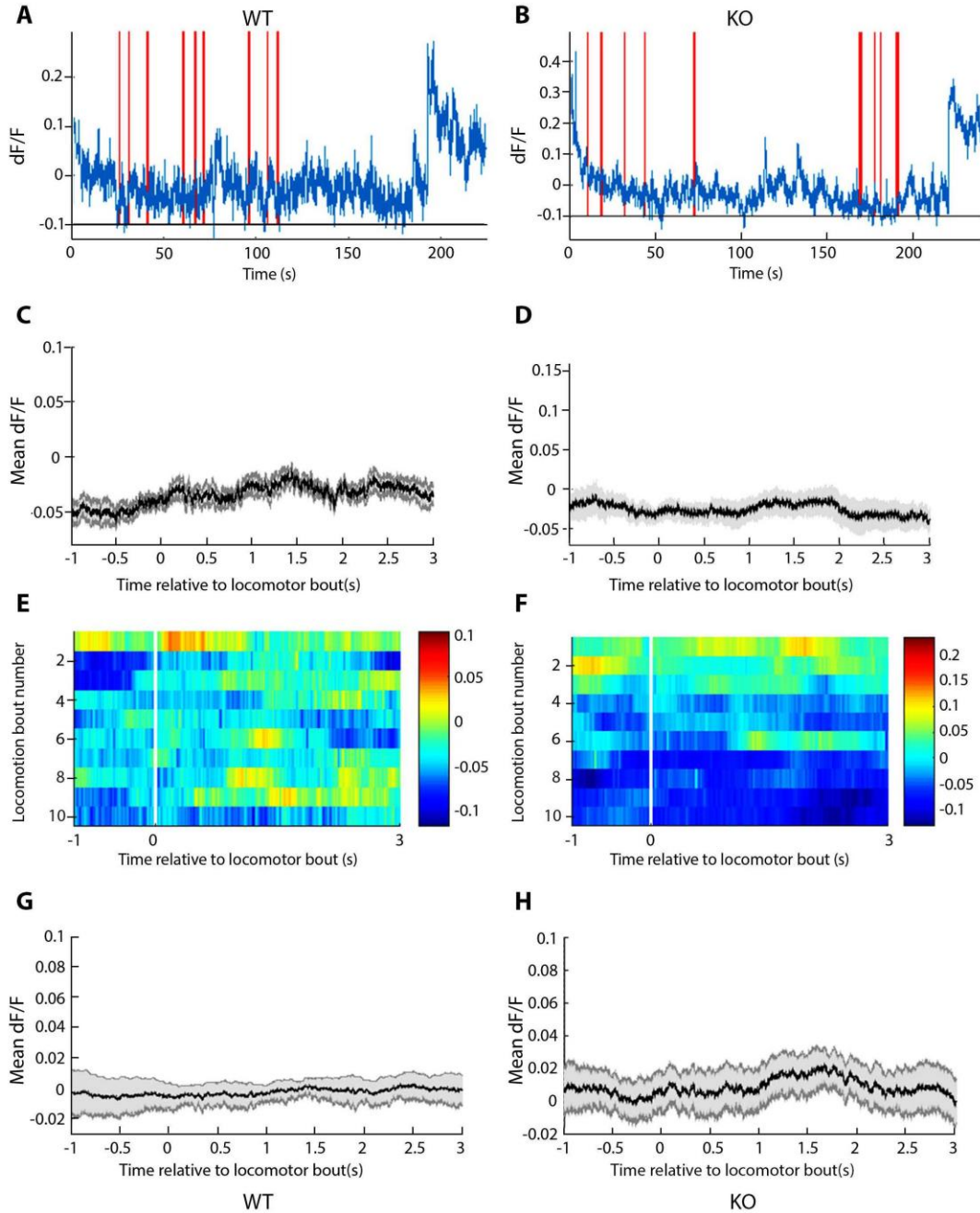


Fig. S6. Modulation of PV neuron activity with locomotion. (A, B) Representative photometry traces from a WT (A) and a CNTNAP2 KO (B) animal. Red lines correspond to locomotor bouts, where the animal initiates movement. (C, D) Corresponding peri-event plots aligned to the start of locomotion from the WT (C) and the KO (D) animal. Black line is the average of first 10 locomotor bouts, gray lines correspond to standard error. (E, F) Representative heatmap time-locked to the beginning of locomotion: warmer colors indicate higher fluorescence signal (E, WT; F, KO). (G, H) Mean peri-event photometry traces for WT (G), and KO (H) mice. Black line is the mean of first 10 locomotor bouts averaged over all animals. Gray lines correspond to standard error. (Paired t-tests: WT ($n = 5$), $p = 0.9974$; KO ($n = 5$), $p = 0.5202$).

Table S1. Raw data of Fig. 1E.

Replicate	Without SSFO	With SSFO
1	250	150
2	300	250
3	300	225
4	225	150
5	200	150
6	150	100
7	200	50
8	150	150

Table S2. Raw data of Fig. 1H.

Replicate	Final steady-state	Initial peak
1	76,31	72,15
2	111,35	108,58
3	48,52	51,43
4	123,13	118,54
5	63,18	63,1
6	39,38	40,16
7	215,71	216,39
8	79,57	78,96

Table S3. Raw data of Fig. 1I.

Replicate	Final steady-state	Initial peak
1	9,14	9,93
2	8,54	9,27
3	6,48	7,84
4	5,93	8,15
5	5,97	3,86
6	7,28	7,01
7	20,49	20,46
8	2	4,6

Table S4. Raw data of Fig. 1K.

Replicate	250 pA	500 pA	750 pA	1000 pA
1	98,6	100	100	100
2	40,2	100	100	100
3	100	100	100	100
4	100	100	100	100
5	100	100	100	100
6	100	100	100	100
7	100	100	100	100

Table S5. Raw data of Fig. 2B.

Replicate	WT		KO	
	Baseline	Stimulation	Baseline	Stimulation
1	5.40	5.58	3.57	6.89
2	9.11	16.88	3.85	16.34
3	6.46	15.47	4.07	14.26
4	5.94	12.15	3.35	10.28
5	10.63	7.66	5.22	7.61
6	3.47	14.72	3.60	14.72
7	9.85	9.30	3.96	13.04
8	22.03	7.62	4.58	6.73
9	4.46	5.27	3.91	10.12
10	6.00	12.22	4.98	N/A
11	8.43	4.02	6.00	N/A
12	6.90	13.50	5.76	N/A
13	N/A	N/A	3.39	N/A
14	N/A	N/A	5.63	N/A
15	N/A	N/A	6.38	N/A
16	N/A	N/A	2.57	N/A
17	N/A	N/A	5.15	N/A

Table S6. Raw data of Fig. 2C.

Replicate	WT		KO	
	Baseline	Stimulation	Baseline	Stimulation
1	59	83	59	64
2	66	59	57	71
3	52	59	31	34
4	40	40	14	61
5	32	36	27	53
6	92	107	28	64

7	91	48	26	47
8	32	91	32	81
9	33	35	64	57
10	109	27	48	N/A
11	54	87	30	N/A
12	N/A	86	17	N/A
13	N/A	30	18	N/A
14	N/A	46	25	N/A
15	N/A	N/A	41	N/A
16	N/A	N/A	21	N/A

Table S7. Raw data of Fig. 2D.

Replicate	WT		KO	
	Baseline	Stimulation	Baseline	Stimulation
1	3.35	3.34	1.19	2.26
2	2.92	2.05	1.86	2.55
3	1.91	2.85	1.53	2.59
4	2.24	2.42	1.69	2.62
5	2.28	1.93	1.45	2.95
6	2.06	3.49	2.25	3.06
7	1.92	2.14	2.01	1.69
8	2.97	1.94	1.60	2.28
9	1.75	2.36	1.85	2.02
10	3.23	2.82	1.17	1.76
11	1.89	2.34	0.95	2.39
12	1.04	3.46	1.08	2.29
13	1.32	1.08	1.29	2.41
14	2.13	2.62	0.98	1.44
15	1.91	2.57	N/A	N/A
16	N/A	0.90	N/A	N/A
17	N/A	1.73	N/A	N/A

Table S8. Raw data of Fig. 2E.

Replicate	WT		KO	
	Baseline	Stimulation	Baseline	Stimulation
1	116	112	149	136
2	113	106	125	122
3	61	112	125	137
4	123	134	147	131
5	116	122	154	110
6	116	130	148	116

7	118	25	160	105
8	122	103	168	14
9	124	105	153	124
10	47	107	130	113
11	108	29	135	N/A
12	81	N/A	127	N/A
13	N/A	N/A	132	N/A

Table S9. Raw data of Fig. 2G.

Replicate	WT		KO	
	Baseline	Stimulation	Baseline	Stimulation
1	2.70	5.84	1.98	7.33
2	3.60	4.48	3.54	10.48
3	8.95	6.79	3.52	6.25
4	12.93	6.43	2.55	8.09
5	2.99	17.64	4.79	2.99
6	13.51	4.62	2.94	7.07
7	5.06	8.31	4.96	3.27
8	3.80	12.41	3.34	4.99
9	2.95	4.62	2.16	8.42
10	12.66	3.10	2.01	9.47
11	3.78	8.92	2.95	4.78
12	2.76	5.60	4.69	3.80
13	22.26	9.59	N/A	5.41
14	N/A	N/A	N/A	4.58

Table S10. Raw data of Fig. 2H.

Replicate	WT		KO	
	Baseline	Stimulation	Baseline	Stimulation
1	29.16	30.46	19.90	43.42
2	20.81	28.92	27.41	37.89
3	59.82	57.77	21.02	68.15
4	42.08	41.96	13.59	61.64
5	61.76	84.62	38.86	82.39
6	70.17	16.64	24.90	41.96
7	35.12	19.02	58.79	40.36
8	38.86	83.69	25.78	47.87
9	46.03	18.47	31.70	61.51
10	46.95	33.33	15.18	37.42
11	91.94	53.18	26.42	37.41
12	47.54	40.72	61.41	32.37

13	90.38	65.86	N/A	44.08
----	-------	-------	-----	-------

Table S11. Raw data of Fig. 2I.

Replicate	WT		KO	
	Baseline	Stimulation	Baseline	Stimulation
1	0.42	0.50	0.34	0.49
2	0.43	0.45	0.37	0.44
3	0.62	0.62	0.38	0.53
4	0.48	0.47	0.18	0.70
5	0.66	1.00	0.38	0.51
6	0.87	0.32	0.32	0.64
7	0.54	0.36	0.53	0.48
8	0.53	0.89	0.32	0.40
9	0.74	0.34	N/A	0.49
10	N/A	N/A	N/A	0.80

Table S12. Raw data of Fig. 2J.

Replicate	WT		KO	
	Baseline	Stimulation	Baseline	Stimulation
1	110	165	208	167
2	117	122	150	152
3	140	140	173	132
4	127	113	150	127
5	135	137	138	145
6	129	104	118	117
7	140	104	137	113
8	106	100	126	162
9	127	106	121	105
10	110	N/A	N/A	113

Details of ANOVA statistical test in Figure 2.

(B) Social exploration duration during the first 10 interaction bouts with and without SSFO stimulation. (First 10 interaction bouts, two-way ANOVA treatment/genotype interaction $F_{1,47} = 3.608$, $p = 0.0636$; genotype effect $F_{1,47} = 0.2339$, $p = 1.454$; treatment effect $F_{1,47} = 15.21$ $p = 0.0003$, followed by Dunnett's pairwise comparisons $WT_{baseline}$ vs. $KO_{baseline}$ $p < 0.05$, $KO_{baseline}$ vs. $KO_{stimulation}$ $p < 0.0001$ ($WT_{baseline}$, $n = 12$; $WT_{stimulation}$, $n = 12$; $KO_{baseline}$, $n = 17$; $KO_{stimulation}$, $n = 9$ pairs) (C) Social exploration duration during the total length of the test with and without SSFO stimulation (Two-way ANOVA treatment/genotype interaction $F_{1,46} = 4.26$, $p = 0.0446$; genotype effect $F_{1,46} = 4.617$, $p =$

0.0370; treatment effect $F_{1,46} = 4.021$, $p = 0.0508$, followed by Dunnett's pairwise comparisons WT_{baseline} vs. KO_{baseline} $p < 0.05$, KO_{baseline} vs. $KO_{\text{stimulation}}$ $p < 0.05$. WT_{baseline} , $n = 11$; $WT_{\text{stimulation}}$, $n = 12$; KO_{baseline} , $n = 16$; $KO_{\text{stimulation}}$, $n = 9$ pairs). **(D)** Mean social exploration duration per interaction bout with and without SSFO stimulation (Two-way ANOVA treatment/genotype interaction $F_{1,59} = 2.427$, $p = 0.1246$; genotype effect $F_{1,59} = 3.515$, $p = 0.0658$; treatment effect $F_{1,59} = 6.775$, $p = 0.0117$, followed by Dunnett's pairwise comparisons WT_{baseline} vs. KO_{baseline} $p < 0.05$, KO_{baseline} vs. $KO_{\text{stimulation}}$ $p < 0.01$. WT_{baseline} , $n = 15$; $WT_{\text{stimulation}}$, $n = 17$; KO_{baseline} , $n = 14$; $KO_{\text{stimulation}}$, $n = 14$ pairs). **(E)** Mean social approach velocity with and without stimulation SSFO stimulation (Two-way ANOVA treatment/genotype interaction $F_{1,34} = 0.3717$, $p = 0.8195$; genotype effect $F_{1,34} = 11.16$, $p = 0.0020$; treatment effect $F_{1,34} = 5.059$, $p = 0.0311$, followed by Dunnett's pairwise comparisons to controls WT_{baseline} vs. KO_{baseline} $p < 0.001$, KO_{baseline} vs. $KO_{\text{stimulation}}$ $p < 0.05$. WT_{baseline} , $n = 12$; $WT_{\text{stimulation}}$, $n = 11$; KO_{baseline} , $n = 13$; $KO_{\text{stimulation}}$, $n = 10$ pairs). **(F)** Social interaction test with 1 s intermittent 473 nm pulse of light to activate SwiChR⁺⁺. **(G)** Social exploration duration during the first 10 interaction bouts with and without SwiChR⁺⁺ activation (Two-way ANOVA treatment/genotype interaction $F_{1,48} = 1.815$, $p = 0.1842$; genotype effect $F_{1,48} = 6.797$, $p = 0.0121$; treatment effect $F_{1,48} = 1.889$, $p = 0.1757$, followed by Dunnett's pairwise comparisons $WT_{\text{stimulation}}$ vs. KO_{baseline} $p < 0.01$, KO_{baseline} vs. $KO_{\text{stimulation}}$ $p < 0.05$. WT_{baseline} , $n = 13$; $WT_{\text{stimulation}}$, $n = 13$; KO_{baseline} , $n = 12$; $KO_{\text{stimulation}}$, $n = 14$ pairs). **(H)** Social exploration duration during the total length of the test with and without SwiChR⁺⁺ activation (Two-way ANOVA treatment/genotype interaction $F_{1,47} = 6.094$, $p = 0.0173$; genotype effect $F_{1,47} = 2.526$, $p = 0.1187$; treatment effect $F_{1,47} = 0.9239$, $p = 0.3414$, followed by Dunnett's pairwise comparisons WT_{baseline} vs. KO_{baseline} $p < 0.05$, KO_{baseline} vs. $KO_{\text{stimulation}}$ $p < 0.05$. WT_{baseline} , $n = 13$; $WT_{\text{stimulation}}$, $n = 13$; KO_{baseline} , $n = 12$; $KO_{\text{stimulation}}$, $n = 13$ pairs). **(I)** Mean social exploration duration per interaction bout with and without SwiChR⁺⁺ activation (Two-way ANOVA treatment/genotype interaction $F_{1,32} = 4.448$, $p = 0.0429$; genotype effect $F_{1,32} = 4.656$, $p = 0.0386$; treatment effect $F_{1,32} = 2.127$, $p = 0.1545$, followed by Dunnett's pairwise comparisons WT_{baseline} vs. KO_{baseline} $p < 0.05$, KO_{baseline} vs. $KO_{\text{stimulation}}$ $p < 0.05$. WT_{baseline} , $n = 9$; $WT_{\text{stimulation}}$, $n = 9$; KO_{baseline} , $n = 8$; $KO_{\text{stimulation}}$, $n = 10$ pairs). **(J)** Mean social approach velocity with and without stimulation SwiChR⁺⁺ activation (Two-way ANOVA treatment/genotype interaction $F_{1,32} = 1.214$, $p = 0.2788$; genotype effect $F_{1,32} = 7.667$, $p = 0.0093$; treatment effect $F_{1,32} = 1.575$, $p = 0.2185$, followed by Dunnett's pairwise comparisons WT_{baseline} vs. KO_{baseline} $p < 0.05$, KO_{baseline} vs. $KO_{\text{stimulation}}$ $p > 0.05$. WT_{baseline} , $n = 10$; $WT_{\text{stimulation}}$, $n = 9$; KO_{baseline} , $n = 9$; $KO_{\text{stimulation}}$, $n = 10$ pairs). 2 separate cohorts included in analyses, presented as mean \pm SEM.

Table S13. Raw data of Fig. 3G.

Replicate	WT			KO		
	Novel	Familiar	Object	Novel	Familiar	Object
1	67.96	34.91	14.64	40.44	33.32	17.76
2	68.27	74.25	14.80	37.68	15.62	23.57
3	76.38	18.65	17.91	14.91	38.14	13.17
4	116.10	18.41	58.10	44.76	60.94	73.88
5	63.45	17.76	16.31	90.75	78.21	24.07
6	101.68	27.16	49.79	75.88	36.30	46.45
7	N/A	N/A	10.07	56.02	60.94	73.56
8	N/A	N/A	14.42	37.06	14.74	13.55
9	N/A	N/A	27.29	49.79	N/A	13.42
10	N/A	N/A	6.45	53.77	N/A	96.93
11	N/A	N/A	N/A	99.19	N/A	17.07
12	N/A	N/A	N/A	18.05	N/A	N/A

Table S14. Raw data of Fig. 3H.

Replicate	WT		KO	
	First 10	Last 10	First 10	Last 10
1	4.24	0.85	1.87	0.95
2	4.83	2.50	1.04	0.91
3	4.78	1.86	1.59	2.18
4	6.49	4.25	7.07	1.47
5	5.63	1.29	5.74	1.08
6	7.44	2.31	2.96	2.73
7	N/A	N/A	0.78	2.62
8	N/A	N/A	4.47	0.82
9	N/A	N/A	3.05	1.71

Movie S1. *CNTNAP2* KO mPFC PV neuron activity during social behavior.

Movie S2. WT mPFC PV neuron activity during social behavior.