Pyramidal cell regulation of interneuron survival sculpts cortical networks

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Complex neuronal circuitries such as those found in the mammalian cerebral cortex have evolved as balanced networks of excitatory and inhibitory neurons. Although the establishment of appropriate numbers of these cells is essential for brain function and behaviour, our understanding of this fundamental process is limited. Here we show that the survival of interneurons in mice depends on the activity of pyramidal cells in a critical window of postnatal development, during which excitatory synaptic input to individual interneurons predicts their survival or death. Pyramidal cells regulate interneuron survival through the negative modulation of PTEN signalling, which effectively drives interneuron cell death during this period. Our findings indicate that activity-dependent mechanisms dynamically adjust the number of inhibitory neurons in nascent local cortical circuits, ultimately establishing the appropriate proportions of excitatory and inhibitory neurons in the cerebral cortex.

In the adult neocortex, approximately one-sixth of neurons are inhibitory γ-aminobutyric acid-containing (GABAergic) interneurons1,2, and this ratio is relatively stable across cortical regions and species regardless of the total number of neurons3–5. The cellular balance between excitation and inhibition is critical for brain function and is likely to be disrupted in a number of neuropsychiatric conditions2–9. However, the mechanisms that regulate the establishment of appropriate numbers of excitatory and inhibitory neurons in the cerebral cortex remain largely unknown.

Programmed cell death, also known as apoptosis, is an essential mechanism that sculpts the central and peripheral nervous systems during development9,10–12. The death of developing neurons is mediated by an evolutionarily conserved signalling pathway that involves the pro-apoptotic BCL2 family member BAX and BAK13. Previous studies have shown that both cortical pyramidal cells and GABAergic interneurons undergo extensive cell death during postnatal development14,15, which suggests that apoptosis may contribute to the establishment of balanced networks of excitatory and inhibitory neurons in the cerebral cortex. However, the temporal relationship and interdependency of the programmed cell death periods for both populations of neurons have not been explored in detail.

Concatenated waves of neuronal death

To determine the developmental sequence that establishes the final ratio of excitatory and inhibitory neurons in the cerebral cortex, we estimated the absolute numbers and relative proportions of pyramidal cells and GABAergic interneurons at different postnatal stages of development using stereological methods in mouse strains in which specific classes of neurons are irreversibly labelled. We chose this method to estimate programmed cell death over the direct quantification of dying cells because classical apoptotic markers such as cleaved caspase-3 have non-apoptotic roles in neurons16 and are expressed only during development using stereological methods in mouse strains in which specific classes of neurons are irreversibly labelled. We chose this method to estimate programmed cell death over the direct quantification of dying cells because classical apoptotic markers such as cleaved caspase-3 have non-apoptotic roles in neurons16 and are expressed only transiently (Extended Data Fig. 1c–e), which are known to undergo programmed cell death during this period19. By contrast, we found that the number of interneurons is steady until P5, drops extensively between P5 and P10 (by about 30%), and then remains constant into adulthood (Fig. 1c–e). Interneuron cell loss follows the normal maturation sequence of MGE and POA interneurons20, with deep layer interneurons adjusting their numbers ahead of superficial layer interneurons (Fig. 1f). These results revealed that consecutive waves of programmed cell death adjust the final ratio of excitatory and inhibitory neurons in the developing cerebral cortex.

Interneuron activity predicts cell death

Our results indicated that the adjustment of interneuron numbers is preceded by a wave of pyramidal cell death, which suggest that these two processes might be directly linked. As previous work has shown that neuronal activity and apoptosis rates are inversely correlated in the developing brain21–23, we hypothesized that pyramidal cells may impact interneuron survival by increasing the activity of the cells to which they connect. We tested this idea by monitoring the activity of MGE and POA interneurons in the superficial layers of the barrel cortex (S1BF) during the period of interneuron cell death. To this end, we generated mice expressing the fluorescent reporter tdTomato and the genetically encoded calcium sensor GCaMP6s in MGE and POA interneurons (Nkx2-1-Cre;RCLtdT/GCaMP6s mice)24 and performed long-term Ca2+ imaging in the same interneurons from layer 2/3 in S1BF of awake, head-restrained pups (Fig. 2a). To select the most appropriate time for these experiments, we estimated interneuron cell death in S1BF during postnatal development and found comparable dynamics to the rest of...
characteristic (ROC) analysis revealed that the event rate at P7 performed significantly better than chance at discriminating between these two populations (Fig. 2f). These results suggested that interneurons with relatively low levels of activity immediately before the period of interneuron cell death have an increased probability of undergoing apoptosis.26,27.

Pyramidal cells regulate interneuron death

The previous experiments led us to hypothesize that interneurons receiving abundant or particularly strong inputs during the period of interneuron cell death would have increased chances of survival. As PV+ and SST+ interneurons receive most of their inputs from local pyramidal cells during the first postnatal week,28, we reasoned that modification of the activity of cortical excitatory neurons during the period of interneuron cell death would influence interneuron survival. To test this idea, we transiently modified the activity of pyramidal cells using a chemogenetic approach based on Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) that induce neuronal activation or inhibition.29 We injected the primary somatosensory cortex (S1) of P0 NcxCre+;Fucci2 mice with an adeno-associated virus (AAV) encoding mutant G-protein-coupled receptors that induce neuronal activation (hM3Dq) or inhibition (hM4Di) following administration of the pharmacologically inert molecule clozapine-N-oxide (CNO) (Fig. 3a). We then injected pups with CNO twice daily between P5 and P8, and examined the distribution of calcium events at P7 for the four numbered neurons in c. Box plots illustrating event rates for P7 interneurons that lived past P8 (magenta) and interneurons that died by P8. In box plots, the central mark indicates the median, and the bottom and top edges of the box indicate the 25th and 75th percentiles, respectively. Two-sided Mann–Whitney test, P = 0.03; n = 18 for cells that died at P8 and 153 for cells that lived beyond P8, from three pups.

**Pyramidal cells regulate interneuron death**

1. **Consecutive waves of programmed cell death of pyramidal cells and interneurons in the early postnatal cortex.** a, Coronal sections through the primary somatosensory cortex (S1) of NcxCre+;Fucci2 (a) and Nkx2-1–Cre;RCLtdTomato (c) mice during postnatal development. b, Total number of pyramidal cells in the entire neocortex of NcxCre+;Fucci2 mice (ANOVA, F = 4.17, *P = 0.02; n = 4 (P2 and P5), 3 (P7) and 5 (P10 and P21) mice). c, Total number of MGE and POA interneurons in the entire neocortex of Nkx2-1–Cre;RCLtdTomato mice (ANOVA, F = 26.80, *P = 0.01; n = 4 mice per age). e, Temporal percentage variation in pyramidal cells and MGE and POA interneurons. f, Total number of MGE and POA interneurons in superficial (L1–L4) and deep layers (L5 and L6) of the neocortex (two-way ANOVA, Finteraction = 1.01, *P = 0.03 and **P = 0.002; n = 3 animals per age). Data are shown as mean ± s.e.m. Scale bars, 100 µm.

2. **Interneuron activity levels predict cell death.** a, Schematic of experimental design. b, Total number of MGE and POA interneurons in layer 2/3 S1BF of Ncx2-1–Cre;RCLtdTomato (n = 3 animals per age). Data are shown as mean ± s.e.m. c, ROI imaged at P7 (left) and P8 (right); individual neurons numbered. d, Raster plots showing the occurrence of calcium events at P7 for the four numbered neurons in c. e, Box plots illustrating event rates for P7 interneurons that lived past P8 (magenta) and interneurons that died by P8. In box plots, the central mark indicates the median, and the bottom and top edges of the box indicate the 25th and 75th percentiles, respectively. Two-sided Mann–Whitney test, P = 0.03; n = 18 for cells that died at P8 and 153 for cells that lived beyond P8, from three pups. f, ROC analysis showing the ability of P7 event rates to discriminate between cells that died by P8 and cells that lived past P8. AUC (area under the curve) = 0.65, *P = 0.025. Scale bar, 15 µm.
homogeneously distributed across layers containing PV+ and SST+ interneurons (Extended Data Fig. 3c, d). CNO administration did not cause a redistribution of interneurons from neocortical areas adjacent to the injection site (Extended Data Fig. 3e, f). Instead, we observed a prominent increase in the density of cleaved caspase-3-positive cells following inhibition of the activity of pyramidal cells during the normal period of interneuron cell death (Extended Data Fig. 4a-c). Notably, control experiments revealed that CNO did not modify the density of PV+ or SST+ interneurons in pups not infected with AAV-expressing DREADDs (Extended Data Fig. 4d, e). Similarly, CNO administration between P10 and P13 in mice injected with hM3Dq or hM4Di induced no significant changes in the density of PV+ and SST+ interneurons at P21 (Extended Data Fig. 5). Together, these results demonstrate that pyramidal cell activity is an essential regulator of interneuron survival during the normal period of interneuron cell death.

**Interneurons match pyramidal cell numbers**

The previous experiments suggest that pyramidal cells ‘rescue’ appropriate numbers of corticospinal neurons from programmed cell death through an activity-dependent mechanism. Drawing on this idea, we reasoned that modification of the number of pyramidal cells before the period of interneuron cell death should also influence the number of surviving interneurons. To test this hypothesis, we generated conditional mice in which pyramidal cells specifically lack Bak and Bax, whose combined function is critical for apoptosis. As expected, we observed that the number of excitatory neurons in the cerebral cortex of NexCre+/−;Bak−/−;Baxfl/fl mutant mice did not decline between P2 and P21 (Extended Data Fig. 6). Consequently, NexCre+/−;Bak−/−;Baxfl/fl (PV+) and SST+ mutant mice had approximately 12% more pyramidal cells than control mice (Fig. 1b and Extended Data Fig. 6).

We next quantified PV+ and SST+ interneurons in S1 of control and NexCre+/−;Bak−/−;Baxfl/fl mutant mice at P21. The density of both PV+ and SST+ interneurons was roughly 30% higher in NexCre+/−;Bak−/−;Baxfl/fl mutant mice than in controls (Fig. 4a, c), which suggests that interneuron cell death is suppressed when pyramidal cell death is prevented. This increase was homogeneously distributed across layers containing PV+ and SST+ interneurons (Extended Data Fig. 7a), and was also observed in other neocortical areas (Extended Data Fig. 7c, d). To evaluate whether the increase in the number of PV+ and SST+ interneurons represented the entire population of cells that should normally have died through programmed cell death, we generated conditional mice lacking Bak and Bax in MGE and POA interneurons. We found that the density of PV+ and SST+ interneurons was also approximately 30% higher in Nkx2-1-Cre;Bak−/−;Baxfl/fl mutant mice.
PTEN regulates interneuron cell death

We next investigated the molecular mechanisms through which pyramidal cell activity prevents programmed cell death in cortical interneurons. In the developing nervous system, the serine–threonine kinase AKT is a critical mediator of neuronal survival that is antagonized by the activity of the phosphatase and tensin homologue PTEN. Consistent with this, we observed that the relative levels of activated AKT (P-AKT/AKT ratio) increased transiently in the neocortex during the period of interneuron cell death (Fig. 5a). Notably, PTEN levels are very heterogeneous among MGE and POA interneurons during the same period (Fig. 5b). PTEN levels were transiently elevated in sparse interneurons in deep and superficial layers of S1, and this increase was concurrent with the peak of pyramidal cell death (Fig. 6a, b). These observations led us to hypothesize that high PTEN levels during this period may drive interneurons towards cell death, which reinforces the idea that excitatory input from pyramidal cells antagonizes the activity of the phosphatase and tensin homologue PTEN (Fig. 5c, d). 

PTEN regulates cell death in Lhx6-Cre;Ptenfl/fl mice

We next investigated the molecular mechanisms through which pyramidal cell activity prevents programmed cell death in cortical interneurons. In the developing nervous system, the serine–threonine kinase AKT is a critical mediator of neuronal survival that is antagonized by the activity of the phosphatase and tensin homologue PTEN. Consistent with this, we observed that the relative levels of activated AKT (P-AKT/AKT ratio) increased transiently in the neocortex during the period of interneuron cell death (Fig. 5a). Notably, PTEN levels are very heterogeneous among MGE and POA interneurons during the same period (Fig. 5b). PTEN levels were transiently elevated in sparse interneurons in deep and superficial layers of S1, and this increase was concurrent with the peak of interneuron cell death in these layers (Fig. 5c, d). These observations led us to hypothesize that high PTEN levels during this period may drive interneurons towards cell death, and that pyramidal cells might influence this process by regulating PTEN in interneurons.
To test this hypothesis, we generated mice in which we conditionally deleted Pten from postmitotic MGE interneurons.\(^{55,56}\) We observed that Lhx6-Cre;Pten\(^{-/-}\) mutant mice had abnormally large jaws and reduced body weight compared to their littermates by P16, probably owing to the embryonic expression of Lhx6 in the first branchial arch,\(^{37}\) which prevented their analysis at later developmental stages. We nevertheless found that Pten conditional mutants had a significantly higher density of PV\(^{+}\) and SST\(^{+}\) interneurons in S1 than control mice (Fig. 5e, f, g and Extended Data Fig. 8c, d), without any difference in relative distribution across layers (Extended Data Fig. 8e). As Lhx6-Cre drives recombination in endothelial cells in addition to MGE interneurons,\(^{53}\) we examined whether a change in the organization of neocortical blood vessels might contribute to increased survival of interneurons in conditional Pten mutants. We found that the density of blood vessels was higher in conditional Pten mutants than in controls (Extended Data Fig. 8c, d). However, this change did not affect the density of pyramidal cells (Extended Data Fig. 8c, d), which rules out an indirect effect of blood vessels on interneuron survival through an increase in pyramidal cell density. To rule out a direct effect of blood vessels on interneuron survival, we carried out a second series of experiments using acute pharmacological inhibition of PTEN. We injected the PTEN inhibitor bpV(pic) systemically at P7 and P8 into wild-type mice and analysed blood vessel density in S1 at P10 (Extended Data Fig. 9b, c). Mice injected with the PTEN inhibitor did not exhibit increased blood vessel coverage (Extended Data Fig. 9b, c). By contrast, transient PTEN inhibition during the period of interneuron cell death increased the density of MGE interneurons compared to control mice (Extended Data Fig. 9a, d, e). Mice injected with the PTEN inhibitor outside the normal window of interneuron programmed cell death showed similar densities of PV\(^{+}\) and SST\(^{+}\) interneurons to controls (Extended Data Fig. 9f–h). These results revealed that PTEN is likely to be required cell-autonomously for interneuron apoptosis during the normal period of interneuron cell death.

Finally, we examined whether pyramidal cell activity influences the survival of interneurons by non-cell-autonomously regulating the expression of PTEN in these cells during the period of interneuron cell death. To this end, we carried out DREADD experiments similar to those that led to an increased number of cortical interneurons following transient activation of pyramidal cells between P5 and P8 (Fig. 3), but here we analysed PTEN levels in cortical interneurons at P8 (Fig. 5h). We found that PTEN levels were significantly decreased in GABAergic interneurons following the activation of pyramidal cells (Fig. 5i, j). These results strongly suggest that pyramidal cells influence the normal programmed cell death of interneurons through the activity-dependent inhibition of PTEN, which tips the balance between survival and apoptotic signalling pathways in developing interneurons.

**Discussion**

Our results suggest that programmed cell death in interneurons has evolved as a mechanism responsible for adjusting the final ratio of excitatory and inhibitory neurons in the cerebral cortex, a critical milestone in the assembly of cortical circuits.\(^{38}\) Although synaptic mechanisms are known to stabilize excitatory–inhibitory ratios in cortical circuits,\(^{39–41}\) this effectively requires that the relative proportions of pyramidal cells and interneurons are within certain parameters.\(^{42–44}\) Considering the disproportionate expansion of neocortical areas during human evolution,\(^{45,56}\) it is tempting to speculate that the dependency of interneuron survival on pyramidal cells provided an evolutionary advantage for the preservation of appropriate ratios of excitatory and inhibitory cells during the rapid increase in pyramidal cell numbers in the primate lineage.

Our work indicates that interneuron cell death is non-cell-autonomously regulated by pyramidal cells, which seem to be able to rescue connected interneurons from their intrinsically determined cell death,\(^{14}\) by inhibiting the activity of PTEN during a critical window in postnatal development. It is worth noting that a sizable proportion of individuals with autism spectrum disorders (ASD) and macrocephaly carry deleterious mutations in the Pten gene.\(^{47,48}\) Our observations indicate that loss of PTEN function is sufficient to disrupt programmed interneuron cell death, which may in turn alter the cellular balance of excitation and inhibition in the cerebral cortex. This mechanism may contribute to deregulation of cortical information processing and social dysfunction in individuals with ASD who carry PTEN mutations.

The rate of apoptosis in pyramidal cells varies among functionally different neocortical areas and even across layers within the same cortical area.\(^{49}\) This suggests that the proposed mechanism might sculpt the heterogeneous patterns of interneuron distribution that exist across the cerebral cortex.\(^{50}\) Consequently, the regulation of programmed cell death in interneurons by pyramidal cells is likely to contribute to the cytoarchitectonical specialization of cortical areas.

**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/s41586-018-0139-6.

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Author contributions F.K.W., K.B., V.S., and O.M. designed experiments. F.K.W., K.B., V.S., and O.M. performed and analysed DREADDs experiments, except for the analysis of PTEN levels, which was carried out by K.B. F.K.W. analysed Bax/Bak mutant mice. K.B. performed western blots, examined interneuron PTEN levels and analysed Pten mutant mice. F.K.W. performed in vivo pharmacological PTEN inhibition experiments. F.K.W., K.B., V.S., and O.M. wrote the manuscript.

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Metal head-post (Luigs and Neumann) was attached over the left hemisphere with cyanoacrylate glue (Henkel). A thin protective layer of glue was applied over the skull. The glue was allowed to dry for 10 min. Dental cement (Paladur) was used to reinforce the attachment of the head-post to the skull. The mouse was injected with buprenorphine (2 μg/g of a 50 μg/ml solution) and returned to its home cage. At P7, the animal was anesthetized and head-restrained in a custom-made head holder. A 3-mm craniotomy was opened over the posterior–lateral neocortex. This cranial window encompassed the primary somatosensory cortex (S1). Care was taken not to damage the dura mater. A circular coverslip (3-mm diameter, Harvard Apparatus) was placed over the craniotomy, and its edges were sealed with cyanoacrylate glue and reinforced with dental cement. Following surgery, dexamethasone (5 μg/g of a 38 μg/ml solution) was injected subcutaneously. The animal was allowed to recover for at least 2 h in its home cage, following which we commenced imaging at P7.

Imaging sessions lasted for 40–60 min and we imaged the same field of view for consecutive days in three mice. tdTomato and GCaMP6s were excited using a Ti-Sapphire laser (Coherent Chameleon) tuned to λ = 930 nm. The emitted photons were collected by two GaAsP detectors through a 20× objective (Olympus, 1.0 NA). The field of view (FOV) measured 385 × 385 μm (512 × 512 pixels). The scan speed was set to 30 Hz and image sequences were obtained in sweeps of 1 min (1800 images per channel per min). The average excitation power was between 40 and 50 mW, and this was kept constant over all imaging days.

To correct for motion artefacts, image registration was carried out using custom-written spatial cross-correlation methods on the tdTomato channel. In brief, on every 1-min sweep, a part of the tdTomato image sequence, where the animal movement was minimal, was averaged to generate a moving image reference. Every frame of the tdTomato image sequence was spatially cross-correlated to this reference image and offset along the x- and y-axes to match the cross-correlation peak. The offsets obtained for each tdTomato frame were applied to the corresponding GCaMP6s frame.

**Calcium imaging analysis.** Circular ROIs (diameter 20 μm) were manually drawn around tdTomato-expressing cell bodies. The mean GCaMP6s fluorescence intensity in time was extracted. Changes in fluorescence signal were calculated as ΔF/Δt, where the baseline fluorescence (F0) is the mode of a kernel density estimate of F (kodensity function in MatLab). Calcium events were detected by setting a threshold of 3% change in fluorescence from baseline.

**Receiver-operating characteristic curves.** To identify whether the calcium event rate (events per min) at P7 could act as a binary classifier in distinguishing cells that would be alive or dead at P8, we plotted the ROC curve by varying the discrimination threshold (in this case, the P7 event rate) and calculated the AUC. To test for statistical significance, the cell labels were randomly shuffled 5000 times. On each shuffle, we calculated the ROC curve and the corresponding AUC. We then compared our observed AUC to the distribution of shuffled AUCs. The P-value is the proportion of shuffled AUCs ≥ observed AUC.

**Intracranial injections.** pAAV8-hsyn-Dio-Hm3D(Gq)-mCherry and pAAV8-hsyn-Dio-Hm4D(Gq)-mCherry were gifts from B. Roth (Addgene plasmids #44361 and #44362). P0 mice were anesthetized with isoflurane and mounted in a stereotoxic frame. Pups were injected with 600 nl of virus diluted in PBS and coloured with 0.5% Fast Green (Sigma). Injections were targeted for the somatosensory cortex with an injection rate of 10 nl/s.

**Drugs.** For DREADD experiments, CNO (Tocris) was dissolved in 5% dimethyl sulfoxide (Sigma) and then diluted with 0.9% saline to either 0.1 mg/ml or 0.5 mg/ml. Pups were injected with vehicle (0.05% DMSO) or CNO (10 μl per g) subcutaneously for 4 days, twice daily. For the PTEN inhibitor experiments, dipotassium bis-peroxovanadimidodicarbonyl dehydrate (bpV(pic), Sigma) was dissolved in 0.9% saline to 0.2 mg/ml. Pups were injected with vehicle (0.9% saline) or bpV(pic) (10 μl per g) intraperitoneally for 2 days, twice daily. All treatments for CNO and PTEN inhibitor experiments were randomly assigned.

**Western blotting.** Mouse somatosensory cortex tissue was homogenized in RIPA lysis buffer containing 50 mM Tris pH 8, 150 mM NaCl, 2 mM EDTA, 0.5% sodium deoxycholate, 0.1% SDS, 1% NP-40 and 1 μg protease inhibitor cocktail (Complete, Sigma). Samples were sonicated in Laemmlı sample buffer and run on 10% SDS–PAGE gels. Separated proteins were electrophoretically transferred onto PVDF membranes. Membranes were blocked with 5% BSA in TBST (20 mM Tris–HCl pH 7.5, 150 mM NaCl and 0.1% Tween20) for 1 h and probed with rabbit anti-P-Akt (Ser473, Cell Signalling, 1:1000) overnight at 4°C, followed by an HRP-conjugated donkey anti-rabbit antibody (Thermo Fisher, 1:10,000). The blots were developed using ECL western blotting detection reagents and following read-out, they were stripped (Thermo Fisher). After confirming stripping efficiency, an HRP-conjugated mouse anti-Akt antibody (Cell Signalling, 1:1000) was used. The blots were developed using ECL western blotting detection reagents, the signals were registered and, following stripping, an HRP-conjugated rabbit anti-Actin (Sigma, 1:20,000) was added for 1 h at room temperature. Pico ECL western blotting reagent was used to detect actin levels.
Signals were read on a Li-COR Odyssey Imaging Band and intensities were analysed using ImageStudioLite.

**Image acquisition.** Images used for analysis were obtained from an ApoTome (Zeiss), epifluorescence microscope (Leica), or SP8 confocal microscope (Leica). ApoTome images were taken using the ApoTome function in Zen2 software. Images obtained with the confocal and epifluorescence microscope were taken using LAS AF software.

**Cell counting.** Cortical layers were identified on the basis of their distinct histological characteristics. Layer 1 was identified as a sparsely populated cell layer. The border between layers 2/3 and 4 was distinguished by the higher nuclei density of layer 4. Layer 5 was identified as the layer basal to layer 4 and above layer 6, which contains less densely packed nuclei. Cell density, within cortical layers, was quantified either manually or using custom routines written in Matlab (MathWorks). For manual quantification, all analyses were conducted blind and cells were counted in a rectangular area, 551.5 µm wide at the pia surface within the somatosensory cortex, auditory cortex or motor cortex. Cells were counted without using pseudocolour in Fiji. Automatic quantification was carried out blinded and using morphological operations for image segmentation.

To identify PTEN staining intensity in tdTomato+ or GABA+ interneurons, self-designed CellProfiler pipelines were used. In brief, tdTomato+ or GABA+ interneurons were identified as primary objects using the global Otsu thresholding method and any objects outside the pre-set diameter range (25–100 pixels) were excluded. PTEN intensity was measured under this cell mask.

**Blood vessel analysis.** The fraction of the total area covered by blood vessels and the average vessel diameter were quantified blind using Vessel Analysis, an ImageJ plugin (http://imagej.net/Vessel_Analysis; N. Govindaraju and M. Elfarnawany).

**Statistical analyses.** Unless specified, results were plotted and tested for statistical significance using Prism 7. The samples were tested for normality using the Shapiro–Wilk normality test. Unpaired comparisons were tested using matched pairs analyses with Student’s t-tests (normally distributed) and Mann–Whitney tests (not normally distributed). Multiple comparisons were conducted using one-way ANOVA with post hoc Tukey’s test (comparing the mean of each column with the mean of every other column) or Dunnett’s test (comparing the mean of each column with the mean of a control column) for normally distributed samples. For samples with nonparametric distribution, either Kruskal–Wallis (single measures) or Friedman’s test (repeated measures) was performed followed by the post hoc Dunn’s test. For multiple comparisons with more than one variable, a two-way ANOVA with post hoc Sidak’s test was used. The cumulative distributions of PTEN intensity levels were compared using the Kolmogorov–Smirnov test. Analysis of calcium events rate was carried out in Matlab. In box plots, the central mark indicates the median, and the bottom and top edges of the box indicate the 25th and 75th percentiles, respectively. The whiskers extend to the most extreme data points not considered outliers. No statistical methods were used to predetermine sample size. Sample sizes were calculated on the basis of similar published studies. All experiments were replicated at least in two different litters. Unless otherwise stated, the experiments were not randomized (that is, assignments were based on genotypes) and the investigators were not blinded to allocation during experiments and outcome assessment.

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

**Data availability.** All data and/or analyses generated during the current study are available from the corresponding author upon reasonable request.

**Code availability.** For automatic quantification, the code was written in Matlab (Mathworks) and is available from the corresponding author on reasonable request.

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Extended Data Fig. 1 | Extensive cell death in layer 2–6 pyramidal cells.

**a**, Coronal sections through the S1 cortex of P4 *NexCre*+;*Fucci2* (left) and P7 *Nkx2-1-Cre;RCLtdTomato* (right) mice immunostained for cleaved caspase-3 (yellow) and mCherry (green, left) or tdTomato (magenta, right). b, Quantification of density of cleaved caspase-3 cells in pyramidal neurons (left, green) and MGE interneurons (right, magenta) during postnatal development (for pyramidal neurons, ANOVA, $F = 73.6$, ***$P = 0.003$ (P2 versus P4), ***$P = 0.00006$ (P4 versus P7), $n = 3$ mice for all ages; for MGE interneurons, ANOVA, $F = 16.91$, *$P = 0.027$ (P5 versus P7), **$P = 0.0029$ (P7 versus P10), $n = 3$ animals for all ages). c, Coronal sections through the barrel cortex of *NexCre*+;*Fucci2* mice during postnatal development immunostained for mCherry (green) and CTGF (yellow). d, Total number of pyramidal cells excluding subplate cells in the neocortex of *NexCre*+;*Fucci2* mice (ANOVA, $F = 4.83$ and *$P = 0.03$; $n = 3$ mice for P2 and P5, and 4 mice for P3, P4 and P21). e, Temporal variation in the percentage of pyramidal cells excluding the subplate contribution during postnatal development. Data are shown as mean ± s.e.m. Scale bars, 100 μm.
Extended Data Fig. 2 | Interneuron cell loss in the barrel field during postnatal development.  
a, Coronal sections through S1BF of Nkx2-1-Cre;RCLtdTomato mice (magenta, MGE interneurons) during postnatal development counterstained with DAPI (grey).  
b, Total number of MGE and POA interneurons in S1BF of Nkx2-1-Cre;RCLtdTomato mice during postnatal development (ANOVA, $F = 6.40$ and $*P = 0.03$; $n = 4$ animals for each age). Data are shown as mean ± s.e.m. Scale bar, 100 μm.
Extended Data Fig. 3  | See next page for caption.
Extended Data Fig. 3 | Alteration of pyramidal cell activity affects interneuron density but not distribution. a, Coronal sections through S1BF cortex immunostained for GABA (magenta) and NeuN (green) and counterstained with DAPI (grey) from P21 NexCre+ mice injected with hM3Dq-mCherry virus followed by vehicle or CNO treatment. b, Quantification of the density of GABA (left) and NeuN+ but GABA- (right) cells in P21 mice injected with hM3Dq-mCherry followed by vehicle (grey) or CNO (magenta) treatment (two-tailed Student's unpaired t-test, **P = 0.005 (GABA), P = 0.68 (NeuN+ GABA-), n = 4 animals for vehicle, n = 3 animals for CNO conditions). c, d, Quantification of the distribution of PV+ (left) and SST+ neurons (right) in P21 NexCre+ mice injected at P0 with hM3Dq-mCherry (c) or hM4Di-mCherry (d) and treated with vehicle (grey) or CNO (magenta) during P5–P8 (two-way ANOVA, F_treatment = 0.48, P = 0.50 (hM3Dq PV), F_treatment = −0.04, P = 0.99 (hM3Dq SST), F_treatment = 0.88, P = 0.37 (hM4DI PV), F_treatment = 0.79, P = 0.39 (hM4DI SST); for PV, n = 7 animals for hM3Dq and hM4DI −CNO, 6 animals for hM3Dq +CNO, and 5 animals for hM4DI +CNO; for SST, n = 9 animals for hM3Dq −CNO, 7 animals for hM3Dq +CNO and hM4DI −CNO, and 5 animals for hM4DI +CNO). e, Coronal sections through auditory cortex immunostained for PV (magenta) or SST (magenta) and counterstained with DAPI (grey) from P21 NexCre+ mice injected with hM3Dq-mCherry virus followed by vehicle or CNO treatment. f, Quantification of the density of PV+ (right) and SST+ neurons (left) in auditory cortex in P21 mice injected with hM3Dq-mCherry virus followed by vehicle (grey) or CNO (magenta) treatment (two-tailed Student's unpaired t-test, P = 0.574 (PV), P = 0.419 (SST), n = 4 animals for both). Data are shown as mean ± s.e.m. Scale bars, 100µm.
**Extended Data Fig. 4 | CNO control experiments.** a, Schematic of experimental design. b, Coronal sections through S1 of P8 NexCre mice injected with AAV8-dio-hM4Di-mCherry at P0 and treated with (+) or without (−) CNO between P5 and P8, immunostained for cleaved caspase-3 (magenta) and counterstained with DAPI (grey). c, Quantification of the density of cleaved caspase-3 cells in P8 mice injected with AAV8-dio-hM4Di-mCherry and treated (magenta) or not treated (grey) with CNO between P5 and P8 (two-tailed Student’s unpaired t-test, ***P = 0.009, n = 8 animals for −CNO, and n = 7 animals for +CNO). d, Schematic of experimental design for CNO control experiments. e, Quantification of the density of PV+ (left) and SST+ (right) cells in P21 mice injected with hM3Dq-mCherry or hM4Di-mCherry and not treated with CNO (grey), or not injected with viruses and treated with CNO (magenta) between P5 and P8 (ANOVA, P = 0.24 (PV+) and P = 0.65 (SST+)); for PV, n = 7 animals for hM3Dq and hM4Di−CNO, 4 animals for non-injected +CNO; for SST, n = 9 animals for hM3Dq −CNO, 7 animals for hM4Di −CNO, and 4 animals for non-injected +CNO). Data are shown as mean ± s.e.m. Scale bar, 100 µm.
Extended Data Fig. 5 | Alteration of pyramidal cell activity beyond the normal period of interneuron cell death does not affect interneuron survival or distribution. a, Schematic of experimental design. b, c, Coronal sections through S1BF immunostained for PV (b) or SST (c) and counterstained with DAPI (grey) from P21 NexCre/+ mice injected with hM3Dq-mCherry (left) or hM4Di-mCherry (right) viruses followed by vehicle or CNO treatment. d, g, Quantification of the density of PV+ (d) and SST+ (g) cells in P21 hM3Dq-mCherry injected mice (left bars) and hM4Di-mCherry injected mice (right bars) followed by vehicle (grey bars) and CNO (magenta bars) treatment at P10–P13 (for PV, two-tailed unpaired Student’s t-test, *P = 0.99 and *P = 0.087, respectively; for SST, two-tailed unpaired Student’s t-test, *P = 0.56 and *P = 0.37, respectively; n = 4 animals for hM3Dq –CNO and 3 animals for all other groups). e, f, h, i, Quantification of the distribution of PV+ (e, f) and SST+ cells (h, i) in mice injected with hM3Dq-mCherry (e, h) or hM4Di-mCherry (f, i) followed by vehicle (grey bars) or CNO (magenta bars) treatment at P10–P13 (two-way ANOVA, *F<sub>treatment</sub> = 0.15, *P = 0.71 (hM3Dq PV), *F<sub>treatment</sub> = 0.60, *P = 0.48 (hM3Dq SST), *F<sub>treatment</sub> = 1.00, *P = 0.37 (hM4DI PV), *F<sub>treatment</sub> = 1.78, *P = 0.25 (hM4DI SST); n = 4 animals for hM3Dq –CNO and 3 animals for all other groups). Data are shown as mean ± s.e.m. Scale bar, 100 µm.
Extended Data Fig. 6 | Loss of BAK and BAX prevents programmed cell death in pyramidal cells. a, Coronal sections through S1BF from P2 and P21 NexCre^+/−;Bak^−/−;Bax^fl/fl;Fucci2 mice immunostained for mCherry (green) and CTGF (yellow). b, Total number of pyramidal cells (excluding subplate cells) in the neocortex of P2 and P21 NexCre^+/−;Bak^−/−;Bax^fl/fl;Fucci2 mice (two-tailed Student's unpaired t-test, \( P = 0.30; n = 3 \) animals for both ages). Data are shown as mean ± s.e.m. Scale bar, 100 µm.
Extended Data Fig. 7 | Loss of BAK and BAX in pyramidal cells or MGE and POA interneurons affects densities but not lamination of MGE and POA interneurons. a, Quantification of the distribution of PV+ (left) and SST+ (right) interneurons in P30 control (grey), NexCre;Bak−/−;Baxfl/fl (dark magenta) and Nkx2-1-Cre;Bak−/−;Baxfl/fl (light magenta) mice (two-way ANOVA, F_{treatment} = 3.56, P = 0.10 (NexCre PV), F_{treatment} = 0.44, P = 0.53 (Nkx2-1-Cre PV), F_{treatment} = 0, P = 0.99 (NexCre SST), F_{treatment} = 0.44, P = 0.54 (Nkx2-1-Cre SST), n = 4 animals for NexCre;Bak−/−;Baxfl/fl (PV) and 5 animals for all other groups). b, Quantification of the fold change in the density of PV+ (top) and SST+ (bottom) interneurons in NexCre;Bak−/−;Baxfl/fl (dark magenta) and Nkx2-1-Cre;Bak−/−;Baxfl/fl (light magenta) mice compared to their respective controls (two-tailed Student’s unpaired t-test, *P = 0.90 (PV), *P = 0.67 (SST); for PV, n = 4 animals for NexCre;Bak−/−;Baxfl/fl, 6 animals for Nkx2-1-Cre;Bak−/−;Baxfl/fl; for SST, n = 5 animals for both NexCre;Bak−/−;Baxfl/fl and Nkx2-1-Cre;Bak−/−;Baxfl/fl). c, Coronal sections through the motor cortex of P30 Bak+/−;Baxfl/fl and NexCre;Bak−/−;Baxfl/fl mice immunostained for parvalbumin (PV, left) and somatostatin (SST, right) and counterstained with DAPI (grey). d, Quantification of the density of PV+ (left) and SST+ (right) cells in the motor cortex of control and pyramidal cell-specific Bax/Bak double mutant mice at P30 (two-tailed Student’s unpaired t-test, *P = 0.02 (PV), +P = 0.01 (SST); for PV, n = 4 animals for both; for SST, n = 3 animals for both). Data are shown as mean ± s.e.m. Scale bar, 100 μm.
Extended Data Fig. 8 | See next page for caption.
Extended Data Fig. 8  |  PTEN expression in deep layer cortical interneurons and effects of loss of PTEN function on neurons and blood vessels. a, Coronal sections through layer 5 of S1BF from Nkx2-1-Cre;RCLtdTomato mice at P5, P7, P8 and P10, immunostained for PTEN and counterstained with DAPI (grey). PTEN expression is shown as a custom LUT in tdTomato-masked cells. b, Cumulative distribution of mean PTEN intensity in layer 5 and 6 MGE and POA interneurons (Kruskal–Wallis test, ***P = 0; n = 7,270 cells (P5), 4,544 cells (P7), 6,780 cells (P8) and 5,043 cells (P10) from 3 mice at each age). c, Coronal sections through S1BF from Ptenfl/fl and Lhx6-Cre;Ptenfl/fl mice at P16 immunostained for GABA (red, left), NeuN (green, middle) and isolectin B4 (IB4, cyan, right) and counterstained with DAPI (grey). d, Quantification of the density of GABA+ (far left) and NeuN+ GABA− (left) cells and vessel area (right) and diameter (far right) in P16 Ptenfl/fl (grey) and Lhx6-Cre;Ptenfl/fl (magenta) mice (two-tailed unpaired Student’s t-test, **P = 0.0035 (GABA), *P = 0.0326 (vessel area), P = 0.0810 (vessel diameter); Kolmogorov–Smirnov test, P = 0.1000 (NeuN+ GABA− cells), n = 3 mice for both genotypes). e, Quantification of the distribution of PV+ (left) and SST+ (right) cells in P16 Ptenfl/fl (grey) and Lhx6-Cre;Ptenfl/fl (magenta) mice (two-way ANOVA, Fgenotype = 0.29, P = 0.61 (PV); Fgenotype = 0.0004, P = 0.98 (SST); n = 4 Ptenfl/fl mice and 3 Lhx6-Cre;Ptenfl/fl mice). Data are shown as mean ± s.e.m. Scale bars, 100 µm.
Extended Data Fig. 9 | Pharmacological inhibition of PTEN during the interneuron cell death period increases interneuron survival.

a, f, Schematics of experimental design. b, Coronal sections through S1BF from P10 mice injected at P7–P8 with vehicle (left) or BpV(pic) (right) stained for isolectin B4 (IB4, cyan) and DAPI (grey). c, Quantification of blood vessel area (left) and diameter (right) in P10 mice treated with vehicle (grey) or BpV(pic) (magenta) (Kolmogorov–Smirnov test (vessel area), \(P = 0.60\); two-tailed unpaired Student’s t-test (vessel diameter), \(P = 0.58\), \(n = 3\) animals for each group). d, g, Coronal sections through S1BF from P21 mice injected at P7–P8 (d) or P12–P13 (g) with vehicle (left) or BpV(pic) (right) and immunostained for PV and SST and counterstained with DAPI. e, h, Quantification of the density of PV+ (left) and SST+ (right) cells in S1BF from P21 mice injected at P7–P8 (e) or P12–P13 (h) with vehicle (grey) or BpV(pic) (magenta) (P7–P8 groups: two-tailed unpaired Student’s t-test, \(*P = 0.04\) (PV), \(*P = 0.03\) (SST); \(n = 7\) mice for each group; P12–P13 groups: two-tailed unpaired Student’s t-test, \(P = 0.84\) (PV), \(P = 0.82\) (SST), \(n = 5\) animals for each group). Data are shown as mean ± s.e.m. Scale bars, 100 µm.
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Experimental design

1. Sample size
   Describe how sample size was determined.

   There was no specific statistical method to determine sample size. Each experimental condition was carried out with a minimum three biological replicates from two independent experiments. Variations between samples were also used to determine the suitability of the sample size (i.e., a large biological variation between samples would imply a larger number of biological replicates required). In addition, sample sizes were also based on previously published data using similar techniques.

2. Data exclusions
   Describe any data exclusions.

   In the DREADDs experiments, brains that were not highly infected (e.g., the infection zone was limited only to a few sections and not across all layers) or centered in the somatosensory cortex were excluded. For the analysis of PTEN fluorescence intensity in DREADDs experiments, 1 vehicle and 1 CNO brains were excluded due to the presence of fluorescence aggregates in the tissue.

3. Replication
   Describe the measures taken to verify the reproducibility of the experimental findings.

   All attempts at replication were successful and all data is shown. For each brain, a minimum of 4 coronal sections were used in the analysis. For each treatment or time point, a minimum three biological replicate were included. The exact number of each biological replicate can be obtained from the figure legend.

4. Randomization
   Describe how samples/organisms/participants were allocated into experimental groups.

   All treatments for DREADDs and for PTEN inhibitor experiments were randomly chosen within the litter. All other groups were assigned based on genotypes.

5. Blinding
   Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

   For Bak/Bax mutants, DREADDs and PTEN inhibitor experiments, image acquisition and analysis were done blind by randomly assigning the brains to a series of numbers. The identity of the mutants and treatments were revealed at the end of the analysis. For the analysis of Lhx6-Cre;Ptenfl/fl mice, image acquisition was done blindly and the analysis was performed using custom routines written in Matlab.

Note: all in vivo studies must report how sample size was determined and whether blinding and randomization were used.
6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

| n/a | Confirmed |
|-----|-----------|
| ✗   | The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.) |
| ✗   | A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly |
| ✗   | A statement indicating how many times each experiment was replicated |
| ✗   | 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 any assumptions or corrections, such as an adjustment for multiple comparisons |
| ✗   | Test values indicating whether an effect is present |
| ✓    | Provide confidence intervals or give results of significance tests (e.g. P values) as exact values whenever appropriate and with effect sizes noted. |
| ✓    | A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range) |
| ✓    | Clearly defined error bars in all relevant figure captions (with explicit mention of central tendency and variation) |

See the web collection on statistics for biologists for further resources and guidance.

7. Software

Describe the software used to analyze the data in this study.

Statistical analyses were done on Prism 7 or Matlab. Please see methods for specific details. For the PTEN intensity experiments, the Cell Profiler pipeline was used to extract the data on fluorescence intensity on masked TdTomato and GABA-positive cells respectively. ImageStudioLite was used to analyse and measure band intensities for the western blots. All image processing was done on ImageJ or Fiji. Microscope images were obtained using the Zen (Zeiss) and LAS AF (Leica) microscopes.

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). Nature Methods guidance for providing algorithms and software for publication provides further information on this topic.

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a third party.

No unique material was used in this paper.
9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

The following antibodies were used as previously validated in the literature:

- goat anti-CTGF (L-20, 1:200, Santa Cruz, SC-14939), rabbit anti-DsRed (1:500, Clontech 632496), goat anti-mCherry (1:500, antibodies-online, ABIN1440057), rabbit anti-GABA (1:2000, Sigma, A2052), mouse anti-GABA (1:500, Sigma, A0310), mouse anti-parvalbumin (1:1000, Swant, PV 235), rabbit anti-parvalbumin (1:5000, Swant, PV-27), rat anti-somatostatin (YC7, 1:300, Milipore, MAB354), rabbit anti-PTEN (Y1841:500, Abcam, ab32199), rabbit anti-pAKT (Ser473, Cell Signalling, 1:1000, 4060), HRP-conjugated mouse anti-AKT (Cell signalling, 1:1000, 4298), rabbit anti-actin (Sigma, 1:20000, A3854) and HRP-conjugated donkey anti-rabbit antibody (Thermo Fisher, 1:10000, SA1-200).

The following secondary antibodies were used:

- donkey anti-goat IgG (H+L) 488 (1:400, A-11055)
- donkey anti-goat IgG (H+L) 555 (1:400, A-21424)
- donkey anti-goat IgG (H+L) 647 (1:400, A-21447)
- donkey anti mouse IgG (H+L) 488 (1:400, A-21202)
- goat anti mouse IgG (H+L) 555 (1:400, A-21424)
- donkey anti-mouse IgG (H+L) 487 (1:400, A-31571)
- donkey anti-rabbit IgG (H+L) 488 (1:400, A-21206)
- donkey anti-rabbit IgG (H+L) 555 (1:400, A-31572)
- donkey anti-rabbit IgG (H+L) 647 (1:400, A-31573)
- donkey anti-rat IgG (H+L) 488 (1:400, A-21208)
- goat anti-rat-IgG (H+L) 555 (1:400, A-21434)
- goat anti-rat IgG (H+L) 647 (1:400, A-21247)

10. Eukaryotic cell lines

- a. State the source of each eukaryotic cell line used.
  - No eukaryotic cell lines were used
- b. Describe the method of cell line authentication used.
  - No eukaryotic cell lines were used
- c. Report whether the cell lines were tested for mycoplasma contamination.
  - No eukaryotic cell lines were used
- d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.
  - No eukaryotic cell lines were used

Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals

Provide all relevant details on animals and/or animal-derived materials used in the study.

All experiments were carried out following the guidelines of King’s College London Biological Service Unit and in accordance with the European Community Council Directive of November 24, 1986 (86/609/EEC). Animal work was carried out under licence from the UK Home Office in accordance with the Animals (Scientific Procedures) Act 1986.

We used the following mouse lines in our study:

- NexCre/Cre (Goebbels et al. 2006), Fucci2Fl/Fi mice (Lester et al. 2007), Nkx-2-1Cre/+ (Xu et al. 2008, JAX008661), RCltdTomato/ttdTomato (JAX07909), GCaMP5F/Fi (Madsen et al. 2015, JAX024106), Bak-/-;BaxFi/Fi (Takeuchi et al. 2005, JAX006329), Lhx6Cre/+ (Fogarty et al. 2007), PtenFi/+ (JAX006440) and CD1s obtained from Jackson Laboratories. Please refer to the method sections for more details.

Both male and female mice were used indiscriminately throughout the study. For the stereology, mice from the NexCre;Fucci were collected at the following stages P2, P3, P4, P5, P7, P10 and P21 while for the Nkx-2-1Cre; RCltdTomato (including tissue for PTEN expression levels) tissues were collected at P2, P5, P7, P8, P9, P10 and P21. For the Dereads experiment, tissues were collected at P8 (for PTEN analysis) and P21. For the BaxBak experiments (for MGE interneuron expression), all tissues were collected at P30. For the western blot analysis, tissues were collected from wildtype animals from the following ages - P5, P6, P7, P8, P9 and P10. For the PTEN inhibitor experiments, tissue were collected at P16. For the PTEN inhibitor experiments, tissue were collected at both P10 and P21 animals.
Policy information about studies involving human research participants

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.

No human research participants were used.