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# Neocortical synaptic engrams for remote contextual memories

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## SUPPLEMENTARY INFORMATION

**Supplementary Table 1 Summary of statistical analysis: t-test\***

Data	t-test	Response variable	Groups	DF**	t value	P value
<a href="#">Fig. 1c</a>	Unpaired	Freezing time	HC (10 mice) CFC (11 mice)	17	-1.05	0.308
<a href="#">Fig. 1e</a>	Unpaired	tdTomato+ cell density	HC (8 mice) CFC (7 mice)	6	-3.04	0.023
<a href="#">Fig. 1e</a>	Unpaired	c-Fos+ cell density	HC (8 mice) CFC (7 mice)	12	-1.43	0.179
<a href="#">Fig. 1e</a>	Unpaired	Fos+ proportion among tdT+ cells	HC (8 mice) CFC (7 mice)	12	-6.13	< 0.001
<a href="#">Fig. 1g</a>	Unpaired	tdTomato+ cell density	HC (6 mice) CFC (7 mice)	8	-2.51	0.036
<a href="#">Fig. 1g</a>	Unpaired	c-Fos+ cell density	HC (6 mice) CFC (7 mice)	9	-1.68	0.128
<a href="#">Fig. 1g</a>	Unpaired	Fos+ proportion among tdT+ cells	HC (6 mice) CFC (7 mice)	9	-5.35	< 0.001
<a href="#">Fig. 3f</a>	Unpaired	Freezing time	eYFP (13 mice) MutCREB (15 mice)	25	2.42	0.023
<a href="#">Fig. 3j</a>	Unpaired	AMPA EPSC	E-E synapses (26 cells) E-NE synapses (24 cells)	47	-1.11	0.273
<a href="#">Fig. 3j</a>	Unpaired	AMPA/NMDA ratio	E-E synapses (32 cells) E-NE synapses (28 cells)	57	-0.88	0.384
<a href="#">Fig. 4i</a>	Paired	qEPSC amplitude	tdT- vs tdT+ mPFC neurons (16 pairs of neurons)	15	0.29	0.776
<a href="#">Fig. 4j</a>	Unpaired	qEPSC (E-E) - qEPSC (E-NE)	7 days after CFC (16 pairs) 28 days after CFC (27 pairs)	32	-2.30	0.028
<a href="#">Fig. 5c</a>	Unpaired	tdTomato+ cell density	Casp3- (11 mice) Casp3+ (10 mice)	18	4.18	< 0.001
<a href="#">Fig. 5e</a>	Unpaired	Fos+ proportion among tdT+ cells	Casp3- (8 mice) Casp3+ (9 mice)	13	3.32	0.006
<a href="#">Fig. 5f</a>	Unpaired	Fos+ proportion among tdT+ cells	Casp3- (8 mice) Casp3+ (9 mice)	13	5.09	< 0.001
<a href="#">Fig. 5h</a>	Unpaired	Freezing time	Casp3- (4 mice) Casp3+ (5 mice)	3	7.53	0.005
<a href="#">Fig. 5j</a>	Paired	qEPSC amplitude (DG: Casp3+ group)	tdT- (E-NE) vs tdT+ (E-E) neurons (20 pairs of neurons)	19	-0.04	0.972
<a href="#">Fig. 5j</a>	Paired	qEPSC amplitude (DG: Casp3- group)	tdT- (E-NE) vs tdT+ (E-E) neurons (16 pairs of neurons)	15	-2.92	0.011
<a href="#">Fig. 6k</a>	Unpaired	Freezing time	dCA1: Casp3- (6 mice) dCA1: Casp3+ (6 mice)	6	3.14	0.020
<a href="#">Fig. 6k</a>	Unpaired	Freezing time	RSC: Casp3- (9 mice) RSC: Casp3+ (10 mice)	16	3.41	0.004
<a href="#">Fig. 7e</a>	Unpaired	AMPA/NMDA ratio	E-E synapses (20 cells) E-NE synapses (19 cells)	31	-3.64	0.001

Data	t-test	Response variable	Groups	DF**	t value	P value
<b>Fig. 8g</b>	Paired	AMPA/NMDA ratio	tdT- vs tdT+ mPFC neurons (13 pairs of neurons)	12	-5.00	< 0.001
<b>Ext. Data Fig. 1b</b>	Unpaired	Freezing time	Same context (9 mice) Different context (9 mice)	15	1.95	0.070
<b>Ext. Data Fig. 2e</b>	Paired	AMPA EPSC amplitude	tdT- (NE-NE) vs tdT+ (NE-E) neurons (13 pairs of neurons)	12	-2.24	0.043
<b>Ext. Data Fig. 2e</b>	Paired	AMPA/NMDA ratio	tdT- (NE-NE) vs tdT+ (NE-E) neurons (14 pairs of neurons)	13	-2.68	0.019
<b>Ext. Data Fig. 2h</b>	Paired	AMPA EPSC amplitude	tdT- (NE-NE) vs tdT+ (NE-E) neurons (14 pairs of neurons)	13	-1.09	0.295
<b>Ext. Data Fig. 2h</b>	Paired	AMPA/NMDA ratio	tdT- (NE-NE) vs tdT+ (NE-E) neurons (15 pairs of neurons)	14	-3.82	0.002
<b>Ext. Data Fig. 2k</b>	Paired	AMPA EPSC amplitude	tdT- (NE-NE) vs tdT+ (NE-E) neurons (12 pairs of neurons)	11	-0.15	0.886
<b>Ext. Data Fig. 2k</b>	Paired	AMPA/NMDA ratio	tdT- (NE-NE) vs tdT+ (NE-E) neurons (12 pairs of neurons)	11	0.06	0.954
<b>Ext. Data Fig. 3d</b>	Unpaired	Freezing time	mCherry (9 mice) hM4Di (9 mice)	15	2.68	0.017
<b>Ext. Data Fig. 3e</b>	Unpaired	Freezing time	mCherry (13 mice) hM4Di (10 mice)	20	-0.06	0.952
<b>Ext. Data Fig. 4d</b>	Paired	AMPA EPSC amplitude	tdT- (NE-NE) vs tdT+ (NE-E) neurons (11 pairs of neurons)	10	-1.11	0.293
<b>Ext. Data Fig. 4d</b>	Paired	AMPA/NMDA ratio	tdT- (NE-NE) vs tdT+ (NE-E) neurons (12 pairs of neurons)	11	-1.31	0.219
<b>Ext. Data Fig. 4e</b>	Unpaired	AN (E-E) - AN (E-NE)	Engram inputs (30 pairs) Nonengram inputs (12 pairs)	37	2.92	0.006
<b>Ext. Data Fig. 5e</b>	Paired	AMPA EPSC	tdT- (E-NE) vs tdT+ (E-E) neurons (16 pairs of neurons)	15	-3.52	0.003
<b>Ext. Data Fig. 5e</b>	Paired	AMPA/NMDA ratio	tdT- (E-NE) vs tdT+ (E-E) neurons (13 pairs of neurons)	12	-4.31	0.001
<b>Ext. Data Fig. 5j</b>	Paired	AMPA EPSC	tdT- (E-NE) vs tdT+ (E-E) neurons (13 pairs of neurons)	12	-1.52	0.155
<b>Ext. Data Fig. 5j</b>	Paired	AMPA/NMDA ratio	tdT- (E-NE) vs tdT+ (E-E) neurons (13 pairs of neurons)	12	0.00	0.996
<b>Ext. Data Fig. 6d</b>	Paired	qEPSC amplitude	tdT- (E-NE) vs tdT+ (E-E) neurons (20 pairs of neurons)	19	-3.37	0.003
<b>Ext. Data Fig. 6f</b>	Paired	mEPSC amplitude	tdT- vs tdT+ neurons (11 pairs of neurons)	10	-0.42	0.681
<b>Ext. Data Fig. 6i</b>	Paired	qEPSC amplitude	tdT- (E-NE) vs tdT+ (E-E) neurons (14 pairs of neurons)	13	-0.17	0.870
<b>Ext. Data Fig. 7d</b>	Paired	qIPSC amplitude	tdT- vs tdT+ mPFC neurons (24 pairs of neurons)	23	3.35	0.003
<b>Ext. Data Fig. 8c</b>	Paired	AMPA/NMDA ratio	tdT- (NE-NE) vs tdT+ (NE-E) neurons (13 pairs of neurons)	12	-0.20	0.848
<b>Ext. Data Fig. 8g</b>	Paired	qEPSC amplitude	tdT- (E-NE) vs tdT+ (E-E) neurons (20 pairs of neurons)	19	-0.57	0.574

Data	t-test	Response variable	Groups	DF**	t value	P value
<a href="#">Ext. Data Fig. 8h</a>	Unpaired	tdTomato+ cell density	Casp3- (5 mice) Casp3+ (6 mice)	4	5.01	0.007
<a href="#">Ext. Data Fig. 8i</a>	Unpaired	tdTomato+ cell density	Casp3- (7 mice) Casp3+ (9 mice)	7	7.78	< 0.001
<a href="#">Ext. Data Fig. 9f</a>	Unpaired	Freezing time	eYFP (11 mice) PSAM4 (9 mice)	17	2.13	0.048
<a href="#">Ext. Data Fig. 9g</a>	Unpaired	Freezing time	eYFP (6 mice) PSAM4 (8 mice)	11	-0.20	0.845
<a href="#">Ext. Data Fig. 9j</a>	Paired	AMPA/NMDA ratio	tdT- vs tdT+ BLA neurons (12 pairs of neurons)	11	-3.99	0.002
<a href="#">Ext. Data Fig. 9k</a>	Paired	AMPA/NMDA ratio	tdT- vs tdT+ BLA neurons (16 pairs of neurons)	15	-0.73	0.478

\* All t-tests were two-sided.

\*\* DF: degree of freedom

## Supplementary Table 2 Summary of statistical analysis: ANOVA

Data	ANOVA	Response variable	Factors	DF*	F value	P value			
Fig. 1f	Repeated measures two-way	Freezing time (%)	Groups	(1,20)	2.74	0.114			
			ChR2 (13 mice)						
			eYFP (9 mice)						
			Behavioral session	(1,20)	4.91	0.038			
			Laser on						
			Laser off						
			Subject	(20,20)	7.18	< 0.001			
			Interaction (group x behavioral session)	(1,20)	9.14	0.007			
			** Post hoc comparisons						
			ChR2: laser on versus laser off: P = 0.003						
eYFP: laser on versus laser off: P = 1.000									
ChR2 / laser on versus eYFP / laser on: P = 0.021									
Fig. 2e, 2h	Two-way	AMPA EPSC	Test day	(1,92)	17.17	< 0.001			
			28 days after CFC (Fig. 2e)						
			7 days after CFC (Fig. 2h)						
			Synapses	(1,92)	8.44	0.005			
			E-E synapses						
			E-NE synapses						
			Interaction (test day x synapses)	(1,92)	4.09	0.046			
			** Post hoc comparisons						
			28 days after CFC: E-E versus E-NE synapses: P < 0.001						
			7 days after CFC: E-E versus E-NE synapses: P = 1.000						
E-E synapses: 28 days versus 7 days after CFC: P < 0.001									
Fig. 2e, 2h	Two-way	AMPA/NMDA ratio	Test day	(1,93)	0.97	0.327			
			28 days after CFC (Fig. 2e)						
			7 days after CFC (Fig. 2h)						
			Synapses	(1,93)	10.08	0.002			
			E-E synapses						
			E-NE synapses						
			Interaction (test day x synapses)	(1,93)	8.10	0.005			
			** Post hoc comparisons						
			28 days after CFC: E-E versus E-NE synapses: P < 0.001						
			7 days after CFC: E-E versus E-NE synapses: P = 1.000						
E-E synapses: 28 days versus 7 days after CFC: P = 0.049									
Fig. 3d	One-way	AMPA/NMDA ratio	Synapses	(2,46)	10.41	< 0.001			
			E-NE synapses (18 cells)						
			E-E/MutCREB- synapses (13 cells)						
			E-E/MutCREB+ synapses (18 cells)						
			** Post hoc comparisons						
			E-NE versus E-E/MutCREB- synapses: P < 0.001						
			E-E/MutCREB- versus E-E/MutCREB+ synapses: P = 0.001						
			E-NE versus E-E/MutCREB+ synapses: P = 1.000						
			Fig. 4e, 4g	Two-way	EPSC amplitude	EPSC	(1,47)	2.14	0.148
						qEPSC (Fig. 4e)			
mEPSC (Fig. 4g)									
Postsynaptic neurons	(1,47)	4.88				0.032			
tdT- neurons									
tdT+ neurons									
Cell pairs	(47,47)	3.80				< 0.001			
qEPSC (27 pairs of tdT- / tdT+ neurons)									
mEPSC (22 pairs of tdT- / tdT+ neurons)									
Interaction (EPSC x postsynaptic neurons)	(1,47)	4.39				0.042			
** Post hoc comparisons									
qEPSC: tdT- (E-NE) versus tdT+ (E-E): P = 0.014									
mEPSC: tdT- versus tdT+: P = 1.000									

\* DF: degree of freedom

\*\* Post hoc Bonferroni's simultaneous multiple comparisons

Data	ANOVA	Response variable	Factors	DF*	F value	P value
Fig. 5d	Repeated measures two-way	Freezing time (%)	Groups	(1,24)	28.99	< 0.001
			Casp3- (9 mice)			
			Casp3+ (17 mice)			
			Recall session	(1,24)	4.88	0.037
			Recent memory			
			Remote memory			
			Subject	(24,24)	1.55	0.145
			Interaction (group x recall session)	(1,24)	25.73	< 0.001
			** Post hoc comparisons			
			Recent memory recall: Casp3- versus Casp3+: P = 1.000			
Remote memory recall: Casp3- versus Casp3+: P < 0.001						
Fig. 7e	Repeated measures two-way	AMPA EPSC	BLA neurons	(1,24)	15.30	< 0.001
			tdT- neurons			
			tdT+ neurons			
			Photostimulation intensity	(2,24)	10.56	0.003
			6.3, 13.4, and 20.5 mW/mm <sup>2</sup>			
			Cell	(27,24)	7.11	< 0.001
Fig. 7i	Repeated measures two-way	AMPA EPSC	Interaction (BLA neurons x photostim)	(2,24)	0.41	0.669
			mPFC neurons	(1,60)	0.00	0.997
			mCherry- neurons			
			mCherry+ neurons			
			Photostimulation intensity	(3,60)	32.33	< 0.001
			2.8, 6.3, 13.4, and 20.5 mW/mm <sup>2</sup>			
Fig. 8g	Repeated measures two-way	AMPA EPSC	Cell	(20,60)	8.19	< 0.001
			Interaction (mPFC neurons x photostim)	(3,60)	0.09	0.964
			mPFC neurons	(1,56)	158.02	< 0.001
			tdT- neurons			
			tdT+ neurons			
			Photostimulation intensity	(2,56)	45.60	< 0.001
Ext. Data Fig. 3c	Repeated measures two-way	AP firing number	6.3, 13.4, and 20.5 mW/mm <sup>2</sup>			
			Cell	(28,56)	30.35	< 0.001
			Interaction (mPFC neurons x photostim)	(2,56)	3.23	0.047
			** Post hoc comparisons			
			6.3 mW/mm <sup>2</sup> : tdT- versus tdT+: P < 0.001			
			13.4 mW/mm <sup>2</sup> : tdT- versus tdT+: P < 0.001			
			20.5 mW/mm <sup>2</sup> : tdT- versus tdT+: P < 0.001			
			CNO treatment	(1,54)	26.17	< 0.001
			Pre-CNO			
			Post-CNO			
Ext. Data Fig. 10d	Repeated measures two-way	Freezing time (%)	Current injection	(3,54)	17.84	< 0.001
			100, 200, 300, and 400 pA			
			Cell (9 cells)	(8,54)	4.89	< 0.001
			Interaction (CNO x current injection)	(3,54)	2.2	0.098
			Groups	(1,13)	24.63	< 0.001
			mCherry (8 mice)			
Ext. Data Fig. 10f	Repeated measures two-way	Freezing time (%)	hM4Di (7 mice)			
			Recall session	(1,13)	59.50	< 0.001
			Recall 1 (+Tam)			
			Recall 2 (+CNO)			
			Subject	(13,13)	5.71	0.002
			Interaction (group x recall session)	(1,13)	9.51	0.009
			** Post hoc comparisons			
			Recall 1 (+Tam): mCherry versus hM4Di: P = 1.000			
			Recall 2 (+CNO): mCherry versus hM4Di: P < 0.001			
			Ext. Data Fig. 10f	Repeated measures two-way	Freezing time (%)	Groups
mCherry (9 mice)						
hM4Di (10 mice)						
Recall session	(1,17)	4.63				0.046
Recall 1 (+Tam)						
Recall 2 (+CNO)						
Ext. Data Fig. 10f	Repeated measures two-way	Freezing time (%)	Subject	(17,17)	2.19	0.058
			Interaction (group x recall session)	(1,17)	0.01	0.934