**HAUNTING NEWBORN NEURONS IN THE HUMAN ADULT BRAIN**

Flor-García M 1,2,3†; Terreros-Roncal J 1,2,3,†; Moreno-Jiménez E.P 1,2,3†; Llorens-Martín M 1,2,3,\*.

1 Department of Molecular Neuropathology, Centro de Biología Molecular “Severo Ochoa”, CBMSO, CSIC-UAM, Madrid (Spain). Phone number: +34-911964592.

2 Department of Molecular Biology, Faculty of Sciences, Universidad Autónoma de Madrid, Madrid (Spain). Phone number: +34-911964592.

3 Center for Networked Biomedical Research on neurodegenerative diseases (CIBERNED), Madrid (Spain). Phone number: +34-911964592.

† These authors contributed equally to this work as first author.

\*Correspondence to: María Llorens-Martín. *Centro de Biología Molecular “Severo Ochoa”*, *Universidad Autónoma de Madrid (campus de Cantoblanco)*, *c/Nicolás Cabrera 1*, 28049, Madrid (Spain). Phone number: +34-911964592. [m.llorens@csic.es](mailto:m.llorens@csic.es).

GENERAL INTRODUCTION

The hippocampus is a brain region tightly related to memory processing. Moreover, this region hosts one of the most unique phenomena that occurs in the adult mammalian brain, namely the continuous generation of new neurons during lifetime. This phenomenon is named adult hippocampal neurogenesis (AHN). AHN is crucial for learning and memory, and it confers an unparalleled degree of plasticity to the entire hippocampal circuitry. Even though AHN has been extensively characterized in rodents during the last decades, direct evidence in human has remained elusive.

INTRODUCTION TO STUDY

During the last decade, our laboratory has focused on studying human AHN during physiological and pathological aging. In particular, we have studied a neurodegenerative disease that severely targets the hippocampus, namely Alzheimer´s disease (AD). Given the outstanding regenerative potential of generating new neurons during adulthood, determining the occurrence of this phenomenon in the human brain is a crucial question with remarkable therapeutic potential.

METHODOLOGY

Given the existence of contradictory evidences either supporting or questioning the occurrence of human AHN, we aimed to determine whether methodological differences could underlie these discrepancies. We used *post-mortem* human brain samples obtained under tightly controlled conditions and state-of-the-art tissue processing methodologies. Moreover, we tested the influence of using different fixative solutions (either formalin or paraformaldehyde (PFA), fixation time, *post-mortem* delay (time elapsed between *exitus* and sample immersion in fixative), and histological procedures on the detection of different proteins (so-called markers) related to AHN. The influence of these factors was analyzed on brain samples obtained from 13 neurologically healthy subjects (between 42 and 87 years of age) and 45 patients with AD. We analyzed not only the presence of immature neurons but also different parameters related to the maturation of these cells in the human hippocampus during physiological and pathological aging.

RESULTS

Our results show the unambiguous detection of thousands of immature neurons (expressing the gold-standard marker of newly generated neurons Doublecortin (DCX)) in the human DG up to the tenth decade of life. Moreover, we confirmed our initial hypothesis that certain tissue processing methodologies completely abolished the detection of these cells. We concluded that short fixation in PFA, combined with novel histological procedures allow the unambiguous detection of AHN markers in the human hippocampus. In contrast, formalin, the most commonly used fixative in brain banks worldwide, completely abolished their detection.

Importantly, we observed a decrease in the number of DCX positive neurons through physiological aging. Moreover, we determined whether immature neurons at distinct maturation stages could be found in the human hippocampus. To this end, we first analyzed the colocalization between DCX and other markers characteristic of different maturation stages of this process. Based on both the percentages of double-labeled cells and their morphological features, we have outlined the first proposed model of the maturation stages of AHN in humans.

Next, we wondered whether AHN was impaired in patients with AD. Remarkably, our data revealed both a progressive reduction in the number of immature neurons and a dramatic impairment in their maturation. Importantly, these alterations are observed at early stages of the disease, although a progressive worsening is observed as the disease advances.

CONCLUSIONS

The use of the most suitable preservation and tissue processing methodologies have brought to light the existence of a dynamic population of immature neurons in the human DG throughout aging until the tenth decade of life. Despite a slight decrease in the number of immature neurons throughout physiological aging, these cells go through different maturation stages during AHN in humans. Finally, our data reveal a marked early and progressive reduction in the number of immature neurons, accompanied by an alteration in their maturation, in patients with AD.

GENERAL CONCLUSIONS AND FUTURE PERSPECTIVES

Our findings suggest unexplored mechanisms of circuit plasticity in the aging human hippocampus, revealing that AHN occurs and persists through life. Strikingly, our results highlight the importance of lately disregarded methodological aspects of histological procedures to detect this dynamic population of immature neurons in the DG. This message should be taken into account by future studies aimed to expand our current knowledge on this fascinating process that occurs in the human brain. Moreover, the putative detection of AHN impairments by non-invasive techniques might turn this phenomenon into a relevant biomarker of disease progression. Finally, given that AHN can be regulated extrinsically (at least in rodents), increasing AHN in humans emerges as a potential therapeutic target for preventing, or slowing down, neurodegenerative diseases.