

1 **Title: VIP interneurons selectively enhance weak but behaviorally-relevant stimuli.**

2

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7

8 **Abstract**

9

10 Vasoactive intestinal peptide-expressing (VIP) interneurons in cortex regulate feedback  
11 inhibition of pyramidal neurons through suppression of somatostatin-expressing (SST)  
12 interneurons and, reciprocally, SST neurons inhibit VIP neurons. Here, we show that VIP neurons  
13 in mouse primary visual cortex have complementary contrast tuning to SST neurons and respond  
14 synergistically to front-to-back visual motion and locomotion. Network modeling indicates that this  
15 VIP-SST mutual antagonism regulates the gain of cortex to achieve both sensitivity to  
16 behaviorally-relevant stimuli and network stability.

17

18 **Main Text**

19

20 Inhibitory interneurons play a major role in establishing the dynamics of cortical  
21 microcircuits.<sup>1,2</sup> In layer 2/3 of cortex, vasoactive intestinal peptide-expressing (VIP) interneurons  
22 regulate feedback inhibition of pyramidal neurons through suppression of somatostatin-  
23 expressing (SST) interneurons.<sup>3</sup> Through this disinhibitory mechanism, VIP interneurons are  
24 believed to modulate network dynamics based on the behavioral state of the animal; for instance,  
25 VIP neurons in mouse primary visual cortex (V1) are reliably active during periods of locomotion.<sup>4</sup>  
26 Moreover, VIP neurons in V1 are a target of top-down inputs and mediate enhancement of local

27 pyramidal cell activity in response to activation of those inputs.<sup>5</sup> VIP neurons also receive  
28 reciprocal inhibition from SST neurons, creating a circuit motif of mutual inhibition between VIP  
29 and SST neurons with unknown implications for cortical processing. Behaviorally, mouse V1 is  
30 necessary for the detection of low contrast visual stimuli,<sup>6</sup> and the optogenetic activation of VIP  
31 neurons in mouse V1 increases contrast sensitivity whereas the activation of SST or PV neurons  
32 decreases it.<sup>7</sup> This suggests that the perception of low contrast stimuli is strongly influenced by  
33 VIP neuron activity in V1. Although the activity of VIP neurons has been shown to be suppressed  
34 below baseline in response to high contrast full-field grating stimuli,<sup>8</sup> the responses of VIP neurons  
35 to low contrast visual stimuli are not known. To this end, we investigated the influence of stimulus  
36 contrast and motor behavior (i.e. locomotion) on the visual responses of VIP, SST, and pyramidal  
37 neurons in mouse V1. SST neurons responded exclusively at high contrast whereas VIP neurons  
38 responded exclusively at low contrast with strong preference for front-to-back motion that is  
39 congruent with self-motion during locomotion. As a population, layer 2/3 – but not deeper layer –  
40 pyramidal neurons responded more strongly at low contrast than high contrast and showed a  
41 slight, but significant, bias for front-to-back motion. Finally, we made novel extensions of stabilized  
42 supralinear network (SSN) models to incorporate the diversity of inhibitory interneuron types and  
43 used these models to demonstrate that VIP-driven disinhibition at low contrast can drive large  
44 increases in pyramidal neuron activity, despite the relatively low activity of both SST and  
45 pyramidal neurons in this contrast regime. The selective enhancement of front-to-back motion  
46 could increase detection of obstacles approaching head-on during locomotion. Based on these  
47 results, we conclude that VIP neurons amplify responses of pyramidal neurons to weak but  
48 behaviorally-relevant stimuli.

49 We recorded responses to drifting gratings at eight directions and six contrasts during  
50 calcium imaging of mouse Cre lines for *Vip* and *Sst* as well as pyramidal neurons across cortical  
51 layers (*Cux2*: layer 2/3; *Rorb*: layer 4; *Rbp4*: layer 5; *Ntsr1*: layer 6) transgenically expressing  
52 GCaMP6f and computed the response to each stimulus condition. The majority of neurons were

53 tuned for grating contrast and direction (bootstrapped  $\chi^2$  test,  $p < 0.01$ ; Supplementary Figure 1a;  
54 Supplementary Table 1). We observed direction- or orientation-tuned neurons that responded  
55 preferentially either to high contrast gratings or low contrast gratings (Figure 1a). Substantial  
56 differences in contrast and direction tuning were apparent across Cre lines (Figure 1b-g). Virtually  
57 all VIP neurons responded only at low (<20%) contrast to front-to-back motion (0 degrees; nasal-  
58 to-temporal) or an adjacent direction, yielding the greatest direction bias among Cre lines as  
59 quantified by the vector sum of direction preferences (Figure 1c). The direction of bias was  
60 consistent across all VIP mice ( $n=6$ ; Supplementary Figure 2a) and did not result from stimulus  
61 direction-selective running behavior (Supplementary Figure 2b). High contrast gratings of all  
62 directions suppressed VIP neuron activity (Figure 1e), consistent with a previous report<sup>9</sup> of a small  
63 population of neurons that were active during locomotion but suppressed by the presentation of  
64 full-field high contrast gratings. SST neurons had high contrast selectivity, weak direction  
65 selectivity, and varied direction preference (Figure 1c,d,g), resulting in an average population  
66 response that was strong at high contrast across all directions, complementing the non-direction  
67 selective suppression at high contrast observed in VIP neurons. Unlike inhibitory interneurons,  
68 pyramidal neurons exhibited substantial direction and orientation selectivity and tiled all eight  
69 possible direction preferences (Figure 1b,f,g). Contrast preference among pyramidal neurons  
70 systematically varied across cortical layers, exhibiting a progression from a mixture of low and  
71 high contrast-preferring neurons in layer 2/3 to almost exclusively high contrast-preferring  
72 neurons in layers 5 and 6 (Figure 1b,d; Supplementary Figure 3). Like VIP neurons, CUX2  
73 neurons in layer 2/3 showed direction bias toward front-to-back motion at 5% and 10% contrast  
74 but not higher contrasts (Figure 1c); pyramidal neurons in deeper layers did not have direction  
75 bias. Taken together, concerted changes in response magnitude near 20% contrast across all  
76 Cre lines and layers indicates the presence of a phase transition in cortical dynamics between a  
77 low contrast regime exemplified by relatively inactive SST neurons and a high contrast regime  
78 exemplified by highly active SST neurons.

79 To assess circuit-wide effects of locomotion on cortical dynamics, we examined the  
80 average activity of each neuron population as a whole. We focus here on the responses at low  
81 contrast in layers 2/3 and 4, but not layers 5 and 6 which did not respond at low contrast. VIP,  
82 SST, and pyramidal populations in both layers 2/3 and 4 all had increased activity during  
83 locomotion compared with stimulus presentations during which the mouse was stationary (Figure  
84 2). During locomotion, the low contrast and front-to-back direction selectivity that was common to  
85 nearly all VIP neurons resulted in an average VIP population response that had tuning closely  
86 resembling the tuning of any individual VIP neuron (Figure 2a,e,i,m). By comparison, the VIP  
87 population did not respond to motion of any direction or contrast when the mice were stationary.  
88 Running also increased the SST population response to high contrast gratings, which also had  
89 the highest average response to front-to-back motion but responded strongly as a population to  
90 other directions as well (Figure 2b,f,j,n). The CUX2 population in layer 2/3 responded broadly  
91 across directions but more strongly, and with greater running enhancement, at low than high  
92 contrast (Figure 2c,g,k,o), whereas the Rorb population in layer 4 had comparable response  
93 magnitude and running enhancement across contrasts (Figure 2d,h,l,p).

94 Anatomical and optogenetic perturbation experiments suggest that VIP neurons disinhibit  
95 pyramidal neurons through inhibition of SST neurons.<sup>3,5,10</sup> However, VIP neurons only respond to  
96 one direction of low contrast grating and SST neurons have very weak responses to low contrast  
97 gratings of any direction, potentially limiting the magnitude of SST activity that is available to be  
98 inhibited by VIP neurons and, consequently, limiting the magnitude of disinhibition of pyramidal  
99 neurons. Evidence that visual cortex has higher gain at low contrast than high contrast<sup>11,12,13</sup>  
100 suggests that a small reduction in feedback inhibition (e.g. disinhibition) is capable to drive a large  
101 increase in pyramidal neuron activity. Stabilized supralinear network (SSN) models have been  
102 proposed to account for a variety of contrast-dependent response properties in visual cortex,<sup>14,15</sup>  
103 including the transition from a high gain regime at low contrast to a feedback inhibition dominated  
104 low gain regime at high contrast.<sup>16,17</sup> To investigate the distinct roles of each interneuron type, we

105 extended the SSN model from one homogeneous population of interneurons to three populations  
106 corresponding to VIP, SST, and parvalbumin-expressing (PV) neurons to model layer 2/3 of  
107 mouse V1 (Figure 3a; Supplementary Methods). Briefly, the network is a ring model in which each  
108 CUX2 pyramidal neuron receives external (“sensory”) excitatory input that has Gaussian tuning  
109 with mean (i.e. peak/preferred direction) corresponding to the neuron’s position on the ring and  
110 standard deviation of 30 degrees; PV neurons also receive external input which is not tuned. The  
111 strength of external input is intended to represent a monotonically-increasing function of stimulus  
112 contrast, though no specific relationship is claimed here. Connections from CUX2 neurons (i.e.  
113 excitatory connections) also have Gaussian tuning that depends on the difference between the  
114 orientation preferences of the pre- and post-synaptic neurons whereas connections from inhibitory  
115 neurons (i.e. inhibitory connections) are not tuned (Figure 3b). All neurons are modeled as rate  
116 units with rectified quadratic transfer function. This model is able to qualitatively reproduce the  
117 population direction and contrast tuning we observed for VIP, SST, and CUX2 neurons as well as  
118 make a prediction for the tuning of PV neurons (Figure 3c). Model VIP neurons are most active  
119 at a low level of external input corresponding to the highest gain (“supralinear”) regime for CUX2  
120 and PV activity (Figure 3d: left). Ablating the VIP-to-SST inhibitory connection, the only output of  
121 VIP neurons contained in the model, results in a large reduction in the gain and activity of VIP,  
122 CUX2, and PV populations at low input (Figure 3d: right). These results indicate that VIP  
123 disinhibition is capable of producing substantial increases in gain at low contrast despite low  
124 activity of the intermediate SST neuron population.

125 This survey of contrast tuning in mouse V1 revealed two distinct regimes of cortical  
126 dynamics in layer 2/3. At high contrast, SST neuron activity is high, VIP neuron activity is  
127 suppressed, and layer 2/3 pyramidal neuron activity is reduced compared with the low contrast  
128 regime; at low contrast, SST neuron activity is low, VIP neuron activity is direction tuned and  
129 gated by locomotion, and layer 2/3 pyramidal neuron activity is higher and more enhanced by  
130 locomotion. Measurements of size tuning have shown that SST neurons prefer large gratings,

131 suggestive of a role mediating surround suppression, whereas VIP neurons only respond to  
132 gratings smaller than those that drive SST neurons.<sup>18,19</sup> In mouse primary auditory cortex, VIP  
133 neurons are selective for lower sound intensities than SST or PV neurons.<sup>20</sup> Taken together, a  
134 parsimonious explanation of these results is that VIP neuron activity supports a high gain regime  
135 that increases sensitivity to weak inputs, whereas SST neuron activity promotes a low gain regime  
136 that decreases sensitivity to strong inputs and maintains network stability. Heightened sensitivity  
137 to detect low contrast objects or obstacles approaching head-on during locomotion might be more  
138 behaviorally relevant than other directions of motion. This ability of VIP neurons to promote high  
139 gain in the local microcircuit might be indicative of a more general role at the nexus of top-down  
140 (e.g. attention) and bottom-up (e.g. saliency) processes.

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## 175 **Author Contributions**

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177 Conceptualization, M.A.B. and S.E.J.dV. Methodology, D.J.M., G.K.O., J.A.L., and

178 S.E.J.dV. Investigation, S.C, I.K., J.D.L., E.K.L., J.L., T.V.N., C.N., K.N., S.S., and C.W. Formal

179 Analysis, D.J.M., G.K.O., and S.E.J.dV. Writing – Original Draft, D.J.M. Writing – Review &

180 Editing, D.J.M., G.K.O., M.A.B., and S.E.J.dV. Supervision, J.A.L., R.C.R., M.A.B., and S.E.J.dV.

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186



1 **Figure 1:** Contrast and direction preferences are cell-type and layer specific. **a)** Two examples of  
2 single cells, a low-pass (top) and a high-pass (bottom) contrast response function. Left: Heatmaps  
3 show the mean response of the cell averaged over all presentations of a drifting grating of a given  
4 direction and contrast. Right: Mean response to gratings of each contrast at the cell's peak  
5 direction (top) as well as each direction at the cell's peak contrast (bottom). Error bars are SEM.  
6 **b)** Waterfall plots showing the response significance at each contrast and direction of all  
7 responsive cells ( $\chi^2$  test;  $p < 0.01$ ) from mice of each Cre line. Cells are ordered by direction  
8 preference at the cell's peak contrast. Response significance for each condition is obtained by  
9 comparing the mean condition response minus mean blank (i.e. zero contrast) response to a null  
10 distribution of such differences that is generated by shuffling responses across trials (see  
11 methods); responses below the median of the shuffle distribution are blue (i.e. suppressed),  
12 responses above the median of the shuffle distribution are red (i.e. enhanced). **c)** Radial plot of  
13 the average direction preference of cells of each Cre line at each contrast. Arrows are the vector  
14 sum of all responsive cells at a given contrast. Gray shaded region indicated a 90% confidence  
15 interval of the vector sum for population with uniformly-distributed direction preferences. Scale:  
16 The distance between each pair of concentric dashed rings is 25%. **d)** Cumulative distribution of  
17 contrast preferences (center-of-mass of a cell's contrast response function; CoM) across Cre  
18 lines. **e)** Fraction of all cells of each Cre line that are suppressed by contrast. The mean response  
19 to all grating directions at 80% contrast must be significantly below mean blank response  
20 (bootstrapped distribution of mean response differences; family-wise type 1 error  $< 0.05$ ; see  
21 methods). **f)** Cumulative distribution of global orientation selectivity indices (gOSI) across Cre  
22 lines. **g)** Cumulative distribution of direction selectivity indices across Cre lines.  
23  
24

25 **Figure 2:** Average population responses of inhibitory, but not excitatory, cells are strongly biased  
26 toward front-to-back visual motion which is enhanced during locomotion. **a-d)** Mean blank-  
27 subtracted response magnitude of all cells from mice of each superficial Cre line during stationary  
28 (top) and running (bottom) periods. Gray boxes in Rorb plots indicate insufficient run and  
29 stationary data. **e-h)** Mean population contrast response tuning during stationary (faint lines)  
30 and running (bold lines) periods. **i-l)** Mean population direction response tuning at low (5-10%)  
31 contrast as in **e-h**. insets: mean population direction response tuning at high (60-80%) contrast.  
32 **m-p)** Mean single-cell direction tuning (i.e. aligned to each cell's peak direction) as in **e-l**. All error  
33 bars are SEM. Sample size indicates number of cells with number of experiments in parenthesis.  
34  
35

36 **Figure 3:** A stabilized supralinear network model with three interneuron populations reproduces  
37 contrast and direction tuning of multiple cell types and implicates Vip cells in enhancement of  
38 network gain for weak inputs. **a)** Top: The network architecture is a ring corresponding to the  
39 peak of each Cux2 pyramidal cell's direction tuning curve. The entire ring spans 180 degrees of  
40 direction. Bottom: A schematic illustrates the connectivity among cell types. **b)** Top: The  
41 distribution of excitatory connection strength from Cux2 pyramidal cells onto each cell type is  
42 Gaussian with mean equal to the difference in orientation preference of pre- and post-synaptic  
43 cells. The distributions of recurrent connections onto Cux2 cells and connections onto Vip cells  
44 are narrow (standard deviation of 30 degrees) compared to the distributions onto PV and Sst  
45 cells (standard deviation of 100 degrees). Bottom: Inhibitory connection weights do not vary as  
46 a function of the difference between the peak directions of pre- and post-synaptic cells. **c)** The  
47 average population responses across direction and contrast conditions qualitatively reproduce  
48 experimental data for Cux2, Sst, and Vip cells shown in Figure 2. **d)** Left: The steady state firing  
49 rates are shown for model cells of each type with peak direction tuning of zero degrees in  
50 response to an input "stimulus" of zero degrees. Right: The steady state firing rates of the same  
51 model cells in response to an input of zero degrees with the Vip-to-Sst connection strength set  
52 to zero demonstrates that this connection is necessary for high gain of Cux2 and PV cells at the  
53 low input levels for which Vip cells are most responsive.  
54

55 **Supplementary Table 1:** The total number of cells and mice per Cre line.

56

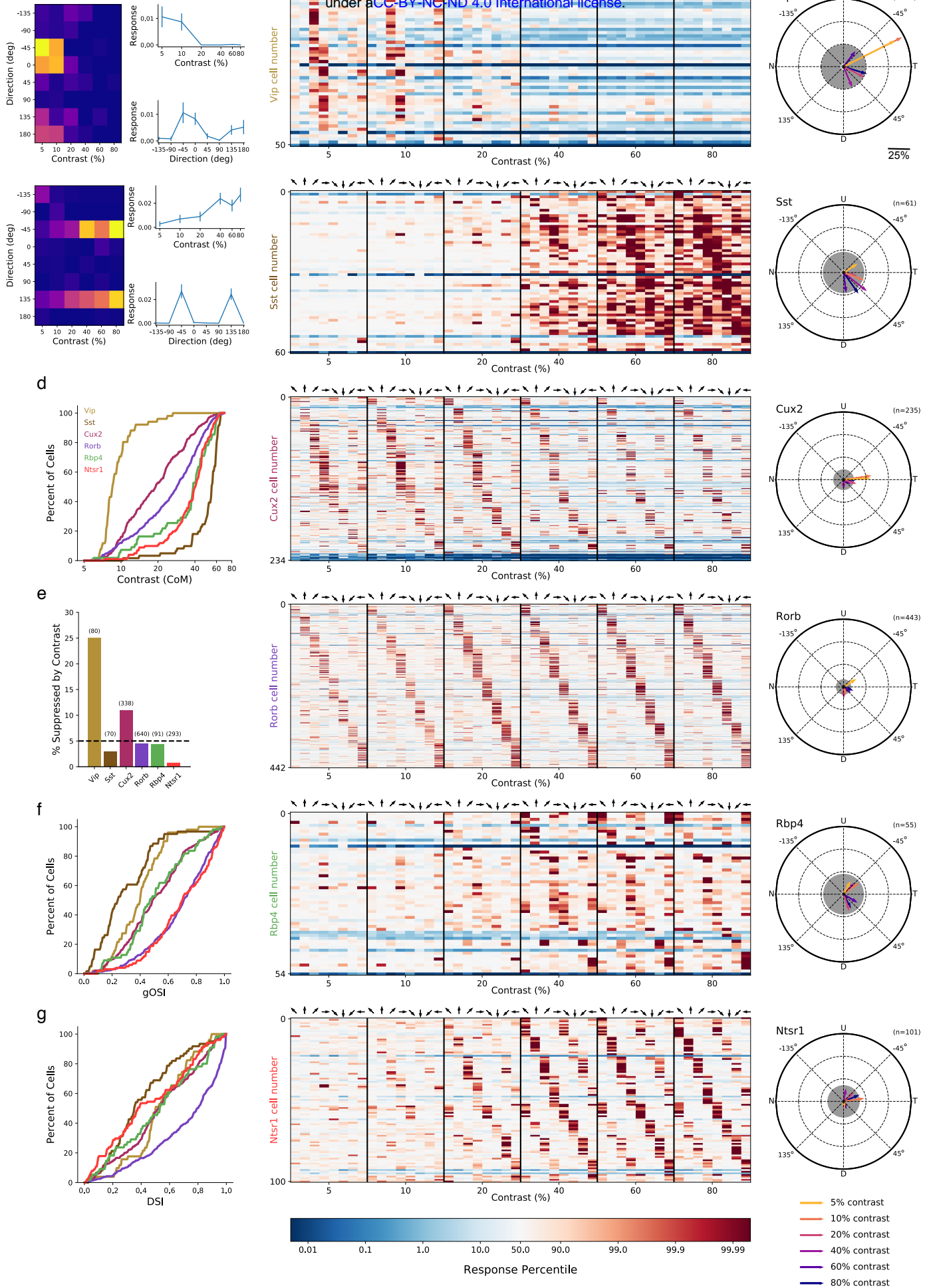
57 **Supplementary Figure 1:** The fraction of imaged cells that were significantly responsive to the  
58 gratings stimulus (bootstrapped  $\chi^2$  test,  $p < 0.01$ ).  
59

60 **Supplementary Figure 2:** The direction of bias was consistent across all VIP mice and did not  
61 result from stimulus direction-selective running behavior. **a)** Vector sums for each of the six Vip-  
62 Cre mice. **b)** Performance of a linear support vector classifier trained to decode the direction of  
63 grating (1-of-8 classification) from the running speed of the mouse. The average validation  
64 performance for three-fold cross-validation is shown. Each dot is the performance for one  
65 mouse; bars are the mean across mice of a given Cre line.  
66  
67

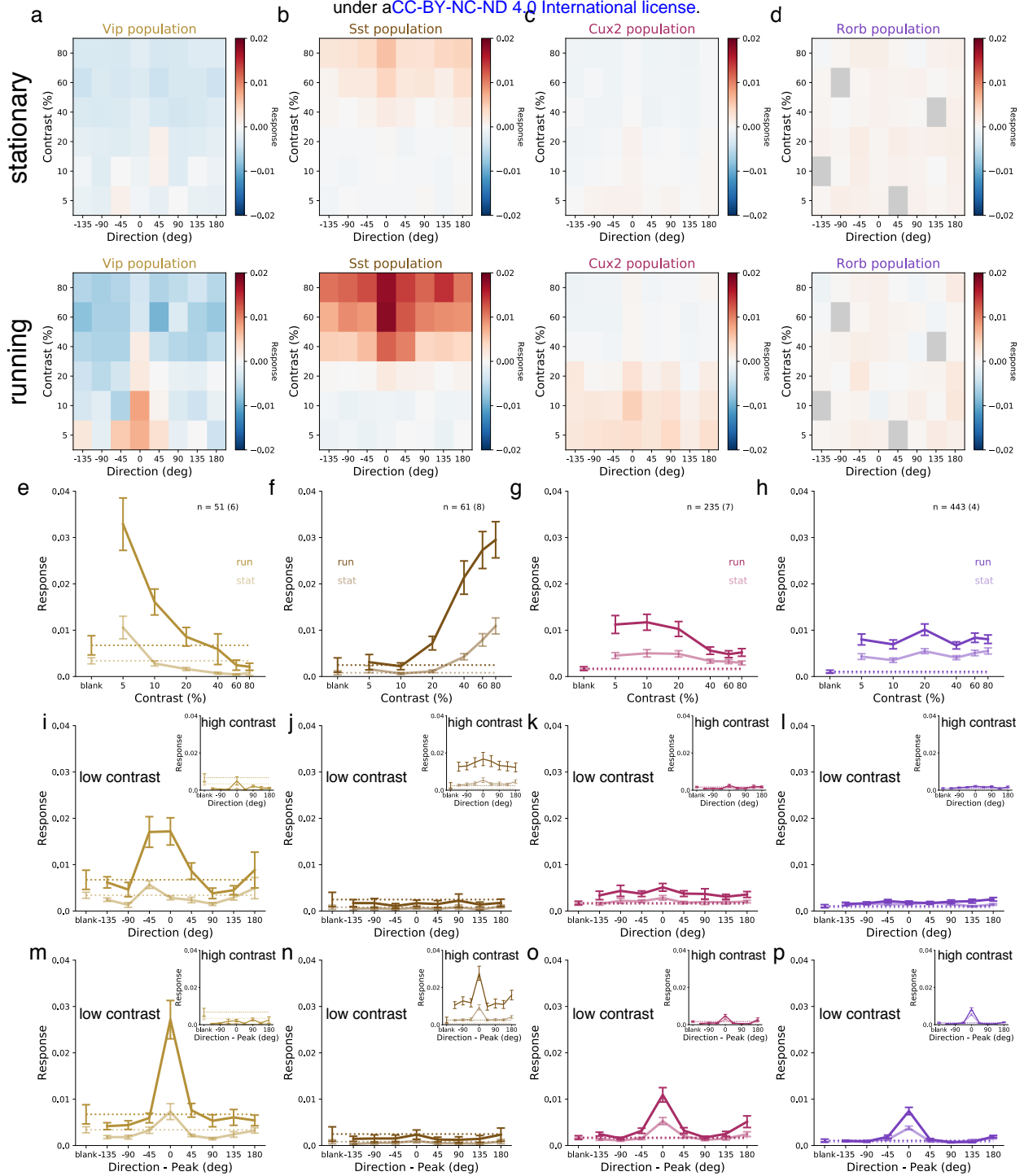
68 **Supplementary Figure 3:** Distribution of contrast response types by Cre line determined by fitting  
69 of rising sigmoid (high pass), falling sigmoid (low pass), or the product of rising and falling  
70 sigmoids (band pass). See methods.

# Figure 1

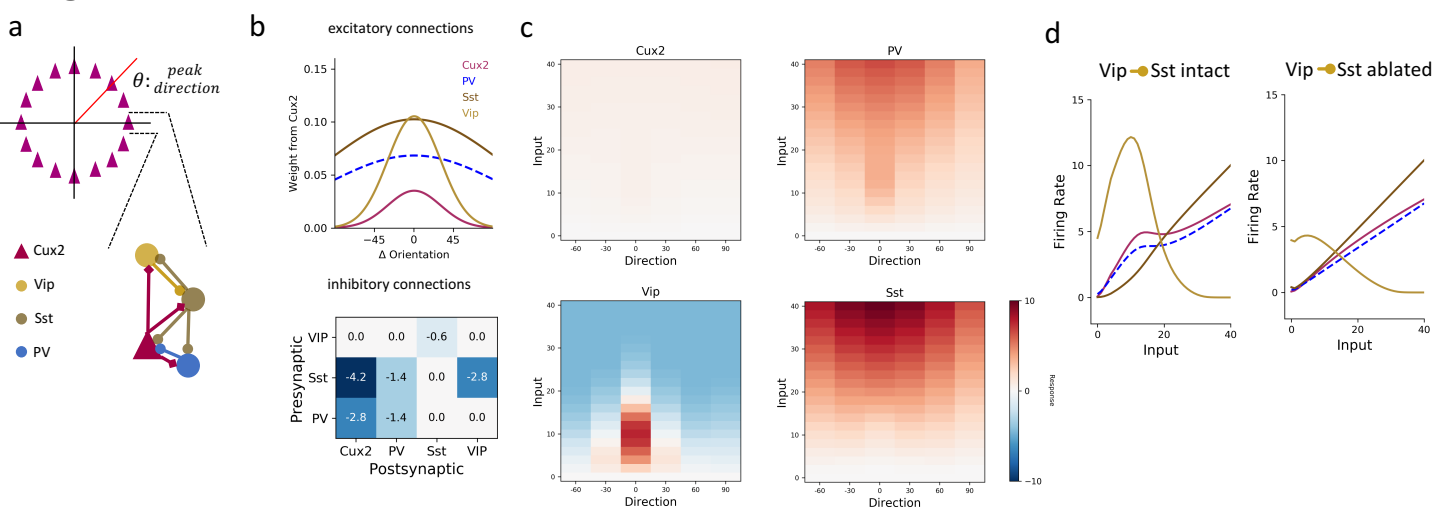
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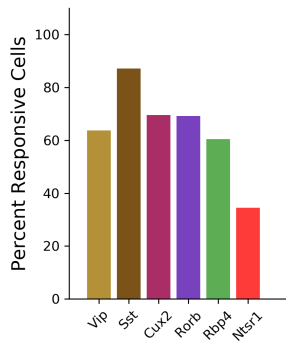
# Figure 3



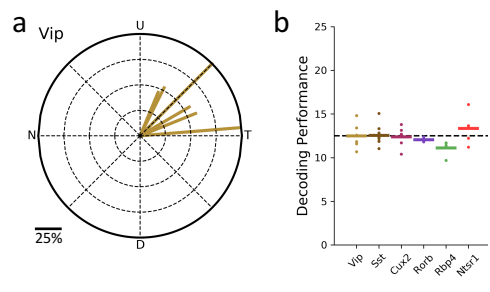
# Supplementary Table 1

	Cells (Mice)
<b>Cux2</b>	338 (7)
<b>Rorb</b>	640 (4)
<b>Rbp4</b>	91 (4)
<b>Ntsr1</b>	293 (6)
<b>Sst</b>	70 (8)
<b>Vip</b>	80 (6)

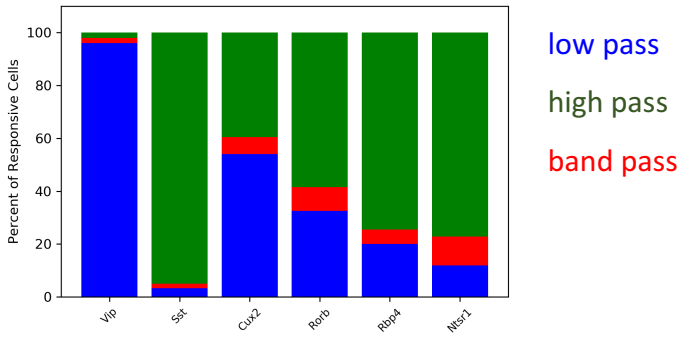
# Supplementary Figure 1



# Supplementary Figure 2



# Supplementary Figure 3



## 1 **Methods**

### 2 3 *Experimental Animals*

4  
5 All animal procedures were approved by the Institutional Animal Care and Use Committee  
6 (IACUC) at the Allen Institute for Brain Science. Six double or triple transgenic mouse lines were  
7 used to drive expression of GCamp6/f in genetically-defined cell types, including four excitatory  
8 (Cux2-CreERT2;Camk2a-tTA;Ai93, Rorb-IRES2-Cre;Camk2a-tTA;Ai93, Rbp4-  
9 Cre\_KL100;Camk2a-tTA;Ai93, and Ntsr1-Cre\_GN220;Ai148) and two inhibitory (Vip-IRES-  
10 Cre;Ai148 and Sst-IRES-Cre;Ai148) mouse lines. Mice were habituated to head fixation and  
11 visual stimulus presentation for two weeks prior to data collection (See de Vries, Lecoq, Buice *et*  
12 *al.* for further Cre line, surgical, and habituation details).

### 13 14 *Two photon imaging platform and image processing*

15  
16 Data was collected using the same data collection pipeline as the Allen Brain  
17 Observatory and processed using the same image processing and event detection methods. All  
18 analyses of cell responses were performed on L0 penalized detected events (See de Vries,  
19 Lecoq, Buice *et al.* for further imaging and image processing details).

20  
21 Briefly, two-photon imaging data was collected from the retinotopic center of primary visual  
22 cortex that was identified through mapping during widefield intrinsic signal imaging. Cux2-  
23 CreERT2;Camk2a-tTA;Ai93, Vip-IRES-Cre;Ai148, and Sst-IRES-Cre;Ai148 mice were imaged at  
24 175 um below the cortical surface in layer 2/3; Rorb-IRES2-Cre;Camk2a-tTA;Ai93 mice were  
25 imaged at 275 um below the cortical surface in layer 4; Rbp4-Cre\_KL100;Camk2a-tTA;Ai93 mice  
26 were imaged at 375 um below the cortical surface in layer 5; and Ntsr1-Cre\_GN220;Ai148 mice  
27 were imaged at 550 um below the cortical surface in layer 6. (These Cre lines and imaging depths  
28 match those used in the Allen Brain Observatory.)

### 29 30 *Visual Stimulus*

31  
32 As experimental sessions took place on the same data collection pipeline as the Allen  
33 Brain Observatory, visual stimulus monitor calibration and positioning was identical (See de Vries,  
34 Lecoq, Buice *et al.* for further visual stimulus presentation details). The stimulus consisted of a  
35 full field drifting sinusoidal grating that was presented at a single spatial frequency (0.04  
36 cycles/degree) and temporal frequency (1 Hz), 8 directions uniformly distributed in 45 degree  
37 increments (0 degrees = horizontal front-to-back motion), and 6 contrasts (5%, 10%, 20%, 40%,  
38 60%, and 80%). Direction of motion was always orthogonal to the orientation of the grating. Each  
39 grating was presented for 2 seconds, followed by 1 second of mean luminance gray before the  
40 next grating. Each grating condition (direction, contrast combination) was presented 15 times.  
41 Trials were randomized with 30 randomly interleaved blank (i.e. mean luminance gray, zero  
42 contrast) trials.

## 43 **Analysis**

### 44 45 46 *Statistical test for responsiveness*

47  
48 A chi-square test for independence was used to determine significantly responsive cells  
49 to the drifting grating stimulus set. A chi-square test statistic was computed  $\chi^2 = \sum_{i=0}^n \frac{(E_i - O_i)^2}{E_i}$ ,

50 where  $O_i = \frac{1}{m_i} \sum_{j=0}^{m_i} R_{i,j}$  is the observed average response ( $R$ ) of the neuron over  $m$  presentations  
51 of a grating stimulus of a particular condition (i.e. direction-by-contrast pair or blank,  $n = 49$  total  
52 conditions), and  $E_i = \frac{\sum_i^n \sum_j^{m_i} R_{i,j}}{\sum_i^n m_i}$  is the expected (grand average) response per stimulus  
53 presentation. A p-value was then calculated for each cell by comparing the test statistic against a  
54 null distribution of 200,000 test statistics, each computed from the cell's responses after shuffling  
55 (with replacement) cell responses across all presentations.

56

### 57 *Response Significance by Stimulus Condition and test for suppression by contrast*

58

59 The distribution of responses to stimulus presentations varied substantially across cells.  
60 To facilitate the visualization of responses across all cells and stimulus conditions simultaneously  
61 (figure 1b), a statistical measure was used to normalize response magnitudes. The mean blank-  
62 subtracted response to a given stimulus condition was calculated as:  $\bar{R} = \frac{1}{m_i} \sum_{j=0}^{m_i} R_{i,j} -$   
63  $\frac{1}{m_{blank}} \sum_{j=0}^{m_{blank}} R_{blank,j}$ . Then, a bootstrapped null distribution of such mean (blank-subtracted)  
64 condition responses was generated by sampling with replacement from all of the cell's responses  
65 across all stimulus presentations. The percentiles of each cell's observed mean condition  
66 response within its own bootstrapped distribution are the values plotted in Figure 1b. Cells were  
67 determined to be suppressed by high contrast if this percentile for the peak direction grating  
68 condition at 80% contrast was below 0.05.

69

### 70 *Orientation and Direction Selectivity Metrics*

71

72 Global orientation selectivity was computed from mean extracted event responses to  
73 drifting gratings, at the cell's preferred contrast, as

$$74 \quad gOSI = \frac{\sum R_{\theta} e^{i\theta}}{\sum R_{\theta}}$$

75 where  $\theta$  is the direction of grating movement, and  $R_{\theta}$  is the mean response to that direction of  
76 motion.

77

78 Direction selectivity was computed from mean extracted event responses to drifting  
79 gratings, at the cell's preferred contrast, as

$$80 \quad DSI = \frac{R_{pref} - R_{null}}{R_{pref} + R_{null}}$$

81 where  $R_{pref}$  is a cell's mean response in its preferred direction (i.e. largest response-evoking  
82 direction) and  $R_{null}$  is its mean response to the opposite direction.

83

### 84 *Contrast Preference Metric*

85

86 Contrast preference was computed from mean extracted event responses to drifting  
87 gratings, at the cell's preferred direction, as

$$88 \quad c_{CoM} = e^{\left( \frac{\sum R_c \ln c}{\sum R_c} \right)}$$

89

90 where  $c$  is the contrast of the drifting grating,  $R_c$  is a cell's mean response at contrast  $c$ , and  $c_{CoM}$   
91 is the log-scaled center of mass of the cell's contrast response tuning.

92



93 *Bias in population direction preference*

94

95 The direction and magnitude of bias in direction preference for a population of cells (e.g.  
96 all cells recorded from one mouse or all cells recorded from all mice of a particular Cre line) was  
97 calculated as the direction and magnitude of the vector sum of the direction preferences of the  
98 cells that comprise the population, at a particular contrast, as

99

100 
$$\theta_{bias} = \tan^{-1} \left( \frac{\sum \sin \theta_i}{\sum \cos \theta_i} \right)$$

101

102 
$$r_{bias} = \frac{1}{n_{cells}} \sqrt{\left( \sum \cos \theta_i \right)^2 + \left( \sum \sin \theta_i \right)^2}$$

103

104 where  $\theta_i$  is the preferred direction of cell  $i$ ,  $n_{cells}$  is the number of cells in the population,  $\theta_{bias}$  is  
105 the direction of the vector sum over the population, and  $r_{bias}$  is the magnitude of the vector sum  
106 over the population.

107

108 *Stimulus Tuning conditioned on locomotion behavior*

109

110 As part of the standardized pipeline for the Allen Brain Observatory, mice were held on a  
111 running wheel during experimental sessions and locomotion behavior was recorded (See de  
112 Vries, Lecoq, Buice *et al.* for further run speed measurement details). The mean running speed  
113 was calculated for each trial over the same time window as the mean cellular response was  
114 calculated. Trials for which the mean running speed was greater than or equal to 1cm/s were  
115 categorized as running trials, whereas trials for which the mean running speed was below 1cm/s  
116 were categorized as stationary trials. The mean and standard error of the mean event magnitude  
117 for each contrast and direction condition shown in Figure 2 was calculated separately for running  
118 and stationary trials. The criterion for a cell to be included in the calculation for a given direction-  
119 by-contrast condition was that the mouse had to be running for a minimum of four trials *and* be  
120 stationary for a minimum of four trials of that condition.

121

122 *Contrast Response Function Fitting and model comparison*

123

124 Event responses as a function of contrast, at a cell's preferred direction, were fit to a rising  
125 sigmoid ("high pass"), a falling sigmoid ("low pass"), and the product of one rising and one falling  
126 sigmoid ("band pass").

127 
$$R_{high\ pass}(c; h, b, s, c_{50}^r) = b + h \frac{1}{1 + e^{-s(c - c_{50}^r)}}$$

128 
$$R_{low\ pass}(c; h, b, s, c_{50}^f) = b + h \frac{1}{1 + e^{s(c - c_{50}^f)}}$$

129 
$$R_{band\ pass}(c; h, b, s, c_{50}^r, c_{50}^f) = b + h \left( \frac{1}{1 + e^{-s(c - c_{50}^r)}} \right) \left( \frac{1}{1 + e^{s(c - c_{50}^f)}} \right)$$

130 where  $c$  is the contrast,  $c_{50}^r$  is the contrast at which the response rises halfway between the base  
131 and height,  $c_{50}^f$  is the contrast at which the response falls halfway between the base and height,  
132  $b$  is the lowest response,  $h$  is the response amplitude, and  $s$  is the slope of the sigmoid (fixed at  
133  $s = 10$ ). The best fit model was determined by calculating the Akaike Information Criterion (AIC)  
134 for each model and selecting the model with lowest AIC.

135 The AIC can be calculated as

136 
$$AIC = 2k - 2 \ln \mathcal{L}$$

137 
$$\mathcal{L} = \prod_{contrasts} \prod_{trials} \mathcal{N}(R_c^i | \mu = \hat{R}_c, \sigma_R^2)$$

138 
$$\ln \mathcal{L} = -\frac{1}{2\sigma_R^2} \sum_{contrasts} \sum_{trials} (R_c^i - \hat{R}_c)^2 + constant$$

139 where  $k$  is the number of parameters fit in the model,  $\mathcal{L}$  is the likelihood of observing the responses  
140 given the fitted model and response distribution,  $R_c^i$  is the cell's response to a grating stimulus of  
141 contrast  $c$  (at the cell's preferred direction) on trial  $i$ ,  $\hat{R}_c$  is the response predicted by the model to  
142 a grating stimulus of contrast  $c$ ,  $\sigma_R^2$  is the variance of all of the cell's responses, and  $\mathcal{N}$  is the  
143 normal distribution. In practice, it is more convenient to directly calculate the log-likelihood than  
144 to calculate the likelihood and subsequently take the log, and the constant can be ignored for  
145 model selection since the same constant applies to all models being compared.

146 Due to the non-normal response distribution, possibly arising from calcium imaging as well  
147 as an underlying non-normal spiking distribution, we bootstrapped the log-likelihood rather than  
148 assume normality. Therefore, the likelihood was calculated numerically by shuffling responses  
149 across trials 1000 times and calculating the sum of square residuals from the predicted responses  
150 as  $SS = \sum_{contrasts} \sum_{trials} (R_c^i - \hat{R}_c)^2$  for each shuffle. The likelihood was taken as the fraction of  
151 shuffles for which  $SS$  was greater than the observed  $SS$ .

### 152 *Stabilized Supralinear Network (SSN) Model*

153 The SSN was modelled as a ring network, largely maintaining the basic architecture and  
154 dynamics described in Rubin et al. (2015) but deviating primarily in the diversity of inhibitory  
155 neurons and distributions of connections between neuron populations (including untuned  
156 inhibitory connections, described below). Our network consisted of one excitatory population  
157 (representing layer 2/3 CUX2 pyramidal neurons) and three inhibitory populations (representing  
158 PV, SST, and VIP interneurons, respectively). The ring network structure was imposed by  
159 providing each excitatory neuron with external ("sensory") excitatory input that had Gaussian  
160 tuning with mean (i.e. peak/preferred direction) corresponding to the neuron's position on the ring  
161 and standard deviation of 30 degrees; PV neurons also received external input which was not  
162 tuned (i.e. all PV cells receive input of equal strength). The entire network covered 180 degrees  
163 of orientation (or direction). The strength of external input was intended to represent a  
164 monotonically-increasing function of stimulus contrast, though no specific relationship between  
165 input magnitude and contrast is claimed here.

166 Connections from CUX2 neurons (i.e. excitatory connections) also had Gaussian tuning  
167 that depended on the difference between the orientation preferences of the pre- and post-synaptic  
168 neurons, whereas connections from inhibitory neurons (i.e. inhibitory connections) were not tuned  
169 (Figure 3b). The distributions of recurrent connections onto Cux2 cells and connections onto Vip  
170 cells were narrow (standard deviation of 30 degrees) compared to the distributions onto PV and  
171 Sst cells (standard deviation of 100 degrees).

172 The network consisted of 184 excitatory neurons, 40 PV neurons, 15 SST neurons, and  
173 15 VIP neurons. The excitatory population had 180 neurons with uniform 1-degree spacing of  
174 peak directions to tile the ring, plus 4 extra neurons with peak direction of zero degrees to capture  
175 the slight bias of the CUX2 neurons. All model VIP neurons had a peak direction of zero degrees  
176 to capture the strong bias for front-to-back motion observed for VIP neurons. In addition, all SST  
177 and PV model neurons also had a peak direction of zero degrees, though the very broadly-tuned  
178 inputs to these neurons results in a much weaker bias of net input to these neurons than the bias  
179 to VIP neurons. All neurons were implemented as rate models with firing rate that was a rectified  
180 quadratic function of the summed input to the neuron,  
181  
182

183

$$r_{ss}(I) = \begin{cases} kI^2 & I > 0 \\ 0 & I \leq 0 \end{cases}$$

184 where  $I$  is the input strength,  $r_{ss}$  is the steady state firing rate, and  $k$  is a constant of proportionality.  
185 For ease of comparison with the SSN models developed by Rubin et al. (2015), we used  $k = 0.04$   
186 for all models.

187 For a given external input, the firing rates of all neurons in the network were obtained by  
188 evolving the network in time, with dynamics:

189

$$\dot{r} = r_{ss}(I_{sum}(t)) - r(t)$$
$$I_{sum}^j(t) = I_{sp}^j + \sum_i W_{i,j} r^i(t)$$

190

191 where  $r(t)$  is the time-dependent firing rate,  $\dot{r}$  is the time derivate of the neuron's firing rate,  $r_{ss}$  is  
192 the steady state firing rate that varies in time based on the inputs to the neuron,  $I_{sum}^j$  is the net  
193 input to neuron  $j$ ,  $I_{sp}^j$  is a constant spontaneous input to neuron  $j$ , and  $W_{i,j}$  is the connection  
194 strength from presynaptic neuron  $i$  onto postsynaptic neuron  $j$ . To provide spontaneous activity  
195 to the network, and account for the higher spontaneous activity of VIP neurons<sup>1</sup>, we set  $I_{sp}^{CUX2} =$   
196  $I_{sp}^{PV} = I_{sp}^{SST} = 2$  and  $I_{sp}^{VIP} = 10$ . The network is evolved with Euler integration with updates of  $\Delta r^j =$   
197  $\frac{\Delta t}{\tau^j} \dot{r}^j$  at each time step of  $\Delta t = 0.1$  ms, where the time constants of the different neuron types are  
198  $\tau^{CUX2} = \tau^{SST} = \tau^{VIP} = 20$  ms and  $\tau^{PV} = 10$  ms.

199

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201

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