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Response to Comment on "Impact of neurodegenerative diseases on human adult hippocampal neurogenesis"

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Alvarez-Buylla and colleagues provide an alternative interpretation of some of the data included in our manuscript and question whether well-validated markers of adult hippocampal neurogenesis (AHN) are related to this phenomenon in our study. In Terreros-Roncal *et al*., reconstruction of the main stages of human AHN revealed its enhanced vulnerability to neurodegeneration. Here, we clarify ambiguities raised by these authors.

Alvarez-Buylla *et al*. asserted undetectable neurogenesis in the adult human dentate gyrus (DG), as they did not visualize well-validated adult hippocampal neurogenesis (AHN) markers [such as nestin or doublecortin (DCX)] in this structure (*1*). The authors now acknowledge the presence of cells positive for these and other markers (*2*) while disbelieving that they are related to AHN in our study (*3*). No published data support their suggestion that the human DCX$^+$ dentate granule cells (DGCs) described in numerous studies, including ours (*3*–*5*), have a developmental origin. Conversely, the adult-born nature of DCX$^+$ cells is consistent with bromodeoxyuridine and ^{14}C birthdating approaches (*6*, *7*), which support the continuous addition of new neurons to the human DG. Our study (*3*) reveals the presence of DCX$^+$ immature DGCs at distinct differentiation stages in this structure. DCX$^+$ cells colabeled with calretinin, neuronal nuclei, or calbindin (which identify sequential stages of AHN) show morphologies and positioning [figure 1, F to J, and figure S2H of (*3*)] matching those observed in rodents. Contrary to Alvarez-Buylla *et al*.'s view that DCX$^+$ cells in (*3*) are large, the size of these cells varies during their maturation [figures 1J and S2H of (*3*)] but remains significantly smaller than that of mature DGCs [figure 2m of (*5*) and figure 6K of (*4*)]. Alvarez-Buylla *et al*. allege that DCX$^+$ DGCs in (*3*) are exclusively located in the granule cell layer (GCL), despite our quantitative data revealing the abundant presence of these cells in the subgranular zone (SGZ). In fact, DCX$^+$ cells are distributed in a maturation gradient between the GCL and SGZ, the most immature (those that coexpress calretinin or polysialylated neural cell adhesion molecule) being located in the SGZ [figures 1G and S2B of (*3*)]. These data support the notion that human DCX$^+$ DGCs undergo a dynamic maturation process characteristic of AHN in numerous mammalian species.

Alvarez-Buylla *et al*. postulate that the putative presence of DCX$^+$ cells in non-neurogenic regions challenges the authenticity of human AHN. However, a recent study (*8*) revealed that the DCX signal observed in the macaque cortex is artifactual, thereby calling for caution when interpreting DCX staining in non-neurogenic regions of the primate brain. Conversely, control experiments showed that DCX protein is present in neurogenic niches of macaques (*8*) and humans [see the use of monoclonal antibodies and preadsorption with blocking peptides in extended data figure 4 of (*5*)]. Their suggestion that DCX is reexpressed by mature DGCs goes against experimental evidence (*9*). The expression of the DGC marker Prox1 by ~90% DCX$^+$ cells [figure 1E of (*3*)] and the absence of DCX protein in glia, vasculature, and interneurons [figure S5 of (*3*)] also contest Alvarez-Buylla *et al*.'s view that DCX may be detected in non-DGCs.

Nestin$^+$ cells are present in the adult human DG (*10*). In (*3*), we observed a population of nestin$^+$ cells that express a panel of radial glia–like cell markers [such as SRY-box transcription factor 2 (Sox2) and vimentin] while lack-

ing S100 calcium-binding protein-B (S100β) expression. Although these criteria are widely used to phenotypically identify neural stem cells (NSCs) by immunohistochemistry (*11*), Alvarez-Buylla *et al.* suggest that the nestin$^+$ S100β$^-$ cells identified in (*3*) are astrocytes. The morphology of nestin$^+$ S100β$^-$ NSCs differs from that of nestin$^+$ S100β$^+$ astrocytes [figures 1 and S3 of (*3*)]. Moreover, it is consistent with that of hippocampal NSCs in aged rodents (*12*). Nestin$^+$ S100β$^-$ cells show distally branched [figure S3E of (*3*)] long apical processes that transverse the GCL [figure S3, A, C, D, and F, of (*3*)], and ~97% of their somas are located in the SGZ [figure S3G of (*3*)]—features also evident with vimentin staining (Fig. 1). Furthermore, the number of nestin$^+$ cells does not exhibit variations correlative to those of S100β$^+$ astrocytes either in control or diseased individuals [figure S16E of (*3*)]. These observations refute Alvarez-Buylla *et al.*'s interpretation that the nestin$^+$ S100β$^-$ NSCs identified in (*3*) are astrocytes.

Alvarez-Buylla *et al.* challenge the nature of the proliferative cells observed in (*3*), suggesting that ~10,000 phospho-histone 3$^+$ mitotic cells per mm^3 do not constitute an actual proliferative niche. These authors overlooked the fact that, in (*3*), we also used ELAV-like proteins HuC/HuD [which are transiently expressed by intermediate progenitors and proliferative neuroblasts immediately after cell division (*13*)] to phenotypically characterize human DG proliferative cells. About 90% of these cells expressed DCX and ~85% were located in the SGZ [figure S4 of (*3*)]. These data contest their suggestion that HuC/HuD labels mature neurons and support the notion that most proliferative cells in the human DG correspond to transit-amplifying progenitors and neuroblasts located in the SGZ. With respect to calretinin$^+$ cells, only those double-labeled with DCX were studied in (*3*) and (*5*), thereby excluding putative calretinin$^+$ interneurons.

Several interpretations by Alvarez-Buylla *et al.* with respect to neurodegenerative diseases are inaccurate. Regarding frontotemporal dementia (FTD), they mention two studies that found DGC loss in patients with FTD-Tau, which is characterized by DG nuclear inclusions and atrophy. However, these features are far from constant in other FTD variants (*14*). Given the absence of patients with FTD-Tau in (*3*), this comment is irrelevant to our study. These authors mention a study suggesting absence of major DG alterations in patients with dementia with Lewy bodies. However, our study included patients not only with that condition but also with Parkinson's disease. These α-synucleinopathies have different clinical, neuropathological, and molecular features, thereby triggering distinct hippocampal signatures (*3*). Alvarez-Buylla *et al.* suggest that the fluctuations in the number of RGL and proliferative cells observed in neurodegenerative diseases point to these cells not being related to AHN. The altered neuronal differentiation and exacerbated apoptosis [figure S8 of (*3*)] likely account for unchanged numbers of mature neurons even in the presence of increased proliferative cells and/or NSCs in diseased individuals. Moreover, independent regulation of individual AHN stages has been extensively demonstrated (*15*).

We appreciate the interest that our new data have raised in our colleagues and are confident that our study will contribute to a greater understanding of how AHN persists throughout human life.

REFERENCES

1. S. F. Sorrells, M. F. Paredes, A. Cebrian-Silla, K. Sandoval, D. Qi, K. W. Kelley, D. James, S. Mayer, J. Chang, K. I. Auguste, E. F. Chang, A. J. Gutierrez, A. R. Kriegstein, G. W. Mathern, M. C. Oldham, E. J. Huang, J. M. Garcia-Verdugo, Z. Yang, A. Alvarez-Buylla, Human hippocampal neurogenesis drops sharply in children to undetectable levels in adults. *Nature* **555**, 377–381 (2018). doi:10.1038/nature25975 Medline
2. A. Alvarez-Buylla, A. Cebrian-Silla, S. F. Sorrells, M. A. Nascimento, M. F. Paredes, J. M. Farcía-Verdugo, Z. Yang, E. J. Huang, Comment on "Impact of neurodegenerative diseases on human adult hippocampal neurogenesis". *Science* **376**, abn8861 (2022).
3. J. Terreros-Roncal, E. P. Moreno-Jiménez, M. Flor-García, C. B. Rodríguez-Moreno, M. F. Trinchero, F. Cafini, A. Rábano, M. Llorens-Martín, Impact of neurodegenerative diseases on human adult hippocampal neurogenesis. *Science* **374**, 1106–1113 (2021). doi:10.1126/science.abl5163 Medline
4. M. Flor-García, J. Terreros-Roncal, E. P. Moreno-Jiménez, J. Ávila, A. Rábano, M. Llorens-Martín, Unraveling human adult hippocampal neurogenesis. *Nat. Protoc.* **15**, 668–693 (2020). doi:10.1038/s41596-019-0267-y Medline
5. E. P. Moreno-Jiménez, M. Flor-García, J. Terreros-Roncal, A. Rábano, F. Cafini, N. Pallas-Bazarra, J. Ávila, M. Llorens-Martín, Adult hippocampal neurogenesis is abundant in neurologically healthy subjects and drops sharply in patients with Alzheimer's disease. *Nat. Med.* **25**, 554–560 (2019). doi:10.1038/s41591-019-0375-9 Medline
6. P. S. Eriksson, E. Perfilieva, T. Björk-Eriksson, A.-M. Alborn, C. Nordborg, D. A. Peterson, F. H. Gage, Neurogenesis in the adult human hippocampus. *Nat. Med.* **4**, 1313–1317 (1998). doi:10.1038/3305 Medline
7. K. L. Spalding, O. Bergmann, K. Alkass, S. Bernard, M. Salehpour, H. B. Huttner, E. Boström, I. Westerlund, C. Vial, B. A. Buchholz, G. Possnert, D. C. Mash, H. Druid, J. Frisén, Dynamics of hippocampal neurogenesis in adult humans. *Cell* **153**, 1219–1227 (2013). doi:10.1016/j.cell.2013.05.002 Medline
8. R. X. Liu, J. Ma, B. Wang, T. Tian, N. Guo, S. J. Liu, No DCX-positive neurogenesis in the cerebral cortex of the adult primate. *Neural Regen. Res.* **15**, 1290–1299 (2020). doi:10.4103/1673-5374.272610 Medline
9. J. P. Brown, S. Couillard-Després, C. M. Cooper-Kuhn, J. Winkler, L. Aigner, H. G. Kuhn, Transient expression of doublecortin during adult neurogenesis. *J. Comp. Neurol.* **467**, 1–10 (2003). doi:10.1002/cne.10874 Medline
10. S. Cipriani, I. Ferrer, E. Aronica, G. G. Kovacs, C. Verney, J. Nardelli, S. Khung, A.-L. Delezoide, I. Milenkovic, S. Rasika, P. Manivet, J.-L. Benifla, N. Deriot, P. Gressens, H. Adle-Biassette, Hippocampal Radial Glial Subtypes and Their Neurogenic Potential in Human Fetuses and Healthy and Alzheimer's Disease Adults. *Cereb. Cortex* **28**, 2458–2478 (2018). doi:10.1093/cercor/bhy096 Medline
11. J. M. Encinas, G. Enikolopov, Identifying and quantitating neural stem and progenitor cells in the adult brain. *Methods Cell Biol.* **85**, 243–272 (2008). doi:10.1016/S0091-679X(08)85011-X Medline
12. S. Martín-Suárez, J. Valero, T. Muro-García, J. M. Encinas, Phenotypical and

functional heterogeneity of neural stem cells in the aged hippocampus. *Aging Cell* **18**, e12958 (2019). doi:10.1111/acel.12958 Medline

13. N. Marichal, G. García, M. Radmilovich, O. Trujillo-Cenóz, R. E. Russo, Enigmatic central canal contacting cells: Immature neurons in "standby mode"? *J. Neurosci.* **29**, 10010–10024 (2009). doi:10.1523/JNEUROSCI.6183-08.2009 Medline
14. M. Bocchetta, J. E. Iglesias, M. A. Scelsi, D. M. Cash, M. J. Cardoso, M. Modat, A. Altmann, S. Ourselin, J. D. Warren, J. D. Rohrer, Hippocampal Subfield Volumetry: Differential Pattern of Atrophy in Different Forms of Genetic Frontotemporal Dementia. *J. Alzheimers Dis.* **64**, 497–504 (2018). doi:10.3233/JAD-180195 Medline
15. T. Plümpe, D. Ehninger, B. Steiner, F. Klempin, S. Jessberger, M. Brandt, B. Römer, G. R. Rodriguez, G. Kronenberg, G. Kempermann, Variability of doublecortin-associated dendrite maturation in adult hippocampal neurogenesis is independent of the regulation of precursor cell proliferation. *BMC Neurosci.* **7**, 77 (2006). doi:10.1186/1471-2202-7-77 Medline

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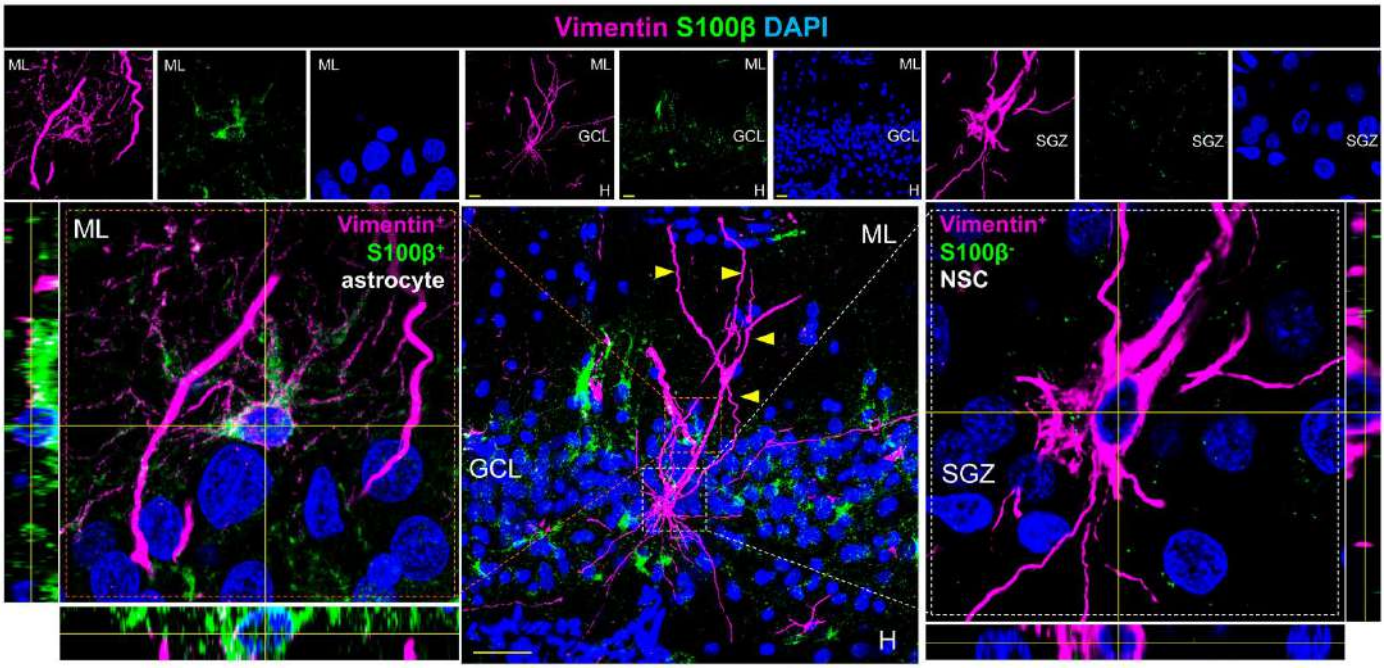

Fig. 1. Vimentin and S100β staining in the human DG. ML, molecular layer; GCL, granule cell layer; SGZ, subgranular zone; DAPI, 4′,6-diamidino-2-phenylindole. Scale bar, 10 μm.

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