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Primary cilia are required in a unique subpopulation of neural progenitors

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The apical domain of embryonic (radial glia) and adult (B1 cells) neural stem cells (NSCs) contains a primary cilium. This organelle has been suggested to function as an antenna for the detection of morphogens or growth factors. In particular, primary cilia are essential for Hedgehog (Hh) signaling, which plays key roles in brain development. Their unique location facing the ventricular lumen suggests that primary cilia in NSCs could play an important role in reception of signals within the cerebrospinal fluid. Surprisingly, ablation of primary cilia using conditional alleles for genes essential for intraflagellar transport [kinesin family member 3A (*Kif3a*) and intraflagellar transport 88 (*Ift88*)] and *Cre* drivers that are activated at early [*Nestin*; embryonic day 10.5 (E10.5)] and late [human glial fibrillary acidic protein (*hGFAP*); E13.5] stages of mouse neural development resulted in no apparent developmental defects. Neurogenesis in the ventricular–subventricular zone (V-SVZ) shortly after birth was also largely unaffected, except for a restricted ventral domain previously known to be regulated by Hh signaling. However, *Kif3a* and *Ift88* genetic ablation also disrupts ependymal cilia, resulting in hydrocephalus by postnatal day 4. To directly study the role of B1 cells' primary cilia without the confounding effects of hydrocephalus, we stereotaxically targeted elimination of *Kif3a* from a subpopulation of radial glia, which resulted in ablation of primary cilia in a subset of B1 cells. Again, this experiment resulted in decreased neurogenesis only in the ventral V-SVZ. Primary cilia ablation led to disruption of Hh signaling in this subdomain. We conclude that primary cilia are required in a specific Hh-regulated subregion of the postnatal V-SVZ.

adult neurogenesis | subependyma | ventricular zone | olfactory bulb | Gli1

The primary cilium, a minute elongated organelle with a (9+0) microtubular cytoskeleton (axoneme) on the surface of most cells, is essential for signal transduction and particularly for Hedgehog (Hh) signaling (1–4). The primary cilium, therefore, has very important functions during vertebrate development (5, 6), including the development of the central nervous system (7–9). Primary cilia are required for the expansion of progenitor pool during cerebellar development (10, 11) and in the formation of neural stem cells (NSCs) and progenitors in the adult hippocampus (12–14). Moreover, it has been shown that primary cilia regulate dendritic refinement and synaptic integration of adult-born hippocampal neurons (15). Recent evidence also shows that Arl13b in primary cilia is essential for the early polarization of the neuroepithelium and the formation of radial glia (16). In addition, primary cilia and Arl13b regulate migration and placement of interneurons in the developing cerebral cortex (17, 18).

The walls of the lateral ventricles retain an active germinal niche in the ventricular–subventricular zone (V-SVZ) that continues generating neurons and glial cells in the postnatal brain of many mammals (19). The astroglia-like NSCs (B1 cells) give rise to intermediate progenitor cells (C cells), which in turn generate neuroblasts (A cells) (20–22). These young neurons migrate along the rostral migratory stream to the olfactory bulb (OB). B1 cells retain epithelial characteristics, including an apical domain that contacts the lateral ventricle (23). This apical process contains a primary cilium and is surrounded by multiciliated ependymal (E1) cells in a pinwheel-like organization (23). Given their location and the important functions that primary cilia have in the processing of extracellular signals, B1 cells' primary cilia could have key roles in the reception of ventricular signals for the regulation of adult neurogenesis (24, 25). However, the function of B1 cells' primary cilia remains unknown. Genetically ablating primary cilia—by removing essential components of the intraflagellar transport (IFT) system (26)—inevitably eliminate the motile cilia of E1 cells, resulting in disruption of cerebrospinal fluid (CSF) flow and hydrocephalus. Because E1 cells and CSF are thought to play important roles in the regulation of B1 cell proliferation, it is not possible to dissociate non–cell-autonomous effects of disruption of ependymal cilia from direct effects of primary cilia removal in B1 cells.

Here we used various approaches to genetically ablate primary cilia in NSCs at different developmental stages and in different locations. Surprisingly, we found that primary cilia removal during fetal development had strikingly little effect on the development of the telencephalon. During early postnatal life, primary cilia were also dispensable in most B1 cells, but were essential in a specific Hh-regulated subdomain of the V-SVZ. Our results suggest that primary cilia function is tightly linked to Hh signaling within a restricted domain of the postnatal neurogenic region.

Results

Primary Cilia Removal in Fetal NSCs Results in No Major Developmental Forebrain Defects. To investigate whether primary cilia are required for normal telencephalon development, we conditionally ablated

Significance

The primary cilium, an antenna-like extension on the surface of many cells, is considered essential for growth factor and morphogen reception and transduction. Here we asked, what is the function of primary cilia that projects from the apical surface of neural stem cells (NSCs) into the ventricle? Surprisingly, the removal of primary cilia from fetal and early postnatal NSCs had little effect on forebrain development. Primary cilia, however, were essential for the function of NSCs in a restricted region of a major postnatal germinal niche, the ventricular–subventricular zone. Our data are consistent with primary cilia being essential for the normal proliferation of a subset of Hedgehog-responsive postnatal progenitors.

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the primary cilia in neural progenitors by crossing *Nestin::Cre* mice (27, 28) with mice homozygous for conditional alleles of the kinesin family member 3A (*Kif3a*) gene ($Kif3a^{fl/fl}$), which is essential for the IFT system that maintains the primary cilia (29). The *Nestin* promoter drives expression of *Cre* throughout the embryonic neural tube as early as embryonic day 10.5 (E10.5) (27, 28). $Nestin::Cre;Kif3a^{fl/+}$ mice had primary cilia on the apical surface of their radial glia, as visualized by using antibodies against the microtubule subunit acetylated tubulin (AcTub) and the primary cilia marker adenylyl cyclase III (ACIII) (Fig. S1 *A–C*). In contrast, $Nestin::Cre;Kif3a^{fl/fl}$ mice had lost most of their radial glial primary cilia by E14.5 (Fig. S1*A′*), with only a few cells retaining a primary cilium in the posterior regions of the V-SVZ. By E16.5 (Fig. S1*B′*) and postnatal day 0 (P0) (Fig. S1*C′*), no primary cilia were observed in V-SVZ wholemounts of $Nestin::Cre;Kif3a^{fl/fl}$ mice.

Consistent with the observation that the primary cilium was removed from many of the ventricular zone NSCs in $Nestin::Cre;Kif3a^{fl/fl}$ mice, we found that most cells in the cortex, striatum, and OB were devoid of a primary cilium (Fig. S1 *D′–F′*). Surprisingly, there was little, if any, defect in the neonatal cerebral cortex of $Nestin::Cre;Kif3a^{fl/fl}$ mutants. At P0, the mutant telencephalon was normal in size, with dorsal–ventral (D-V) and medial–lateral (M-L) dimensions (D-V: 4.03 ± 0.18 mm; M-L: 2.87 ± 0.14 mm) similar to $Nestin::Cre;Kif3a^{fl/+}$ controls (D-V: 3.80 ± 0.37 mm; M-L: 2.89 ± 0.16 mm) (Fig. S2 *A* and *D*). Hematoxylin and eosin staining showed that cortical layer organization was similar in $Nestin::Cre;Kif3a^{fl/fl}$ mice and controls (Fig. S2*B*). This result was confirmed by immunostaining for Tbr1 (deep layer cortical marker) (30) and Satb2 (upper layer cortical marker) (ref. 31; Fig. S2 *E–G* and *K–M*). In both $Nestin::Cre;Kif3a^{fl/fl}$ and control neonates, Cux1 was expressed in cells throughout the developing cortical layers (32), whereas Ctip2 was highly expressed within cortical layer 5/6, as well as in many cells in the striatum (33) (Fig. S2 *H–J* and *N–P*). The OB diameter was also similar between $Nestin::Cre;Kif3a^{fl/fl}$ (1.67 ± 0.04 mm) and control (1.61 ± 0.05 mm) mice (Fig. S2 *C* and *D*). The $Nestin::Cre;Kif3a^{fl/fl}$ V-SVZ also appeared surprisingly normal at birth, with the ventricular lumen comparable in size to controls (Fig. S2 *A* and *D*). Consistently, we observed normal expression of the cortical layer markers Tbr1, Satb2, Cux1, and Ctip2 in another cilia mutant mouse that we generated: $Nestin::Cre;Ift88^{fl/-}$ (Fig. S2 *Q–V*). Similar to Kif3a, intraflagellar transport 88 (IFT88) is an essential component of the IFT system that maintains the primary cilia. At P0, most of the cells in the forebrain of $Nestin::Cre;Ift88^{fl/-}$ mice also had no primary cilia (Fig. S1 *G′–I′*). These results suggest that genetic ablation of primary cilia does not result in major telencephalic developmental defects.

Early Removal of Primary Cilia Results in Reduced Proliferation in a Specific Subregion of the Postnatal V-SVZ. The above observations suggest that removal of primary cilia using *Nestin::Cre* (27, 28) and the conditional alleles $Kif3a^{fl/fl}$ or $Ift88^{fl/-}$ had little effect in cortical, striatal, and septal development. However, these animals developed hydrocephalus starting at P4, coinciding with ciliogenesis of E1 cells. Before hydrocephalus develops (P0–P4), the V-SVZ is highly proliferative and produces a large number of young neurons destined for the OB (34, 35). We therefore investigated the effect of primary cilia removal in $Nestin::Cre;Kif3a^{fl/fl}$ mice before confounding effects of hydrocephalus were apparent. At P0, basal bodies were clearly observed on the apical surface of V-SVZ progenitors (Fig. 1 *B–E*, red), and in controls each basal body was next to a primary cilium (Fig. 1 *B–E*, green). In contrast, in $Nestin::Cre;Kif3a^{fl/fl}$ mice, basal bodies were correctly localized in the apical domain, but we could not detect primary cilia by ACIII staining (Fig. 1 *B′–E′*).

In the absence of primary cilia, proliferation in the neonatal anterior cortical (AC), anterior dorsal (AD), anterior medial (AM), posterior cortical (PC), posterior dorsal (PD), posterior

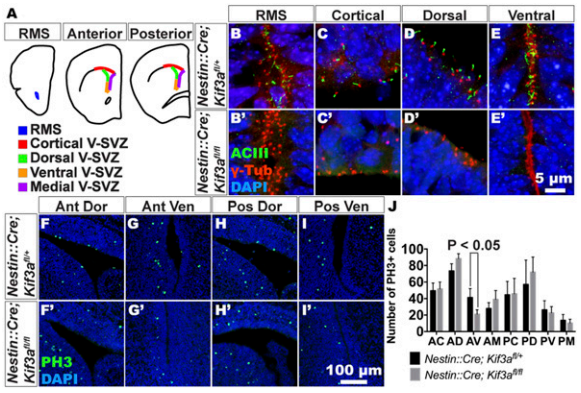

Fig. 1. Cilia ablation results in reduced proliferation in the anterior ventral (AV) V-SVZ in neonatal $Nestin::Cre;Kif3a^{fl/fl}$ mice. (*A*) For all analyses in this study, the ventricular wall is divided into cortical (red), dorsal (green), ventral (orange), and medial (purple) regions and is studied at two anterior–posterior levels. (*B–E*) Immunostaining for the primary cilia marker ACIII shows each radial glia extending a primary cilium toward the ventricular lumen in $Nestin::Cre;Kif3a^{fl/+}$ mice (*B–E*), whereas no primary cilium is observed in the $Nestin::Cre;Kif3a^{fl/fl}$ mice at P0 (*B′–E′*). Note that basal bodies visualized using anti–γ-tubulin antibodies are still present in the radial glia of $Nestin::Cre;Kif3a^{fl/fl}$ mice (*B′–E′*). (*F–I*) In the $Nestin::Cre;Kif3a^{fl/+}$ V-SVZ, antibodies against PH3 label the mitotic cells. (*F′–I′*) Although cilia ablation has occurred in all regions of the $Nestin::Cre;Kif3a^{fl/fl}$ V-SVZ, only the AV region has a decreased number of $PH3^+$ cells. (*J*) Bar graph shows mean ± SD from at least three mice per experimental group. *P* values are calculated using Student's *t* test.

ventral (PV), and posterior medial (PM) V-SVZ regions was similar in $Nestin::Cre;Kif3a^{fl/fl}$ and control mice (Fig. 1 *F* and *H–J* and Fig. S3 *A* and *C–E*). However, a subtle but significant decrease in phosphohistone H3 (PH3) staining (Fig. 1 *G′* and *J*) and 5-bromo-2′-deoxyuridine (BrdU) labeling (Fig. S3 *B′* and *E*) was observed in the anterior ventral (AV) region. Very few activated caspase-3^+ apoptotic cells were observed in both $Nestin::Cre;Kif3a^{fl/fl}$ and control animals (Fig. S3*F*), suggesting that the decreased numbers of proliferating cells in the AV V-SVZ was unlikely due to increased apoptosis. Similar observations of primary cilia ablation throughout the V-SVZ (Fig. S3 *G′–J′*), but reduced proliferation only in the AV region, were obtained when analyzing the $Nestin::Cre;Ift88^{fl/-}$ mice using PH3 staining (Fig. S3 *K–O*). These results suggest that primary cilia are not required for proliferation in most regions of the V-SVZ, but are essential for normal proliferation in the AV V-SVZ.

The reduced proliferation observed in the AV V-SVZ of $Nestin::Cre;Kif3a^{fl/fl}$ mice could be due to defects in the early embryonic specification of progenitor cells in this ventral domain (e.g., number of primary progenitors are reduced). We therefore investigated the effects of removal of *Kif3a* with *hGFAP::Cre* (36, 37) on V-SVZ proliferation. The human glial fibrillary acidic protein (*hGFAP*) promoter (and Cre) first becomes active dorsally (in cortex) at approximately E13.5 (36, 37), and then between P0 and P10 (38) Cre expression spreads ventrally in the V-SVZ facing the striatum. Consistently, we observed that *hGFAP::Cre;Ai14* reporter mice showed a dorsal-restricted pattern of Cre recombination at P0 (Fig. S4 *A* and *B*). Confocal microscopy revealed that, whereas most $Nestin^+$ cells in the cortical V-SVZ expressed tdTomato, very few $tdTomato^+$ $Nestin^+$ cells were observed in the dorsal and ventral V-SVZ (Fig. S4 *C* and *D*). In accordance with this Cre recombination pattern, $hGFAP::Cre;Kif3a^{fl/fl}$ mice had lost primary cilia only in the cortical V-SVZ at P0 (Fig. S4 *E′–H′*). These animals did not exhibit any significant change in the numbers of activated caspase-3^+ cells (Fig. S4*I*), $PH3^+$ cells (Fig. S4*J*), or $BrdU^+$ cells (Fig. S4*K*) in any of the V-SVZ subregions compared with $hGFAP::Cre;Kif3a^{fl/+}$ controls. This result is consistent with the above observations suggesting

that primary cilia are not required for normal proliferation in AC and PC V-SVZ.

By P10, *hGFAP::Cre* recombination (assessed by *Ai14* reporter expression) had extended ventrally (Fig. S4 *L* and *M*). Both endogenous GFAP and transgenic *Ai14* reporter expression were observed in all regions (cortical, dorsal, and ventral) of the V-SVZ (Fig. S4 *N–P*). In *hGFAP::Cre;Kif3a*$^{fl/+}$ controls, ACIII^{+} primary cilia were present dorsally and ventrally (Fig. S4 *Q–T*). By this stage, radial glia had differentiated into CD24^{+} E1 cells (Fig. S4 *N–P*) (39) or B1 cells. E1 cells' motile cilia were not stained by ACIII. The sparse distribution of ACIII staining was a reflection of B1 cells at the center of pinwheels (23). Consistent with the pattern of *Ai14* recombination, almost all primary cilia in the *hGFAP::Cre;Kif3a*$^{fl/fl}$ V-SVZ were ablated (Fig. S4 *Q′–T′*). The rare exceptions occurred generally in the most ventral V-SVZ, consistent with the observation that this region was the last to undergo recombination. When BrdU was injected daily from P0 to P10 (Fig. 2*A*), the numbers of BrdU^{+} cells in AC, AD, AM, PC, PD, PV, and PM regions of the V-SVZ were similar between *hGFAP::Cre;Kif3a*$^{fl/fl}$ mice and controls (Fig. 2 *B–D*). However, the AV region had significantly lower numbers of BrdU-labeled cells in *hGFAP::Cre;Kif3a*$^{fl/fl}$ mice compared with controls (Fig. 2 *B* and *E*). Consistent with this finding, when BrdU was injected twice at P0–P1 (Fig. S4*U*), by P10, we observed significantly higher numbers of BrdU-labeled cells in the AV V-SVZ (Fig. S4*V*). This increase was not due to reduced neuroblast migration and/or reduced cell death because the number of BrdU+ migrating neuroblast (Fig. S4*W*) and activated caspase-3^{+} cells (Fig. S4*X*) in the V-SVZ were similar in *hGFAP::Cre;Kif3a*$^{fl/fl}$ mice and controls. The increase in BrdU-labeled cells in the AV V-SVZ, when BrdU was only injected at P0–P1, was likely due to reduced proliferation and decreased dilution of the BrdU (40) between P1 and P10. However, by P10, *hGFAP::Cre;Kif3a*$^{fl/fl}$ mice had already developed hydrocephalus (Fig. 2 *C′* and *D′*), and changes in CSF composition and intraventricular pressure as a result of hydrocephalus could affect the proliferation of V-SVZ progenitors.

Selective Postnatal Ablation of Primary Cilia in B1 Cells Leads to Fewer Ventrally Derived Deep Granule Cells in the OB. To specifically ablate primary cilia in B1 cells while maintaining the integrity of the ependymal layer, we microinjected adenovirus expressing Cre (Ad::*Cre*) into *Kif3a*$^{fl/fl}$*;Z/EG* mice at P0 using developed methods (41, 42) (Fig. 3*A*). *Kif3a*$^{fl/fl}$*;Z/EG* mice are heterozygous for the *Z/EG* reporter, which constitutively expresses *LacZ* and conditionally expresses EGFP upon Cre recombination (43). By using stereotaxic technique, Ad::*Cre* was targeted at the distal ends of restricted populations of radial glial basal processes. The virus is transported to the radial glial cell body (41) where it recombines the floxed alleles (*Kif3a*$^{fl/fl}$*;Z/EG*), resulting in selective labeling and genetic recombination of a restricted group of radial glia. We used stereotaxic coordinates that allowed specific recombination of dorsal (Fig. S5 *A* and *B*) or ventral (Fig. S5 *C* and *D*) V-SVZ radial glia. The distribution of GFP^{+} cells in V-SVZ wholemounts (Fig. S5 *A* and *C*) and coronal sections (Fig. S5 *B* and *D*) confirmed that a restricted group of radial glia was targeted by these microinjections. By P40, B1 (Fig. 3*B*) and E1 (Fig. S5*G*) cells derived from Ad::*Cre*-infected *Kif3a*$^{fl/fl}$*;Z/EG* radial glia became organized as pinwheels similar to those in *Kif3a*$^{fl/+}$*;Z/EG* controls. The V-SVZ patches of affected cells had a mosaic distribution of recombined and wild-type cells, indicating that only a subpopulation of the radial glia within an injected region was infected by the Ad::*Cre*. In most cases, only one or two cells within a pinwheel was recombined. We frequently observed single GFP^{+} B1 cells with no primary cilia, but other GFP^{-} B1 cells within the same pinwheel retaining their cilia (Fig. 3*B′*). Quantification confirmed that the majority of recombined B1 cells in *Kif3a*$^{fl/fl}$*;Z/EG* mice had no primary cilia (Fig. S5 *E* and *F*). In contrast, primary cilia were observed in the GFP^{+} and GFP^{-} B1 cells of *Kif3a*$^{fl/+}$*;Z/EG* control mice (Fig. S5 *E* and *F*). GFP^{+} E1 cells in *Kif3a*$^{fl/fl}$*;Z/EG* mice had also lost their motile cilia (Fig. S5*G′*). Because the number of E1 cells affected was very small, these animals did not develop hydrocephalus.

Interestingly, it took >30 d for cilia to be ablated following the Ad::*Cre* injection at P0. We therefore injected BrdU to the mice at P40–P45 and analyzed their OB at P60 (Fig. 3*A*) to allow time for BrdU-labeled cells to migrate and differentiate. OB neurons that were GFP^{+} and BrdU^{+} (Fig. 3 *C* and *D*) were identified as the progeny of B1 cells that had undergone Cre recombination. Previous work has shown that dorsal- and ventral-targeted radial glia give rise to different populations of granule cells in the OB—superficial and deep granule cells, respectively (41, 44). Consistently, in both *Kif3a*$^{fl/fl}$*;Z/EG* and control mice, dorsal Ad::*Cre* targeting of V-SVZ progenitor cells gave rise to mostly GFP^{+} superficial granule cells (Fig. 3*E* and Fig. S5*H*), and ventral injections generated mostly GFP^{+} deep granule cells (Fig. 3*F* and Fig. S5*I*). This result suggests that loss of B1 cells' primary cilia does not change the specification of the types of neurons they produce. However, primary cilia loss in the ventral, but not the dorsal, B1 cells had a dramatic effect on the number of neurons generated: injection of Ad::*Cre* at P0, followed by BrdU administration at P40 resulted in much fewer adult-born (GFP^{+} BrdU^{+}) deep granule cells derived from the ventral V-SVZ (Fig. 3*F*), but no significant effect on the numbers of progeny neurons derived from the dorsal V-SVZ (Fig. 3*E*). These results indicate that primary cilia are not required for dorsal V-SVZ neurogenesis, but are essential in the ventral V-SVZ. This finding is consistent with observations made above using the *Nestin* and *hGFAP::Cre* lines.

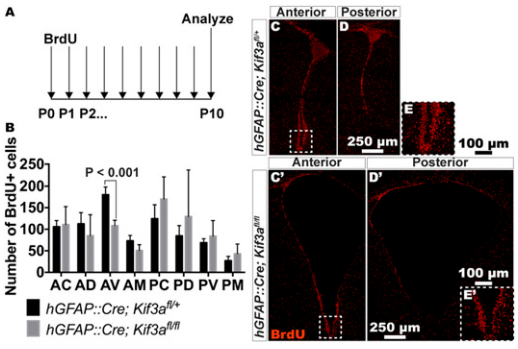

Fig. 2. Cilia removal leads to decreased proliferation in the AV V-SVZ in P10 *hGFAP::Cre;Kif3a*$^{fl/fl}$ mice. (*A*) *hGFAP::Cre;Kif3a*$^{fl/fl}$ mice retain most of their cilia at birth and lose almost all of their V-SVZ cilia by P10 (Fig. S4); therefore, we administered BrdU to these mice every day from P0–P10 and analyzed their brains at P10. (*B–E*) Compared with *hGFAP::Cre;Kif3a*$^{fl/+}$ controls, *hGFAP::Cre;Kif3a*$^{fl/fl}$ mice have decreased BrdU labeling in the AV V-SVZ (*C′*; magnified in *E′*), suggesting decreased proliferation in this specific subdomain. (*C′* and *D′*) Note that *hGFAP::Cre;Kif3a*$^{fl/fl}$ mice have enlarged ventricles, indicative of hydrocephalus. (*B*) Bar graph shows mean ± SD from at least three mice per experimental group. *P* values are calculated using the Student's *t* test. Multiple high-magnification images are tiled to form the final images in panels *C* and *D*.

Primary Cilia Are Required for Sonic Hh Signaling in the AV V-SVZ. Studies in the developing neural tube, limb bud, cerebellum, and dentate gyrus have shown that primary cilia are required for Sonic Hh (Shh) signaling (1–4, 11–13, 45–47). Notably, the AV V-SVZ—which exhibited reduced proliferation in *Nestin::Cre;Ift88*$^{fl/-}$, *Nestin::Cre;Kif3a*$^{fl/fl}$, *hGFAP::Cre;Kif3a*$^{fl/fl}$, and Ad::*Cre*-infected *Kif3a*$^{fl/fl}$*;Z/EG* mice—coincides with the V-SVZ domain with high Shh signaling (42, 44, 48). Indeed, in situ hybridization (ISH) showed an enriched expression of *Gli1*, a Shh target gene,

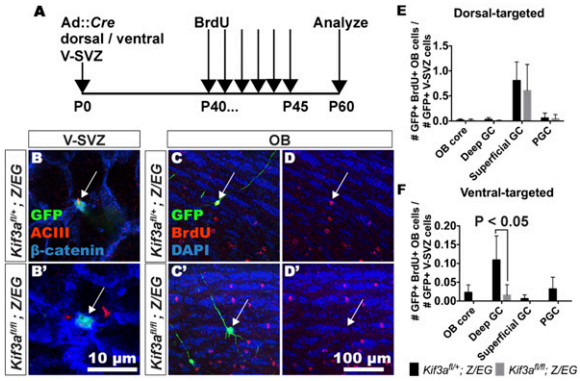

Fig. 3. Specific elimination of primary cilia in a subpopulation of B1 cells in the ventral V-SVZ results in reduced OB neurogenesis. (*A*) Ad::*Cre* was injected into *Kif3a*$^{fl/fl}$*;Z/EG* mice to remove *Kif3a* from a subpopulation of radial glia at P0. BrdU was administered to the same animals at P40–P45, a time point after the removal of primary cilia from B1 cells (Fig. S5). The OBs of the mice were analyzed at P60. (*B*) V-SVZ wholemount shows a recombined (GFP$^+$) B1 cell with a primary cilium (red) in a *Kif3a*$^{fl/+}$*;Z/EG* control. (*B′*) In contrast, a recombined (GFP$^+$) B1 cell in a *Kif3a*$^{fl/fl}$*;Z/EG* mouse lacks a primary cilium. Note that adjacent nonrecombined (GFP$^-$) B1 cells in the same pinwheel core still have a primary cilium. (*C* and *D*) Confocal microscopy images showing a GFP$^+$ BrdU$^+$ granule cell, generated after Ad::*Cre* recombination in a *Kif3a*$^{fl/+}$*;Z/EG* animal (*C* and *D*), and a GFP$^+$ BrdU$^-$ cell, likely generated before primary cilia ablation, in a *Kif3a*$^{fl/fl}$*;Z/EG* animal (*C′* and *D′*). (*E*) The numbers of BrdU$^+$ GFP$^+$ OB cells derived from the dorsal V-SVZ are unaffected in *Kif3a*$^{fl/fl}$*;Z/EG* mice. (*F*) However, the number of BrdU$^+$ GFP$^+$ deep granule cells generated from the ventral V-SVZ is significantly decreased, suggesting that ablation of primary cilia in the ventral V-SVZ results in reduced neurogenesis in the adult brain. (*E* and *F*) Bar graphs show mean ± SD from at least three mice per experimental group. *P* values are calculated using two-way ANOVA with Sidak's multiple comparisons test.

in the AV V-SVZ of *Nestin::Cre;Ift88*$^{fl/+}$ mice at P0 (Fig. 4 *A–D*). This *Gli1* expression was abolished in *Nestin::Cre;Ift88*$^{fl/-}$ cilia mutants (Fig. 4 *A′–D′*). ISH signal was also reduced in the brain parenchyma of *Nestin::Cre;Ift88*$^{fl/-}$ sections (Fig. 4 *A′–D′*), likely due to elimination of low-level *Gli1* expression in neurons and glial cells (49). This result suggests that primary cilia in the brain parenchyma, which are reduced in the *Nestin::Cre;Ift88*$^{fl/-}$ mice (Fig. S1 *G′–I′*), are also required for this low-level Gli1 expression. Moreover, the AV V-SVZ of neonatal *Nestin::Cre;Ift88*$^{fl/-}$ mice had drastically decreased numbers of cells expressing Nkx2.1 (Fig. 4 *E*, *F*, and *I*), a marker for Shh-responsive cells. This decrease was not observed in *hGFAP::Cre;Kif3a*$^{fl/fl}$ mice (Fig. 4 *G*, *H*, and *J*), which still had primary cilia in the ventral V-SVZ (Fig. S4*H′*). These results suggest that primary cilia-mediated Shh signaling is required for the regulation of proliferation and neurogenesis in the AV subdomain of the postnatal V-SVZ.

Discussion

The V-SVZ harbors the largest population of NSCs in the adult brain. Like radial glia during development, B1-cell apical endings have a primary cilium that projects into the ventricle, which has been suggested to contain signaling factors (24, 25). Here we have generated cilia mutant mice using *Cre* lines driven by *Nestin* or *hGFAP* promoters to remove the conditional alleles *Kif3a*$^{fl/fl}$ and *Ift88*$^{fl/-}$. Genetic disruption of ciliary genes is inevitably confounded by the simultaneous removal of ependymal cilia that leads to severe hydrocephalus. Therefore, we have additionally adapted a method developed to target region-specific subpopulations of neonatal radial glia (41, 42) to selectively ablate primary cilia in NSCs by stereotaxic injection of Ad::*Cre* into neonatal brains of *Kif3a*$^{fl/fl}$*;Z/EG* mice. This process resulted in minimal disruption to the ependymal layer, and the animals did not develop hydrocephalus. We provide evidence that removal of primary cilia has a subdomain-specific effect on V-SVZ neurogenesis. In the AV region, elimination of primary cilia from NSCs led to decreased proliferation and production of deep granule neurons. In contrast, neurogenesis in posterior and dorsal regions was unaffected. Furthermore, we show that ablation of primary cilia disrupted Shh signaling and decreased Nkx2.1$^+$ cells in the AV V-SVZ, suggesting that primary cilia may act through a Shh-dependent mechanism to regulate neurogenesis in this ventral region.

When we removed primary cilia early in development using *Cre* driven by *Nestin* or *hGFAP* promoters, we found no major defects in forebrain development. This observation was surprising because it has been suggested that primary cilia play key roles not only in the Hh pathway, but also in other signaling events: Wnt (50–54); serotonin receptor 6 (5-HT$_6$) (55); platelet-derived growth factor receptor α (PDGFR-α) (56, 57), and insulin-like growth factor-1 receptor (IGF1R) (16, 58). Consistent with our observations, a recent report disrupting Arl13b, a small GTPase important for ciliary signaling, found no significant telencephalic developmental defects using the same *Nestin::Cre* and *hGFAP::Cre* lines (16). A recent study also found that removal of primary cilia from gonadotrophin-releasing hormone (GnRH) neurons in *GnRH::Cre Kif3a*$^{fl/fl}$ mice did not affect their extensive migration or most of their functions (59). In contrast, removal of Arl13b during early neuroepithelial expansion (using *Foxg1:Cre*) does result in a dramatic polarity inversion of early neuroepithelial cells (16). Furthermore, a recent study using a different *Nestin::Cre* (*NesCre8*) line that is activated earlier at E8.5 (60) showed that *NesCre8;Kif3a*$^{fl/fl}$ mice develop enlarged and bumpy cortices (61). We did not observe these phenotypes in our *Nestin::Cre; Kif3a*$^{fl/fl}$ mice, in which *Cre* expression is first noticed at E10.5 (27, 28). Notably, *NesCre8;Kif3a*$^{fl/fl}$ mice lose their V-SVZ cilia around E12.5, whereas in our *Nestin::Cre;Kif3a*$^{fl/fl}$ mice, cilia ablation occurred around E14.5. These observations highlight the importance of developmental timing in genetic ablation experiments. Together, our findings suggest that ablation of primary cilia after the beginning of the neurogenic period surprisingly does not result in major abnormalities. Compensatory signaling mechanisms may exist to ensure relatively normal forebrain development in the absence of primary cilia. Alternatively, the short stub that is left following conditional ablation of *Kif3a* (13, 61) or *Ift88* (62) may be sufficient for reception and transduction of the above signals.

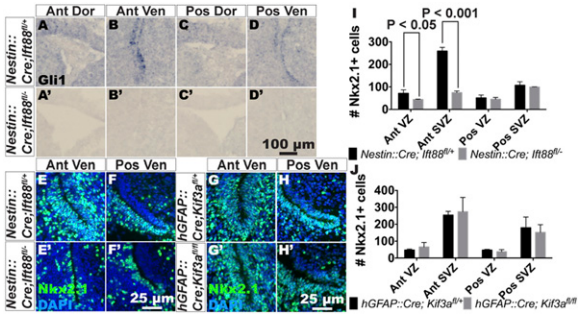

Fig. 4. Cilia ablation disrupts Shh signaling in the AV V-SVZ. (*A–D*) ISH shows enriched expression of *Gli1*, a Shh target gene, in the AV V-SVZ (*B*) in *Nestin::Cre;Ift88*$^{fl/+}$ mice at P0. (*A′–D′*) However, *Gli1* expression is abolished in *Nestin::Cre;Ift88*$^{fl/-}$ mice. (*E*, *F*, and *I*) *Nestin::Cre;Ift88*$^{fl/-}$ mice also have decreased numbers of the Shh-responsive Nkx2.1$^+$ cells in the AV V-SVZ (*E′*). (*G*, *H*, and *J*) In contrast, *hGFAP::Cre;Kif3a*$^{fl/fl}$ mice, which have not lost the cilia in the ventral V-SVZ at P0 (Fig. S5), have normal numbers of Nkx2.1$^+$ cells in the AV (*G′*) and PV (*H′*) regions. (*I* and *J*) Bar graphs show mean ± SD from at least three mice per experimental group. *P* values are calculated using the Student's *t* test.

It is also surprising that loss of primary cilia affected neurogenesis only in a specific subdomain (AV region) of the V-SVZ but had such little effect in all other regions of this major neurogenic area. This finding is especially intriguing in light of the other signaling pathways involved in the regulation of adult V-SVZ proliferation [e.g., Wnt (63), PDGFR-α (64, 65), 5-HT (66), and IGF2 (58)] and for which the primary cilia have been implicated (see above). It will be important to determine whether these pathways are not required or whether primary cilia function is substituted for these other signaling pathways by other signal-transduction mechanisms. Equally surprising is that removal of an organelle that has been so tightly linked to the cell cycle has so few effects on the proliferation of a large population of dividing progenitors in development and in the adult. It has been shown that primary cilia are regulated dynamically throughout the cell cycle, that primary cilia are present in G_0 and G_1 cells and usually in S/G2 cells, but get resorbed before mitotic entry to reappear postcytokinesis (67–69). The presence of a fully developed primary cilium appears to be dispensable for the proliferation of a large number of primary progenitors in development and in the adult. Interestingly, at 30 d postinjection of Ad::*Cre* into *Kif3a*$^{fl/fl}$*;Z/EG* mice, many of the recombined V-SVZ cells still had a primary cilium. By P40, most of the recombined cells had lost their cilia. We do not know why it takes so long for cilia removal in Ad::*Cre*-injected *Kif3a*$^{fl/fl}$*;Z/EG* mice, but it could be due to the low turnover of ciliary components, possibly associated to quiescence in postnatal NSCs.

It has been shown that essential Hh signaling components—Patched1, Smoothened (Smo), Suppressor of fused, and Gli transcription factors—are enriched in primary cilia (2–4). Our findings indicate that the proliferative defects we observed are tightly associated with regions of the juvenile and adult V-SVZ that are regulated by Hh (42, 44). Indeed, previous studies using *Smo* conditional knockout mice (70, 71) reported a temporal requirement for Hh signaling in telencephalic development, similar to the time during which primary cilia are required (16). *Nestin::Cre;Smo*$^{fl/-}$ mice, which lose *Smo* by E12, showed largely unperturbed forebrain patterning (70). In contrast, removing *Smo* by E9 in the *Foxg1::Cre;Smo*$^{fl/-}$ mice resulted in gross ventral patterning defects accompanied by complete dorsalization of the telencephalon (71). It has been suggested that Shh secreted by the choroid plexus into the CSF signals in a paracrine manner to stimulate the proliferation of cerebellar granule neuron precursors (72). A similar mechanism might be at play in the V-SVZ, given the strategic location of B1 cells' primary cilia. However, the precise origin of Shh destined for the primary cilia in B1 cells remains unknown (24, 25, 44). It will be interesting to determine whether primary cilia are required for the production of the four recently identified OB interneuron types derived from the AV V-SVZ, a region with high Gli1 expression (42).

We describe here a proliferative defect on neurogenic progenitors that is very strictly spatially defined in the AV region of the V-SVZ, despite removing primary cilia in a large subset of progenitor cells in the brains of *Nestin::Cre;Ift88*$^{fl/-}$, *Nestin::Cre;Kif3a*$^{fl/fl}$, and *hGFAP::Cre;Kif3a*$^{fl/fl}$ mice. Injection of Ad::*Cre* into *Kif3a*$^{fl/fl}$*;Z/EG* mice also reduced neurogenesis only in the ventral region, where Shh signaling is high (42, 44). This finding lends support to the idea that primary cilia in B1 cells act mainly through the Shh pathway to regulate adult NSC activity. Brain abnormalities and cognitive deficits are prominent features associated with human ciliopathies (73, 74), and primary cilia constitute a major apical compartment of adult NSCs. Our results show that primary cilia are essential for the regulation of adult NSCs, specifically in the ventral region of the V-SVZ, and that cilia dysfunction leads to disruption of Shh signaling in this region of the postnatal brain. These findings provide insights into the regulation of postnatal neurogenesis in the V-SVZ.

Materials and Methods

Mice. All animal procedures were carried out in accordance with the guidelines from the University of California, San Francisco, Institutional Animal Care and Use Committee. The genetically modified mice used—*Nestin::Cre* (27, 28), *hGFAP::Cre* (36, 37), *Ift88*$^{fl/fl}$ (75), *Ift88*$^{+/-}$ (76), *Kif3a*$^{fl/fl}$ (77), *Ai14* (78), and *Z/EG* (43)—have been described. Mice with the genotypes *Nestin::Cre;Kif3a*$^{fl/+}$, *Kif3a*$^{fl/fl}$, and *Kif3a*$^{fl/+}$ had the same phenotypes and were pooled together as controls to their *Nestin::Cre;Kif3a*$^{fl/fl}$ littermates. Similarly, *Nestin::Cre;Ift88*$^{fl/+}$, *Ift88*$^{fl/-}$, and *Ift88*$^{fl/+}$ mice were pooled together as controls to their *Nestin::Cre;Ift88*$^{fl/-}$ littermates, and *hGFAP::Cre;Kif3a*$^{fl/+}$, *Kif3a*$^{fl/fl}$, and *Kif3a*$^{fl/+}$ mice were pooled together as controls to their *hGFAP::Cre;Kif3a*$^{fl/fl}$ littermates. BrdU was administered at a concentration of 50 mg per kg of body weight. Neonatal pups were injected s.c.; adult mice were injected intraperitoneally.

Tissue Preparation and Staining. Mice were perfused with 4% (wt/vol) paraformaldehyde. Brains were postfixed overnight at 4 °C, cryoprotected in 30% (wt/vol) sucrose, embedded in Tissue-Tek Optimal Cutting Temperature compound (Sakura), and cut into 12-μm sections. V-SVZ wholemount dissections were performed as described (23). For immunostaining, sections were incubated with primary antibodies overnight at 4 °C, followed by secondary antibodies at room temperature for 2 h. Primary antibodies used were rabbit anti-ACIII (polyclonal) (1:500; Santa Cruz), mouse anti–γ-tubulin (GTU88) (1:500; Abcam), rabbit anti-PH3 (polyclonal) (1:500; Millipore), rat anti-BrdU [BU1/75(ICR1)] (1:600; Novus), chicken anti-Nestin (polyclonal) (1:600; Lifespan Biosciences), mouse anti-GFAP (GA5) (1:1,000; Millipore), rat anti-CD24 (M1/69) (1:500; BD Pharmingen), rabbit anti-activated caspase-3 (polyclonal) (1:1000; Cell Signaling), chicken anti-GFP (polyclonal) (1:500; Aves Labs), mouse anti-AcTub [6-11B-1] (1:1,000; Sigma), mouse anti–β-catenin [14/β-catenin] (1:500; BD Transduction), rabbit anti–β-catenin (polyclonal) (1:500; Sigma), rabbit anti-Nkx2.1 (polyclonal) (1:500; Santa Cruz), rabbit anti-Tbr1 (polyclonal) (1:500; Millipore), mouse anti-Satb2 (SATBA4B10) (1:500; Abcam), mouse anti-Cux1 (M222) (1:500; Abcam), rat anti-Ctip2 (25B6) (1:500; Abcam), and rabbit anti-DCX (polyclonal) (Cell Signaling). Secondary antibodies used were conjugated to Alexa Fluor dyes (Invitrogen/Molecular Probes). ISH was performed by using standard protocols (13, 44) and antisense riboprobe from *Gli1* cDNA (gift from A. Ruiz I Altaba, University of Geneva Medical School, Geneva).

Microscopic Analysis and Quantification. Images were taken with an Olympus AX70 light microscope or a Leica SP5 confocal microscope. Counting of cells was performed by using the Cell Counter plugin in ImageJ (National Institutes of Health) or the Imaris software (Bitplane). Sections at comparable rostrocaudal levels (Fig. 1*A*) of each mouse were used. For the quantification of PH3$^+$ cells, three sections were counted per rostrocaudal level per animal. Measurements of OB interneuron localization were carried out as described (41, 44), with normalization to granular layer width.

Stereotaxic Injections of Neonatal Pups. Mice were anesthetized by hypothermia for 4 min, positioned in the head mold, and injected with 20 nL of Ad::*Cre* as described (41, 42). Injections were carried out with a beveled pulled glass micropipette (Wiretrol 5 μL, Drummond Scientific) with a 40-μm diameter tip positioned at an angle of 45°. Using the center of the eyeball to zero the *x* (anterior–posterior) and *y* (M-L) coordinates, and the skin surface to zero the *z* (depth) coordinate, the injection coordinates were (x, y, z; in millimeters): dorsal V-SVZ (1.20, 1.85, −2.30) and ventral V-SVZ (1.10, 2.90, −3.20).

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