Regulation of postnatal cortical development by neuronal primary cilia

Kostandina Bicja[1,*], Juan Yang[1], Brendon Lewis[1,#], Liyan Qiu[1], and Xuanmao Chen[1]

[1] Department of Molecular, Cellular, and Biomedical Sciences, College of Life Sciences and Agriculture, University of New Hampshire, Durham, NH 03824

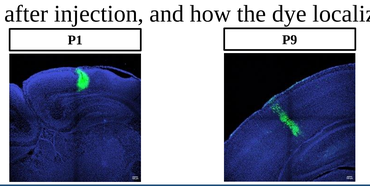
[*] UNH Neuroscience and Behavior and [#] Biochemistry Undergraduate Programs

Abstract

Neuronal primary cilia (NPC) are small, centriole-based surface projections known to regulate embryonic neurodevelopment by multiple signaling pathways. However, whether NPC modulate postnatal brain development is not clear. We utilized transgenic mice overexpressing the ciliary protein Arl13b, which display elongated cilia in all regions of the hippocampus. In the postnatal dentate gyrus, primary cilia showed a loosely opposite orientation. They were generally perpendicular to the lamina of the granule cell layer. This result is consistent with our previous research showing opposite cilia orientation in the hippocampal CA1 region. This supports that cilia directionality is opposite in compact laminated structure in the forebrain. Additionally, the ratio of north-oriented cilia to all cilia decreases with age in both control and Arl13b+ mice, suggesting a reverse movement of late-born neurons. We determined cortical differences among control mice and forebrain-specific cilia knockout (KO) (Ift88 lox/lox, Emx1-Cre) mice. Interestingly, KO brains displayed a thicker cortex than controls in posterior and middle regions. Using injected CFSE dye, which accumulates inside neurons, neuronal positioning in the cortex can be tracked more closely in future experiments. These preliminary results suggest that NPC regulate postnatal cortical development.

Methods

Brain tissue was immunofluorescence stained and imaged with a Nikon A1R HD confocal microscope. In addition to ciliary protein Arl13b, transgenic Arl13b+ mice also overexpressed Centrin2, a protein marking the centriole base of cilia that was necessary for orientation measurement. ImageJ software was used to quantify cilia orientation and length in the dentate gyrus. Orientation was measured from base-to-tip relative to the laminar structure using an angle tool, and length was measured using a freehand line tool. A Zeiss 610 Versa X-ray micro-CT scanner was used to visualize whole-brain sizes of Arl13b+, control, and KO mice. Micro-CT sample preparation included dehydration with 30%, 50%, 70%, 80%, and 90% ethanol (2 hours per dilution), staining with 1% iodine in 90% methanol (7 days), rehydration with 30% and 70% ethanol (1 hour per dilution), and embedding in 1% agarose gel (stored at 5°C). Dragonfly software was used for tomographic reconstruction. For CFSE dye injection, pups were anesthetized by hypothermia and manually injected with 5 ul of 0.25 mM CFSE dye into the cortex using either a needle or glass pipette. Pups were recovered on a heating mat for 10 minutes. The images below show the cortex at P1 right after injection, and how the dye localizes by P9.



Conclusions

1. Arl13b+ mice have longer primary cilia in the hippocampus.
2. Arl13b+ mice show oppositely oriented primary cilia in CA1 and dentate gyrus, perpendicular to the lamina.
3. The proportion of north-oriented cilia decreases with age in WT and Arl13b+ mice, suggesting a reverse movement of late-born neurons
4. Forebrain-specific cilia knockout mice have a thicker cortex than WT mice, due to altered postnatal development.

Figure 1

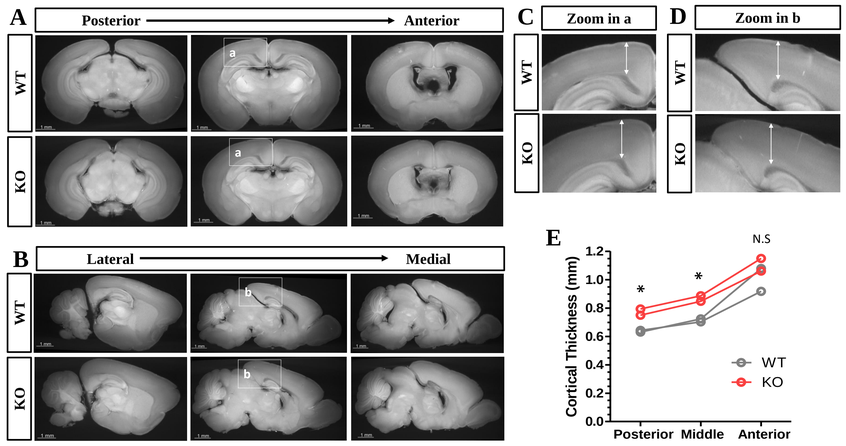


Fig. 1. Cerebral cortex is thicker in forebrain-specific cilia KO mice. *(A-B)* Micro-CT images of coronal and sagittal sections of postnatal day 20 (P20) WT and cilia KO brains. *(C-D)* Zoomed-in squares *a* and *b* showing cortical thickness differences. Arrows represent the measured region. *(E)* Line graph of cortical thickness in WT and KO brains measured from posterior, middle, and anterior coronal sections. The cortex is thicker in posterior and middle sections of cilia KO mice. N.S., not-significant, * $p < 0.05$; two-way ANOVA.

Figure 2

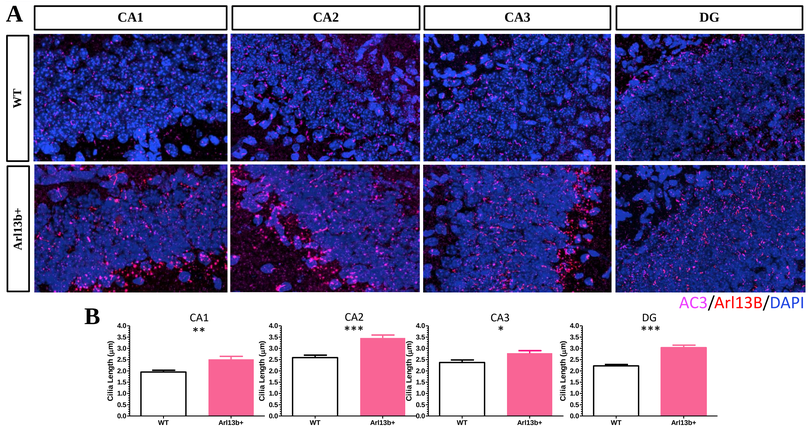


Fig. 2. Arl13b+ mice show elongated cilia in all regions of the hippocampus. *(A)* Confocal images showing hippocampal CA1, CA2, CA3, and dentate gyrus (DG) regions. *(B)* Bar charts comparing cilia lengths of WT and Arl13b+ mice. All have elongated cilia in transgenic Arl13b+ mice compared to WT controls as early as P5. WT stands for wild-type mice; Arl13b+ stands for transgenic Arl13b-mcherry; Centrin2-GFP mice. AC3 staining labels cilia in WT mice. Arl13b-mcherry mice have red fluorescent cilia. DAPI labels cell bodies. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; Student's t-test.

Acknowledgements:

This study is supported by National Institutes of Health Grants K01AG054729, P20GM113131-7006, R15MH126317, R15MH125305, and COLE Neuroscience Research Awards and UNH CoRE PRP awards to X.C. K.B. is supported by a Research Experience Apprenticeship Program (REAP) and a Summer Undergraduate Research Fellowship (SURF) award from the Hamel Center for Undergraduate Research of UNH. J. Y. is supported by a Summer Teaching Assistant Fellowship (STAF) and a Dissertation Year Fellowship (DYF) from UNH Graduate School. We thank the University Instrumentation Center for A1R HD confocal imaging service.

Figure 3

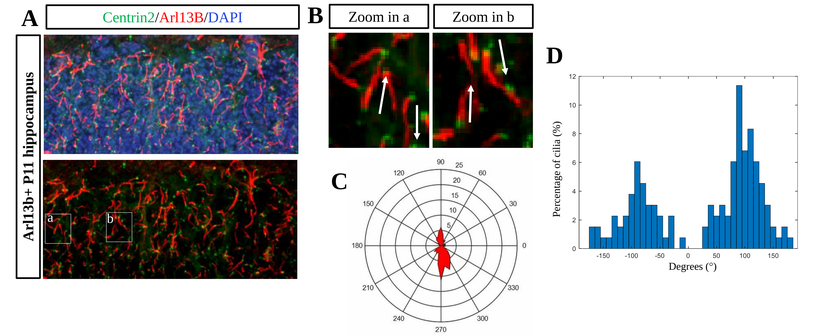


Fig. 3. Cilia in the dentate gyrus are oppositely oriented and perpendicular to the lamina. *(A-B)* Arl13b+ P11 hippocampal dentate gyrus with green centrioles marking the base of primary cilia. *(C-D)* Polar plot and histogram showing cilia orientation pattern and their corresponding percentages. Most primary cilia axonemes point south (180º - 360º) in polar plot (C). The histogram plot (binned in 10-degree intervals)(D) presents the percentage of cilia at different angles. The degrees in polar plots are converted to histogram plots by subtracting 180º.

Figure 4

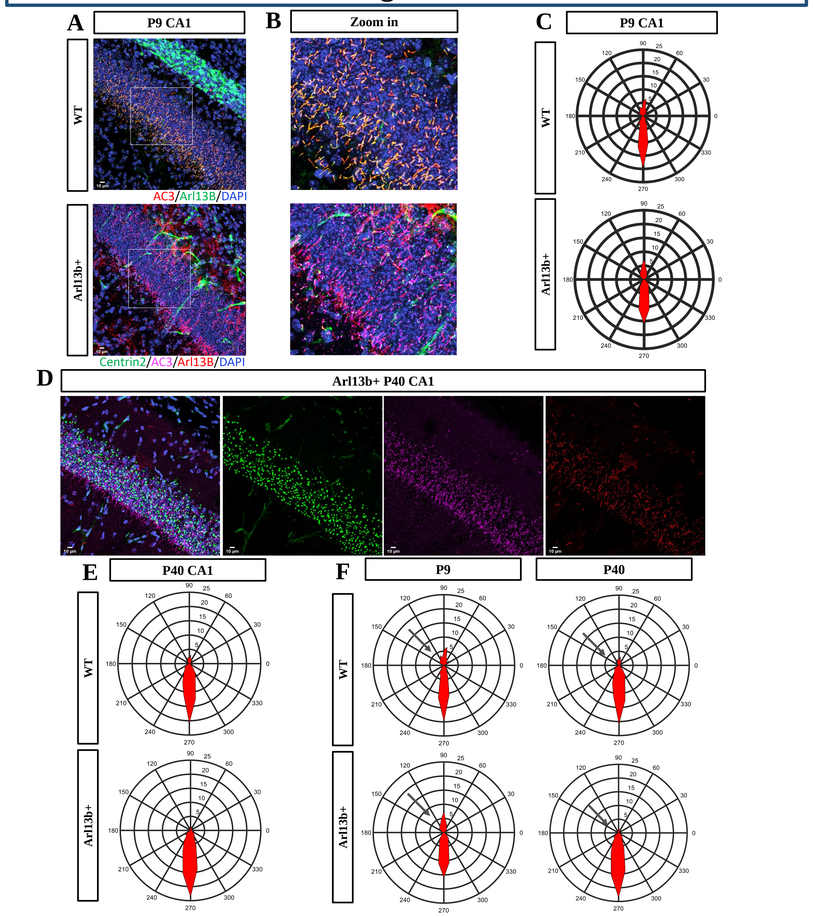


Fig. 4. Cilia in the ventral CA1 are oppositely oriented and perpendicular to the lamina, and proportion of north-oriented cilia decreases with age. *(A-C)* P9 CA1 cilia are oppositely oriented, with most pointing south. *(D-F)* A smaller proportion of cilia point north between P9 and P40. This pattern is observed in both WT and Arl13b+ mice.