From mice to humans: a need for comparable results in mammalian neuroplasticity

Marco Ghibaudi, Enrica Boda, Luca Bonfanti*

Brain plasticity—A universal tool with many variations: The study of brain plasticity has been gaining interest since almost a century and has now reached a huge amount of information (> 80,000 results in PubMed). Overall, different types of plasticity, including stem cell-driven genesis of new neurons (adult neurogenesis), cells in arrested maturation (dormant neurons), neuro-glial and synaptic plasticity, can coexist and contribute to grant plastic changes in the brain, from a cellular to system level (Benedetti and Couillard-Despres, 2022; Bonfanti et al., 2023). Most of the current knowledge is based on laboratory rodents and largely deals with cellular and molecular mechanisms aimed at exploiting a potential for brain repair. Comparative approaches have also been used, spanning from simple organisms (e.g., *drosophila*, zebrafish) to the direct study of human brains (either on postmortem tissue or through non-invasive imaging). The finding of common aspects in the entire animal world leads to consider neural plasticity as a shared biological tool to allow structural and functional changes as an adaptive mechanism. Conversely, due to adaptation itself, different types of plasticity emerged in animal groups living in widely different ecological niches (Barker et al., 2011). Consequently, the occurrence of the main types of plastic changes (i.e., synaptic plasticity, adult neurogenesis, and immature, or "dormant" neurons; Benedetti and Couillard-Despres, 2022; Bonfanti et al., 2023) can remarkably vary depending on the animal species or age considered, regarding their anatomical location, spatial extension, and rate (Paredes et al., 2016; La Rosa et al., 2020; **Figure 1A**). For instance, the genesis of new neurons is abundant, topographically widespread, and consistent through the lifespan in fish, whereas it appears quite reduced in mammals, both in space and time (Bonfanti, 2011). Another important difference between non-mammalian and mammalian brain plasticity concerns its ultimate role, which encompasses striking regenerative processes allowing brain repair in the former, while being mostly aimed at refining the neural circuits through postnatal brain development in the latter (Bonfanti, 2011). Though the different types of brain plasticity can be found in all species, comparative research started to reveal significant variation among mammals, particularly concerning different types of neurogenic processes (with and without division; Benedetti and Couillard-Despres, 2022) that can show either high or low rates depending on brain size, gyrencephaly, and longevity of the species considered (Bonfanti et al., 2023). These interspecies variations underline the notion that plasticity is not a brain function, rather a tool that can be used to perform remarkably differing functions among animal species (Barker et al., 2011). There are multiple explanations for the adaptive significance of adult neurogenesis and how particular ecological needs and evolutionary pathways have directed its function in each animal group. These adaptive processes are at the basis of a trade-off in different types of plasticity, determining a high interspecies heterogeneity that must be known for the correct translation of experimental results obtained in laboratory rodents (Bonfanti et al., 2023). In summary, besides data indicating evolutionary conservation of cellular/molecular mechanisms and local circuit connectivity motifs in mice and humans, the existence of remarkable differences in neuroanatomy, global connectivity, and, particularly, neurogenic plasticity, increases the need for multispecies comparative studies (Bonfanti et al., 2023; **Figure 1A**). Yet, such kind of approach, in addition to obvious technical and ethical difficulties, entails the problem of obtaining comparable results.

The variables involved and the need for "comparable" results: Any comparative approach spanning from laboratory animal models to humans is a complex one, not only under the profile of tissue collecting and processing, but also concerning the reproducibility and interpretation of results. In the study of brain plasticity, attention has been often focused on single technical aspects (e.g., tissue fixation or cell counting method; Zhao and van Praag, 2020), bypassing or underestimating other important variables (Ghibaudi et al., 2023; **Figure 1B**). Some possible sources of error, such as microglia proliferation, doublecortin labeling of astrocytes in the hippocampus of epileptic patients, or non-specific 5′-bromo-2′-deoxyuridine (BrdU) labeling due to DNA repair, have been considered elsewhere (Dennis et al., 2016; Ammothumkandy et al., 2022). In addition, the recent awareness

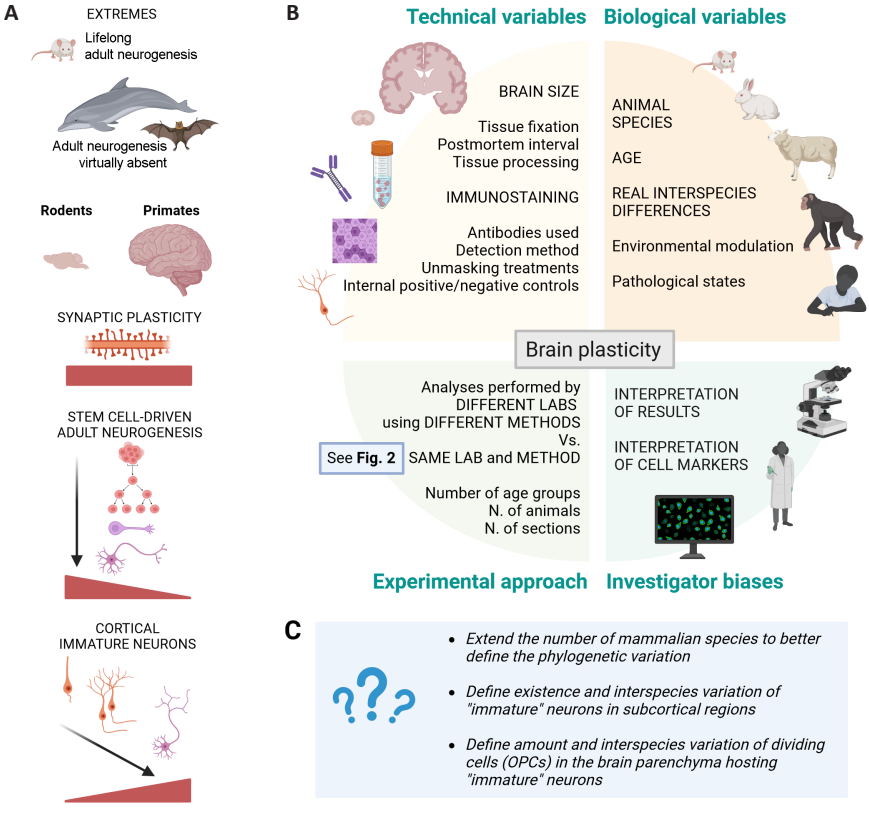

Figure 1 | **Interspecies heterogeneity in brain plasticity and variables to be considered in comparative studies.** (A) Interspecies variation in brain neurogenic plasticity. Unlike synaptic plasticity, which is considered relatively widespread and homogeneous across the animal world, neurogenic processes show remarkable variation, depending on the species. In addition to some extreme situations (top), e.g., the robust and lifelong canonical adult neurogenesis of mice in contrast with the substantial absence of this process in dolphins and some bat species (microchiroptera), most mammalian species display different gradients of neurogenic events because of different adaptations (bottom; reviewed in Barker et al., 2011; Bonfanti et al., 2023). The focus has been put on mice and primates, as representative of small brains usually endowed with smooth, less extended cortices (left) and large brains with a highly convoluted, expanded neocortex (right). Defining differences in the rate, spatial and temporal extension of plasticity can be important to avoid mistakes in the translation of preclinical data to humans. (B) Variables encountered in comparative analyses of widely different mammalian species and involved in correctly determining the interspecies differences illustrated in A. (C) Future perspectives to extend comparable results for the mapping of immature neurons in mammals. Created with BioRender.com.

for substantial interspecies differences in the types and rates of brain plasticity asks for a novel attitude, first avoiding the search for confirmation of results as already described in mice when directly studying larger brains. Even after reaching an optimal result in the conditions required by a single animal species, data are often not comparable with other species due to the different experimental approaches set by each laboratory (**Figure 1B**). Despite the same types of structural changes are shared by widely different species, their temporal extension can remarkably vary, resulting as a lifelong process in some species and dropping at very young ages in others (Paredes et al., 2016; Bonfanti et al., 2023). This point can be a very important nodus in sampling, especially when comparing species characterized by highly different lifespans. The general age-related drop in neurogenic plasticity during juvenile stages of mice is even more evident in large-brained mammals (Paredes et al., 2016) and can substantially affect the interpretation of results obtained in non-rodent, long-living species. Heterochronic changes in brain evolution within its basic developmental allometry can be studied to provide an empirical basis to recognize equivalent maturational states across animals. Despite these efforts, comparing events occurring at specific ages in animal species characterized by distant lifespans (e.g., mice and humans), it is not an easy task, since the age-related reduction of specific plastic processes can follow evolutionary adaptations (Bonfanti et al., 2023). A striking example is represented by the widely different persistence of neurogenic activity in the lateral ventricle subventricular zone: persisting at high rates in aging mice and dropping at two years in humans (Bonfanti et al., 2023). For these reasons, when trying to obtain "comparable" data from the analysis of many species, different age groups representing the main steps of animal lifespans should be considered, an approach that can also provide internal positive controls for processes decreasing with age (e.g., actively dividing stem cells during youth, then entering quiescence).

Besides the technical and ethical problems that make comparative approaches difficult and time consuming (summarized in **Figure 1B**), even when the best conditions have been established to detect some markers in a single animal species, these conditions might not be suitable for other species, for other antigens or using the same antibodies (Ghibaudi et al., 2023). In addition, most comparative studies do concern only one species or a small number of species. One might consider these problems solved by directly studying the human brain, yet, investigating how plastic processes have been shaped by evolution and reconstructing their adaptation in phylogeny (e.g., from rodents to human primates) can help understanding why they can be different in humans.

To address multiple variables in comparative neuroplasticity, we recently developed an approach to simultaneously study the brains of widely different mammals (including gyrencephalic, long-living species) at different age groups, by applying the same type of analyses and tissue processing, and by minimizing the impact of variables which cannot completely be standardized, e.g., postmortem interval and tissue fixation. We applied this method to obtain comparable results on the occurrence, topographical extension, and amount of a given cell population, namely, the cortical immature neurons (cINs; La Rosa et al., 2020; **Figure 2**). Other than finding remarkable interspecies variation concerning the amount of cINs and their spatial distribution (far more abundant and extending to the whole neocortex in gyrencephalic species), this approach also allowed to reveal age-related differences (a substantial maintenance of cINs at advanced ages in gyrencephalic mammals when compared with their faster drop in rodents; **Figure 2**, bottom).

A further pitfall raised by recent discoveries concerns the purported specificity of some makers in detecting the origin of specific, undifferentiated cell populations. Most results coming from postmortem brain tissues are obtained by detecting immunocytochemical markers linked to the maturational stages of the cells (e.g., doublecortin, a cytoskeletal protein considered for long time as a marker for newborn cells). Nevertheless, recent findings stemming out from the study of "dormant" neurons revealed that immaturity marker detection and neurogenesis can be unrelated processes, thus stultifying the specificity of some antigens for neurogenesis: doublecortin-positive neurons are newly born elements in the neurogenic sites, while they are not in the cerebral cortex layer II (Benedetti and Couillard-Despres, 2022). It seems obvious to conclude that reaching a decision on whether a doublecortin-positive neuron would fall into either an active neurogenic process or an immature neuron persistence would require the coexistence of the immaturity marker with other markers/tools identifying cell division. Nevertheless, in all cases where it is difficult to obtain convincing double-stained specimens to characterize the dividing cells, the picture can be further complicated by the existence of widespread, non-neuronal cell proliferation.

Oligodendrocyte progenitor cell division—a confounding element: A certain number of proliferative events can be detected in the brain parenchyma of adult rodents and humans, based on the labeling for nuclear proteins expressed during the cell cycle (e.g., Ki67 or PH3) or the incorporation of elements/base analogs (e.g., the thymidine analog BrdU) in the newly duplicated DNA. Besides a negligible number of endothelial cells, oligodendrocyte progenitor cells (OPCs), the most immature stage along the oligodendroglial lineage, are the major dividing cell population in the adult brain parenchyma in physiological conditions (Yeung et al., 2014; Boda et al., 2015). Sparse duplets of newly generated "sister" OPCs can be detected throughout the mouse brain parenchyma, as identified by the expression of typical markers such as Olig2, the platelet-derived growth factor alpha receptor, and the NG2 chondroitin sulfate proteoglycan. In both rodents and humans, OPCs persist in the adult and aged nervous tissue thanks to a low but almost constant turnover sustained by local cell proliferation (Yeung et al., 2014; Boda et al., 2015).

A recent study quantified the cells proliferating in all compartments of the mouse brain (Semënov, 2021), comparing their amount in the two neurogenic sites (lateral ventricle–olfactory bulb system; hippocampus) and the remaining parenchyma, from 2 to 30 months. The total amount of dividing cells in the parenchyma (around 20,000 at 2 months; mostly glial cells, among which, mostly OPCs; Yeung et al., 2014) are sparse and diluted in a wide volume with respect to the high concentration found in the neurogenic sites (around 45,000 in lateral ventricle; 2700 in hippocampus; mostly neurons), yet they remain substantially stable during lifespan (around 16,000 at 30 months), while dividing cells drop dramatically in the lateral ventricle and hippocampus (10-fold and 37-fold, respectively). This study, supported by other (less systematic) analyses, clearly indicates that parenchymal proliferating OPCs do not follow the substantial reduction observed in neurogenic sites. For this reason, the dividing OPC cell population can be a confounding element when searching for newly generated cells, especially in old brains and in species with substantial age-related reduction of neurogenesis (e.g., humans). Until now, this aspect has been underestimated, being not systematically addressed under a comparative profile considering long-living, gyrencephalic species. The discovery that non-newly generated, immature neurons can coexist with sparse proliferating OPCs in wide areas of brain parenchyma, asks for a better knowledge of the two cell populations in mammals, also considering possible interspecies differences.

Conclusion: Most biomedical research, as most studies on brain plasticity, is commonly carried out using laboratory rodents. Though providing information on the cellular and molecular mechanisms of neural plasticity, this approach can conceal important interspecies differences hampering the correct translation of results to humans (as proven by the failure of most clinical trials based on stem cell therapeutic approaches for neurological disorders; Namiot et al., 2022). As suggested here, a mix of technical, comparative, and interpretative aspects must be taken into consideration to reach an unabridged vision of the extent, features, and role of different types of plasticity in mammals, as they have been shaped by evolution. Due to extant differences in mammalian brain structure and functions, this task requires highly systematic comparative studies to grant comparable results. One possibility consists of analyses performed by the same laboratory, using the same method, on multiple (widely different) species, involving different age groups, and trying to minimize the sources of heterogeneity that cannot be avoided. This kind of approach, recently applied to 80 brains belonging to 10 mammalian species (La Rosa et al., 2020), revealed a remarkable increase in immature neurons retaining neotenic features (doublecortin-expressing cINs) from mouse to gyrencephalic

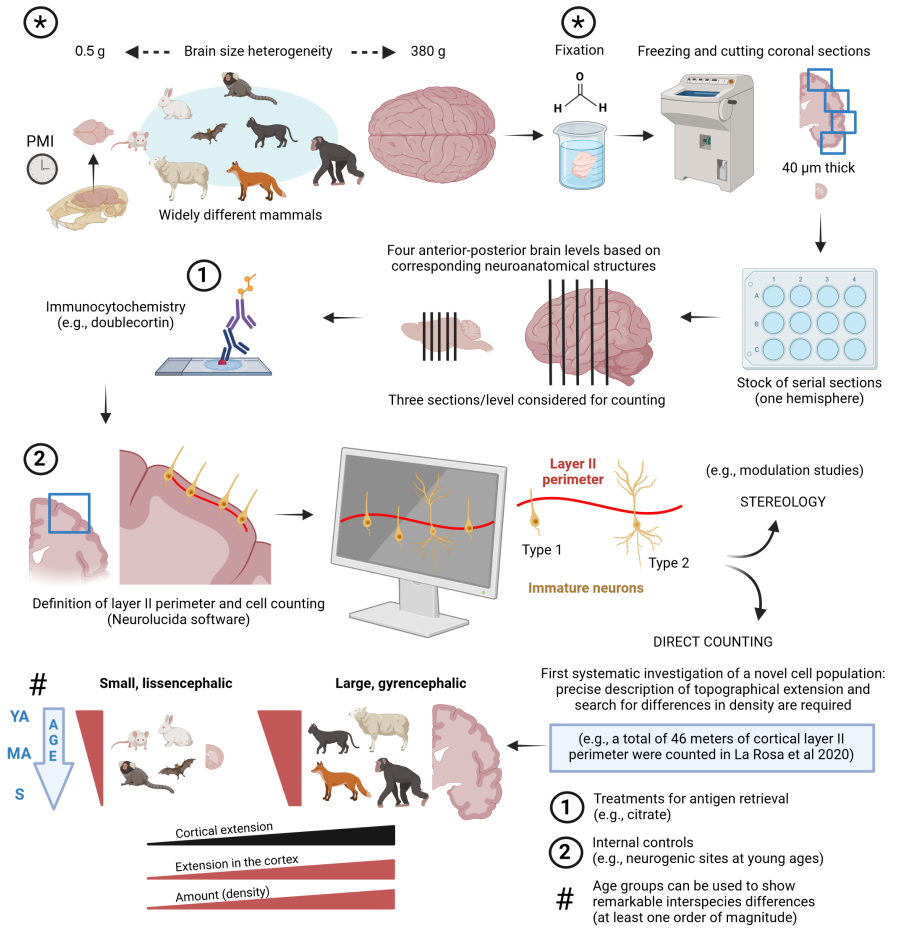

Figure 2 | **How to obtain comparable results: a method used for cortical immature neurons.**
A comparable method was recently used to study the interspecies variation of doublecortin-positive neurons (cINs) in mammals widely differing for brain size, gyrencephaly, lifespan, and ecological niche (La Rosa et al., 2020). Eighty brains (entire hemispheres) from three age groups (young-adult, middle age, and senior; four animals each) were serially cut at the cryostat; corresponding neuroanatomical structures were identified to establish four anterior-posterior comparable brain levels, and twelve sections (three each level) were immunostained for doublecortin to identify and count immature neurons in the cortical layer II, to obtain a linear density (cells/mm of layer II perimeter). Variables that cannot be completely standardized are indicated by circled asterisks; these points can be balanced by actions illustrated with circled numbers (1 and 2). A direct counting of all stained cells with Neurolucida software was performed on a total of 46 meters of the cortical perimeter, to give a whole picture of the neuronal cell population investigated (including the differential occurrence of cell types). This method allowed to compare, at the same time, in widely different mammals (i) the number of cells (cell density, increasing with brain size and gyrencephaly), (ii) their topographical distribution (restricted to paleocortex in rodents and extending to the entire cerebral cortex in gyrencephalic species) and, (iii) their modifications at different ages (generally decreasing with increasing age but more stable in gyrencephalic species with respect to rodents, indicating a substantial maintenance of the immature cell population through lifespan). Created with BioRender.com. cINs: Cortical immature neurons; MA: middle age; PMI: postmortem interval; S: senior; YA: young adult.

mammals (**Figure 2**), and can be used in the future both to obtain comparable results in the cortex of other species (and ages) or to explore the occurrence of the same type of neurons in other brain regions, in order to gain a whole picture of their evolutionary adaptations across the mammalian brains.

This work was supported by Progetto Trapezio, Compagnia di San Paolo (67935-2021.2174) to LB, and Fondazione CRT (Cassa di Risparmio di Torino; RF=2022.0618) to LB.

Marco Ghibaudi, Enrica Boda, Luca Bonfanti*
Neuroscience Institute Cavalieri Ottolenghi, Orbassano, Italy (Ghibaudi M, Boda E, Bonfanti L)
Department of Veterinary Sciences, University of Turin, Turin, Italy (Ghibaudi M, Bonfanti L)
Department of Neuroscience Rita Levi-Montalcini, University of Turin, Turin, Italy (Boda E)
***Correspondence to:** Luca Bonfanti, PhD, luca.bonfanti@unito.it.
https://orcid.org/0000-0002-1469-8898 (Luca Bonfanti)

Date of submission: February 2, 2024
Date of decision: March 4, 2024
Date of acceptance: March 19, 2024
Date of web publication: April 16, 2024

https://doi.org/10.4103/NRR.NRR-D-24-00143
How to cite this article: *Ghibaudi M, Boda E, Bonfanti L (2025) From mice to humans: a need for comparable results in mammalian neuroplasticity . Neural Regen Res 20(2):464-466.*

Open peer reviewer: *Jon I Arellano, Yale University School of Medicine, USA.*
Additional file: *Open peer review report 1.*

References

Ammothumkandy B, Ravina K, Wolseley V, Tartt AV, Yu PN, Corona L, Zhang N, Nune G (2022) Altered adult neurogenesis and gliogenesis in patients with mesial temporal lobe epilepsy. Nat Neurosci 25:493-503.

Barker JM, Boonstra R, Wojtowicz, JM (2011) From pattern to purpose: how comparative studies contribute to understanding the function of adult neurogenesis. Eur J Neurosci 34:963-977.

Benedetti B, Couillard-Després S (2022) Why would the brain need dormant neuronal precursors? Front Neurosci 16:877167.

Boda E, Di Maria S, Rosa P, Taylor V, Abbracchio MP, Buffo A (2015) Early phenotypic asymmetry of sister oligodendrocyte progenitor cells after mitosis and its modulation by aging and extrinsic factors. Glia 63:271-286.

Bonfanti L (2011) From hydra regeneration to human brain structural plasticity: a long trip through narrowing roads. Sci World J 11:1270-1299.

Bonfanti L, La Rosa C, Ghibaudi M, Sherwood CC (2023) Adult neurogenesis and "immature" neurons in mammals: an evolutionary trade-off in plasticity? Brain Struct Funct doi: 10.1007/s00429-023-02717-9.

Dennis CV, Suh LS, Rodriguez ML, Kril JJ, Sutherland GT (2016) Human adult neurogenesis across the ages: an immunohistochemical study. Neuropathol Appl Neurobiol 42:621-638.

Ghibaudi M, Amenta A, Agosti M, Riva M, Graïc JM, Bifari F, Bonfanti L (2023) Consistency and variation in doublecortin and Ki67 antigen detection in the brain tissue of different mammals, including humans. Int J Mol Sci 24:2514.

La Rosa C, Cavallo F, Pecora A, Chincarini M, Ala U, Faulkes CG, Nacher J, Cozzi B, Sherwood CC, Amrein I, Bonfanti L (2020) Phylogenetic variation in cortical layer II immature neuron reservoir of mammals. eLife 9:e55456.

Namiot ED, Niemi JVL, Chubarev VN, Tarasov VV, Schiöth HB (2022) Stem cells in clinical trials on neurological disorders: trends in stem cells origins, indications, and status of the clinical trials. Int J Mol Sci 23:11453.

Paredes MF, Sorrells SF, Garcia-Verdugo JM, Alvarez-Buylla A (2016) Brain size and limits to adult neurogenesis. J Comp Neurol 524:646-664.

Semënov MV (2021) Proliferative capacity of adult mouse brain. Int J Mol Sci 22:3449.

Yeung MS, Zdunek S, Bergmann O, Bernard S, Salehpour M, Alkass K, Perl S, Tisdale J, Possnert G, Brundin L, Druid H, Frisén J (2014) Dynamics of oligodendrocyte generation and myelination in the human brain. Cell 159:766-774.

Zhao X, van Praag H (2020) Steps towards standardized quantification of adult neurogenesis. Nat Commun 11:4275.

P-Reviewer: Arellano JI; C-Editors: Zhao M, Liu WJ, Qiu Y; T-Editor: Jia Y