Environment complexity stimulates visual cortex neurogenesis: death of a dogma and a research career
Michael S. Kaplan

Introduction by the Editor
Over the past few years, the classic idea that no new nerve cells are born in the adult mammalian brain has finally and conclusively been refuted by the scientific community. Yet, the first indications that neurogenesis occurs in the brain of adult mammals were obtained using light and electron microscopy over two decades ago. Why this went unrecognized is described in a personal account by the researcher who pioneered those studies: Michael Kaplan.

Last year, Dr Charles Gross found me in Baltimore and requested my insight into the origins of the central dogma that neurogenesis did not occur in adult brain: we are now in the midst of a paradigm shift, he explained, and our conception of the plasticity in the adult mammalian brain has forever altered – neurogenesis in the adult brain is finally being accepted by the scientific community.

This paradigm shift has been a long time coming and is the result of over twenty years of increasingly irrefutable evidence. Indeed, as early as 1974, at the beginning of my career at Tulane University, my major Professor, Dr J.W. Harper, and I believed that we had found evidence that complex environments could stimulate neurogenesis in adult visual cortex (Fig. 1). Our work using light microscopic radiography appeared to show that in a variety of microscopic radiography appeared to

The beginning: a few lucky breaks and the first hints of things to come
My interest in this controversial topic got off of to a flying start when in 1970 as an undergraduate student at Tulane University (1970–1975), I worked closely with J.W. Harper. This was an extremely fortunate collaboration because Harper was a keen believer in the light microscopic work of Dr Joseph Altman who showed neurogenesis. Harper’s goal was to investigate this further and determine whether enriched environments would increase the rate of neurogenesis in the visual cortex. We used a simple yet elegant procedure using tritiated thymidine, which is incorporated into DNA being manufactured during mitosis. Because non-mitotic neurons are not affected, mitotic cells are selectively labeled. Our key experiment was as follows: 30-day-old Sprague-Dawley rats were injected with 3H-thymidine intraperitoneally (5 mcg body weight), and allowed to survive for 30 days while being exposed to a ‘complex environment’. The environment was enriched using various methods such as introduction of wheels, balls and other rodent toys into the cage. When tissue from the visual cortex was processed for light microscopic autoradiography using the 100× objective, we identified a large number of labeled cells that had the characteristics of neurons. These cells – as many as eight in one field were light (oligodendrocytes are dark), had a regular appearance and were arranged in Layer IV of the visual cortex in a pattern characteristic of granules (Fig. 1). As an undergraduate student in 1971, this novel finding was unbelievably exciting and intriguing; was I really seeing neurogenesis in adult brain? The evidence was certainly strong enough for me to believe that this was a story worth pursuing; we concluded that it was highly likely that not only was neurogenesis present in the brain of the young adult rat, but that the rate of cell proliferation to complex environments. This made perfect sense: it was clear that humans who are exposed to culture, supportive family or excellent teachers would flourish in enriched environments, and why should this not involve neurogenesis?

I submitted this undergraduate research in 1975. During my graduate application process, again luck was on my side: impressed by my enthusiasm, Dr James Hinds at Boston University took me on as a graduate student and helped me design a series of experiments to better define the rate of cell proliferation (gliogenesis) in the adult mammalian brain. In the late 1970s, the neuroscience community accepted gliogenesis as a postnatal process without the need for evidence from ultra-structural radiography. Hinds and I were the first to identify gliogenesis in the neocortical gray matter of the adult rat using electron microscopic analysis of light autoradiographs.

Fig. 1. Labeled stellate neurons in layer IV of the adult rat visual cortex. Small silver grains are observed over the nucleus. Multiple labeled neurons are seen in a ‘horizontal’ arrangement typical of visual cortex layer IV neurons. The cells display relatively evenly dispersed nuclear chromatin, abundant nissl substance, and an apparent dendrite is seen in some labeled cells. These features and the cell orientation are typical for stellate granule neurons in the visual cortex.
found labeled, apparently mitotic, neurons (neuroblast) in both the visual cortex and olfactory bulb, similar to my light microscopic work at Tulane. These cells were, in my opinion (and the editor of J. Neurosci.), clearly neurons because they had synapses, premature axons and contained vesicles onto the cell bodies and axons of the mitotic cells. Hinds was more speculative, but allowed me to publish the visual cortex data as a sole author. However, as an expert on the olfactory bulb, Hinds supported all my findings of neurogenesis in this region. In our first peer reviewed published paper, again using 3H-thymidine to stain mitotic cells, we demonstrated that 0.0307% of the total granule cells in this region were newly formed. This paper also presented a mathematical model that proposed that in a 3–12 month period, the number of neurons in this region could increase by up to 55% (Ref. 13). This speculative model was later confirmed using statistical analysis of an accurate count of the mitotic/non-mitotic granule cells in the olfactory bulb in volumetric studies.

One might expect the concept of neurogenesis in adult brain, at least in the rat, to be considered less speculative following the identification of mitotic neuroblasts in the olfactory bulb and cortex. But in any revolution, whether political or scientific, there are crusades and battles: not all are winners. In the midst of a revolution one must choose allegiances, and during the 1960s and 1970s, those who chose to support the notion of neurogenesis in the adult brain were ignored or silenced.

**Dogma stands firm despite mounting evidence**

The importance of these findings—and others showing the ultrastructural identification of thymidine-labeled mitotic neuroblasts—had accumulated over two decades of research without recognition, primarily as a result of the existence of considerable conceptual obstacles that needed to be overcome.

Undaunted, and continuing my work with Hinds, further efforts to gather evidence for neurogenesis followed: after finishing my postdoctoral fellowship, investigating regrowth of olfactory sensory axons into transplanted neural tissues. I then returned to my interest in neurogenesis and showed ultrastructural evidence of a 35% increase in the number of neurons in the adult rodent hippocampus. Similarly, in 1983, we showed that in the olfactory bulb around a quarter of the new neurons generated in early adulthood (three months postnatal) survive up to six months, and a half of the new neurons that divide at 24 months survive beyond six months. This has been further substantiated by recent 1998 data that has shown that these new cells might remain viable for periods from a few days to the lifetime of the animal.

Yet again, however, there was tremendous skepticism and frank disbelief in these data, and I was finding it increasingly difficult to move forward. I was beginning to realize that it might become impossible for me to continue in this particular career direction. I hoped that the identification of mitotic cells in the adult primate CNS (Refs 20, 21), a step up the evolutionary ladder from the rat, might be more persuasive. But despite ultrastructural evidence demonstrating the existence of proliferative stem cells and mitotic cells in the sub-ependymal layer of an adult primate, the dogma of no neurogenesis in adult brain remained unshaken.

**The final countdown**

One of the most fervent supporters of the dogma was Dr Pasko Rakic. In 1984, Rakic and I were presenters at a meeting ‘Hope for a New Neurology’ [Ann. New York Acad. Sci. (1984) for the proceedings]. This conference turned out to be more important to this story than we expected. At the time, Dr Rakic was a prominent figure in the Neuroscience community and a full professor at Yale Medical Center; I had just recently finished my postdoctoral training. In a lecture about neurogenesis, Rakic proclaimed that, based on his research: identification of neurogenesis in rats was phylogenetically specific to lower mammalian forms; the rates of neurogenesis in rats were too small to have any significance; that the ultrastructure of mitotic neuroblast identified one year earlier in the J. Neurosci. would not meet the criteria for a neuroblast at Yale University. This final point was key – Rakic and colleagues had found labeled mitotic cells, but believed them to be glia rather than neurons – all neurons, he believed were post-mitotic. A neuroblast was a post-mitotic cell until 1984 (Ref. 10). He and most others agreed neuroblasts were not capable of cell division before that paper. This was a crucial blow to progress towards overturning the dogma. A further setback occurred during the subsequent year in an autoradiographic study on adult Rhesus monkeys published by this same group. Despite examining all major structures and subdivisions of the brain, again they found not one heavily labeled cell in any of the animals that they believed had the morphological characteristics of a dividing neuron. He concluded that the existing dogma was the correct one: all neurons of the Rhesus monkey brain are generated during prenatal and early postnatal life.

This paper directly contradicted my data, and I was curious about why our findings were so different. Had we obtained similar data but were interpreting what we saw differently, or was my data unique? Did we agree on the criteria for a neuroblast? The key issue appeared to be that Rakic and I differed in our opinion over whether the 3H-thymidine-labeled cells we were seeing were neuronal or glial – Rakic did not believe that the labeled cells could be definitively identified as neurons. I would have liked to discuss this with Rakic and ask if he might allow me to look at his slides in the hope that I might find some of what I had found with my own data. Unfortunately he refused: ‘It would be like removing a page from a book’.

**Hopes from humans**

While I was a non-tenured faculty member at the University of New Mexico Medical Center during the 1980s, I submitted a ‘dose searching’ protocol to infuse 3H-thymidine into the tumor beds of terminally ill brain tumor patients in an effort to obtain evidence that might convince respected but skeptical researchers such as Rakic (Fig. 2). The aim of this study was simply to obtain evidence of ongoing neurogenesis in adult human brain. Although this proposal was funded, neither the dean nor my chairman felt able to support this young postdoctorate as the coordinator of a small clinical trial.

With the political death of this project and my controversial beliefs quashed—catastrophic for the research career of a young post-doc—I decided to abandon this career path in favor of medicine, which I have found tremendously fulfilling. But I
Everywhere, people were talking about was like entering a new world. The meeting was astonishing – it was not only had the skeptics gone, but also had the dogmas of neurogenesis as if it had always been that way. It was clear that the old dogma had died and a paradigm shift had occurred. At one seminar I saw movies of dividing neurons with ‘neuronal-like processes’: twenty years ago those cells would have been called fibroblasts, I thought wryly, and the neuronal-like processes would be ignored without ultra-structural analysis. My daughter and I were impressed by the great number of cell-type specific markers that had been developed for the neuronal cells under certain conditions, and there had been conflicting reports on the specificity of neuronal markers, it did not seem to inhibit enthusiasm about neurogenesis: there almost seemed to be a mood of intoxication. It appeared that not only had the skeptics gone, but critical scientific questioning had diminished too (perhaps too far of a swing to the other side?). It was gratifying to be in an environment where my undergraduate slides (Fig. 1) would no longer appear controversial, but fit entirely with the other data that supported environmental stimulation of the neurogenesis in certain areas of the post-natal cortex. Indeed, my undergraduate work from 1974 is now the first evidence that the environment might increase the rate of neurogenesis in young adult mammals visual cortex.

The high rate of turnover of new neurons has made it more difficult to speculate their functional significance, yet we now know that every day, thousands of new neurons are added to the mammalian brain. This astonishing continual addition over a lifetime implies significant structural change, and the magnitude of this process signifies its tremendous significance in CNS functioning, including, for example, short and long term learning and memory.

References
Perspectives on brain evolution in primates

Evolutionary Anatomy of the Primate Cerebral Cortex
£50 (344 pages) ISBN 0 521 64271 X

This timely volume comprises a series of papers that were presented at a symposium in honor of Harry J. J. L. Jenison at an annual meeting of the American Association of Physical Anthropologists. Jenison’s classic monograph, Evolution of the Brain and Intelligence, established the precedent for subsequent studies of brain evolution by concentrating on one variable that is easily measured in both living and extinct mammals, namely brain size. We know from this early work that independent lines of evolution commonly lead to larger brains. But are separately evolved larger brains bigger and “better” in similar or different ways? In this volume, arguments for both alternatives are convincingly presented, suggesting that brains have often evolved in similar ways while also specializing differently for specific functional roles.

Larger brains probably resemble each other in overall organization for two basic reasons. First, there might be limited ways in which the course of development can be altered to produce differences in brain size. Thus, we repeatedly see the same types of enlargements. Most notably, larger brains have proportionately more neocortex, apparently because late-maturing areas grow even more significantly compared with early-maturing areas. Second, larger brains have more neurons and longer connection distances. Thus, it becomes increasingly difficult to maintain the connections of each neuron with the same proportion of other neurons, and maintain action potential conduction timing over longer distances. Therefore, similar methods of reorganizing brains to reduce these connection problems are probable.

However, there is also convincing evidence from comparative studies of the brains of living mammals, and from brain casts of fossil skulls, that brains vary greatly both in global features and in local structural detail. At the global level, the two cerebral hemispheres of humans and chimpanzees differ in shape, supporting other types of evidence that there are functional differences between the hemispheres. At the local level, many differences in cortical microanatomy in homologous subdivisions of neocortex, such as primary visual cortex, are apparent across primate species. The chapters that address these issues convince the reader that further studies of both the overall trends and the many divergent specializations in brain evolution would be productive.

This book will be of value to those interested in the evolution of different types and sizes of neocortex, the structure most responsible for making humans and mice what they are. But, more importantly, the book gives us a sense of where we are today and how far we need to go in understanding the evolution of primate brains from the small but still impressive brains of the earliest primates to those, including our own, extant today. As Jenison concludes in the book’s last chapter, ‘more research is needed’.

Michael S. Kaplan
816 Frederick Road, Catonsville, MD 21228, USA.
e-mail: marjiesk@home.com

References
1. Jon H. Kaas
Department of Psychology, Vanderbilt University, 301 Wilson Hall, 111 21St Avenue South, Nashville, TN 37240, USA.
e-mail: jon.h.kaas@vanderbilt.edu

Reference