Stable neuron numbers from cradle to grave

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The human cerebral cortex is the main organ responsible for our cognitive abilities, e.g., our abilities to use language and math, to reason and theorize, and to read and write articles such as this one, essentially defining who we are and what we do, and, most importantly, storing a lifetime of experience. The critical cell type underlying these functions is the neuron. The human neocortex contains $\approx 10^{10}$ neurons (1), and, understandably, the question of how and when these crucial cells come into being is fundamental to both normal and pathological conditions and behaviors. The modern era of understanding the time of origin of the neurons of this remarkable structure began with the use of a by-product of the atomic age, i.e., the radioactive nucleotide tritiated thymidine, to sequence the production of the cortical layers in mice (2) by exploiting its ability to incorporate into the DNA of proliferating cells. In this issue of PNAS, Bhardwaj *et al*. (3) exploit the ability of another by-product of this era, i.e., the dramatic elevation in atmospheric ^{14}C levels from the early aboveground nuclear tests, to incorporate into DNA (4) to resolve one of the hottest controversies in modern neuroscience. The question is: Are the neurons of the adult human cortex produced prenatally and then stably retained for our entire lifespan? Or are some new neurons produced in the adult and then integrated into the preexisting circuitry of the cortex? In essence, the issue is "How old are the neurons in our cortex?" Are they constantly renewed like the cells of the skin or the gut, or must the cortex make do for a lifetime with an initial set of neurons?

This issue was first addressed by Rakic (5), who examined the brains of adult rhesus monkeys that had been exposed once or even multiple times to tritiated thymidine from 3 days to 6 years before analysis. In neocortex, he found labeled glial cells but no labeled neurons and concluded that "the full complement of neurons in the primate central nervous system seems to be attained during a restricted developmental period." The introduction of BrdU immunohistochemistry as a nonradioactive marker for DNA synthesis in the brain (6, 7) led to an explosion of studies on adult neurogenesis, eventually reaching consensus that two areas of the adult mammalian brain, the subgranular zone of the dentate gyrus and the subventricular zone lining the lateral ventricles, continue to produce neurons destined for the dentate gyrus and olfactory bulb, respectively, in adult rodents (8) and to a lesser extent in adult nonhuman primates (9). In humans, only the dentate gyrus population contributes new neurons (10, 11). Continued production of neurons for numerous other areas of the brain has been suggested, but the correctness of these suggestions can be questioned for a variety of reasons, including technical aspects of the BrdU method (12–14). The most controversial is the suggestion that a large number of new neurons are contributed daily to the adult primate neocortex (15, 16). However, efforts to replicate these results have not been successful in either primates (17, 18) or rodents (19), and the issue of possible adult neurogenesis in the neocortex has been vigorously debated (12, 20, 21).

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A Two-Pronged Approach

Bhardwaj *et al*. (3) address this key issue for the human cortex using two approaches to determine whether there is (*i*) continuous production of a long-lived population of new neurons over the lifespan of the adult human, or (*ii*) a production of new neurons in the adult that have only a transient existence.

The first method used by the authors (3) is an ingenious one. They exploit the fact that the aboveground nuclear tests in the 1950s and early 1960s produced a dramatic elevation of ^{14}C levels in the atmosphere. Since the cessation of the aboveground tests, the levels of ^{14}C in the atmosphere have fallen exponentially because of equilibration with the oceans, dispersion in the biomass, etc. Because metabolically active ^{14}C levels in plants and animals are in equilibrium with atmospheric ^{14}C and the half-life of ^{14}C is long (>5,000 years), any molecules synthesized during the period of rapidly changing ^{14}C levels can be dated by the proportion of ^{14}C they contain. DNA in nonproliferating cells is stable, so ^{14}C levels in DNA reflect the level of atmospheric ^{14}C at the time of DNA synthesis (i.e., at the time of cell "birth"). This is the essential fact that the authors have grasped and exploited. They reasoned that if the neocortical neurons were produced during the developmental period, then the age of the DNA in these neurons would be the same as the individual (i.e., it would date to the approximate birth date of the human from which it was taken). They have validated this approach in a variety of tissues (4, 22), and now they apply the technology in individuals born (in northern Europe) before, during, and after the above-ground test period using four different regions of the neocortex: prefrontal, premotor (frontal), parietal, and temporal.

The oldest individual analyzed was 72 years old and, thus, was ≈20 years old when the atmospheric levels of ^{14}C started to rise. Five additional individuals were born during or before 1953, the year of the first aboveground nuclear tests and the onset of the rise in atmospheric ^{14}C. The tissue was collected from autopsies performed in 2003–2004; thus, the results from these specimens effectively integrate ≈50 years of neuron production, i.e., over most of the expected lifespan of a human. The effect of this integration is that it makes the analysis quite sensitive to the continuous production of even a small number of cells. Both neurons and nonneurons, distinguished by cell sorting, and an unsorted control were analyzed. The results show that the average age of the neurons (with respect to the age of the individual) is age 0.0 ± 0.4 years, i.e., the same as the age of the individual. In contrast, the nonneuronal cells have an average birth date of 4.9 ± 1.1 years after the birth of the individual. Importantly, the unsorted samples do not differ from the nonneuronal samples, which makes sense because glia outnumber neurons by ≈10:1 in the adult. The dating of the neurons to the same time as the birth of the individual argues strongly that there are no new neurons produced after birth; however, given the precision of the dating methods used (22), it is estimated that the limit on the possible number of new neurons made during this 50-year period is <1% of the total (22). In other words, >99% of the

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human neocortex is produced during the fetal period.

To settle the issue of the possible existence of a transient population of new neurons that would not be detected by the ^{14}C method, Bhardwaj *et al*. (3) did a second experiment using autopsy material collected from cancer patients who had received injections of BrdU (10) for diagnostic purposes from 4.2 months to 4.3 years before death. They found BrdU-labeled cells in the neocortex, but none of the labeled cells were neurons or neuron-like. As a positive control, they examined the dentate gyrus of the same patients, where they found clear evidence of neuron production. From this second experiment, they conclude that if the neocortex contains new neurons with a short lifespan, those new neurons survive for no more than ≈4 months, i.e., the length of the shortest survival span they examined. Importantly, the comparison with the dentate gyrus indicates that any transient new neurons in the neocortex must be considerably less prevalent than they are in the dentate gyrus, where ≈22% of the BrdU-labeled cells apparently have a neuronal phenotype (10). In addition, their results mean that transient cells do not persist in an undifferentiated state and later become neurons, at least within the ≈4-year span examined.

Functional Considerations

Both of the experiments of Bhardwaj *et al*. (3) indicate that there are no new neurons, either long-lived or transient, produced in the adult human for the neocortex. Importantly, these experiments are quantitative and indicate a theoretical maximum limit of 1% on the proportion of new neurons made over a 50-year period. This proportion provides a maximum limit as to the possible rate of production, i.e., 0.02% per year. With respect to the function of the neocortex, this maximum rate of production should be considered in the context of the cortical column, i.e., the physiologically functional unit of information processing in the cortex (23, 24). In the neocortex, the minicolumn, also known as an ontogenetic column (25), is the fundamental anatomical and processing unit that is iteratively arrayed across the neocortical surface; each minicolumn contains 80–100 neurons but may be 2.5 times that size in the visual cortex (23, 24). The crucial relationship here is that,

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regardless of the size of the cortical column, the estimated limit on the number of new neurons produced is extremely small. At the hypothetical maximum limits of production, the larger columns in the visual cortex might receive one new neuron every 20–30 years, whereas the smaller columns in the rest of the neocortex might receive one new neuron every 50 years. In other words, even the maximum theoretical production of new neurons is so small as to make clear that the potential contribution of new neurons to neocortical function is minuscule to nonexistent. In contrast, the BrdU experiment of Bhardwaj *et al*. (3) indicates that there is a constant production of new glial cells in adults. This finding is consistent with their ^{14}C data that reflect an average birth date of ≈5 years after the birth of the individual for the nonneuronal cells. The fact that there is continuous glial cell turnover might be important for pathology and treatment of injury (13).

Stability Reigns

Bhardwaj *et al*. (3) settle a hotly contested issue, unequivocally. The two-pronged experimental approach clearly establishes (*i*) that there is little or no continuous production of new neurons for long-term addition to the human neocortex and (*ii*) that there are few if any new neurons produced and existing transiently in the adult human neocortex. Importantly, the results are quantitatively presented, and a maximum limit to the amount of production of the new neurons can be established from the data presented. The data show that virtually all neurons (i.e., >99%) of the adult human neocortex are generated before the time of birth of the individual, exactly as suggested by Rakic (5), and the inescapable conclusion is that our neocortical neurons, the cell type that mediates much of our cognition, are produced prenatally and retained for our entire lifespan. New neuron production in the dentate gyrus may have a hypothetical role in formation of certain types of memory (26), but its absence in the neocortex penetrates to the essence of how we think and learn. If neuron number in the neocortex is not incremented, then synaptic changes and other forms of plasticity must dominate; i.e., when we learn a new task or fact, then the storage of the new information must entail a reorganization of existing circuitry. The retention of the neuronal population for decades is consistent with our need to retain information for our long lifespan. In short, the cultural complexity of humans requires not only the constant acquisition of new facts and skills but also the retention of others, most notably language, for many decades, and a stable complement of neurons in the neocortex would seem to be essential for these abilities. This could be a reason for the evolutionary choice for a stable cellular composition of our cognitive machinery over a more dynamic pattern.

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