Supplementary data for:

"Layer 5 circuits in V1 differentially control visuomotor behavior"
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Figures S1-S5
Table S1

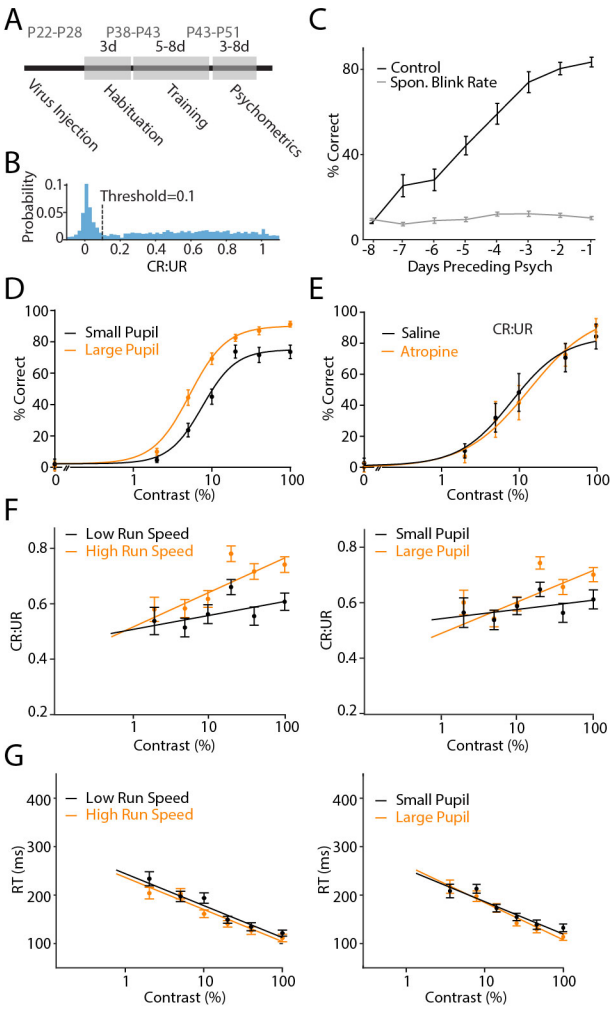


Figure S1. Behavioral state is correlated with visual task performance (related to Figure 1).

(A) Timeline of experimental procedures.
(B) Distribution of blink response magnitudes (CR:UR) across all animals, illustrating the cut-off value used to distinguish correct versus incorrect trials.
(C) Learning curve for animals shown in Figure 1, displayed for training days relative to the transition to psychometric testing. The spontaneous blink rate is shown in gray.
(D) Contrast-dependent performance separated into large (orange) and small (black) pupil trials (n=39 mice). Dots and error bars represent average ± SEM over mice. R_{Max}-Large Pupil 85.5%, R_{Max}-Small Pupil 69.9%; p<0.0001, Permutation Test.
(E) Contrast-dependent performance measured 30 minutes after application of saline (black) or atropine (orange) to the eye (n=9 mice). Dots and error bars represent average ± SEM over mice. R_{Max}-Saline 72.4%, R_{Max}-Atropine 76.0%; p=0.365, Permutation Test.
(F) Left, relative amplitude of conditioned blinks (CR:UR) separated into high (orange) and low (black) locomotion speed trials (n=39 mice). Dots and error bars represent average ± SEM over mice. Slope-High Speed 0.126, Slope-Low Speed 0.055; p=0.0063, Permutation Test. Right, as in left panel for large and small pupil diameter. Slope-Large Pupil 0.115, Slope-Small Pupil 0.038; p=0.0034, Permutation Test.
(G) Left, response time (RT) separated by high and low running speed (n=39 mice). Dots and error bars represent average ± SEM over mice. Slope-High Speed -0.067, Slope-Low Speed -0.065; p=0.338, Permutation Test. Right, as in left panel for large and small pupil diameter. Slope-Large Pupil -0.066, Slope-Small Pupil -0.060; p=0.290, Permutation Test.

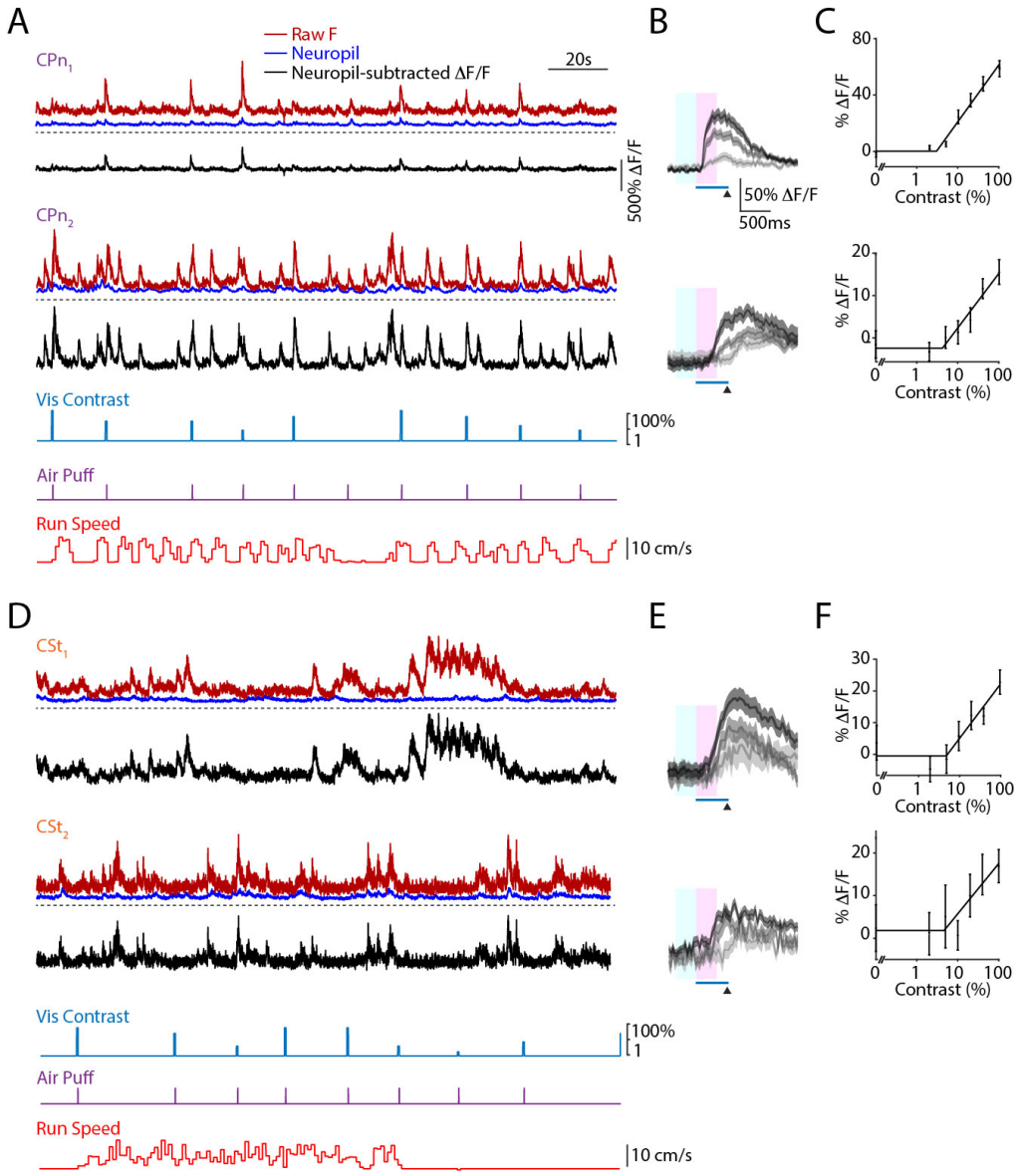
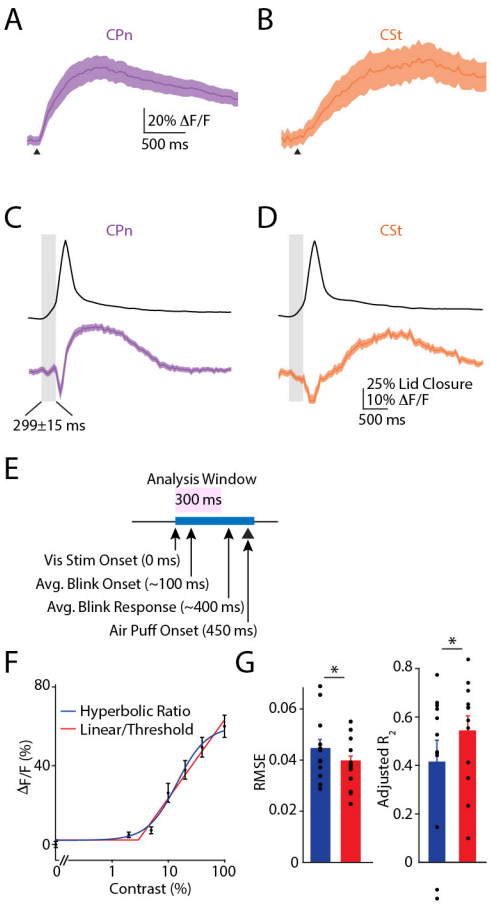


Figure S2. Example calcium imaging traces and visual responses from CPn and CSt neurons (related to Figure. 2).

(A) Examples of two CPn neurons. Raw fluorescence (red), neuropil fluorescence (blue), and neuropil-subtracted ΔF/F (black) are shown for each. Timing for visual stimuli, air puff, and running speed are shown below. Dashed line indicates zero raw fluorescence.
(B) Visual responses at 5%, 20%, and 100% contrast (light, medium, dark gray) for each cell in (A). Timing of visual stimulation (blue bar), air puff (arrowhead), baseline analysis period (light blue window), and response measurement period (pink window) are indicated. Lines and shadings represent average ± SEM over trails at each contrast level.
(C) Contrast-dependent response magnitudes for the example neurons, fit with a rectified linear curve. Dots and error bars represent average ± SEM over trails at each contrast level.
(D-F) Same as A-C for two example CSt neurons.



Supplemental Figure 3

Figure S3. Neuronal calcium transients in response to air puff and spontaneous blinks (related to Figure 3).

(A) Average ± SEM calcium transient (purple with shading) in response to air puff alone across all CPn neurons. Air puff timing indicated by arrowhead.
(B) As in (A) for CSt neurons.
(C) Average lid closure for spontaneously detected blinks (black) and average ± SEM calcium transient (purple with shading) for visually-responsive CPn neurons. Period between blink onset and calcium transient onset indicated by gray bar.
(D) As in (C) for CSt neurons.
(E) Schematic showing analysis window for calcium transients on single trials. Data were analyzed for a 300 ms window (pink bar) following visual stimulus onset and ending prior to the blink-dependent response onset.
(F) Example visual responses for a single neuron across multiple contrasts. Data are fit with either a hyperbolic ratio function (blue) or rectified linear function (red). Dots and error bars represent average ± SEM over trials.
(G) Left, population root-mean-squared error (RMSE) for the two fitting functions (n=204 cells). Bars represent average ± SEM over trials over visually-responsive neurons. Hyperbolic Ratio 0.044±0.004, Linear/Threshold 0.038±0.003; n=11mice, p=0.001, Wilcoxon Matched Pairs Test. Right, as in left panel for adjusted R^2. Hyperbolic Ratio 0.42±0.09, Linear/Threshold 0.54±0.06, n=11 mice; p=0.015, Wilcoxon Matched Pairs Test.

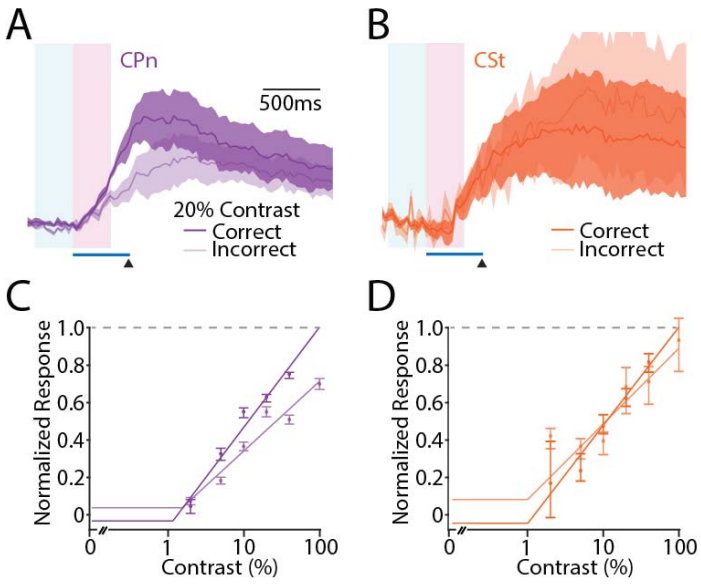


Figure S4. Comparison of normalized activity in CPn and CSt neurons (related to Figure 4).

(A-B) Averaged visual responses at 20% contrast for CPn (A) and CSt (B) neurons separated by correct (dark) and incorrect (light) trials. Timing of the visual stimulus (blue bar) and air puff (arrowhead), and analysis windows (baseline, light blue; response, pink) are shown. Lines and shadings represent average ± SEM over animals, normalized to the correct response amplitude within animal. Underlying data are the same as in Figure 4A-B.
(C-D) Population contrast-dependent response magnitudes for CPn (C) and CSt (D) neurons separated by correct (dark) and incorrect (light) trials. Dots and error bars represent average ± SEM over animals, normalized to the correct response amplitude at 100% contrast within animal. Underlying data are the same as in Figure 4C-D.

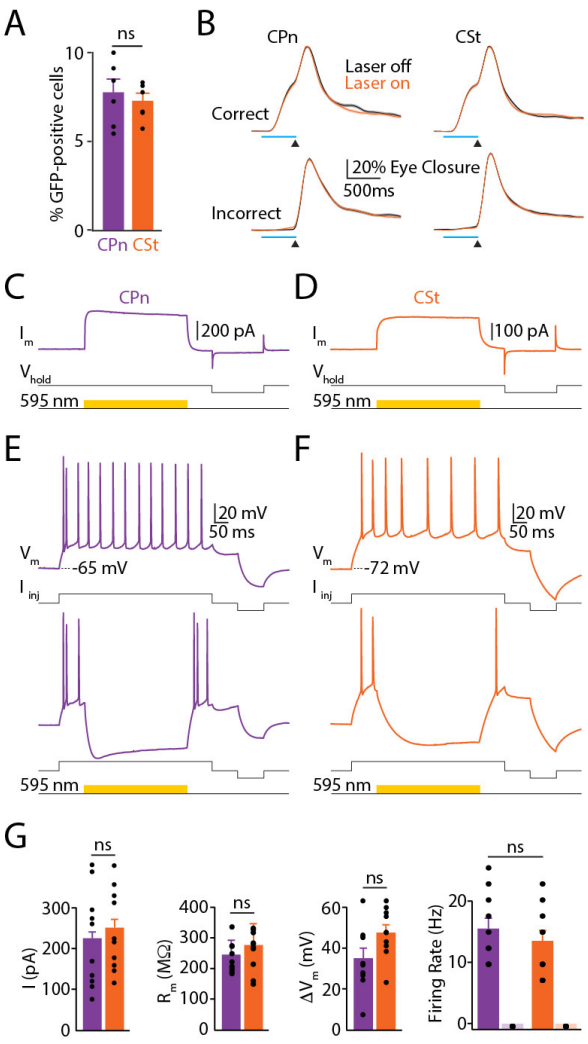


Figure S5. Functional expression of ArchT does not differ between CPn and CSt populations (related to Figure 5).

(A) Proportion of NeuN-identified layer 5 neurons expressing ArchT-GFP in either CPn (purple) or CSt (orange) cohorts. Bars represent average ± SEM over mice. CPn proportion 7.76±0.75%, n=6 mice; CSt proportion 7.28±0.42%, n=6 mice; p=0.589, Mann-Whitney U test.
(B) Lid closure traces averaged across contrasts, separated by correct (upper) and incorrect (lower) trials and for laser-off (black) or laser-on (orange) trials, for CPn (left, n=11 mice) and CSt (right, n=12 mice) cohorts. Lines and shadings represent average ± SEM over mice.
(C) Example outward current evoked in a CPn neuron voltage clamped at -70 mV by 595 nm light through the objective.
(D) As in (C) for an example CSt neuron.
(E) Example CPn neuron showing action potentials evoked by a depolarizing current pulse alone (upper) or with concurrent illumination with 595 nm light (lower). Spikes are completely suppressed by light.
(F) As in (E) for an example CSt neuron.
(G) Population data for CPn (purple) and CSt cells (orange), showing light-evoked current (245±46.7 vs. 276.4±70.0 pA, p=0.92), resting input resistance (225.3±15.3 vs. 251.4±20.5 MOhms, p=0.32), light-evoked hyperpolarization (35.0±4.9 vs. 47.6±3.8 mV, p=0.078), and suppression of firing rate (15.5±1.7 vs. 13.5±1.7 Hz, p=0.38). Mann-Whitney U Test, n=10 cells per group for all comparisons.

Table S1. Summary of all statistical analyses (related to Figures 1-5 and S1-S5).

Figure	Comparison	Test	Statistic A	Value A	Statistic B	Value B	Test statistic	95% Confidence Interval	p-value
Behavior									
Fig.1D	Muscimol vs. Saline performance (n=11 mice)	Permutation	Muscimol Rmax	28.2%	Saline Rmax	86.5%	A/B=0.33	[0.83,1.19]	<0.0001
		Paired t-test	Muscimol 100% contrast	28.9±5.4%	Saline 100% contrast	89.3±2.9%			<0.0001
Fig.1F	High-speed vs. Low-speed performance (n=39 mice)	Permutation	High speed Rmax	88.7%	Low speed Rmax	71.1%	A/B=1.24	[0.94,1.10]	<0.0001
		Paired t-test	High speed 100% contrast	92.1±3.1%	Low speed at 100% contrast	73.1±4.2%			0.0013
Fig.S1D	Large pupil vs. Small pupil performance (n=39 mice)	Permutation	Large pupil Rmax	85.5%	Small pupil Rmax	69.9%	A/B=1.10	[0.94,1.06]	<0.0001
		Paired t-test	Large pupil 100% contrast	91.0±1.9%	Small pupil 100% contrast	72.1±4.4%			0.00011
Fig.S1E	Atropine vs. Saline performance (n=9 mice)	Permutation	Atropine Rmax	76.0%	Saline Rmax	72.4%	A/B=1.05	[0.79,1.27]	0.365
		Paired t-test	Atropine at 100% contrast	76.0±7.6%	Saline at 100% contrast	74.2±8.1%			0.820
Fig.S1F	High speed vs. Low speed CR:UR (n=39 mice)	Permutation	High speed Slope	0.126	Low speed Slope	0.055	A/B=2.31	[0.54,1.94]	0.0063
		Paired t-test	High speed 100% contrast	0.741±0.028	Low speed 100% contrast	0.607±0.031			<0.0001
Fig.S1F	Large pupil vs. Small pupil CR:UR (n=39 mice)	Permutation	Large pupil Slope	0.115	Small pupil Slope	0.038	A/B=3.03	[0.54,2.11]	0.0034
		Paired t-test	Large pupil 100% contrast	0.704±0.026	Small pupil 100% contrast	0.614±0.034			0.0012
Fig.S1G	High speed vs. Low speed RT (n=39 mice)	Permutation	High speed Slope	-0.067	Low speed Slope	-0.065	A/B=1.03	[0.71,1.29]	0.338
		Paired t-test	High speed at 100% contrast	0.111±0.008	Low speed at 100% contrast	0.122±0.006			0.230
Fig.S1G	Large pupil vs. Small pupil RT (n=39 mice)	Permutation	Large pupil Slope	-0.066	Small pupil Slope	-0.060	A/B=1.09	[0.74,1.39]	0.290
		Paired t-test	Large pupil 100% contrast	0.114±0.007	Small pupil 100% contrast	0.133±0.008			0.023
Imaging									
Fig.S3G	Linear/threshold vs. hyperbolic ratio fit of contrast response function (n=11 mice)	Wilcoxon Matched Pairs Test	Hyperbolic RMSE	0.044±0.004	Linear/Threshold RMSE	0.038±0.003			0.001
Fig.S3G	Linear/threshold vs. hyperbolic ratio fit of contrast response function (n=11 mice)	Wilcoxon Matched Pairs Test	Hyperbolic Adjusted R^2	0.42±0.09	Linear/Threshold Adjusted R^2	0.54±0.06			0.002
Fig.3B	CPn vs. CSt visually-responsive neurons (n=6 mice, 449 cells vs. 6 mice, 274 cells)	Mann-Whitney U Test	CPn % Vis responsive	34.9±10.5%	CSt % Vis responsive	19.5±5.5%			0.39
Fig.3C	CPn vs CSt visual response slope (n=6 mice vs. 6 mice)	Mann-Whitney U Test	CPn Slope	0.20±0.06	CSt Slope	0.10±0.02			0.016
Fig.3D	CPn vs. CSt coefficient of variation AUC (n=6 mice vs. 6 mice)	Mann-Whitney U Test	CPn AUC	29.2±3.3	CSt AUC	37.9±1.4			0.0411
Fig.3E	CPn vs CSt noise correlation (9453 pairs vs. 2145 pairs)	Kolmogorov-Smirnov test	CPn median	0.114	CSt median	0.072			<0.0001
Fig.3F	CPn vs CSt locomotion modulation index on CRF Slope (159 cells vs. 51 cells)	One-sided t-test	CPn mean	0.36±0.07	Null	0			<0.0001
		One-sided t-test	CSt mean	0.26±0.03	Null	0			0.0038
		t-test	CPn mean	0.36±0.07	CSt mean	0.26±0.03			0.120
Fig.4C	CPn correct vs. incorrect visual response slope (n= 6 mice)	Permutation	Slope correct	0.16	Slope incorrect	0.10	A/B=1.61	[0.79,1.29]	0.0004
Fig.4D	CSt correct vs. incorrect visual response slope (n=6 mice)	Permutation	Slope correct	0.12	Slope incorrect	0.09	A/B= 1.37	[-0.49,1.68]	0.142
Fig.4G (Orig)	CPn vs. CSt decoder d' (n=6 mice)	Mann-Whitney U Test	CPn decoder d'	0.88±0.12	CSt decoder d'	0.24±0.24			0.026
Fig.4G (Orig)	CPn vs. CSt decoder ROC (n=6 mice)	Mann-Whitney U Test	CPn decoder ROC	0.70±0.03	CSt decoder ROC	0.60±0.05			0.031

not shown	CPn vs. CSt decoder d' using adaptive window (n=6 mice)	Mann-Whitney U Test	CPn decoder d'	0.94±0.31	CSt decoder d'	0.04±0.31			0.002
not shown	CPn vs. CSt decoder ROC using adaptive window (n=6 mice)	Mann-Whitney U Test	CPn decoder ROC	0.82±0.07	CSt decoder ROC	0.73±0.06			0.006
Fig.4G (Shuffled)	CPn vs. CSt decoder d' (n=6 mice)	Mann-Whitney U Test	CPn decoder d'	0.75±0.16	CSt decoder d'	0.22±0.25			0.18
Fig.4G (Shuffled)	CPn vs. CSt decoder ROC (n=6 mice)	Mann-Whitney U Test	CPn decoder ROC	0.69±0.04	CSt decoder ROC	0.57±0.06			0.03
Fig.4H	CPn single neuron decoder d' (n=159 cells)	One-sided t-test	CPn d' mean	0.43±0.03	CPn chance d'	-0.01±0.001			<0.0001
	CSt single neuron decoder d' (n=51 cells)	One-sided t-test	CSt d' mean	0.09±0.05	CSt chance d'	-0.02±0.002			0.088
	CPn vs. CSt single neuron decoder d' (n=159 vs. 51 cells)	t-test	CPn d' mean	0.43±0.03	CSt d' mean	0.09±0.05			<0.0001
	CPn vs. CSt single neuron decoder d' (n=6 mice)	Mann-Whitney U Test	CPn d' animal-wise mean	0.37+-0.08	CSt d' animal-wise mean	-0.012+-0.08			0.015
Optogenetics									
Fig. S5A	CPn vs. CSt GFP-positive L5 cells (% NeuN-labeled cells) (n=6 vs 6 mice)	Mann-Whitney U Test	CPn proportion	7.76±0.75%	CSt proportion	7.28±0.42%			0.589
Fig.5C	CPn Laser on vs. Laser off Performance (n=11 mice)	Permutation	Laser on Rmax	60.5%	Laser off Rmax	80.2%	A/B=0.75	[0.87,1.16]	<0.0001
		Paired t-test	Laser on 100% Contrast	63.9±7.3%	Laser off 100% Contrast	79.7±3.5%			0.011
Fig.5D	CSt Laser on vs. Laser off Performance (n=12 mice)	Permutation	Laser on Rmax	79.3%	Laser off Rmax	77.7%	A/B=1.02	[0.91,1.09]	0.325
		Paired t-test	Laser on 100% Contrast	85.2±4.6%	Laser off 100% Contrast	80.7±4.6%			0.256
Fig. S5B	CPn Laser on vs. Laser off UR Amplitude (n=11 mice)	Paired t-test	Laser on UR	0.91±0.02	Laser off UR	0.89±0.02			0.178
	CSt Laser on vs. Laser off UR Amplitude (n=12 mice)	Paired t-test	Laser on UR	0.91±0.01	Laser off UR	0.92±0.01			0.748
Fig. S5G	CPn vs. CSt ArchT-evoked current (pA, n=10 vs. 10 cells)	Mann-Whitney U Test	CPn current mean	245.2±46.7	CSt current mean	276.4±70.0			0.912
Fig. S5G	CPn vs. CSt Input Resistance (MOhm, n=10 vs. 10 cells)	Mann-Whitney U Test	CPn Input resistance mean	225.3±15.3	CSt Input resistance mean	251.4±20.5			0.315
Fig. S5G	CPn vs. CSt ArchT-evoked hyperpolarization (mV, n=10 vs. 10 cells)	Mann-Whitney U Test	CPn hyperpolarization mean	35.0±4.9	CSt hyperpolarization mean	47.6±3.8			0.078
Fig. S5G	CPn vs. CSt ArchT-evoked reduction in firing rate (Hz, n=10 vs. 10 cells)	Mann-Whitney U Test	CPn reduction in firing rate	15.5±1.7	CSt reduction in firing rate	13.5±1.7			0.376