nature
SUPPLEMENTARY INFORMATION
https://doi.org/10.1038/s41586-018-0078-2
In the format provided by the authors and unedited.

A midline thalamic circuit determines reactions to visual threat

Lindsey D. Salay[1], Nao Ishiko[1] & Andrew D. Huberman[1,2,3,4]*

[1]Department of Neurobiology, Stanford University School of Medicine, Palo Alto, CA, USA. [2]Stanford Neurosciences Institute, Stanford University School of Medicine, Palo Alto, CA, USA. [3]BioX, Stanford University School of Medicine, Palo Alto, CA, USA. [4]Department of Ophthalmology, Stanford University School of Medicine, Palo Alto, CA, USA. *e-mail: adh1@stanford.edu

Figure	Description	Sample size (figure order)	Normality test	Statistical test	Treatment effect	P value	Significance
Figure 1b	% of time	*n*= 15, 15, 5 mice					
	freezing		not passed	Kruskal-Wallis one-way ANOVA	Kruskal-Wallis statistic = 17.69	*P* = 0.0001	***
				Dunn's multiple comparisons test	Mean rank diff.		
	none vs. above				-11.67	*P*=0.0003	***
	below vs. above				-11.67	*P*=0.0162	*
	none vs. below				0	*P*>0.9999	ns
	hiding		not passed	Kruskal-Wallis one-way ANOVA	Kruskal-Wallis statistic = 6.533	*P* = 0.0381	*
				Dunn's multiple comparisons test	Mean rank diff.		
	none vs. above				-5.9	*P*=0.2810	ns
	below vs. above				6.033	*P*=0.6763	ns
	none vs. below				-11.93	*P*=0.05	ns
	ambulatory		not passed	Kruskal-Wallis one-way ANOVA	Kruskal-Wallis statistic = 28.181	*P* <0.0001	***
				Dunn's multiple comparisons test	Mean rank diff.		
	none vs. above				19.8375	*P*<0.0001	***
	below vs. above				11.4	*P*=0.1017	ns
	none vs. below				8.4375	*P*=0.3407	ns
	running		not passed	Kruskal-Wallis one-way ANOVA	Kruskal-Wallis statistic = 7.527	*P* =0.0232	*
				Dunn's multiple comparisons test	Mean rank diff.		
	none vs. above				-5.833	*P*=0.0308	*
	below vs. above				-5.833	*P*=0.2087	ns
	none vs. below				0	*P*>0.9999	ns
	tail rattling		not passed	Kruskal-Wallis one-way ANOVA	Kruskal-Wallis statistic = 5.825	*P* =0.0543	ns
				Dunn's multiple comparisons test	Mean rank diff.		
	none vs. above				-4.667	*P*=0.0719	ns
	below vs. above				-4.667	*P*=0.3312	ns
	none vs. below				0	*P*>0.9999	ns

Figure 1c	number of c-Fos+ cells in the vMT	*n*= 7,10, 5 mice	passed	one-way ANOVA	$F_{2,19}$=11.63	*P*=0.0005	***
	above vs. none			Tukey's multiple comparisons test		*P*=0.0005	***
	above vs. below			Tukey's multiple comparisons test		*P*=0.0061	**
	none vs. below			Tukey's multiple comparisons test		*P*=0.9239	ns

Figure 1d	number of c-Fos+ cells in the Xi	*n*= 7,10, 5 mice	passed	one-way ANOVA	$F_{2,19}$=19.75	*P*<0.0001	***
	above vs. none			Tukey's multiple comparisons test		*P*<0.0001	***
	above vs. below			Tukey's multiple comparisons test		*P*=0.0001	***
	none vs. below			Tukey's multiple comparisons test		*P*=0.7852	ns

Figure 2i	% of time	*n*= 24, 9, 15 mice					
	freezing		not passed	Kruskal-Wallis one-way ANOVA	Kruskal-Wallis statistic = 1.905	*P* = 0.3858	ns
				Dunn's multiple comparisons test	Mean rank diff.		
	controls vs. hM4D				-1.517	*P*>0.9999	ns
	controls vs. hM3D				5.367	*P*=0.7220	ns
	hM4D vs. hM3D				6.883	*P*=0.6750	ns
	hiding		not passed	Kruskal-Wallis one-way ANOVA	Kruskal-Wallis statistic = 1.574	*P* = 0.4552	ns
				Dunn's multiple comparisons test	Mean rank diff.		

	controls vs. hM4D				2.554	P>0.9999	ns
	controls vs. hM3D				5.39	P=0.6356	ns
	hM3D vs. hM4D				-2.836	P>0.9999	ns
	ambulatory		not passed	Kruskal-Wallis one-way ANOVA	Kruskal-Wallis statistic = 11.51	P =0.0032	**
				Dunn's multiple comparisons test	Mean rank diff.		
	controls vs. hM4D				-1.113	P>0.9999	ns
	controls vs. hM3D				-14.98	P=0.0033	**
	hM3D vs. hM4D				13.87	P=0.0449	*
	running		not passed	Kruskal-Wallis one-way ANOVA	Kruskal-Wallis statistic = 1.639	P =0.4407	ns
				Dunn's multiple comparisons test	Mean rank diff.		
	controls vs. hM4D				2.942	P>0.9999	ns
	controls vs. hM3D				-3.458	P>0.9999	ns
	hM4D vs. hM3D				-6.4	P=0.6283	ns
	tail rattling		not passed	Kruskal-Wallis one-way ANOVA	Kruskal-Wallis statistic = 14.58	P =0.0007	***
				Dunn's multiple comparisons test	Mean rank diff.		
	controls vs. hM4D				6.229	P=0.5169	ns
	controls vs. hM3D				-11.57	P=0.0112	*
	hM4D vs. hM3D				-17.8	P=0.0010	***

Figure 2j	number of events	*n*= 24, 9, 15 mice					
	running		not passed	Poisson GLM	$X^2_{2,46}$=7.2	P=0.02851	*
				pairwise comparisions			
	controls vs. hM4D					P=0.51525	ns
	controls vs. hM3D					P=0.03103	*
	hM4D vs. hM3D					P=0.0438	*
	tail rattling		not passed	Quasi-Poisson GLM	$F_{2,46}$=16.075	P=0.000005	***
				pairwise comparisions			
	controls vs. hM4D					P=0.164	ns
	controls vs. hM3D					P=0.0003	***
	hM4D vs. hM3D					P=0.0000004	***

Figure 2k	% of mice rattling	*n*= 24, 9, 15 mice					
	controls vs. hM4D			Fisher's exact test		P=0.1747	ns
	controls vs. hM3D			Fisher's exact test		P=0.0079	**
	hM4D vs. hM3D			Fisher's exact test		P=0.0020	**

Figure 2l	% of rattling events in the open	*n*= 14, 0, 53 rattles					
	controls vs. hM3D			Fisher's exact test		P<0.0001	***

Figure 2m	% of running in the open	*n*=11, 3, 16 runs					
	controls vs. hM4D			Fisher's exact test		*P*>0.9999	ns
	controls vs. hM3D			Fisher's exact test		*P*=0.0003	***
	hM4D vs. hM3D			Fisher's exact test		*P*=0.0206	*

Figure 2n	% time motile	*n*= 24, 9, 15 mice					
			not passed	Kruskal-Wallis one-way ANOVA	Kruskal-Wallis statistic = 15.58	*P* =0.0004	***
				Dunn's multiple comparisons test	Mean rank diff.		
	controls vs. hM4D				-0.3042	*P*>0.9999	ns
	controls vs. hM3D				-17.29	*P*=0.0006	***
	hM3D vs. hM4D				16.98	*P*=0.0093	**

Figure 3q	% of time	*n*= 14, 8,8, 5 mice					
	freezing		not passed	Kruskal-Wallis one-way ANOVA	Kruskal-Wallis statistic = 7.012	*P* = 0.0715	ns
				Dunn's multiple comparisons test	Mean rank diff.		
	controls vs. vMT-BLA				-7.598	*P*=0.2503	ns
	controls vs. vMT-PFC				-3.286	*P*>0.9999	ns
	controls vs. vMT-PFC terminals				6.664	*P*=0.5895	ns
	hiding		not passed	Kruskal-Wallis one-way ANOVA	Kruskal-Wallis statistic = 3.153	*P* = 0.3686	ns
				Dunn's multiple comparisons test	Mean rank diff.		
	controls vs. vMT-BLA				6.045	*P*=0.3174	ns
	controls vs. vMT-PFC				0.607	*P*>0.9999	ns
	controls vs. vMT-PFC terminals				4.107	*P*>0.9999	ns
	ambulatory		not passed	Kruskal-Wallis one-way ANOVA	Kruskal-Wallis statistic = 6.577	*P* =0.0867	ns
				Dunn's multiple comparisons test	Mean rank diff.		
	controls vs. vMT-BLA				4.786	*P*=0.7688	ns
	controls vs. vMT-PFC				5.973	*P*=0.4695	ns
	controls vs. vMT-PFC terminals				-6.464	*P*=0.5762	ns
	running		not passed	Kruskal-Wallis one-way ANOVA	Kruskal-Wallis statistic = 8.423	*P* =0.0380	*
				Dunn's multiple comparisons test	Mean rank diff.		
	controls vs. vMT-BLA				3.83	*P*=0.9332	ns
	controls vs. vMT-PFC				1.768	*P*>0.9999	ns
	controls vs. vMT-PFC terminals				-9.707	*P*=0.0869	ns
	tail rattling		not passed	Kruskal-Wallis one-way ANOVA	Kruskal-Wallis statistic = 10.83	*P* =0.0127	*
				Dunn's multiple comparisons test	Mean rank diff.		
	controls vs. vMT-BLA				2.741	*P*>0.9999	ns
	controls vs. vMT-PFC				-3.759	*P*>0.9999	ns

	controls vs. vMT-PFC terminals				-12.87	P=0.0160	*

Figure 3r	number of events	n= 14, 8,8, 5 mice					
	running		not passed	Poisson GLM	$X^2_{3,31}$= 7.8	P=0.04935	*
				pairwise comparisions			
	controls vs. vMT-BLA					P=0.3378	ns
	controls vs. vMT-PFC					P=0.6699	ns
	controls vs. vMT-PFC terminals					P=0.0453	*
	tail rattling		not passed	Quasi-Poisson GLM	$F_{3,31}$=5.2864	P=0.004626	**
				pairwise comparisions			
	controls vs. vMT-BLA					P=0.4183	ns
	controls vs. vMT-PFC					P=0.0275	*
	controls vs. vMT-PFC terminals					P=0.0133	*

Figure 4e	% of time	n= 36, 17, 15 mice					
	freezing		not passed	Kruskal-Wallis one-way ANOVA	Kruskal-Wallis statistic = 5.587	P = 0.0612	ns
				Dunn's multiple comparisons test	Mean rank diff.		
	controls vs. co-activate				13.13	P=0.0363	*
	controls vs. pre-activate				3.825	P>0.9999	ns
	pre-activate vs. co-activate				-9.3	P=0.4929	ns
	hiding		not passed	Kruskal-Wallis one-way ANOVA	Kruskal-Wallis statistic = 1.201	P =0.5486	ns
				Dunn's multiple comparisons test	Mean rank diff.		
	controls vs. co-activate				-6.119	P=0.8195	ns
	controls vs. pre-activate				-2.006	P>0.9999	ns
	pre-activate vs. co-activate				4.114	P>0.9999	ns
	ambulatory		not passed	Kruskal-Wallis one-way ANOVA	Kruskal-Wallis statistic = 3.257	P =0.1962	ns
				Dunn's multiple comparisons test	Mean rank diff.		
	controls vs. co-activate				-10.42	P=0.2134	ns
	controls vs. pre-activate				-3.172	P>0.9999	ns
	co-activate vs. pre-activate				7.251	P=0.8911	ns
	running		not passed	Kruskal-Wallis one-way ANOVA	Kruskal-Wallis statistic = 10.48	P =0.0053	**
				Dunn's multiple comparisons test	Mean rank diff.		
	controls vs. co-activate				-16.84	P=0.0041	**
	controls vs. pre-activate				-7.797	P=0.4663	ns
	co-activate vs. pre-activate				9.045	P=0.4585	ns

	tail rattling		not passed	Kruskal-Wallis one-way ANOVA	Kruskal-Wallis statistic = 7.781	*P* =0.0204	*
				Dunn's multiple comparisons test	Mean rank diff.		
	controls vs. co-activate				-14.86	*P*=0.0236	*
	controls vs. pre-activate				-9.472	*P*=0.3145	ns
	co-activate vs. pre-activate				5.392	*P*>0.9999	ns

Figure 4f	number of events	*n*= 36, 17, 15 mice					
	tail rattling		not passed	Quasi-Poisson GLM	$F_{2,65}$=5.1249	P=0.008576	**
				pairwise comparisions			
	controls vs. co-activate					*P*=0.00342	**
	controls vs. pre-activate					*P*=0.04222	*
	pre-activate vs. co-activate					*P*=0.45556	ns
	running		not passed	Poisson GLM	$X^2_{2,65}$= 11.7	P=0.002832	**
				pairwise comparisions			
	controls vs. co-activate					*P*=0.000999	***
	controls vs. pre-activate					*P*=0.034170	*
	pre-activate vs. co-activate					*P*=0.362390	ns

Figure 4g	% of time	*n*= 36, 17, 15 mice					
	motile		not passed	Kruskal-Wallis one-way ANOVA	Kruskal-Wallis statistic = 8.806	*P* =0.0122	*
				Dunn's multiple comparisons test	Mean rank diff.		
	controls vs. co-activate				-16.71	*P*=0.0099	**
	controls vs. pre-activate				-7.855	*P*=0.5561	ns
	pre-activate vs. co-activate				8.851	*P*=0.5748	ns

Figure 4k	% of time freezing in response to sweep	*n*= 15, 10 mice					
	controls vs. co-activate		not passed	Mann-Whitney test (two-tailed)	U=37.5	*P* =0.0306	*

Figure 5c	relative pupil size (constant light)	*n*= 12 mice ChR2 activate; *n*=8 mice controls (dashed line)	not passed	Friedman's test	Friedman's statistic = 12.5	*P* =0.0019	**
				Dunn's multiple comparisons test	Mean rank diff.		
	before vs. laser on (during vMT activation)				-15	*P*=0.0044	**
	before vs. laser off (after vMT activation)				-15	*P*=0.0044	**

Figure 5e	relative pupil size (light pulse)	*n*= 11 mice, each tested with and without vMT activation					
	0s, laser off vs on (vMT activate)		passed	Paired *t*-test (two-tailed)	t=5.007 df=10	*P* =0.0005	***
	5s, off v on		passed	Paired *t*-test (two-tailed)	t=3.192 df=10	*P* =0.0096	**
	10s, off v on		passed	Paired *t*-test (two-tailed)	t=0.8286 df=10	*P* =0.4267	ns
	15s, off v on		passed	Paired *t*-test (two-tailed)	t=0.4587 df=10	*P* =0.6562	ns
	20s, off v on		passed	Paired *t*-test (two-tailed)	t=0.1668 df=10	*P* =0.8708	ns
	25s, off v on		passed	Paired *t*-test (two-tailed)	t=0.2063 df=10	*P* =0.8407	ns
	30s, off v on		passed	Paired *t*-test (two-tailed)	t=0.4083 df=10	*P* =0.6917	ns
	35s, off v on		passed	Paired *t*-test (two-tailed)	t=0.1032 df=10	*P* =0.9199	ns
	40s, off v on		passed	Paired *t*-test (two-tailed)	t=1.764 df=10	*P* =0.1083	ns
	45s, off v on		passed	Paired *t*-test (two-tailed)	t=2.737 df=10	*P* =0.0210	*
	50s, off v on		passed	Paired *t*-test (two-tailed)	t=3.419 df=10	*P* =0.0066	**
	55s, off v on		passed	Paired *t*-test (two-tailed)	t=3.855 df=10	*P* =0.0032	**
	60s, off v on		passed	Paired *t*-test (two-tailed)	t=4.044 df=10	*P* =0.0023	**
Figure 5f	relative pupil size constant light						
	vMT-to-PFC with v without CNO	n= 12 vMT-to-PFC	passed	Paired *t*-test (two-tailed)	t=2.821 df=11	*P* =0.0166	*
	vMT-to-BLA with v without CNO	n= 9 vMT-to-BLA	passed	Paired *t*-test (two-tailed)	t=0.01326 df=8	*P* =0.9897	ns

Figure 5h	relative heart rate	*n*= 8 mice ChR2 activate; *n*=13 mice XFP controls					
	20s, ChR2 v XFP		passed	Unpaired *t*-test (two-tailed)	t=3.244 df=19	*P* =0.0043	**
	30s, ChR2 v XFP		passed	Unpaired *t*-test (two-tailed)	t=2.923 df=19	*P* =0.0087	**
	40s, ChR2 v XFP		passed	Unpaired *t*-test (two-tailed)	t=3.862 df=19	*P* =0.0010	**
	50s, ChR2 v XFP		passed	Unpaired *t*-test (two-tailed)	t=3.653 df=19	*P* =0.0017	**
	60s, ChR2 v XFP		passed	Unpaired *t*-test (two-tailed)	t=3.859 df=19	*P* =0.0011	**
	70s, ChR2 v XFP		passed	Unpaired *t*-test (two-tailed)	t=2.977 df=19	*P* =0.0078	**
	80s, ChR2 v XFP		passed	Unpaired *t*-test (two-tailed)	t=1.651 df=19	*P* =0.1153	ns
	90s, ChR2 v XFP		passed	Unpaired *t*-test (two-tailed)	t=0.6433 df=19	*P* =0.5277	ns
	100s, ChR2 v XFP		passed	Unpaired *t*-test (two-tailed)	t=0.3988 df=19	*P* =0.6945	ns
	110s, ChR2 v XFP		passed	Unpaired *t*-test (two-tailed)	t=0.3878 df=19	*P* =0.7025	ns
	120s, ChR2 v XFP		passed	Unpaired *t*-test (two-tailed)	t=0.7396 df=19	*P* =0.4686	ns

Figure 5i	relative breathing rate	*n*= 8 mice ChR2 activate; *n*=14 mice XFP controls					
	ChR2 v XFP		passed	Unpaired *t*-test (two-tailed)	t=1.866 df=20	*P* =0.0768	ns

Figure 5l	RTPP	*n*= 14 mice ChR2 activate; *n*=17 mice XFP controls					

	ChR2 v XFP		passed	Unpaired *t*-test (two-tailed)	t=2.92 df=29	*P* =0.0067	**

Figure 6c	relative firing rate	*n*= 23 cells, mice = 4					
	pre loom v loom		not passed	Wilcoxon matched-pairs signed rank test (two-tailed)	W=276	*P* =0.04	*

Figure 6d	relative firing rate per behavior	rattle, *n*= 47 cells; ambulatory, *n*= 73 cells; run, *n*= 56 cells; freeze *n*= 67 cells; all relative to pre-loom, *n*=87 total cells from 4 mice	not passed	Kruskal-Wallis one-way ANOVA	Kruskal-Wallis statistic = 22.92	*P* =0.0001	***
				Dunn's multiple comparisons test	Mean rank diff.		
	rattling vs pre loom				7.84	*P* >0.9999	ns
	ambulatory vs pre loom				47.21	*P* =0.0064	**
	run vs pre loom				-0.5	*P* >0.9999	ns
	freeze vs pre pre loom				-27.46	*P* =0.2969	ns

Figure 6e	relative firing rate	*n*= 67 cells from 4 mice					
	motile vs immotile		not passed	Wilcoxon matched-pairs signed rank test (two-tailed)	W=-1276	*P* <0.0001	***

Figure 6g	relative firing rate	*n*= 87 cell from 4 mice	not passed	Kruskal-Wallis one-way ANOVA	Kruskal-Wallis statistic = 7.842	*P* =0.0198	*
				Dunn's multiple comparisons test	Mean rank diff.		
	loom day 1 v 2				11.48	*P* =0.3202	ns
	loom day 1 v 3+				17.04	*P* =0.0155	*
	loom day 2 v 3+				5.56	*P* >0.9999	ns

Ext Figure 1e	number of c-Fos+ cells in the vMT	*n*= 6 ,5, 8 mice	passed	one-way ANOVA	$F_{2,16}$=30.82	*P*<0.0001	***
	hM4D/CNO vs GFP/CNO			Tukey's multiple comparisons test		*P*=0.04	*
	hM3D/CNO vs GFP/CNO			Tukey's multiple comparisons test		*P*=0.0003	***
	hM3D/CNO vs hM4D/CNO			Tukey's multiple comparisons test		*P*<0.0001	***

Ext Figure 2a	number of events	*n*= 15, 9, 9, 17, 5 mice					
	tail rattling		not passed	Quasi-Poisson GLM	$F_{4,50}$=1.9375	*P*=0.1187	ns
				pairwise comparisions			
	no treatment vs XFP/CNO					*P*=0.635	ns
	no treatment vs CAV/XFP/CNO					*P*=0.635	ns
	no treatment vs CAV/hM3D/without CNO					*P*=0.139	ns
	no treatment vs XFP/optrode					*P*=0.103	ns

Ext Figure 2b	number of events	*n*= 15, 9, 9, 17, 5 mice					

	running		not passed	Poisson GLM	$X^2_{4,50}$= 1.1	*P*=0.8938	ns
				pairwise comparisions			
	no treatment vs XFP/CNO					*P*=0.5875	ns
	no treatment vs CAV/XFP/CNO					*P*=0.8703	ns
	no treatment vs CAV/hM3D/without CNO					*P*=0.5210	ns
	no treatment vs XFP/optrode					*P*=0.9584	ns

Ext Figure 2c	% of time	*n*= 15, 9, 9, 17, 5 mice					
	freezing		not passed	Kruskal-Wallis one-way ANOVA	Kruskal-Wallis statistic = 5.802	*P* = 0.2144	ns
				Dunn's multiple comparisons test	Mean rank diff.		
	no treatment vs XFP/CNO				2.356	*P*>0.9999	ns
	no treatment vs CAV/XFP/CNO				3.3	*P*>0.9999	ns
	no treatment vs CAV/hM3D/without CNO				-12.2	*P*=0.5208	ns
	no treatment vs XFP/optrode				6.418	*P*=0.9835	ns

Ext Figure 2d	% of time	*n*= 15, 9, 9, 17, 5 mice					
	hiding		not passed	Kruskal-Wallis one-way ANOVA	Kruskal-Wallis statistic = 3.249	*P* =0.5170	ns
				Dunn's multiple comparisons test	Mean rank diff.		
	no treatment vs XFP/CNO				0.2778	*P*>0.9999	ns
	no treatment vs CAV/XFP/CNO				2.333	*P*>0.9999	ns
	no treatment vs CAV/hM3D/without CNO				11.33	*P*=0.5649	ns
	no treatment vs XFP/optrode				-2.02	*P*>0.9999	ns

Ext Figure 2i	% of time M v F controls +CNO	*n*= 10, 9 mice					
	freezing M v F		not passed	Mann-Whitney test (two-tailed)	U=44.5	*P* =0.9781	ns
	hiding M v F		not passed	Mann-Whitney test (two-tailed)	U=39	*P* =0.6254	ns
	ambulatory M v F		not passed	Mann-Whitney test (two-tailed)	U=38.5	*P* =0.6132	ns
	running M v F		not passed	Mann-Whitney test (two-tailed)	U=45	*P* >0.9999	ns
	rattling M v F		not passed	Mann-Whitney test (two-tailed)	U=45	*P* >0.9999	ns

Ext Figure 2j	% of time M v F hM3D +CNO	*n*= 5, 10 mice					
	freezing M v F		not passed	Mann-Whitney test (two-tailed)	U=14	*P* =0.2065	ns
	hiding M v F		not passed	Mann-Whitney test (two-tailed)	U=16.5	*P* =0.2674	ns
	ambulatory M v F		not passed	Mann-Whitney test (two-tailed)	U=16.5	*P* =0.3243	ns
	running M v F		not passed	Mann-Whitney test (two-tailed)	U=22.5	*P* =0.7855	ns
	rattling M v F		not passed	Mann-Whitney test (two-tailed)	U=19	*P* =0.4922	ns

Ext Figure 2k	% of time M v F controls+ sham stim	*n*= 8, 9 mice					
	freezing M v F		not passed	Mann-Whitney test (two-tailed)	U=33	*P* =0.8002	ns
	hiding M v F		not passed	Mann-Whitney test (two-tailed)	U=31.5	*P* =0.6522	ns
	ambulatory M v F		not passed	Mann-Whitney test (two-tailed)	U=33	*P* =0.7957	ns
	running M v F		not passed	Mann-Whitney test (two-tailed)	U=33.5	*P* >0.9999	ns
	rattling M v F		not passed	Mann-Whitney test (two-tailed)	U=32.5	*P* =0.7501	ns

Ext Figure 2j	% of time M v F controls+ sham stim	*n*= 8, 9 mice					
	freezing M v F		not passed	Mann-Whitney test (two-tailed)	U=25.5	*P* =0.2643	ns
	hiding M v F		not passed	Mann-Whitney test (two-tailed)	U=24.5	*P* =0.2788	ns
	ambulatory M v F		not passed	Mann-Whitney test (two-tailed)	U=26.5	*P* =0.3814	ns
	running M v F		not passed	Mann-Whitney test (two-tailed)	U=30.5	*P* =0.6857	ns
	rattling M v F		not passed	Mann-Whitney test (two-tailed)	U=30.5	*P* =0.6072	

Ext Figure 3g	% of time vMT-to-NA	*n*= 14, 3 mice					
	freezing vMT-to-NA vs controls		not passed	Mann-Whitney test (two-tailed)	U=20	*P* =0.9324	ns
	hiding vMT-to-NA vs controls		not passed	Mann-Whitney test (two-tailed)	U=20	*P* =0.9471	ns
	ambulatory vMT-to-NA vs controls		not passed	Mann-Whitney test (two-tailed)	U=12.5	*P* =0.3118	ns
	running vMT-to-NA vs controls		not passed	Mann-Whitney test (two-tailed)	U=14.5	*P* =0.5368	ns
	rattling vMT-to-NA vs controls		not passed	Mann-Whitney test (two-tailed)	U=15	*P* =0.5412	ns

Ext Figure 4b	number of vMT cells	*n*= 4, 4 mice					
	PFC vs BLA		not passed	Mann-Whitney test (two-tailed)	U=7	*P* 0.8857	ns

Ext Figure 5a	% of mice rattling	*n*= 14, 8,8, 5 mice					
	controls vs. vMT-BLA			Fisher's exact test		*P*=0.6106	ns
	controls vs. vMT-PFC			Fisher's exact test		*P*>0.9999	ns
	controls vs. vMT-PFC terminals			Fisher's exact test		*P*=0.4028	ns

Ext Figure 5b	% of rattling events in the open	*n*= 14, 1,16, 13 rattles					
	controls vs. vMT-BLA			Fisher's exact test		*P*=0.1333	ns
	controls vs. vMT-PFC			Fisher's exact test		*P*=0.0860	ns
	controls vs. vMT-PFC terminals			Fisher's exact test		*P*<0.0001	***

Ext Figure 5c	% of running in the open	*n*= 5, 1, 2, 6 runs					
	controls vs. vMT-PFC terminals			Fisher's exact test		*P*=0.0152	*

Ext Figure 5d	% of mice rattling	*n*= 17, 17, 15 mice					
	controls vs. co-activate			Fisher's exact test		*P*=0.2818	ns
	controls vs. pre-activate			Fisher's exact test		*P*=0.2907	ns
	pre-activate vs. co-activate			Fisher's exact test		*P*>0.9999	ns

Ext Figure 5e	% of rattling events in the open	*n*= 21, 67, 46 rattles					
	controls vs. co-activate			Fisher's exact test		*P*>0.9999	ns
	controls vs. pre-activate			Fisher's exact test		*P*=0.0364	*
	pre-activate vs. co-activate			Fisher's exact test		*P*=0.0064	**

Ext Figure 5f	% of running in the open	*n*= 7, 23,15 runs					
	controls vs. co-activate			Fisher's exact test		*P*=0.0242	*
	controls vs. pre-activate			Fisher's exact test		*P*=0.0225	*
	pre-activate vs. co-activate			Fisher's exact test		*P*>0.9999	ns

Ext Figure 6b	% of time freezing (cat odor) controls v chR2 activate	*n*= 7, 9 mice	not passed	Mann-Whitney test (two-tailed)	U=10	*P* =0.0212	*

Ext Figure 6c	% of time avoiding (cat odor) controls v chR2 activate	*n*= 7, 9 mice	passed	Unpaired *t*-test (two-tailed)	t=2.82 df=14	*P* =0.0136	*

Ext Figure 6e	% shallow choice	*n*= 14, 15, 9 mice	not passed	Kruskal-Wallis one-way ANOVA	Kruskal-Wallis statistic = 2,248	*P* =0.3250	ns
				Dunn's multiple comparisons test	Mean rank diff.		
	controls vs. hM4D				4.786	*P*=0.4423	ns
	controls vs. hM3D				3.786	*P*=0.9567	ns
	hM3D vs. hM4D				-1	*P* >0.9999	ns

Ext Figure 6g	relative activity controls v chR2 activate	*n*= 7, 7 mice	not passed	Mann-Whitney test (two-tailed)	U=11	*P* =0.0973	ns

Ext Figure 6i	average tail rattling events	*n*= 14, 7 mice; tested 2 times	not passed	Mann-Whitney test (two-tailed)	U=38	*P*=0.2800	ns

Ext Figure 6j	% mice attacking	*n*= 14, 7 mice		Fisher's exact test		*P*>0.9999	ns

Ext Figure 6k	latency to attack	*n*= 7, 3 mice	not passed	Mann-Whitney test (two-tailed)	U=10	*P*>0.9999	ns

Ext Figure 6l	% time in center						
	ChR2, laser on v off	*n*= 10 mice Chr2	passed	Paired *t*-test (two-tailed)	t=0.624 df=9	*P* =0.5481	ns

	XFP, laser on v off	*n*= 10 mice XFP	passed	Paired *t*-test (two-tailed)	t=0.1483 df=9	*P* =0.8854	ns

Ext Figure 6n	% time in center						
	ChR2, laser on v off	*n*= 10 mice Chr2, relative to XFP (dashed line)	passed	Paired *t*-test (two-tailed)	t=1.742 df=9	*P* =0.1154	ns

Ext Figure 7a	relative pupil size (light pulse)in controls	*n*= 14 mice, each tested with and without CNO					
	0s, XFP CNO v no CNO		passed	Paired *t*-test (two-tailed)	t=0.6874 df=13	*P* =0.5039	ns
	5s, XFP CNO v no CNO		passed	Paired *t*-test (two-tailed)	t=1.066 df=13	*P* =0.3058	**
	10s, XFP CNO v no CNO		passed	Paired *t*-test (two-tailed)	t=1.859 df=13	*P* =0.0858	ns
	15s, XFP CNO v no CNO		passed	Paired *t*-test (two-tailed)	t=1.47 df=13	*P* =0.1652	ns
	20s, XFP CNO v no CNO		passed	Paired *t*-test (two-tailed)	t=1.574 df=13	*P* =0.1394	ns
	25s, XFP CNO v no CNO		passed	Paired *t*-test (two-tailed)	t=1.498 df=13	*P* =0.1580	ns
	30s, XFP CNO v no CNO		passed	Paired *t*-test (two-tailed)	t=1.773 df=13	*P* =0.0996	ns
	35s, XFP CNO v no CNO		passed	Paired *t*-test (two-tailed)	t=1.489 df=13	*P* =0.1603	ns
	40s, XFP CNO v no CNO		passed	Paired *t*-test (two-tailed)	t=1.401 df=13	*P* =0.1847	ns
	45s, XFP CNO v no CNO		passed	Paired *t*-test (two-tailed)	t=0.0605 df=13	*P* =0.9257	ns
	50s, XFP CNO v no CNO		passed	Paired *t*-test (two-tailed)	t=0.4354 df=13	*P* =0.6704	ns
	55s, XFP CNO v no CNO		passed	Paired *t*-test (two-tailed)	t=0.6405 df=13	*P* =0.5330	ns
	60s, XFP CNO v no CNO		passed	Paired *t*-test (two-tailed)	t=0.1749 df=13	*P* =0.8639	ns

Ext Figure 7b	relative pupil size (light pulse) in hM3D mice	*n*= 15 mice, each tested with and without CNO					
	0s, hM3D CNO v no CNO		passed	Paired *t*-test (two-tailed)	t=4.172 df=14	*P* =0.00094	***
	5s, hM3D CNO v no CNO		passed	Paired *t*-test (two-tailed)	t=3.865 df=14	*P* =0.00017	**
	10s, hM3D CNO v no CNO		passed	Paired *t*-test (two-tailed)	t=4.498 df=14	*P* =0.0005	***
	15s, hM3D CNO v no CNO		passed	Paired *t*-test (two-tailed)	t=4.696 df=14	*P* =0.00034	***
	20s, hM3D CNO v no CNO		passed	Paired *t*-test (two-tailed)	t=4.392 df=14	*P* =0.00061	***
	25s, hM3D CNO v no CNO		passed	Paired *t*-test (two-tailed)	t=4.341 df=14	*P* =0.00068	***
	30s, hM3D CNO v no CNO		passed	Paired *t*-test (two-tailed)	t=5.334 df=14	*P* =0.00011	***
	35s, hM3D CNO v no CNO		passed	Paired *t*-test (two-tailed)	t=4.782 df=14	*P* =0.00029	***
	40s, hM3D CNO v no CNO		passed	Paired *t*-test (two-tailed)	t=2.52 df=14	*P* =0.0244	*
	45s, hM3D CNO v no CNO		passed	Paired *t*-test (two-tailed)	t=3.087 df=14	*P* =0.0080	**
	50s, hM3D CNO v no CNO		passed	Paired *t*-test (two-tailed)	t=1.633 df=14	*P* =0.124	ns

	55s, hM3D CNO v no CNO		passed	Paired *t*-test (two-tailed)	t=0.9465 df=14	*P* =0.359	ns
	60s, hM3D CNO v no CNO		passed	Paired *t*-test (two-tailed)	t=0.5985 df=14	*P* =0.559	ns

Ext Figure 7c	relative pupil size (light pulse) in hM4D mice	*n*= 9 mice, each tested with and without CNO					
	0s, hM4D CNO v no CNO		passed	Paired *t*-test (two-tailed)	t=0.3019 df=8	*P* =0.7705	ns
	5s, hM4D CNO v no CNO		passed	Paired *t*-test (two-tailed)	t=0.6862 df=8	*P* =0.5120	ns
	10s, hM4D CNO v no CNO		passed	Paired *t*-test (two-tailed)	t=0.8249 df=8	*P* =0.4333	ns
	15s, hM4D CNO v no CNO		passed	Paired *t*-test (two-tailed)	t=1.118 df=8	*P* =0.2960	ns
	20s, hM4D CNO v no CNO		passed	Paired *t*-test (two-tailed)	t=0.9909 df=8	*P* =0.3508	ns
	25s, hM4D CNO v no CNO		passed	Paired *t*-test (two-tailed)	t=1.205 df=8	*P* =0.2627	ns
	30s, hM4D CNO v no CNO		passed	Paired *t*-test (two-tailed)	t=1.205 df=8	*P* =0.2584	ns
	35s, hM4D CNO v no CNO		passed	Paired *t*-test (two-tailed)	t=1.596 df=8	*P* =0.1491	ns
	40s, hM4D CNO v no CNO		passed	Paired *t*-test (two-tailed)	t=1.088 df=8	*P* =0.3084	ns
	45s, hM4D CNO v no CNO		passed	Paired *t*-test (two-tailed)	t=2.228 df=8	*P* =0.0565	ns
	50s, hM4D CNO v no CNO		passed	Paired *t*-test (two-tailed)	t=3.487 df=8	*P* =0.0082	**
	55s, hM4D CNO v no CNO		passed	Paired *t*-test (two-tailed)	t=3.885 df=8	*P* =0.0046	**
	60s, hM4D CNO v no CNO		passed	Paired *t*-test (two-tailed)	t=1.037 df=8	*P* =0.3300	ns

Ext Figure 7e	relative pupil size constant light	*n*= 15 hM3D, 14 XFP, 9 hM4D mice	not passed	Kruskal-Wallis one-way ANOVA	Kruskal-Wallis statistic = 11.29	*P* =0.0035	**
				Dunn's multiple comparisons test	Mean rank diff.		
	post CNO hM3D v XFP				7.545	*P*=0.0819	ns
	post CNO hM3D v hM4D				11.38	*P*=0.0046	**
	post CNO XFP v hM4D				3.833	*P* =0.9895	ns
Ext Figure 7f	relative pupil size constant dark	*n*= 15 hM3D, 14 XFP, 9 hM4D mice	passed	one-way ANOVA	$F_{2,35}$=9.718	*P*=0.0004	***
	post CNO hM3D v XFP			Tukey's multiple comparisons test		*P*=0.0008	***
	post CNO hM3D v hM4D			Tukey's multiple comparisons test		*P*=0.0060	**
	post CNO XFP v hM4D			Tukey's multiple comparisons test		*P*=0.9668	ns

Ext Data Fig 7g	relative pupil size (constant dark)	*n*= 11 mice ChR2 activate; *n*=12 mice XFP controls					
	ChR2 v XFP		passed	Unpaired *t*-test (two-tailed)	t=3.375 df=21	*P* =0.0029	**

Ext Figure 8b	number of c-Fos+ cells in the vMT	*n*= 7,6, 10, 5 mice	passed	one-way ANOVA	$F_{3,24}$=10.36	*P*=0.0001	***

	above vs. none			Tukey's multiple comparisons test		*P*=0.0002	***
	above vs. below			Tukey's multiple comparisons test		*P*=0.0031	**
	above vs. habituate			Tukey's multiple comparisons test		*P*=0.002	**
	none vs. below			Tukey's multiple comparisons test		*P*=0.9735	ns
	none vs. habituate			Tukey's multiple comparisons test		*P*=0.9627	ns
	below vs. habituate			Tukey's multiple comparisons test		*P*>0.9999	ns

Ext Figure 8c	number of c-Fos+ cells in the Xi	*n*= 7,10, 5 mice	passed	one-way ANOVA	$F_{2,19}$=19.75	*P*<0.0001	***
	above vs. none			Tukey's multiple comparisons test		*P*<0.0001	***
	above vs. below			Tukey's multiple comparisons test		*P*<0.0001	***
	above vs. habituate			Tukey's multiple comparisons test		*P*<0.0001	***
	none vs. below			Tukey's multiple comparisons test		*P*=0.8767	ns
	none vs. habituate			Tukey's multiple comparisons test		*P*=0.7388	ns
	below vs. habituate			Tukey's multiple comparisons test		*P*=0.9973	ns

Ext Figure 10a	arousal levels	*n*= 9, 7, 10 mice					
	tail rattling		not passed	Quasi-Poisson GLM	$F_{2,23}$=14.842	P=0.00007	***
				pairwise comparisions			
	rattling in mice with low v moderate arousal					*P*=0.02043	*
	rattling in mice with low v high arousal					*P*=0.00225	**
	rattling in mice with moderate v high arousal					*P*=0.05603	ns

Ext Figure 10b	arousal levels	*n*= 9, 7, 10 mice					
	running		not passed	Poisson GLM	$X^2_{3,31}$= 17.7	P=0.000138	***
				pairwise comparisions			
	running in mice with low v moderate arousal					*P*=0.06065	ns
	running in mice with low v high arousal					*P*=0.00191	**
	running in mice with moderate v high arousal					*P*=0.069227	ns

Ext Figure 10c	arousal levels	*n*= 9, 7, 10 mice	not passed	Kruskal-Wallis one-way ANOVA	Kruskal-Wallis statistic = 0.915	*P* =0.6329	ns
	freezing			Dunn's multiple comparisons test	Mean rank diff.		
	freezing in mice with low v moderate arousal				3.079	*P*>0.9999	ns
	freezing in mice with low v high arousal				2.322	*P*>0.9999	ns
	freezing in mice with moderate v high arousal				-0.7571	*P*>0.9999	ns

Ext Figure 10d	arousal levels	*n*= 9, 7, 10 mice	not passed	Kruskal-Wallis one-way ANOVA	Kruskal-Wallis statistic = 2.002	*P* =0.3676	ns
	hiding			Dunn's multiple comparisons test	Mean rank diff.		
	hiding in mice with low v moderate arousal				-5.31	*P*=0.4770	ns
	hiding in mice with low v high arousal				-1.917	*P*>0.9999	ns
	hiding in mice with moderate v high arousal				3.393	*P*>0.9999	ns

Ext Figure 10e	arousal levels	*n*= 9, 7, 10 mice					
	% of mice tail rattling						
	low v moderate			Fisher's exact test		*P*=0.3147	ns
	low v high			Fisher's exact test		*P*=0.0031	**
	moderate v high			Fisher's exact test		*P*=0.1544	ns

Ext Figure 10f	arousal levels	*n*= 9, 7, 10 mice					
	% of mice running						
	low v moderate			Fisher's exact test		*P*=0.0406	*
	low v high			Fisher's exact test		*P*=0.0007	***
	moderate v high			Fisher's exact test		*P*=0.4118	ns

Ext Figure 10g	arousal levels	*n*= 9, 7, 10 mice					
	% of mice freezing						
	low v moderate			Fisher's exact test		*P*>0.9999	ns
	low v high			Fisher's exact test		*P*>0.9999	ns
	moderate v high			Fisher's exact test		*P*>0.9999	ns

Ext Figure 10h	arousal levels	*n*= 9, 7, 10 mice					
	% of mice hiding						
	low v moderate			Fisher's exact test		*P*=0.3077	ns
	low v high			Fisher's exact test		*P*>0.9999	ns
	moderate v high			Fisher's exact test		*P*=0.3382	ns