NEURODEGENERATION

A fresh look at adult neurogenesis

Improved protocols for the visualization of immature neurons in the human brain provide evidence for generation of neurons in the adult hippocampus and uncover reduced neurogenesis in Alzheimer's disease.

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While most neurons are generated before birth, new neurons are continuously generated in discrete areas of the brain throughout life via a process called adult neurogenesis. One of these areas is the hippocampus (Fig. 1a). In animal models, decreased adult hippocampal neurogenesis leads to impaired learning and memory as well as altered affective behavior[1]. These findings have posed the question of whether altered adult neurogenesis may be implicated in neurological and psychiatric diseases in humans. Adult neurogenesis is, however, challenging to study in humans, and knowledge about the extent of this process, as well as how it is affected in pathological situations, is limited. Moreno-Jiménez et al.[2] report in *Nature Medicine* an improved strategy for the visualization of adult neurogenesis, which they use to reveal the presence of a surprisingly high number of immature neurons in the human hippocampus and a progressive reduction of adult neurogenesis in Alzheimer's disease (AD).

When a new neuron is generated from a progenitor cell, it goes through distinct maturational stages, which can be defined by protein markers. The most common methodology used to study adult neurogenesis in humans is immunohistochemical detection of markers for immature neurons, also called neuroblasts; it is assumed that some of these cells will eventually become mature neurons. Several studies have demonstrated the presence of immature neurons throughout life in the human hippocampus, although the quantitative estimates have slightly varied[3–5]. One study published last year failed to detect immature neurons beyond adolescence in the human hippocampus[6], reviving an old debate about the extent of adult neurogenesis in humans[3,7].

It is well known that tissue handling is essential for the preservation and detection of neuroblast markers[7], and it is likely that the use of different protocols, at least in part, explains the varying results between studies. Immunofluorescence analysis is compromised by very prominent background fluorescence, known as autofluorescence, in the tissue of the adult human brain, resulting in a poor signal-to-noise ratio and reducing the sensitivity for detection of many markers[7]. Moreno-Jiminez et al.[2] optimized tissue fixation, autofluorescence quenching, epitope retrieval and antibody selection, resulting in the most distinctive visualization of cells at different maturational stages in the neurogenic process in the adult human brain to date. Furthermore, they selected brains from subjects with a short post-mortem delay, contributing to remarkably well-preserved tissue.

After establishing these optimized conditions, Moreno-Jiménez et al.[2] visualized doublecortin (DCX), the most commonly used neuroblast marker, in combination with other proteins associated with different maturational stages, in the dentate gyrus of the hippocampus in 13 healthy individuals. The number of these cells declined with age, but persisted at least into the ninth decade of life, corroborating previous studies[4,8]. Moreno-Jiménez et al.[2] report a several-fold-higher density of DCX-positive cells in the dentate gyrus than previously reported[4,5]. They suggest that the high number of neuroblasts detected in their study is the result of an optimized protocol with higher sensitivity and that previous studies might have underestimated the number of neuroblasts in this region of the brain. An additional factor contributing to the high neuroblast numbers they report may be that they focused on the anterior hippocampus, where neurogenesis is thought to be most prolific[5]. A strength of their study is that they demonstrated the co-existence of several neuroblast-associated markers in individual cells, which lends strong support to the notion that these cells indeed are immature neurons. Nevertheless, profiling the cells more thoroughly, for example via single-cell RNA-sequencing, will be valuable for better characterization of neuroblasts and their progression through the neurogenic lineage.

The number of neuroblasts serves as a proxy marker for adult neurogenesis, as it does not identify new mature neurons per se. Since it is not known how long a cell remains in the neuroblast phase and what proportion of neuroblasts give rise to a new neuron, it is not possible to directly infer how many new neurons are generated. Other strategies that take advantage of incorporation of the synthetic nucleotide bromodeoxyuridine (BrdU) or nuclear-bomb-test-derived ^{14}C have demonstrated that there is substantial neurogenesis throughout life in the human hippocampus[8,9].

Next, Moreno-Jiménez et al.[2] assessed the number of DCX-positive cells in the hippocampus from individuals with

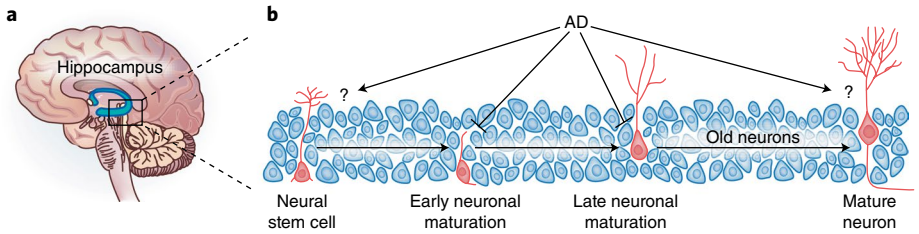

Fig. 1 | The number of immature neurons in the human hippocampus decreases in AD. a, The hippocampus is located in each hemisphere of the human brain and has a role in memory. **b**, The stages of neuronal maturation in the adult neurogenesis process among mature granule neurons (shown in blue). Cells at different maturation stages (shown in red) are affected in AD. Whether the neural stem cell population is affected and to what extent new mature granule neurons are generated in AD remain to be resolved (shown as question marks).

AD. AD is the most common cause of dementia, and the hippocampus is often severely affected. Previous studies using immunohistochemistry have shown varying results, including increased or unaltered neurogenesis[10,11] and faulty maturation of young neurons[12]. Moreno-Jiménez et al.[2] found that the number of DCX-positive cells was significantly lower in individuals with AD compared with healthy controls and that later stages of AD were accompanied by neurogenesis that was further reduced. Moreover, when assessing the expression of markers related to specific stages of neuronal maturation, the authors found that the number of cells expressing early neuronal maturation markers and of those expressing late neuronal maturation markers was reduced in late AD (Fig. 1b). The number of cells expressing a marker for early neuronal maturation was significantly reduced at an earlier stage of AD as well.

Moreno-Jiménez et al.[2] provide support for active adult neurogenesis in the human hippocampus and suggest that neurogenesis is impaired in AD at different stages of maturation. Looking ahead, this poses new questions. Is the neural stem cell population affected in AD? How many of the newly generated neurons actually survive and integrate in the long term in the healthy brain and the AD brain? Adult neurogenesis appears to be affected in the early stages of AD; could the decline in neurogenesis in fact precede the onset of the disease? Further characterization of adult neurogenesis through assessment of the dynamics of the stem and progenitor cells that give rise to neuroblasts, as well as quantification of the integration of mature neurons, will provide valuable information for understanding its implication in disease, and hopefully for the future development of targeted therapies. ❐

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Competing interests

The authors declare no competing interests.

STROKE

Divergent effects of lipids on stroke

A large study provides causal evidence of the opposing effects of plasma low-density lipoprotein (LDL) levels on ischemic and hemorrhagic stroke in a Chinese population and suggests there is a a net benefit associated with LDL lowering.

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Lipid-modifying therapies, specifically 'statins' (3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors), are a mainstay of prevention of stroke and heart disease. Statins have multiple effects, but they, along with newer agents such as proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors, are used chiefly to reduce LDL, or 'bad' cholesterol, levels. LDL is a plasma cholesterol component that promotes atherosclerosis and thereby contributes to the development of ischemic stroke and heart disease. While most incident stroke cases globally are ischemic, approximately 30% are hemorrhagic, and the rate of hemorrhagic stroke is higher in China and other countries that are less economically developed[1]. The relationship between LDL concentration and hemorrhagic stroke — specifically intracerebral hemorrhage (ICH) — is poorly understood. In this issue of *Nature Medicine*, Sun et al.[2] provide novel evidence that a lower LDL concentration is likely associated with ICH risk, though it reduces ischemic stroke risk.

Plasma LDL concentration is a well-studied risk factor for ischemic stroke. Previous observational studies identified a correlation between elevated plasma LDL levels and ischemic stroke, particularly that due to atherosclerotic cerebrovascular disease, although the associations were weaker than for coronary heart disease[3]. The causal nature of this relationship can be inferred from large clinical trials in which individuals randomized to statins and PCSK9 inhibitors for plasma LDL reduction have a reduced risk of ischemic stroke[4,5]. Many multicenter studies have enrolled individuals worldwide, including some from China, but the study populations have largely been Western.

Mendelian randomization studies provide an alternative approach to clinical trials for assessing causality. They use 'genetically instrumented' variables, or genetic measures that correlate with the risk factor of interest — LDL, in this case. The measures are derived from genetic polymorphisms that are known to be strongly associated with LDL. This design facilitates investigation of causal relationships between lifelong exposure — since individuals are born with the genetic variants — and outcomes of interest. These studies overcome the potential confounding by unmeasured confounders seen in observational studies and possible off-target effects of pharmaceutical agents found in randomized trials. Prior Mendelian randomization studies provided complementary evidence that elevated LDL levels cause ischemic stroke[6].

In contrast, previous data regarding LDL levels and ICH risk were limited. Prior observational data from studies, including those of Asian populations, with small numbers of ICH cases suggested that lower levels of LDL were associated with an increased risk of ICH[7]. Further, individual randomized trials in largely Western populations did not demonstrate an increased risk of ICH with therapeutic plasma LDL lowering, though a meta-analysis suggested an excess risk of hemorrhagic stroke with LDL reduction[8]. Additionally, in a secondary prevention clinical trial of high-intensity statin therapy after stroke among primarily North Americans and Europeans, randomization to high-dose statin therapy was associated with an increased risk of hemorrhagic stroke[9]. These data have led to persistent