OPINION

How widespread is adult neurogenesis in mammals?

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Abstract | It is now widely accepted that neurogenesis occurs in two regions of the adult mammalian brain — the hippocampus and the olfactory bulb. There is evidence for adult neurogenesis in several additional areas, including the neocortex, striatum, amygdala and substantia nigra, but this has been difficult to replicate consistently other than in the damaged brain. The discrepancies may be due to variations in the sensitivity of the methods used to detect new neurons.

In the 1960s, Altman used ^{3}H-thymidine labelling and light microscopy to show that new cells with neuronal morphologies could be found in the olfactory bulb, hippocampus and neocortex of adult rats and cats[1–3]. This method involves injecting animals with radiolabelled thymidine, a nucleoside which is picked up by cells synthesizing DNA in preparation for division. The ^{3}H-thymidine-labelled cells are identified at later time points using autoradiography. Altman's ^{3}H-thymidine evidence for adult neurogenesis in the mammalian brain was corroborated by Kaplan, who found new cells with the ultrastructural characteristics of neurons in the olfactory bulb, hippocampus and neocortex of adult rats[4–6]. However, this work had little impact on the field. Shortly after the last of these papers was published, Rakic reported that there was no neurogenesis in the brains of adult monkeys[7]. This study used the same methods as Altman but found no evidence of adult neurogenesis in "all major structures and subdivisions of the brain including the … neocortex, hippocampus, olfactory bulb…." The field then entered a dormant phase that lasted more than ten years, during which few papers addressed this subject in any mammalian species.

In the 1980s, Nottebohm and his colleagues published a series of elegant studies showing that a substantial number of new neurons are produced in the song system of adult birds. The new cells were generated from the lining of the ventricles[8,9] and migrated through mature parenchyma to their final destinations[8,9], where they attained morphological and ultrastructural characteristics of neurons[10,11], extended axons into appropriate target regions[10,11], received synaptic input[11] and were activated by auditory stimuli[12]. These studies on birds facilitated the 'rediscovery' of hippocampal neurogenesis in adult rodents in the early 1990s (REFS 13–16). Subsequently, evidence was shown for adult neurogenesis in the hippocampus of the tree shrew, marmoset and macaque[17–20]. Around this time, bromodeoxyuridine (BrdU) labelling came into use for studies of adult neurogenesis. BrdU labelling is conceptually similar to labelling with ^{3}H-thymidine — the thymidine analogue BrdU is injected into adult animals where it is picked up by cells synthesizing DNA in preparation for division. BrdU-labelled neurons are visualized using immunocytochemistry, and this method can be combined with several other techniques to ascertain the neuronal identity of the new cells.

Interest in adult neurogenesis markedly increased when it was demonstrated that new neurons were produced in the dentate gyrus of adult humans[21]. The study examined the brains of patients with cancer injected with BrdU for diagnostic purposes (to label proliferating cells in their tumours). This provided a unique opportunity to search for evidence of adult neurogenesis in human autopsy tissue. The authors found evidence for BrdU-labelled cells in the adult human hippocampus. The new neurons were identified on the basis of BrdU labelling combined with markers for neuron-specific proteins. Given that very small doses of BrdU were used and that the patients were sick and relatively old, it was readily acknowledged that these findings were likely to underestimate the number of neurons produced throughout adulthood. This study was a turning point in the field as it stimulated many investigations in experimental animals which produced further data regarding the neuronal identity of the new cells[22–25].

Additional studies advanced our understanding of adult neurogenesis in the rodent olfactory bulb[26,27], but the possibility that this characteristic extended to humans remained in question until recently. Two studies published in 2004 on this subject reached different conclusions — one reported that, as in rodents, new neurons are generated in the subventricular zone and migrate, via the rostral migratory stream, to the olfactory bulb[28], whereas the other did not find such evidence[29]. However, a recent paper convincingly demonstrated that adult neurogenesis in the olfactory bulb not only occurs in humans but is a robust phenomenon, comparable in magnitude to that of rodents[30]. The negative report may have missed this evidence because the human rostral migratory stream is oriented in a different plane to that of rodents. It should be noted that much literature now exists on adult neurogenesis in the mammalian brain; due to space limitations, only a subset of the relevant papers are referenced here.

Around the time that adult neurogenesis in the dentate gyrus and olfactory bulb became accepted, the terms 'neurogenic' and 'non-neurogenic' came into use for this field[21,31]. In this context, 'neurogenic' refers to brain regions that produce or recruit new neurons under normal conditions (in the absence of damage). Conversely, 'non-neurogenic' refers to brain regions that do not produce or recruit new neurons. At present, one view is that the only areas in the adult mammalian brain that are neurogenic are the dentate gyrus and the subventricular zone/olfactory bulb, and the remainder of the brain has been designated as non-neurogenic

(FIG. 1). This article considers the arguments for and against the unique neurogenic status of the dentate gyrus and olfactory bulb, with an emphasis on the methodological factors underlying discrepancies in the evidence for adult neurogenesis in the neocortex.

The case for the neocortex

Several investigators have reported evidence for adult neurogenesis in the neocortex (TABLE 1). In addition to the early work by Altman[3] and Kaplan[4], more recent studies have reported evidence for adult neurogenesis in the hamster[32], rat[33,34] and macaque neocortex[33,35–37]. Below, I discuss whether these results might be false positives — new neurons reported in the adult neocortex where none exists — taking each of the experimental approaches used in turn.

^{3}H-thymidine autoradiography. The earliest reports of adult neurogenesis in the mammalian brain used ^{3}H-thymidine autoradiography. Using this method, some evidence for adult neurogenesis in the neocortex was found. Altman acknowledged that some of the apparently ^{3}H-thymidine-labelled neurons he found were actually ^{3}H-thymidine-labelled non-neuronal satellite cells in close proximity to neurons — if the satellite cell were positioned directly over the nucleus of the neuron, the two cells might appear to be a single ^{3}H-thymidine-labelled neuron[3]. Although Altman used relatively thin tissue sections (7 μm), he still found examples of this type of 'false positive' in the adult neocortex. However, other apparently ^{3}H-thymidine-labelled neurons did not seem to be satellite cells, prompting him to conclude that some of these cells were newly generated neurons[3].

Subsequently, Kaplan[4] examined ^{3}H-thymidine-labelled cells in the neocortex of adult rats with an electron microscope. This method rules out the possibility that proposed ^{3}H-thymidine-labelled neurons are labelled non-neuronal cells lying over neurons, and provides additional information to support the neuronal identity of the new cells. Kaplan[4] reported new cells with the ultrastructural characteristics of neurons, including axons, dendrites and synapses, and concluded that "relatively small labelled neurons and their synapses are found in at least three brain regions (olfactory bulb, dentate gyrus, and visual cortex) in a normal adult rodent". Thus, this approach ruled out the involvement of satellite cells and provided further evidence for the neuronal identity of ^{3}H-thymidine-labelled cells.

Bromodeoxyuridine labelling. The more recent studies that reported adult neurogenesis in the neocortex used labelling with BrdU and antibodies that detect neuronal markers, principally NeuN (neuronal nuclei, a protein expressed in the nucleus and sometimes the cytoplasm of mature neurons). They found BrdU and NeuN double-labelled cells in the adult neocortex of several mammalian species, including hamsters[32], rats[33,34] and macaques[33,35–37]. Many fewer BrdU and NeuN double-labelled cells were observed in the neocortex than in the dentate gyrus. As discussed for the ^{3}H-thymidine autoradiography method, BrdU labelling can falsely identify a new satellite cell lying over the nucleus of a mature neuron as a single double-labelled cell. Such examples have been reported in the adult neocortex[33]. This possibility can be ruled out by using a confocal microscope to examine very thin optical sections through a BrdU and NeuN double-labelled cell, reconstructing the neuron and rotating it to examine orthogonal views. These methods were used to rule out false double labelling in a number of the reports of new cortical neurons[33,34,37]: in each case, individual cells that were clearly BrdU and NeuN double-labelled were found in the adult neocortex.

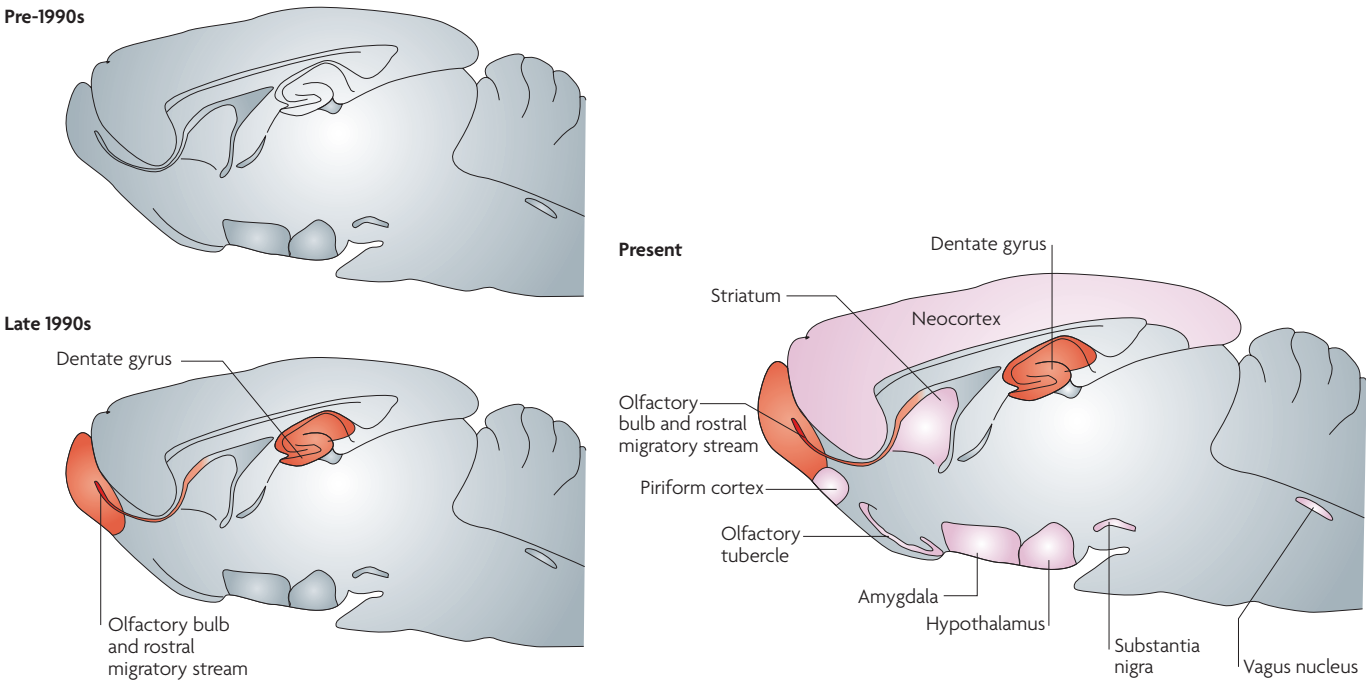

Figure 1 | **Changes in the view of adult neurogenesis in the mammalian brain over the past 15 years shown on a schematic diagram of the adult rat brain.** In the pre-1990s, all regions were categorized as 'non-neurogenic' (grey). In the late 1990s, only the dentate gyrus and olfactory bulb (as well as the subventricular zone, which gives rise to the rostral migratory stream) were categorized as 'neurogenic' (red). Today, the two known neurogenic regions are shown in red, and areas for which there is controversial evidence for low-level adult neurogenesis are shown in pink. It should be noted that since not all of these brain regions are present on the same sagittal plane, their location is approximated on this diagram.

Table 1 | **Evidence for and against adult neurogenesis in brain regions other than the dentate gyrus and olfactory bulb**

Brain Region	Positive	Negative	Positive, with damage or other experimental manipulation
Neocortex	^{3}H-thymidine + Nissl: rat, cat[3]; ^{3}H-thymidine + EM: rat[4]; BrdU + neuronal markers: hamster, rat, macaque[32–37]	^{3}H-thymidine + Nissl: macaque[7]; BrdU + neuronal markers: mouse, rat, macaque, human[45–48,51]	BrdU + neuronal markers: mouse, rat[50,62]
Striatum	BrdU + neuronal markers: rat, rabbit, macaque[34,63,64,92]	^{3}H-thymidine + Nissl: macaque[7]; BrdU + neuronal markers: mouse, rat[77,78,81,84]	BrdU + neuronal markers: mouse, rat[77–81,83,84]
Amygdala	BrdU + neuronal markers: vole, macaque[36,65,67]	BrdU + neuronal markers: rat[61]	BrdU + neuronal markers: rat[61]
Piriform cortex	BrdU + neuronal markers: rat[73]		
Olfactory tubercle	BrdU + neuronal markers: squirrel monkey, macaque[72]		
Hypothalamus	BrdU + neuronal markers: hamster, vole, mouse[32,65,67,68]		BrdU + neuronal markers: mouse, rat[68,81]
Substantia nigra	^{3}H-thymidine + EM, BrdU + neuronal markers: mouse[70]	BrdU + neuronal markers: mouse, rat[74–76]	BrdU + neuronal markers: rat[82]
Brainstem	BrdU + neuronal markers: rat[71]		

BrdU, bromodeoxyuridine; EM, electron microscopy.

The presence of BrdU and NeuN double-labelled cells in the adult neocortex has also raised questions about whether BrdU and/or NeuN can nonspecifically label cells. Some investigators have pointed out that BrdU may be incorporated into cells that are synthesizing DNA for reasons other than mitosis. Indeed, studies of damaged tissues have reported BrdU labelling in the absence of mitosis, particularly in cells that are dying or undergoing DNA repair[38,39]. However, no examples of this have been reported in the intact adult brain[40]. The absence of BrdU and NeuN double-labelled cells at short time points after BrdU injection further argues against this interpretation, and instead supports a scenario in which progenitor cells incorporate BrdU, divide and then differentiate into neurons.

Similarly, it is conceivable that NeuN nonspecifically labels non-neuronal cells, but there is no evidence for this possible confound in the intact brain. In fact, if anything, NeuN staining probably underestimates the number of neurons because certain neuronal populations do not express this marker at all, and other neurons seem to stain for the marker with relatively low intensity[41]. Some studies that have reported evidence for adult neurogenesis in the neocortex also used additional neuronal markers to corroborate the BrdU and NeuN results. One study found double labelling of newly generated cells with numerous pan-neuronal and interneuronal markers, including human-neuron-specific RNA binding proteins (HuC/D), neuron-specific enolase (NSE), excitatory amino acid carrier 1 (EAAC1), γ-aminobutyric acid (GABA), glutamic acid decarboxylase 67 (GAD67) and the calcium binding proteins calbindin and calretinin[34]. These markers are all expressed by neurons, although some are also expressed by glia, particularly after damage. However, expression of several neuronal markers, including NeuN, NSE and HuC/D, for which there is no evidence for glial expression in the intact brain, provides strong evidence that these new cells are neuronal. It should be noted that some studies have reported co-labelling of neuronal markers by cells that express NG2 (REF. 34,42). NG2 is a proteoglycan which was originally believed to mark only oligodendrocyte progenitors. However, more recent studies have reported evidence that NG2-positive cells give rise to both neurons and glia[42,43]. Indeed, a subpopulation of NG2-positive cells has even been shown to generate what appear to be immature action potentials in response to depolarization[44]. Thus, the co-labelling of neuronal markers with NG2 probably reflects the multipotent nature of NG2 progenitors, and is not evidence that glial cells nonspecifically express neuronal markers.

On the other hand, several studies have been unable to replicate findings of adult neurogenesis in the neocortex (TABLE 1). In addition to the early negative ^{3}H-thymidine autoradiographic study in the adult macaque[7], some recent studies using BrdU labelling have also found no evidence of BrdU and NeuN double-labelled cells in the neocortex of adult mice[45], rats[46], macaques[47] or humans[48].

What might explain these discrepancies? One possibility is that differences in histological protocols produce variations in the limit of detectability of BrdU and NeuN double-labelled cells. Such variations might be particularly important when searching for BrdU and NeuN double-labelled cells in a brain region where the expected density of such cells is relatively low, such as the neocortex. Because the BrdU antibodies only bind to BrdU in single-stranded DNA, all BrdU immunohistochemical protocols require denaturing steps that are universally damaging to tissue[49]. Variations in this step are common and some variations might compromise histological quality more than others. For instance, hydrochloric acid is used for denaturing DNA in nearly all BrdU studies, but the incubation times used range from 15 minutes to 2 hours, and the temperature at which denaturation is carried out ranges from room temperature to 37°C[49,50]. In addition to treatment with hydrochloric acid, many BrdU protocols include additional denaturation steps such as incubation for 2 hours at 65°C in formamide[21,45–48], a chemical with solvent-like properties which can damage tissue. Many BrdU protocols also include the incubation of tissue in an alkaline borate buffer, which also has the potential to damage tissue. Finally, BrdU protocols differ in whether or not they involve freezing tissue before it is cut[21,45–48,51]. Even with appropriate cryoprotection, freezing tissue inevitably causes some damage and reduces histological quality. Thus, it is possible that differences in histological protocols contribute to some of the discrepancies in the literature. There is evidence that several of these steps not only reduce tissue quality but also diminish the signal-to-noise ratio for NeuN staining

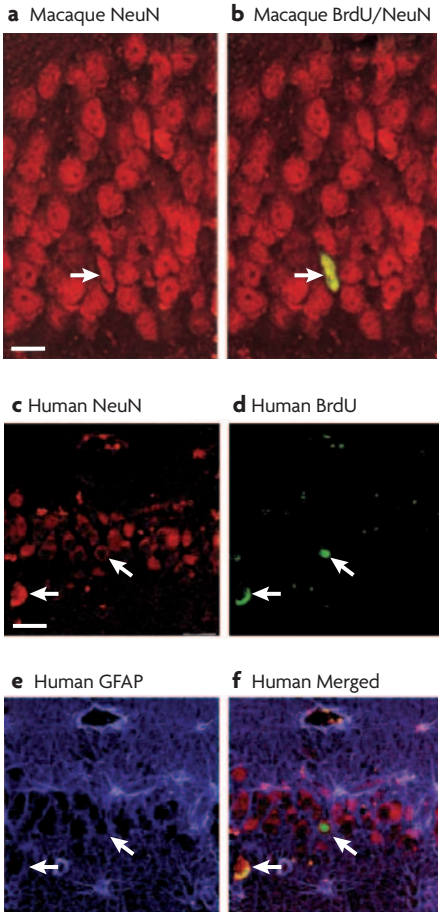

Figure 2 | **Studies reporting no adult neurogenesis in the neocortex have presented unconvincing evidence for adequate BrdU and NeuN double labelling in the dentate gyrus.** Evidence of new neurons (bromodeoxyuridine (BrdU) and NeuN double-labelled cells) in the adult hippocampus has been presented as a positive control for studies reporting the absence of adult neurogenesis in the neocortex. Inadequate quality of these control images suggests that neocortical neurogenesis may lie below the limit of detectability for a given staining procedure, since the rate of new cell addition in the neocortex is much lower than in the hippocampus. **a**,**b** | The only published example of a BrdU (green) and NeuN (red) double-labelled cell (arrow) in the adult macaque hippocampus by this group[20]. It was used to substantiate claims of no adult neurogenesis in the macaque neocortex[47]. This cell lacks the characteristic shape and staining pattern of surrounding granule cells and is therefore not a convincing positive control for double labelling of new neurons. Scale bar, 10 µm. **c**–**f** | The only published example of a BrdU and NeuN double-labelled cell in the adult human hippocampus[21]. It was used to substantiate claims of no adult neurogenesis in the human neocortex[48]. This cell does not have NeuN staining in the nucleus, suggesting possible tissue damage. Blue cells in panel **e** are stained with glial fibrillary acidic protein (GFAP), a marker of astrocytes. Scale bar, 25 µm. Panels **a**,**b** reproduced, with permission, from REF. 20 © (1999) National Academy of Sciences. Panels **c**–**f** reproduced, with permission, from REF. 21 © (1998) Macmillan Publishers Ltd.

in the adult neocortex (for more information see the laboratory website in the Online links box).

Because of the potential for false negatives produced by histological problems, it is crucial to provide positive evidence for adult neurogenesis in a known neurogenic region when reporting negative findings in the neocortex. Such evidence serves as an indication that the methods used would have detected BrdU and NeuN double-labelled cells in the neocortex were they present. Studies reporting no adult neurogenesis in the neocortex of monkeys[47] and humans[48] have referred back to earlier studies of adult neurogenesis in the dentate gyrus of the same experimental brains for positive controls, each providing a single example of a BrdU and NeuN double-labelled cell in the dentate gyrus[20,21]. In both cases, although double-labelled cells were found, the examples provided are not convincing in that one does not have a granule cell morphology[20] and the other does not have NeuN staining in the nucleus[21] (FIG. 2, compare with FIG. 3). As the quality of the histology and the resulting images were not optimal, the possibility that histological procedures interfered with the detection of BrdU and NeuN double-labelled cells in the neocortex cannot be ruled out. That is, if there is not good BrdU and NeuN double-label staining in the dentate gyrus, the absence of such staining in the neocortex or elsewhere cannot be used as evidence that no new neurons exist.

Discrepancies between studies of BrdU and NeuN labelling in the adult neocortex might also result from differences in the microscopic analysis of stained tissue. Such studies often involve searching for presumptive double-labelled cells first with a fluorescent microscope, followed by closer examination of each selected cell with three-dimensional confocal analysis[47]. However, the use of a dual-band filter set for the initial tissue examination may exclude double-labelled cells that are small and have little or no NeuN-stained cytoplasm around the BrdU and NeuN double-labelled nucleus. Double labelling is often difficult to detect with this approach when both labels stain the same cellular components. Studies of adult neurogenesis in the neocortex have often reported that BrdU and NeuN double-labelled cells are relatively small[33,34], and many do not have stained cytoplasm. Therefore, a stepwise approach of viewing BrdU-labelled neocortical cells first with a conventional fluorescent microscope and selecting only certain cells for confocal analysis, as opposed to directly performing a confocal analysis on every BrdU-labelled cell, might exclude *a priori* many of the double-labelled cells.

It is also possible that differences in the criteria used to identify labelled cells as neurons account for some discrepancies between studies of BrdU and NeuN double-labelled cells in the adult neocortex. This issue can be illustrated by examining a paper that is often cited as evidence against adult neurogenesis in the macaque neocortex[51]. The authors report finding BrdU and NeuN double-labelled cells in the prefrontal cortex of the adult macaque (FIG. 4); however, these cells were classified as non-neuronal because "the oval, triangular, or spindle-shaped nuclei of these cells were significantly smaller than of any cortical neurons in the same tissue"[51]. However, examination of the figures in this paper reveals many small NeuN-positive cells that are not co-labelled with BrdU (FIG. 4d). This indicates a bias against designating a neocortical cell as a neuron on the basis of the size and shape of its cell body. Although many neocortical

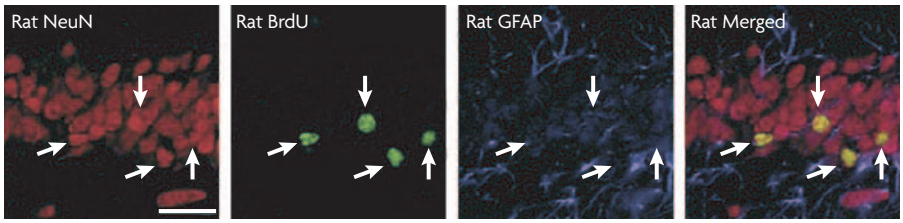

Figure 3 | **Convincing evidence for adequate BrdU and NeuN double-labelled staining in the adult rat hippocampus.** Used for comparison with human staining[21] (FIG. 2). The morphology and staining patterns of the double-labelled cells in these images are dissimilar from those presented in FIG. 2. Scale bar, 25 µm. BrdU, bromodeoxyuridine, green; GFAP, glial fibrillary acidic protein, blue; NeuN, a neuron-specific marker, red. Reproduced, with permission, from REF. 21 © (1998) Macmillan Publishers Ltd.

neurons have large cell bodies, a considerable number are very small. In fact, several subclasses of cortical interneurons (for example, calretinin-positive cells, one class of parvalbumin-positive cell and one class of calbindin-positive cell) have cell body diameters in the range of 5–13 μm[52,53]. Thus, it seems inappropriate to define small, NeuN-positive cells in the neocortex as non-neuronal on the basis of their size. Indeed, in the article described above[51], the BrdU and NeuN double-labelled cells defined as neurons in the olfactory bulb (FIG. 4a) are very similar to those defined as non-neuronal in the prefrontal cortex (FIG. 4b–d), casting doubt on the idea that one population is more convincingly neuronal than the other. This example shows the importance of using the same criteria for adult neurogenesis in all brain regions, whether they have been designated neurogenic or not.

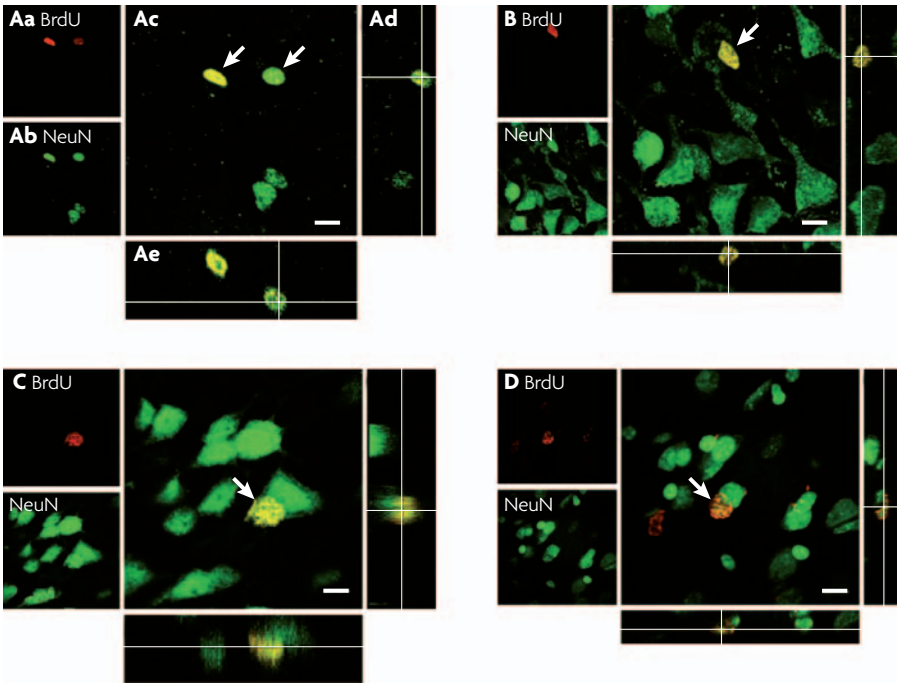

Figure 4 | **The standards of evidence of adult neurogenesis are not applied evenly across brain areas.** A widely cited paper[51] claiming no evidence of adult neurogenesis in the primate neocortex reported bromodeoxyuridine (BrdU) and NeuN double-labelled neurons in the olfactory bulb (**A**), yet categorized similar examples in the prefrontal cortex (**B**–**D**) as 'non-neuronal'. **A** | BrdU and NeuN double-labelled cells in the olfactory bulb. **Aa** | BrdU stain (red). **Ab** | NeuN stain (green). **Ac** | BrdU and NeuN double-labelled cells (arrows). **Ad,e** | orthogonal views of panel **Ac**, showing BrdU and NeuN double-labelled cells. **B**–**D** | The BrdU and NeuN double-labelled cells (arrows) in the prefrontal cortex were characterized as non-neuronal on the basis of their size, shape and staining patterns. However, they do not appear less 'neuronal' than those in panel **A**. Observe the small NeuN-positive cells in panel **D** that are not co-labelled with BrdU, which demonstrate the small size of some cortical neurons. Scale bar, 8 μm. Reproduced, with permission, from REF. 51 © (2003) Society for Neuroscience.

Endogenous markers of immature neurons. Given the controversy over the results of ^{3}H-thymidine and BrdU labelling studies, other techniques might help to determine whether neurogenesis occurs in the adult neocortex. Several studies have reported the expression of molecules used as markers for immature neurons in the dentate gyrus, such as polysialic acid–neural cell adhesion molecule (PSA–NCAM), collapsin response mediator protein 4 (CRMP4) and doublecortin in the adult neocortex[51,54–57]. However, this staining has been interpreted as due to either nonspecific labelling[51] or expression of these proteins by mature neurons that are structurally plastic rather than due to adult neurogenesis[56,57]. It is important to note that these markers do not definitively indicate that the cells are new or that, if so, they will survive and incorporate into existing circuits. However, their presence is suggestive of new neuron formation.

Two newer approaches, retroviral labelling and ^{14}C labelling, have been used to search for adult neurogenesis in the neocortex[48,58]. These studies failed to find positive evidence for cortical neurogenesis in adults, but the results are far from conclusive as each method has limitations in sensitivity that increase the likelihood of false-negative findings.

Retroviral labelling. The use of retroviruses to drive the expression of fluorescent proteins in new cells has some advantages over BrdU labelling. First, because the fluorescent protein is distributed throughout the entire infected neuron, an extensive picture of the morphology of the new cells, including their dendritic trees and axonal arborizations, can be obtained[23,24]. This can be used to determine whether the fluorescent cells are neurons or glia, as most neurons have morphologies that are readily distinguishable from those of glia (despite overlap in the size of their cell bodies). Although some glia may share certain morphological characteristics with neurons, the presence of a labelled axon (although not necessary to establish neuronal identity) would definitively rule out a glial phenotype. Second, retroviruses can be used to identify adult-generated neurons in living slice preparations[23], thus enabling electrophysiological characterization of the new cells. Although receiving synaptic input, vesicular release of transmitters and voltage-gated ion channels are features of both neurons and glia[59,60], a demonstration that retrovirus-labelled cells generate action potentials would provide strong evidence that a new cell is a neuron. However, the retroviral method is not ideal for studies of neurogenesis in brain regions where it is likely to occur at a relatively low rate, because retroviral infection is sporadic and unpredictable, so negative results are not definitive. As mentioned above, for a method to be useful for ruling out adult neurogenesis in a brain region, it must be sensitive enough to detect neurogenesis at a relatively high level in a known neurogenic region. The problem with retroviral labelling is evident in studies in the rodent dentate gyrus[23,24], where the rate of adult neurogenesis is relatively high[22]. Even with more efficient retroviruses, the number of labelled cells in the dentate gyrus is lower than the number found with BrdU labelling[23,24].

Additional problems make it difficult to interpret the results of retroviral labelling studies. Spurious labelling of neurons that were not generated around the time of the retroviral infection can occur as a result of the fusion of an infected microglial cell with a mature neuron[58]. As microglial fusions are not seen in uninfected brain sections, this finding indicates that either the virus or the injections used in this method cause immune-mediated side effects. The fact

Box 1 | **Damage-induced neurogenesis**

Numerous reports published over the past 10 years have suggested that adult neurogenesis in known 'neurogenic' regions can be stimulated by damage. Damage to the hippocampus, by lesions, ischaemia or seizures, increases the number of newly born neurons in the dentate gyrus and olfactory bulb[66,93,94]. This indicates that even the relatively high levels of adult neurogenesis reported in the rodent dentate gyrus and olfactory bulb are not maximal; under certain conditions, the rate of neuron production can be greatly increased. The overall story for other brain regions appears to be similar; adult neurogenesis has been reported in a number of brain regions following damage. There are reports of damage-induced neurogenesis in the rodent neocortex as a result of selective lesion of neurons[50], as well as following ischaemia[62]. Similar findings have been reported for other brain regions, including the striatum, amygdala, hypothalamus and substantia nigra, where experimental manipulations such as ischaemia, growth factor infusion or pharmacological manipulations lead to the production of new neurons[61,77–84]. Is damage-induced neurogenesis an enhancement of a low level of adult neurogenesis or an awakening of a latent potential for neurogenesis in brain regions that are normally incapable of supporting this process? This issue may be difficult to resolve with current techniques, because insensitive methods which detect the presence of bromodeoxyuridine (BrdU) and NeuN double-labelled cells in situations where the levels of adult neurogenesis are relatively high may be incapable of identifying a very small number of such cells. In some of the cases of induced neurogenesis, evidence for adult neurogenesis was also detected in the control animals, although it is difficult to assess whether these are examples of neurogenesis occurring in intact brain regions or a milder form of damage-induced neurogenesis.

that retroviruses must be directly injected into the brain, near the site of the neuronal progenitor cells, presents another problem because brain damage is inevitable with this delivery method, and injury-induced neurogenesis has been observed in many brain regions[50,61,62] (BOX 1). Without the development of methods that can deliver viruses to specific brain regions without causing damage, positive or negative retroviral results of adult neurogenesis will be difficult to interpret.

^{14}C labelling. At present, adult neurogenesis in the human brain can be studied only in individuals who have had an exogenous, detectable substance incorporated into their DNA at discrete time points. Studies of the brains of patients with cancer who have been injected with BrdU are limited to those patients who have subsequently died as a result of terminal illness. However, it is also possible to analyse the incorporation of radioactive carbon into DNA in the human brain[48]. This method relies on the fact that large changes in atmospheric ^{14}C occurred between the advent of nuclear bomb testing in 1955 and the test ban treaty of 1963, causing enough variability in radioactivity to assess the age of cell populations in humans who were alive during or after this time period. An advantage of this method over BrdU labelling is that the incorporation of ^{14}C into DNA occurs naturally, as opposed to through medical intervention. Thus, it is possible to use this method to look for evidence of adult neurogenesis in healthy humans who died as a result of an accident instead of a lengthy illness.

The authors of a study that used this technique isolated nuclei from the cortical cells of adult humans, and sorted them on the basis of whether they bound NeuN antibodies. They then used accelerator mass spectrometry to determine the levels of ^{14}C in the NeuN- and non-NeuN-expressing cell populations. The authors estimated that the mean birthdate of the cortical neurons was around the time of the birth of the individual, providing no evidence for cortical neurogenesis at later time points. However, they discussed the fact that this method can only detect relatively large numbers of new cells generated at a given time point, and estimate the detectable limit to be about 1% of the total population of neurons[48]. This is a particularly important point when interpreting data from the neocortex, as studies that described evidence for adult neurogenesis in the neocortex of experimental animals all reported small numbers of BrdU and NeuN double-labelled cells[32–37]. There seem to be at least 30 times as many BrdU- and NeuN-positive cells in the dentate gyrus as in the neocortex of macaques[33]. The ^{14}C study did not report results for the human dentate gyrus[48], so it is difficult to determine what the results would be in a region that is known to have a high level of neurogenesis. However, if there are only one-thirtieth as many adult-generated neurons in the human neocortex as in the dentate gyrus, the new cortical neurons may well fall considerably below the limits of detection for this technique. It is important to note that the ^{14}C method could detect a level of cell production that was lower than 1% of the total population of cells, providing the new cells survived for long periods of time — until the time of the individual's death. In this regard, it is worth noting that as in the dentate gyrus, the BrdU-labelled neurons that have been found in the adult neocortex decline in the period of time following their generation, suggesting that many die[33]. Hence, the ^{14}C method would not allow for the detection of neurons that were produced at relatively low levels and not permanently incorporated. So, on the basis of available evidence it seems premature to conclude that the human neocortex does not produce neurons after birth.

Other non-neurogenic regions

The debate about adult neurogenesis in regions outside of the dentate gyrus and olfactory bulb does not focus exclusively on the neocortex. Over the past few years, there have been several reports of adult neurogenesis in regions other than the neocortex, including the striatum[34,63,64,65], the amygdala[36,65,67], the hypothalamus[32,68,69] and the substantia nigra[70], as well as in the brainstem[71], olfactory tubercle[72] and piriform cortex[73] (TABLE 1). However, as in the case of the neocortex, conflicting data exist. The positive evidence includes co-labelling with BrdU and neuronal markers, ^{3}H-thymidine labelling with electron microscope analysis and the presence of immature neuronal markers (PSA–NCAM and CRMP4). However, there are also studies that have failed to confirm these findings[7,74–76], or have found such evidence only after damage, growth factor infusion or other pharmacological manipulations[77–84] (TABLE 1). It is difficult to point to any one reason for the discrepant results, but the same issues that apply to the neocortex studies apply to these studies as well. And, like the neocortex, these regions all differ from the dentate gyrus and olfactory bulb in that any addition of new neurons occurs at a much lower rate, again raising the question of whether the negative results are due to a lack of sensitivity of the techniques used.

Discussion and future directions

Two decades ago, neurogenesis anywhere in the adult primate brain was claimed to be non-existent[7]. As a result of new technologies, a number of studies have now produced evidence for adult neurogenesis in the primate hippocampus[85] and olfactory bulb, even in humans[21,28,30]. For other parts of the brain, the situation is still not clear. There have been a number of recent reports of adult neurogenesis in several brain areas, including the neocortex. The number of

new neurons in these areas has invariably been fewer than those reported for the hippocampus and olfactory bulb. However, this should not be grounds for rejecting the possibility that these new neurons may have a functional role, because there is considerable evidence that even a small number of neurons can influence behaviour[86,87].

Several studies have linked adult-generated neurons in the rodent hippocampus to learning and memory[88–90]. It has also been suggested that adult neurogenesis in the hippocampus may be related to the therapeutic effects of antidepressants[91]. Although these issues remain unresolved, the search for a functional role for adult neurogenesis in the hippocampus is currently an active area of investigation. The potential function of new neurons in brain regions other than the hippocampus and olfactory bulb, however, remains unexplored.

Before we can begin to decipher their functions, we must develop more reliable methods to detect new neurons in areas where they are relatively sparse, such as in the neocortex. One approach may be to assess the identity of new cells with transgenic animals that express fluorescent proteins only in neurons. As fluorescent proteins would label the entire neuron, dendritic morphology (as well as the presence of an axon) could be assessed, thereby eliminating the problem of relying on staining of just the nucleus and cell body to determine neuronal identity (as in the case of NeuN). To be useful in this regard, the fluorescent protein must be both ubiquitously expressed among neurons and compatible with birth dating methods. Moreover, conclusive evidence that a new cell has become a functioning neuron requires the demonstration that it receives synaptic input, generates action potentials and communicates with its target. Obtaining such electrophysiological evidence will be valuable but arduous. It may require identifying circumstances under which low-level neuron production can be augmented. Given that in the past the claims of 'no new neurons' first in the adult mammalian brain and then in the adult primate brain turned out to be incorrect, we should be cautious about other claims of 'no new neurons' and, instead, seek new, more sensitive and more reliable techniques, while keeping an open mind to the possibility of a more widely neurogenic adult brain.

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FURTHER INFORMATION
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