Differential tuning of excitation and inhibition shapes direction selectivity in ferret visual cortex

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To encode specific sensory inputs, cortical neurons must generate selective responses for distinct stimulus features. In principle, a variety of factors can contribute to the response selectivity of a cortical neuron: the tuning and strength of excitatory¹–³ and inhibitory synaptic inputs⁴–⁹, dendritic nonlinearities⁷–⁹ and spike threshold¹⁰,¹¹. Here we use a combination of techniques including in vivo whole-cell recording, synaptic- and cellular-resolution in vivo two-photon calcium imaging, and GABA (γ-aminobutyric acid) neuron-selective optogenetic manipulation to dissect the factors that contribute to the direction-selective responses of layer 2/3 neurons in ferret visual cortex (V1). Two-photon calcium imaging of dendritic spines¹²,¹³ revealed that each neuron receives a mixture of excitatory synaptic inputs selective for the somatic preferred or null direction of motion. The relative number of preferred- and null-tuned excitatory inputs predicted a neuron's somatic direction preference, but failed to account for the degree of direction selectivity. By contrast, in vivo whole-cell patch-clamp recordings revealed a notable degree of direction selectivity in subthreshold responses that was significantly correlated with spiking direction selectivity. Subthreshold direction selectivity was predicted by the magnitude and variance of the response to the null direction of motion, and several lines of evidence, including conductance measurements, demonstrate that differential tuning of excitation and inhibition suppresses responses to the null direction of motion. Consistent with this idea, optogenetic inactivation of GABAAergic neurons in layer 2/3 reduced direction selectivity by enhancing responses to the null direction. Furthermore, by optogenetically mapping connections of inhibitory neurons in layer 2/3 in vivo, we find that layer 2/3 inhibitory neurons make long-range, intercolumnar projections to excitatory neurons that prefer the opposite direction of motion. We conclude that intracortical inhibition exerts a major influence on the degree of direction selectivity in layer 2/3 of ferret V1 by suppressing responses to the null direction of motion.

Using sparse expression of the ultrasensitive fluorescent indicator protein GCaMP6s and two-photon calcium imaging¹³, we first determined a neuron's somatic preferred direction using drifting grating stimuli (example cell, Fig. 1a) and then presented the somatic preferred and null directions while imaging spines on apical and basal dendrites. Individual dendritic branches contained spines that responded preferentially to either the somatic preferred or null directions (example branch, Fig. 1b); this diversity existed throughout the dendritic fields of single neurons (Fig. 1c). By quantifying spine responses using a direction selectivity index (DSI, see Methods) we found that spines tuned for the somatic preferred and null directions had different DSI values (Fig. 1d; Wilcoxon rank-sum, P = 0.002, n = 384 preferred spines, n = 233 null spines from n = 17 cells from 10 animals), and in 8 of 12 direction-selective cells (soma DSI > 0.3), more spines were tuned to the somatic preferred direction than to the null direction (Fig. 1e). Similar fractions of spines from basal (300 of 498) and apical (84 of 119) dendrites responded more strongly to the somatic preferred direction (60.2% and 70.6%, respectively). We then computed bootstrapped sums of normalized spine responses (see Methods) to assess the relation between the DSI of a neuron's inputs and of its soma. Summed excitatory synaptic input was weakly tuned (summed spine DSI = 0.1 ± 0.1, median ± interquartile range (IQR), n = 17 cells) and we found no correlation between summed spine and somatic DSI across our sample (Fig. 1f; r = −0.08, P = 0.75, n = 17 cells), regardless of how we assessed synaptic input direction selectivity (see Methods, Extended Data Fig. 1a, b). Together, these data emphasize that the functional specificity of excitatory synaptic inputs converging onto individual layer 2/3 neurons is sufficient to account for somatic direction preference, but fails to explain the degree of somatic direction selectivity. Previous work has suggested that spike threshold amplifies weak biases in excitatory synaptic inputs to enhance the spiking direction selectivity of neurons in layer 4 of cat V1¹⁴. To investigate whether subthreshold membrane potential (Vₘ) responses reflect the weak biases in excitatory inputs demonstrated by our spine imaging, we made whole-cell patch-clamp recordings from layer 2/3 neurons using a K⁺-based internal solution (n = 76 cells from 23 animals; example, Fig. 2a, b). Most cells (78%, n = 54 of 69) showed strong spiking direction selectivity (Extended Data Fig. 2). Unexpectedly, nearly half of the cells with direction-selective spiking responses (48%, n = 26 of 54) showed strong direction tuning in their Vₘ responses (Fig. 2c), and the degree of Vₘ selectivity was correlated with spiking selectivity (n = 69 cells, r = 0.56, P = 5.67 × 10⁻³). The strong direction selectivity evident in Vₘ responses is in sharp contrast to the weak selectivity predicted by the distribution of excitatory synaptic inputs and forced us to consider factors that might contribute to the strong direction tuning of Vₘ responses. In principle, the emergence of strong subthreshold tuning from broadly tuned excitatory inputs could reflect mechanisms that enhance the effectiveness of excitatory inputs tuned to the preferred direction, diminish the effectiveness of excitatory inputs tuned to the null direction or a combination of these factors. To distinguish among these alternatives, we investigated whether there was a consistent relationship between subthreshold selectivity and subthreshold response amplitude to preferred and null direction stimuli. We found no correlation between Vₘ DSI and subthreshold response amplitude to the preferred direction (Fig. 2d; r = −0.001, P = 0.99, n = 76). By contrast, we found a strong anticorrelation between Vₘ DSI and null direction response amplitude (Fig. 2e; r = −0.69, P = 5.07 × 10⁻¹², n = 76). These results indicate that factors that influence null direction responses are important in determining Vₘ selectivity.

We then considered the degree to which inhibitory inputs contribute to subthreshold responses to the null direction of motion⁵,¹⁵,¹⁶. Theoretical models predict that when levels of inhibition are high relative to excitation, not only is there a reduction in the level of depolarization, but also a reduction in the ‘noise’ or Vₘ variability¹⁷,¹⁸. We therefore examined the relationship between subthreshold DSI and Vₘ noise (see Methods) for the preferred direction and observed no significant correlation (Fig. 2f, r = −0.10, P = 0.39, n = 76 cells). Instead, we uncovered a strong anticorrelation between subthreshold DSI and Vₘ noise at the null direction (Extended Data Fig. 3; Fig. 2g, r = −0.61, P = 0.002, n = 76 cells), consistent with the idea that subthreshold direction selectivity is driven by the null direction of motion. These results are consistent with theoretical predictions, and support the idea that subthreshold direction selectivity is driven by the null direction of motion.
P = 6.94 × 10^{-9}, n = 76 cells), consistent with a significant role for inhibition to the null direction in shaping the subthreshold direction selectivity of layer 2/3 neurons.

The idea that inhibition contributes to direction selectivity contrasts with a number of demonstrations that inhibition normalizes cortical activity^{19,20}. These studies showed that excitation and inhibition are generally co-tuned^{4,14,20,21}, whereas our observations suggest that the tuning of excitatory and inhibitory inputs onto direction-selective neurons is dissimilar. To directly measure excitatory (\(G_e\)) and inhibitory (\(G_i\)) synaptic conductances underlying direction selectivity, we performed whole-cell patch-clamp recordings using a Cs^+–based internal solution^{14} and recorded \(V_m\) responses to drifting gratings at different spatial frequencies to extract synaptic conductances and their direction tuning (Fig. 3a, b; see Methods). We observed a wide range of direction selectivity in synaptic conductances and found that excitatory and inhibitory DSIs were not correlated (Fig. 3c, \(r = 0.043, P = 0.91, n = 10\) cells from 7 animals) and therefore not co-tuned. In half of our recorded neurons, excitation and inhibition preferred opposite directions (\(\Delta \theta > 135^\circ\)) and across the population there was a significant bias towards preferring opposite directions (Extended Data Fig. 4a, Monte Carlo significance test, \(P = 0.023\)). Despite a lack of co-tuning, excitation and inhibition shared similar tuning bandwidth (Extended Data Fig. 4b).

Understanding the effect of inhibitory conductances on subthreshold responses requires consideration of co-occurring excitatory conductances. Thus, we measured the relative strength of inhibition as the ratio of inhibitory to excitatory conductance (\(G_i/G_e\)) and found that \(G_i/G_e\) was systematically larger for null direction than for preferred direction stimuli (Fig. 3e; Wilcoxon sign–rank, \(P = 0.037, n = 10\)). Moreover, the direction selectivity of predicted \(V_m\) from empirically measured synaptic conductances^{22} (population tuning curves in Extended Data Fig. 4c) was significantly correlated with the \(G_i/G_e\) ratio at the null direction (Fig. 3f; \(r \approx 0.81, P = 0.008, n = 10\) cells from 7 animals) but not the preferred direction (Extended Data Fig. 5). Consistent with our spine imaging data (Fig. 1f), predicted \(V_m\) direction selectivity was not correlated with the direction tuning of excitation alone (Fig. 3d; \(r = 0.49, P = 0.15, n = 10\)). Our measurements of synaptic conductances suggest that relatively stronger inhibitory input at the null direction enhances somatic direction selectivity.

If relatively greater \(G_i/G_e\) at the null direction contributes to direction selectivity in layer 2/3, inactivation of GABAergic neurons in layer 2/3 would reduce suppression at the null-direction and reduce selectivity, as suggested by previous pharmacological studies^{23}. To test this hypothesis, we optogenetically suppressed layer 2/3 GABAergic neurons by expressing GaCr2 under the control of the mouse Dlx5/6 (\(m\)Dlx) enhancer and measured direction selectivity using whole-cell patch-clamp recordings with K^+–based internal solution. Optogenetic inactivation of GABAergic neurons (Extended Data Fig. 6) increased evoked response amplitude (Fig. 3g) and reduced \(V_m\) DSI (Fig. 3h; \(P = 0.004\), Wilcoxon sign–rank, \(n = 16\) cells from 4 animals) and spiking DSI (Extended Data Fig. 7a). Notably, changes in subthreshold direction selectivity were not related to the absolute \(V_m\) depolarization induced by GABAergic photoinhibition in individual neurons (Extended Data Fig. 7b). Instead, the degree to which null-direction responses were modulated by GABAergic suppression (see Methods) depended on the cell’s \(V_m\) DSI (Fig. 3i; \(r = 0.56, P = 0.025, n = 16\)) whereas no such relationship was observed for modulation of preferred-direction responses (Fig. 3i; \(r = 0.20, P = 0.46, n = 16\)). On the basis of these results, we conclude that inhibition enhances subthreshold direction selectivity through null-direction suppression, and we predict that GABAergic neurons preferring the opposite direction would contribute to this suppression.

GABAergic neurons in ferret V1 are direction-tuned and form direction columns^{24} aligned with the underlying intrinsic signal direction preference map (Extended Data Fig. 8a, b). For GABAergic neurons to innervate oppositely tuned excitatory cells, their projections must extend beyond the local direction domain and into adjacent cortical columns. This would be inconsistent with studies from mouse V1, where excitatory neurons receive inhibitory input from local (within 100–200\(\mu\)m) GABAergic neurons^{25,27}. However, in carnivore V1, it has been shown that GABAergic neurons make axonal projections that span longer distances^{26,29}. To test whether GABAergic neurons project beyond their local cortical columns, we labelled axon projections with punctate injections of AAV2/1-mDix-GCaMP6s and characterized the direction tuning of axon projections at sites distal to the injection location (Fig. 4a–d). A substantial fraction (60.5%) of long-range projecting individual boutons exhibited direction-selective responses...
null direction response and $V_m$ DSI (Fig. 2). We found an unexpected abundance of direction-selective GABAergic boutons tuned to the direction opposite to direction domains (Fig. 4f). Furthermore, individual bouton preferences were significantly different from the map (Monte Carlo significance test, $P < 0.001$, $n = 493$ boutons), providing an anatomical substrate for the synaptic inhibition observed in our previous measurements.

To examine whether individual neurons receive inhibitory synaptic input from distant GABAergic neurons, we developed a technique called somatically targeted optogenetic membrane potential mapping (STOMPM) to directly map the spatial connectivity of inhibitory neurons onto excitatory neurons in vivo. We localized channelrhodopsin-2 to the soma and proximal dendrites of GABAergic neurons using a Kv2.1 targeting motif (Fig. 4g) to prevent stimulation of the neuropil and to enhance our functional resolution. As the direction preferences of GABAergic neurons are smoothly mapped in a columnar fashion (Extended Data Fig. 8), we used patterned photostimulation driven by a digital light processing projector to activate GABAergic neurons in local cortical regions (~100–200 µm, Fig. 4g) while recording from single neurons to measure inhibitory postsynaptic potentials (IPSPs) (example cell, Extended Data Fig. 9). Optical stimulation evoked robust IPSPs (Fig. 4h) even at spots distant from recorded cells. Neurons received inhibitory synaptic input from long distances (Fig. 4i); inhibitory input fields often exceeded 1 mm along their major axes (Fig. 4i–k; major axis length $930 ± 278$ µm, median ± IQR, $n = 21$ cells from 7 animals) and many inputs arrived from distances greater than 500 µm (Extended Data Fig. 10). We recognize that these measures are likely to underestimate the total extent of input field size (see Methods). Finally, we aligned our stimulation grid with the intrinsic signal direction preference map (Fig. 4d) to characterize the functional origin of evoked IPSPs. Neurons with direction-selective $V_m$ ($n = 7$ cells, mean tuning curve in Fig. 4m) received almost equivalent inhibitory synaptic input from null-tuned as from preferred-tuned direction domains (Fig. 4n).

Previous studies suggest that inhibition and excitation are generally co-tuned (4, 14, 15, 24, 31) (Fig. 4o), as shown for orientation selectivity in mouse V1 and layer 4 simple cells of cat V1, albeit with distinct temporal dynamics, acting to scale or gate overall responses.

Fig. 3 | Differential tuning between excitation and inhibition enhances direction selectivity. a, Estimated excitatory (blue, $G_e$) and inhibitory (red, $G_i$) synaptic conductances driven by gratings from an example cell; line is bootstrapped mean and error bars are bootstrapped s.d. b, Tuning of peak (see Methods) synaptic conductances and predicted $V_m$ (dashed line) for cell in a; data are bootstrapped mean and s.d. c, Comparison of $G_e$ and $G_i$ DSI ($n = 10$ from 7 animals). d, Predicted $V_m$ DSI (see Methods) compared to $G_e$ DSI ($n = 10$). e, Comparison of $G/G_i$ at null and preferred directions ($n = 10$). f, Predicted $V_m$ DSI compared to null direction $G/G_i$; grey line is least-squares fit ($n = 10$). g, Example $V_m$ during visual stimulation and inactivation of GABAergic neurons expressing GtACR2 (cyan) or without inactivation (black); dashed line is resting $V_m$. h, Comparison of $V_m$ DSI with and without inactivation; black line indicates population means ($n = 16$ from 4 animals). i, Optogenetic modulation of preferred direction response versus $V_m$ DSI ($n = 16$). j, Optogenetic modulation of null direction response versus $V_m$ DSI; grey line is least-squares fit ($n = 16$).
By contrast, we find that cortical inhibition can suppress responses to specific stimuli through differential tuning with excitation (Fig. 4o). Such differential tuning can arise through multiple combinations of excitation and inhibition, such that null-direction suppression is driven by either null-biased or equivalent inhibitory inputs for both directions (Fig. 4o). Differential tuning can enhance subthreshold selectivity, which is further augmented through spike threshold11 (Fig. 4o). Our findings are conceptually similar to those in retinal ganglion cells32, but differ in exact circuit implementation as ganglion cell direction selectivity arises through inhibitory input mediated by starburst amacrine cells. One factor we did not consider is the temporal interplay between excitation and inhibition, which could have an important contributing role in enhancing selectivity31. Together with the results of previous studies, our findings indicate that the selective responses of cortical neurons are built with a broadly tuned palette of excitatory synaptic inputs that is further refined by enhancing responses to the preferred stimulus7,8,12 and suppressing responses to non-preferred stimuli.

**Online content**

Any Methods, including any statements of data availability and Nature Research reporting summaries, along with any additional references and Source Data files, are available in the online version of the paper at https://doi.org/10.1038/s41586-018-0354-1

**Fig. 4 | Inhibitory interneurons make long-range, intercolumnar projections onto excitatory neurons.**

a. Epifluorescence image of injection site with GABAergic axon imaging sites highlighted; example field of view (FOV) in red. b. Intrinsic signal polar direction map for a. c. Example bouton FOV. d. GABAergic boutons overlaid on direction preference map; direction preference of boutons and intrinsic signal pseudocoloured as in b, bi-directional boutons coloured grey. e. Example bouton tuning curve (box in d); data are mean ± s.e.m. f. Distribution of direction preference difference between GABAergic boutons (n = 493) and corresponding intrinsic signal direction preference map. g. Top, Flag staining of cells expressing AAV1-mDlx-ChR2-Flag-Kv2.1-p2a-H2b-CyRFP; bottom, experimental design; neurons in different cortical columns are optogenetically activated. h. Example single spot illumination and Vm responses. i. Mean IPSP waveforms evoked by sampled spots. j. Map of IPSP amplitudes. k. Distribution of IPSP-field major axis lengths across cells (n = 21). l. Example stimulation grid aligned to intrinsic signal polar direction map. m. Peak-aligned average direction tuning curve for cells with direction-tuned membrane potential (DSI > 0.3, black; individual cells in grey, n = 7). n. Fraction of spots tuned to a cell's preferred (<45°) or null (>135°) direction (grey bars, mean ± s.e.m.). o. Cartoon model of co-tuning (top) and differential tuning (bottom) of excitation (Ge) and inhibition (Gi) for direction. Subthreshold direction selectivity is inherited from synaptic conductances when co-tuned. Differential tuning of Ge and Gi, whereby there is greater Gi/Ge at the null direction, can preferentially suppress excitation and enhance subthreshold selectivity. With differential tuning, inhibition can be either bidirectional or oppositely tuned for direction relative to Ge.

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METHODS

All procedures were performed according to NIH guidelines and were approved by the Institutional Animal Care and Use Committee at Max Planck Florida Institute for Neuroscience.

Constructs. pAAV-mDlx-GmCP6F6-plasmid-21 was gifted to G. Fishell (Addgene plasmid # 83889). fUGW-hGtACR2-EYFP was a gift from J. Spudich (Addgene plasmid # 67887). pCAG-CyRFP1 was a gift from R. Yasuda (Addgene plasmid # 84356). pAAV-mDlx-ChR2-Flag-Kv2.1.p2a.H2B-CyRFP was a gift from M. Bolton.

Intrinsic signal imaging. Female ferrets aged about 30 days (~P30; Marshall Farms) were anesthetized with ketamine (50 mg/kg) and isoflurane (1–3%, delivered in O2), then intubated and artificially respired. Atropine was administered to reduce secretions and a 1:1 mixture of lidocaine and bupivacaine administered subcutaneously into the scalp. Animals were placed on a feedback-controlled heating pad to maintain internal temperature at 37°C. Under sterile surgical conditions, a small craniotomy (0.8 mm diameter) was made over the visual cortex ~7–8 mm lateral and ~2–3 mm anterior to the lambda.

For spine imaging, we injected 52 nl per depth) a mixture (1:100,000) of AAV1-hSyn-Cre and AAV1-Syn-FLEX-GmCP6F6 (U Penn, –1 × 1013 GC/ml) at 400 and 200 µm below the pia through bevelled glass micropipettes (10–15 µm outer diameter). For imaging GABAergic axons or somata, we injected 5–30 nl of AAV1-mDlx-GmCP6F6 at 400 and 200 µm below the pia. For opticogenetic inactivation experiments, we injected 1 µl of AAV1-mDlx-GtACR2-eYFP (titre >1 × 1013 GC/ml, custom preparation from Vigen) at 400 and 200 µm below the pia through bevelled glass micropipettes (15–20 µm outer diameter). For STOMP M (see below) we injected 1 µl of AAV1-mDlx-ChR2-Flag-Kv2.1.p2a.H2B-CyRFP (titre >1 × 1013 GC/ml, custom preparation from Vigen) through bevelled glass micropipettes (15–20 µm outer diameter). To prevent dural regrowth and adhesion, the craniotomy was filled with sterile 1% w/v agarose (Type IIIa, Sigma-Aldrich).

Cranial window. After ~5 weeks of expression, ferrets were anaesthetized with 50mg/kg ketamine and 1–3% isoflurane. Atropine and bupivacaine were administered to the scalp to allow the internal solution of the pipette to dialyse the cell, eliminating action potentials and depolarizing the resting membrane potential as expected with current clamp recording. For STOMP M experiments, a fibre (1 mm, NA .63) coupled to a 455 nm LED light source (Pricimatix) was lowered to ~3–5 mm above the cortical surface. Power density at the cortical surface ranged from 1 to 4 mW/mm2. Optogenetic stimulation either coincided with visual stimulation, or began with a brief ramp (100 to 300 ms) before visual stimulation was turned off.

For measurements of synaptic conductances, the internal solution contained (in mM) 135 Cs-MeSO4, 10 QX-314, 4 TEA-Cl, 2 EGTA, 2 MgATP, 10 HEPES, 10 Na2-phosphocreatine (pH 7.3, 295 mOsm) and pipettes were typically 6–9 MΩ. Capacitance compensation was corrected online and series resistance was corrected online or offline. Conductance measurements typically began around 30 min after break-in to allow the internal solution of the pipette to dialyse the cell, eliminating action potentials and depolarizing the resting membrane potential as expected with the use of Cs+ and QX-314.

Connectivity mapping. Connectivity mapping (STOMP M) was performed on a custom-built microscope based on previously published designs. A digital light processing projector (X600, Optoma) with its colour wheel removed was inserted into the agarose or muscle. Recordings were performed by inserting a pipette through an agarose-filled craniotomy or by using a glass cover slip with a hole drilled for pipette access. A silver–silver chloride reference electrode was inserted into the agarose or muscle. Recordings were made in current clamp mode and current pulses delivered by custom Labview software.

For measurements of membrane potential tuning, spike tuning, effects of optogenetic inhibition and connectivity mapping, pipettes of 5–8 MΩ resistance were pulled using borosilicate glass (King Precision Glass) and filled with an intracellular solution containing (in mM) 135 K gluconate, 4 KCl, 10 HEPES, 10 Na2-phosphocreatine, 4 Mg-ATP, 0.3 Na3-GTP, 0–1 Alexa 594 or 488, pH 7.2, 295 mOsm. Neurons were recorded from layer 2/3 (100 to 800 µm below the pia) using a Multiclamp 700B (Molecular Devices). Series resistance and pipette capacitance were corrected online. Series resistance for recordings typically ranged from 20 to 80 MΩ. Analogue signals were digitized using Spike2 (CED). For optogenetic inactivation experiments, a fibre (1 mm, Na., 63) coupled to a 455 nm LED light source (Pricimatix) was lowered to ~3–5 mm above the cortical surface. Power density at the cortical surface ranged from 1 to 4 mW/mm2. Optogenetic stimulation either coincided with visual stimulation, or began with a brief ramp (100 to 300 ms) before visual stimulation was turned off.

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Connectivity mapping. Connectivity mapping (STOMP M) was performed on a custom-built microscope based on previously published designs. A digital light processing projector (X600, Optoma) with its colour wheel removed was mounted to a tilt platform (Siskiyou) and linear stage (Thorlabs). A 50 mm f/1.4 SLR lens (Nikon) was mounted as close as possible to the projector and coupled to an achromatic doublet (AC508-150-A). Light passed through a blue dichroic filter (52–532, Edmund Optics) and was reflected on the sample using a dichroic mirror (T495LPXR, Chroma), and focused onto the sample using a 35 mm f/2.0 SLR lens (Nikon). Emission light passed through a 105 mm f2.0 lens (Nikon) and was focused onto the sample using a 50 mm f/1.4 SLM fluorescence microscope (Nikon) controlled by Micromanager (http://www.micro-manager.org). Single pixels on the DMD corresponded to ~4 µm at the sample. Diffuse background light was <0.1 mW/mm². Opsin was restricted to the soma using the Kv2.1 targeting motif. Before obtaining whole-cell recordings, we focused excitation light on the cortical surface. Upon break-in, we first measured the direction tuning of the cell using a grating protocol. Then, we centred a stimulation grid on the pipette and delivered 25–50 trials of random grid stimulation. Spots were typically 100–200 µm full width at half-maximum (FWHM), ~1–3 mW power, and displayed for 100 ms. We used positive current injection to depolarize the cell and increase the driving force for IPSPs (reversal potential ~75–70 mV, Extended Data Fig. 9). We probably underestimated input field sizes owing to limitations in the spatial spread of virus injection, light blue absorption in blood vessels and experimental geometry in which the large patch pipette interferes with light stimulation.

Intrinsic signal imaging. Intrinsic signal imaging was performed on the STOMP M microscope or on the Thorlabs Bergamo II. The cortex was illuminated with blue light to obtain a blood vessel map, after which collimated 630 nm light from an LED (Thorlabs) was directed onto the surface of the brain to measure intrinsic haemodynamic responses. Visually evoked responses were collected at ~50 Hz using an Andor Xyla camera. Visual stimuli were broadband grating stimuli (8 s on, 8 s off, 0.06–0.1 cycles per degree, 16 directions).

Fixation and immunostaining. Upon completion of imaging, isoﬂurane was raised to 5% and 0.5 ml Ethalosin given IV. The animal was transcardially perfused with 100 ml of 0.9% NaCl (w/v) and then 500 ml of 4% paraformaldehyde in 0.1 M phosphate buffer (PB), blocked in phospho-saline (PBS), blocked in blocking solution containing 1% BSA, 2% normal goat serum, and 0.3% Triton X-100 in PBS for 1 h, and then incubated in mouse anti-FlagM2 at 1:5000 (Sigma cat# F1804) overnight at room temperature. After three washes in buffer, 10 min each, the sections were
incubated in Alexa goat anti-mouse 488 at 1:500 (Thermo Fisher, cat# A32723) for 2 h at room temperature. After incubation, sections were washed twice in 0.1M PBS for 10 min each, followed by one wash in 0.1M PB. Sections were then mounted on Superfrost Plus slides (VWR, West Chester, PA) and coverslipped with SlowFade Gold (Thermo Fisher cat# S36936). Control slides were treated without the primary antibody. These control sections showed no labelling. To test the specificity of secondary antibodies, the secondary antibody was applied to the tissue without a primary antibody; no staining was observed in these controls.

Analysis. Calcium imaging. Imaging data were excluded from analysis if motion along the z-axis was detected. Dendrite images were corrected for in-plane motion via a 2D cross-correlation based approach in MATLAB. Axon images were corrected for in-plane motion using a piecewise non-rigid motion correction algorithm. ROIs were drawn in ImageJ; dendritic ROIs spanned contiguous dendritic segments and bouton/spine ROIs were circular. Mean pixel values for single ROIs were computed over the imaging time series and imported into MATLAB via MFI. \( \Delta F/F_0 \) was computed by defining \( F_0 \) using a 60 s percentile filter (typically 10th percentile), which was then low-pass filtered at 0.01 Hz. Bouton and somatic responses were computed as the average response to the visual stimulus and were included for analysis of direction selectivity if \( \Delta F/F_0 \) exceeded 10% and I–CV was \( >0.1 \). \( \Delta F/F_0 \) traces were median filtered with a 3-sample window. For spine signals, we subtracted a scaled version of the dendritic signal to remove backpropagating action potentials as previously performed [11, 14]. \( \Delta F/F_0 \) traces were synchronized to stimulus triggers sent from Psychopy and collected by Spike2. Spines were included for analysis if the average response exceeded 2 median absolute deviations above the baseline noise (measured during the blank) and were weakly correlated with the dendritic signal (Spearman's correlation, \( r = 0.3 \)). Some spine traces contained negative events after subtraction, so we excluded negative \( \Delta F/F \) values when computing Spearman's correlation between the spine and the dendrite. Because the amplitude of NMDA receptor mediated calcium transients are not necessarily correlated with EPSP amplitude at the soma [44], we normalized each spine's responses so that each spine had equal weight when computing summed spine inputs. Summed spine inputs were computed as the average spine response to each stimulus, bootstrapped 100 times. We also compared tuning of populations of spine inputs to somatic output by including response amplitude in the calculation and by computing the fraction of spines that preferentially respond to the preferred direction. DSI was computed as:

\[
\text{Preferred – Null} = \frac{\text{Preferred}}{\text{Null}}
\]

Whole-cell recording. Membrane potential recordings were median filtered with a 30 to 100 sample window to remove action potentials and binned to 5 ms. Responses to individual stimulus cycles were extracted for \( V_m \) and spikes separately. Mean (F0) and modulation amplitudes (F1 and F2) of each cycle were computed via Fast Fourier Transform (MATLAB). \( V_m \) and spiking peak responses were computed as previously described [12]. Some cells exhibited \( V_m \) modulation at F2, so we also included the F2 component when computing \( V_m \) responses. For computing \( V_m \) noise, we aligned cycle responses across trials, then took the standard deviation for each time point. \( V_m \) standard deviation was computed as the mean of this standard deviation value for each stimulus.

Conductance measurements were made in current-clamp mode [11, 14]. Multiple current steps depolarized or hyperpolarized the neuron close to the reversal potential for inhibition and excitation, respectively. Leak-subtracted synaptic conductances were computed by estimating \( G_{\text{leak}} \) using the blank stimulus and then performing a linear fit of measured membrane potential responses at different current injections. Mean and standard deviation of synaptic conductances were computed with a bootstrap (100 iterations). Cells were excluded from further analysis if negative conductances were extracted across multiple stimuli. To predict membrane potential responses from empirically measured synaptic conductances, we computed stimulus-dependent responses as previously described [22],

\[
\Delta V(\theta) = \frac{g_{R_1}(\theta) R_1 + g(\theta) R_2}{g_{R_1}(\theta) + g(\theta)} - V_r
\]

in which \( R_1 \) is ~50 mV, \( V_r \) is ~50 mV, \( R_2 \) is 0 mV, \( R_1 \) is ~70 mV, \( \theta \) is the direction of motion, \( g(\theta) \) is the measured leak conductance, and \( g(\theta) \) and \( g(\theta) \) are measured synaptic conductances.

Optogenetic stimulation experiments compared visually evoked subthreshold responses under blue light stimulation with the responses obtained without blue light stimulation. Modulation ratio was computed as the response amplitude with blue light on divided by the response amplitude with blue light off.

For connectivity mapping, membrane potential traces were median filtered with a time window of 1.2 ms. We defined the prestimulus membrane potential as the membrane potential in the 9 ms before IPSP onset. Because of spontaneous activity in vivo, single trials were excluded if cells showed large depolarizations (>5 mV) relative to the prestimulus membrane potential. Significant IPSPs were defined as IPSPs exceeding three standard deviations below the mean of the prestimulus membrane potential. We used the centroid of the significant IPSP field for distance measurements from single cells. Ellipse fits of the binarized significant IPSP array were computed using the MATLAB function ‘regionprops.m’.

Intrinsic signal imaging. Single-condition maps were computed by comparing whether reflectance changes evoked by a single-stimulus condition could be discriminated from reflectance changes evoked across all presented stimuli [23]. To discriminate a single-condition stimulus at each pixel, reflectance changes across all stimuli were combined into a normalized histogram, and then a pixel’s single condition response was computed non-parametrically as the probability of the area under a ROC curve (using the trapezoidal rule). Maps were filtered as previously described using a bandpass filter [22]. Single bouton direction preferences were compared to the direction preference of the intrinsic signal direction preference map contained within the 100 µm two photon field of view. Somatic direction preference was compared to the direction preference of the intrinsic map at the location of the cell. For STOMPM, stimulation grids were aligned to blood vessel reference maps for intrinsic signal imaging using an affine transform. We computed binary masks for each stimulation spot, and used these masks to measure intrinsic signal direction preference at single stimulation spots.

Statistics. Sample sizes are similar to others used in the field. No statistical methods were used to predetermine sample size. Inclusion criteria for each experiment are detailed for methods. The experiments were not randomized. The experimenter was blind to location in the direction preference map when performing map-related experiments; otherwise, the investigators were not blinded to allocation during experiments and outcome assessment. To test whether two distributions of direction preference were significantly different from random, we compared the median difference with a null distribution generated from Monte Carlo simulations (\( n = 1,000 \)). For each Monte Carlo simulation, we calculated the median difference between two randomly sampled distributions of direction preferences drawn from a uniform distribution ranging from 0° to 359° with sample sizes equivalent to the measured distributions. Statistical tests were non-parametric and two-sided, except for the Monte Carlo significance tests, which were one-sided. All correlations values reported were computed using Spearman’s correlation.

Code availability. Analyses were performed using MATLAB using standard functions. Custom code is available from the corresponding author upon reasonable request.

Data availability. Source data are provided for graphical data representations in Figs. 1d–f, 2c–g, 3c–f, h–j, and 4f, k, m, n. Data are available from the corresponding author upon reasonable request.

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Extended Data Fig. 1 | Summed spine inputs fail to predict somatic direction selectivity, regardless of the method used to compute the sum. a, No significant correlation between the DSI of summed spine inputs (with amplitude included) and somatic DSI. Spearman’s $r = -0.11$, $P = 0.68$, $n = 17$. b, No significant correlation between the fraction of spines that respond more strongly to the preferred direction and somatic DSI. Spearman’s $r = -0.082$, $P = 0.75$, $n = 17$. 
Extended Data Fig. 2 | Distribution of spiking DSI. Dashed line indicates cutoff of DSI > 0.3; \( n = 69 \) cells with spiking responses.
Extended Data Fig. 3 | Example of noise suppression at null stimulus relative to blank. Figure shows responses to preferred, null and blank.
Extended Data Fig. 4 | Direction tuning fits for excitatory and inhibitory conductances. a, Difference in direction preference of excitation and inhibition are significantly greater than chance; Monte Carlo significance test, \( P = 0.023 \); difference in direction preference, \( 135° ± 95° \), median ± IQR, \( n = 10 \) cells from 7 animals. b, FWHM of excitation and inhibition were not significantly different. FWHM 61° ± 46° and 61° ± 110° for excitation and inhibition, respectively; median ± IQR, \( n = 10 \), Wilcoxon sign-rank \( P = 0.70 \). c, Individual (grey) and population average (coloured) tuning curves for \( G_e \), \( G_i \) and predicted \( V_m \), peak-aligned to excitation.
Extended Data Fig. 5 | $I/E$ ratio at preferred direction is not correlated with simulated subthreshold direction selectivity. Spearman's $r = 0.0061$, $P = 1$, $n = 10$ cells from 7 animals.
Extended Data Fig. 6 | Putative GABAergic neuron directly suppressed by blue light. Error bars, mean ± s.e.m.
Extended Data Fig. 7 | Additional data related to blue light photoinhibition of GABAergic neurons. a, Optogenetic suppression of GABAergic neurons significantly reduces spiking direction selectivity; Wilcoxon sign-rank, n = 14 cells with spiking responses, P = 0.0049. Black line, mean; grey lines, single cells. b, Absolute V_m depolarization induced by blue light is not related to optogenetic changes in V_m direction selectivity (computed as the difference in DSI between light off and light on conditions); Spearman’s r = 0.11, P = 0.70, n = 14 cells with spiking responses from 4 animals.
Extended Data Fig. 8 | Alignment of GABAergic neurons with intrinsic signal polar direction map. 

**a**, Underlying intrinsic signal polar direction map with direction-tuned GABAergic neurons overlaid. **b**, Direction preferences of inhibitory neurons and intrinsic signal direction preference map are significantly more similar than chance; $P < 0.001$, Monte Carlo significance test, $n = 76$ direction-selective neurons from 3 planes in 1 animal.
Extended Data Fig. 9 | Reversal potential of optogenetically evoked PSPs is consistent with inhibition. Grey points are individual data points; black is mean ± s.e.m. Data come from individual stimulation trials from one cell.
Extended Data Fig. 10 | Relationship of IPSP amplitude and distance. Grey points are individual data points; black is binned mean ± s.e.m. Data come from trial-averaged stimulation responses from $n = 21$ cells from 7 animals.
Reporting Summary

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Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. figure legend, table legend, main text, or Methods section).

| Item                                                                 | Confirmed |
|----------------------------------------------------------------------|-----------|
| n/a                                                                  | Confirmed |
| The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement |           |
| An indication of whether measurements were taken from distinct samples or whether the same sample was measured repeatedly |           |
| The statistical test(s) used AND whether they are one- or two-sided |           |
| Only common tests should be described solely by name; describe more complex techniques in the Methods section. |           |
| A description of all covariates tested |           |
| A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons |           |
| A full description of the statistics including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals) |           |
| For null hypothesis testing, the test statistic (e.g. F, t, r) with confidence intervals, effect sizes, degrees of freedom and P value noted |           |
| Give P values as exact values whenever suitable. |           |
| For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings |           |
| For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes |           |
| Estimates of effect sizes (e.g. Cohen's d, Pearson's r), indicating how they were calculated |           |
| Clearly defined error bars |           |
| State explicitly what error bars represent (e.g. SD, SE, CI) |           |

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

| Data collection | Scanimage, Spike2, Micromanager, Psychopy, custom Labview code |
|-----------------|----------------------------------------------------------------|
| Data analysis   | Analysis code was written in Matlab                            |

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

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For a reference copy of the document with all sections, see nature.com/authors/policies/ReportingSummary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

| Sample size | No statistical methods were used to pre-determine sample sizes. Sample sizes are consistent with comparable studies in the field. |
|-------------|------------------------------------------------------------------------------------------------------------------|
| Data exclusions | Data exclusions are described in the text/methods. |
| Replication | All attempts at replication were successful. |
| Randomization | No randomization was applied. |
| Blinding | The only blinding applied was with that the experimenter was blind to location in the intrinsic signal direction preference map while imaging or performing electrophysiology experiments. |

Reporting for specific materials, systems and methods

| Materials & experimental systems | Methods |
|---------------------------------|---------|
| n/a | n/a |
| ☒ | ☐ | Involved in the study |
| ☐ | ☒ | Involved in the study |
| ☒ | ☐ | Antibodies |
| ☒ | ☐ | Antibodies |
| ☐ | ☒ | Eukaryotic cell lines |
| ☒ | ☐ | Palaeontology |
| ☒ | ☐ | Animals and other organisms |
| ☐ | ☒ | Human research participants |
| ☒ | ☐ | Flow cytometry |
| ☒ | ☐ | MRI-based neuroimaging |

Antibodies

Antibodies used | FLAG antibody (Sigma F1804)

Validation | This commercially available antibody is listed under RRID 262044. The manufacturer validated the antibody by Western dot and dot blot. IHC validation was performed in the lab by testing various concentrations on ferret tissue. Negative controls were performed by adding secondary antibody to tissue not treated with primary antibody.

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals | Female ferrets (Marshall Farms) were typically 4-5 weeks old at virus injection and P50+ at the time of terminal experiment.

Wild animals | The study did not involve wild animals.

Field-collected samples | The study did not involve field-collected samples.