Neuromodulatory control of localized dendritic spiking in critical period cortex

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Sensory experience in early postnatal life, during so-called critical periods, restructures neural circuitry to enhance information processing1. Why the cortex is susceptible to sensory instruction in early life and why this susceptibility wanes with age are unclear. Here we define a developmentally restricted engagement of inhibitory circuitry that shapes localized dendritic activity and is needed for vision to drive the emergence of binocular visual responses in the mouse primary visual cortex. We find that at the peak of the critical period for binocular plasticity, acetylcholine released from the basal forebrain during periods of heightened arousal directly excites somatostatin (SST)-expressing interneurons. Their inhibition of pyramidal cell dendrites and of fast-spiking, parvalbumin-expressing interneurons enhances branch-specific dendritic responses and somatic spike rates within pyramidal cells. By adulthood, this cholinergic sensitivity is lost, and compartmentalized dendritic responses are absent but can be re-instated by optogenetic activation of SST cells. Conversely, suppressing SST cell activity during the critical period prevents the normal development of binocular receptive fields by impairing the maturation of ipsilateral eye inputs. This transient cholinergic modulation of SST cells, therefore, seems to orchestrate two features of neural plasticity—somatic disinhibition and compartmentalized dendritic spiking. Loss of this modulation may contribute to critical period closure.

An important and unanswered question is what distinguishes the engagement of plasticity during critical periods of early postnatal development from that in the adult cortex. In the adult cortex, the necessary components of plasticity include attention and/or reinforcement2, disinhibition of pyramidal cell bodies3,4, and various forms of dendritic potentiation, including localized dendritic spiking5–7. Less is understood about the engagement of plasticity in the developing cortex, where sensory experience exerts an extremely robust and permanent influence on cortical circuitry. Neuromodulation and inhibition are key players in this plasticity8–10, but their joint influence on dendritic integration and somatic firing in pyramidal cells is not known. Dendritic compartmentalization is particularly relevant to the establishment of binocular receptive fields in the primary visual cortex, which depends on a strengthening of initially weak, subthreshold ipsilateral eye input11,12 and the matching of their receptive field tuning properties to the contralateral eye13. Local dendritic spiking would enhance this process, driving the functional clustering of synaptic inputs14,15 as well as the potentiation of weak, but coactive inputs16–18. To obtain a more informed understanding of how vision drives plasticity during critical periods and why this influence is lost with age, we investigated the modulation of pyramidal neurons and the three major types of inhibitory neurons in the primary visual cortex: dendrite-targeting SST cells; soma-targeting parvalbumin-expressing cells; and cells expressing vasoactive intestinal peptide (VIP)19. We gauged cell-type-specific changes in activity as a function of neuromodulation by imaging spontaneous and visually evoked changes in the fluorescence of the genetically encoded calcium indicator GCaMP6 via resonant scanning two-photon microscopy. These measures were made in alert, head-fixed mice running or resting on a spherical treadmill. Measurements were taken at two developmental ages—4 weeks of age (postnatal day (P)28), and 8 weeks of age (P56). P28 is the age of greatest sensitivity to the instructive influence of vision, and P56 is well beyond critical period closure20, in addition to being a commonly used age of study for adult mice.

At P28, the spontaneous activities of SST cells increased during periods of locomotion, but by P56 this positive correlation was significantly reduced (Fig. 1a, b). Visually evoked responses of SST cells followed a similar trend. At P28, the median change in fluorescence (ΔF/F) of the visually evoked ultrasensitive GCaMP6s sensor was larger during locomotion than at rest, and by P56 this state-dependence was absent (Fig. 1c, d, Extended Data Fig. 1). These measures suggest that there is an age-dependent loss in the sensitivity of SST cells to neuromodulators released into the cortex during running, because the reticular activating system is engaged during locomotion21,22. We tested this hypothesis in acute cortical slices by measuring the evoked firing rates of SST cells in layer 2/3 of the primary visual cortex to the cholinergic agonist carbachol (CCh). Supporting earlier work23–25, we found that P28 SST cells responded robustly to bath application of 2 mM CCh, when synaptic signalling of local excitatory and inhibitory neurons was blocked. Notably, this direct cholinergic response was not present at P56, despite unchanged intrinsic properties (Fig. 1e, f, Extended Data Fig. 2). Expanding on these results in vivo, we found that optogenetic stimulation of cholinergic cells in the basal forebrain drove time-locked GCaMP6s responses in SST cells in the visual cortex of P28, but not P56 mice (Fig. 1g–j). Collectively, these measures indicate that at the peak of the critical period—and not in adulthood—SST cell responses are enhanced by acetylcholine released from basal forebrain cortical projections.

Because SST cells primarily receive inhibition from VIP cells26,27, we also measured VIP interneuron activity at both developmental time points. No age-dependent changes in the influence of behavioural state or sensitivity to CCh were observed on VIP cell responses (Extended Data Fig. 3), consistent with previous findings23,25,26. Presumptive VIP input to SST cells also appeared to be constant with age: in acute slices of binocular visual cortex, CCh-mediated inhibitory currents were evident in SST cells at both ages with similar amplitudes (Extended Data Fig. 4).

SST interneurons send inhibitory input to all other cell types, including fast-spiking parvalbumin cells26,29. When we examined the spontaneous responses of parvalbumin cells, we found no influence of locomotive state in P28 mice; by P56, however, parvalbumin responses increased when mice ran (Fig. 2a, b). Similarly, running enhanced visually evoked parvalbumin cell responses at P56, but not at P28 (Fig. 2c, d). This increase at P56 is unlikely to be caused by direct actions of acetylcholine on parvalbumin cells, as whole-cell recordings from parvalbumin cells in acute cortical slices showed no direct responses to CCh at either P28 or P56 (Fig. 2e, f). This is consistent with previous studies, which show that the modulation of parvalbumin responses is
Fig. 1 | SST cells lose cholinergic sensitivity after critical period closure. a. Heat map time series of z-scored GCaMP6s spontaneous responses of SST cells recorded in a P28 (left) and a P56 (right) mouse. Scale bar, −1 to 5. b. Cumulative distribution of correlation coefficients to running for all SST cells recorded in all mice at P28 (orange) and P56 (grey) (P28: n = 200 cells, 6 mice, 6 fields of view; P56: n = 100 cells, 7 mice, 7 fields of view). P = 5.73 × 10−7, Mann–Whitney U-test. c. As in a, but for visually evoked SST responses to non-repeating natural movie scenes (see Methods). Grey bars indicate times when visual stimulation occurred. Scale bar, −1 to 4. d. Plot of the visually evoked median ΔF/F of each cell as a function of behavioural state, for all recorded cells (P28: n = 94 cells, 4 mice, 4 fields of view; P56: n = 102 cells, 5 mice, 5 fields of view). P28 run to P56 run, P = 4.63 × 10−6, Mann–Whitney U-test. e. Left, schematic of whole-cell current clamp recordings of SST cell responses to bath application of carbacoch (CCh) in the presence of synaptic blockers (6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) and GABAA antagonist GABAzine). Middle, example of responses recorded from a P28 mouse; scale bar, 20 mV and 10 s. Inset shows evoked SST waveform; scale bar, 10 mV and 0.5 s. Right, example from a P56 mouse.

Inset shows a healthy response to current injection despite lack of response to CCh; scale bar, 100 ms and 20 mV. In both plots, the onset of CCh application is denoted by the yellow square. f. Box plot of SST cell firing rates evoked by CCh as a function of age (P28: n = 11 cells, 5 mice, 11 slices; P56: n = 9 cells, 4 mice, 9 slices). ***P = 2.90 × 10−4, Mann–Whitney U-test. For these plots, the red central mark denotes the median and the outer edges correspond to the top and bottom percentiles, with whiskers extending to 2σ. g. Schematic of in vivo optical activation of the nucleus basalis with concurrent imaging in V1. V1 SST cells labelled with GCaMP6s (green) and visible ChR2+ (red) buttons from the nucleus basalis. Scale bar, 60 μm. h. Representative SST cell activity after nucleus basalis stimulation (blue) in a P28 and a P56 mouse. The median response is shown in red (P28: n = 40 cells, 1 mouse, 1 field of view; P56: n = 67 cells, 1 mouse, 1 field of view). Scale bar, 20% ΔF/F and 1 s. i. Box plot of median percentage change in ΔF/F for SST cells after nucleus basalis stimulation (P28: n = 101 cells, 3 mice, 3 fields of view; P56: n = 345 cells, 4 mice, 4 fields of view). ***P = 2.89 × 10−3, Mann–Whitney U-test. Box plot parameters as described in f.

Fig. 2 | Inhibitory drive onto parvalbumin cells weakens after critical period closure. a. Representative heat map time series of z-scored spontaneous parvalbumin cell responses from P28 (left) and P56 (right) mice. Scale bar, −1 to 5. b. Cumulative distribution of correlation coefficients to running for all recorded parvalbumin cells (P28: blue: n = 73 cells, 4 mice, 4 fields of view; P56: grey: n = 103 cells, 4 mice, 4 fields of view). P = 3.91 × 10−4, Mann–Whitney U-test. c. As in a, but showing parvalbumin cell responses to non-repeating natural movie scenes. Scale bar, −1 to 4. d. Plot of the visually evoked median ΔF/F of each cell as a function of behavioural state, for all recorded cells (P28: blue: n = 101 cells, 5 mice, 5 fields of view; P56: grey: n = 162 cells, 5 mice, 5 fields of view). P28 run to P56 run, P = 8.82 × 10−5. Mann–Whitney U-test. e. Example traces of parvalbumin cell response to CCh application at P28 (top) and P56 (bottom). Recordings made under current clamp with synaptic blockers. Coloured box denotes time of CCh application. Scale bars, 10 mV and 10 s. f. Plot of CCh-evoked responses for all parvalbumin cells recorded in slices taken from P28 and P56 mice (P28: n = 8 cells, 4 mice, 8 slices; P56: n = 9 cells, 3 mice, 9 slices). P = 0.8346, Mann–Whitney U-test. g. Examples of voltage-clamped whole-cell recordings of parvalbumin cell responses to bath application of CCh at P28 (top) or P56 (bottom). Scale bar, 50 pA and 10 s. h. Plot of median inhibitory postsynaptic current (IPSC) amplitude evoked by CCh application for all recorded parvalbumin cells at P28 (P28: n = 40 cells, 3 mice, 3 slices; P56: n = 9 cells, 4 mice, 9 slices). **P = 0.0012, Mann–Whitney U-test.
indirect. Supporting this view, CCh induced large GABA (γ-aminobutyric acid)-mediated inhibitory currents in parvalbumin cells at P28 but not at P56 (Fig. 2g, h). These measures indicate that cholinergic action on SST and VIP cells drives a strong inhibition of parvalbumin cells at P28, but by P56, this inhibition is lessened—a developmental shift that is opposite to what we found in SST cells.

Given these reciprocal differences of the most prominent sources of inhibition to pyramidal neurons, an intriguing question is whether arousal state differently affects visually evoked dendritic and somatic responses in the critical period versus adult mice. In the adult cortex, branch-specific dendritic activity arises during active learning and decision-making, and these localized responses facilitate

P56: n = 48 branch pairs), ***P = 0.0104 (P28), P = 0.05 (P56), Wilcoxon signed-rank test. f. Example responses to visual stimulation from a pyramidal cell soma at P28 (left) and P56 (right). For each age, three time-series plots are shown: locomotion is plotted at the top, the z-scored GCaMP6s response in the middle, and the temporally deconvolved spike probability at the bottom. Scale bars, 20 s and 2 s.d. (for GCaMP6s signals). Deconvolved spike probability scale bar, 1 AU. g. Plot of cumulative distributions of the correlation coefficients of GCaMP6s signals to running for all recorded pyramidal neurons (P28, green: n = 1,064 cells, 6 mice, 9 fields of view; P56, grey: n = 778 cells, 8 mice, 13 fields of view). P = 5.03 × 10^{-22} (P56), Wilcoxon signed-rank test. h. Histogram of median spike probability per second measured during running in P28 (green) and P56 (grey) mice (P28: n = 1,105 cells, 6 mice, 9 fields of view; P56: n = 821 cells, 8 mice, 13 fields of view). P = 0.0114, Mann–Whitney U-test.

Control still to run, P = 0.9535; stimulation still to run, ***P = 0.0044, Wilcoxon signed-rank test. e. As in a, with the exception that pyramidal cell somas are recorded. f. Representative time series heat map of pyramidal cell responses in a P56 mouse with optogenetic stimulation of SST cells. The z-scores from individual cells are plotted per frame (15.5 frames per second (fps)); colour scale is –1 to 5 z-scores. Top, black trace denotes locomotion; blue bars denote optogenetic stimulation. g. Box plots of somatic spike probabilities as a function of behavioural state in P56 mice before or during optogenetic SST cell stimulation (n = 79 cells, 5 mice, 11 fields of view). Control still to run, ***P = 1.14 × 10^{-11}, stimulation still to run, ***P = 1.10 × 10^{-11}, stimulation run to control run, ***P = 6.00 × 10^{-3}, Wilcoxon signed-rank test with post hoc Bonferroni correction.
compartmentalized synaptic plasticity. With this in mind, we measured visually evoked dendritic Ca$^{2+}$ responses (using the fastest sensor GCaMP6f) along the apical dendrites of layer 2/3 pyramidal neurons from both age groups. We recorded from sister dendrites, in which a bifurcation from a parent dendrite was identifiable within the imaging plane. Calcium signals were extracted from dendritic branches and temporally deconvolved, giving a measure proportional to spike rate in arbitrary unitsCNO, but for ipsilateral responses in control groups (controls, n = 283 cells, DREADD, n = 226 cells, 4 mice, 8 fields of view). P = 0.0325, Mann–Whitney U-test. c. Box plots of the inferred firing rate of contralateral responses in control or experimental groups (controls, n = 283 cells, DREADD, n = 226 cells). *P = 0.0145, Mann–Whitney U-test. d. As in a, but for ipsilateral responses. e. As in b, but for ipsilateral receptive fields for either control or experimental groups (controls, n = 283 cells, DREADD, n = 226 cells). P = 0.0234, Mann–Whitney U-test. f. As in c, but for ipsilateral responses in control or experimental groups (controls, n = 283 cells, DREADD, n = 226 cells). ***P = 1.1637 × 10^{-4}, Mann–Whitney U-test. g. Example binocular neurons showing matched (left) or unmatched (right) receptive fields. Plots are individually normalized to show the maximum response in the spatial frequency (cycles per degree) and orientation (degrees) domains. Discrepancy in preferred orientation in degrees (Δθ) is noted. h. Histograms of probability distribution of Δθ in binocular neurons in control (top) and experimental (bottom) groups (controls, n = 283 cells, 5 mice, 9 fields of view; DREADD + CNO, n = 226 cells, 4 mice, 8 fields of view). P = 2.67 × 10^{-5}, Mann–Whitney U-test.

branch-specific dendritic inhibition and somatic disinhibition—two hallmarks of cortical plasticity3–5,57. The presence of local calcium activity within functionally distinct dendritic compartments and enhanced somatic spiking may be the substrate through which sensory experience engages plasticity in the very young cortex. The engagement of these components is distinct from the adult cortex, in which these properties emerge during learning and decision-making5,56—states that involve attention and reinforcement.

Notably, direct stimulation of SST cells in the adult cortex was not sufficient to drive an increase in branch-specific dendritic activity and somatic spiking. These events only occurred when SST stimulation was concurrent with running. One possibility is that acetylcholine acts in parallel on SST cells and pyramidal cells to evoke compartmentalized dendritic plasticity in the developing cortex. Local dendritic activity, back-propagations, and acetylcholine combined have powerful
implications for inducing plasticity within specific dendritic compartments and could strengthen initially weak inputs in the developing cortex. The loss of the cholinergic co-activation of excitatory cells and SST-mediated inhibition would shift plasticity rules, in which inhibition becomes inverted along the somatodendritic axis, and may have a role in the closure of the critical period.

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Competing interests J.T.T. is a co-owner of Neurolabware LLC.

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**METHODS**

**Animals.** All procedures were done in compliance with the Office of Animal Research Oversight, the Institutional Animal Care and Use Committee, at the University of California, Los Angeles. Cre lines were used to selectively express GCaMP6p in specific cell types. The following mouse lines were used from Jackson Laboratories: SST-IRESCre (stock no. 018973), PV-Cre (stock no. 08069), VIP-IRESCre (stock no. 010908), and ChAT-Cre (stock no. 006410). For optical identification of interneurons, mice were crossed with Ai9—expressing mice (stock no. 007905). All mice were heterozygous for their respective transgenes, and both male and female mice were used. Mice were group housed under a normal 12 h light/dark cycle. The experiments were not randomized, and investigators were not blinded to allocation during experiments and outcome assessment.

**Cranial window surgeries.** All two-photon imaging was carried out through a cranial window. For all ages, surgical preparation included administration of anti-inflammatory carprofen, the affixation of an aluminium headbar to the skull, the removal of bone over the primary visual cortex, injection of viral vectors, and the placement of a glass coverslip over the exposed region. Body temperature was maintained at 37 °C. Under isoflurane anaesthesia, the scalp was retracted and Vetbond was used to coat the skull surface and the edges of the exposed area, followed by a layer of black dental acrylic. The aluminium headbar was affixed with dental acrylic to this surface, caudal to the area of interest over V1. A 3-mm diameter area over V1 (centred at 1.5 mm from bregma and 3 mm from the midline) was removed using a high-speed drill. After virus injection, the craniotomy was sealed with a coverglass using Vetbond to create an imaging window. All remaining exposed regions of skull and surgery margins were sealed with Vetbond and acrylic.

For experiments that were free to move on a spherical treadmill, animals were given a 1-week period or more for recovery before imaging. For adult mice, injections and surgeries were performed altogether, 2 weeks before imaging at P56. For imaging at P28, mice were injected with virus via a small burrhole over V1 at P14, with the headbar and craniotomy surgeries performed at P21.

**Virus injections.** For calcium imaging, either CaMKII-GCaMP6p or flex-GCaMP6s (UPenn Vector Core: AAV1.CamKII.GCaMP6p.WPRE.SV40, number AV-1-PV3435, and AAV1.Syn.Flex.GCaMP6s.WPRE.SV40, number AV-1-PV2821) were expressed in cortical neurons (titre: 10^12 genomes per ml). For all age groups, virus was injected 2 weeks before imaging. Virus injection was done using a glass micropipette and a PicoSprizer III (Parker) (15 p.s.i., 10 ms pulses, 3 s between pulses). For imaging Cre-dependent interneuron classes, virus containing flex-GCaMP6s was used, injected at a beginning depth of 350 μm and moving up every 50 μm, with the last injection depth being 100 μm below the pial surface, for a total of approximately 1.5 μl of injected virus across cortical layer 2/3. For imaging pyramidal cell dendrites, a viral vector containing CaMKII-GCaMP6p was used, and approximately 50 nl of undiluted virus was injected at a depth of 300 μm from the cortical surface in the centre of V1. The low volume for these experiments was beneficial to achieving optimal sparsity for observing pyramidal cell morphology. In some experiments, the P28 and P56 animals were injected and also in neurons expressing ChAT, an enzyme responsible for the synthesis of acetylcholine. In each mouse, a local injection of flex-ChR2 was made into nucleus basalis, which was measured by a power meter (Thorlabs). LED triggering was synced to laser scanning through Scanbox, and activation of the LED silenced a gated photomultiplier (Hamamatsu) before LED light delivery, so that light from the LED was not collected and the integrity of the PMT was preserved. Blue light was delivered in 10-ms pulses, repeated 25 times at 15 Hz. Stimulation was done during both periods of stillness and movement, and when done within the same imaging session, at least 30 s was left between stimulation periods.

To selectively stimulate choline acetyltransferase (ChAT)-positive neurons and axons from the nucleus basalis, mice were generated to express Cre in SST cells and also in neurons expressing ChAT, an enzyme responsible for the synthesis of acetylcholine. In each mouse, a local injection of flex-ChR2 was made into nucleus basalis and, separately, flex-GCaMP6s was injected into visual cortex. Although neurons expressing ChAT or SST are present in both areas, SST neurons of the basal forebrain do not project to the cortex and, cortical ChAT-positive neurons make up less than 1% of all cortical interneurons. A fibre-optic cannula (Prizmatix) was implanted unilaterally 1–2 weeks before imaging, after virus injections. Cannulae were 4.3 mm long and were inserted at a 30° angle (2 mm rostral of injection site) to allow space for the object and were approximately 0.5 mm away from the nucleus basalis. Similar to full-field stimulus experiments in V1, blue light (approximately 460 nm) was administered in 10-ms pulses, 25 times at 15 Hz at 5–6 mW. To minimize any natural activation of the basal forebrain during these experiments, mice were trained to be motionless and observed a grey screen throughout recordings.

**DREADD manipulation of SST cells during development.** To suppress SST cell activity over a prolonged period during development, we used a Cre-dependent inhibitory hM4Di-DREADD receptor in SST-Cre mice. To initially validate DREADD-mediated suppression of SST interneurons, flex-GCaMP6s and DREADDs were co-expressed in SST cells. After the craniotomy and a period of recovery, evoked activity of SST cells was recorded using natural movie scenes. CNO (C8982, Sigma Aldrich) was dissolved in saline to make a 0.5 mg ml^-1 solution in saline. After 2 h of equilibration (DMSO) to prevent sedimentation. After dosing, neurons expressing ChAT or SST are present in both areas, SST neurons of the basal forebrain do not project to the cortex and, cortical ChAT-positive neurons make up less than 1% of all cortical interneurons. A fibre-optic cannula (Prizmatix) was implanted unilaterally 1–2 weeks before imaging, after virus injections. Cannulae were 4.3 mm long and were inserted at a 30° angle (2 mm rostral of injection site) to allow space for the object and were approximately 0.5 mm away from the nucleus basalis. Similar to full-field stimulus experiments in V1, blue light (approximately 460 nm) was administered in 10-ms pulses, 25 times at 15 Hz at 5–6 mW. To minimize any natural activation of the basal forebrain during these experiments, mice were trained to be motionless and observed a grey screen throughout recordings.

**DREADD manipulation of SST cells during development.** To suppress SST cell activity over a prolonged period during development, we used a Cre-dependent inhibitory hM4Di-DREADD receptor in SST-Cre mice. To initially validate DREADD-mediated suppression of SST interneurons, flex-GCaMP6s and DREADDs were co-expressed in SST cells. After the craniotomy and a period of recovery, evoked activity of SST cells was recorded using natural movie scenes. CNO (C8982, Sigma Aldrich) was dissolved in saline to make a 0.5 mg ml^-1 solution in saline. After 2 h of equilibration (DMSO) to prevent sedimentation. After dosing, neurons expressing ChAT or SST are present in both areas, SST neurons of the basal forebrain do not project to the cortex and, cortical ChAT-positive neurons make up less than 1% of all cortical interneurons. A fibre-optic cannula (Prizmatix) was implanted unilaterally 1–2 weeks before imaging, after virus injections. Cannulae were 4.3 mm long and were inserted at a 30° angle (2 mm rostral of injection site) to allow space for the object and were approximately 0.5 mm away from the nucleus basalis. Similar to full-field stimulus experiments in V1, blue light (approximately 460 nm) was administered in 10-ms pulses, 25 times at 15 Hz at 5–6 mW. To minimize any natural activation of the basal forebrain during these experiments, mice were trained to be motionless and observed a grey screen throughout recordings.
long the effect lasted. In a separate group, CNO was administered to mice without DREADD expression to determine any unexpected effects of clozapine on SST cells.

For experiments in which SST cell activity was suppressed for a period of days, hM4Di-DREADD receptors were expressed in SST cells concurrently with GCaMP6f in pyramidal neurons. This was accomplished by injecting flex-DREADD along with CaMKII-GCaMP6f in the binocular zone of primary visual cortex in SST-Cre mice at postnatal day 10 and allowing 2 weeks for expression. Craniotomies were performed on P21, and mice received CNO (2.5 mg kg\(^{-1}\)) every 8–12 h from P24–P27. Twelve or more hours from the last dose of CNO, the ipsilateral and contralateral inputs to binocular neurons were tested. Control groups, either expressing DREADD and receiving saline, or not expressing DREADD and receiving CNO, underwent the same procedure.

**Acute slice preparation.** Whole-cell recordings were done in acute slices from P28 and P56 mice. tdTomato-expressing mouse lines (SST-Cre/Ai9 mice; PV-Cre/Ai9; VIP-Cre/Ai9) were used for targeting specific interneuron subtypes. Animals were anaesthetized with isoflurane and perfused with ice-cold sucrose-based artificial cerebrospinal fluid (ACSF). Coronal slices (300-μm thick) from the visual cortex were cut on a vibratome in ice-cold sucrose-ACSF solution. Slices were incubated in regular ACSF for 30 min at 25 °C before recording at room temperature. Sucrose-ACSF was perfused with carbogen and contained (in mM): sucrose, 222; glucose, 11; NaHCO\(_3\), 26; NaH\(_2\)PO\(_4\), 1; KCl, 3; MgCl\(_2\), 7; and CaCl\(_2\), 0.5. Regular ACSF contained (in mM): sucrose, 4; MgCl\(_2\), 2; CaCl\(_2\), 2.5; and NaCl, 124. A low-chloride K-glucuronate intracellular pipette solution was used to record cells in current clamp mode, containing (in mM): K-glucuronate, 126; KCl, 4; HEPES, 10; ATP-Mg, 4; GTP-Na, 0.3; and Na-phosphocreatine, 10. pH 7.4, 300 mOsm. A high-chloride internal solution was used to record IPSCs in voltage-clamp mode, containing (in mM): KCl, 120; HEPES, 10; Mg-ATP, 4; GTP-Na, 0.3; and Na-phosphocreatine, 10; pH 7.4, 295 mOsm. Cholinergic responses were tested through bath application of the cholinergic agonist CCh, in which CCh was added in small amounts (0.3–0.5 μl of 2 mM). Excitatory and inhibitory synaptic activity were blocked using bath-applied CNQX (10 μM) and GABA\(_z\)ine (10 μM).

**Intracellular recording and analysis.** Cells in layer 2/3 were visualized with an Olympus BX61WI microscope coupled with a 40× water immersion lens (Olympus), infrared-DIC optics and CCD camera (Qimaging). Slices were screened for cell bodies containing tdTomato using a custom fluorescence filter. Glass pipettes (4–7 MΩ) were pulled with a Sutter Instruments P1000 puller. Data were collected and acquired with a MultiClamp 700B amplifier and a Digidata 1440A system (Molecular Devices), with WinWCP software (Strathclyde). For all cells, response to current steps, input resistance, and access resistance was measured before drug application and after washout (>30 min) to verify the health of each cell. Only cells without notable changes in current-step responses were used for further analysis (Extended Data Fig. 2). Firing rate and changes in membrane potential were analysed using Clampfit software.

**Statistics.** No statistical methods were used to predetermine sample size. All statistical analyses were done using non-parametric procedures in MATLAB. Significance levels were set to \(\alpha < 0.05\) for all two-group comparisons. Mann–Whitney \(U\)-tests were used for testing differences between independent groups, and groups with repeated measures were compared with the Wilcoxon signed-rank test. In comparisons involving more than two groups, custom-written MATLAB code was used to tailor an analysis of variance (ANOVA) to non-normally distributed data with unequal variances, for independent and non-independent groupings: \(F\)-statistics were computed as the ratio between the sum of squares among groups and the sum of squares within groups, and \(P\) values were calculated by comparing the \(F\)-statistic derived from the data and the average \(F\)-statistic generated from resampling the shuffled data 10,000 times. Our data required two types of ANOVA: two-way ANOVA with repeated measures, and one-way ANOVA with repeated measures (Kruskal–Wallis test). ANOVAs were followed by post hoc comparisons with either the Wilcoxon signed-rank test or the Mann–Whitney \(U\)-test where justified. The Bonferroni method was used to correct for multiple comparisons.

**Reporting summary.** Further information on research design is available in the Nature Research Reporting Summary linked to this paper.

**Data availability**
The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.
**Extended Data Fig. 1 | Analysis of ΔF/F during still or running events.**

Example GCaMP6s responses of a P28 SST cell during natural scenes with variable running activity. Treadmill motion and corresponding GCaMP6s signal are shown (15.5 fps), with grey bars denoting periods of visual stimulation. The z-score of the GCaMP signal is denoted by the colour map, and the trace denotes ΔF/F. Each presentation of the visual stimulus is classified as either a run (green) or still (black) trial. ΔF/F of GCaMP6s from all run (left) or still (right) trials are plotted. The median (red) of the median (black) ΔF/F response is taken to give an average value for still or run trials.
Extended Data Fig. 2 | Current injection responses for SST, parvalbumin and VIP cells before and after carbachol application. 
a, Example current injection responses showing distinct waveform from a SST cell. b, Change in voltage with 20 pA current steps for each cell type at P28 or P56. No significant change in excitability was found between age groups (P28: n = 11; P56: n = 9). P > 0.00625, two-way repeated-measures ANOVA followed by Mann–Whitney U-test, Bonferroni-corrected alpha. c, Evoked spiking for all cells before CCh application (open boxes) and after washout (yellow boxes), for respective cell types (n = 19). No significant change in response to current injection was found after CCh application; P > 0.0055, repeated-measures ANOVA followed by Wilcoxon signed-rank test, Bonferroni-corrected alpha. d, Example parvalbumin cell waveform. e, As in b, but for parvalbumin cells (P28: n = 8; P56: n = 9). No significant change in excitability was found between age groups; P > 0.0071, two-way repeated-measures ANOVA followed by Mann–Whitney U-test, Bonferroni-corrected alpha. f, As in c, but for parvalbumin cell responses before and after CCh washout (n = 17). No significant change was found; P > 0.0062, repeated-measures ANOVA followed by Wilcoxon signed-rank test, Bonferroni-corrected alpha. g, Example VIP cell waveform. h, As in b, but for VIP cells (P28: n = 10; P56: n = 8). No significant change in excitability was found between age groups; P > 0.0083, two-way repeated-measures ANOVA followed by Mann–Whitney U-test, Bonferroni-corrected alpha. i, As in c, but for VIP cell responses before and after carbachol washout (n = 18). No significant change was found; P > 0.0083, repeated-measures ANOVA followed by Wilcoxon signed-rank test, Bonferroni-corrected alpha. Box plot parameters as in Fig. 1.
Extended Data Fig. 3 | VIP cells show no age-dependent changes in cholinergic modulation. a, As in Fig. 1, example VIP cell populations from P28 and P56. b, Plot of the visually evoked median ΔF/F of each cell as a function of behavioural state, for all recorded cells (P28: n = 220; P56: n = 305). Still to run, P = 1.63 × 10−64 (P28), P = 1.75 × 10−60 (P56), Wilcoxon signed-rank test. c, Example traces of VIP cell response to CCh application at P28 (left) and P56 (right). Recordings were made under current clamp and in the presence of synaptic blockers. Coloured box denotes time of CCh application. Insets show evoked VIP cell waveform. Scale bars, 0.25 s and 10 mV. d, Box plot of VIP cell firing rates evoked by CCh as a function of age (P28: n = 10; P56: n = 8). P = 0.9804, Mann–Whitney U-test. Box plot parameters as in Fig. 1.
Extended Data Fig. 4 | CCh-induced IPSCs on SST cells are present across development. a, Example whole-cell recordings of SST cell responses to CCh in voltage-clamp mode at P28 (left) or P56 (right). b, Median IPSC amplitudes evoked by CCh application for all recorded SST cells at P28 and P56 (P28, n = 6; P56, n = 8). P = 0.4136, Mann–Whitney U-test. Box plot parameters as in Fig. 1.
Extended Data Fig. 5 | Deconvolution and ΔF/F comparisons produce analogous findings in sister dendrites. a, Top, time series showing ΔF/F and concurrent deconvolution for still and run epochs in a sister dendrite from a P28 mouse. Note the deconvolution is based on significant changes in slope. Spearman’s correlation coefficients (rₛ values) are shown. ΔF/F has been filtered for clarity. Bottom, as in the top panel for sister dendrites from a P56 mouse. Scale bar, ΔF/F = 1 and 1 s. Event probability = 1 AU.

b, Median percentage change in ΔF/F from still to running, for all P28 and P56 branches (P28: n = 36 branches; P56: n = 96 branches). P = 0.6608, Mann–Whitney U-test.
c, Correlation of ΔF/F between sister dendrites to movement at P28 or P56 (P28: n = 18 branch pairs; P56: n = 48 branch pairs). Still to run, ***P = 5.36 × 10⁻⁴ (P28), P = 0.043 (P56), Wilcoxon signed-rank test. N.S., not significant. Box plot parameters as in Fig. 1.
Extended Data Fig. 6 | Example visually evoked P28 and P56 sister dendrite activity. Examples of temporally deconvolved GCaMP6f traces showing event probability in sister dendrites from a P28 and P56 mouse. Grey bars mark periods of locomotion across traces. Left, P28 sister dendrites show decorrelated activity during movement. Right, P56 sister dendrites maintain synchronized activity across run and still epochs. Scale bar indicates event probability = 1 AU.
Extended Data Fig. 7 | P28 and P56 modulation of pyramidal cell somas and dendrites during spontaneous activity. a, Plot of the visually evoked median ΔF/F of each cell as a function of behavioural state, for all recorded cells, at P28 and P56, in the absence of visual stimulation, measured at the soma (P28: n = 563 cells; P56: n = 723 cells). Still to run, P = 4.31 × 10^{-60} (P28), P = 1.94 × 10^{-89} (P56), Wilcoxon signed-rank test. b, Plot of cumulative distributions of the correlation coefficients of GCaMP6f signals to running for all recorded pyramidal neurons while viewing a grey screen (P28: n = 860 cells; P56: n = 513 cells). P = 0.993, Mann–Whitney U-test. c, Box plots of temporally deconvolved event probabilities of dendrites during grey screen viewing at P28 and P58 during in running and still conditions (P28: n = 36 branches; P56: n = 96 branches). Still to run, **P = 0.0044 (P28), ***P = 4.36 × 10^{-6} (P56), Wilcoxon signed-rank test. d, Box plots of the fold change between sister branches as a function of age and behavioural state (P28: n = 18 branch pairs; P56: n = 56 branch pairs). Still to run, P = 0.987 (P28), P = 0.02 (P56), Wilcoxon signed-rank test. e, Box plots of the correlation coefficients between event probability time series of sister branches as a function of age and behavioural state (P28: n = 18 branch pairs; P56: n = 56 branch pairs). Still to run, P = 0.2914 (P28), **P = 0.0042 (P56), Wilcoxon signed-rank test. Box plot parameters as in Fig. 1.
Extended Data Fig. 8 | Verification of ChR2-driven SST activity in vivo.
a. SST cells expressing both ChR2-tdTomato and GCaMP6s.
b. Representative time series heat map of SST cell GCaMP6s responses to optogenetic stimulation. The z-scores from individual cells are plotted per frame (15.5 fps); colour scale is −1 to 5 z-scores. Top, blue bars denote LED light pulses. Bottom, inset showing z-scored traces and significant increase in GCaMP6s signal for 14 cells.
Extended Data Fig. 9 | Verification of chemogenetic control of SST cells using DREADDs. a, Top, example evoked responses of a SST cell expressing DREADD receptors. Bottom, evoked responses from the same SST cell 4 h after intraperitoneal administration of CNO (2.5 mg kg\(^{-1}\)). Scale bar, 10% ΔF/F and 30 s. b, Measurement of change in median evoked ΔF/F in SST cells after CNO administration over an 8 h period, in mice with or without DREADD expression (DREADD−/CNO+: n = 25 cells; DREADD+/CNO+: n = 21 cells). 2 h, DREADD− to DREADD+: ***P = 1.41 \times 10^{-4}; 4 h, DREADD− to DREADD+: ***P = 7.98 \times 10^{-5}, Wilcoxon signed-rank test. Error bars denote s.e.m.
Reporting Summary

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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).

- **n/a**
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- The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
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- The statistical test(s) used AND whether they are one- or two-sided
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- 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 |
|-----------------|
| All calcium imaging data was collected using Scanbox software, and all electrophysiological recordings were acquired using WinWCP. |

| Data analysis |
|-----------------|
| Custom-written MATLAB code was used to determine averages, correlations, relative change, and rates for all collected data. Built-in functions in Matlab were used for all statistical comparisons between two groups, and custom-written code was used for statistical tests involving more than two groups (two-way mixed ANOVAs or one-way ANOVAs). |

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.

**Data**

**Policy information about availability of data**

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:
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All relevant data collected and analyzed is shown in all main figures. Data and code are available upon reasonable request from the corresponding author.
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Life sciences study design

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

| Sample size | Sample size was determined based on cell count: following data collection, all within-group samples were pooled and tested for outliers. Sample size was not predetermined, but general field standards were applied to achieve statistical power: for calcium imaging data, more than 100 cells per group was preferable, taken from at least 3 animals, and for electrophysiology, between 8 and 20 cells per group were included. |
|-------------|
| Data exclusions | Steps were taken to ensure the health of recorded neurons in vivo and in vitro. Animals without dynamic GCaMP6 signals were either not recorded or not included. Cells without healthy responses to current injection following pharmacological application were not included. |
| Replication | All attempts at replication were achieved providing cell health was preserved. |
| Randomization | For studies involving multiple groups (figure 5), allocation into experimental group was randomized. Experiments were sorted by animals which received saline and animals which received CNO, intermixed with positive and negative controls. |
| Blinding | Neither data collection or data analysis were blinded, as experiments were age-specific. |

Reporting for specific materials, systems and methods

| Materials & experimental systems | Methods |
|----------------------------------|---------|
| n/a | n/a |
| ☑ | ☑ |
| ☐ Unique biological materials | ChiP-seq |
| ☑ Antibodies | Flow cytometry |
| ☑ Eukaryotic cell lines | MRI-based neuroimaging |
| ☑ Palaeontology | |
| ☑ Animals and other organisms | |
| ☑ Human research participants | |

Animals and other organisms

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

| Laboratory animals | Methods |
|--------------------|---------|
| The following mouse lines were used from Jackson Laboratories: SST-IRES-Cre (stock no. 018973), PV-Cre (stock no. 08069), VIP-IRES-Cre (stock no. 010908), and ChAT-Cre (stock no. 006410). For optical identification of interneurons, mice were crossed with A9-expressing mice (stock no. 007905). Two age groups were used: P28 and P56. All mice were heterozygous for their respective transgenes, and both male and female mice were used. |

| Wild animals | |
|--------------|-----|
| This study did not involve wild animals. |

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