Molecular Psychiatry (2020) 25:2207–2209
https://doi.org/10.1038/s41380-018-0337-5

CORRESPONDENCE

Limits to human neurogenesis—really?

Paul J. Lucassen[1] · Nicolas Toni[2] · Gerd Kempermann[3] · Jonas Frisen[4] · Fred H. Gage[5] · Dick F. Swaab[6]

Received: 15 November 2018 / Accepted: 26 November 2018 / Published online: 7 January 2019

In the adult rodent hippocampus, an important brain region for learning and memory, new neurons are born throughout life. Hippocampal stem cells undergo proliferation and neuronal differentiation before they are functionally incorporated in the adult hippocampal network. In contrast to rodents, little is known about neurogenesis in the human brain.

Sorrells et al. [1] collected a unique series of hippocampal postmortem samples from 37 control cases and 22 neurosurgical specimens from epilepsy patients. They used immunocytochemistry to study proteins marking proliferation or proxy markers often inferred to reflect neurogenesis and concluded that neurogenesis drops to negligible levels during adulthood. Based on this early decline observed in postmortem tissues, they question the functional relevance of neurogenesis in the adult human brain.

While the tissues they used are rare and the images of high quality, we disagree with their conclusion that, based on these negative data, human neurogenesis is insignificant.

First, their conclusion is mainly based on the relative *absence* of two neurogenesis markers, i.e., doublecortin (DCX) and polysialic acid neural cell adhesion molecule (PSA-NCAM), although proliferation is still detected (see, e.g., their Figs. 2A, 4a in Sorrells et al.). A main concern is that they provide very little patient information about perimortem factors, inherent to human postmortem brain, that affect protein preservation.

First, thorough clinical documentation should be available for each subject, including ethical approval, permission to use brain tissue for research purposes, and a systematic neuropathological investigation to confirm control status. Secondly, factors like agonal state (indicated by tissue or cerebrospinal fluid pH), fixation duration and postmortem delay (PMD), i.e., the time between death and brain fixation [2], should be available to interpret each individual's results. In their cohort, PMD was <48 h for most cases, a delay that has likely caused protein breakdown [2, 3], especially of DCX, which disappears quickly in a rodent PMD brain series (Fig. 8b–e in [4]). While Sorrells et al. detected positive DCX and PSA-NCAM signals in the brains of young children, this is expected for a variety of reasons, as early neonatal brains differ from adult brains in many ways; (a) often neonates die from unknown and different causes, (b) they often die in hospitals, allowing PMDs to be shorter, (c) premature death in children is unlikely to be comparable to chronic conditions in adult, (d) autofluorescent signal is low in tissues from children but often high in adults, which may affect signal-to-noise ratio for immunofluorescence studies, and (e) the water and glia content, including myelination, is different in small brains, leading to a different level of fixation.

Moreover, Sorrells et al. conclude that an elongated morphology of a DCX-positive cell was evidence of young neurons. However, with increasing PMD, DCX+ cells in rat brain rapidly lose dendritic signal, whereas the soma becomes more similar to mature neurons [4]. Also, non-specific DCX signal appears outside the dentate, as also shown by Sorrells et al. in white matter and cortex. Since DCX is unlikely to be produced in those areas, the morphological features of cell types have likely changed with PMD. Furthermore, DCX and PSA-NCAM expression do not co-localize in Sorrells et al. (ext Fig. 5d), even though

✉ Paul J. Lucassen
p.j.lucassen@uva.nl

[1] Brain Plasticity Group, Swammerdam Institute for Life Sciences, Center for Neuroscience, University of Amsterdam, Amsterdam, The Netherlands

[2] Lausanne University Hospital, Department of Psychiatry, Center for Psychiatric Neurosciences, Lausanne, Switzerland

[3] German Center for Neurodegenerative Diseases (DZNE) Dresden, and CRTD—Center for Regenerative Therapies Dresden, Technische Universität, Dresden, Germany

[4] Department of Cell and Molecular Biology, Karolinska Institute, Stockholm, Sweden

[5] Laboratory of Genetics, The Salk Institute for Biological Studies, La Jolla, CA, USA

[6] Netherlands Institute for Neurosciences, Meibergdreef, Amsterdam, The Netherlands

both markers overlap in rodent brain after perfusion fixation, i.e., when no PMD is present.

PMD and agonal state also affect post-translational modifications of proteins. Studies examining the effects of prolonged PMDs on human hippocampi revealed that most cytoskeletal proteins showed a drastic loss, based on immunohistochemistry and Western blot [5]. In particular, for PSA-NCAM, its poly-sialic group is unstable because of the intramolecular self-cleavage of the glycosidic bonds of sialic acid [6] that occurs under mildly acidic conditions. This is relevant for patients who died of bronchopneumonia or related conditions that result in hypoxia and a pronounced lactic acidosis of the brain. Moreover, polysialation disappears after inflammation [6], whereas adult neurogenesis is potently suppressed by environmental factors like sickness/inflammation [7, 8].

DCX is also particularly sensitive to stress; its signal drops sharply upon 30 min after capture in wild-caught bats [9], another species in which adult neurogenesis was previously thought to be absent [10]. Similarly, stress hormones in humans peak around the time of death [11], which may affect DCX signal in human brain as well [2, 4, 12]. In the absence of detailed individual patient information in Sorrells et al., it is hard to evaluate to what extent environmental or methodological factors influenced their results.

As a possible control for PMD, Sorrells et al. studied resected tissues from epilepsy patients fixed within minutes after surgery, hence lacking a PMD. However, surgery is only done on patients with severe epilepsy who have likely been exposed to many seizures and antiepileptic drugs. In rodents, these factors reorganize the hippocampal network, promoting astrogliosis and/or depleting proliferating precursors [13]; resected tissue also shows an injury response [14]. Hence, neurogenesis is most likely hard to detect in resected epilepsy tissue. Moreover, for obvious reasons, resected tissue from controls is unavailable; therefore, comparing these results is difficult.

Furthermore, Sorrells et al. studied only three, randomly sampled tissue sections per hippocampus per patient. This fact is not trivial, because neurogenesis differs considerably between the dorsal and ventral hippocampus [15]. Also, resected human material is generally taken from the anterior part of the temporal lobe, complicating the comparison of these findings. A more representative sample selection throughout the entire hippocampus would have been informative, as done by Boldrini et al. [12], who studied similar human tissues, with shorter PMDs, using stereological estimates and reported thousands of DCX+ cells up into old age.

Sorrells et al. support their arguments by providing electron microscope images. Here, the morphological criteria used to identify cell types and their maturation stages depend even more strongly on PMD and are complicated by the absence of 3D reconstructions. Global cell shape cannot be inferred from individual sections, and transmission electron microscopy requires the preparation of ultrathin specimens (typically of 50 nm thickness). Brain cells display a 10–30 µm diameter soma and a complex shape, with processes that span distances of several millimeters. Thus, depending on the section plane, the contour of a cell in one random ultra-thin section may be extremely variable and may not include axon, dendrite or myelin sheath.

Characteristics such as process length and soma roundness can therefore not be inferred from individual sections but only from full serial sections encompassing the entire cell, which was not done in their experiment (Fig. 4, extended data Figure 4). Furthermore, other criteria that the authors used for the identification of cell types or cell maturity stage, in search of immature neurons or precursor cells, included chromatin condensation, soma shape, and darkness or irregular contour of the membranes (extended data Figs. 4, 7; Figure 4). However, the cell death that occurs upon tissue resection or after PMD modifies chromatin density, cytoplasm darkness, soma structure, membrane integrity, or organelle density within minutes [16]. Such criteria are therefore difficult to use for identifying cell types, let alone for evaluating the absence of a specific cell type (extended data Figure 4).

To summarize, factors like PMD, agonal state, fixation and/or anatomical differences may have precluded optimal detection of neurogenesis by Sorrells et al. Without detailed individual patient information, it is impossible to judge whether the absence of signal is due to environmental or methodological aspects, or whether it can nevertheless be interpreted as a proxy for (an absence of) neurogenesis. As evidence for adult human neurogenesis is supported by studies that applied what could be considered gold-standard methods, i.e., BrdU and C14, which study the neurons directly and not precursor stages as a proxy, one negative finding based on marker expression alone cannot prove the absence of neurogenesis [17] as also discussed in more detail recently [18, 19].

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Publisher's note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

References

1. Sorrells SF, Paredes MF, Cebrian-Silla A, Sandoval K, Qi D, Kelley KW, et al. Human hippocampal neurogenesis drops sharply in children to undetectable levels in adults. Nature. 2018;555:377–81.

2. Bao A-M, Swaab DF. The art of matching brain tissue from patients and controls for postmortem research. Handb Clin Neurol. 2018;150:197–217.
3. Gonzalez-Riano C, Tapia-González S, García A, Muñoz A, DeFelipe J, Barbas C. Metabolomics and neuroanatomical evaluation of post-mortem changes in the hippocampus. Brain Struct Funct. 2017;222:2831–53.
4. Boekhoorn K, Joels M, Lucassen PJ. Increased proliferation reflects glial and vascular-associated changes, but not neurogenesis in the presenile Alzheimer hippocampus. Neurobiol Dis. 2006;24:1–14.
5. Blair JA, Wang C, Hernandez D, Siedlak SL, Rodgers MS, Achar RK, et al. Individual case analysis of postmortem interval time on brain tissue preservation. PLoS ONE. 2016;11:e0151615.
6. Sumida M, Hane M, Yabe U, Shimoda Y, Pearce OM, Kiso M, et al. Rapid trimming of cell surface polysialic acid (PolySia) by exovesicular sialidase triggers release of preexisting surface neurotrophin. J Biol Chem. 2015;290:13202–14.
7. Ryan SM, Nolan YM. Neuroinflammation negatively affects adult hippocampal neurogenesis and cognition: can exercise compensate? Neurosci Biobehav Rev. 2016;61:121–31.
8. Lucassen PJ, Oomen CA, Naninck EF, Fitzsimons CP, van Dam AM, Czeh B, et al. Regulation of adult neurogenesis and plasticity by (early)stress, glucocorticoids, and inflammation. Cold Spring Harb Perspect Biol. 2015;7:a021303.
9. Chawana R, Alagaili A, Patzke N, Spocter MA, Mohammed OB, Kaswera C, et al. Microbats appear to have adult hippocampal neurogenesis, but post-capture stress causes a rapid decline in the number of neurons expressing doublecortin. Neuroscience. 2014;277:724–33.
10. Amrein I, Dechmann DK, Winter Y, Lipp HP. Absent or low rate of adult neurogenesis in the hippocampus of bats (Chiroptera). PLoS ONE. 2007;2:e455.
11. Erkut ZA, Klooker T, Endert E, Huitinga I, Swaab DF. Stress of dying is not suppressed by high-dose morphine or by dementia. Neuropsychopharm. 2004;29:152–7.
12. Boldrini M, Fulmore CA, Tartt AN, Simeon LR, Pavlova I, Poposka V, et al. Human hippocampal neurogenesis persists throughout aging. Cell Stem Cell. 2018;22:589–99.
13. Jessberger S, Parent JM. Epilepsy and adult neurogenesis. Cold Spring Harb Perspect Biol. 2015;7:a020677.
14. Verwer RW, Sluiter AA, Balesar RA, Baayen JC, Speijer D, Idema S, et al. Altered loyalties of neuronal markers in cultured slices of resected human brain tissue. Brain Pathol. 2016;26:523–32.
15. Wu MV, Sahay A, Duman RS, Hen R. Functional differentiation of adult-born neurons along the septotemporal axis of the dentate gyrus. Cold Spring Harb Perspect Biol. 2015;7:a018978.
16. Puyal J, Ginet V, Clarke PG. Multiple interacting cell death mechanisms in the mediation of excitotoxicity and ischemic brain damage: a challenge for neuroprotection. Prog Neurobiol. 2013;105:24–48.
17. Kempermann G, Gage FH, Aigner L, Song H, Curtis MA, Thuret S, et al. Human adult neurogenesis: evidence and remaining questions. Cell Stem Cell. 2018;S1934-5909:30166–8.
18. Paredes MF, Sorrells SF, Cebrian-Silla A, Sandoval K, Qi D, Kelley KW, et al. Does Adult Neurogenesis Persist in the Human Hippocampus? Cell Stem Cell. 2018;23:780–1.
19. Tartt AN, Fulmore CA, Liu Y, Rosoklija GB, Dwork AJ, Arango V, et al. Considerations for Assessing the Extent of Hippocampal Neurogenesis in the Adult and Aging Human Brain. Cell Stem Cell. 23:782–3.