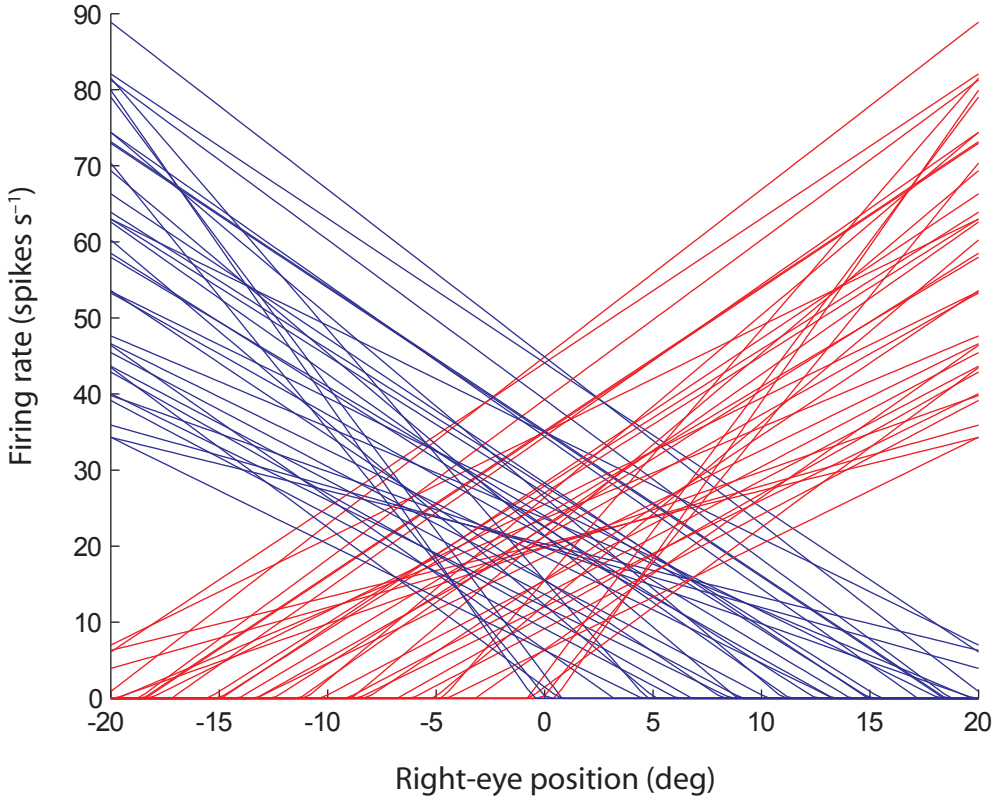
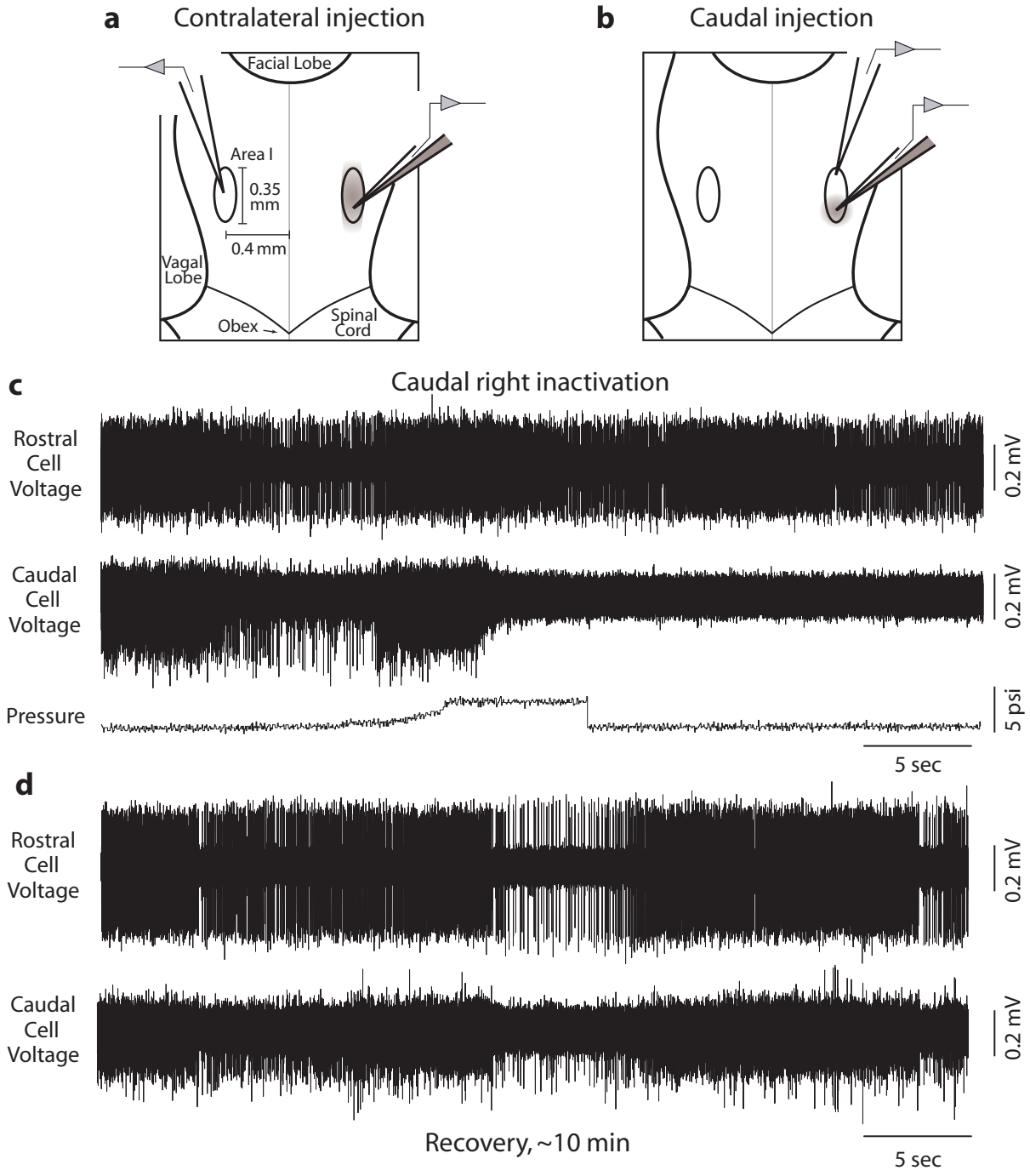


Supplementary Figure 1: Model tuning curves defined by experimentally measured rate vs. position relationships

Neuronal firing rates as a function of eye position were taken from experimental fits to firing rates recorded during eye fixations at different eye positions. To produce model cells for both populations, each recorded neuron (N=36) was used to generate a model neuron on the recorded side and a model neuron with a mirror-image firing rate relationship on the opposite side. Red: right-side neurons; blue: left-side neurons.



Supplementary Figure 2: Method for functional dissection of a circuit
(**a**) Schematic of the hindbrain and electrode placement for contralateral injections. A recording electrode (left) was placed in one area I to monitor changes in firing rate drift during inactivation of contralateral area I with a second electrode (right). Placement for injection was guided by recording extracellular signals from position neurons through the one side of the double-barreled glass electrode; efficacy of the pharmacological agent (gray) was ascertained by loss of neuronal discharge. (**b**) Schematic for caudal injections. Neuronal firing rate drift the rostral end of area I was monitored with one electrode while cells at the caudal end were inactivated using the double-barreled electrode as above. (**c**) Recorded voltages from two electrodes positioned as in (b) during pressure injection of lidocaine. Recordings on the caudal electrode were abolished, while the quality of the recording from the cell recorded rostrally was unaltered. Modifications to drift dynamics began and were assessed within seconds. (**d**) Recovery of discharge on the caudal recording approximately 10 minutes after injection in (c).

Supplementary Table 1: Change in position drift for each complete left inactivation

Position drift difference in the left and right oculomotor-range halves for the right (top) and left (bottom) eyes during a complete inactivation of the left population (1:10 lidocaine, 11:21 muscimol)

Experiment #	*Difference left*	*P left*	*Difference right*	*P right*
1	0.45	0.00	–0.33	0.02
2	0.61	0.00	–0.74	0.00
3	0.84	0.00	–0.50	0.00
4	2.09	0.00	0.39	0.00
5	0.89	0.00	–0.24	0.01
6	1.40	0.00	0.18	0.00
7	1.49	0.00	0.03	0.54
8	1.13	0.00	–0.27	0.00
9	1.44	0.00	0.47	0.00
10	1.38	0.00	0.02	0.72
11	0.66	0.00	0.44	0.00
12	1.91	0.00	3.23	0.00
13	1.41	0.00	0.33	0.00
14	0.35	0.00	–0.06	0.49
15	2.01	0.00	–0.02	0.90
16	1.01	0.00	1.03	0.00
17	0.53	0.00	–0.22	0.01
18	0.37	0.00	0.65	0.00
19	0.36	0.00	0.07	0.02
20	0.28	0.00	0.20	0.00
21	1.01	0.00	0.17	0.01

Experiment #	*Difference left*	*P left*	*Difference right*	*P right*
1	–0.69	0.00	0.19	0.05
2	–0.08	0.31	0.85	0.00
3	–0.87	0.00	0.67	0.00
4	–0.91	0.00	–0.22	0.00
5	–1.53	0.00	0.15	0.20
6	–0.96	0.00	–0.13	0.07
7	–1.90	0.00	0.14	0.00
8	–1.67	0.00	0.03	0.55
9	–0.62	0.00	0.06	0.19
10	–0.49	0.00	0.20	0.04
11	–0.27	0.00	–0.10	0.12
12	–2.33	0.00	–2.66	0.00
13	–1.70	0.00	–0.67	0.00
14	–0.70	0.00	–0.40	0.00
15	–4.17	0.00	–0.24	0.15
16	–2.01	0.00	–0.67	0.00
17	–1.44	0.00	–0.26	0.01
18	–1.51	0.00	–0.67	0.00
19	–0.97	0.00	–0.23	0.00
20	–0.97	0.00	–0.15	0.00
21	–0.80	0.00	–1.05	0.00

Supplementary Table 2: Change in rate drift for each complete left inactivation

Rate drift difference in the left and right oculomotor-range halves for a right-side neuron during a complete inactivation of the left population (1:10 lidocaine, 11:21 muscimol)

Experiment #	*Difference left*	*P left*	*Difference right*	*P right*
1	2.69	0.00	–2.86	0.00
2	2.82	0.00	–3.02	0.00
3	3.49	0.00	–1.83	0.19
4	—	—	0.24	0.67
5	1.35	0.10	0.47	0.56
6	—	—	0.08	0.93
7	—	—	–2.44	0.00
8	2.70	0.04	–0.48	0.73
9	2.47	0.00	0.12	0.62
10	2.34	0.03	4.21	0.00
11	0.09	0.84	1.31	0.01
12	5.54	0.00	8.43	0.00
13	1.72	0.06	–2.03	0.01
14	—	—	3.44	0.00
15	13.16	0.00	0.64	0.85
16	0.82	0.51	4.44	0.03
17	2.77	0.05	–2.43	0.29
18	–2.25	0.16	1.79	0.03
19	1.46	0.00	0.49	0.11
20	3.07	0.00	–0.20	0.67
21	1.50	0.05	1.59	0.01

Supplementary Table 3: Change in rate drift for each caudal right inactivation

Rate drift difference in the left and right oculomotor-range halves for a right-side neuron during a caudal right inactivation (22:29 lidocaine, 30:37 muscimol)

Experiment #	*Difference left*	*P left*	*Difference right*	*P right*
22	—	—	–1.95	0.00
23	—	—	–4.19	0.00
24	—	—	–3.22	0.00
25	—	—	–8.02	0.00
26	–0.47	0.50	–1.43	0.00
27	–0.01	0.96	–1.44	0.00
29	0.60	0.49	–4.34	0.00
29	–0.25	0.62	–1.98	0.00
30	—	—	–2.49	0.00
31	—	—	–3.22	0.00
32	—	—	–7.11	0.00
33	3.36	0.04	–2.74	0.00
34	4.03	0.00	1.58	0.38
35	–1.27	0.36	–1.35	0.44
36	—	—	–3.80	0.00
37	—	—	–6.97	0.00

Supplementary Table 4: Values of η for the model simulations

For the traditional model (column 2), values are listed in the order of recruitment of neurons above the eye position threshold $E_{th,i}$ as the eyes move from left to right. For the high-threshold (column 3) and bistable-dendrites (column 4) models, values are instead listed in the order in which neurons cross the firing rate threshold for activating postsynaptic targets, r_{th} or r_{on}, respectively. These orderings correspond to the neuron numbers given in the construction of the cumulative-input plots (Figs. 1c bottom, 7a right, and 7d right).

i	$\eta_{i,Fig.1D}$	$\eta_{i,Fig.7B}$	$\eta_{i,Fig.7E}$
1	1.81	2.99	0.30
2	2.53	2.54	0.30
3	2.00	3.53	0.30
4	3.09	3.51	0.30
5	3.77	2.42	0.30
6	1.89	1.31	0.30
7	1.84	1.98	0.30
8	0.46	2.11	0.30
9	0.34	0.67	0.30
10	0.30	0.40	0.30
11	0.23	0.43	0.30
12	1.59	0.34	0.30
13	1.30	0.72	0.33
14	0.31	1.35	0.30
15	0.29	0.60	0.30
16	0.65	0.40	0.30
17	0.48	0.25	0.30
18	0.46	0.23	0.74
19	0.89	0.91	0.87
20	0.56	0.36	0.30
21	0.26	0.53	0.63
22	0.92	1.05	0.30
23	0.50	1.19	0.76
24	0.18	2.78	0.30
25	0.29	2.46	0.30
26	0.54	1.31	1.04
27	0.77	0.15	0.68
28	0.39	1.23	0.51
29	0.19	1.11	0.60
30	0.20	0.41	0.57
31	0.18	0.15	0.71
32	0.15	0.60	0.46
33	0.15	3.91	2.00
34	0.15	4.94	0.34
35	0.15	2.58	2.00
36	0.34	2.58	2.00

Supplementary Methods

Animal preparation

Goldfish (*Carassius auratus*, 3–5" tip to peduncle, 25–50 g) were purchased from a commercial supplier (Hunting Creek Fisheries, Thurmont, MD) and kept at 20–22°C in a 50 gallon aquarium with daily exposure to light. For experiments, fish were placed in a 15" diameter cylindrical acrylic tank, where they were stabilized by attaching a head-mounted lug to a post and by placing the trunk between two supported sponges. Eye movements were measured with the scleral-search-coil technique. Animal care, procedure for head fixation, surgical preparation, details of the experimental holding tank, and search coil method were as previously described[1] (ACSF, in mM: 140 NaCl, 2.44 KCl, 5 $NaHCo_3$, 0.42 Na_2HPO_4, 5 Hepes, 1.16 $CaCl_2$, 1.0 $MgCl_2$; pH 7.2, 270 mOsm). Water temperature during experiments was between 20–22°C. Eye movements and microelectrode voltage during area I recordings were simultaneously digitized (Digidata 1200, Clampex 7, Axon Instruments, Burlingame, CA).

Pharmacological inactivation

Pressure injections of pharmacological agents into area I were performed with double-barreled glass electrodes (2B150F, World Precision Instruments, Sarasota, FL) that had one barrel loaded with lidocaine (L5647, Sigma) or muscimol (M1523, Sigma) and the other with recording solution. The standard lidocaine solution was 70 mM lidocaine dissolved in ACSF reduced to 70 mM NaCl, and the standard muscimol solution was 1 mM muscimol dissolved in ACSF. In a few experiments more concentrated solutions (140 mM lidocaine with 0 mM NaCl ACSF; 5 mM muscimol) were used to further reduce injected volume. The recording solution consisted of 2 M NaCl, 5 mM Hepes, and was set to pH=7.4 with NaOH. Fast Green (2 mM, F-99, Fisher) was added to all injection and recording solutions. The injection barrel was attached to air pressure lines, while the other barrel contained a chlorided silver wire connected to a recording headstage (Neurodata IR-283, Cygnus Technologies, Delaware Water Gap, PA). The electrode was beveled (BV-10, Sutter Instruments) with the injection barrel against the plate to achieve tip diameters (perpendicular to the length of the glass) of 3–5

microns for the injection barrel and 1–2 microns for the recording barrel; this brought the resistance of the recording barrel to under 5 MOhm. Injection tips were calibrated by pressure injecting into a fluorinated hydrocarbon (Fluorinert F77, Sigma) or mineral oil and measuring drop volume (5 psi pressure for 5 seconds through a 5 micron tip ejected ~1 nL). A second single-barreled electrode, as previously described[1], was used to monitor the activity of position neurons distal to the injection site.

Localization of area I was as previously described[1]. Recordings were predominantly from large (>0.5 mV) biphasic action potentials indicative of proximity to a soma[1]. During recording, electrode position was frequently adjusted in few micron steps to provide a high signal-to-noise ratio recording of a given cell, allowing a single neuron to be monitored for tens of minutes. For injection, the experimental tank was sealed from light so that the optokinetic system could not stabilize fixations, and baseline neuronal activity was determined with a 2–5 minute recording. Pharmacological agents were injected with steady pressure (5 psi) for brief periods (**Supplementary Fig. 2c**). Efficacy of lidocaine or muscimol injections was determined by confirming loss of spiking activity on the recording barrel of the injection electrode, combined with drift of the eyes toward the contralateral field when positioned to fixate in the ipsilateral field (relative to the injection side). In ~1/5 of the experiments, repeated attempts at injection were made before achieving inactivation, consistent with transient blockage of the injection tip.

For contralateral inactivation (**Supplementary Fig. 2a**), the extent of inactivation in the dorso-ventral axis was determined by scanning vertically with the injection electrode; injections proceeded until a 400 micron vertical extent centered on area I was inactivated. The electrode was then removed from the brain and injection was performed in the same manner at a location 200–400 microns more rostral, eliminating any residual activity in area I. Complete contralateral inactivation was typically achieved in under 3 minutes.

Caudal injections producing partial unilateral inactivations (**Supplementary Fig. 2b,c,d**) were typically achieved in less than 30 seconds. Rostral cells were recorded at locations 300–400 microns away. Separate experiments (not shown) placing the recording electrode closer to the injection site to assess the extent of inactivation

indicated that the typical caudal injection silenced ~30% of one population. Injection at the caudal end minimized the possibility of direct effects on the rostral cell because position neurons have dendrites that largely course rostrally[1]. Even so, approximately a third of the time injections induced inactivation of the monitored cell, indicating either spread of injected solution to the recorded site or a recording from a neuronal process. In these cases the experiment was not included in the analyzed data set and the fish was allowed to recover for a period of one hour or more.

To understand better the potential for direct drug effects on the monitored cell, action potential width and the range of saccade-related firing rate were compared during control and inactivation. In 3/8 cases of ipsilateral lidocaine injection, action potential width abnormally increased by as much as 25%. In 1/8 cases of ipsilateral muscimol injection, the rate range during saccades decreased by 20%. Thus, caudal inactivations, in addition to silencing a subset of one population and removing a possible source of input to rostral cells, might have also altered rostral cells to the point of modifying potential cellular mechanisms of persistence. Changes in width or range were not seen with contralateral injections; thus, contralateral inactivations apparently only removed a possible source of input.

Data analysis

All analysis was done using Matlab (MathWorks, Natick, MA). Saccades were identified by use of a velocity threshold. Action potential detection and firing rate calculations were as previously described[1] ("method three", 0.15 s smoothing window). The underlying trend in rate drift was approximated by a cubic smoothing spline[2] to firing rate over an inter-saccadic period (0.2 seconds after one saccade to 0.2 seconds before the next). The cubic smoothing spline achieves a balance between a simple linear least-squares fit of a straight line (80% weighting) and a full cubic spline interpolation (20% weighting).

Three parameters were set to define a fixation for subsequent analysis. One (t_a) was used to ignore a period of time immediately after saccades when there is significant "postsaccadic slide" in eye position and firing rate in naive goldfish[1]. Another (t_b) was used to ignore a period immediately before saccades. The inter-saccadic region not

excluded by these limits defined the period of fixation. Those fixations shorter than a threshold length (t_f) were ignored. Different controls had different average values for slide magnitude and saccade frequency. This necessitated a choice of values for these parameters in every experiment to avoid throwing out data. The range of values for the parameters were t_a: 0.3–0.6 s, t_b: 0.1–0.3 s, t_f: 0.4–1.0 s. For any given experiment, a fixed combination of three values was used across baseline and injection data sets.

For qualifying fixations, mean eye position, drift in eye position, firing rate, and drift in firing rate were determined for non-overlapping segments of length 0.3 seconds between t_a and t_b. Firing rate was determined by taking the mean value of the spline segment, and firing rate drift was taken to be the slope of the best-fit line to the spline segment; rate drift was only calculated for a segment if the average rate during the segment was greater than 1 sp/s. For each experiment, the range of eye position was normalized to +/– 15 degrees for the control period, then the same scaling and offset values were applied to the inactivation period. In the coarse drift analysis for individual experiments a drift value was only assigned to an oculomotor-range half if more than 10 segments contributed. In the fine drift vs. position analysis data were grouped into bins of 5 degrees (–20:5:20) after consolidating all experiments; averages were assigned to a bin only if there were more than 5 cells that provided data in that bin. Differences between inactivation and control states were calculated on a bin-by-bin basis. In many cases the oculomotor range during inactivation was larger than during control: if so, inactivation data in a bin beyond 15 (–15) degrees were compared to control data located in the range 10 to 15 (–10 to –15) degrees. Slopes and standard errors of the slope (s.e.) on difference plots were calculated using a least-squares algorithm weighted by the inverse of the variance associated with each data point.

References

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