Conserved properties of dentate gyrus neurogenesis across postnatal development revealed by single-cell RNA sequencing

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The dentate gyrus of the hippocampus is a brain region in which neurogenesis persists into adulthood; however, the relationship between developmental and adult dentate gyrus neurogenesis has not been examined in detail. Here we used single-cell RNA sequencing to reveal the molecular dynamics and diversity of dentate gyrus cell types in perinatal, juvenile, and adult mice. We found distinct quiescent and proliferating progenitor cell types, linked by transient intermediate states to neuroblast stages and fully mature granule cells. We observed shifts in the molecular identity of quiescent and proliferating radial glia and granule cells during the postnatal period that were then maintained through adult stages. In contrast, intermediate progenitor cells, neuroblasts, and immature granule cells were nearly indistinguishable at all ages. These findings demonstrate the fundamental similarity of postnatal and adult neurogenesis in the hippocampus and pinpoint the early postnatal transformation of radial glia from embryonic progenitors to adult quiescent stem cells.

The dentate gyrus, part of the hippocampus, is involved in learning, episodic memory formation, and spatial coding. It receives unidirectional input from the entorhinal cortex through the perforant path and projects to the CA3, forming the first step of the hippocampal trisynaptic circuit. The dentate gyrus is composed of three layers: the cell-sparse molecular layer and the hilus, separated by a dense layer of granule neurons whose dendrites extend into the overlying molecular layer.

The majority of granule cells are born perinatally, with substantial numbers of cells generated until around postnatal day (P) 10 (Fig. 1a). Radial glia are neuronal stem cells during embryonic development and are still abundant in the first postnatal weeks. Radial glia-like cells differentiate into proliferating progenitors (neuronal intermediate progenitor cells or nIPCs) and exit the cell cycle to become migrating neuroblasts, then settle in the granule cell layer and mature around postnatal week 3 into fully functional excitatory granule cells.

In adult mammals, radial glia-like cells are neuronal stem cells (NSCs) in the subventricular zone and continue to generate granule cells from the dentate gyrus subgranular zone. Thus, a small number of progenitor cells in the subgranular zone continue to generate new granule cells throughout life, albeit at low rates. The process is believed to contribute to learning and memory formation, and it is sensitive to stress and antidepressants, as well as to enrichment and exercise. Upon differentiation in the adult, newborn neuroblasts go through a several-weeks-long process of maturation, acquiring mature membrane properties after about 3–4 weeks. While adult NSCs and early postnatal radial glia-like cells show many similarities, they act in different environments, perhaps requiring different regulatory mechanisms to maintain their neurogenic potential. However, it is not currently known whether NSCs are simply radial glia-like cells that persist or whether there are any molecular properties that make adult NSCs unique. Similarly, it is not currently known whether early postnatal and adult neurogeneses proceed through identical intermediate states or whether the adult process is different beyond timing from the initial postnatal waves.

Recent work using single-cell RNA-seq and targeting the neurogenic populations has suggested that neurogenesis forms a continuum of molecular states. However, we suspected that the limited number of cells in those studies, and the targeted sampling using specific markers, might have masked the presence of distinct cell types. We therefore performed larger-scale single-cell RNA-seq and unbiased sampling to delineate the cellular states along the granule cell lineage. We examined perinatal and juvenile development, as well as adult mice, and we used two complementary platforms to rule out batch effects and obtain both wide and deep sampling of cells and time points. We unraveled a branching lineage including all major neural cell types: astrocytes, oligodendrocytes, granule cells, and hippocampal pyramidal neurons or mossy cells. We observed a clear sequence of distinct steps that constitute granule cell development from quiescent stem or progenitor cells to mature neurons, and we validated these findings using in situ hybridization. Comparing cells across time from embryonic day (E) 16.5 to P132, we demonstrate a perinatal transformation of neurogenesis in the dentate gyrus, from an embryonic to a postnatal configuration. During the second postnatal week, radial glia shifted sharply from an embryonic to an adult state, and during the same time, cycling intermediate precursor cells shifted from a less to a more neurogenic state. A similar stepwise maturation of the granule cells was observed around the third postnatal week, establishing a distinct postadolescent granule cell identity. In contrast, neuroblasts and immature granule cells were indistinguishable at all ages.

Results

Cell census of the developing dentate gyrus. To address unanswered questions about the postnatal development of the dentate gyrus, we performed single-cell RNA-seq on microdissected mouse dentate gyrus. Dataset A comprised four timepoints: P12, P16, P24, and P35, including a total of 5,454 cells using droplet-based single-cell RNA-seq.
Fig. 1 | Transcriptional architecture of the mouse dentate gyrus. a, Sampling timepoints (top) along postnatal dentate gyrus development (schematic bottom). Left: perinatal migration of NPCs, generating outer granule layer (green). Middle: early postnatal radial migration of NPC.s from the neurogenic niche (orange). Right: adult neurogenesis from NPCs retained in the subgranular zone (red). b, Heatmap of 22 clusters from 5,454 cells (all sampled timepoints), represented by their top marker genes (648 genes), expression normalized by gene. Heatmap colors as in c. c, Dendrogram and correlation matrix of the 22 clusters identified. Yellow boxes in the dendrogram indicate main cell classes. VLMC, vascular and leptomeningeal cell; PVM, perivascular macrophage; OL, oligodendrocyte; NFOL, newly formed oligodendrocyte; OPC, oligodendrocyte precursor cell. d, t-SNE visualization of 5,454 cells, colored by cluster annotation. e, Structure of the dentate gyrus and previously described spatial organization of cell types in relation to clusters identified in this dataset (ML, molecular layer; GL, granule layer; H, hilus; CA3, hippocampus CA3 pyramidal layer; CR, Cajal–Retzius).

cell RNA-seq (10x Genomics Chromium; see Methods). To rule out batch effects, we include a set of experiments (Dataset B) using an orthogonal single-cell RNA-seq technology (valve-based microfluidics chips; Fluidigm C1), sampled at 17 developmental timepoints, with 2,303 cells. Based on these initial findings, we acquired a third, larger dataset (Dataset C, 10x Chromium), extending to earlier and later timepoints, comprising 24,185 cells from E16.5, P0, P5, P18, P19, P23, P120, and P132. We analyzed the datasets separately because they were obtained using different technologies (A and C vs. B) and kit versions (A vs. C). All datasets are summarized in Supplementary Fig. 1. We provide an online browsable resource (http://linnarssonlab.org/dentate) where gene expression can be visualized in individual cells and clusters of all three datasets.

Focusing first on Dataset A (Fig. 1a), graph-based clustering analysis (Markov clustering26 of the mutual k nearest neighbor graph; see Methods) revealed 22 distinct types of cells (Fig. 1b), which we arranged in four major categories (Fig. 1c): vascular (endothelial, pericytes, and vascular leptomeningeal cells), immune (microglia and perivascular macrophages), glial, and neuronal cell types. Visualizing these cells using t-distributed stochastic neighbor embedding (t-SNE27; Fig. 1d) revealed the entire architecture of the postnatal developing dentate gyrus in detail (Fig. 1e). At center, dominating the structure, was the granule cell lineage, starting with radial glia-like cells (RGLs), via nIPCs, two neuroblast stages (Neuroblast 1 and 2), immature granule cells, and mature granule cells. RGLs were closely related to, but clearly distinct from, astrocytes. Oligodendrocytes also formed a three-stage lineage (oligodendrocyte precursor cells, newly formed oligodendrocytes, and mature oligodendrocytes28), while all remaining cell types formed distinct and separated clusters. We found Reelin (Reln)-expressing Cajal–Retzius cells, which are required for proper morphogenesis and migration in both the cortex and the hippocampus, enriched in P10 and P16 samples. We found...
Fig. 2 | Dissecting the molecular progression through early neurogenesis. a. Mutual k nearest neighbor (KNN) graph of astrocytes and early neurogenesis-related clusters: RGLs (n = 165), nIPCs (n = 88), and Neuroblasts 1 (n = 97) and 2 (n = 777). Nodes represent cells (total n = 1,127 cells); edges connect mutual k nearest neighbors (k = 40). Examples of markers enriched in each type are indicated. b. Heatmap of genes with differential expression during early neurogenesis, sorted by their peak cluster. Full list of genes and expression provided in Supplementary Table 1. Above, the frequency of each cluster per age is depicted by circles (proportional to area). P24* and P35* include an estimated 85% of P24 or P35 cells, while 15% are contributed from the P16 and P12 samples, respectively, due to sex-based sample separation inaccuracy (see Methods). Heatmap colors as in Fig. 1c. c, d, e. Single-cell expression of representative top enriched markers for each stage (number of molecules). Numbers on the right indicate maximum detected expression. d, e. Mutual KNN graphs, as in a, stained for the expression of genes indicated. e. Mutual KNN graph with nIPC-specific genes removed (Supplementary Fig. 3). nIPCs were divided by their position along the transition between RGL and NB, as indicated by the line. Numbers of cells as in a, f. Distribution of the percentage of NB-like or RGL-like nIPCs positive for genes associated with cell cycle, neurogenesis, or progenitor cells.
Fig. 3 | Validation and spatial localization of early neurogenic markers across age. a, Schematic of the dentate gyrus and higher-magnification image of the granule layer. b, RNAscope multiplex in situ hybridizations of dentate gyrus sections from mice aged P10, P16, P24, and P37; representative images are shown. In a, Schematic of the dentate gyrus and higher-magnification image of the granule layer. In b, cell-type specific markers are highlighted. c, Proportion of cells positive for markers validated here (top) and other common markers from literature (bottom) for astrocytes and cell types in early neurogenesis (left) and other cell types summarized by class (right, gray circles). The circles’ areas are proportional to the frequency of cells with expression >0 within the cluster. ‘Other’ cell-class expression circles are outlined in red when the gene expression is enriched compared to neurogenic cell types. d, Schematic summarizing neurogenesis validation in the granule layer across timepoints. RGL and nIPC are represented by colored circles, as in c, yellow shaded area illustrates area enriched in mature granule cells (Pki5 staining in b), and pink shaded area is enriched in neuroblasts (Igfbpl1 staining in b).

that in addition to the more broadly expressed Reln, Cajal–Retzius cells highly specifically expressed homeobox domain genes such as Lhx1 and Lhx5 (Supplementary Fig. 2)⁹, as well as cell death regulators Tp73 (encoding p73) and Diabolo.

Radial glia-like cells are different from astrocytes and nIPCs. We first sought to identify the key cell types of early neurogenesis, i.e., RGLs, nIPCs, neuroblasts, and granule cells (Fig. 2). RGLs were identified by their close similarity to astrocytes (Gfap, Hes5, and Sox9) and distinct expression of recently described neuronal stem cell marker Lpar1 (also expressed in oligodendrocytes; Fig. 3c). They almost completely lacked expression of the aquaporin-encoding gene Aqp4, characteristic of mature astrocytes. They expressed both Nes (nestin) and Prom1 (also known as CD133), commonly used stem cell markers, although both genes were more highly expressed in endothelial cells (Fig. 3c). The complete absence of expression of cell-cycle genes such as Cdk1, Top2a, and Aurkβ showed that these cells are quiescent, in contrast to the actively dividing nIPCs.

nIPCs were an actively cycling progenitor population, with a strong cell cycle machinery gene signature (for example, Cdk1, Aurkβ, Top2a, and many more). In addition, these cells had taken on an early neuronal fate, expressing the known neurogenic transcription-factor-encoding genes Neurog2, Eomes, and Neurod4. This is consistent with previous findings in the developing neocortex, where Eomes (also known as Tbr2) is not expressed by RGL cells; only when entering a proliferating intermediate progenitor state is Tbr2 switched on. In the t-SNE plot, which arranges cells based on their overall transcriptional similarity, nIPCs were located at the root of a trajectory leading to mature granule cells (Fig. 1d).

We further found two neuroblast populations, here defined as the first step of differentiation after nIPCs exit the cell cycle. The first population, Neuroblast 1, retained expression of Eomes (shared with nIPCs), Tlx2, and Calb2 (which encodes calretnin), while the second, Neuroblast 2, expressed Gal, and both populations shared expression of Sox11 and doublecortin (Dcx). Both neuroblast stages also expressed Igfbpl1, a marker that we found to be more specific than the widely used Dcx and Sox11 markers (Fig. 2). Together, our findings identify these cells as two sequential steps in the maturation of migrating neuroblasts.⁹
To identify possible transitions between these cell types, we reanalyzed astrocytes and the early neurogenesis clusters (RGL, nIPCs, and Neuroblasts 1 and 2) using a graph representation that shows pairs of mutual $k$ nearest neighboring cells in gene expression space (Fig. 2a). In this representation, cells are linked by graph edges when they are each other’s nearest neighbors in the high-dimensional gene expression space, suggesting possible transitions in expression space. Conversely, the absence of edges suggests disallowed paths through expression space. Graph edges confirmed the order of the trajectory from RGLs, via nIPCs to Neuroblast 1 and then Neuroblast 2. Assuming parsimony in gene expression, it also confirmed that neurogenesis proceeds essentially linearly through a sequence of steps and that the two neuroblast populations are sequential, not parallel. It also demonstrated the close relationship between quiescence and active division, and how is it related to Neuroblast 2. Assuming parsimony in gene expression, it also confirmed that neurogenesis proceeds essentially linearly through a sequence of steps and that the two neuroblast populations are sequential, not parallel. 

With dentate gyrus neurogenesis dissected into several molecularly distinct steps, we performed an unbiased search for cell-type-specific gene expression. Focusing on transcription factors, we rediscovered genes encoding previously known key regulators Hes5 (RGLs and astrocytes), Neurog2 (nIPCs), Neurod4 and Eomes (Neuroblast 1), and Sox11 (Neuroblast 2). In addition, using the wide scope of the current study, we identified several highly specific markers and present an expression sequence of single and combinatorial genes (Figs. 2a–c and 3c, Supplementary Fig. 3, and Supplementary Tables 1 and 2), marking the progression from RGL via nIPC to Neuroblasts 1 and 2. For instance, we found the transcription-factor-encoding Thap2c specifically expressed in quiescent RGLs and nIPCs and propose it as a marker in the context of early neurogenesis. Vmn1, Rhcg, and Wnt8b are further examples of genes more narrowly expressed in RGL cells than commonly used markers.

A fundamental outstanding question in dentate gyrus neurogenesis concerns the distinction between quiescent and active progenitors or stem cells. Is quiescence a distinct transcriptional state, or is it just the absence of cell division? What is the nature of the transition from quiescence to active division, and how is it related to...
to neuronal commitment? As mentioned, nIPC was the only cluster of cells that was proliferating, but this cluster already expressed neurogenic transcription factors. Both nIPCs and neuroblasts were depleted with age (Fig. 2b), whereas RGLs were maintained. But how could RGLs be maintained in the absence of cell division? We speculated that a small population of proliferating RGLs might have been clustered with nIPCs, which would not be unexpected since cell division involves the activation of large numbers of genes, which would dominate and drive clustering. To further dissect the proliferating nIPCs, we removed all genes expressed specifically in nIPCs (which would include cell-cycle genes), but not those shared with either RGLs or neuroblasts. We then asked whether individual nIPC cells were now more similar to neuroblasts or to RGLs. After recomputing the k nearest neighbor graph, we found that most nIPCs were now intermingled with the earliest neuroblasts (Fig. 2e and Supplementary Fig. 4), consistent with their commitment to neuronal differentiation. However, notably, a small number of cells that was proliferating, but this cluster already expressed neurogenic transcription factors. Both nIPCs and neuroblasts were depleted with age (Fig. 2b), whereas RGLs were maintained. But how could RGLs be maintained in the absence of cell division? We speculated that a small population of proliferating RGLs might have been clustered with nIPCs, which would not be unexpected since cell division involves the activation of large numbers of genes, which would dominate and drive clustering. To further dissect the proliferating nIPCs, we removed all genes expressed specifically in nIPCs (which would include cell-cycle genes), but not those shared with either RGLs or neuroblasts. We then asked whether individual nIPC cells were now more similar to neuroblasts or to RGLs. After recomputing the k nearest neighbor graph, we found that most nIPCs were now intermingled with the earliest neuroblasts (Fig. 2e and Supplementary Fig. 4), consistent with their commitment to neuronal differentiation. However, notably, a small number of cells remained close to RGLs in gene expression (expressing, for example, Hes5, Ascl1, Lpar1, and Nes) and did not express most neurogenic transcription-factor-encoding genes (Neurod1, Tbr1, Eomes, Neurod2, and Neurod4), but were still actively proliferating (many genes, including Cdk1, Top2a, Cerpe, Aurbk, Mcm2-8, and Mkk67). We confirmed this observation by splitting nIPCs into RGL-like and neuroblast-like subsets (Supplementary Fig. 4) and measuring the enrichment of progenitor-, cell-cycle-, and neuroblast-specific genes (Fig. 2e,f). This analysis suggests the existence of a small population of dividing RGLs that had not entered the neurogenic program. Thus RGLs were mostly quiescent, but could be maintained by occasional proliferation without differentiation (or, alternatively, by occasional asymmetric cell division). This also consistent with the observation in the subventricular zone that most stem cells are quiescent (91.4% based on the hGFAP:GFP reporter).

To validate genes expressed specifically in each of the steps of early neurogenesis (Fig. 2) and locate cell types in the tissue, we performed multiplexed RNA staining of the dentate gyrus (Fig. 3a). Ascl1+Tfap2c+Cdk1+ RGLs were almost entirely confined to the subgranular zone at all ages, as were the proliferating Ascl1+Tfap2c+Cdk1+ nIPCs (Fig. 3b). Neuroblasts 1 (Igfblpl1+Eomes+) or 2 (Igfblpl1+Eomes+) accumulated just above the subgranular zone, spanning almost the entire thickness of the granule cell layer at P10, but were transient and replaced by mature granule cells (Plks+Eomes+) at the later ages (Fig. 3b,d). These findings confirm the existence of distinct RGL, nIPC, and neuroblast cell types and reveal the spatial and temporal dynamics of these cells (Fig. 3d). Further, we showed that the separate or combinatorial expression of these genes labeled the cell types more specifically than previously used markers such as Glap, Hes5, or Sox2 (shared with astrocytes), Prom1 and Nes (shared with vascular endothelial cells), or Lpar1 (also expressed in oligodendrocytes) (Fig. 3c).

Rapid maturation of granule cells around postnatal week 3. Noting the rapid maturation of neuroblasts into mature granule
cells between P16 and P24 (compare *Igfbpl1* and *Plk5* stainings in Fig. 3b), we next focused on the later stages of neurogenesis. As granule cells matured, they transitioned from the still glia-like neuroblasts (Fig. 1d,e) into an immature granule cell state and finally to mature granule cells (Fig. 3b), we next focused on the later stages of neurogenesis. As granule cells matured, they transitioned from the still glia-like neuroblasts (Fig. 1d,e) into an immature granule cell state and finally to mature granule cells (Fig. 3b), we next focused on the later stages of neurogenesis. As granule cells matured, they transitioned from the still glia-like neuroblasts (Fig. 1d,e) into an immature granule cell state and finally to mature granule cells (Fig. 3b), we next focused on the later stages of neurogenesis. As granule cells matured, they transitioned from the still glia-like neuroblasts (Fig. 1d,e) into an immature granule cell state and finally to mature granule cells (Fig. 3b), we next focused on the later stages of neurogenesis. As granule cells matured, they transitioned from the still glia-like neuroblasts (Fig. 1d,e) into an immature granule cell state and finally to mature granule cells (Fig. 3b), we next focused on the later stages of neurogenesis. As granule cells matured, they transitioned from the still glia-like neuroblasts (Fig. 1d,e) into an immature granule cell state and finally to mature granule cells (Fig. 3b), we next focused on the later stages of neurogenesis. As granule cells matured, they transitioned from the still glia-like neuroblasts (Fig. 1d,e) into an immature granule cell state and finally to mature granule cells (Fig. 3b), we next focused on the later stages of neurogenesis. As granule cells matured, they transitioned from the still glia-like neuroblasts (Fig. 1d,e) into an immature granule cell state and finally to mature granule cells (Fig. 3b), we next focused on the later stages of neurogenesis. As granule cells matured, they transitioned from the still glia-like neuroblasts (Fig. 1d,e) into an immature granule cell state and finally to mature granule cells (Fig. 3b), we next focused on the later stages of neurogenesis. As granule cells matured, they transitioned from the still glia-like neuroblasts (Fig. 1d,e) into an immature granule cell state and finally to mature granule cells (Fig. 3b), we next focused on the later stages of neurogenesis. As granule cells matured, they transitioned from the still glia-like neuroblasts (Fig. 1d,e) into an immature granule cell state and finally to mature granule cells (Fig. 3b), we next focused on the later stages of neurogenesis. As granule cells matured, they transitioned from the still glia-like neuroblasts (Fig. 1d,e) into an immature granule cell state and finally to mature granule cells (Fig. 3b), we next focused on the later stages of neurogenesis.
Fig. 4e). A similar abrupt disappearance of immature mossy cells (Mossy-Calb2 cells) and appearance of mature mossy cells (Mossy-Sv2b cells) was observed, suggesting shared initiating processes.

Thus, our results so far show that the dentate gyrus granule cell lineage proceeds through a sequence of distinct cellular stages, with characteristic and stereotypical spatial distribution from the subgranular zone to the outer granule cell layer. The early cell types—RGLs, nIPCs, and neuroblasts—are clearly distinct states. The presence of a small fraction of dividing RGLs is consistent with self-renewal of the RGL pool, whereas the active cell division of all nIPCs, and their decline over time, support the notion that these cells are a transient amplifying cell type. As granule cells mature, they transition around the third postnatal week from an immature to a mature state, associated with a shift in gene expression involving large numbers of neuronal-related genes. Together, these findings refine and extend previous literature, and we provide extensive lists of cell-type-specific gene expression (Fig. 4b and Supplementary Fig. 6).

**Adult neurogenesis resembles early postnatal development.** A second fundamental question in postnatal neurogenesis concerns the relationship between embryonic development and adult neurogenesis. To what extent are these processes related? Is adult neurogenesis fundamentally different, relying on a distinct type of adult stem cell that differentiates through distinct cellular states to become mature granule cells, or are they one and the same, with developmental stem cells persisting into adulthood and reiteration of the developmental differentiation program? Notably, since these questions are about similarities, not differences, they cannot be adequately addressed using markers alone (i.e., the fact that a marker is shared does not rule out other molecular differences). In contrast, single-cell RNA-seq strongly limits the possibility of unobserved differences, and thus can reveal similarities with much greater confidence.

To address these questions, we generated a third, larger, dataset (Dataset C: 10x Chromium), spanning perinatal (E16.5–P5), juvenile (P18–P23), and adult (P120–P132) animals (Fig. 5a–d and Supplementary Figs. 1 and 7). Notably, this dataset extended the range of ages sampled to both embryonic and true adult stages. We again confirmed the previous findings: RGLs, nIPCs, and neuroblasts were distinct cell states conserved between juvenile and adult mice, and granule cells matured abruptly around P20. RGLs were also confirmed to be clearly distinct from juvenile and (adult) astrocytes (Fig. 5e), particularly expressing transcription-factor-encoding genes Sox4 and Ascl1, as well as Thesp (also known as SPOT14), a regulator of adult neurogenesis via lipid metabolism\(^{3,8}\) that is expressed in the quiescent radial and nonradial cells that give rise to the neuronal lineage in adult mice.

Overall, the t-SNE visualization revealed a similar process to that of Datasets A and B. We found all the same non-neuronal clusters and GABAergic neurons and the same trajectory from RGLs to nIPCs, to neuroblasts, to immature granule cells and then to mature granule neurons. These clusters also largely expressed the same enriched markers as previously observed (Fig. 5d). However, because the dissection at the earlier timepoints necessarily included some hippocampus proper, we now also observed immature pyramidal neurons (Fig. 5a). Notably, these cells branched from the granule cell lineage only at the late postmitotic neuroblast stage, indicating a shared differentiation trajectory.

Furthermore, the extension to perinatal time points (E16.5, P0, and P5) revealed the existence of a perinatal-specific early neurogenesis program (Fig. 5b,c). Perinatal radial glia (RG) were distinct from juvenile and adult RGLs, with greater expression of Sox4 and Sox11 (which are jointly required to generate the hippocampus\(^{13}\)), Vim at the younger ages, and an increased expression of Notch2 and Padi2 in the juvenile and adult (Fig. 5d). Both cell types were largely quiescent as indicated by the absence of reliable cell-cycle genes, including Top2a and Cdk1.

We also found perinatal-specific cycling radial glia (RG-cycling; Fig. 5d), which differed from nIPCs in their lower expression of genes encoding neurogenic transcription factors such as Eomes, Elavl2, Elavl4, Neurog2, Neurod4, Sox4, and Sox11. Although RG-cycling were only rarely observed after P5, they would be analogous to the cycling radial glia-like nIPCs (Fig. 2e,f) found in juvenile and adult animals, likely representing RGLs occasionally entering the cell cycle without differentiating. Thus, the stage of initiation of neurogenesis (RG and RG-cycling) showed a clear shift in the second postnatal week from an embryonic form (RG, RG-cycling) to a juvenile and adult form (RGL and cycling radial glia-like nIPCs).

In contrast, nIPCs spanned the full range of ages, from E16.5 to P132 (Fig. 5d), as did neuroblasts and immature granule cells. That is, adult forms of these cell types did not generate separate clusters from those of the perinatal or juvenile stages, in contrast to the clear distinction between perinatal and juvenile forms of radial glia. This was true both for cells sampled without selection and for hGFAP-GFP\(^{+}\) cells sorted by fluorescence-activated cell sorting (FACS, Supplementary Fig. 1g; compare also the result from Dataset B in Supplementary Fig. 5).

To quantify this observation (Fig. 6), we performed pairwise comparisons of perinatal versus juvenile and adult types (where we would expect significant differences for RG only), as well as juvenile versus adult (where we would expect minor differences). Comparing perinatal RG to juvenile RGLs (Fig. 6a), we found many genes that were both statistically significantly differentially expressed (red circles) and showed greater than twofold differences (outside the dashed lines), including Thesp in (2.48-fold, \(P = 1.7 \times 10^{-10}\)) the juvenile and Vim (2.79-fold, \(P = 7.7 \times 10^{-13}\)) in the perinatal. In contrast, comparing juvenile to adult RGLs (Fig. 6d), we found few genes that were significantly differentially expressed (e.g., Aldolc, \(q = 4.2 \times 10^{-14}\) and only three (Pla2g7, Sparc1, and Aldoc) showed greater than twofold change. Thus, RG cells, which initiate neurogenesis, switch their molecular identity after P5 and then maintain it until at least P132.

In contrast, both nIPCs and neuroblasts at all ages were near indistinguishable. nIPCs differed by only two genes between perinatal and juvenile (expressed at least twofold; Fig. 6b) and by three between juvenile and adult (Fig. 6c). In neuroblasts, not a single gene showed twofold difference between perinatal and juvenile or juvenile and adult mice (Fig. 6c,f). We conclude that although the initiation stage of neurogenesis differs between juvenile and adult compared to perinatal and embryonic, the differentiation trajectory of cells committed to neuronal fate is conserved from the nIPCs stage onwards.

One unique feature was observed during development that was absent in the adult. Immature granule cells were found only in young animals, while in adults these were almost absent (Supplementary Figs. 1 and 5d). This was validated in situ, where detection of intermediate marker Fxyd7 was strongly decreased, but not absent, in P24 and P37 (Fig. 4e). This does not necessarily indicate that adult neurogenesis skips a step, but more likely that the great abundance of mature granule cells (which accumulate by age) and the decreasing rate of neurogenesis at this age makes transient intermediate stages more difficult to observe.

**Discussion**

We have described the perinatal development and persistent adult neurogenesis of the dentate gyrus using single-cell RNA-seq. Our data strongly supports a unified process of early postnatal and adult neurogenesis, with a set of clearly defined, rather than continuous, cell types and transitions. Both the cell types and transitions were invariant over time, but with changes in the number of cells of each type as well as their spatial distribution (Figs. 2 and 5). The
long-lived stem cell of the dentate gyrus is the RGL, as supported by clonal analysis of Nes+ RGLs\(^8\). We found that RGLs were predominantly quiescent, even during development (Fig. 2), and that in that state they were marked by a very small number of highly enriched markers, which included Rhcg, Vnn1, and Lpar1.

After RGLs entered the cell cycle and the nIPC state, a large number of neurogenic transcription-factor-encoding genes, including Neurod1, Neurod2, Neurod4, and Eomes were induced, after which the cells likely would proceed irreversibly to a neuronal fate. However, a small proportion of cycling cells retained features of RGL and did not show robust activation of the neurogenic factors (Supplementary Fig. 4). Thus the pivotal event in dentate neurogenesis is the fate choice occurring as an RGL begins to divide.

Once nIPCs have committed to a neuronal fate, they either continue to divide or exit the cell cycle as neuroblasts. Data from the SVZ suggests that the average nIPC divides about four times\(^8\), resulting in an approximately eightfold amplification from RGLs to the neuroblast stage. Since we observed a decrease in actively cycling nIPCs with age—likely explained by a reduced amplification rate—depletion of neuroblasts should be expected. At the same time, the gradual reduction of nIPCs and neuroblasts (Fig. 5) confirms that they must be transient cellular states, while the abundances of RGLs and astrocytes remained roughly stable in adult animals. We thus speculate that once neurogenic transcription factors are induced, cells proceed unidirectionally and irreversibly through nIPC, Neuroblasts 1 and 2, and immature and mature granule cell stages.

Extending the analysis to late embryonic and early postnatal stages (perinatal, E16.5–P5), we found that the differentiation lineage was unchanged from the nIPC stage through neuroblast and immature granule cell stages, which were indistinguishable between E16.5 and P132. This reinforces the fundamental importance of the induction of neurogenesis, which once induced will proceed through a stereotyped differentiation trajectory. In contrast, on and before P5, including in the embryo at E16.5, the initiation stage of neurogenesis differed markedly compared with that in juveniles and adults. The quiescent radial glia differed, with a perfect separation at the second postnatal week: no perinatal RGs were found after P20, when mature granule cells were formed rapidly (a similarly sharp transition was observed for mossy cells). A parsimonious interpretation is that before P20, permissiveness for maturation of granule cells is absent, resulting in the accumulation of an immature granule cell type; these cells mature at all once when the environment becomes permissive. A shift in the neurogenic profile around P20 is consistent with early studies by Altman and colleagues, in which the transition of developmental to adult neurogenesis was seen to occur between P10 and P30 in the rat\(^1\). In Nestin-eGFp mice, a strong drop in GFP+ neuronal progenitors was reported between P14 and P28\(^8\).

Major efforts have been invested into isolating and studying populations of stem and progenitor cells, using a wide range of animal models and marker genes\(^4\). We showed that several of these marker genes are not specific to quiescent or cycling progenitors, greatly restricting their utility for such purposes. For instance, Gfap, Hes5, and Sox2 expression were shared with astrocytes, while markers like Prom1, Lpar1, and Nes were highly coexpressed by entirely different cell types (endothelial cells and oligodendrocytes; Fig. 3c), and even Rehn expression by Cajal–Retzius cells is not exclusive to this population (Supplementary Fig. 2).

The wide scope and unbiased nature of our study allowed us to suggest an array of sequential or combinatorial markers that are more narrowly expressed in the relevant cell types and stages of early and late neurogenesis. One such marker gene is Tja2pc, highly specific to RGL and nIPC in our dataset. Tja2pc (also known as Tja2pc and AP2γ) encodes a transcription factor that plays a key role in early mammalian extraembrionic development and organogenesis. A murine knockout for this gene is embryonic lethal as early as E7.5\(^{44,45}\). Recently, Tja2pc was shown to be involved directly in hippocampal neurogenesis and suggested to regulate the key neurogenic genes\(^4,12\). Our data suggest that Tja2pc is an exclusive marker for the hippocampus stem cell niche, and we speculate that one role could be to ensure a certain level of proliferation\(^4,45\), while at the same time determining the fate of subgranular zone RGLs by pre-specification. This would be similar to its function in the developing cortex where it specifies onset of a Tbr2 NeuD neurogenic lineage program specifically generating certain layer II/III neurons\(^44\).

Our findings establish the essential unity of perinatal, juvenile, and adult neurogenesis in the mammalian hippocampus (Supplementary Fig. 8), but with sharp changes at around the second (RGLs) and the third (granule cells) postnatal weeks; demonstrate that neurogenesis proceeds through distinct discrete states; show that the intermediate progenitor and neuroblast stages are molecularly conserved from E16.5 to P132; provide markers with high specificity for each state; show their spatial distribution during development; and suggest a simple model for hippocampal neurogenesis driven by the choice RGLs make to differentiate, or not, as they divide.

Methods

Methods, including statements of data availability and any associated accession codes and references, are available at https://doi.org/10.1038/s41593-017-0056-2.

Received: 20 April 2017; Accepted: 22 November 2017;
Published online: 15 January 2018

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Acknowledgements
The authors thank P. Enfors for critical discussion of the data and manuscript and A. Johnsson for lab management. The study was supported by the Knut and Alice Wallenberg foundation (2015.0041 to S.L.) and the Human Frontiers Science Program to A.Z. DNA sequencing was performed at the National Genomics Infrastructure, and single-cell RNA sequencing at the Eukaryotic Single-cell Genomics Facility, both at Science for Life Laboratory, Stockholm, Sweden.

Author contributions
H.H., A.Z., and S.L. designed the study. H.H. and A.Z. performed all experiments, analyzed the data, and prepared the figures. P.L. analyzed the data. H.H. and S.L. wrote the paper, with input from all authors.

Competing interests
The authors declare no competing financial interests.

Additional information
Supplementary information is available for this paper at https://doi.org/10.1038/s41593-017-0056-2.

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Methods

Animals. We used male and female wild type CD-1 mice (Charles River) and C57Bl/6 mice and hFGAP-FGP reporter mice on embryonic day E16.5 and between postnatal days P0–P12. Details on the mice used per experiment are found in Supplementary Tables 5 and 6. All experimental procedures followed the guidelines and recommendations of Swedish animal protection legislation and were approved by the local ethical committee for experiments on laboratory animals (Stockholms Norra Djurförsöksnämnd, Sweden).

Single cell dissociation. Dissection and single-cell dissociation were carried out as previously described. Briefly, mice were killed by an overdose of isoflurane, followed by transcardial perfusion through the left ventricle with artificial cerebrospinal fluid (aCSF, in mM: 87 NaCl, 2.5 KCl, 1.25 NaH₂PO₄, 26 NaHCO₃, 75 sucrose, 20 glucose, and varying concentrations of CaCl₂ and MgSO₄). The concentrations of CaCl₂ and MgSO₄ were adapted according to sampling age: 2 mM for each age except, on average, approximately 2.360 (A), and 2.450 (C) mM CaCl₂ and 1.5 mM MgSO₄, respectively. Cells were kept on ice or at 4 °C at all steps except enzymatic digestion. The brain was removed, 300-μm vibratome sections were collected, and the dentate gyrus was microdissected. Single-cell suspensions were prepared using the Papain kit (Worthington) with 25–35-min enzymatic digestion (depending on age), followed by manual trituration using a BSA-coated p1000 pipette or fire-polished Pasteur pipettes.

FAC sorting. hFGAP-FGP mice were killed for analysis and single cell suspensions were removed, 300-μm vibratome sections were collected, and the dentate gyrus was microdissected. Single-cell suspensions were prepared using the Papain kit (Worthington) with 25–35-min enzymatic digestion (depending on age), followed by manual trituration using a BSA-coated p1000 pipette or fire-polished Pasteur pipettes.

Fluidigm C1. For processing on Fluidigm C1, cells were resuspended in aCSF with 5% DNaCe, and diluted to 500–800 cells/μl for cell load on a 4°C on a microscope-sized chip. All capture sites were imaged and manually evaluated for the presence of a single, healthy cell; other wells were excluded from library preparation. cDNA synthesis, with unique molecular identifiers (UMIs) and 5′ sequencing library preparation by tagmentation followed by multiplexing, was carried out using the C1-STRT protocol, as described before. An overview of Fluidigm C1 experiments is found in Supplementary Table 6.

10x Genomics Chromium. Dataset A sampling was carried out on two experimental days (Supplementary Table 5), pooling samples from male and female mice in a single experiment each day to further reduce the risk of batch effects. The dentate gyrus of male (day 1, P35; day 2, P24) and female (day 1, P12; day 2, P16) mice were dissected and dissociated separately. Suspensions were pooled at equal ratios after visual inspection and counting. Dataset C was sampled as indicated in Supplementary Table 5. We then resuspended 300–1,000 cells/μl in aCSF and added to the RT mix to aim for sampling of around 3,000–5,000 cells. All downstream cDNA synthesis (13 PCR cycles), library preparation, and sequencing were carried out as instructed by the manufacturer (10x Genomics Chromium Single Cell Kit Version 1 (Dataset A) or Version 2 (Dataset C)).

Sequencing, QC, and data analysis. Libraries were sequenced on an Illumina HiSeq2000, 2500, or 4000 to an average depth of approximately 56,700 reads per cell (raw, Dataset A) or 200,000 and 43,000 reads per cell (mapped, Datasets B and C), yielding on average, approximately 4,660 (B), and 3,650 (C) distinct molecules and 1,340 (A), 2,580 (B), and 1,640 (C) genes per cell. Fluidigm C1 library reads were aligned and converted to mRNA molecule counts as described previously. 10x Genomics Chromium samples were aligned to the reference genome and converted to mRNA molecule counts using the cellranger pipeline (Dataset A: v.1.1, Dataset C: v.1.3) provided by the manufacturer.

Data analysis on 10X Chromium Datasets A and C, clustering, and visualization. 10X Chromium Dataset A (Dataset C) data files were loaded and merged to one dataset. For more uniform representation of timepoints, Dataset C data files were downsampled as indicated in Supplementary Table 5 (‘Cells_analysed’). Valid cells were defined as having >600 (C: >800) total genes, between 100 and 500,000 (C: 30,000–200,000) unique molecular identifiers, and no more genes >1.2. This resulted in 6,090 (C: 25,546) cells. Next, we removed 46 (C: 732) doublet cells based on coexpression (Pearson’s r > 0.5, and 0.5 < |r| < 0.8) and the threshold for doublet cells was set to minimum total molecules set to 1,500; for doublets remaining, C1qc (as described before) was used for exclusion was set more stringently to >0 detected molecules; for KNN, k = 10; and (iv) perplexity in t-SNE was set to 60. The number of clusters after MCL was 39; these were manually inspected and partly merged, resulting in 17 final clusters. Here, we started out with 4,425 valid cells and removed 719 suspected doublets, as well as cells belonging to small cliques, resulting in 2,706 cells. Clusters suspected to be low quality (lack of specific marker genes) were removed, resulting in 2,303 cells in the final dataset.

Dataset A: allocation of sampling age to cell. Dataset A sampling was carried out on two experimental days, each day pooling samples from male and female mice in a single experiment to reduce the risk of batch effects. Thus, the dentate gyrus of male (day 1, P35; day 2, P24) and female (day 1, P12; day 2, P16) mice were dissected and dissociated separately, but pooled at equal ratios to a single Chromium well. To separate male and female cells (and thus timepoints), the sampling timepoint (P12, P16, P24, or P35) was assigned to each cell according to sample name (10×_43_1 or 10×_46_1) and expression of sex-specific genes: Ddx3y, Ufy, and Eif2c5y as male-derived and Xist and Tifx for female-derived cells. We thus allocated each cell to a female (P12, P16) or male (P24, P37) source (i.e., if the sum of female genes was higher than that of the male genes, the cell was defined as female and vice versa). For zero expression of either gene sets, the sex was labeled unknown. Male was most likely, male (P24 or P37), as the male (P24 or P37) sex genes (mainly Xist) was detected in 85% of cells coming from similar samples of known female–only origin (data not shown). In these cases, we labeled the cells P24* or P37*, and expect that about 15% of cells classified were false-positives.

Analysis of early neurogenesis and granule cell maturation. To analyze early neurogenesis (Fig. 2) and granule cell maturation (Fig. 4), we generated separate mutual KNN graphs and lists of peak cluster enriched genes. For this, we loaded data only from relevant cell clusters (early neurogenesis: astrocytes, RGL, nIPC, NB1, and NB2; GC maturation: NB2, immature granules, and mature granules). Low or widely expressed genes (expressed in less than 20 cells or in more than 60% of all cells) were filtered, and the 5,000 most differentially expressed genes were selected by plotting log(CV) vs. log(mean) (as above). All cells were normalized to 10,000 molecules. PCA projections were calculated with 100 components, and correlation based on the PCA coordinates was calculated. A mutual KNN graph (k = 80) was calculated based on correlation as distance, and weights in the mKNN graph were replaced by the Jaccard distance (ratio of shared neighbors). Cliques (connected components) of fewer than 10 cells were removed. The graph was calculated (Matlab force option), and original clusters were visualized on the new layout. For analysis of early neurogenesis without nIPC-specific genes, the same pipeline was used, with the additional removal of genes significantly specific to nIPC using the rank-sum test (FDR 5%) after feature selection.

To identify enriched genes per peak cluster, we loaded data from relevant cell clusters (as above). For each transition identified by the KNN (for example, astrocytes to RGL), we then calculated significantly expressed genes using rank-sum tests and fold enrichment as the log mean average in the population divided by the log mean average in all groups. Significantly expressed genes were defined as having a FDR < 0.01 or in at least 50% of the populations, fold enrichment >1.5, and followed the requirement that fold enrichment in the first or last population be <0.1 (early neurogenesis) or <0.4 (GC maturation).
Pairwise cluster comparison. Prior to comparison, cells were normalized to 5,000 molecules. To remove nonrelevant genes potentially adding noise and false positives in later testing, rough-feature (gene) selection was performed, testing clusters of interest against all other clusters. Here, only genes passing FDR 20% after t test on log2((x+1)-transformed data were selected. Next, the top 3,000 differential genes were selected based on log2(CV) vs. log2(mean) (as above). Finally, we performed gene-by-gene rank-sum testing for each pairwise group, recording q values (FDR corrected P values) and log2(x)-fold change. The number of cells included in each cluster by age is listed in Supplementary Table 7.

RNAscope. CD-1 mice (Charles River) were killed with an overdose of isoflurane and perfused through the left ventricle with PBS. Brains were immediately dissected out, embedded in OCT on dry ice and stored at –80 °C. We collected 10-μm cryostat sections covering the anterior–posterior axis of the dentate gyrus. The sections were either fixed in 4% PFA for 5 min, rinsed in PBS, and stored at –80 °C until staining, or immediately frozen at –80 °C and fixed after thawing. RNAscope hybridizations were carried out according to the manufacturer’s instructions, using the RNAscope Multiplex Fluorescent (Advanced Cell Diagnostics) for fresh frozen sections. Briefly, thawed sections were dehydrated in sequential incubations with ethanol, followed by 30 min Protease IV treatment and washing in PBS. Appropriate combinations of hybridization probes (all Advanced Cell Diagnostics, here gene target name (mouse) and catalogue number: Ednrb 473801-C1, Cdk1 476081-C2, Aldoc 429531-C3, Ryr2 479981-C1, Dcx 478678-C2, Pks 479971-C3, Neurod6 444851-C1, Sirtb 479998-C3, Gad1 400951-C3, Ascl1 313291-C1, Tuj1 488861-C3, Eomes 429641-C1, Igf1r 488851-C2, Cad2 513641-C3, Fxyd7 431141-C1, Prx1 488591-C2, Ngfl 488781-C3, Lhx1 488581-C1, and Bdnf 405981-C2) were incubated for 2 h at 40 °C, followed by four amplification steps (according to protocol), DAPI counterstaining, and mounting with Prolong Gold mounting medium (P36930, Thermo Fisher Scientific). Images were acquired using a Nikon Ti-E with motorized stage.

Immunohistochemistry. CD-1 mice (Charles River) were killed with an overdose of isoflurane and perfused through the left ventricle with PBS, followed by 4% PFA. Brains were dissected out, postfixed in 4% PFA for 16 h, cryoprotected in 10% and 30% sucrose, embedded in OCT, and frozen and stored at –80 °C. We cut 16-μm sections on a cryostat, covering the anterior–posterior axis of the dentate gyrus, and frozen at –80 °C until staining. Before staining, thawed sections were treated with 1x Target Retrieval solution (Dako), according to the manufacturer’s instructions. Primary antibodies (goat anti-GFP (Rockland 600-101-215, 1:1,000), rabbit anti-GFAP (Dako Z-0334, 1:500), rabbit anti-GFP (Molecular Probes A-11122, 1:1,000), goat anti-AldolaseC (Santa Cruz Biotech. N-14 SC-12065, 1:500), and goat anti-PDGFRα (R&D AF1062, 1:200)) were incubated O/N, at 4°C, in PBS with 5% normal goat or donkey serum, 3% BSA, 0.5% sodium azide and 0.3% Triton X-100, followed by washing and incubation with appropriate secondary antibodies (donkey anti-goat Alexa Fluor 488, donkey anti-rabbit Alexa Fluor 555, donkey anti-rabbit Alexa Fluor 488, donkey anti-goat Alexa Fluor 555; all Molecular Probes, 1:1,000), overnight at 4 °C in PBS, counterstained with Hoechst (Molecular Probes), and mounted with ProLong Gold (Molecular Probes).

Statistics and reproducibility. No statistical methods were used to predetermine sample sizes; our sample sizes were determined iteratively. The study did not include any intervention, so no randomization was performed and data collection and analysis were not performed blind to the conditions of the experiments. The conclusions drawn from scRNA-seq data presented in this study are based on three independent experimental series performed on three different scRNA-seq protocols. We provide details of the number of experiments contributing to each dataset in Supplementary Tables 5 and 6. To identify genes enriched to specific cell types, we performed two-sided Wilcoxon rank-sum tests (no underlying distribution assumed), with correction for multiple testing (FDR), as detailed in the Method sections “Analysis of early neurogenesis and granule cell maturation” and “Pairwise cluster comparison.” For validation of marker genes identified in scRNA-seq, in situ hybridizations (see “RNAscope”) were carried out on 6 sections each of 2 mice per timepoint. Similar results were repeatedly obtained and representative images are presented. Immunohistochemistry was carried out on sections from two animals, with similar results. Further details on experimental design are found in the Life Sciences Reporting Summary.

Life Sciences Reporting Summary. Further information on experimental design is available in the Life Sciences Reporting Summary.

Data and code availability. The raw datasets generated in the current study are available in the NCBI Gene Expression Omnibus (GEO) repository, accession number GSE95753. We provide an online browsable resource of single-cell expression data for all three datasets at http://linnarsonlab.org/dentate/. The code used to perform analyses in this paper is available on GitHub at https://github.com/linnarsson-lab/DG_paper_public.

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50. Zeisel, A. et al. Brain structure. Cell types in the mouse cortex and hippocampus revealed by single-cell RNA-seq. Science 347, 1138–1142 (2015).
Experimental design

1. Sample size

Describe how sample size was determined.

No statistical methods were used to pre-determine sample sizes. Our sample sizes were determined iteratively, generating a total of three datasets. The number of animals and number of cells analyzed was set to ensure multiple animals contributed to each reported cell type (cluster).

2. Data exclusions

Describe any data exclusions.

Valid cells were defined to have >600 (C: >800) total genes, between 800-20,000 (C: 1000-30,000) molecules, and a ratio of molecules to genes >1.2. This resulted in 6,090 (C: 25546) cells. Next, we removed 46 (C: 732) doublet cells based on coexpression (>1 molecule) of any pair of the following marker genes: Stmn2 (neurons), Mog (oligodendrocytes), Aldoc (astrocytes), C1qc (microglia), Cldn5 (endothelial). These criteria were established during exploratory data analysis.

3. Replication

Describe whether the experimental findings were reliably reproduced.

The main findings were successfully reproduced in three independent datasets using two technological platforms.

4. Randomization

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

Samples were not randomized for the experiments. The study was exploratory and did not include any intervention.

5. Blinding

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

No blinding was 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 (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section) |
| ☑   | A description of any assumptions or corrections, such as an adjustment for multiple comparisons |
| ☑   | The test results (e.g. \(P\) values) given as exact values whenever possible and with confidence intervals 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 |

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

Software

Policy information about availability of computer code

7. Software

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

scRNA-seq analyses were performed using Cell Ranger v1.1, and v1.3 and MATLAB 2016B. RNAscope and Immunofluorescence imaging was carried out using the NIS-Elements AR (v 4.40) software and analyzed in Fiji/ImageJ (v2.0.0)

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.

Materials and reagents

Policy information about availability of materials

8. Materials availability

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

No restrictions apply

9. Antibodies

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

Commercial antibodies against well-known antigens were used, and validated by the manufacturer:
- goat anti-GFP (Rockland 600-101-215, 1:1000) (Li et al. 2016, RhoA determines lineage fate of mesenchymal stem cells by modulating CTGF–VEGF complex in extracellular matrix.)
- rabbit anti-GFAP (Dako, Z-0334, 1:500) (Zeisel et al. 2015)
- rabbit anti-GFP (Molecular Probes, A-11122, 1:1000) (Johansson et al. 2008. Extensive fusion of haematopoietic cells with Purkinje neurons in response to chronic inflammation.)
- goat anti-AldolaseC (Santa Cruz Biotech. N-14 SC-12065, 1:500) (Zeisel et al. 2015)
- goat anti-PDGFRA (R&D, AF1062, 1:200) (Marques et al. 2016)
10. Eukaryotic cell lines
   a. State the source of each eukaryotic cell line used. No cell lines used
   b. Describe the method of cell line authentication used. No cell lines used
   c. Report whether the cell lines were tested for mycoplasma contamination. No cell lines 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 cell lines 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 details on animals and/or animal-derived materials used in the study.

   Male and female wild type CD-1 mice (Charles River) and C57Bl/6 mice, as well as hGFAP-GFP reporter mice (Zhuo et al. 1997) on embryonic day E16.5, as well as between postnatal days P0-132, were used. All experimental procedures followed the guidelines and recommendations of Swedish animal protection legislation and were approved by the local ethical committee for experiments on laboratory animals (Stockholms Norra Djurförsökssetiska nämnd, Sweden).

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 used