Optimization of interneuron function by direct coupling of cell migration and axonal targeting

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Neural circuit assembly relies on the precise synchronization of developmental processes, such as cell migration and axon targeting, but the cell-autonomous mechanisms coordinating these events remain largely unknown. Here we found that different classes of interneurons use distinct routes of migration to reach the embryonic cerebral cortex. Somatostatin-expressing interneurons that migrate through the marginal zone develop into Martinotti cells, one of the most distinctive classes of cortical interneurons. For these cells, migration through the marginal zone is linked to the development of their characteristic layer 1 axonal arborization. Altering the normal migratory route of Martinotti cells by conditional deletion of Mafb—a gene that is preferentially expressed by these cells—cell-autonomously disrupts axonal development and impairs the function of these cells in vivo. Our results suggest that migration and axon targeting programs are coupled to optimize the assembly of inhibitory circuits in the cerebral cortex.

The assembly of neural circuits involves a series of highly coordinated events, from cell fate specification and neuronal migration to the precise targeting of synaptic connections. While these processes are often studied separately, they must have been efficiently linked during evolution to optimize the formation of neural circuits. For instance, migrating pioneer neurons establish permissive environments for specific brain connections1–4, whereas the allocation of neurons into segregated cell layers facilitates the rapid assembly of functional networks5. However, links between cell fate specification, neuronal migration, and precise axonal targeting remain largely unexplored.

Neural circuits in the cerebral cortex consist of two major classes of neuron, excitatory pyramidal cells and inhibitory GABAergic interneurons. Cortical interneurons are highly heterogeneous, comprising several functional classes with unique morphological, electrophysiological, and molecular features6. Recent transcriptomic analyses in the mouse adult neocortex have identified over 20 molecularly distinct classes of interneurons7,8. Although different classes of interneurons cannot be distinguished based on a unique criterion9, axonal arborization is a major classification feature since it largely determines the function of interneurons in neural circuits5,6,10.

Cortical interneurons can also be classified based on their developmental origin and expression of key molecular markers9. Most interneurons derive from the medial ganglionic eminence (MGE) and belong to two major groups, parvalbumin-expressing (PV+) and somatostatin-expressing (SST+) interneurons11. This later group encompasses at least two major classes of cells, which can be distinguished by the presence (Martinotti cells) or absence of a dense axonal plexus in layer 112–15. The remaining classes of cortical interneurons originate in the caudal ganglionic eminence (CGE) and in the preoptic area (POA)16.

Interneurons reach the embryonic cortex via two highly stereotyped routes, the marginal zone (MZ) and the subventricular zone (SVZ)17, but the logic behind the segregation of interneurons into different migratory streams remains unclear. One possibility is that interneurons are specified into distinct classes before reaching the cortex, and the selection of a particular migratory route is part of an unfolding program of neuronal differentiation. This hypothesis is supported by interspecies transplantation experiments, which showed that the ability of interneurons to use distinct migratory routes differs between species18. Alternatively, interneuron specification might be influenced by the local environment in the cortex19, and so migratory route allocation might be independent of interneuron specification.

Here we found that different types of embryonic SST+ interneurons use distinct routes of migration through the embryonic cortex. In particular, Martinotti cells display a strong preference for migration through the MZ, a behavior that seems to be linked to the development of their prominent axonal arbor in layer 1. Translaminar PV+ interneurons also migrate preferentially through the MZ, which suggest that this might be a general mechanism for interneurons with axon arbors spanning across multiple cortical layers. These results suggest that interneurons are committed to distinct cell fates before their arrival in the cortex and reveal an unexpected degree of cell-autonomous coordination between different developmental programs during the assembly of neural circuits.

Results
Migratory route choice varies among different classes of interneurons. We investigated whether migratory route preference (SVZ versus MZ) varies during embryonic development using VgatCre;RCE mice, in which all GABAergic interneurons are labeled with GFP. Most interneurons (~75%) migrated via the SVZ, and

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Articles in Nature Neuroscience describe the migratory behavior of interneurons across various regions of the subpallium. The study highlights the existence of specific migratory route preferences for different classes of interneurons, as evidenced by the analysis of HttCre;RCE embryos. The immunohistochemical analysis of Gad65-GFP mice, repeated with similar results in 3 animals and showing distribution of different groups of GABAergic interneurons, supports this finding. The experiments using tamoxifen (Supplementary Fig. 1)21 suggest that cortico-interneurons when crossed with a reporter line in the absence of a migratory path, remain in the same migratory route once they have made a choice. This indicates that the fate of a specific class of interneurons is linked to migratory route choice, with this preference remaining constant throughout development (Fig. 1a–d).

Fig. 1 | Different interneurons exhibit migratory route choice biases. a–c. Coronal sections through the neocortex (NCx) showing immunohistochemistry for GFP in VgatCre;RCE embryos at various stages, repeated with similar results in 3 animals. d, Bar graphs represent mean ± s.e.m. for fraction of interneurons in the MZ (n=3 animals per stage). e,f,h,i. Coronal sections through the E15.5 cortex of Nkx2-1Cre;RCE (e), Gad65-Gfp (f), SstCre;Ai9 (h), and Dlx1/2CreER;Ai9 (i) mice, repeated with similar results in 3 animals and showing distribution of different groups of GABAergic interneurons. g. Cumulative distribution of fraction of interneurons in the MZ for each genotype at all stages examined (n=12 sections per animal, 3 animals per genotype and stage). j. Bar graphs represent mean ± s.e.m. for fraction of interneurons in the MZ for each genotype at all stages examined (n=3 animals per genotype and stage). Two-way ANOVA, post hoc Tukey HSD (honest significant difference) test, **P < 0.001. H, Hippocampus; LGE, lateral ganglionic eminence. Scale bars, 200 μm.

This preference remained relatively constant during development (Fig. 1a–d). We asked whether interneurons originating in different regions of the subpallium—MGE, CGE, or POA—have distinct migratory route preferences. We quantified the SVZ/MZ ratio for interneurons derived from the MGE or POA and from the CGE using Nkx2-1-Cre;RCE and Gad65-GFP mice, respectively. The fractions of MGE- or POA-derived and CGE-derived interneurons migrating through the MZ were very similar (~25%) and remained constant at all stages (Fig. 1e–g). These observations confirmed that migratory route choice is not determined by the place of origin of interneurons.

We next wondered whether specific classes of interneurons prefer different migratory routes. Because SST is the only neurochemical marker that is expressed by migrating interneurons embryonically, we analyzed the SVZ/MZ ratio for SST+ interneurons using SstCre;Ai9 mice. We found a much higher proportion of SST+ interneurons migrating through the MZ (~50%) than expected from the analysis of Nkx2-1-Cre;RCE mice (Fig. 1g,h,i). Similar results were obtained using Dlx1/2CreER;Ai9 mice (Fig. 1g,i), a ‘leaky’ transgenic strain that labels sparse SST+ interneurons (along with some prospective PV+ interneurons) when crossed with a reporter line in the absence of tamoxifen (Supplementary Fig. 1). These results suggest that cortical interneurons are not randomly allocated to any migratory route.

Migratory route choice is stable and cell-autonomous. We reasoned that if the fate of a specific class of interneurons is linked to a migratory route, then interneurons should remain in the same migratory route once they have made a choice. To test this idea, we electroporated the MGE of embryonic day (E) 13.5 Nkx2-1-Cre mouse embryos with a plasmid conditionally encoding Kikume Green-Red, a photoconvertible green-to-red fluorescent protein. Forty-eight hours later, we photoconverted individual interneurons in the MZ and tracked their migratory behavior for 14 h (Supplementary Fig. 2a and Supplementary Video 1). Most interneurons migrating through the MZ remained within this route during this period ( Supplementary Fig. 2b,e), even though many should have abandoned this route during that time based on their normal migratory speed (Supplementary Fig. 2d,e).

We devised a transplantation experiment to test whether migrating interneurons are cell-autonomously committed to a particular migratory route (Supplementary Fig. 2f). We obtained cell suspensions by physically separating the MZ and the SVZ in E16.5 embryos. In control experiments, we verified the efficiency of this protocol in Calb2CreER;Ai9 embryos, in which the first fraction obtained was enriched in Cajal–Retzius cells (tdTomato*), while the fourth and fifth fractions were enriched in SVZ Tbr2+ cells (Supplementary Fig. 3). We transplanted MZ or SVZ cells from Nkx2-1-Cre;Ai9 embryos into the pallial–subpallial boundary of acute slices obtained from wild-type embryos (Supplementary Fig. 2f). We found that migrating interneurons had a strong inclination to use the same route from which they were isolated (Supplementary Fig. 2g–i), which reinforced the idea that interneurons are committed to a particular route of migration.

SST+ interneurons in the MZ and SVZ unfold distinct molecular programs. Migrating interneurons have identical morphology regardless of their origin or migratory route21,44. To investigate whether SST+ cells migrating through different routes are
molecularly distinct, we carried out RNA-seq analyses using SST\(^+\) cells isolated from the MZ and SVZ using the method described above (Supplementary Fig. 3). We found 81 genes differentially expressed (DEseq R analysis, false discovery rate < 5%) between both populations of cells, of which 24 genes were upregulated in SST\(^+\) interneurons migrating through the MZ compared to the SVZ (Fig. 2a,d and Supplementary Fig. 4).

We wondered whether the genes enriched in either MZ or SVZ SST\(^+\) cells correspond to genes that are differentially expressed by distinct classes of interneurons in the adult cortex. To answer this question, we took advantage of a recent single-cell RNA-seq study that unbiasedly identified six different groups of SST\(^+\) cells in the visual cortex of adult mice\(^1\). We assessed mean \(z\)-scores for the 81 differentially expressed genes in different groups of adult SST\(^+\) interneurons (Fig. 2b and Supplementary Fig. 5). We found that genes enriched in SST\(^+\) interneurons migrating through the embryonic MZ had mean positive \(z\)-scores that mostly clustered within three main subtypes of SST\(^+\) interneurons, Cdk6\(^+\), Cbln4\(^+\), and Myh8\(^+\) cells (Fig. 2b,c). In contrast, these genes had negative \(z\)-scores for SST\(^+\) interneurons characterized by the expression of Chodl (Fig. 2b,c). Conversely, genes enriched in SST\(^+\) cells migrating through the embryonic SVZ had mean positive \(z\)-scores in Chodl\(^+\) interneurons, but negative scores in the other five groups of SST\(^+\) interneurons (Fig. 2c and Supplementary Fig. 5a,b). These results strongly suggest that SST\(^+\) interneurons migrating through the embryonic MZ and SVZ of the developing cortex correspond to different populations of SST\(^+\) interneurons in the adult cortex.

We also assessed mean \(z\)-scores for individual genes enriched in the MZ and found that only 3 of 24 genes were positively enriched in one population of SST\(^+\) interneurons and were selectively de-enriched in the other five populations (Fig. 2c). Specifically, Mafb, Maf, and Chl1 were found to be particularly enriched in the Cbln4\(^+\) population, which expresses a gene enriched in Martinotti cells\(^5\). In addition, we also observed that Chrm2, which encodes a specific marker of layer 5 Martinotti cells\(^6\), was highly enriched in both Cdk6\(^+\) and Myh8\(^+\) cell populations (Supplementary Fig. 5c). This analysis prompted us to test whether SST\(^+\) cells migrating through the embryonic MZ are prospective Martinotti cells.

**Martinotti cells preferentially migrate through the MZ.** Electrophysiological and morphological studies have shown that SST\(^+\) interneurons comprise at least two functionally distinct classes of interneurons: (i) layer 1-targeting Martinotti cells and (ii) locally projecting SST\(^+\) interneurons\(^7,8\). Unbiased labeling of interneuron progenitor cells at E14.5 in Pvalb\(^{Cre}\) embryos (Fig. 3a) revealed that 60% of SST\(^+\) interneurons populating layer 2/3 had large axonal arbors in layer 1, a characteristic feature of Martinotti cells (Fig. 3b,e), while the remainder were locally projecting SST\(^+\) interneurons (Fig. 3c,f). To test whether prospective Martinotti cells preferentially migrate through the MZ, we carried out pial-surface electroperoration experiments (Supplementary Fig. 6)\(^8\) using a conditional reporter plasmid to specifically target SST\(^+\) cells in this stream (Fig. 3c). To compare these results with the retroviral labeling experiments, we targeted the cortex of Pvalb\(^{Cre}\) pups immediately after birth, when a large fraction of layer 2/3 MGE-derived interneurons is still migrating tangentially\(^8\). As predicted from our biochemical analyses, we found that the vast majority of SST\(^+\) cells migrating through the MZ at this stage gave rise to interneurons with the morphology of Martinotti cells (90%; Fig. 3d,g,e). We also observed a substantial number of labeled SST\(^+\) interneurons in layer 5, the majority of which displayed Martinotti cell morphology (87%; Supplementary Fig. 7). In contrast, the fraction of labeled SST\(^+\) cells found in layer 4, which mostly contains locally projecting SST\(^+\) interneurons\(^9\), was very small (Supplementary Fig. 7). These experiments indicate that SST\(^+\) cells migrating through the MZ became Martinotti cells.

**Translaminar PV\(^+\) interneurons also migrate through the MZ.** We next wondered whether other interneurons with translaminar axons similar to those of Martinotti cells would also preferentially migrate through the MZ. Although most PV\(^+\) interneurons are basket cells whose axons arborize in the same layer in which the cell soma is located\(^1\), recent studies have described a small population of deep-layer PV\(^+\) interneurons with ascending translaminar axons\(^2,9\). Consistently, unbiased labeling of interneuron progenitor cells at E12.5 in Pvalb\(^{Cre}\) embryos revealed that about 10% of PV\(^+\) cells populating layers 5 and 6 were translaminar (Fig. 3h,i,l). By contrast, pial-surface electroporation in neonatal Pvalb\(^{Cre}\) pups revealed a much higher proportion of deep-layer translaminar PV\(^+\) cells (> 50%; Fig. 3j,k,l). Similarly to Martinotti cells, deep-layer translaminar PV\(^+\) interneurons preferentially migrated through the MZ.

**Martinotti cells leave their nascent axon in the MZ when they enter the cortex.** The previous results suggested that interneurons with translaminar axons use the MZ as their migratory route. We hypothesized that this might be directly linked to their axonal morphology and focused our attention again on Martinotti cells to test this idea. Since the developing MZ becomes layer 1 in the adult cortex and Martinotti cells have an axon in layer 1, we hypothesized that the migration of these cells through the MZ may influence their axonal arborization. To test this hypothesis, we performed time-lapse imaging experiments in slice cultures. Using Dlk1/2\(^{Cre}\)/Ai9 mice to sparsely label a small population of SST\(^+\) cells (Supplementary Fig. 1), we monitored the migration of prospective Martinotti cells at early postnatal stages as they ‘dove’ into the cortical plate. We observed that most (11 of 13) SST\(^+\) cells that entered the cortical plate from the MZ left behind a trailing process (Fig. 4a and Supplementary Video 2), whereas most presumptive PV\(^+\) basket cells labeled in Nkx2-1\(^{Cre}\)/Ai9 mice (Supplementary Fig. 1) using the same migratory stream did not (1 of 8; Fig. 4c and Supplementary Video 3). We measured the speed of the trailing process in both sets of experiments and found that only the trailing process of the prospective Martinotti cells remained stationary (Fig. 4b). Moreover, the final distance between the trailing process and the cell soma was significantly larger in SST\(^+\) than in presumptive PV\(^+\) basket cells (Fig. 4d). Finally, to determine the molecular composition of the trailing process in SST\(^+\) cells, we performed pial-surface electroporation with a conditional plasmid encoding membrane tdTomato and a truncated form of kinesin family member 5 (Kif5C\(^{A560}\)) that localizes to nascent axons\(^8\) (Fig. 4e). We found that Kif5C\(^{A560}\) labeled the tips of the processes that remained in the MZ (11 of 14 cells, with 4 nonpolarized; Fig. 4f). This finding confirms that the process left in the MZ by Martinotti cells was the nascent axon.

**Abnormal route choices in conditional Mafb mutants.** We next screened for migration defects in mouse mutants carrying loss-of-function alleles for some of the genes preferentially expressed by MZ-migrating SST\(^+\) interneurons (Fig. 2d and Supplementary Fig. 4). We observed no major changes in the fraction of SST\(^+\) cells taking the MZ or SVZ migratory routes in Neto1 and Elfn1 mutant mice (Supplementary Fig. 8). In contrast, conditional deletion of Mafb from SST\(^+\) interneurons led to a small decrease (~20%) in the fraction of SST\(^+\) interneurons migrating through the MZ compared to control mice, along with a corresponding increase in SST\(^+\) cells in the SVZ (Fig. 5a–d). Despite these changes in route choice, the laminar distribution of SST\(^+\) cells in the neocortex of postnatal day (P) 21 conditional Mafb mutants and control mice was comparable (Fig. 5e–g). Together, these experiments suggested that postmitotic MafB expression is required for migratory route allocation by a fraction of prospective Martinotti cells.
**Fig. 2** | Different classes of SST⁺ interneurons migrate through distinct routes. **a**, Heatmap representing relative levels (z-scores) of genes differentially expressed in SST⁺ cells migrating through the MZ or SVZ at E17.5, with false discovery rate (FDR) < 5% using the Benjamini–Hochberg method (n = 3 litters of embryos). **b**, Heatmap representing relative levels (z-scores) of MZ-enriched genes (from a) in different classes of SST⁺ interneurons from the adult visual cortex (data from ref. 8). **c**, Bar graph representing mean ± s.e.m. z-scores for all MZ-enriched (from b) and SVZ-enriched genes (Supplementary Fig. 5) expressed in SST⁺ interneurons (n = 41 cells for Chodl, 19 for Cdk6, 68 for Cbln4, 14 for Tascst2d, 41 for Myh8, and 33 for Th). Red boxes indicate molecularly distinct classes of SST⁺ interneurons with positive mean z-scores in MZ-enriched genes. **d**, Coronal sections through the E17.5 telencephalon illustrating mRNA expression of Mafb, Elfn1, and Neto1, repeated in 2 animals with similar results. **e**, Violin plot showing individual cells (dots) of z-scores for individual MZ-enriched genes at E17.5. Five genes are highlighted based on expression (n = 41 cells for Chodl, 19 for Cdk6, 68 for Cbln4, 14 for Tascst2d, 41 for Myh8, and 33 for Th). Five genes are highlighted based on expression. Three genes show positive mean z-scores uniquely in SST⁺Cbln4⁺ cells (putative Martinotti cells) and negative or zero mean z-scores in all other populations of SST⁺ interneurons. H, hippocampus; NCx, neocortex; SP, subplate; Str, striatum. Scale bar, 250 µm.
Layer 1 axon defects in Martinotti cells from conditional Mafb mutants. Although the final number of SST+ interneurons in the neocortex of conditional Mafb mutants was normal, our previous analysis showed that a fraction of these interneurons reached the cortex through an abnormal route. To test whether this may impact the development of axonal arbors by Martinotti cells, we analyzed the density of layer 1 axons (labeled with GFP from the 

![Image](image1.png)

Fig. 3 | Martinotti cells and PV+ translaminar interneurons preferentially migrate through the MZ. a.h, Schematic of experimental design for the labeling of interneuron progenitor cells using conditional retroviruses in Cre-expressing embryos. c.j, Schematic of experimental design for the labeling of interneurons migrating through the MZ using pial-surface electroporation of conditional reporter plasmids in Cre-expressing embryos. b.d.f.g, Representative images of SST+ interneurons at P21 obtained through viral labeling at E14.5, repeated with similar results in 30 cells from 5 animals (b,f) or pial-surface electroporation at P0, repeated with similar results in 40 cells from 10 animals (d,g). e, Quantification of the proportion of Martinotti cells found in viral labeling and pial surface electroporation experiments (n = 30 and 40 cells from 5 and 10 animals for viral injections and electroporation, respectively; two-tailed Fisher’s exact test, **P = 0.004). Error bars represent binomial proportion confidence intervals. i.k, Representative images of translaminar PV+ interneurons (i, left panel; k, both panels) and intralaminar PV+ interneurons (i, right panel) at P21 obtained through viral labeling at E12.5, repeated with similar results in 60 cells from 7 animals (g) or pial surface electroporation at P0, repeated with similar results in 39 cells from 8 animals (i). l, Quantification of the proportion of translaminar PV+ interneurons found in viral labeling and pial-surface electroporation experiments (n = 60 and 39 cells from 7 and 8 animals for viral injections and electroporation, respectively; Fisher’s exact test, ***P = 1.108 × 10^-5). Scale bar, 50 µm.

There are three alternative explanations for the observed phenotypes: (i) loss of MaB changed the fate of Martinotti and these cells did not migrate through the MZ; (ii) loss of MaB disrupted both migration and layer 1 axonal arborization independently; and (iii) layer 1 axonal arborization was secondary to the migration phenotypes caused by the loss of MaB. We use the co-expression of calretinin (CR)+ and calbindin (CB) (Supplementary Fig. 5c) to molecularly identify prospective Martinotti cells and found comparable fractions of SST+CR+ and SST+CB+ interneurons in conditional Mafb mutants and controls (Supplementary Fig. 10). To reinforce these results, we performed whole-cell patch-clamp recordings from layer 2/3 SST+ cells in the somatosensory cortex. Analysis of SST+ cells with ascending axonal projections into layer 1, identified by biocytin labeling in patched cells, revealed no significant differences in the intrinsic properties and excitability of Martinotti cells in controls and conditional mutants (P > 0.05 for all six parameters examined; Supplementary Fig. 11a–g). Together, these results suggest that loss of Mafb did not impact the specification of Martinotti cells.

To distinguish between the other two possibilities, we carried out a new series of experiments. Since a fraction of SST+ cells migrated normally through the MZ in conditional Mafb mutant mice (Fig. 5a–d), these cells should exhibit normal layer 1 axonal morphologies if the defects observed in many Martinotti cells were secondary to defective migration through the MZ. To test this, we performed pial-surface electroporation experiments in control and conditional Mafb mutant (Fig. 6d). We found that Martinotti cells that had migrated through the MZ developed similar layer 1 axonal arborizations in both control and Mafb mutants (Fig. 6e,i). These results suggest that loss of Mafb affected the development of Martinotti cells by disrupting their normal migration choice and, secondarily, their axonal arborization.
Defective inhibition of pyramidal cells in Mafb conditional mutants. We analyzed the functional consequences of the disruption of layer 1 axons in Martinotti cells. To examine the function of SST⁺ interneurons, we injected adeno-associated viruses engineered to express channelrhodopsin-2 (ChR2) in SST⁺ interneurons following Cre-mediated recombination in the somatosensory cortex in control and conditional Mafb mutant mice at P2. We then prepared acute slices at P50–P60 for whole-cell patch-clamp recordings in pyramidal cells. In initial experiments, we found that the minimum light intensity required to evoke an inhibitory postsynaptic current (IPSC) was similar between both genotypes (Supplementary Fig. 11h–k), which, together with the lack of difference in intrinsic membrane properties, suggests that both genotypes expressed comparable levels of ChR2. We next used subcellular ChR2-assisted circuit mapping to trigger localized synaptic release from SST⁺ interneurons at predefined locations while recording from pyramidal cells. We focused on layer 3 pyramidal cells to have sufficient spatial resolution to discriminate between SST⁺ inputs at different locations within the dendritic arbor of pyramidal cells (Fig. 6). We found a prominent reduction in IPSC amplitudes in layer 3 pyramidal cells in the mutant Mafb slices compared to controls (Fig. 6k,l). These results demonstrated a functional impairment of SST⁺ interneurons in conditional Mafb mutant mice.

In vivo functional impairments in Mafb conditional mutants. SST⁺ interneurons exhibit the highest level of orientation selectivity...
among the different classes of interneurons\(^{35,36}\). Orientation selectivity depends on synaptic inputs, which in the case of layer 2/3 SST\(^+\) interneurons is derived primarily from adjacent layer 2/3 pyramidal cells\(^{35,36}\). SST\(^+\) interneurons in turn influence layer 2/3 pyramidal cells by inhibiting their apical dendrites\(^{37}\). Since inhibition from SST\(^+\) interneurons modulates the visual tuning properties of pyramidal cells\(^{35,36}\), we reasoned that the reduced inhibitory drive observed in conditional \(Mafb\) mutant mice would likely impact the tuning responses of both pyramidal cells and SST\(^+\) interneurons. We measured visually evoked responses in individual neurons using calcium imaging in awake, headfixed mice (Fig. 7a,b). SST\(^+\) interneurons were labeled by injecting conditional adeno-associated viruses encoding GCaMP6s into V1 of control and conditional mutant \(Mafb\) mice. As previously reported\(^{35}\), we confirmed that layer 2/3 SST\(^+\) interneurons responded to drifting gratings and exhibited a broad range of orientation tuning (Fig. 7c). We found similar proportions of SST\(^+\) interneurons responding to drifting gratings in \(Mafb\) mutants and controls \((P=0.709, Kruskal–Wallis test)\), indicating that basic functional properties were preserved. This is consistent with the in vitro findings that intrinsic properties of SST\(^+\) neurons were not affected in \(Mafb\) mutants (Supplementary Fig. 11a–g). We then assessed the selectivity of these visual responses. We averaged the stimulus-evoked response for each drifting grating across all trials and found that the responses evoked by the preferred orientation in conditional \(Mafb\) mutants were
Fig. 6 Abnormal development of Martinotti cell layer 1 axons in conditional Mafb mutant mice. 

a, Schematic of experimental design for labeling interneuron progenitor cells using conditional retroviruses in Cre-expressing embryos. b–c, Coronal sections through P21 neocortexes, showing the morphology of Martinotti cells labeled through retroviral infection in Sst<sup>Cre</sup>;Mafb<sup>loxP/+</sup> (b) and Sst<sup>Cre</sup>;Mafb<sup>loxP/loxP</sup> (c) mice, repeated with similar results for n = 29 cells from 8 mice and 37 cells from 10 mice, respectively. d, Schematic of experimental design for the labeling of interneurons migrating through the MZ using pial-surface electroporation of conditional reporter plasmids. e–f, Coronal sections through P21 neocortex showing the morphology of Martinotti cells labeled by pial electroporation in Sst<sup>Cre</sup>;Mafb<sup>loxP/+</sup> (e) and Sst<sup>Cre</sup>;Mafb<sup>loxP/loxP</sup> (f) mice, repeated with similar results for n = 36 and 32 Martinotti cells from 9 and 8 mice, respectively.

g–h, Boxplots represent median, first and third quartiles, and 1.5 IQR of length of Martinotti cell axons labeled by retroviral infection in layer 1 (g) and layers 2–6 (h) at P21 (n = 29 and 37 Martinotti cells from 8 and 10 control and mutant mice, respectively; two-tailed Student’s t test with Bonferroni correction, ***P = 0.0005). Cumulative frequency plots for the length of Martinotti cell axons in layer 1 (g) and layers 2–6 (h) are shown next to the corresponding histograms.

i, Boxplots represent median, first and third quartiles, and 1.5 IQR of length of Martinotti cell axons labeled by pial-surface electroporation in layer 1 and layers 2–6 at P21 (n = 36 and 32 Martinotti cells from 9 control and 8 mutant mice, respectively; two-tailed Student’s t test with Bonferroni correction, P = 0.681 and P = 0.911, respectively).

j, Schematic of experimental design for the subcellular ChR2-assisted circuit mapping (sCRACM) of SST<sup>+</sup> interneuron outputs. Each circle depicts the target of a 70-μm<sup>2</sup> laser spot used to evoke inhibitory input onto a single recorded pyramidal cell, whose position is indicated by a white triangle. IPSC amplitude from each inhibitory spot is plotted as a heatmap in the overlaying image. R1–R5, rows 1–5.

k, Representative laminar profiles of mean IPSCs recorded in layer 3 pyramidal cells from control and mutant mice. For these profiles, heatmaps were collapsed into one dimension by averaging evoked IPSC amplitude at each spot per row. The position of the recorded cells is indicated by a triangle. L, Boxplots represent median, first and third quartiles, and 1.5 IQR of IPSCs for each input row (n = 21 cells from 10 Sst<sup>Cre</sup>;Mafb<sup>loxP/+</sup> mice and 19 cells from 11 Sst<sup>Cre</sup>;Mafb<sup>loxP/loxP</sup> mice; two-factorial ANOVA, mean IPSC vs. genotype–row, **P = 0.004).
Fig. 7 | Impaired visual responses of SST⁺ interneurons in Mafb mutant mice. a, Schematic of experimental design for the expression of GCaMP6s in SST⁺ interneurons using conditional adenovirus-associating virus (AAV) plasmids in Cre-expressing mice. b, Experimental setup for two-photon calcium imaging in V1 of awake, headfixed mice and representative two-photon images of layer 2/3 SST⁺ interneurons labeled with GCaMP6s in SstCre;Mafb^{lox/lox} and SstCre;Mafb^{+/+} mice. PMT, photomultiplier tubes. c, Calcium transients (ΔF/ΔF₀) of representative SST⁺ interneurons imaged during the presentation of eight oriented drifting gratings (0 to 315°; arrows and numbers indicate the angle of drift direction). For each genotype, the upper trace shows a single trial with gray regions indicating the periods of visual stimulation. The lower traces show the responses for all trials (gray) and the average response across trials (black). The orientation-tuning polar plot is also shown for each neuron. d, Average tuning curve for all SST⁺ interneurons in SstCre;Mafb^{lox/lox} and SstCre;Mafb^{+/+} mice, calculated from the average stimulus-evoked response (SER) for each of the eight directions of drifting gratings and normalized to the preferred orientation. Light gray shading indicates s.e.m. (n = 61 neurons from 7 SstCre;Mafb^{lox/lox} mice and 79 neurons from 7 SstCre;Mafb^{+/+} mice; Kruskal–Wallis test, ***P = 0.001, **P = 0.005; orthogonal orientations, *P = 0.063). e, Boxplots represent median, first and third quartiles, and 1.5 IQR of OSI for each genotype. Red crosses indicate the average across all fields of view (n = 7 fields of view for each genotype for SST⁺ interneurons and 6 fields of view for each genotype for pExc neurons; Kruskal–Wallis test, *P = 0.016). f, Boxplots represent median, first and third quartiles, and 1.5 IQR for percentage of orientation selective neurons (OSI > 0.25) for each field of view per genotype (n = 7 fields of view for each genotype; Kruskal–Wallis test, *P = 0.015). g, Pairwise correlation (Pearson correlation coefficient) matrices for all SST⁺ interneurons in one representative field of view for each genotype (n = 61 neurons in SstCre;Mafb^{lox/lox} mice and 79 neurons in SstCre;Mafb^{+/+} mice; Kruskal–Wallis test, **P = 0.009). h, Boxplots represent median, first and third quartiles, and 1.5 IQR for percentage of orientation selective neurons (OSI > 0.25) for each field of view per genotype (n = 7 fields of view for each genotype; Kruskal–Wallis test, *P = 0.015). i, Middle: the average of all pairwise correlations for each field of view, between SST⁺ interneurons (left; P = 0.035) and between pExc (right; P = 0.016), for each genotype. Red crosses indicate the average across all fields of view (n = 7 fields of view for each genotype for SST⁺ interneurons and 6 fields of view for each genotype for pExc neurons; Kruskal–Wallis test). Left and right: quantifications of the distributions of pairwise correlations between SST⁺ interneurons (left; n = 61 neurons from 7 SstCre;Mafb^{lox/lox} mice and 79 neurons from 7 SstCre;Mafb^{+/+} mice) and pExc neurons (right; n = 1,321 neurons from 7 SstCre;Mafb^{lox/lox} mice and 1,424 neurons from 7 SstCre;Mafb^{+/+} mice). j, Classification accuracy of a template-matching decoder trained on the activity of SST⁺ interneurons or pExc neurons to decode grating identity. Red crosses indicate the average across all fields of view. Dashed line indicates chance level of decoding accuracy (n = 7 fields of view for each genotype for SST⁺ interneurons, **P = 0.008; and 6 fields of view for each genotype for pExc neurons, P = 0.032; Kruskal–Wallis test). Scale bar, 100µm. All boxplots represent median, first and third quartiles, and 1.5 IQR.
significantly lower (Fig. 7d) and more variable (Fig. 7e) than in controls. We observed no significant differences in orthogonal orientations (P = 0.063 and P = 0.314, respectively). In line with these observations, we found that the orientation selectivity index (see Methods) of SST\(^+\) interneurons was significantly lower in conditional Mafb mutants compared to controls (Fig. 7f,g). The proportion of selective SST\(^+\) interneurons (orientation selectivity index > 0.25) was also greatly reduced in conditional Mafb mutants compared to controls (Fig. 7h).

We then tested the impact of the impaired activity of SST\(^+\) interneurons on the visual properties of layer 2/3 excitatory neurons. We used the same experimental approach but labeled all neurons with GCaMP6s and employed an activity-independent marker (tdTomato) to identify SST\(^+\) interneurons. Using this approach, we imaged calcium transients in both SST\(^+\) and SST\(^-\) neurons (putative excitatory (pExc) neurons). We found no significant difference in the average orientation selectivity of pExc neurons between controls and conditional Mafb mutants (n = 1,321 and 1,424 neurons for controls and mutants, respectively; Kruskal–Wallis test, P = 0.163). However, we found significantly higher trial-by-trial variability of evoked responses for all orientations (n = 1,321 and 1,424 neurons for controls and mutants, respectively; Kruskal–Wallis test, P = 0.043).

We next evaluated the impact of these single-cell functional impairments on the population-level representations of visual stimuli in V1. By calculating pairwise correlations between all neurons within a single field of view, we determined how strongly the activity of a cell was correlated with that of all other cells in one field of view (Fig. 7i). We found that both SST\(^+\) interneurons and pExc neurons displayed lower pairwise correlations in conditional Mafb mutants than in controls (Fig. 7i). Lower pairwise correlations in mutants may reflect differences in information coding of visual stimuli at the population level. To test this idea, we quantified how well neuronal populations represented a visual stimulus in both genotypes by using a decoder\(^39\). In brief, we used a template-matching decoder to predict which oriented grating was presented to the mouse for each trial. This prediction was calculated from the calcium responses of all cells imaged in a single field of view. The ability of the decoder to correctly classify the presented grating per trial was significantly lower in conditional Mafb mutants than in controls (Fig. 7k) for both SST\(^+\) interneurons and pExc neurons (Fig. 7k). These results suggest that population-level representations of visual stimuli were impaired in conditional Mafb mutants.

Discussion
Two general principles on the assembly of inhibitory neocortical circuits emerge from our experiments. First, interneuron wiring and function is optimized through a complex sequence of developmental events that begin long before the first synaptic contacts are established. Second, interneurons are fated to develop into specific classes by the time they invade the embryonic cortex. These observations suggest that different classes of interneurons are specified early in development through intrinsic programs that unfold over a protracted period of time.

Optimization of interneuron function through migratory route selection. Interneurons are generated in the subpallium and migrate tangentially to reach the developing cortex\(^40\). In spite of their relatively uniform appearance\(^44\), migrating interneurons soon segregate into two parallel streams (MZ and SVZ) as they disperse through the embryonic cortex\(^7\). Although this seems to be a unique feature of mammalian cortical development\(^8\), its impact on neural circuit assembly has remained unclear.

Our results suggest that migratory route selection for cortical interneurons is linked to specific fates. Time-lapse imaging and transplantation experiments revealed that interneurons are committed to particular migratory routes. This suggests that interneurons migrating through the MZ or SVZ express different guidance molecules that contribute to their sorting into specific streams, as shown previously for striatal interneurons\(^41\). Although previous reports have suggested that migratory route allocation is independent of the place of origin of interneurons\(^8\), our experiments reveal that different types of cortical interneurons favor particular routes. For instance, SST\(^+\) Martinotti cells migrate preferentially through the MZ, whereas SST\(^-\) non-Martinotti cells disperse via the SVZ. Thus, at least for some types of interneurons, route choice is an early decision that must be molecularly encoded in the different genetic programs of these cells. Consistent with this idea, a recent transcriptomic analysis of CGE interneurons suggests that cell identity in this region is also linked to specific route choices\(^42\).

The migration of Martinotti cells through the MZ is linked to the development of their axon arbors in layer 1. In contrast to other migrating interneurons\(^35\)\(^–\)\(^38\), time-lapse imaging experiments revealed that prospective Martinotti cells leave their nascent axon in the marginal zone while they invade the developing cortical plate. This process is highly reminiscent of the development of cerebellar granule cells, which also leave their prospective axons in the molecular layer while migrating from the external granule cell layer to their final position in the cerebellum\(^43\). The precise molecular mechanisms linking the migration of Martinotti cells through the MZ and the normal targeting of their axons in layer 1 remain to be identified. It is conceivable that the embryonic MZ creates a permissive microenvironment for early synaptic stabilization, perhaps with the participation of Cajal–Retzius cells that are known to shape the architecture of layer 1\(^4\).

We found that MaB is an important factor controlling the migration of Martinotti cells through the MZ, although other molecules are likely involved. Our experiments revealed that only Martinotti cells that fail to migrate through the MZ have very poorly developed axonal arbors in layer 1, which suggests that migratory route is linked to axonal targeting. Although we cannot exclude other roles for MaB, our results indicate that normal development of axonal arbors in layer 1 impacts their ability to regulate the function of pyramidal cells. Considering the prominent role of Martinotti cells in neural circuits in the visual cortex\(^40\)\(^–\)\(^44\), the findings of (i) a deficit in SST\(^+\) axonal arborization in layer 1, (ii) a reduction in inhibition in layer 2/3 pyramidal neurons, and (iii) impaired tuning of visual responses are all consistent with each other and indicate that defects in a subset of Martinotti cells disrupt population-level representations of visual stimuli.

Early fate specification of distinct types of cortical interneurons. Several models have been proposed to explain the mechanisms underlying the diversity of cortical interneurons. One model proposes that interneuron identity is established shortly after cells become postmitotic\(^41\), even though many of the characteristics that distinguish a particular type of interneuron are not evident until adulthood. Implicit in this model is that a limited number of genetic factors are necessary to prime the development of newborn cells toward a particular fate\(^45\)\(^–\)\(^48\). Alternatively, the genetic information for cellular identity of a particular type of interneuron is only acquired once the cells arrive at their final position in the cortex\(^1\). In this model, newborn interneurons acquire a potentially uniform intrinsic program, only segregating into general or ‘cardinal’ classes. The specification of particular cell types would then require their interaction with the cortical environment, possibly through activity-mediated mechanisms\(^49\).

Our results strongly support the hypothesis that cells are fated to become specific types of cortical interneurons very early in development. Using layer 2/3 SST\(^+\) interneurons as a model, we found that cells within this main group of interneurons are specified to become
either Martinotti or non-Martinotti cells at least by the time they choose a migratory route to reach the embryonic cortex. Several lines of evidence support this conclusion. First, many of the genes that distinguish SST+ cells during their migration are enriched in specific types of SST+ interneuron in the adult cortex2,3, a strong indication that cell identity is established molecularly before morphological and electrophysiological properties emerge. Second, targeted electroporation of the MZ revealed that the vast majority of SST+ cells migrating through this route become Martinotti cells, which implies that non-Martinotti cells migrate through the SVZ. Third, despite the layer 1 axonal defects, Martinotti cells that fail to migrate through the MZ in conditional 

\[ \text{Mbp} \] mutants retain molecular (CR and CB co-expression) and electrophysiological (intrinsic properties) features of Martinotti cells, which suggests that they are specified before interacting with local cues in the MZ. Consistent with this idea, a recent single-cell RNA-seq study has shown that different groups of SST+ interneurons can be readily identified shortly after birth in the ganglionic eminences4. Nevertheless, additional experiments would be required to unequivocally demonstrate that Martinotti and non-Martinotti SST+ interneurons are specified before their arrival in the cortex. In any case, our results demonstrate that fate potential, at least for distinct subtypes of SST+ interneurons, precedes the emergence of distinctive interneuron properties.

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Author contributions

L.L. conceived and developed the project and performed most wet-lab experiments and analyses, with help from S.E.B. A.L. performed transplantation experiments. M.M.S. performed electrophysiological experiments with advice and support from A.M.-S. J.M.P.P. and N.L.R. designed and performed in vivo imaging experiments. L.L. and O.M. conceived the study and wrote the paper with input from all authors.

Competing interests

The authors declare no competing interests.

Additional information

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Methods

Mice. The following transgenic mouse lines were used in this study: A19 (B6.Gt(Rosa)26Sor<tm1(R220W)Kij>;Cba129S7); A19 (B6.Gt(Cba129S7);Ddx2/CreERT2(Tg(112cβ-eRT2-ALP);P2a-Cre);Elm1); Gal4-Cre; Mafbx<loxP/loxP> (B6.129-Nclod<tm1(Bv)>;Nkx2.1-Cre (C57BL/6-Tg(Nkx2.1-Cre)Sank)); Ddx2/CreERT2(Tg(112cβ-eRT2-ALP);P2a-Cre);Elm1); Gal4-Cre; Mafbx<loxP/loxP> (B6.129-Nclod<tm1(Bv)>;Nkx2.1-Cre (C57BL/6-Tg(Nkx2.1-Cre)Sank)); RCE ((B6.Gt(Rosa)26Sor<tm1(R220W)Kij>;Cba129S7);Ddx2/CreERT2(Tg(112cβ-eRT2-ALP);P2a-Cre);Elm1); Gal4-Cre; Mafbx<loxP/loxP> (B6.129-Nclod<tm1(Bv)>;Nkx2.1-Cre (C57BL/6-Tg(Nkx2.1-Cre)Sank)); RCE ((B6.Gt(Rosa)26Sor<tm1(R220W)Kij>;Cba129S7);Ddx2/CreERT2(Tg(112cβ-eRT2-ALP);P2a-Cre);Elm1); Gal4-Cre; Mafbx<loxP/loxP> (B6.129-Nclod<tm1(Bv)>;Nkx2.1-Cre (C57BL/6-Tg(Nkx2.1-Cre)Sank)); RCE ((B6.Gt(Rosa)26Sor<tm1(R220W)Kij>;Cba129S7);Ddx2/CreERT2(Tg(112cβ-eRT2-ALP);P2a-Cre);Elm1); Gal4-Cre; Mafbx<loxP/loxP> (B6.129-Nclod<tm1(Bv)>;Nkx2.1-Cre (C57BL/6-Tg(Nkx2.1-Cre)Sank)); RCE ((B6.Gt(Rosa)26Sor<tm1(R220W)Kij>;Cba129S7);Ddx2/CreERT2(Tg(112cβ-eRT2-ALP);P2a-Cre);Elm1); Gal4-Cre; Mafbx<loxP/loxP> (B6.129-Nclod<tm1(Bv)>;Nkx2.1-Cre (C57BL/6-Tg(Nkx2.1-Cre)Sank)); RCE ((B6.Gt(Rosa)26Sor<tm1(R220W)Kij>;Cba129S7);Ddx2/CreERT2(Tg(112cβ-eRT2-ALP);P2a-Cre);Elm1); Gal4-Cre; Mafbx<loxP/loxP> (B6.129-Nclod<tm1(Bv)>;Nkx2.1-Cre (C57BL/6-Tg(Nkx2.1-Cre)Sank)). All adult mice were housed in groups and kept on a reversed light/dark cycle (12/12) regardless of genotypes. Only time-mated pregnant female mice that had undergone in utero surgery were housed individually. Both male and female mice were used in all experiments. For developmental and histology studies, mice ages ranged from E14.5 to P30. For electrophysiology, mice ages ranged from P20–P28 for intrinsic properties recordings and P90–P60 for optogenetic stimulation. For in vivo two-photon imaging studies, mice ages ranged from P62–P75 at the time of imaging. All procedures were approved by the Institute of Neurosciences, King’s College London and the University of Edinburgh animal welfare committees and were performed under UK Home Office project licenses.

Histology. For in situ hybridization, embryonic mice were perfused transcardially with 4% paraformaldehyde (PFA) in PBS treated with DEPC, and the dissected brains were fixed overnight at 4°C in the same solution. Brains were embedded in 4% low-melting-point agarose and sectioned at 60 μm with a vibratome. Free-floating coronal sections were mounted on Super Frost slides and subsequently hybridized with digoxigenin-labeled probes, as described previously.

For immunohistochemistry, postnatal mice were perfused transcardially with 4% PFA in PBS and the dissected brains were fixed for 2h at 4°C in the same solution. Brains were sectioned at 60 μm on a vibratome or 40 μm on a freezing microtome and free-floating coronal sections were then subsequently used for immunohistochemistry as previously described. Embryonic mice were dissected and fixed overnight in 4% PFA in PBS. Brains were sectioned at 100 μm (E13.5 to E15.5) or 60 μm (E16.5 to E17.5) on a vibratome. The cortexes of E16.5 or E17.5 animals were dissected in 0.2 M sucrose solution (70 mM sucrose, 86 mM NaCl, 4 mM KCl, 1 mM NaH2PO4, 7 mM glucose, 0.05 mM kynurenic acid, 0.05 mM APV, 0.09 M NaSO4, 0.03 M K2SO4, and 0.014 M MgCl2. During digestion, the solution was oxygenated by bubbling 5% carbogen (95% O2/5% CO2). Cells were washed three times with DEPC-treated water, and processed for immunohistochemistry.

All primary antibodies used in this study were previously published: chicken anti-γ (H) antibodies (Molecular Probes) were conjugated with Alexa Fluor dyes and used at 1:500 (Clonetech, cat. no. 632496), rat anti-Somatostatin (1:200, Millipore, cat. no. AB1549), mouse anti-Parvalbumin (1:2,000, Swant, cat. no. 235), rabbit anti-Calretinin (1:2,000, Swant, cat. no. 7699/9H), rabbit anti-Calbindin (1:1,000, Swant, cat. no. CB-38a), rabbit anti-Thr-2β (1:500, Abcam, cat. no. ab23345), and rabbit anti-MafB (1:500, Atlas Antibodies, cat. no. HPA005653). Secondary antibodies (Molecular Probes) were conjugated with Alexa Fluor dyes and used at 1:500: chicken Alexa Fluor 488-conjugated anti-IgG (H+L), Alexa Fluor 647-conjugated mouse anti-IgG1, Alexa Fluor 488-conjugated rat anti-IgG (H+L), Alexa Fluor 546-conjugated rat anti-IgG (H+L), Alexa Fluor 555-conjugated rabbit anti-IgG (H+L), and Alexa Fluor 488-conjugated rabbit anti-IgG (H+L).

Cloning. Overexpression plasmids RV-CAG-dio-Kikume, RV-CAG-dio-mdtTomato, RV-CAG-dio-mEYFP, and RV-CAG-dio-Kif5C were cloned by PCR or overlapping PCR into a conditionally expressing retroviral backbone (Addgene 87662) using Ascl and Pacl as cloning sites. Kif5C560 was sequenced using pBA-KIF5C (Addgene 64211). In situ hybridization probes | www.nature.com/natureneuroscience

Time-lapse imaging. Slices were kept at 37°C and 5% CO2 for the duration of the time-lapse experiments. For photoconversion experiments, a plasmid encoding conditionally expressed Kikume (RV-CAG-dio-Kikume) was electroporated in the MGE of slices obtained from E13.5 Nkx2-1-Cre embryos. Two days after electroporation, Kikume-expressing cells in the MZ were visualized with a high-magnification objective (40× NA 0.9, Leica) on an inverted confocal microscope (SP2, Leica). Regions of interest were drawn manually around the somata of 10–12 cells per slice, and photoconversion was achieved by using 3× 3 average frame scan with a 405-nm laser set at 10 mW. Converted cells were verified by emission from GFP to RFP. Time-lapse images were taken at intervals of 2h per frame for a total of 16h per slice. At each time point, 4 or 5 fields of view were acquired with a z-stack interval of 10 μm, spanning 100 μm to cover both the dorsal and ventral telencephalon.

For early postnatal time-lapse imaging, time-mated E14.5 females from Nkx2.1<Cre/+>A9 were injected intraperitoneally with single doses of 2 mg/kg of tamoxifen diluted in corn oil. At P0–P1, brains from either Nkx2.1<Cre/+>A9 or Ddx2/CreERT2(Tg(112cβ-eRT2-ALP);P2a-Cre);Elm1); Gal4-Cre; Mafbx<loxP/loxP> (B6.129-Nclod<tm1(Bv)>;Nkx2.1-Cre (C57BL/6-Tg(Nkx2.1-Cre)Sank)) neonate mice were cut in 250-μm thick coronal slices in HBSS and cultured on Millicell (Millipore) organotypic membrane inserts in Neurobasal/B27. Slices were kept at 37°C and 5% CO2 for 6h to settle before imaging. Time-lapse images were taken at intervals of 1 to 1.5h per frame for 60–70h using a 25× water immersion objective (NA 0.95) in an inverted confocal resonant scanning microscope coupled with a hybrid CMOS-sensor and phase-contrast detection (SP2, Leica). At each time point, the acquired image included the cortical plate and the pial surface with z-stack intervals of 1.25 μm, spanning 60–70 μm.

Electrophysiology. For analysis of intrinsic properties, P28–P23

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decapitation, the brain was quickly removed and glued to a cutting platform before being submerged in ice-cold sucrose solution. Coronal slices (300 µm) were cut using a vibratome (Leica) and placed in cutting artificial cerebrospinal fluid (ACSF; in mM: 124 NaCl, 1.25 NaH2PO4, 3 KCl, 26 NaHCO3, 10 glucose, 2 CaCl2, 1 MgCl2, oxygenated with 95% O2/5% CO2) at 32 °C for 1 h, and then kept at room temperature (22–25 °C for recording.

Cells were visualized with an upright microscope (Olympus). Patch pipettes (3–5 MΩ) were made from borosilicate glass capillaries using a vertical puller (P10, Narishige) and contained 0.2% neurobiotin (Vector Laboratories, United Kingdom). Traces were recorded using a Multiclamp 700B amplifier (Molecular Devices, United Kingdom), sampled at 20 kHz and filtered at 3 kHz. Cells were excluded from analysis if the access resistance exceeded 25 MΩ.

For subcellular ChR2-assisted circuit mapping (cCRACM), PZ SstCre;MafbloxP/loxP or SstCre;MafbloxP/loxP mice were anesthetized with isoflurane and injected in the somatosensory cortex with AAV9-E1a-dio-ChETA (E121T/H134R)-eYFP-VPRE-bGH (Biophorum Operations Group). For quantification of axonal length, the maximum projection of z-stack images of single cells stained with fluorescent protein markers was quantified using a customized script written in Matlab (Mathworks). For each z-projection, axons were segmented using a low-intensity threshold, and the soma and dendrites were segmented by setting a high-intensity threshold.

RNA-seq analysis. RNA-seq data from MZ and SVZ FACS cells were mapped to mouse genome version mm9 (USCD), and count data were analyzed with Bioconductor (version 3.10). False discovery rates were calculated using the Benjamini–Hochberg method. DAPI counterstaining and defined as ROIs.

Quantification. Histology. Images were taken using fluorescence microscopes (DM5000B, CTR5000, and DMIURF from Leica or ApoTope.2 from Zeiss) coupled to digital cameras (DC500 or DFC350FX, Leica; OrcaR2, Hamamatsu) with the appropriate emission-filter sets or in inverted confocal microscopes (Leica TCS SP8 and Zeiss LSM800 Airyscan). Images stained with reporter proteins (GFP or DsRed) or specific markers were quantified using custom software written in Matlab (Mathworks; see code availability below). In brief, cell bodies were segmented using disk morphological shape function, size, and intensity thresholding. Background and high-density noise were removed using filtering. Anatomical regions such as the MZ, CP, and SVZ were determined using DAPI counterstaining and defined as regions of interest (ROI). An animal was considered a biological replicate. For each brain, 10–12 images spanning rostral to caudal regions of the somatosensory cortex were taken and treated as technical replicates.

For quantification of axonal length, the maximum projection of z-stack images of single cells stained with fluorescent protein markers was quantified using a customized script written in Matlab (Mathworks). For each z-projection, axons were segmented using a low-intensity threshold, and the soma and dendrites were segmented by setting a high-intensity threshold.

The resulting axonal binary image was achieved by subtracting the low-intensity threshold from the high intensity threshold images. Binary pixels were converted to 8-bit values and anatomical boundaries of cortical layers were determined by DAPI counterstaining and defined as ROIs.

Electrophysiology: Intrinsic properties were recorded in current-clamp mode and analyzed using Clampfit 10.7 (Molecular Devices, United Kingdom). The membrane potential was assessed directly after establishing a whole-cell recording. Afterwards, the membrane potential was biased to –70 mV for subsequent recordings. Input resistance was calculated from five increasing 5-pA, 300-ms current steps. The false discovery rates were calculated using the Benjamini–Hochberg method. DAPI counterstaining and defined as ROIs.

**RNA-seq analysis.** RNA-seq data from MZ and SVZ FACS cells were mapped to mouse genome version mm9 (USCD), and count data were analyzed with Bioconductor (version 3.10). False discovery rates were calculated using the Benjamini–Hochberg method. DAPI counterstaining and defined as ROIs.

**In vivo two-photon imaging.** Adeno-associated viral (AAVs) injections and cranial window implantations were performed as previously described. In brief, mice were anesthetized with isoflurane and AAVs were injected in V1 at three different depths (~250, 400, and 600 µm from cortical surface) with intracranial solution containing (in mM) 5 KCl, 130 potassium-gluconate, 10 HEPES, 2.5 MgCl2, 4 NaATP, 0.4 NaGTP, 10 sodium-phosphocreatine, 0.6 EDTA (pH 7.2–7.3, 285–295 mOsm) for current clamp, or in solution containing (in mM) 115 NaMeSO4, 20 CsCl, 10 HEPES, 2.5 MgCl2, 4 NaATP, 0.4 NaGTP, 10 sodium-phosphocreatine, 0.6 EDTA (pH 7.2–7.3, 285–295 mOsm) for voltage clamp. Intracellular solutions also contained 2% neurobiotin (Vector Laboratories, United Kingdom). Traces were recorded using a Multiclamp 700B amplifier (Molecular Devices, United Kingdom), sampled at 20 kHz and filtered at 3 kHz. Cells were excluded from analysis if the access resistance exceeded 25 MΩ.

Time-lapse imaging analysis. Time-lapse images were analyzed using the ImageJ (1.32, sequential image analysis). Neuronal cell bodies were selected manually based on downsampled frames (2 Hz), as well as maximum-intensity projections for each trial. Fluorescence traces were calculated as the mean pixel intensity for each cell soma. Fluorescence traces were calculated as the mean pixel intensity for each cell soma.

**Electrophysiology.** Intrinsic properties were recorded in current-clamp mode and analyzed using Clampfit 10.7 (Molecular Devices, United Kingdom). The membrane potential was assessed directly after establishing a whole-cell recording. Afterwards, the membrane potential was biased to –70 mV for subsequent recordings. Input resistance was calculated from five increasing 5-pA, 300-ms current steps. The false discovery rates were calculated using the Benjamini–Hochberg method. DAPI counterstaining and defined as ROIs.

**ScCRACM, analyses were done with using customized software written in Matlab (Mathworks). Responses were analyzed as the average response amplitude of the three repeats if a response was detected in at least two sweeps. Attenuation correction was applied. Attenuation, I( ¯g), was estimated as I( ¯g) = 1 – (r − c), where r is the distance from the soma of the stimulated spot. We used λ = 365, as has been reported previously for cesium-based intracellular solutions.**

In vivo two-photon imaging analysis. Imaging data were corrected for motion artifacts using discrete Fourier transformation-based image alignment (SIMA 1.3.2, sequential image analysis). Neuronal cell bodies were selected manually based on downsampled frames (2 Hz), as well as maximum-intensity projections for each trial. Fluorescence traces were calculated as the mean pixel intensity for each cell soma. Fluorescence traces were calculated as the mean pixel intensity for each cell soma.

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The variability across trials was calculated for each drifting period as the s.e.m. of the SER across all trials. The preferred orientation (SERpref) was defined for each neuron by calculating the average ΔF/Δt for each drifting direction of each orientation and by taking the orientation with the maximum SER value. Orientation tuning was quantified by calculating an orientation selectivity index (OSI, difference between SERpref and SERshort, where SERshort is the average response to the orientation orthogonal to the preferred one).
The correlation coefficient of two neurons is a measure of their linear dependence. For each field of view with \( N \) neurons, we calculated the Pearson correlation coefficient:

\[
    \rho(A, B) = \frac{1}{N-1} \sum_{i=1}^{N} \left( \frac{A_i - \mu_A}{\sigma_A} \right) \left( \frac{B_i - \mu_B}{\sigma_B} \right)
\]

where \( \mu_A \) and \( \sigma_A \) are the mean and s.d. of neuron A, respectively, and \( \mu_B \) and \( \sigma_B \) are the mean and s.d. of neuron B. The pairwise correlation coefficient matrix of all neurons is then the matrix of correlation coefficients for each pairwise variable combination.

To quantify the accuracy by which the four orientations could be classified based on the neuronal population activity, a template-matching decoder was employed, which compares the population activity to response templates of the different stimulus types. These templates are generated by taking the mean \( \Delta F/F_0 \) across trials during the presentation of each oriented grating \( \theta \) for each neuron in a single field of view, resulting in a template of population activity. The similarity of this template \( R^\theta \) to the actual population activity \( R^{stim} \) is given by:

\[
    I_\theta = \frac{\sum_{i=1}^{N} R^{stim}_i \cdot R^\theta_i}{|R^{stim}| |R^\theta|}
\]

where \( i \) indexes the \( N \) elements (neurons) of \( R \). The similarity index \( I \) is calculated for all orientations, and the decoded output is determined by taking the orientation with the highest similarity to the population activity. Decoder accuracy is given by the percentage of correctly decoded trials.

**Statistical analysis.** Error bars in all graphs indicate s.e.m. unless otherwise stated in the legends. For each experiment, the sample size and statistical tests used are summarized in Supplementary Table 1. No statistical methods were used to predetermine sample sizes, but our sample sizes are similar to those reported in previous publications. Mice were numbered randomly and selected sequentially. With the exception of time-lapse experiments, data collection and analysis were not performed blind to the conditions of the experiments. All other imaging and physiology data were collected with a reference number, with the experimentalist blinded to the genotype. Data analyses were performed in Matlab and R with custom-written code, and sample genotype information was compiled at the end of analysis. For all parametric statistical tests, data were tested for normality with Shapiro–Wilk tests and for equal variances using the F-test. No data point was excluded in the study.

**Accession codes.** RNA-sequencing data and the analysis pipeline used in this study are available at NCBI GEO under accession number GSE111150.

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

**Code availability.** Custom-written code in Matlab (Mathworks, USA) and R are available and accessible via public web based servers. Live versions of all codes can be found on GitHub. The project for in vivo calcium imaging analysis can be located at https://github.com/rochefort-lab/fissa. The codes used to generate and analyze sCRACM and histological images can be located in the corresponding folders at https://github.com/OscarMarinlab/Papers/tree/master/Lim2018/Figs_codes/Matlab_scripts.

**Data availability.** Data supporting the findings of this study are available along with its corresponding analysis code at https://github.com/OscarMarinlab/Papers/tree/master/Lim2018/Figs_codes. Raw RNA-sequencing data and the analysis pipeline used in this study are available at NCBI GEO under accession code GSE111150.

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Reporting Summary

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Statistical parameters

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

☐ n/a

☑ Confirmed

☐ The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement

☐ An indication of whether measurements were taken from distinct samples or whether the same sample was measured repeatedly

☐ The statistical test(s) used AND whether they are one- or two-sided

☐ Only common tests should be described solely by name; describe more complex techniques in the Methods section.

☐ A description of all covariates tested

☐ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons

☐ 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

Images were collected on confocal or fluorescence microscope using software by the manufacturer. For confocal & fluorescence, Leica LAS software was used to acquire the images. For Apotome images, Zeiss Zen blue software was used.

For electrophysiology recording, clampfit was used for data collection, Clampfit 10.7 (Molecular Devices)

In vivo two-photon calcium imaging was performed using a custom-built resonant scanning two-photon microscope running LabVIEW based software (version 8.2; National Instruments, UK).

Data analysis

Histological data such as cell counting, axon morphology:

Images are segmented in Matlab customized codes and statistical analysis were done in codes written in R. R codes are denoted with "R" and Matlab codes are denoted with "m".

RNAseq data:

MZ and SVZ FACS cells were mapped to mouse genome version mm9 (USCD), and count data were analyzed with Bioconductor software DESeq2 in R.

Slice time-lapse imaging data:

Time-lapse images were analyzed using ImageJ Mtrack2 plugin.

Electrophysiology: Intrinsic properties were recorded in current clamp and analyzed using Clampfit 10.7 (Molecular Devices, United Kingdom)

sCRACM analyses were done with using customized software written in MATLAB (Mathworks).

In vivo Ca2+ imaging experiments: Imaging data were corrected for motion artifacts using SIMA 1.3.2, sequential image analysis. Delta F/
F0 traces were generated and corrected for local neuropil contamination using the FISSA toolkit. All codes and corresponding data are deposited and published on GitHub. The links to the repositories are stated on the manuscript.

**Data**

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:
- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

Data availability statement:

Data supporting the findings of this study are available along with its corresponding analysis code at https://github.com/OscarMarinlab/Papers/tree/master/Lim2018/Figures_codes. Raw RNA-sequencing data and the analysis pipeline used in this study are available at the NCBI/GEO server with accession numbers GSE111150.

**Field-specific reporting**

Please select the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

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- [ ] Ecological, evolutionary & environmental sciences

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

**Life sciences study design**

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

**Sample size**

Sample size was calculated based on similar studies in the literature.

**Data exclusions**

No data was excluded.

**Replication**

All experiments were replicated by different using a different animal of the same genotype. For all histology experiments, 8 to 12 sections from the same animal were quantified to achieve a mean. This is then repeated for different animals and the reported measurements represent the mean of individual animals. For experiments where the n is cell numbers, cells are from at least 3 different animals. For slice culture experiments, time-lapse were performed on slices from different animals independently replicated on different experimental days with independently prepared solutions/ culturing medium. All attempts at replication have been successful.

**Randomization**

Animals were tagged with numbers randomly and selected by sequence of animal number.

**Blinding**

Images / data were analyzed with Matlab and R custom-written code and output files were blinded and compiled during analysis.

**Reporting for specific materials, systems and methods**

**Materials & experimental systems**

| n/a | Involved in the study |
|-----|-----------------------|
|     | Unique biological materials |
|     | Antibodies |
|     | Eukaryotic cell lines |
|     | Palaeontology |
|     | Animals and other organisms |
|     | Human research participants |

**Methods**

| n/a | Involved in the study |
|-----|-----------------------|
|     | ChIP-seq |
|     | Flow cytometry |
|     | MRI-based neuroimaging |
**Unique biological materials**

Policy information about **availability of materials**

**Obtaining unique materials**

All plasmids have been deposited on Addgene and will be released once the manuscript is accepted.

- RV-CAG-dio-Kikume - Addgene 87663
- RV-CAG-dio-mTdTm Tomato - Addgene 87665
- RV-CAG-dio-mEYFP - Addgene 87666
- RV-CAG-dio-Klf5c-2A-TdTomato - Addgene 87668
- T7T3-pacl-Neto1 - Addgene 86672
- T7T3-PacI-elfn1_459to1199 - Addgene 86671
- T7T3-Pacl_MafB_JSH - Addgene 87670

**Antibodies**

**Antibodies used**

Primary antibodies used are:
- chicken-anti-GFP (1:1000, Aves Lab)
- rabbit-anti-DsRed (1:500, Clonetech)
- rat-anti-Somatostatin (1:200, Millipore)
- mouse-anti-Parvalbumin (1:200, Swant)
- rabbit-anti-Calretinin (1:1000, Swant)
- rabbit-anti-Calbindin (1:1000, Swant)
- rabbit-anti-Tbr2 (1:500, Abcam)
- Rabbit-anti-MafB (1:500, Atlas antibody).

Secondary antibody used were all conjugated with Alexafluor dyes, used at 1:500, and purchased from Molecular Probes -
- Anti-Chicken IgG (H+L) 488
- Anti-Mouse IgG1 647
- Anti-Rat IgG (H+L) 488
- Anti-Rat IgG (H+L) 546
- Anti-Rabbit IgG (H+L) 555
- Anti-Rabbit IgG (H+L) 488

**Validation**

All antibodies used have been previously published and cited in the manuscript.

**Eukaryotic cell lines**

Policy information about **cell lines**

**Cell line source(s)**

Hek293FT

**Authentication**

Hek293FT were directly purchase from ATCC

**Mycoplasma contamination**

Cell lines were tested for mycoplasma contamination 4 times a year.

**Commonly misidentified lines**

(See ICLAC register)

None of the cell lines used is listed as commonly misidentified.

**Animals and other organisms**

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

**Laboratory animals**

The following transgenic mouse lines were used in this study: Ai9[B6.129Gt(Rosa)26Sortm9(CAG-tdTomato)Hze]51, Calb2Cre [B6(Cg)-Calb2tm1(cre)J2h)52, Dlx1/2-CreERT2 [Tg(I12b-cre/ERT2,-ALPP)37Fsh]53, Elfm154, Gad65-Gfp55, Mafbfl/fl 56, Neto1 [B6N.129-Neto1tm1.1(Mci)]57, Nkx2-1-Cre [C57Bl/6J-Tg(Nkx2-1-Cre)2Sand]58, Nkx2-1CreERT2[Nkx2-1tm1.1(cre/ERT2)J1h]59, RCE [(B6.129Gt(Rosa)26Sortm1.1(CAG-EGFP)Fsh]58, SstCre [B6N.Cg-Ssttm2.1(cre)J2h]52 and VgatCre [B6.FVB-Tg(Slc32a1-cre)2.1Hzo] 59. All adult mice were housed in groups and kept on reverse light/dark cycle (12/12 h) regardless of genotypes. Only time-mated pregnant female mice that have undergone in utero surgeries were house individually. Both male and female mice were used in all experiments. For developmental and histology studies, mice ages range from E14.5 to P30. For electrophysiology, mice ages range from P20-P28 for intrinsic properties recordings and P50-P60 for optogenetic stimulation. For in vivo two-photon imaging studies, mice ages range from P62-P75 at the time of imaging.

**Wild animals**

This study did not involve wild animals.

**Field-collected samples**

This study did not involve field-collected samples.