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Neurogenesis in adult primate neocortex: an evaluation of the evidence

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Reports of continuous genesis and turnover of neurons in the adult primate association neocortex — the site of the highest cognitive functions — have generated great excitement. Here, I review the available evidence, and question the scientific basis of this claim.

Although the cells of the intestinal epithelium are replaced every 2 weeks and those of our skin are renewed every few months, it has generally been accepted that neurons in most structures of the mammalian brain are generated during restricted developmental periods[1]. So, under normal conditions, the brain is considered to be a non-renewable organ composed of fully differentiated neurons[2]. Unambiguous evidence for adult neurogenesis in mammals has so far been limited to the granule cells of the dentate gyrus and olfactory bulb[3,4]. Although the function and longevity of these cells are still being investigated, the recent report that the macaque monkey neocortex acquires streams of new neurons throughout adulthood[5] stunned the scientific community and circled the globe in the popular press. The uncommon interest in this report relates to the claim that substantial numbers of neurons are being added daily to the primate prefrontal, parietal and temporal lobes — sites of the highest cognitive functions. An independent claim of similarly large additions that alternate with comparable losses of neurons in the human neocortex during the first years of postnatal life, published at about the same time[6], has amplified the perceived significance of neuronal turnover. These two reports have been celebrated in the lay press as "the most startling discovery" of the decade[7]. The desire to cure neurological disorders using replacement therapy might help to explain the readiness to accept these claims uncritically[4]. As this subject has broad biomedical implications, it is essential to examine the scientific basis of these claims. Here, I address the question of whether or not neurogenesis in the cerebral neocortex continues throughout adult life in the Old World primate macaque monkey — a species that is phylogenetically closely related to the human.

Previous evidence

Because of the obvious implications for human brain formation, in the mid-1970s, I initiated a longitudinal study that was designed to examine the time of neuron origin in non-human primates. This project, which was started at Harvard University and continued at Yale, has involved 26 researchers from seven countries. It has examined extensive autoradiographic material from a total of 127 macaque monkeys exposed to ^{3}H-thymidine (^{3}H-dT) at various pre- and postnatal stages from 25 embryonic (E) days to 17 years of age. ^{3}H-dT is commonly used in this kind of study because it is readily incorporated into DNA during the S phase of the cell cycle; intensive radiolabelling therefore indicates the time of a neuron's 'birthday' (FIG. 1). Most of the collaborators engaged in this project, including myself, expected neurogenesis in non-human primates to continue after birth, as brain maturation occurs over a prolonged period in these animals. However, with the exception of the granule cells of the cerebellum and hippocampus, which showed neurogenesis after birth and even into the juvenile stage, more than 100 classes of neuron examined in 34 structures were generated during specific and highly restricted gestational and neonatal periods. The results of this project have been published in more than 25 papers, some of which are cited here[8–18]. The overall conclusion was that the capacity for continuous neurogenesis in adult macaques is severely limited, and we suggested that this capacity diminished over the course of vertebrate evolution[19]. We were aware of, and have acknowledged, the technical difficulties in identifying the nature of ^{3}H-dT-labelled granule cells in the adult dentate gyrus: "if neurogenesis has been missed as a result of methodological limitations, the number of newly generated neurons must be very small and thus hardly comparable to massive levels of neurogenesis reported in birds and rodents"[20].

Examination of the cerebral cortex — the main subject of the present article — revealed that neurogenesis in the ten cytoarchitectonic areas examined, begins almost simultaneously (~E38–40), and ends between E70 (in the limbic cortex) and E102 (in the visual cortex). Cortical neurons are generated in the proliferative zones near the cerebral ventricle and then migrate to the developing cortex[21]. In each area, neurons settle into a distinct inside–outside gradient, in which the neurons generated earlier occupy the deep layers and the neurons generated later are situated more superficially (FIG. 2). The sole exception to this general rule is layer I, in which neurons derived from the ganglionic eminence are added throughout the entire period of corticogenesis[22].

In contrast to neurogenesis, gliogenesis in the primate cerebrum, which also begins

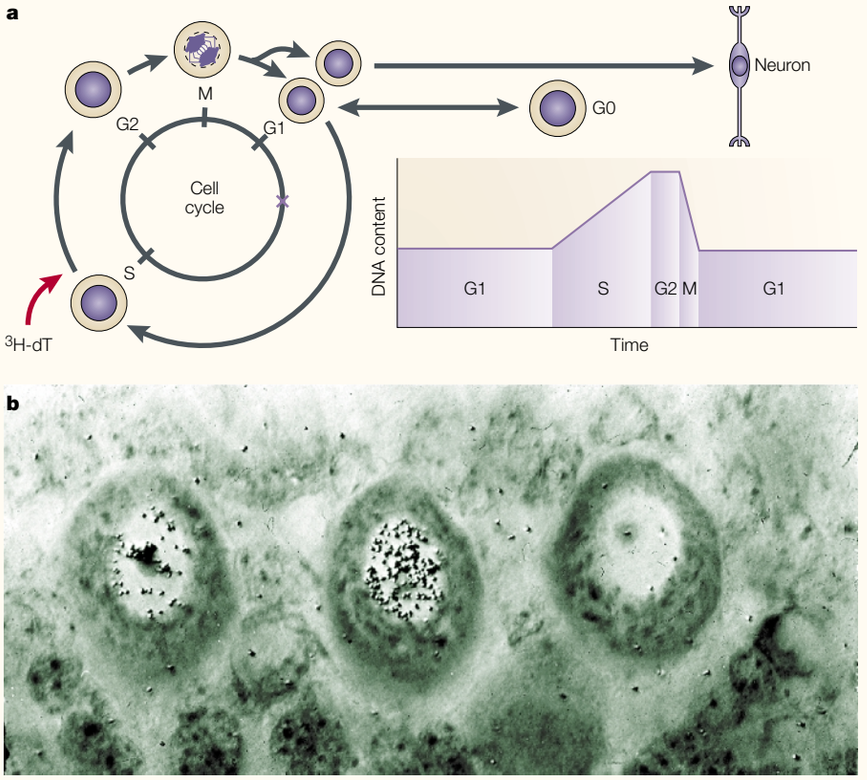

Figure 1 | **Incorporation of ^{3}H-dT as a marker of cell division. a** | DNA synthesis during different phases of the cell cycle (G1 and G2, gap phases; M, mitotic division; S, synthesis phase). The method of ^{3}H-thymidine (^{3}H-dT) incorporation after a single injection of the isotope is stoichiometric, and therefore allows the distinction between mitotic division (when the amount of DNA doubles) and cases in which the cell has not divided (and is therefore labelled less intensely). **b** | Purkinje cells in the middle and on the left can be considered as heavily labelled, whereas the five grains over the cell nucleus on the right might be due to several biological and technological factors, some of which are discussed in the main text. Importantly, if the animal was injected with ten doses of ^{3}H-dT, instead of a single dose, this cell might be as heavily labelled as the one in the middle, and could be falsely interpreted as divided.

before birth[23], accelerates during the neonatal period and continues at a low rate throughout life. In the mature neocortex, neuronal and glial cells can be easily identified, but only glial cells are heavily labelled by ^{3}H-dT (FIG. 3). For example, most oligodendrocytes, which outnumber neurons by several-fold in the primate cerebrum, are generated until puberty, but continue to appear at a slow pace throughout life[24]. Numerous investigations of cortical neurogenesis using ^{3}H-dT autoradiography in other mammalian species — from rodents to carnivores — have also found it to be confined to the developmental period[25–32].

New studies in primates

In view of the extent and consistency of evidence across mammalian species that indicates restriction of cortical neurogenesis to specific developmental periods, the recent report of continuous neurogenesis in the primate association neocortex[5] was unexpected. The existence of these new neurons and the magnitude of their daily invasion of the association neocortex has been considered extraordinary and in need of confirmation by other research groups[33]. Although, more recently, Gould *et al.* have modified their initial position[34], their corrected estimate of the number of new neurons is still staggering. We therefore decided to re-examine the proliferation and phenotypic differentiation of labelled cells in the cerebral cortex of adult macaque monkeys using the thymidine analogue 5-bromodeoxyuridine (BrdU), which was used in the original study to mark DNA replication. We found[35] that many BrdU-labelled cells were present in the subependymal zone of the lateral cerebral ventricle and localized predominantly along its lateral side, similar to the distribution of proliferating cells described in rodents, monkeys and humans[19,36–43]. Some of these cells, also stained with TuJ1 (a marker for immature neurons that detects β-tubulin III) to indicate their neuronal nature, join the rostral migratory stream to the olfactory bulb, where they differentiate into interneurons[36]. The distribution of BrdU/TuJ1-labelled cells is similar to the olfactory migratory pathway that has been described in rodents[42,43]. The BrdU-labelled cells that are situated in the white matter subjacent to the cortex did not co-label with TuJ1, and were distributed along blood vessels or myelinated fibre tracts. These observations indicated that the labelled cells were probably newly generated endothelial cells or oligodendrocytes, respectively, as observed in our previous ^{3}H-dT (REFS 19,41) and BrdU studies[44] in primates and other mammals[37–40].

In the neocortex, BrdU-labelled nuclei were observed in every animal and every cytoarchitectonic area examined[35]. In the prefrontal cortex, which has been reported to receive most of the newly generated neurons[5], labelled cells were distributed individually (FIG. 4a,b) or as 'doublets' (FIG. 4c), which indicates that some of them might have divided in the neocortex (FIG. 4d). The phenotype of BrdU-labelled cells in the cortex was examined by triple labelling for BrdU, NeuN (a marker for mature neurons) and glial fibrillary acidic protein (GFAP; a marker of astrocytes). Although several thousand BrdU-labelled cells were examined, not a single one was co-labelled with NeuN[35]. In fact, close inspection of some ambiguous cases showed that BrdU-labelled nuclei actually belonged to satellite glial cells that are closely apposed to NeuN-positive neurons (FIG. 4e–h; see also REF. 35). For example, when satellite glial cells, such as those illustrated in FIGS 2 and 3a,b are viewed from the side, they can seem to be 'labelled neurons' (FIG. 4e–g). In fact, dividing satellite glia have been described in the adult neocortex of several mammals[19,35,37,38,41,44,45].

Our latest BrdU data[35] are in complete harmony with previous ^{3}H-dT studies in both primate and non-primate species that report cortical neurogenesis during specific developmental periods[9,13,15,18,25–32]. Consistent with these findings, a recent retroviral and BrdU immunofluorescent study also failed to detect cortical neurogenesis in normal adult mice, although this report indicates that neurogenesis might be induced experimentally[46]. Our findings of migrating neuroblasts in the rostral migratory stream, and new neurons in the olfactory bulb and dentate gyrus of the same set of monkeys, attest to the reliability of the method[36,47]. So, the judicious use of BrdU confirms ^{3}H-dT findings that neocortical neurons in primates are generated prenatally and are not normally renewed in adult life — up to 3 decades in macaque monkeys[19].

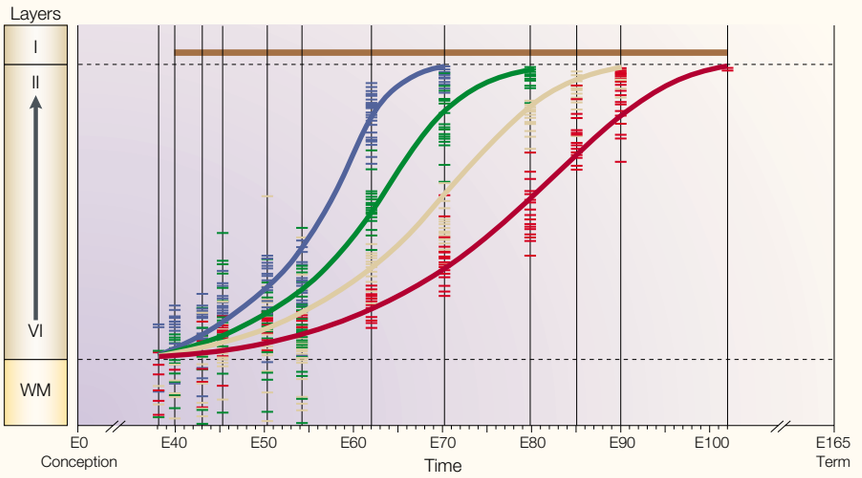

Figure 2 | **Relationship between the time of origin and the final position of cortical neurons in the macaque monkey.** Representation of the positions of heavily labelled neurons in the four representative cytoarchitectonic cortical areas. Each monkey was injected with 10 mCi kg^{-1} of ^{3}H-thymidine (^{3}H-dT) on a selected embryonic day (E) and killed postnatally. A representation of the approximate position of layers I– VI and the white matter (WM) is on the left. Embryonic days are represented on the horizontal axis, starting with the beginning of the second fetal month (E34) and ending at term (E165). Positions of the vertical lines indicate the embryonic day on which an animal received a pulse of ^{3}H-dT. On each vertical line, short horizontal markers indicate positions of the heavily labelled neurons encountered in a 2.5-mm-long strip of cortex. Blue, Brodmann area (BA) 24; green, BA 11; yellow, BA 46; red, BA 17. Layer I neurons in the primates are generated throughout the entire period of neurogenesis in each area (brown).

Criteria and methodological caveats

How can diametrically opposed findings that are obtained by the same method in the same species be reconciled? First, it should be emphasized that some experts in this field met the report of large additions of neurons to the adult primate neocortex with scepticism. For example, Nowakowski[33], who introduced the use of BrdU to developmental neurobiology[48], was very critical of the use of this procedure to detect labelled cells, and identify them as migrating and mature neurons. Although BrdU immunohistochemistry has advantages, it also has some drawbacks that can lead to erroneous conclusions if potential technical problems are ignored[49].

^{3}H-dT/BrdU labelling is not sufficient. It is essential to recognize that ^{3}H-dT and BrdU are not markers of cell division, as is commonly assumed, but indicators of DNA synthesis. Therefore, the conclusion that a given cell in the adult brain is 'new' depends on detecting the duplication of DNA during its last mitotic division. This is usually done by supplying exogenous DNA precursors — ^{3}H-dT or its analogue BrdU — and assessing their incorporation by autoradiography or immunoreactivity, respectively (FIGS 1, 4 and 5). With ^{3}H-dT autoradiography, radiolabelled neurons are considered to be heavily labelled only if, in animals injected with a single dose of ^{3}H-dT, the number of overlying silver grains is at least 50% of that recorded in maximally labelled nuclei in the same specimen[8,9] (FIG. 1). As the amount of label is halved every time a cell passes through the cell cycle, the presence of both heavily and lightly labelled cells has been used as a criterion for confirming that the label incorporation is due to cell proliferation[50]. This simple quantitative criterion for DNA replication avoids false-positive data due to background or other artefacts[33,41,50]. However, unlike ^{3}H-dT autoradiography, immunohistochemical detection of BrdU is not stoichiometric and is therefore not well suited to quantification (FIG. 1). This problem is exacerbated by the use of amplification methods that further enhance the intensity of labelling, which gives the impression of a degree of labelling that does not reflect the magnitude of DNA replication[33]. For this reason, high and/or multiple doses of BrdU (or its addition to drinking water) to detect rare cell divisions in adult animals should be avoided. Whereas a dose of less than 50 mg kg^{-1} is commonly used to label dividing neurons in the developing brain, multiple doses of 50–500 mg kg^{-1} that are used in adult animals can produce artefacts and heavily label even low levels of normal DNA turnover or minor DNA repairs (FIG. 1).

The incorporation of BrdU into the nucleus is often taken as an uncontested sign of cell division. However, damaged or degenerating neurons can activate cyclins (cell-cycle-associated proteins) and initiate abortive DNA synthesis without mitosis, as has been observed during the process of cell degeneration[51,52]. In some cases, neurons apparently become tetraploid and this "genetic imbalance may persist for many months before the cells die"[51]. So, some of the 'new neurons' that are reported to survive for a short time might actually be 'old neurons' that synthesize DNA as part of an apoptotic process[51,53,54]. In addition, unscheduled DNA

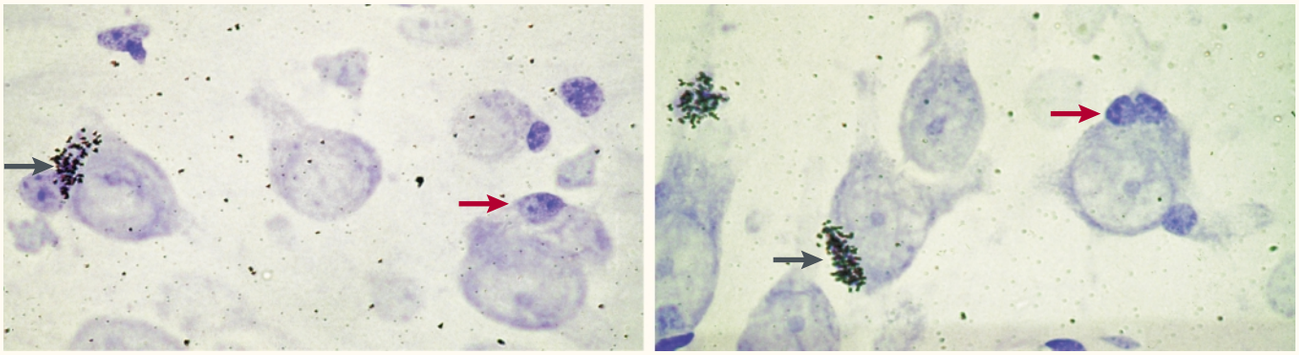

Figure 3 | **Satellite glia labelled with ^{3}H-thymidine.** Photomicrophotographs of heavily labelled satellite glial cells in the frontal lobe of a monkey 35 days after a single injection of ^{3}H-thymidine (10 mCi kg^{-1}). Cresyl violet counterstaining is sufficient to distinguish between neurons and labelled (black arrows) and unlabelled (red arrows) glial cells, even when they are closely apposed to neuronal perikarya.

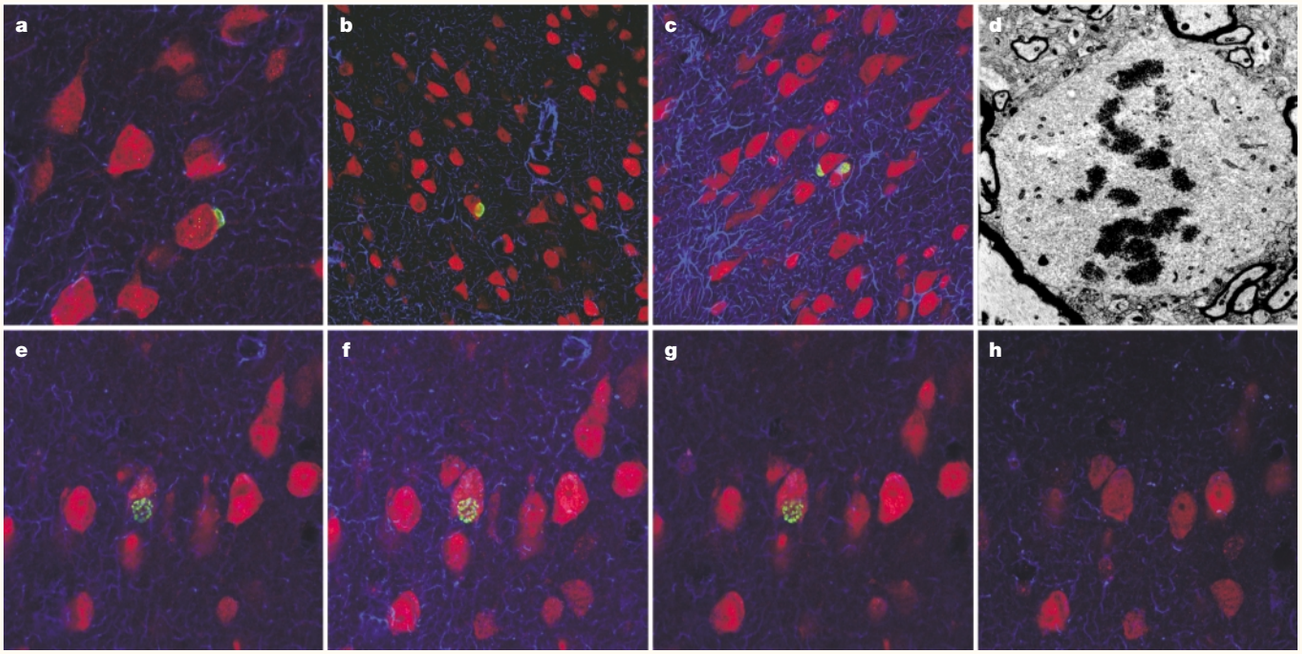

Figure 4 | **BrdU-labelled satellite glial cells. a–c** | 5-Bromodeoxyuridine (BrdU)-labelled nuclei (green) closely associated with the perikarya of NeuN-positive, BrdU-negative neurons (red). **d** | A mitotic figure encountered in the adult monkey motor cortex indicates that a glial doublet such as illustrated in **c** might have divided locally. **e–h** | A series of confocal images taken at 0.8 μm intervals through a pyramidal cell, which appears to be doubly labelled for BrdU and NeuN in **e**, **f** and **g**; however, the image in **h** reveals that these labels belong to two closely apposed cells. The BrdU-labelled nucleus (yellow) is located in a different focal plane than the NeuN-positive neuronal cell body (red). The BrdU-positive nucleus is visible in **e**, **f** and **g**. The NeuN-positive neuron and its nucleus, shown in **h**, are not BrdU labelled.

synthesis might occur to provide additional gene copies to enhance transcription in more metabolically active cells or in situations of larger demand, as shown in hepatocytes[55]. Some cells are indeed more vulnerable to adverse conditions (for example, excitotoxicity) and show higher levels of DNA repair[56]. All of this means that the claims of an increased or decreased rate of 'neurogenesis', made on the basis of observed BrdU labelling after high and/or multiple doses of BrdU, leave open the question of whether the labelled nuclei belong to old neurons that have incorporated BrdU into their DNA or to newborn cells (FIG. 5).

Cell division must be shown independently. Because BrdU can be incorporated into DNA without cell division, it is essential to show that BrdU incorporation is associated with proliferation by independent means. Other markers that are sometimes used as supplemental evidence for new neurons in the adult brain, such as the proliferating-cell nuclear antigen, can also be expressed by non-proliferating neurons[57] and are not specific. Independent evidence can be obtained in several ways, such as counting mitotic figures, tracing cell histories, or counting the number of neurons and establishing comparable rates of cell death. So, it should be shown that BrdU labels mitotic figures, as this ensures that DNA synthesis is occurring as part of the S phase of the cell cycle. Although the presence of mitotic figures itself cannot establish the phenotypic fate of a cell, an increase in their frequency compared with a control can show that BrdU incorporation is not due to repair or unscheduled DNA synthesis. Counts of mitotic cells are not difficult, as they are readily visible using appropriate nuclear stains. Similarly, tracing cell history after division through the migration and differentiation stages to the mature state can be done by either sequential ^{3}H-dT or BrdU experiments, or with the use of retroviral gene-transfer methods. The use of these procedures is important when a change in BrdU labelling occurs in response to experimental manipulations or exposure to drugs. Finally, continuing neurogenesis should, in principle, lead to a net increase in the number of neurons, particularly in cases in which the suggested rate is large; this increase should be reflected in an increase in neuronal number. Alternatively, when the structure does not grow in size, despite the suggested neurogenesis, an increase in the number of pyknotic cells equivalent to neuronal addition needs to be shown. As BrdU incorporation itself is not a specific marker of cell division, but a sign of DNA synthesis, monitoring the life history of new neurons, and/or providing evidence that their number increases or is maintained by equivalent cell death, are essential, albeit time-consuming, procedures.

Labelling of neurons must be unambiguous. One advantage of BrdU labelling over ^{3}H-dT labelling is that it can be readily combined with the immunohistochemical determination of cell type, neurotransmitter phenotype and even the identity of receptors on the surface of the labelled cell. Double labelling is not necessary for the identification of most neurons, such as Purkinje or pyramidal cells, which are easily recognized, but is essential for the identification of small neurons, such as granule cells, that, in histological preparations, look similar in size, shape and tinctorial properties to glia. Unfortunately, many of the so-called 'neuron-specific' markers also label cells of other classes, particularly developing and reactive glia. For example, the so-called neuron-specific enolase (NSE), microtubule-associated protein 2 (MAP2) or TOAD-64 (turned on after division, 64 kDa; also called TUC-4 and Ulip1) are inappropriate as markers for neurons, as they also react with astrocytes and/or oligodendrocytes[58–60].

The target of the NeuN antibody, which is often used as a definitive neuron-specific marker, has not as yet been identified. This antibody does not stain some neuronal classes

(for example, Purkinje or mitral cells), and can interact positively with various non-neuronal cells — from ependymoma to bone marrow — under certain conditions[61–64]. Furthermore, an antibody that is neuron-specific in rodents might not be equally specific in other species. False-positive identification of neurons can also occur as a result of the nonspecific binding of antibodies. It therefore seems prudent to have appropriate controls and establish antibody specificity when the results are unusual or have extraordinary implications. It is also important to establish basic criteria for when a given cell should be considered a 'neuron'. Is a single antigen to an unknown molecule sufficient to identify a neuronal phenotype? It seems reasonable that the formation of a synaptic connection in a suspected new cell be shown by electron microscopy to prove its neuronal nature[49].

Last, some of the false positives are due to optical problems. For example, the superposition of small satellite glial cells that are labelled with BrdU on an unlabelled neuronal soma can mimic labelling in the neuronal nucleus (see FIG. 3 and FIG. 4e–h). So, stacks of serial optical planes on the confocal microscope should be used to confirm the double labelling of a neuron[35,45]. Even if this is done, examining an insufficient number of focal planes might lead to the false identification of a cell as labelled when its nucleus is situated outside the tissue section being examined.

In summary, the immunohistochemical and immunofluorescent approaches to detecting newly generated neurons are highly sensitive, but also unreliable if proper controls are not carried out. The three basic criteria listed above are proposed as guidelines to increase the reliability of identification of 'new' neurons.

Re-evaluation of evidence

Korr and Schmitz[65] have effectively disputed the claim[6] of a large influx of neurons that alternates with a corresponding loss in the human cerebral cortex during early postnatal years. Nowakowski and Hayes have also questioned the evidence for neurogenesis in the neocortex of adult primates in an incisive review[33]. Here, I focus on only one problem — the source of new cortical neurons, which is the key piece of evidence that new cortical neurons were actually being produced[5]. The local proliferation of mature neurons in the cortex itself as a possible source of new neurons is excluded by short-term survival experiments (case numbers 1 and 2 in REF. 5). Gould *et al.* provide evidence of the source of new neurons by showing migrating, young neurons in figure 5D of REF. 5 (reproduced here in FIG. 6a). After a short survival period, BrdU-labelled cells were situated along the dorsomedial side of the ventricle, under the corpus callosum. The figure shows a stream of "BrdU-labeled cells in the white matter … elongated or fusiform in shape" with "leading and trailing processes characteristic of migrating cells … arrayed in a stream from their likely site of origin in the wall of the lateral ventricle, through the white matter, to their probable destination in the frontal neocortex". We never observed such a migratory stream in our extensive material.

I would argue that some of those BrdU-labelled nuclei belonged to endothelial cells that could easily be misinterpreted as "migrating in a stream through the subcortical white matter". For example, the aligned, labelled cells that are identified as chains of migratory neurons in figure 5D of Gould *et al.*[5] seem to be endothelial cells that line a longitudinally cut capillary. A similar 'migratory stream' that is shown in figure 4C of their subsequent paper[34] might also be a misidentified capillary wall (see FIG. 6b). So, evidence for massive migration to the neocortex of the primate frontal lobe[5] is disputable.

The existence of a migrating stream of cortical neurons in the adult cerebrum was also supported by the observation that some BrdU-labelled cells in the white matter that were co-labelled for TOAD-64 (collapsin-response-mediator protein 4) had a bipolar shape with leading and trailing processes[5]. However, non-neuronal cells, such as immature astrocytes and oligodendrocytes, also adopt bipolar shapes while migrating from the subependymal zone into the cerebral white matter and neocortex[66], and show TOAD-64 immunoreactivity[60,67]. In fact, oligodendrocyte processes can be modulated

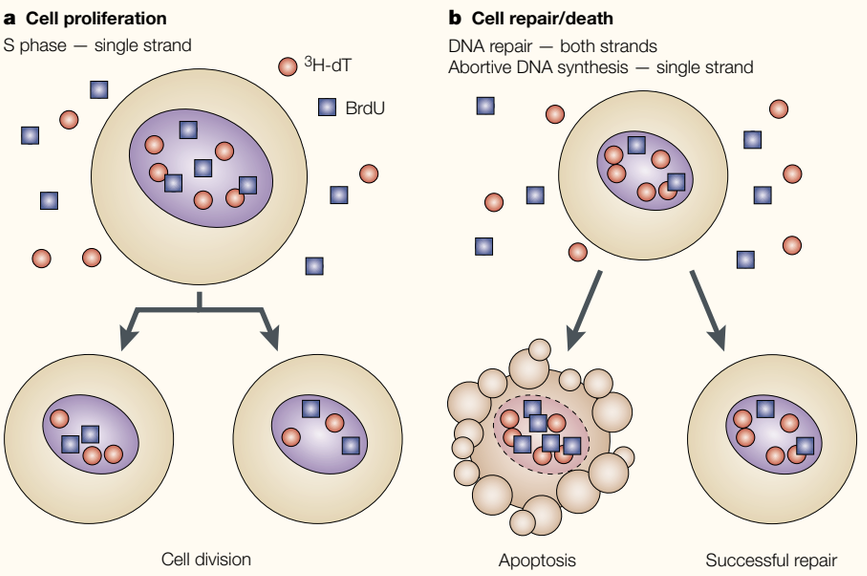

Figure 5 | **Incorporation of exogenous nucleotides into nuclear DNA.** Exogenous ^{3}H-thymidine (^{3}H-dT; red circles) or 5-bromodeoxyuridine (BrdU; blue squares) compete with the natural, endogenous thymidine for incorporation into nuclear DNA. **a** | During the S phase (DNA synthesis) of the cell cycle, incorporation occurs predominately into new stands of DNA; provided that the cell stops dividing, a significant presence of these labels indicates the time of final cell division. **b** | Incorporation of nucleotides occurs at a slower rate as part of DNA turnover or repair (of both strands), or at the higher rate during an abortive cell cycle (predominately in a single strand), which leads to recovery or death.

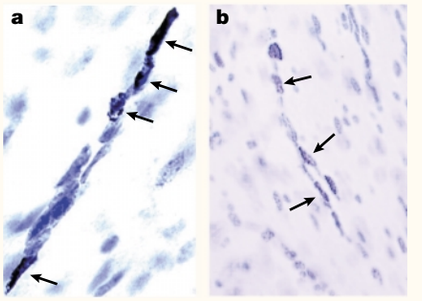

Figure 6 | **Endothelial cells might be misidentified as migrating cells. a**,**b** | Two examples of 5-bromodeoxyuridine-labelled cells (arrows) that seem to migrate in a stream through the subcortical white matter. In both cases, it is possible that the streams correspond to endothelial cells of a longitudinally cut capillary wall. Part **a** reproduced with permission from REF. 5 © 1999 American Association for the Advancement of Science; part **b** reproduced with permission from REF. 34 © 2001 National Academy of Sciences, USA.

by TOAD-64 phosphoproteins[60]. So, the TOAD-64-positive cells identified as migrating neurons are likely to be oligodendrocytes. The recent finding that cells positive for the chondroitin sulphate proteoglycan NG2 in the white matter of the adult human cerebrum are oligodendroglial progenitors[68,69] is consistent with this interpretation[19,41,44] as it is with numerous previous reports[37–40].

The number of migrating cells in the Gould *et al.* study[5], calculated from case numbers 8 and 9, and after a single BrdU injection, is more than 10,000 per day[33]. Even if only 25% of BrdU-labelled cells were neurons, as has been estimated more recently[34], the resulting migratory stream would still be large enough to be readily detected in the frontal lobes with any light microscopic method, but it has never been observed. Moreover, if most new cells degenerate between 2 and 9 weeks after their birth[34], then many pyknotic neurons commensurate with the massive cell death would be expected. This prediction has never been confirmed.

Prospective and perspective

Although a substantial rate of neurogenesis and neuronal turnover in many vertebrate species, including the dentate gyrus and olfactory bulb in mammals, has been established for decades[36,42,43,47,73], it was recently alleged that a 'dogma', originating with Albert von Kölliker, Wilhelm His and Santiago Ramón y Cajal at the turn of twentieth century and perpetuated by developmental biologists, has prevented advances in this subject[74]. I disagree that the term 'dogma' fairly characterizes the standards and practice of science in this field. It is actually instructive to note how careful and responsible the old masters were when interpreting their data, which were obtained with relatively crude methods. The inadequacy of the methods was acknowledged even much later in the title of Altman's 1964 paper: "Are new neurons made in adult mammals?"[75] It turned out that the answer was yes, at least for the granule cells of the dentate gyrus, but the judicious use of a question mark served to indicate that he was aware that the data were not conclusive. The best illustration that 'dogma' is an unfair characterization of this field is that when the convincing evidence was obtained for adult neurogenesis in the dentate gyrus and olfactory bulb in mammals, it was readily accepted by the scientific community. In my own laboratory, David Kornack and I reported neurogenesis in the adult dentate gyrus and olfactory bulb of the macaque monkey when new methods made it possible to follow their fate from cell division to a differentiated state in their final sites of residence[36,47]. Neurogenesis in the dentate gyrus and the olfactory bulb has been observed to a greater or lesser degree in all mammalian species examined, so it is not a phylogenetically new phenomenon. By contrast, a massive neuronal migratory stream to the primate association cortex[5] would be a new evolutionary acquisition not found in other mammals. I would argue that an uncritical acceptance of this phenomenon without proper evidence is more detrimental to scientific advance than requesting that the credible evidence be provided before a long-standing principle of neuroscience is put aside.

It is possible that adult neurogenesis could hold the promise of a potential cure for selective degenerative diseases and brain traumas. The absence of neurogenesis in most structures of the adult primate brain is the very reason for the surge in research on how to induce it after brain lesions or in various neurological disorders. Enhancing neurogenesis as a form of therapy in structures affected by disease and trauma is a promising approach, but the genes and factors that inhibit neurogenesis in most of the adult brain must also be identified, and directed differentiation of stem cells along neuronal lineages must be achieved. Crucially, it should be noted that the diminished capacity for adult neurogenesis in monkeys might indicate that therapies of this type could face special obstacles in humans, and that it will be important to find out why there are species-specific and regional variations in this phenomenon. We should also try to increase the longevity of existing nerve cells, as the replacement of cortical neurons that subserve some of the highest brain functions could be counterproductive. So, Kölliker, His and Ramón y Cajal were not only careful scientists, but also profound thinkers — their conclusion that nerve cells that subserve the most precious human mental functions are irreplaceable under normal conditions has so far been correct.

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TIMELINE

Ricardo Miledi and the calcium hypothesis of neurotransmitter release

Jade-Ming Jeng

Ricardo Miledi has made significant contributions to our basic understanding of how synapses work. Here I discuss aspects of Miledi's research that helped to establish the requirement of presynaptic calcium for neurotransmitter release, from his earliest scientific studies to his classic experiments in the squid giant synapse.

"The arrival of an action potential at an axon terminal causes a rise in the cytosolic Ca^{2+} concentration, which triggers exocytosis of the synaptic vesicles and release of transmitter." — *Molecular Cell Biology*[1]

"Experiments at the squid giant synapse, where the presynaptic nerve terminal is large enough to permit the insertion of microelectrodes, show that an increase in intracellular Ca^{2+} in the absence of depolarization stimulates transmitter release. Thus, Ca^{2+} is both necessary and sufficient for secretion." — *An Introduction to Molecular Neurobiology*[2]

The above excerpts, both taken from current introductory, college-level textbooks, describe a concept that is universally accepted as a basic and fundamental principle in neuroscience: that Ca^{2+} is required at an axon terminal for vesicular neurotransmitter release to occur. Although it can be difficult (and most often an oversimplification) to attribute such advances in scientific knowledge to a single person, Hall[2] highlights the importance of a set of experiments that were carried out in the squid. And in this case, the key experiments, reported in 1973 in the *Proceedings of the Royal Society of London* in a paper entitled "Transmitter release induced by injection of calcium ions into nerve terminals"[3], were indeed carried out and reported by one author — Ricardo Miledi, at present Distinguished Professor in the Department of Neurobiology and Behavior at the University of California, Irvine, and Investigador Titular at the Centro de Neurobiología, Universidad Nacional Autónoma de México.