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Historic Overview

Given the tremendous advance that neuroscience has experienced in recent years, some of the pillars on which it has been sustained have started to collapse. Not so long ago, it was believed that we were born with a fixed number of neurons that died to a greater or lesser extent throughout our lifetime. It was widely accepted that the production of new neurons did not persist after birth in the healthy brain or under pathological conditions. This fact was both recognized and defended by scientists and society as a dogma.

We can now state, however, that adult neurogenesis exists. New neurons are produced during adulthood in all of the vertebrates that have been studied, from fish to mammals, including the human species. In nonmammal vertebrates, it has been described that the degree of neurogenesis is higher and that it affects more regions than in mammals. This fact does not limit the relevance that the presence of neural stem cells in the human brain has to neuroscience. We now know more than we knew decades ago, but we still have to learn how to communicate with stem cells.

The process of learning that neurogenesis actually exists has taken almost a century and has progressed slowly so far. In the late 1800s, scientists worldwide, including the prestigious Spanish researcher, Santiago Ramon y Cajal (1913), maintained that neurogenesis was a process restricted to brain development that ceased after birth. This conclusion was the result of studying the histology of the brain with the techniques of the time, such as Nissl and silver impregnation. Most researchers defined neurons as cells that were characterized by the presence of dendritic arborizations. When dendrites were not well developed, cells were thought to be in the process of differentiation, plastic changes, or the result of a histological artifact.

In the first half of the twentieth century, however, occasional studies described these less differentiated neurons as potential newly formed neurons. This hypothesis was speculation that lacked rigorous scientific work to support it. A remarkable study by Sugita (1918) described an increase in new neurons in the rat cerebral cortex after comparing neonates with 20-day postnatal animals. This result has since been attributed to stereological errors in his analysis that disregarded the increase in brain volume with age.

The observation of mitosis correlates with the existence of active proliferating centers. In the past, however, some authors identified mitosis in the brain as dividing

glial cells or endothelium. Occasionally mitotic figures were observed in the adult lateral ventricles, regions that play an important role during embryonic development. After a few decades, an elegant study drew the interest of scientists back to mitosis (Bryans 1959). This study was performed in rats in which colchicine was injected. Colchicine is a substance that binds to microtubules and disrupts the mitotic spindle, arresting cells in mitosis. After injection, a large amount of mitosis was found in the cells surrounding the lateral ventricles, making it possible for these ventricular walls to be a source of new neurons in the adult brain. Although scientists were on the right track, technical resources were limited at that time, and it was not possible to prove that mitotic cells gave rise to new neurons. This fact led the scientific community to overlook these new findings.

However, in the early 1950s, specific markers of cell proliferation—such as tritiated thymidine—were discovered, and research into adult neurogenesis revived. Tritiated thymidine (3HT) incorporates into nucleic acid during its synthesis in the S-phase of the cell cycle that precedes cell division, and it can be detected later by autoradiography. Altman (1962, 1969a, b), a self-taught postdoctoral student, was a pioneer in the use of this substance in the adult brain, and he published a series of reports showing neurogenesis in diverse brain regions of the young and adult rat brain. Animals were sacrificed shortly after injection in order to label proliferating centers, and at longer times after injection to study the fate of the cells that proliferated in these centers. Altman described neurogenesis in the neocortex, hippocampus, and olfactory bulb, and went on to not only identify the newly formed cells as microneurons, but also to relate neurogenesis to a potential role in memory and learning. Many scientists felt that this type of neurogenesis should be interpreted as being sporadic and residual, i.e., a peculiarity of the rodent brain. Although his work was accurate to a great extent, he received criticism for two reasons: (1) there was no proof of the existence of migration routes from the germinal zones (lateral ventricle wall) toward the final locations of other brain areas, and; (2) the micronuclei—supposedly neuronal—may have been confused with the nuclei of glial cells. Altman had no proof of cell migration pathways other than that toward the olfactory bulb, and he did not know how to functionally explain the meaning of the generation of new neurons. We should not forget the work done in this field by Bayer, who studied neurogenesis by means of the incorporation of 3HT into the third ventricle, the septal nucleus, the hippocampal region, amygdalae, the superior colliculus, and the olfactory bulb (Altman and Bayer 1979a, b, c, 1981a, b; Bayer 1980a, b, 1983). The majority of his work, however, was performed during brain development. Another supporter of adult neurogenesis was Kirsche (1967). By using 3HT, Kirsche demonstrated the existence of active proliferation sites (sulci) in the adult brains of several groups of vertebrates. He attempted to perform a comparative study of homologous regions, but his work was not well accepted. This was because he believed that the brain was immature in nonmammalian vertebrates and had not yet undergone complete growth.

Further interest in neurogenesis can then be attributed to a technical advance, the electron microscope. Electron microscopy (EM) allowed the observation of

cells at high resolution, enabling neurons and glial cells to be differentiated. Using EM, Kaplan studied cells labeled with 3HT in the rodent brain, and confirmed the existence of adult neurogenesis and the presence of synaptic contacts in the newly formed neurons (Kaplan 1981, 1985; Kaplan et al. 1985). He also demonstrated long survival times for these young neurons. These studies were performed in the hippocampus, an area that was becoming increasingly important because of its involvement in learning and memory.

The scientific community started to wonder whether this phenomenon could be extended to all vertebrates and mammals, especially to humans. A number of reports showed that, although neurogenesis is not restricted to mammals, it is present in all vertebrates, and the number of proliferative centers involved is higher the closer they are to the base of the phylogenetic tree. At present, we know that neurogenesis occurs in fish in the whole brain, particularly in the cerebellum, while it is exclusive to the telencephalon in all other vertebrates. Only in amphibians and certain ophidian reptiles can new neurons be found in the optic tectum, probably owing to their relationship with the retina.

By this time, the existence of adult neurogenesis in nonhuman mammals, at least in the dentate gyrus of the hippocampus and the olfactory bulb, was widely accepted. The main concern was elucidating whether adult neurogenesis existed in the human brain and where it occurred. To answer that question, and given the lack of human brain material, studies were performed in species that were closely related to humans, such as primates. In a series of detailed studies using 3HT, Rakic (1985a, b) concluded that there was no adult neurogenesis in primates and possibly not in humans either. He did not find labeled cells in the adult primate brain. He hypothesized that neurogenesis in the brains of lower vertebrates could be the result of immaturity of the brain after birth and the need for new neurons to complete the number of neurons necessary to reach the adult state. Rakic also used physiology to explain this event. New neurons of lower vertebrates could store new data throughout life, thus enabling the learning process. In contrast, and as a result of evolution, primates would be born with mature neuronal units that are ready to store all information after birth. The arrival of newly formed neurons may alter not only these systems but also the existing circuitry, causing the loss of stored data. This hypothesis was accepted up until a few years ago.

During this same period, a satisfactory explanation was given to address the physiological meaning of adult neurogenesis. Fernando Nottebohm (Goldman and Nottebohm 1983; Nottebohm 1985; Paton and Nottebohm 1984), while working with songbirds, demonstrated that adult neurogenesis occurred in a telencephalic center that was directly related to vocal learning. Vocal learning is essential for communication between birds, reproduction and the establishment of territories. He showed that: (1) there were several centers involved, but that only the high vocal center showed neurogenesis; (2) the newly formed neurons received synaptic contacts, their ultrastructure was similar to that in mature neurons, and they were functional; (3) when eliminated, birds were impaired in the learning of new sounds, and; (4) the regions involved were four times larger in males than in females, and

this phenomenon was reverted by injecting testosterone into females. This was accompanied by an increase in neurogenesis in females that was not observed in untreated females.

A few years later, migratory cells were described in the adult reptile brain (Garcia-Verdugo et al. 1986). These cells were located between the lateral ventricles and a region of neuronal cell bodies called the medial cortex. The medial cortex of reptiles has been compared to the dentate gyrus in mammals. By using 3HT and EM, the existence of neurogenesis was also demonstrated in reptiles (Lopez-Garcia et al. 1988). Newly formed neurons in the reptilian brain were born close to the ventricles and migrated radially toward the majority of telencephalic regions. After selective chemical injury, and by increasing neurogenesis, the reptilian cerebral cortex was repaired completely (Font et al. 1997).

Despite the fact that the field of neurogenesis in birds and reptiles advanced rapidly, limited studies were performed on neurogenesis in mammals. However, the use of new cell markers and novel techniques questioned Rakic's hypothesis, as several studies have demonstrated postnatal neurogenesis in primates (Gould et al. 1998, 1999a). A surprising study by Gould et al. (1999a) described neurogenesis in associative regions in primates for the first time. In a recent review of the topic, and after considerable controversy, this work was thoroughly reviewed (Gould 2007). Simultaneously, adult neurogenesis was also found in the human brain. The first demonstration in humans was performed in five cancer patients who were injected with a proliferation marker, bromodeoxyuridine (BrdU), which works similarly to 3HT, for the purpose of determining tumor activity (Eriksson et al. 1998). The brains of these patients were studied after death, and labeled cells were detected in the hippocampal dentate gyrus. Specific neuronal immunohistochemistry markers also stained these cells.

All of this data has aroused the curiosity of the scientific community, and increasing effort has been expended to finally demonstrate the validity of adult neurogenesis. Elucidating the identity of the cells responsible for neurogenesis will permit us to understand the physiological reason for the generation of new neurons, to establish dialogue with them, and therefore to direct them to damaged regions, undoubtedly leading to a fascinating future for this field of research.