

1 Targeted cortical stimulation reveals 2 principles of cortical contextual 3 interactions

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32 Abstract

33 Cross-orientation suppression is a classic form of contextual normalization in visual cortex, yet
34 the degree to which cortical circuits participate in the normalization computation is unclear. We
35 visualized orientation maps of individual ferrets, and provided patterned optogenetic stimulation
36 to both excitatory and inhibitory cells in orientation columns that either matched or were
37 orthogonal to the preferred visual orientation of neurons recorded with electrodes. When visual
38 or optogenetic stimulation of columns preferring one orientation was combined with optogenetic
39 stimulation of columns preferring the orthogonal orientation, we observed less suppression than
40 when orthogonal stimulation was provided visually, suggesting that cortical circuits do not
41 provide a large fraction of visual cross-orientation suppression. Integration of visual and
42 optogenetic signals was linear when neurons exhibited low firing rates and became sublinear
43 when neurons exhibited higher firing rates. We probed the nature of sublinearities in cortex by
44 examining the influence of optogenetic stimulation of cortical interneurons. We observed a range
45 of responses, including evidence for paradoxical responses in which interneuron stimulation
46 caused a decrease in inhibitory firing rate, presumably due to the withdrawal of recurrent
47 excitation. These results are compatible with cortical circuits that exhibit strong recurrent
48 excitation with stabilizing inhibition that provides normalization, albeit normalization that is too
49 weak across columns to account for cross-orientation suppression.

50

51 Introduction

52 Responses of neural circuits depend on context. In visual cortex, neurons respond to bars or grat-
53 ings of a preferred orientation but are also highly influenced by the simultaneous presentation of
54 additional stimuli, such as gratings that are orthogonal to the preferred orientation or flanking stim-
55 uli (Bishop et al., 1973; Morrone et al., 1982; Bonds, 1989; DeAngelis et al., 1992; Das and Gilbert,
56 1999; Cavanaugh et al., 2002; Smith et al., 2006; Busse et al., 2009; MacEvoy et al., 2009). Many of
57 these contextual influences are well-described phenomenologically by a normalization equation
58 that consists of summation and division (Bonds, 1989; Carandini and Heeger, 2011). However, the
59 cortical circuit mechanisms that underlie contextual processing are incompletely understood.
60 A classic form of normalization is cross-orientation suppression: the response of visual cortical
61 neurons to a preferred orientation is suppressed when an orthogonal stimulus is also presented,
62 even if the neuron exhibits no response to the orthogonal stimulus when presented alone. Under-
63 standably, cross-orientation inhibition emerged as an early hypothesis for the circuit implementa-
64 tion of cross-orientation suppression (DeAngelis et al., 1992; Heeger, 1992), but experiments that
65 blocked cortical inhibition or measured the orientation dependence of cortical inhibition did not
66 find evidence consistent with this hypothesis (Anderson et al., 2000; Katzner et al., 2011). Subse-
67 quently, careful consideration of the responses of the feed-forward inputs from the lateral genic-
68 ulate nucleus (LGN) led to a feed-forward hypothesis: that desynchronization of the temporal re-
69 sponse phases of the LGN inputs to a given V1 cell, LGN saturation and rectification, and V1 spike
70 threshold could account, in large part, for the reduced responses observed in cross-orientation
71 suppression (Freeman et al., 2002; Li et al., 2006; Priebe and Ferster, 2006; Priebe, 2016). How-
72 ever, experiments using dichoptic presentation of the two gratings showed a weak cortical com-
73 ponent of cross-orientation suppression (Sengpiel and Vorobyov, 2005), and it remains unclear
74 whether the arguments supporting the feed-forward hypothesis, which were based on analysis of
75 responses to drifting gratings, would apply to the thin bar stimuli used by MacEvoy et al. (2009).
76 In addition, normalization is observed in a variety of visual cortical computations in both V1 and
77 MT (Heuer and Britten, 2002), and experiments and circuit models suggest that recurrent cortical

78 connections, particularly those that operate in an inhibition-stabilized regime (Ozeki et al., 2009;
79 Shushruth et al., 2012; Rubin et al., 2015; Litwin-Kumar et al., 2016; Sato et al., 2016; Palmigiano et
80 al., 2020), have the necessary ingredients to implement normalization that does not involve direct
81 cross-orientation inhibition.

82 We designed experiments to directly probe the possible role of cortical circuits in cross-orientation
83 normalization. Using a custom ProjectorScope apparatus (Huang et al., 2014; Roy et al., 2016), we
84 located orientation maps in ferret visual cortex with intrinsic signal imaging, and then provided
85 patterned optogenetic stimulation directly to different orientation columns. We examined, with
86 electrode recordings, how cortical circuits integrate visual and optogenetic stimuli of different con-
87 trasts and orientations.

88 We observed substantial differences in the interaction of columns depending upon whether stim-
89 ulation was delivered visually or optogenetically. First, optogenetic activation of an orientation
90 column caused spiking activity that spread beyond the orientation columns that were directly stim-
91 ulated (but not when glutamatergic synapses were blocked). Second, paired responses to visual
92 stimulation at the preferred orientation and optogenetic stimulation of the orthogonal columns
93 were smaller than the linear sum of the two stimuli alone, but the paired suppression was sub-
94 stantially less than was observed with purely visual paired stimuli. Further, paired optogenetic
95 stimulation of iso-orientation and orthogonal columns showed far less suppression than visual-
96 opto stimulus pairs. Finally, all 3 combinations of stimuli exhibited different suppression dynamics
97 for marginal increases in the orthogonal stimulus, suggesting that different circuit behaviors un-
98 derlie these different situations.

99 In a second set of experiments, we looked for hallmarks of inhibition-stabilized activity, where
100 providing additional drive to inhibitory neurons causes a “paradoxical” decrease in inhibitory re-
101 sponses instead of the expected increase due to the increased drive (Tsodyks et al., 1997; Ozeki et
102 al., 2009; Sanzeni et al., 2020). In models, the paradoxical decrease is due to a temporary increase
103 in inhibition, which removes excitatory drive from the circuit, which in turn results in weaker acti-
104 vation of inhibitory neurons. Using an inhibitory cell specific promoter (Dimidschstein et al., 2016),
105 we provided optogenetic activation to cortical inhibitory neurons during visual stimulation. We ob-
106 served a range of inhibitory neuron responses, including some that were “paradoxical” and others
107 that were not.

108 **Results**

109 **Direct stimulation to test normalization mechanisms in cortex**

110 Visual cortical neurons exhibit sublinear responses to the simultaneous presentation of two grating
111 stimuli (Adelson and Movshon, 1982; Morrone et al., 1982; Morrone et al., 1987; DeAngelis et al.,
112 1992; Heeger, 1992; Carandini et al., 1997; Simoncelli and Heeger, 1998; Reynolds and Heeger,
113 2009; Popovic et al., 2018). Because some of this suppression is likely present in the inputs to the
114 cortex that arise from LGN (Freeman et al., 2002; Li et al., 2006; Priebe and Ferster, 2006), we
115 designed an experiment to directly provide input to different regions of the cortical circuit itself, in
116 order to separate cortical contributions to normalization from those of its inputs.

117 To do this, we updated a custom-made optical system (Roy et al., 2016), now called ProjectorScope
118 2 (Fig. 1A), that allowed us to use intrinsic signal imaging (Blasdel and Salama, 1986; Grinvald
119 et al., 1986) to identify the locations of orientation columns and also allowed us to provide pat-
120 terned optogenetic stimulation to the cortical surface. We injected viruses to express a variant of
121 ChR2 (Boyden et al., 2005; Berndt et al., 2011) nonspecifically in all neurons (Roy et al., 2016) and
122 prepared the ferrets for intrinsic signal imaging. After the orientation column map was acquired,
123 optogenetic stimulation masks that targeted certain orientation columns were calculated based
124 on the empirical map, and masked images were subsequently projected onto the V1 surface to
125 stimulate the corresponding columns (Fig. 1B and 1C).

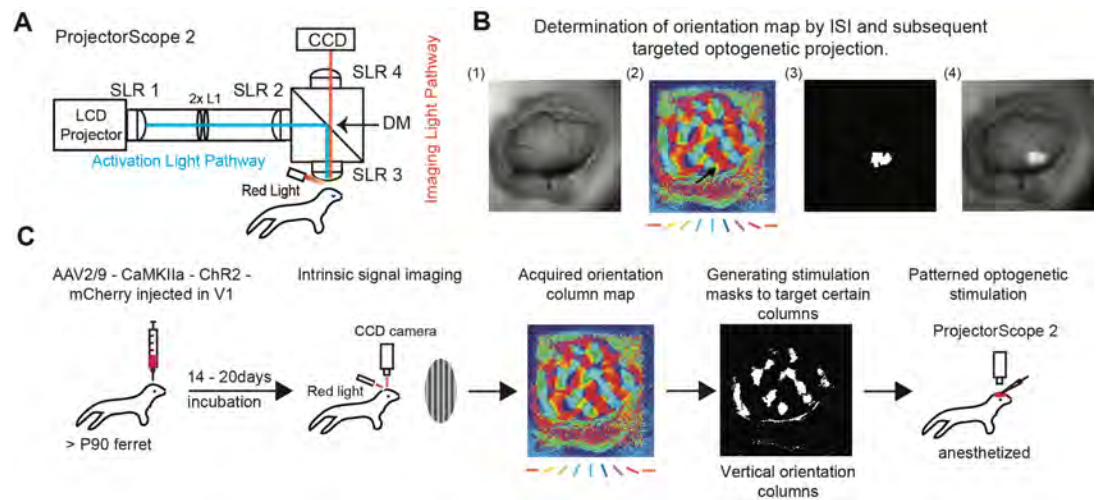


Figure 1. Optogenetic simulation of functionally-identified cortical columns with ProjectorScope 2. **A:** ProjectorScope 2 construction. Patterned light for optogenetic stimulation is generated by an LCD projector and transmitted to the brain surface by three SLR lenses of the same type. Two juxtaposed lenses (2x L1) are used to minify the projection image to the appropriate size, 3mm by 3mm, in order to target several orientation columns in ferret V1. A dichroic mirror (DM) reflects light between 390-460 nm to activate Chr2, while allowing green and red light to pass to the CCD camera for intrinsic signal imaging (ISI). ISI is performed by providing brightfield red light over the brain surface and taking images of the reflected light using the CCD, with an SLR lens to bring the light into focus. **B:** Determination of orientation map by ISI and subsequent targeted optogenetic projection. (1) Brain surface lit by room light. (2) The orientation column map acquired by ISI. The color key indicates the angle that a local region prefers. The arrow points to a region that will be used for targeted projection. (3) A projection mask based on the region pointed by the arrow in (2). (4) A raw image of the projection of the mask onto the brain surface. **C:** Viruses are injected to express Chr2 in V1 of adult ferrets, over 90 days old. After about three weeks, ISI is performed on the transfected ferrets to acquire an orientation column map. Then, the masks that target columns with certain orientation angles are generated based on the map.

126 **Single-column optogenetic stimulation causes wide spread of activity due to hori-**
 127 **zontal connections**

128 A key requirement of our experiment was to demonstrate that we could provide distinct input to
 129 different groups of nearby cortical neurons. While it was clear from visual inspection of the camera
 130 image that the light stimulus was illuminating distinct portions of the cortical circuit, several out-
 131 comes were possible at the neural level. First, providing direct input to a small column of neurons
 132 might only activate the neurons that were stimulated. Second, our direct optogenetic input might
 133 be restricted to distinct groups of neurons, but that activity could spread across columns through
 134 cortical synaptic connections; in this case, we would need to perform additional experiments with
 135 synaptic blockers to show that optogenetic inputs were being provided to distinct groups of neu-
 136 rons. Third, because axonal projections extend for millimeters and dendritic trees extend for a few
 137 hundred microns across the cortical surface, activation of these axons and dendrites might be suf-
 138 ficient to drive spiking in cell bodies over a wide area, which would mean that we would be unable
 139 to provide inputs to distinct groups of nearby neurons even with precise optical stimulation of the
 140 cortical surface.

141 Using electrodes, we characterized the optogenetic receptive zone (ORZ) of single neurons by stim-
 142 ulating the brain surface using circular dots ($\approx 750\mu\text{m}$ in diameter) in a randomized fashion. A 2-D
 143 Gaussian fit was performed on the recorded optogenetic responses over the cortical surface to de-
 144 lineate the local region that could be effectively activated by light (Fig. 2A). The ORZ could therefore
 145 be described as an elliptical shape comprising the interior 63%-tile of the fit (Roy et al., 2016).

146 We found that ORZs were larger in these adult ferrets (about the size of a ferret hypercolumn) than
 147 in our previous work in young ferrets (Roy et al., 2016), so we created stimuli to test the specificity of

148 activation of different orientation columns that were restricted to the ORZ (Fig. 2B). In this manner,
 149 we examined the “tuning” of each neuron to optogenetic activation of various orientation columns
 150 within the ORZ. We found that responses were quite non-specific, indicating that cortical activity
 151 spread substantially across orientation columns (Fig. 2C, left). There are two possible sources for
 152 this spread: either our optical stimulus itself caused widespread activation, or direct optogenetic
 153 activation was restricted to specific regions of the cortical surface and the spread of activity was
 154 due to activity within the cortical network.
 155 To differentiate the two scenarios, we applied synaptic blockers (NBQX and DL-AP5 to block AMPA
 156 and NMDA receptors, respectively) and measured the specificity index (1- circular variance) before
 157 and after the application of synaptic blockers. We found that neurons exhibited more specific
 158 responses to the preferred-column activation in the presence of synaptic blockers (Fig. 2D, t-test,
 159 $P = 0.0387$). This suggests that some of the non-specific column-based optogenetic stimulation is
 160 due to the spread of evoked neural activity via connections intrinsic to the visual cortex, consistent
 161 with previous studies in tree shrew (Huang et al., 2014). Nevertheless, the fact that responses
 162 evoked with optogenetic stimulation in the presence of synaptic blockers were more local showed
 163 that we were able to provide input to distinct regions.

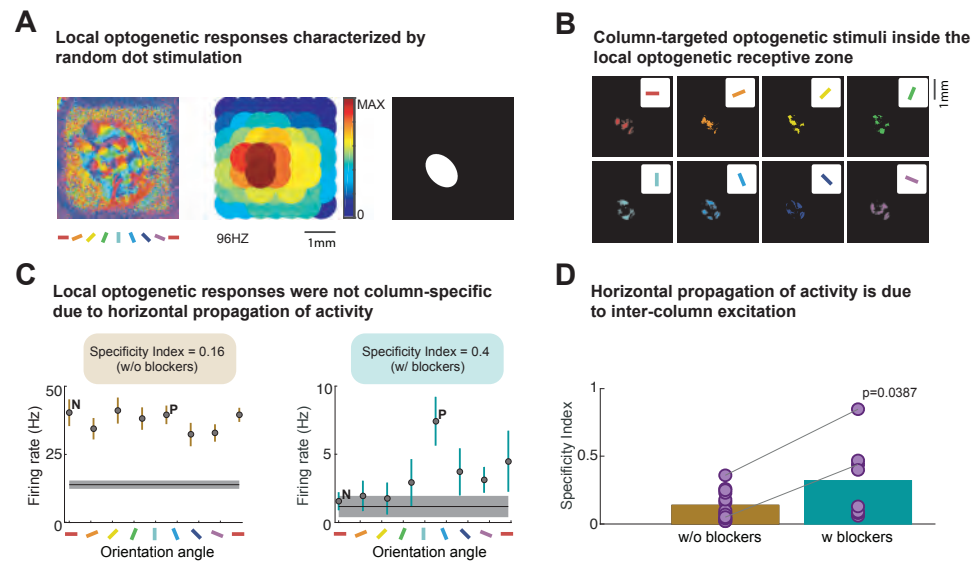


Figure 2. Although optogenetic activation of cortex produced local activity, this activity did not respect boundaries of orientation columns, due to horizontal propagation of activity. **A:** The orientation column map (left); the optogenetic responses of a single unit to spot stimuli (750 μm in diameter) with the heat map indicating the response intensities (middle); the elliptical optogenetic receptive zone (ORZ) characterized by two-dimensional Gaussian fit of the responses to spot stimulation (right). **B:** The masks used to test the orientation column specificity of the optogenetic stimulation were generated inside the optogenetic receptive zone and targeted orientation columns of varying angles with steps in 22.5 degrees. **C:** A neuron’s optogenetic responses of the orientation specificity test before synaptic blockers and after synaptic blockers. The orientation angles shown on the x-axis are the angles of the corresponding column masks. **D:** The average specificity index of all the neurons ($n=12$ without blockers; $n=8$ with blockers) after synaptic blockers are applied is higher than that when no blockers are used ($P<0.05$). Blue lines connect data points acquired from the same neurons. These results imply that ChR2 stimulation of the cortex in animals of this age activates several adjacent orientation columns, in part due to synaptic propagation of signals across the cortex.

164 **Visual, mixed visual and optogenetic stimulation, and paired optogenetic stimula-**
 165 **tion have different normalization properties**

166 Having established that we could provide distinct independent inputs to different groups of cor-
 167 tical neurons, we examined the cortical contributions to normalization by comparing how the re-

168 sponse to the simultaneous presentation of a pair of stimuli was related to a simple linear sum of
 169 the responses to the stimuli independently. In all, we compared cortical integration under three
 170 conditions: visual, combined visual and optogenetic, and all optogenetic (Fig. 3).

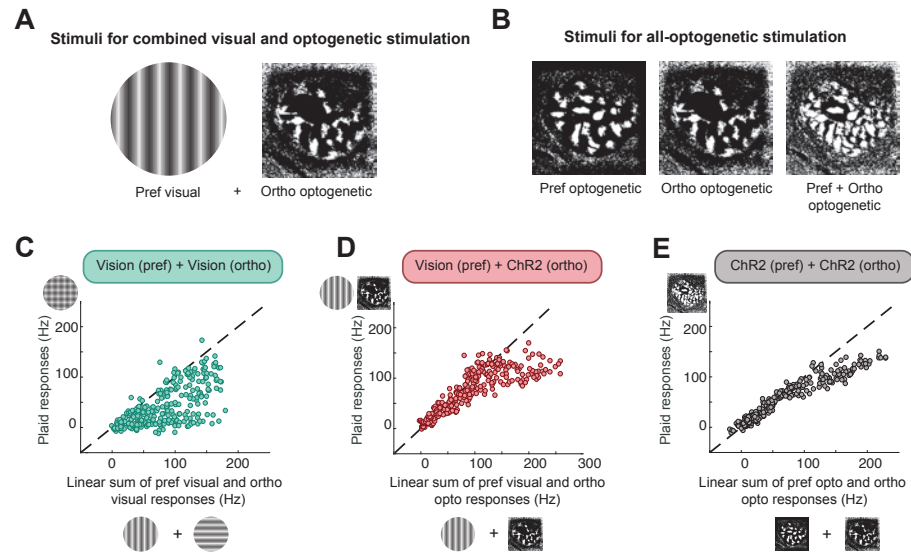


Figure 3. Neurons exhibit sublinear responses to combined visual and optogenetic stimulation. **A:** The stimuli used for hybrid stimulation. The visual stimulus provides the preferred orientation of the recorded neuron and the projection mask provides optogenetic modulation. **B:** Optogenetic stimuli for activation of neuron recorded with an electrode. Left) A strong optogenetic stimulus that includes the electrode region and all columns that prefer the same orientation (within 30°) as the recording site. Middle) A modulating stimulus that excludes the 1-sigma optogenetic receptive zone but includes all columns that prefer the orthogonal orientation (within 30°) as the recording site. Right) Combined stimuli. **C:** Single-unit responses to paired visual stimulation of preferred and orthogonal orientations, compared to the linear sum of preferred and orthogonal responses when those stimuli were presented alone. For each cell (N=23 cells, from 5 animals), responses to 16 combinations of preferred and orthogonal orientations are shown (4 contrast levels each). Responses are clearly sublinear for stimuli that evoked low or high firing rates. **D:** Single unit responses to paired visual and optogenetic stimulation. For each cell, responses to 16 combinations of visual preferred orientation (4 contrast levels) and optogenetic stimulation of the orthogonal orientation columns (4 drive levels) are shown. For stimuli that evoked low firing rates, paired stimulation exhibited mostly linear summation, but this summation became more sublinear for stimuli that evoked higher firing rates. **E:** Single unit responses to optogenetic stimulation of a cell's preferred columns and orthogonal columns. For each cell, responses to 16 combinations of optogenetic drive (4 drive levels for preferred stimulation and orthogonal stimulation) are shown. For stimuli that evoked low firing rates, paired stimulation produced a response that was nearly the same as the linear sum of the two component stimuli when presented alone. For stimuli that evoked larger responses, summation became non-linear.

171 In the first condition (all visual), we examined the classic phenomenon of cross-orientation sup-
 172 pression by comparing the sum of the responses of neurons to visual stimulation at the preferred
 173 orientation or at the orthogonal orientation to the response to a visual "plaid" of the two orienta-
 174 tions presented together, at a variety of stimulus contrasts. We compared these results to stim-
 175 ulation with a visual stimulus at the preferred orientation combined with optogenetic activation
 176 of the orthogonal orientation columns (Fig. 3A). Once again, we varied the relative drive of these
 177 stimuli, by varying contrast in the case of the visual stimulus and by varying optogenetic light in-
 178 tensity in the case of the optogenetic stimulus. Finally, we examined the responses to optogenetic
 179 stimulation of columns that matched the preferred orientation of the recorded cell, optogenetic
 180 stimulation of columns that were orthogonal to the preferred orientation of the recorded cell, and
 181 responses to both stimuli paired, at a variety of optogenetic stimulus intensities (Fig. 3B).
 182 There were obvious differences in the interactions among the different stimulation conditions. Re-
 183 sponses of all recorded cells (N=23) to all contrast combinations are shown in Fig. 3CDE. Cross-

184 orientation visual stimulation showed substantial non-linear summation at all contrast levels and
 185 response intensities (Fig. 3C), while visual-optogenetic or optogenetic-optogenetic stimuli com-
 186 bined linearly at low-to-moderate response levels and then exhibited sublinear summation at high
 187 response levels (Fig. 3DE).

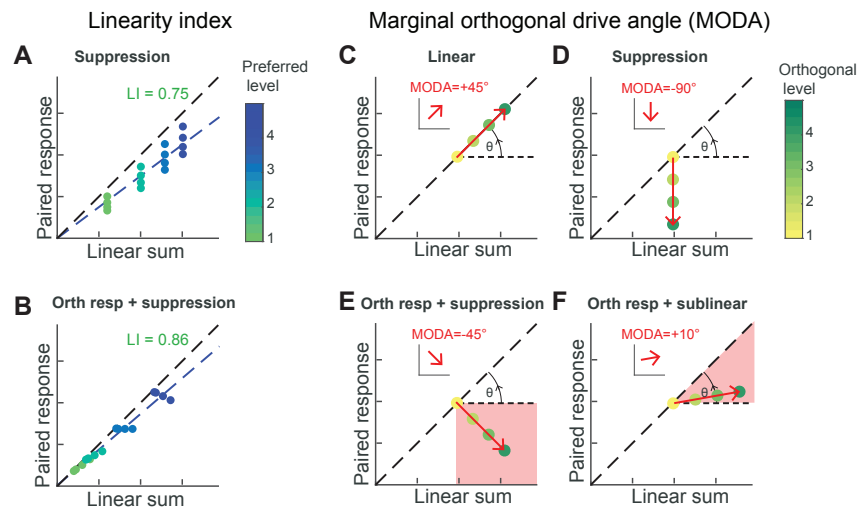


Figure 4. Single unit response schematics. **A:** Responses of a schematized neuron to paired stimulation of a preferred and orthogonal stimulus, as compared to the linear sum of responses to the individual stimuli alone. The schematized neuron was stimulated with the preferred stimulus at 4 levels of drive $\alpha_1 - \alpha_4$, and the orthogonal stimulus also covaried with 4 levels of drive $\beta_1 - \beta_4$. Color indicates the preferred stimulus contrast. We calculated a Linearity Index (LI) as the slope of a linear fit that went through the origin and fit the other points with least squared error. **B:** A second schematized neuron. **C-F:** A neuron being driven with a high-drive (α_4) preferred stimulus paired with an orthogonal stimulus at increasing drive ($\beta_1 - \beta_4$). **C:** In this neuron, the response to paired stimulation is equal to the linear sum of the stimuli presented alone; that is, for marginal increases in orthogonal drive (increases in β), the response moves at an angle of $+45^\circ$ in this space. We call this the Marginal Orthogonal Drive Angle (MODA). **D:** An example of pure suppression. Marginal increases in orthogonal stimulus drive causes a reduction in the paired response, but the orthogonal stimulus does not provide any drive by itself (linear sum of the two stimuli alone is unchanged). MODA is -90° . **E:** A cell where the orthogonal stimulus produces a response alone – the responses move rightward along the X axis with increasing orthogonal drive – but the net impact on paired stimulation is to provide suppression – the response moves downward along the Y axis with increasing orthogonal drive. MODA is between -90° and 0° . **F:** A cell where the orthogonal response produces a response alone and the net impact on paired stimulation is sublinear addition – the response moves rightward and upward with increasing orthogonal drive. MODA is between 0° and $+45^\circ$.

188 To probe these differences further, we developed two quantitative measures. The first measure,
 189 that we termed the Linearity Index (LI), is a measure of the overall linearity of the response and is
 190 the slope of the line that i) must pass through the origin at 0,0, and ii) passes through all responses
 191 to all combinations of paired stimuli with least squared error (Fig. 4AB). For the second measure, we
 192 calculated the directional angle of movement of the joint response vs. the linear sum of responses
 193 when the preferred contrast was high as the orthogonal contrast was increased (see Materials
 194 and Methods). We called this second measure the Marginal Orthogonal Drive Angle (MODA). A
 195 MODA value of $+45^\circ$ indicates linear summation (Fig. 4C), while a MODA value of -90° indicates
 196 that the second stimulus does not exhibit a response on its own but provides suppression of the
 197 response to the first stimulus (Fig. 4D). MODA values that are between -90° and 0° indicate that
 198 the second stimulus does exhibit some response on its own, but that its influence on the paired
 199 stimulation is overall suppressing (Fig. 4E). Finally, MODA values between 0° and $+45^\circ$ indicate that
 200 the second stimulus exhibits some response on its own, and contributes positively but sublinearly
 201 to the response to the paired stimuli (Fig. 4F).

202 Analysis of single cell responses indicated that normalization was substantially different across

203 these different stimulus conditions. In the all-visual condition, responses were highly sublinear
204 across all response intensities, and orthogonal stimuli tended to be primarily suppressive (MODA
205 near -90°), as shown in the example cells in Fig. 5A. In the mixed vision-optogenetics condition,
206 responses were relatively linear for low stimulus response values. Many cells, such as those ex-
207 amples in Fig. 5B, exhibited MODA values between -90° and 0° , indicating that the orthogonal
208 optogenetic stimulus provided some response on its own, but that this orthogonal stimulus most
209 commonly provided suppression to the paired stimulation. In all-optogenetic stimulation (Fig. 5C),
210 MODA values were commonly just slightly positive, indicating that orthogonal stimulation provided
211 a response when presented alone, and that the paired response was increased by orthogonal stim-
212 ulation, albeit in a sublinear manner.

213 Population data across all cells is shown in Fig. 5DEF. In the all-visual condition, the Linearity Index
214 (LI) was statistically constant (Fig. 5Di), being the same for cells that exhibited low firing rates or
215 high firing rates when the preferred stimulus was shown at high contrast ($P=0.6149$). The MODA
216 values in the all-visual condition were clustered around -90° (Fig. 5Dii), indicating that the orthogo-
217 nal visual stimulus did not drive cells by itself, but suppressed responses to preferred visual stimuli.
218 This was true particularly for cells with long Marginal Orthogonal Drive vector lengths that reflect
219 the presence of a strong trend. In the mixed visual and optogenetics measurements, the linearity
220 index was nearly 1 for cells that exhibited lower firing rates to high contrast visual stimulation, but
221 was less than 1 for cells that exhibited higher firing rates to high contrast visual stimulation (Fig.
222 5Ei). MODA values for this condition were variable, with most cells ranging between -90° and 0°
223 (Fig. 5Eii), indicating that the orthogonal optogenetic stimulus drove cells by itself, but suppressed
224 responses to preferred visual stimuli. Finally, for the all-optogenetic condition, linearity indexes
225 were again dependent upon the maximum firing rate of the cell to the preferred stimulus, with
226 cells that exhibited high firing rates exhibiting more sublinear responses (Fig. 5Fi. MODA values
227 were clustered around a slightly positive angle (Fig. 5Fii), indicating that the orthogonal optogenetic
228 stimulus drove cells by itself, and added sublinearly to the response to preferred optogenetic stim-
229 ulation. The different MODA values across the three stimulus pairing types indicated that these results
230 cannot be explained by a simple single-cell saturation mechanism, but rather suggest that cortex
231 is integrating these signals differently.

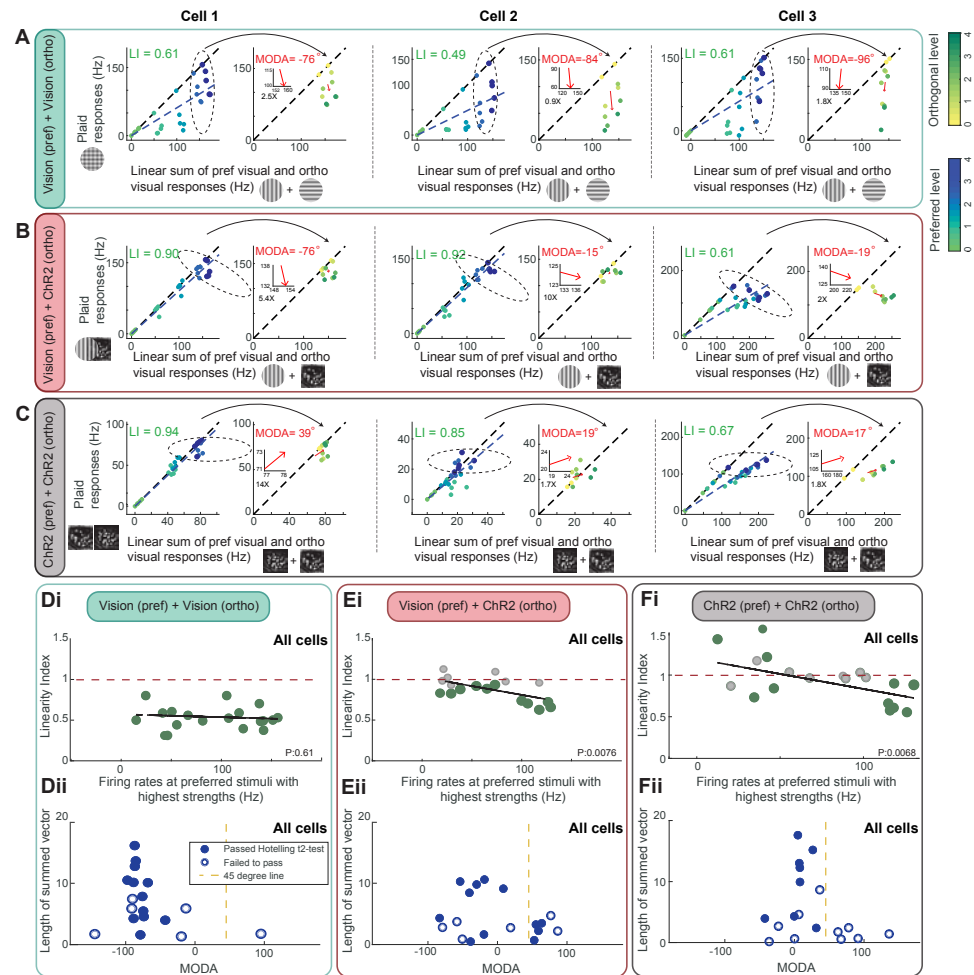


Figure 5. Responses of individual neurons and populations. **A:** Responses to paired visual stimulation of preferred and orthogonal gratings compared to the linear sum of those stimuli delivered alone. **A, left graph:** Cells were shown preferred gratings at 4 different contrast levels (levels, see color key) and orthogonal gratings at 4 different contrast levels (not differentiated). Linearity index (LI) fit line shown with LI value. **A, right graph:** Changes in responses with marginal increases in orthogonal drive. Responses to increasing orthogonal drive are shown paired with the two highest preferred contrasts. Marginal orthogonal drive vector and marginal orthogonal drive angle (MODA) indicated. A zoomed-in view of the MODA vector is provided in the inset, with magnification (NX) indicated. Note how for cell 2 and 3, the MODA is close to 90°, indicating almost pure suppression. **B:** Same, but for combinations of visual and optogenetic stimulation. Cells were driven with visual gratings at the preferred orientation with 4 levels of contrast, and orthogonal orientation columns were driven optogenetically at 4 levels of drive. Cells in B are the same cells in A. MODA values were variable among this population. **C:** Same, but for all optogenetic stimulation. Cells were driven with optogenetic stimulation of the preferred columns at 4 levels of drive, and were driven with optogenetic stimulation of the orthogonal columns at 4 levels of drive. MODA values tended to be above 0°, indicating that orthogonal stimuli provided drive and added sublinearly with the preferred stimulus. **Di, Ei, Fi:** Linearity index values for all cells, plotted against the firing rate that was produced for preferred stimuli at the highest contrast or drive. **Di:** For purely visual stimulation, Linearity Index values were approximately 0.5 and did not exhibit any significant correlation with maximum firing rate (Pearson's correlation, $p=0.615$). Filled dot indicates values that differed significantly from 1 (t-test, $p<0.05$). Open dot in subsequent panels indicates values that did not differ significantly from 1 (t-test, $p<0.05$). **Ei:** For mixed visual and optogenetic stimulation, Linearity Index values were approximately 1 when cells exhibited low firing rates, and decreased when cells exhibited higher firing rates. There was a significant correlation between the Linearity Index and maximum firing rate for visual stimulation at the preferred orientation (Pearson's correlation, $p<0.0076$). **Fi:** Linearity index values when stimulation to preferred and orthogonal columns was provided by optogenetic stimulation. There was a significant correlation between the Linearity Index and maximum firing rate ($p<0.0068$). **Dii, Eii, Fii:** Marginal orthogonal drive vector lengths and angles.

232 **Fig. 5 Caption continuation** Cells that exhibited a vector that differed significantly from 0,0 (Hotelling-T2 test $p < 0.05$) are indicated by filled dots;
233 vectors that did not differ significantly from 0,0 are plotted as open dots. **Dii:** Length and MODA values for visual stimulation. There were a cluster of
234 points with significant vector lengths with MODA values near -90° , indicating that cross-orientation stimulation is primarily suppressing. **Fii:** Values
235 for mixed visual stimulation (preferred orientation) and optogenetic stimulation (orthogonal columns). There was a range of MODA values but the
236 median cell exhibited a MODA value that was between -90° and 0° . **Gii:** Values for optogenetic stimulation (preferred columns and orthogonal
237 columns). MODA values for vectors that had significant length were slightly positive. These results indicate that all three stimulus paradigms
238 exhibited different marginal influences of increasing orthogonal drive.

239 **Circuit models that might underlie these responses**

240 At first inspection, the differences in normalization for these different conditions are difficult to rec-
241 oncile with what is known from previous work on cortical circuits. If we imagine a region H that is
242 selective to horizontal orientations, and a nearby region V that is selective to vertical orientations,
243 then during visual stimulation with horizontal orientations, region H exhibits strong responses,
244 while region V does not respond. Further, synaptic conductance measurements of visually respon-
245 sive neurons in region V indicate that principal neurons in V do not receive strong inhibitory or
246 excitatory inputs when horizontal orientations are presented (Anderson et al., 2001), so the visu-
247 ally responsive neurons in H cannot strongly inhibit or excite the principal neurons of V. On the
248 other hand, if we optogenetically activate region H, then we observe responses in V.
249 How can optogenetic activation of region H cause activity in region V when visual activation of H
250 does not? If we set aside the possibility that the direct optogenetic stimulation is not actually local
251 (see Fig. 2), then one of the simplest ways this can happen is if there are projections across the
252 columns by neurons that are not driven by the visual stimulus used to drive area H. These neurons
253 might not be visually responsive at all, or might have spatial or temporal frequency preferences
254 that were not driven by the visual stimuli used here. A 2-photon imaging study of anesthetized
255 ferrets of a similar age showed that visual responses are relatively sparse, and that many neurons
256 did not exhibit responses to visual stimulation (Smith et al., 2015). These neurons are likely to
257 be activated by optogenetic stimulation, and they may exhibit coupling into the circuit that differs
258 from their nearby visually-active neighbors.
259 There is a wide – but not an unlimited – number of possible circuits that could meet these criteria.
260 Here, we show two examples of circuit models that are inconsistent with our observations on the
261 way to unpacking one example circuit configuration that is consistent with our observations.
262 We began with the ring model of Rubin et al. (2015) (Fig. 6A). Each position in the ring represents
263 a preferred orientation, ranging around the ring from 0° ($= 180^\circ$) to 179° , and is modeled by a pair
264 of E and I cells that are reciprocally- and self-connected. Each pair forms a supralinear stabilized
265 network. The synaptic strengths of the horizontal connections across elements of the ring fall off
266 slowly with distance/orientation in a gaussian manner, and all connection strengths (E-to-E, E-to-I,
267 I-to-E, I-to-I) fall off with the same distance dependence ($\sigma = 32^\circ$, as one moves along the ring). The
268 visual input is tuned to stimulus orientation and falls off in a Gaussian manner ($\sigma = 30^\circ$) as one
269 moves away from the cells that prefer a given orientation, and optogenetic input falls off similarly
270 but is set slightly broader ($\sigma = 45^\circ$). The visual and optogenetic input are provided to both E and I
271 cells. Neurons with different maximum firing rates are simulated by varying the maximum input
272 level provided to different simulations.

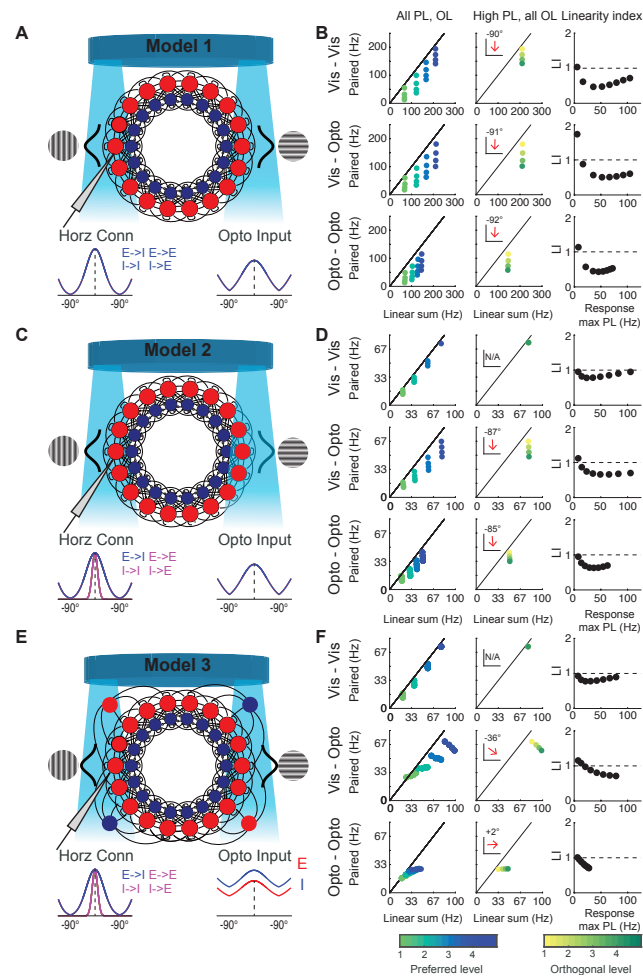


Figure 6. Models consistent with data. Common to all models: Cells are arranged in a ring, with an excitatory (E) and inhibitory (I) unit at each position. Position corresponds to preferred orientation. The units are coupled in a supralinear stabilized network. Visual and optogenetic inputs are centered at the stimulus orientation, are equal to E and I units at a given position, and decrease as a Gaussian function of the difference between the stimulus orientation and the units' preferred orientation. For visual input, the Gaussian has standard deviation 30°; for optogenetic input, the standard deviation is 45°. **A:** Model 1: The ring model of Rubin et al. (2015). Horizontal connectivity among all cortical cells decreases as a Gaussian of standard deviation 32°. **B:** Left) Responses of Model 1 to stimulation with 16 combinations of 4 preferred levels of drive (PL) and 4 orthogonal levels (OL) of drive. Cells were shown preferred gratings at 4 different contrast levels (levels, see color key) and orthogonal gratings at 4 different contrast levels (not differentiated) Top) preferred and orthogonal are visual; Middle) preferred is visual and orthogonal is optogenetic; Bottom) preferred and orthogonal are optogenetic Center) Responses for the strongest preferred stimulus and varying orthogonal drive (see color key). MODA value indicated in inset. Right) Linearity index for cells with different maximum firing rates. Model 1 exhibits stronger normalization than is observed in cortex, and marginal orthogonal drive increases cause only suppression, in contrast to data from cortex for visual-optogenetic and optogenetic-optogenetic conditions. **C:** Model 2: Modified ring model with less cortical normalization. Now, E to I connections fall off as a Gaussian of 25° width; other connections fall off with 10° width. **D:** Same as B, for Model 2. Visual normalization is no longer provided by the cortex. For optogenetic stimulation, this model exhibits weaker normalization, but orthogonal stimuli are still almost purely suppressing, in contrast to data from experiments. **E:** Model 3: Now optogenetic stimulation also activates non-visual E and I neurons (disembodied cells) that are coupled to the entire network, so that the effective optogenetic input is broad. **F:** Same as B, for Model 3. Now, mixed visual and optogenetic input is more linear at low responses, and MODA shows that the orthogonal stimulus provides some response by itself but suppresses responses to the preferred visual stimulus, as in experiment. Pure optogenetic stimulation is more linear at low response rates, and MODA shows that the orthogonal stimulus provides some response by itself and adds sublinearly to the response to the preferred visual stimulus, as in experiment.

273 Responses to combinations of visual and optogenetic inputs at different contrasts in the unmod-
274 ified Rubin et al. (2015) (Model 1) are shown in Fig. 6B. Previous studies (Ahmadian et al., 2013;
275 Rubin et al., 2015) have shown that cross-orientation normalization in this model occurs alongside
276 the paradoxical response of an inhibition-stabilized circuit: cross-orientation inputs to inhibitory
277 neurons causes the firing rates of both E and I neurons to drop, so that overall cross-orientation
278 suppression results in a net reduction of both excitatory and inhibitory synaptic conductances. In
279 this model, linearity indices are near 1 for cells that were only driven weakly by the preferred stim-
280 ulus, and drop to about 0.5 for cells that are driven more strongly by the preferred stimulus. This
281 differs from the actual data from our experiment, where the linearity index was nearly constant
282 (around 0.5) for cells that were driven either strongly or weakly (Fig. 5D). Further, MODA values
283 in the model for all conditions (all-visual, mixed visual and optogenetic, and all-optogenetic) were
284 all around -90° , indicating that the orthogonal stimulus was producing strong suppression without
285 causing a response on its own. These simulation results are inconsistent with our measurements
286 in the mixed and all-optogenetic conditions (where the orthogonal stimulus caused a response and
287 MODA > -90), indicating that the unmodified Rubin et al. model exhibits stronger cross-orientation
288 normalization than we observe in our measurements.

289 The fact that normalization in the original Rubin et al. model was too strong to account for the
290 mixed visual and optogenetics results led us to explore a different cortical circuit model (Model 2).
291 We dropped the requirement that the cortical circuit itself should provide visual cross-orientation
292 suppression; instead, we allowed our model circuit to exhibit little visual cross-orientation suppres-
293 sion, assuming that in the real circuit, much or all of this might occur in feed-forward inputs from
294 the LGN (Freeman et al., 2002; Li et al., 2006; Priebe and Ferster, 2006; Priebe, 2016). We mod-
295 ified the model so that E-to-E, I-to-E, and I-to-I connections were all much more local (10° Gaus-
296 sian fall-off instead of 32° fall-off), while leaving E-to-I connections at a 25° fall-off to allow some
297 within-cortex normalization (Fig. 6C) but much weaker than would be required to account for cross-
298 orientation suppression. Simulations showed that this model still lacked important features of our
299 data: optogenetic cross-orientation stimulation did not, by itself, evoke strong responses in the
300 model as it did in our data, but instead exhibited almost pure suppression (Fig. 6D). In this model,
301 cross-column suppression when using an optogenetic stimulus is due to the broader tuning of the
302 optogenetic input ($\sigma = 45^\circ$).

303 Finally, we examined a circuit (Model 3) that had additional E and I neurons that were not visually
304 active (Fig. 6E). Presumably these non-visual cells are active under specific conditions that might
305 involve other modalities or modulatory states. We made very simple assumptions about these
306 non-visual E and I neurons: they did not receive input from visually-responsive neurons, but they
307 did provide broad, uniform projections to visually-responsive neurons around the ring. This model
308 exhibited several features of our actual data (Fig. 6F). First, single optogenetic stimuli directed at
309 preferred or orthogonal columns evoked responses across the whole ring. Second, mixing a pre-
310 ferred visual stimulus with optogenetic stimulation of the orthogonal orientation columns evoked
311 suppressive responses (MODA angles less than 0°). Third, normalization was relatively linear for
312 neurons that were driven to low firing rates (response ratio approximately 1 or even higher), and
313 the degree of suppression increased for neurons that were driven to high firing rates (response
314 ratio less than 1). Normalization was only prominent when cells were driven to higher firing rates,
315 similar to the actual data. Fourth, paired optogenetic stimulation evoked sublinear responses but
316 exhibited positive MODA angles (greater than 0°), indicating that the paired response was greater
317 than the response to the preferred stimulus alone.

318 **Selective optogenetic stimulation of inhibitory neurons reveals broad classes of** 319 **functional types.**

320 While Model 3 is consistent with our data, the space of possible cortical circuits that might exist in
321 the brain and be consistent with our data is still very large. We sought to look for direct evidence
322 of the inhibition-stabilized dynamics that the model posits, as has been found in the mouse (Sato

323 et al., 2016; Adesnik, 2017; Sanzeni et al., 2020) and for which evidence has been reported for
324 surround suppression in cat (Ozeki et al., 2009) and ferret (Rubin et al., 2015). A key prediction
325 of ISN-type dynamics is the presence of so-called “paradoxical responses” (Ozeki et al., 2009). If
326 network activity is driven strongly by recurrent connections among excitatory and inhibitory cells,
327 then local excitatory cells are responsible for a significant portion of the synaptic drive to inhibitory
328 cells. If one were to selectively deliver an increase in drive to inhibitory neurons, then excitatory
329 neurons would slow down, but this reduction in excitatory drive would in turn reduce the activity
330 of cortical interneurons. Hence, in the model, the inhibitory cells “paradoxically” respond to small
331 increases in activation with overall reductions in activity (as compared to the increase in activity
332 that might be naively expected).

333 This prediction is illustrated by simulations in Fig. 7. Under a parameter regime where excitatory
334 recurrent connections are set so strongly that the network activity would blow up without inhi-
335 bition, and inhibitory synaptic strengths are set to be high enough to stabilize this activity (ISN
336 regime), an external increase in drive to inhibitory neurons results in the described paradoxical
337 decrease in activity in interneurons, until the external optogenetic drive to the inhibitory interneu-
338 rons becomes so strong as to dominate the interneuron responses (Fig. 7ABC, same parameters
339 as a single column of the rings of Rubin et al. 2015 and Model 3). On the other hand, if recurrent
340 connections among excitatory and inhibitory neurons are weak (non-ISN), then external drive to
341 inhibitory interneurons merely serves to monotonically increase the activity of these interneurons
342 (Fig 7DEF).

343 To test these predictions, we prepared ferrets with a virus (AAV9-mDlx-ChR2-mCherry-Fishell-3)
344 that restricts the expression of channelrhodopsin-2 to inhibitory neurons (Dimidschstein et al.,
345 2016). We delivered wide-field white light to stimulate the brain surface, and the light intensity was
346 modulated to achieve different levels of external drive to inhibitory neurons. Optogenetic stimula-
347 tion was presented with and without visual stimulation at the preferred orientation with different
348 contrasts, in order to understand how the activated cortex would be modulated by the external op-
349 togenetic increases in inhibitory drive. In these experiments, a 32-channel probe (Plexon S-probe)
350 was used to achieve better yield of recording both excitatory and inhibitory cells.

351 We observed a variety of response profiles to combined visual stimulation and optogenetic stimu-
352 lation of interneurons. Some cells exhibited no response to optogenetic interneuron stimulation
353 alone, but exhibited strong suppression of visual responses when optogenetic drive was strong.
354 We labeled these neurons as putative excitatory neurons (Fig. 8A, bottom row).

355 We also observed response profiles that we imagined arose from inhibitory neurons. These cells,
356 labeled as putative inhibitory neurons, exhibited strong responses to strong optogenetic stimula-
357 tion when it was presented either with or without visual stimulation (Fig. 8A, top and middle rows).
358 The putative inhibitory neurons always responded directly to light and, on average but not always,
359 exhibited shorter spike duration than the putative excitatory cells (Supplementary Figure 8-1).

360 The putative inhibitory interneurons were not uniform in response profile, but instead exhibited
361 a range of responses. At one extreme, responses from some putative inhibitory interneurons re-
362 sembled the responses from interneurons in an ISN-like circuit (Fig. 8A, top row). During high con-
363 trast visual stimulation, the activity of these interneurons was suppressed for weak optogenetic
364 activation, consistent with the idea that increased drive to interneurons was reducing excitatory
365 activity, which in turn decreased recurrent inhibitory activity. Without simultaneous visual stimu-
366 lation, these were less clear, given the low spontaneous firing rates of most neurons. At another
367 extreme, we also observed several neurons that exhibited monotonic increases in responses to op-
368 togenetic drive (Fig. 8A, middle row). These results suggest that there are multiple ways in which
369 interneurons can be interconnected with cortical circuits. The full range of tuning profiles that we
370 observed is projected onto its first two principle components and plotted in Fig. 8B. Within the pu-
371 tative inhibitory population, the data are more consistent with a continuum rather than discrete
372 clusters.

373 The diversity of responses from putative interneurons raises the question as to the nature of the

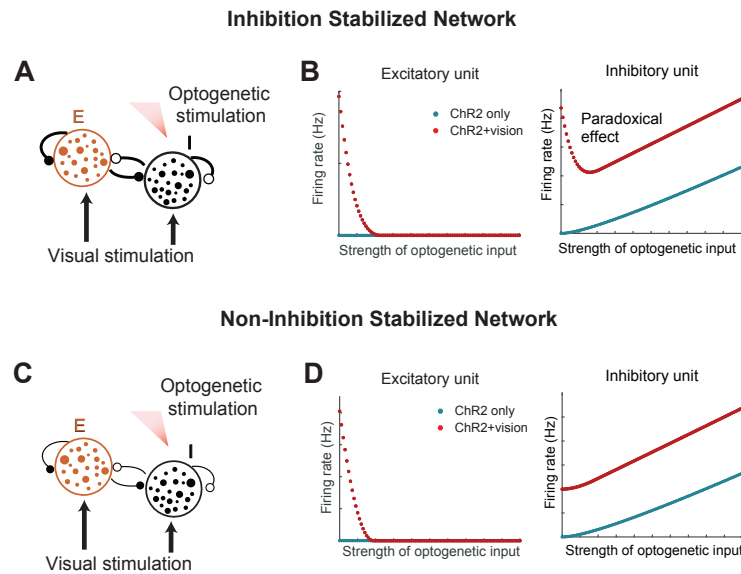


Figure 7. Differential responses to optogenetic interneuron stimulation in ISN and non-ISN models. A: In the simulation of ISN ring model (illustrated here as one pair of E and I), the E and the I units have strong recurrent connections. Visual stimulation provides inputs to both the E and the I units, and optogenetic stimulation provides inputs to the I units only. **B (left):** Excitatory unit responses in the ISN. When the inhibitory units are activated by ChR2 stimulation, the excitatory units have no responses. When visual stimulation is provided together with ChR2 stimulation, the excitatory units respond strongly to visual stimulation when the inhibition is weak and reduce firing rates as the inhibition becomes stronger. **B (right):** Inhibitory unit responses in the ISN. When the inhibitory units are activated by ChR2 stimulation but are not otherwise active in the circuit, they monotonically increase firing rates as the stimulation becomes stronger. When ChR2 stimulation is provided in the presence of visual stimulation, the firing rates decrease before increasing, creating a “dip” shape in the response curve that is characteristic of the ISN. **C:** In the simulation of the non-ISN model, the ring structure is maintained as in the ISN but the recurrent connections are weak. **D (left):** Excitatory unit responses in the non-ISN network. Activation of the interneurons suppresses the excitatory unit activity. **D (right):** Inhibitory unit responses in the non-ISN network. In the non-ISN network, the inhibitory units show direct responses to light in a monotonically increasing manner. When ChR2 stimulation is combined with visual stimulation, the inhibitory units also respond in a monotonically increasing manner and the “dip” is absent.

374 overall impact of cortical interneurons on the circuit. Of course, interneuron connectivity could be
 375 very specific, but as a point of interest we calculated the grand average of the normalized responses
 376 of all the inhibitory cells to combined optogenetic stimulation and visual stimulation (Fig. 8C). This
 377 grand average would reflect the inhibitory influence on the cortical network if interneuron types
 378 were pooled unselectively. The grand average tuning curve exhibits an empirical dip below zero
 379 for weak external input, although no point on the curve is significantly below 0 with a p-value of
 380 less than 0.05.

381 Diversity in interneuron responses to optogenetic stimulation could arise from diversity in the re-
 382 sponse of different inhibitory cell types, due to heterogeneity in neural connections, or due to the
 383 heterogeneity in the opsin expression across inhibitory cells. We created a large-scale EI network
 384 model with heterogeneous, randomly distributed connectivity (Fig. S4). We modeled the photocur-
 385 rent to each cell as a Hill equation as reported experimentally (Asrican et. al. 2018), and the ac-
 386 tivation of each interneuron to vary with depth, as expected from scattering of light across the
 387 cortical tissue (Yona et. al. 2018). In this model, we observed a heterogeneous range of response
 388 profiles in optogenetically-activated interneurons, as in the data. The model not only captures the
 389 initial response of I cells to laser power, but also the negative correlation between the effect of the
 390 laser at low intensity and the effect of the laser at high intensity to each cell: If the cell is initially
 391 excited by the laser, then it tends to quickly saturate for large laser power, whereas if it responds

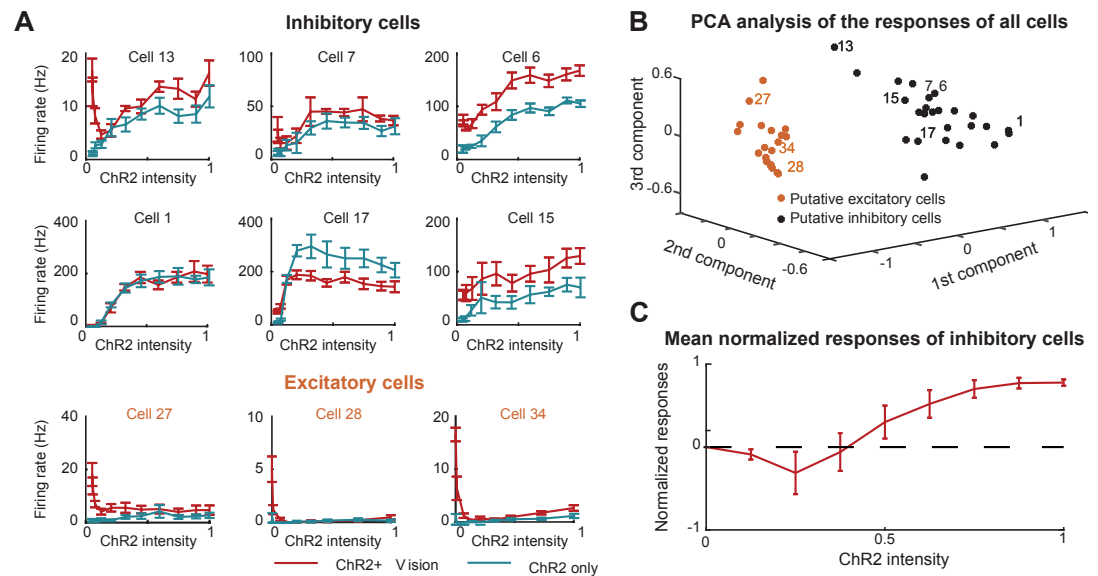


Figure 8. The influence of direct interneuron stimulation on visual responses in putative excitatory and inhibitory interneurons. **A:** Representative unit responses from the optogenetic inhibition experiments. Raw mean firing rates (without any background subtraction) are shown for increasing optogenetic stimulation alone (blue) or in the presence of a high contrast visual stimulus (red). (Top) Putative inhibitory neurons that responded like neurons in the ISN simulation. (Middle) Putative inhibitory neurons that did not respond like that the ISN simulation. Instead, the firing rates either saturated or increased at low light intensities and then decreased at high light intensities. (Bottom) Putative excitatory neurons. **B:** Projection of response profiles to visual stimulation and increasing optogenetic interneuron activation onto the first 3 principle components. Putative excitatory and inhibitory neurons are well separated, but there is diversity of responses within the putative inhibitory population. Numbers indicate the example neurons displayed in **A-C**. **C:** The grand average of the normalized ChR2+Vision responses of all putative inhibitory cells. For every inhibitory cell, the responses were normalized in such way that the average response at 0 light intensity is normalized to 0 and the average maximum response is 1.

392 paradoxically to the laser at low power (i.e. if it has a negative response to weak excitatory input)
 393 then its response does not saturate at large laser power. These results suggest that diversity in
 394 such properties as synaptic connectivity, optogenetic activation strengths, and cellular thresholds
 395 could underlie the variable response profiles we observed in Figure 8.

396 Discussion

397 In this study, we performed combined visual and patterned optogenetic stimulation to test how
 398 cortical circuits responded to multiple inputs. We found that cortical stimulation that was optically
 399 restricted to specific orientation columns caused activity that spread non-selectively to neighbor-
 400 ing columns. We used this protocol to examine the cortical contributions to contextual modula-
 401 tion by stimulating different cortical columns visually and optogenetically. We found evidence for
 402 cross-column cortical normalization, but much less than would be needed for a purely cortical
 403 mechanism to account for cross-orientation suppression. We found a wide range of interneuron
 404 couplings to the circuit, including those that responded to weak optogenetic activation by reducing
 405 their activity, consistent with inhibition stabilized networks.

406 Nonspecific spread of activity with respect to orientation columns

407 We tested the spatial spread of optogenetic responses and found that the column-based patterned
 408 optogenetic stimulation did not respect the boundaries of orientation columns, although local ac-
 409 tivation was typically restricted around the recorded neuron. While it is difficult to exclude the
 410 possibility that this unselective spreading is due in part to activation of passing axons and den-

411 drites of neurons in other columns, blocking the NMDA and AMPA receptors revealed that the
412 influence of horizontal connections played a large role. Viruses that cause strong expression of
413 ChR2 that is targeted to the soma could help manage concerns about passing dendrites, but the
414 one soma-targeting virus we were able to try did not cause sufficient expression to produce strong
415 responses in vivo with our stimulation system (Baker et al., 2016). Our results are consistent with
416 a study that performed sparse stimulation of nearby orientation columns in tree shrew (Huang
417 et al., 2014). One might have expected to find species differences between tree shrew and ferret
418 because the tree shrew primarily exhibits length-summation in layer 2/3 (Chisum et al., 2003) while
419 ferret exhibits both length-summation and surround suppression (Rubin et al., 2015; Popovic et
420 al., 2018), but both species showed non-specific spread of activity in layer 2/3.
421 An important corollary of nonspecific spread is that column-level stimulation of cortical neurons is
422 unlikely to provide an optimal stand-in for visual activation, e.g. in a visual prosthesis. In addition,
423 a given visual stimulus causes only a sparse activation of visual cortical neurons (Rocheffort et al.,
424 2009; Haider et al., 2010; Smith et al., 2015), and there appear to be cells that are not activated
425 by visual stimulation or are only activated in conjunction with some non-visual stimulus (Saleem
426 et al., 2018), whereas an optogenetic stimulus will activate all cells in a column. It is possible that
427 expression of optogenetic channels restricted to LGN axons may allow more specific stimulation
428 of visually-driven neurons in particular.

429 **Linear vs. non-linear interactions of visual and optogenetic signals**

430 Several studies have now examined integration of visual and optogenetic signals.
431 Huang and colleagues (Huang et al., 2014) used AAV viruses to cause very broad expression of
432 channelrhodopsin in excitatory neurons in the tree shrew. In these experiments, optogenetic acti-
433 vation using small spots of light targeted to preferred or orthogonal columns added linearly over
434 a wide range of firing rates. Further, visual stimulation with the preferred orientation combined
435 with optogenetic stimulation of preferred columns also showed linear summation.
436 In macaque, Nassi et al. (2015) used lentivirus to cause more localized expression of C1V1 in exci-
437 tatory neurons of the macaque. These investigators stimulated broadly with an optical fiber and
438 observed a variety of facilitatory and suppressive interactions to joint visual stimulation and opto-
439 genetic stimulation that was not specific to particular columns of the orientation map. The vast
440 majority of these neurons exhibited sublinear summation of visual and optogenetic signals.
441 Histed (2018) used transgenic approaches to cause expression of ChR2 in mouse visual cortical
442 neurons, and found that visual and optogenetic inputs summed in a largely linear manner, though
443 with sublinear summation at higher firing rates. Another study in the mouse, which used optoge-
444 netic antidromic activation of callosal inputs, found that callosal inputs facilitated responses at low
445 visual contrasts but suppressed responses at higher visual contrasts (Sato et al., 2014).
446 We found evidence for nearly linear summation when neurons exhibited low firing rates, which
447 became sublinear as neurons exhibited larger firing rates. These results cannot be explained by
448 a simple process of single cell saturation of firing rate outputs, because marginal increases in or-
449 thogonal drive produced very different responses in the visual-optogenetic stimulation protocol
450 as compared to the optogenetic-optogenetic stimulation protocol. This indicates a contribution of
451 cortical circuits to this integration.

452 **In layer 2/3, cross-column suppression is insufficient to account for cross-orientation 453 suppression to visual stimuli**

454 Cortical neurons exhibit weaker responses to a preferred oriented stimulus when the stimulus is
455 combined with an orthogonally-oriented stimulus (Bishop et al., 1973; Morrone et al., 1982; Bonds,
456 1989; DeAngelis et al., 1992; Cavanaugh et al., 2002; Smith et al., 2006; Busse et al., 2009; MacEvoy
457 et al., 2009). Experiments in the last 20 years have shown that direct cross-column inhibition is
458 unlikely to underlie this phenomenon, as synaptic conductance measurements of both excitatory
459 and inhibitory inputs peak at the preferred orientation and are relatively weak at the orthogonal

460 orientation (Anderson et al., 2000), and both excitation and inhibition are reduced by the addition
461 of an orthogonal grating stimulus to a preferred-orientation grating stimulus (Priebe and Ferster,
462 2006).

463 A more recent model suggested that nonlinear circuit properties induced by supralinear single-cell
464 input/output functions could explain the change from cross-orientation facilitation for weak stim-
465 ulti to cross-orientation suppression for stronger stimuli. Furthermore, because the network was
466 inhibition-stablized for stronger stimuli, the model could explain cross-orientation suppression
467 with a combined reduction of excitatory and inhibitory conductances (Ozeki et al., 2009; Rubin et
468 al., 2015). Cross-column excitatory inputs would cause inhibitory neurons to temporarily increase
469 their firing rates, reducing the firing rates of their neighboring excitatory neurons. Because the
470 neighboring excitatory neurons themselves provide strong input to their inhibitory neighbors, the
471 overall firing rates of both excitatory and inhibitory neurons goes down, along with local excitatory
472 and inhibitory synaptic conductances. Therefore, the model was compatible with the conductance
473 measurements that did not show strong cross-orientation inhibition (Anderson et al., 2000; Priebe
474 and Ferster, 2006).

475 Alternatively, other past models suggested that there was no need for any cortical explanation of
476 cross-orientation suppression. These models suggest that cross-orientation suppression can be
477 largely accounted for by changes in LGN inputs to V1 cells along with V1 spike threshold (Lauritzen
478 et al., 2001; Freeman et al., 2002; Li et al., 2006; Priebe and Ferster, 2006; Priebe, 2016).

479 Our optogenetic stimulation results were inconsistent with a cortical explanation for cross-orientation
480 suppression. Under the hypothesis that optogenetic stimulation is in any way like a visual stim-
481 ulation, stimulation of orthogonal columns should have produced a strong suppression in the
482 orthogonal columns. Instead, when we stimulated a set of orientation columns, we observed
483 a moderate spreading of cortical responses. Combined visual and optogenetic stimulation pro-
484 duced responses very similar to the linear sum of the individual stimuli for moderate response
485 strengths, while paired stimulation of visual stimuli of moderate contrast produced strongly sub-
486 linear responses. In our model that best matched the data, there was some weak cross-column
487 suppression, but the cross-column suppression was much smaller than is required to produce the
488 cross-orientation suppression seen using visual stimuli.

489 **Inhibition-stabilized dynamics and paradoxical responses**

490 Another goal of our study was to examine whether we could find direct evidence of inhibition-
491 stabilized dynamics in ferret visual cortex. In inhibition-stabilized dynamics, the cortical circuit acts
492 as a strong amplifier of external input, such that most of the synaptic drive that impinges on each
493 cortical cell arises from within the cortex itself (Suarez et al., 1995; Tsodyks et al., 1997; Ozeki et
494 al., 2009; Rubin et al., 2015). Evidence for inhibition-stabilized dynamics has been observed in
495 studies of surround suppression in cat visual cortex (Ozeki et al., 2009) and mouse visual cortex
496 (Sato et al., 2016; Adesnik, 2017; Sanzeni et al., 2020), mouse somatosensory cortex (Sanzeni et
497 al., 2020), and mouse motor cortex (Sanzeni et al., 2020). Indirect evidence for inhibition-stabilized
498 dynamics has been observed in ferret visual cortex (Rubin et al., 2015). Sanzeni et al (2020) also
499 looked for paradoxical responses by direct optogenetic stimulation of interneurons, and found that
500 paradoxical responses in awake animals could be evoked by stimulating either all interneurons
501 or just PV neurons when they were targeted in a transgenic manner. Interneuron receptive field
502 properties differ between species that have columnar features beyond retinotopic maps and those
503 that only have retinotopic maps; for example, interneurons in cat and ferret visual cortex can be
504 highly tuned for orientation (Hirsch et al., 2003; Cardin et al., 2007; Wilson et al., 2017), while a
505 majority of interneurons in mouse visual cortex are not (Sohya et al., 2007; Niell and Stryker, 2008;
506 Liu et al., 2009; Kerlin et al., 2010). Here we showed that a subset of ferret interneurons also
507 exhibited characteristic paradoxical behavior with direct stimulation.

508 We observed heterogeneous interneurons responses to increasing optogenetic stimulation. These
509 responses resemble the heterogenous paradoxical/non-paradoxical responses that Sanzeni et al.

510 (2020) and Mahrach et al (2020) observed in experiments using viral-mediated infection of PV+
511 neurons. Sanzeni et al. (2020) observed nearly universal paradoxical responses when they used
512 transgenic methods to express optogenetic channels in either PV+ neurons or all interneurons, via
513 a VGAT promoter. In ferret, we used a viral promoter that caused expression of ChR2 in a wide
514 variety of interneuron classes (Dimidschstein et al., 2016), but interneuron subclass-specific viruses
515 are now becoming available for non-rodents (Mehta et al., 2019; Vormstein-Schneider et al., 2020).
516 Future experiments will be needed to determine if some of the heterogeneity we observed could
517 be due to differences in responses of different interneuron subtypes.

518 **Materials and Methods**

519 **General design.**

520 All experimental procedures were approved by the Brandeis University Animal Care and Use Com-
521 mittee and performed in compliance with National Institutes of Health guidelines. Eleven adult
522 ferrets (*Mustela putorius furo*, Marshall Farms; >P90, female) were used in total: five ferrets were
523 used for patterned optogenetic experiments, four ferrets were used for optogenetic specificity ex-
524 periments, and four ferrets were used for inhibitory optogenetic experiments. Females were used
525 exclusively because co-housing male and female adult ferrets in the same space is stressful for
526 the animals if they are not allowed to mate. For patterned optogenetic and optogenetic specificity
527 experiments, AAV9.CamKIIa.hChr2(E123T/T159C).mCherry.WPRE.hGH was used to express Chr2
528 in neurons.

529 **Virus injection.**

530 All virus injections were achieved by pre-treating ferrets with ketoprofen (1mg/kg, IM) and tramadol
531 (2-5mg/kg, oral) on the morning of surgery. The ferrets were anesthetized with ketamine/xylazine
532 cocktail (20-30mg/kg, 2-3mg/kg) through IM injections and the anesthesia was maintained by ad-
533 ditional injections of ketamine/xylazine (10-50% amount of ketamine/xylazine used during initial
534 anesthesia). Atropine (0.16mg/kg) was given to reduce secretions. Ringer's solution (2.75/ml/kg/hr)
535 was given by subcutaneous injections to prevent dehydration. The body temperature was con-
536 trolled and monitored by a thermostatic controller (TR-200, Fine Science Tools or PhysioSuite, Kent
537 Scientific), and the EKG levels were continuously monitored.

538 Ferrets were held in a stereotaxic apparatus by two ear bars and a bite bar. The heads were
539 shaved and sterilized by alternate applications of Betadine-soaked gauze and 70% isopropanol-
540 soaked gauze three times. Bupivacaine (0.25-0.5ml of 0.25% with a maximum dose of 2mg/kg,
541 IM) was injected around the incisions on the head. Head muscle and skin were retracted and a
542 craniotomy, about 1-2 mm wide, was performed. A small durotomy was made with a 31-gauge
543 needle on a cotton tip applicator. The glass pipettes used to inject viruses were pulled on a vertical
544 puller (PC100, Narishige) and beveled to achieve a tip about 30um in diameter. Virus was delivered
545 by a microinjection device (Nanoject, Drummond Scientific) with 22 pulses of 23 nl/pulse with 10
546 seconds intervals. To achieve a broad expression area (2.5 mm²), two or three locations were
547 injected, two depths (300um and 500um below the brain surface) at each location, for each ferret.
548 After the virus injection, the craniotomy site was covered with an Amniograft membrane (in some
549 experiments) and the removed skull. The scalp incision was closed with non-absorbable sutures
550 and the wound site was covered with Neosporin. Animals were returned to the cage with the rest
551 of the litter after they were ambulatory. Analgesics and antibiotics were administered through 48
552 hours after surgery.

553 **Construction of ProjectorScope 2.**

554 The ProjectorScope 2 (Fig. 1D, Supplementary Fig. 1,2) was built with several modifications of
555 ProjectorScope 1 (Roy et al., 2016) to achieve wide patterned optogenetic stimulation and intrinsic
556 signal imaging. Patterned light for optogenetic stimulation was generated by an LCD projector
557 (NP3250W, NEC) and transmitted onto the brain surface by three single-lens reflex (SLR) lenses of
558 the same type (Nikon, focal length 50 mm, f/1.2). The original projection lens was replaced with
559 one of these SLR lenses to reduce misalignment between the projector and the rest of the optical
560 system. Two juxtaposed lenses (L1; Thorlabs achromat, focal length 30mm, diameter 25mm) were
561 used to minify the projection image to the appropriate size, 3mm by 3mm, in order to cover the
562 exposed area in ferret V1. A dichroic mirror (DM; Semrock FF483/639) reflected light between 390-
563 460 nm to activate Chr2, while allowing green and red light to pass to the CCD camera (Dalsa
564 camera, 1M60) for intrinsic signal imaging. Intrinsic signal imaging was performed by providing
565 690-nm light (halide light, Lumen Dynamics, Xcite 200DC, with a filter, Semrock FF01-675/67-25)

566 over the brain surface and taking images of the reflected light using the CCD, with an SLR lens
567 (Nikon, focal length 135 mm, f/2.8) to bring the image into focus. The maximum power that the
568 system can provide is approximately 10mW/mm² measured at 475 nm by projecting a full-field,
569 100% contrast white image. ProjectorScope 2 allows three-dimensional adjustments for easier
570 focus on a curved brain surface (details seen in Supplementary Fig. 1,2).

571 **Non-survival surgery.**

572 About 4 weeks after the virus injection, the ferrets were sedated with ketamine (20mg/kg, IM). At-
573 ropine (0.16mg/kg, IM) and dexamethasone (0.5mg/kg, IM) were administered to reduce bronchial
574 and salivary secretion and to reduce inflammation, respectively. The animal was anesthetized with
575 a mixture of isoflurane, oxygen, and nitrous oxide through a mask while a tracheostomy was per-
576 formed. Animals were then ventilated with 1.5%–3% isoflurane in a 2:1 mixture of nitrous oxide
577 and oxygen. A cannula was inserted into the intraperitoneal cavity for delivery of neuromuscu-
578 lar blockers and Ringer's solution (3 ml/kg/hr), and the animal was placed in a custom stereotaxic
579 frame that did not obstruct vision. The head was fixed with a custom head plate that allowed pitch
580 adjustments for imaging. All wound margins were infused with bupivacaine. Silicone oil was placed
581 on the eyes to prevent corneal drying. A craniotomy (4 by 4mm) was made in the right hemisphere
582 centered around the virus injection site, and the dura was removed with a 31-gauge needle. A
583 few drops of liquid agarose were applied on the exposed brain surface, and, while the agarose
584 was still liquid, a pre-drilled coverslip (a hole of about 700um in diameter was drilled through the
585 coverslip) was mounted on top of the craniotomy area and held until the agarose became solid
586 (Levy et al., 2012). The coverslip edge was secured using cyanoacrylate glue and excess agarose
587 on the coverslip was removed. Next, the ferrets were paralyzed with the neuromuscular blocker
588 gallamine triethiodide (10–30 mg/kg/hr) through the intraperitoneal cannula to suppress sponta-
589 neous eye movements, and the nitrous oxide-oxygen mixture was adjusted to 1:1. The animal's
590 ECG was continuously monitored to ensure adequate anesthesia, and the percentage of isoflurane
591 was increased if the ECG indicated any distress. Body temperature was maintained at 37°C.

592 **Visual stimulation.**

593 Visual stimuli were created in MATLAB with the Psychophysics Toolbox on a Macintosh Pro running
594 OSX and displayed on a Sony monitor (GDM-520). The monitor was placed 35cm in front of the
595 ferret. Stimuli were full field sine wave gratings with 0.15 spatial frequency and 4Hz temporal
596 frequency.

597 For intrinsic signal imaging experiments, 100% contrast, bidirectional grating stimuli with orien-
598 tations varied from 0° to 135° with a step of 45° were played. Each orientation condition was
599 repeated 20 times with 10s inter-stimulus-interval and 5s stimulus duration.

600 For the optogenetic experiments, visual stimuli were 100% contrast, full-field, with 0.15 spatial
601 frequency, 4Hz temporal frequency with 8 cycles and repeated 5 times with 3-5s inter-stimulus-
602 interval. To measure the orientation selectivity, the orientations were varied from 0° to 157.5°
603 with a step of 22.5°. To measure responses to visual contrast, the orientation was fixed at the
604 preferred angle and the contrasts were varied as 16%, 32%, 64%, or 100%.

605 Intrinsic signal imaging. Intrinsic signal imaging was performed for some optogenetic experiments
606 to obtain the orientation column maps. With ProjectorScope 2, 690-nm light illuminated the brain
607 surface and the reflected light from the brain surface was captured by the camera. The images
608 were acquired at 30 Hz with custom software in LabVIEW and a National Instruments PCI-1426 ac-
609 quisition board. The raw images were averaged for every 0.5s and the image that was 0.5s before
610 the onset of the stimulus was used as the baseline image. The single condition images represent-
611 ing responses for individual orientations were averaged over all repetitions and the orientation
612 column map was generated by calculating the vector summation of responses in the single condi-

613 tion images:

$$P = \sum_{k=1}^N R(\theta_k) e^{\frac{2\pi i \theta_k}{180^\circ}} \quad (1)$$

614 where $R(\theta_k)$ is the responses in a single condition image for a certain orientation stimulus, and P
615 represents the response of each pixel in the map as a vector summation in the complex plane.

616 **Electrophysiology.**

617 In some optogenetic experiments and optogenetic specificity experiments, single barrel carbon
618 fiber electrodes were used (E1011, Kation Scientific). Such carbon fiber electrodes have very small
619 tips of about 5um in diameter so they minimize damage to the brain tissue and do not cast shadows
620 over the stimulated brain area. One electrode was inserted through the hole on the coverslip into
621 ferret V1, and was lowered to a depth that ranged from 100um to 400um below the brain surface.
622 The signals were amplified by RHD2132 and collected by the RHD2000 evaluation board (Intan
623 Technologies).

624 In the inhibitory optogenetic experiments, a custom 32-channel probe (S-probe, Plexon) was used.
625 Instead of using pre-drilled coverslips, the probe was positioned to just touch the brain surface
626 and 2

627 **Optogenetic receptive zone.**

628 We characterized the optogenetic receptive zone (ORZ) for each patterned optogenetic experiment.
629 To measure the spatial range of the effective optogenetic stimulation, we projected small dots,
630 750um in diameter, in a randomized fashion tiling across the entire projection area, 3 by 3 mm²,
631 onto the ferret primary visual cortex that had ChR2 expression. We determined whether a cell's
632 response at any of the stimulus positions was significantly different from the response to a "blank"
633 stimulus by performing an ANOVA test ($P < 0.05$). Responses were fitted by a bivariate Gaussian
634 function to estimate the region over which a cell was strongly activated :

$$R(s) = NR \left\{ \left[\sum_x \sum_y G(x, y, \mu, \Sigma) I_s(x, y) \right], a, c_{50}, n \right\} \quad (2)$$

635 Where $I_s(x, y)$ is the intensity at point x, y for stimulus s , $G(x, y, \mu, \Sigma)$ is the bivariate Gaussian with
636 mean μ and covariance matrix Σ , and $NR(c, a, c_{50}, n)$ is the Naka-Rushton function:

$$NR(c, a, c_{50}, n) = \frac{ac^n}{c^n + (c_{50})^n} \quad (3)$$

637 Where a is the maximum cell response, c is the stimulus intensity, and c_{50} is the intensity of a
638 stimulus that produces half of the maximum response. Variables $a, c_{50}, n, \mu, \Sigma$ were used as free
639 parameters for the fit. The size of the ORZ was taken to be the full width at half-height (FWHH)
640 along the major and minor axes of $G(x, y, \mu, \Sigma)$.

641 **Patterned optogenetic stimulation.**

642 In patterned optogenetic specificity experiments, stimulation masks targeting specific orientation
643 columns were generated based on the map acquired during intrinsic signal imaging (custom soft-
644 ware, MATLAB). The preferred orientation of the recorded neuron was identified as the orientation
645 that evoked the strongest visual responses from the orientation tuning curve. Visual contrast tun-
646 ing curves were initially measured using 16%, 32%, 64%, or 100% contrast. The optogenetic light
647 intensity tuning curve were measured by projecting the mask of the ORZ with varying image inten-
648 sities, 20%, 40%, 60%, 80%. The measurements of these tuning curves were repeated by changing
649 contrasts or intensities until comparable levels between visual and optogenetic responses were
650 found, which was usually achieved after 2-3 iterations. Five levels of visual contrasts or light inten-
651 sities, including 0% contrast and 0% intensity, were chosen for each cell. For the combined visual
652 and patterned optogenetic stimulation, each visual stimulus was paired with an optogenetic mask

653 stimulus and each pair started at the same time with the optogenetic stimulation lasting for 1s and
 654 visual stimulation lasting for 2s. The orientation column masks for a given angle were made by in-
 655 cluding all pixels in the intrinsic signal imaging map that matched the specified angle within some
 656 tolerance. The tolerance (or thickness) of the column masks was varied by changing the range of
 657 orientation angles that each column mask contained: 15°, 30°, or 45° tolerance. We found that a
 658 tolerance of 15° was too small for some cells to evoke enough optogenetic responses and that a tol-
 659 erance of 45° was too big to create distinctly complementary patterns of inputs, so, in the analysis
 660 here, we only report the results based on 30° masks. The stimulation order was randomized.
 661 For all-optogenetic stimulation (Fig. 3A), the masks were created to target either the preferred ori-
 662 entation columns that were revealed in the orientation columns map, the orthogonal orientation
 663 columns, or both. For hybrid stimulation (Fig. 3B), the masks were created to target the orthogonal
 664 orientation columns and the visual stimulus presented the preferred orientation. For the optoge-
 665 netic specificity experiments, only the orientation columns inside the ORZ were included and the
 666 masks were created to target the orientations from 0° to 157.5° with a step of 22.5° and a tolerance
 667 of 15°.

668 Pharmacological blocking experiments.

669 To test the hypotheses of optogenetic specificity, an NMDA antagonist (DL-2-Amino-5-phosphonopentanoic
 670 acid, APV, 1mM- 5mM) and an AMPA antagonist (2,3-Dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[f]quinoxaline-
 671 7-sulfonamide, NBQX, 100uM-1mM) were used to block NMDA and AMPA receptors. For these
 672 experiments, the coverslips were pre-drilled with two holes that were 2-3mm apart, one for the
 673 carbon fiber electrode and one for the blocker pipette. A pulled glass pipette similar to the dimen-
 674 sion used in virus injections was used to apply the blockers and the blocker solution was delivered
 675 by Nanoject, with 69nl/pulse, 4 pulses/min, for 14 min, for a total volume of 3.8ul. The effective-
 676 ness of the blockers was tested by examining visual responses.

677 Data analysis.

678 Extracellular data was extracted using 4-5 standard deviations as the threshold and then clustered
 679 using K-means or KlustaKwik based on either two-point features (for the single barrel carbon fiber
 680 electrode recordings) or the principal components (for the S-probe recordings). In the patterned
 681 optogenetic experiments, the firing rates were calculated as spike counts from the 0.5s-1s after the
 682 onset of the stimuli divided by 0.5s duration, because the firing rates reached the steady states
 683 after 500ms of stimulation. In the inhibitory optogenetic experiments, the calculations of firing
 684 rates were based on the entire 1s duration of paired stimulation.

685 To analyze the optogenetic specificity, the specificity index is defined as 1-circular variance (Ringach
 686 et al., 2002; Mazurek et al., 2014), calculated as:

$$1 - \text{CirVar} = \frac{\sum_k R(\theta_k) e^{2i\theta_k}}{\sum_k R(\theta_k)} \quad (4)$$

687 Where $R(\theta_k)$ is the response to angle θ_k . The comparison between the specificity before and after
 688 blockers applications was based on two-sample t-test.

689 To calculate the Marginal Orthogonal Drive Angle (MODA), we created an weighted vector that
 690 described the influence of adding extra orthogonal drive to the linearity of a cell's response. We
 691 denote the response of a cell to a preferred contrast C_{pref} and orthogonal contrast C_{orth} as $R(C_{\text{pref}}, C_{\text{orth}})$.
 692 The linear prediction for the response of the cell is $LP(C_{\text{pref}}, C_{\text{orth}}) = R(C_{\text{pref}}, 0) + R(0, C_{\text{orth}})$.
 693 The change in linearity was computed for each pair of presented orthogonal contrasts $C_{\text{orth}}^j, C_{\text{orth}}^k$
 694 (where the contrast C_{orth}^k must be greater than C_{orth}^j) as a vector

$$V(C_{\text{pref}}, C_{\text{orth}}^j, C_{\text{orth}}^k) = \begin{bmatrix} \Delta LP \\ \Delta R \end{bmatrix} = \begin{bmatrix} LP(C_{\text{pref}}, C_{\text{orth}}^k) - LP(C_{\text{pref}}, C_{\text{orth}}^j) \\ R(C_{\text{pref}}, C_{\text{orth}}^k) - R(C_{\text{pref}}, C_{\text{orth}}^j) \end{bmatrix} \quad (5)$$

695 To compute an accurate estimate of the Marginal Orthogonal Drive, we computed this quantity
 696 over the 2 highest preferred orientation contrasts, and for all pairs of orthogonal contrasts (there
 697 were 4 orthogonal contrasts, so there are 6 pairs of orthogonal contrasts where the second is
 698 greater than the first). Further, we normalized the contribution of each vector to the overall Marginal
 699 Orthogonal Drive by the change in orthogonal contrast, reasoning that if we added an extra amount
 700 (say, twice) of orthogonal stimulus contrast to one pair of stimuli as compared to another pair, we
 701 ought to divide the contribution by that extra amount (say, by 2) to normalize the contribution of
 702 the contrasts used, so that the vector has units of change in response per unit contrast. The total
 703 Marginal Orthogonal Drive vector was thus:

$$\vec{V} = \sum_{i=1}^2 \sum_{j=1}^3 \sum_{k=j+1}^4 \frac{\vec{V} \left(C_{\text{pref}}^i, C_{\text{orth}}^j, C_{\text{orth}}^k \right)}{C_{\text{orth}}^k - C_{\text{orth}}^j} \quad (6)$$

704 where i represents the two highest contrasts of the preferred stimulus, and j and k represent the
 705 levels of orthogonal contrast from which the starting data point and the ending data point of a
 706 vector are found.

707 The Marginal Orthogonal Drive Length is then

$$\text{MODL} = \sqrt{V[1]^2 + V[2]^2} \quad (7)$$

708 and the Marginal Orthogonal Drive Angle is

$$\text{MODA} = \tan^{-1} \left(\frac{V[2]}{V[1]} \right) \quad (8)$$

709 where $V[i]$ is the i th dimension of the vector V .

710 The Linearity index is the slope of the best linear fit line of R and LP (in the least squares sense)
 711 that passes through all responses under all contrasts and must pass through the origin.

712 Ring model simulations.

713 The ring models consisted of 180 E and I cells, placed along a ring. Each position was given a label
 714 $\theta_i = 0 \dots 179^\circ$. The steady-state response of each cell was given by the equation from Rubin et al.
 715 2015:

$$r_i^{ss} = 0.04 \left[\sum_{j=1}^N w_{ij} r_j + h_i \right]_+^2 \quad (9)$$

716 where w_{ij} is the connection from neuron j to neuron i , and h_i is the sum of visual (hvisual) and
 717 optogenetic (hopto) input to neuron i . The connections from e-to-e neurons had a weight W_{EE} that
 718 fell off in a Gaussian manner with angular distance around the ring as σ_{EE} . Similarly, connections
 719 from i-to-e had a value W_{EI} that fell off with σ_{EI} , connections from e-to-i had a value W_{IE} that fell
 720 off with σ_{IE} , and i-to-i connections had a weight W_{II} that fell off with σ_{II} . The following differential
 721 equation was solved numerically using Euler's method:

$$\tau_i \frac{dr_i}{dt} = -r_i + r_i^{ss} \quad (10)$$

722 where τ_i is 200ms for excitatory cells and 100ms for inhibitory cells. Visual stimulation at an angle
 723 θ was delivered to each neuron with a strength of 1 and a Gaussian falloff of 30° .

724 The details of the connections and optogenetic input differed by model. For Model 1, W_{EE} was
 725 0.018, W_{EI} was -0.0094, W_{IE} was 0.0171, W_{II} was -0.0073, and $\sigma_{EE} = \sigma_{IE} = \sigma_{EI} = \sigma_{II} = 32^\circ$. Opto-
 726 genetic input was delivered to each neuron with a strength of 1 and a Gaussian falloff of 45° from
 727 the angle of columnar stimulation θ .

728 For Model 2, the parameters were the same as Model 1, except that visual and optogenetic inputs
 729 to inhibitory neurons were slightly increased (to 1.1), and $\sigma_{EE} = \sigma_{IE} = \sigma_{II} = 10^\circ$, while σ_{EI} was
 730 dropped to 25°

731 For Model 3, the parameters were the same as Model 2, except that optogenetic input was modeled
 732 differently. The orthogonal input was modeled as the sum of $\frac{1}{2}$ the Gaussian fall off as before and a
 733 constant input of 0.5 at all θ_i , and the preferred input included an additional 0.2 input to inhibitory
 734 neurons to reflect the fact that the preferred optogenetic stimulation included the region right
 735 around the electrode (which was omitted in the orthogonal input). That is, the input to excitatory
 736 neurons was given as the real part of the following equations, and the input to inhibitory neurons
 737 was given as the imaginary part of the following equations:

$$h_{\text{opto}}^{\text{orth}} = (1 + 1.1i)(0.5G(1, 45^\circ) + 0.5) \quad (11)$$

$$h_{\text{opto}}^{\text{pref}} = (1 + 1.1i)(0.5G(1, 45^\circ) + 0.5) + 0.2i \quad (12)$$

738 Heterogeneous network simulations.

739 To simulate the impact of optogenetic stimulation in a heterogeneous network with variable opto-
 740 genetic activation and variable synaptic weights, we simulated a column with 2 populations with N
 741 neurons in it, each with steady-state rate \vec{r} given by:

$$\vec{r} = \phi(W\vec{r} + \vec{h}) \quad (13)$$

742 Where the function ϕ is a modified rectified power law function $\phi(x) = (\alpha x)^n / (1 + (x + \alpha)^n)$, which
 743 saturates for $x \rightarrow \infty$ and behaves similarly to the standard rectified power law or small values
 744 of x . We chose $\alpha = 35$ and $n = 2$. The connectivity elements are sparse with sparsity $p = 0.3$.
 745 The nonzero elements of W are $w_{ee} = 1$, $w_{ei} = 1.1$, $w_{ie} = 0.89$, $w_{ii} = 0.91$ and they scale as $1/N$ with the
 746 network size.

747 The external inputs to each neuron h_i^α have a baseline component and an optogenetic input, such
 748 that

$$h_i^\alpha = h_i^{\text{b},\alpha} + I_i^{\text{Chr2}}(x)\delta_{\alpha I} \quad (14)$$

749 The baseline was uniformly distributed $h^{\text{b},\alpha}$, centered in $\mu_{hb} = 10$ and with a width $\sigma_{hb} = 6$ for both
 750 cell types. The optogenetic input only affects inhibitory neurons and is defined below.

751 Model of Optogenetic Perturbations

752 There were two main sources of photocurrent heterogeneity, one being light dispersion through
 753 the tissue and the second one being the number or Chr2 channels that are expressed in each cell.
 754 Regarding the first one the amount of light that reaches the cells decays exponentially with distance
 755 (Yona et. al. 2016). We assumed that the recorded neurons are homogeneously distributed in the
 756 z axis of the probe. The light that arrived to each neuron was given by

$$I_i(x) = 2x \exp\left(\frac{-z_i}{\lambda}\right) \quad \text{with} \quad z_i \sim U(0, 1) \quad (15)$$

757 Where x is the light intensity at the surface, z_i is the distance of the cell i from the surface, and λ
 758 is the spatial scale (We took $\lambda = 2$). We assumed that, each channel had an input output function
 759 that is given by a Hill Equation and that each channel had a different threshold, given by I_i^0

$$H_i(x) = \frac{(I_i(x) - I_i^0)^\gamma}{1 + (I_i(x) - I_i^0)^\gamma} \quad (16)$$

760 With $I_i^0 = I_i(x_0)$, with $x_0 \sim \mathcal{N}(\mu_{x_0}, \sigma_{x_0})$. The values were chosen to be $\mu_{x_0} = -0.05$ while $\sigma_{x_0} = 0.001$.
 761 The second source of heterogeneity is the number of Chr2 channels that are expressed with the
 762 virus, that we assume to be gaussian distributed. In the simulations, we didn't specify the number
 763 of channels, but we used a normalized number distributed as $\kappa c \sim \mathcal{N}(\mu_c, \sigma_c)$ with $\mu_c = 72$ and $\sigma_c = 8$

764 Each individual channel contributed independently and identically to the photocurrent such that
765 the total input to the cell was

$$I_i^{\text{Chr2}}(x) = \kappa c_i (H_i(x) - H_i(0)) \quad (17)$$

766 Where the subtraction by $H_i(0)$ guaranteed that the effect of the laser at zero intensity ($x=0$) was
767 0.

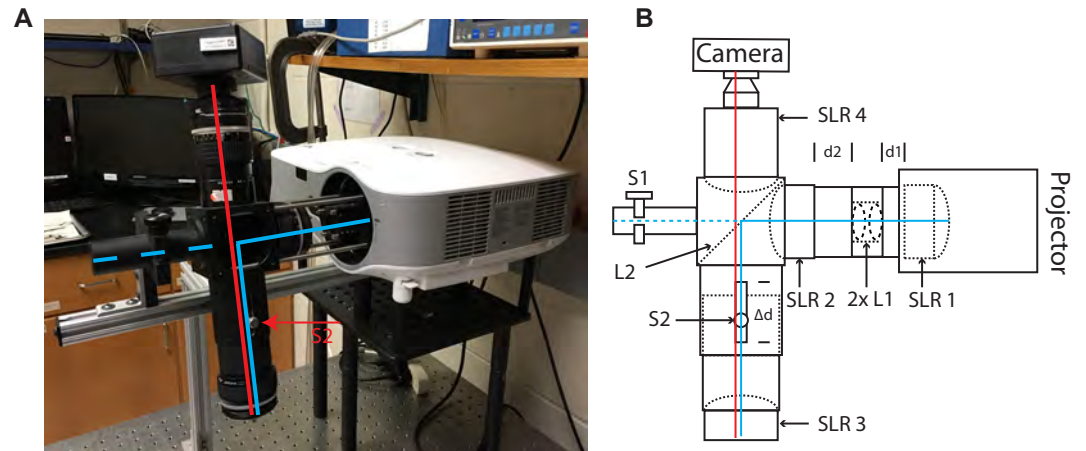


Figure S1. ProjectorScope 2. ProjectorScope 2 on the experimental table. The blue axis represents the direction of the outgoing light from the projector reflected by a dichroic mirror. The red axis represents the direction along which light travels from the brain surface to the camera during intrinsic signal imaging. The red arrow points to the tube that provides translation along the red axis using set screw S2. **B.** A schematic of ProjectorScope 2. SLR, single-reflex lens; S1, screw 1; S2, screw 2; 2x L1, two closely placed achromat lenses; L2, dichroic mirror; $d_1=30\text{mm}$; $d_2=45\text{mm}$; $\Delta d = 6\text{cm}$.

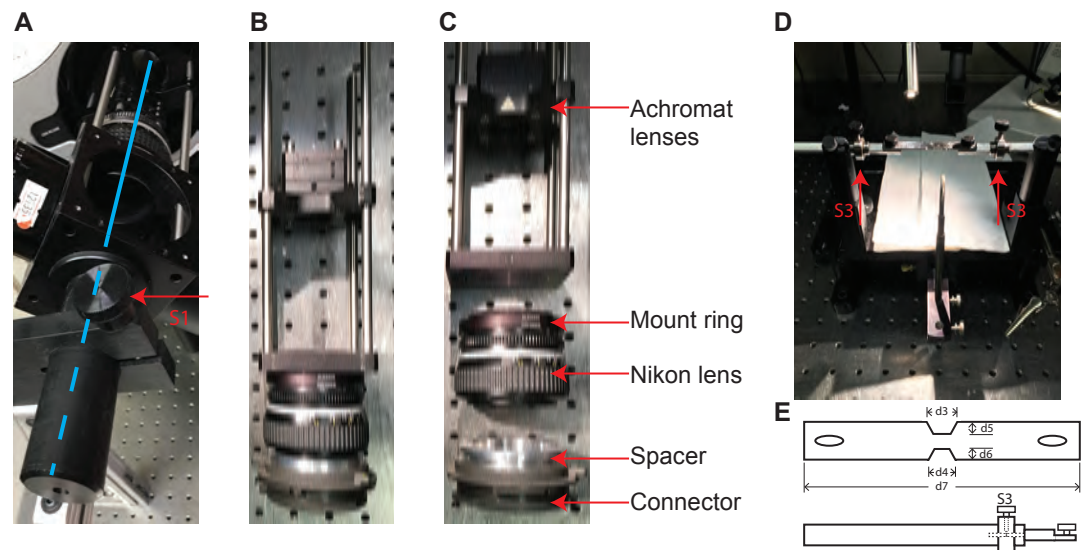


Figure S2. Components of ProjectorScope 2 and the custom head-plate and head-bars. **A.** The cylindrical bar that allows the cube to rotate around the blue axis. The knob S1 controls the rotation of the bar. **B.** The replaced projection lens with all components connected. **C.** A disconnected version of B, which separately displays the connector to the projector, the spacer, the Nikon lens, and the custom-made mount ring that connects the Nikon lens to the Thorlabs rectangular connector. **D.** The rotatable headplate. The red arrows point to the rotatable parts, S3, that can adjust the animal's head pitch angle. Combining the controllable parts in S1, S2, and S3, ProjectorScope 2 allows three-dimensional adjustments for easier focus on a curved brain surface. **E.** Schematics of the head-plate and the head-bar. The head-plate, 1mm thick, has two half-hexagon notches to easily fit the curved skull surface. $d_3=7\text{mm}$, $d_5=2\text{mm}$, $d_4=5\text{mm}$, $d_6=1.5\text{mm}$, $d_7=6.5\text{cm}$. The head-bar provides rotation by adjusting S3.

Supplementary Figure 8-1

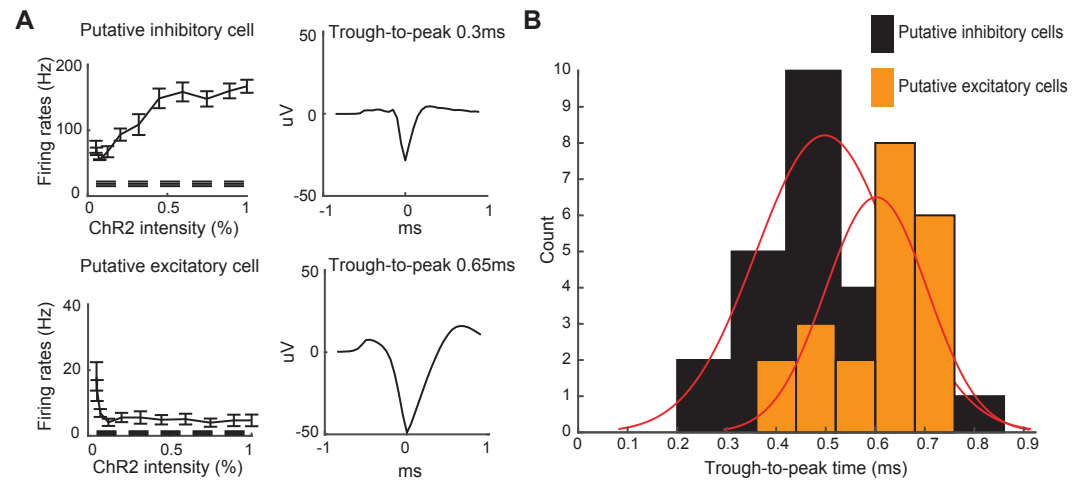


Figure S3. Optogenetic stimulation of ferrets expressing mDlx-ChR2 revealed neurons with putative excitatory and inhibitory signatures. **A.** Left) Responses of an example putative inhibitory cell (top) that shows direct activation by light stimulation, and a putative excitatory cell (bottom) that is strongly inhibited by light. Raw responses are shown, without visual stimulation, and without any subtraction of background firing rates (dashed line). Right) Zoomed-in views of the average action potential waveform from each neuron along with the trough-to-peak time. **B.** Histograms of trough-to-peak times of putative inhibitory cells and putative excitatory cells. Cells' identities were first determined by their response profiles to optogenetic stimulation and then the trough-to-peak times were calculated and summarized. Gaussian density fits to the histograms are shown in red. On average, the putative excitatory cells showed longer spike durations than the inhibitory cells, although there were putative inhibitory cells that exhibited wider spikes, consistent with the variety of interneuron types found in cortex (fast-spiking and regular-spiking).

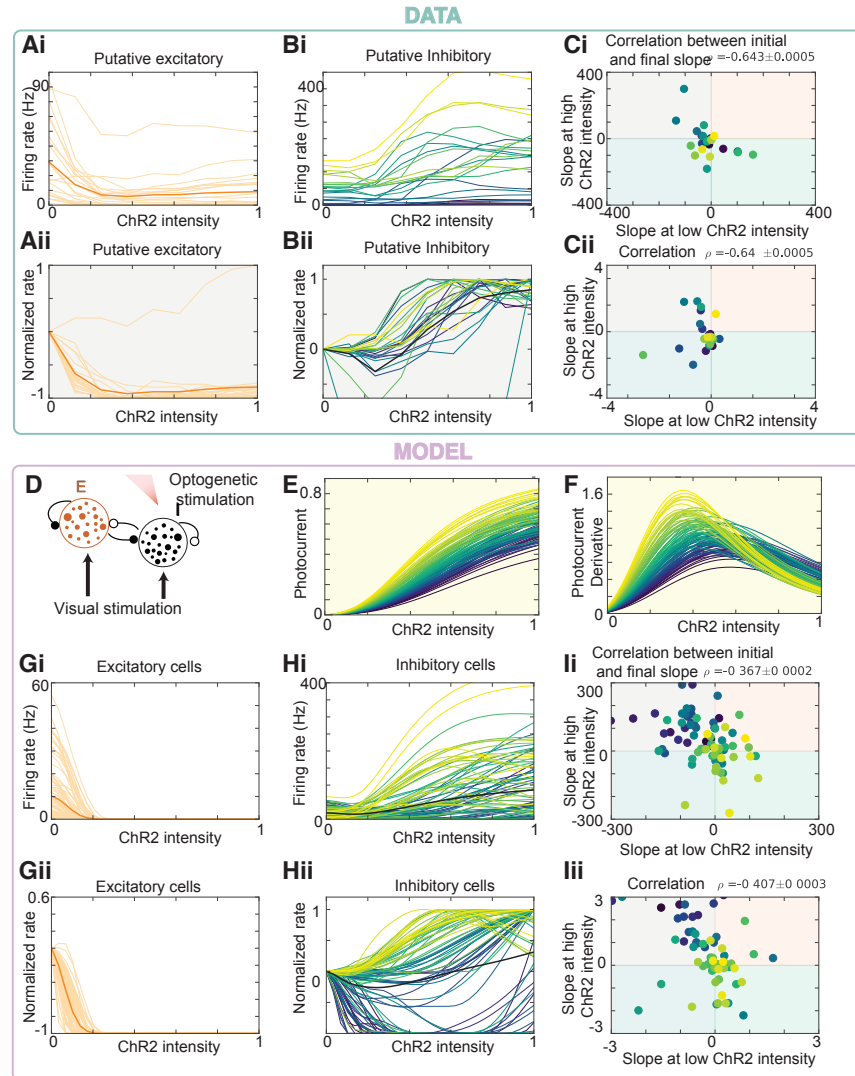


Figure S4. Optogenetic stimulation of ferrets expressing mDlx-ChR2 revealed neurons with putative excitatory and inhibitory signatures. TOP: **Ai**: Putative excitatory cells (as defined in Fig. S3.) as a function of the intensity of the optogenetic input to the inhibitory cells. **Aii**: Same as Ai but normalized such that the curves start from zero and were maximal at 1 (as in Fig. 8c). **Bi**: and **Bii**: Putative inhibitory cells. The paradoxical effect (i.e. that some cells decrease their activity upon optogenetic drive) is better revealed by normalizing the cells. **Ci**: The slope of each cells response to a weak laser input is negatively correlated with the slope at a large laser input. In other words, the cells that did not respond paradoxically tended to saturate at smaller laser intensities. **Cii**: Negative correlation for normalized responses. BOTTOM: **D**: Heterogeneous model with N neurons ($N=500$ in simulations). **E**: Model of optogenetic current for each cell as a function of laser intensity as given by Eq. 17. **F**: Derivative of the photocurrent. **Gi**: Excitatory cells as a function of the intensity of the optogenetic input to the inhibitory cells. **Gii**: Same as Ai but normalized such that the curves start from zero and are maximal at 1. **Hi**: and **Hii**: Inhibitory cells. The paradoxical effect in the model is better revealed by normalizing the cells, as in the data. **Ii**: and **Iii**: The model captures the negative correlation between the slope in response to a weak laser and the slope at a large laser input for both normalized and non-normalized responses.

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