of the thousands of categories that humans can distinguish. Given these facts, it is highly implausible that the tuning functions for these neuronal populations could be restricted to single categories. Voxels that responded maximally to one of the two animate categories, human faces and four-legged animals, often responded significantly to the other animate category, suggesting that they are more coarsely tuned to animate objects and may also respond to other animate categories such as birds, fish, insects and so on. The spots that responded maximally to cars and sculptures must also respond to other categories, but further experiments will be necessary, with careful, theory-driven sampling of categories, to fully characterize the real tuning functions of these cortical spots.

High-resolution imaging shows a fine-scale structure, with a spatial frequency of 1 mm to 5 mm, but is that the fundamental scale for the neural code for faces and objects? What are the organizing principles for the spatial organization or topography of this code in ventral occipitotemporal cortex? Neural populations that respond to similar stimulus properties tend to be found in nearby locations, such as orientation-selective columns in early visual coretex and face orientation columns in inferior temporal cortex[13]. The mosaic of intermixed cortical spots in the results of Grill-Spector *et al.* does not reveal any obvious relationship between neighboring spots, but one may well exist. Indeed, the higher concentration of face-selective spots in the FFA, relative to the LOC, suggests a more macroscopic level of organization that also underlies the topography of face and object representations.

Understanding the code for faces and objects also requires investigation of the tuning functions of individual neurons within these cortical spots. Single-unit recording in a highly face-selective spot in the monkey brain (identified with fMRI) showed that individual neurons also were highly face selective but had different coarse tuning to individual faces, providing sufficient information as a population to distinguish individual faces[14].

Thus, the topography of the neural code for faces and objects seems to exist at multiple spatial scales (**Fig. 1**): a coarse scale with a spatial frequency of 3 mm to 20 mm, as revealed by standard-resolution imaging of category-selective areas and distributed patterns of response; a fine scale, represented by clustered neuronal populations in small spots, 1 mm to 6 mm across, that are highly selective for a single category or a small number of presumably related categories; and an even finer scale represented by individual neurons within these spots that are tuned to different examples of the preferred category or categories. Different methods are optimal for investigation of these different scales of organization. The new paper by Grill-Spector *et al.* demonstrates that the mid-level scale can now be investigated noninvasively in humans with high-resolution functional brain imaging. Their results, though consistent with standard-resolution imaging, are inconsistent with many key features of the most influential models that were based on standard-resolution findings and, thereby, represent a major advance in our understanding of the neural code for faces and objects.

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Adult cortical neurogenesis: nuanced, negligible or nonexistent?

Edmund Au & Gord Fishell

Can the adult neocortex in primates generate new neurons? A new study uses a clever way of determining the age of postmortem human neurons to conclude that there is very little postnatal cortical neurogenesis—or none at all.

Neurodegenerative diseases such as Alzheimer disease and Parkinson disease are typified by a progressive loss of neuronal populations in the brain, neurons that are never meaningfully replaced. Given the grim course of these diseases, it is an appealing idea that there might be an endogenous mechanism capable of replacing dying neurons. Endogenous adult neurogenesis might be harnessed and possibly augmented to enable effective treatment of neuronal loss due to injury or disease. However, leaving aside technical hurdles such as the formation of proper functional connections, it remains uncertain whether cortical neurogenesis ever occurs postnatally in humans. Recently, Frisén and colleagues have published two studies[1,2] that strongly suggest that there is little, if any, neurogenesis in the human cortex after birth.

Postnatal neurogenesis clearly occurs in lower mammalian species[3]. It is well established that neurons are generated in rodents throughout adulthood[4], supplying two regions: the olfactory bulb and the hippocampus[5,6]. The extent of neurogenesis in humans and primates has remained more questionable[7,8]. Some data suggest that whereas new neurons are generated for the hippocampus[9], this may not be the case for the olfactory bulb[10]. In other areas of the brain, such as the cortex, the issue is even more controversial. Analysis in primates using 5-bromodeoxyuridine (BrdU) and neuronal markers have argued both for[7,11] and against[8,12] cortical neurogenesis.

Aside from the obvious ethical issues that limit prospective experimental manipulations, the greatest difficulty with studying neurogenesis in humans lies in the long lifespan of the subjects. The Frisén group has elegantly overcome this obstacle by taking advantage of an event that affected all life on earth during the middle of the 20th century and enables the prospective birth dating of neurons in the human cortex.

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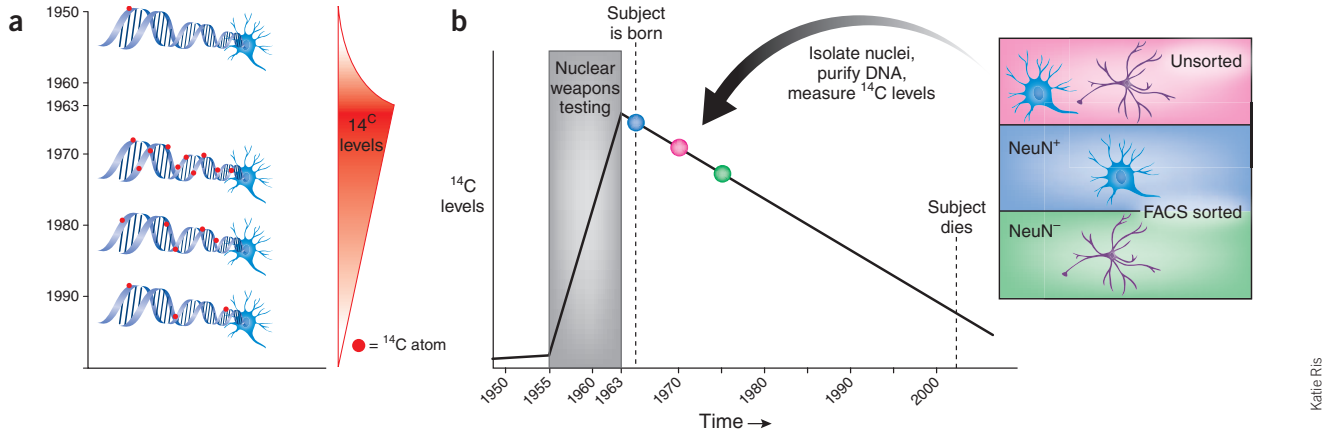

Figure 1 Dynamic levels of ambient ^{14}C serve to birth-date postmitotic cells in the brain. (**a**) Owing to nuclear weapons testing, ^{14}C spiked during the middle of the 20th century and dropped after the 1963 nuclear test ban treaty. Incorporation of ^{14}C into neuronal DNA reflects ambient levels of ^{14}C in the atmosphere. Because ^{14}C levels dropped off at a predictable rate, it is possible to birth-date postmitotic cells by measuring ^{14}C levels in their DNA. (**b**) A sample human subject born shortly after cessation of nuclear weapons testing, whose cortex was processed postmortem for ^{14}C birth dating. Unsorted cells' DNA ^{14}C levels indicated an age about five years younger than the subject (pink). Using fluorescence-activated cell sorting (FACS), cortical cells were sorted into NeuN-positive neurons (blue) and NeuN-negative non-neuronal cells (green). Sorted neurons (blue) were birth-dated to approximately the same time as the individual, whereas non-neuronal cells (green) were on average ten years younger than the individual. Therefore, non-neuronal cells skewed the average in the unsorted sample, and the vast majority of cortical neurons were generated at or before the birth of the individual.

From 1955 to 1963, extensive surface testing of nuclear weapons led to massively elevated levels of the ^{14}C isotope in the atmosphere. This ^{14}C was incorporated into CO_2, taken up by plants, consumed by humans and metabolized. In this fashion, elevated ^{14}C levels were eventually incorporated into human DNA, labeling all cells born during this time period (**Fig. 1a**). After the nuclear test ban treaty of 1963, atmospheric ^{14}C levels dropped rapidly, with most of the excess ^{14}C equilibrating into the world's oceans.

Once the high exposure of ^{14}C was over, the isotope was rapidly depleted in all short-lived cellular components of postmitotic cells such as neurons, but high levels of ^{14}C were conserved in DNA, which does not turn over. Therefore, given the absence of significant DNA synthesis (beyond that which accompanies DNA nick repair for instance), nuclear ^{14}C should provide a reliable indicator of whether and when a specific neuron was generated during this period (**Fig. 1a**).

Frisén and colleagues[2] analyzed postmortem brain samples from individuals born between 1933 and 1977 (with individuals born before the period of high exposure acting as controls) and, by correlating the level of ^{14}C in DNA with the birth date of the subject, determined whether any new neurons were generated postnatally (**Fig. 1b**). The authors found on average that the cells in the cortex were approximately five years younger than the subject, suggesting that many are generated after birth. However, when the cells were sorted into two populations, neuronal and non-neuronal by virtue of neuronal nuclear antigen (NeuN) expression, this result seemed to be explained entirely by the postnatal generation of non-neuronal cells in the cortex. Non-neuronal cells were on average ten years younger than the subject, reflecting the high gliogenesis that occurs during childhood. By contrast, the average age of NeuN-positive neurons was approximately the same as that of the subject, consistent with the idea that we are born with all of the cortical neurons we will ever have.

In the earlier study[1], the Frisén group used this method to examine samples taken from the occipital cortex. In the present work[2], they examined all areas of the cortex: prefrontal (including premotor), parietal, temporal and occipital regions. Throughout, they found that non-neuronal cells were generated postnatally, but cortical neurons were born at or just before the time of birth. Moreover, to test for neurogenesis over shorter time frames, they also analyzed samples from cancer patients that had been given BrdU for diagnostic purposes. The authors did not detect any BrdU-labeled cells in the cortex that expressed neuronal markers such as NeuN or neurofilament. Previous work had suggested that cortical neurons generated in adulthood were a transient population[11], such that new neurons would be constantly generated but had a limited lifespan and would not be detected by ^{14}C analysis. By correlating the time of BrdU administration and the time of death, Bhardwaj and colleagues[2] could address whether such a putative transient population could be detected. They failed to find BrdU-labeled neurons in human brain tissue at even 4.2 months, the shortest time period after BrdU administration that they examined. This suggests that if cortical neurogenesis occurred in the areas they examined, the neurons generated would be either extremely rare or remarkably short lived.

These findings correlate well with previous evidence that argues against significant postnatal neurogenesis in the cortex of humans and primates. However, this study, though compelling, cannot entirely dismiss postnatal cortical neurogenesis as nonexistent—indeed, the authors state that their limit of resolution with ^{14}C analysis is approximately 1% and that there could still be a very small population of cortical neurons generated after birth. In addition, there are limits to the temporal resolution of ^{14}C birth dating. For example, one could envision a potentially significant population of neurons born in early childhood—close enough to the time when most cortical neurons are generated embryonically—that could be overlooked, lost in the calculated average age of all cortical neurons. However, for argument's sake, what if a minute percentage of cortical neurons were generated in adulthood? One still has to question whether they would have any meaningful functional impact. Similarly, if a population of cortical neurons were born perinatally, could this have any relevance for the treatment of neurodegenerative diseases whose onset is typically late in adulthood?

It would have been very encouraging to find a neurogenesis mechanism already in place, one that would generate neurons capable of functionally integrating into the milieu of the adult cortex. If newly generated neurons could,

under normal circumstances, successfully integrate into the complex circuitry of the cortex, the feasibility of cell replacement therapies for brain repair would immediately appear less daunting. The new findings[2], unfortunately, argue strongly against this possibility. This is not to say that we should abandon hope, however. There is evidence suggesting that human adult neural stem cells retain the latent ability to generate neurons in culture[10]. Moreover, the adult rodent brain may be coaxed into generating new cortical[13] and striatal[14] neurons in response to injury. Of course, experience to date clearly shows that findings in rodents cannot automatically be translated to humans, but nonetheless, the authors' plans to use the same approach to analyze individuals that have suffered strokes and other brain injuries should certainly prove interesting. Even if these studies fail to reveal significant neurogenesis, work on cell replacement approaches for brain repair should and will most certainly continue. Hopefully it will proceed with appropriate caution: evolution has already weighed in on the relative advantages of permitting the generation and integration of neurons into the postnatal brain of higher nervous systems. For reasons that are not yet evident, the risks seem to outweigh the benefits.

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Huntingtin's critical cleavage

John D Fryer & Huda Y Zoghbi

The early pathogenic events leading to neurodegeneration in Huntington disease are not clear. A recent paper shows that mutating a caspase-6 cleavage site in the huntingtin protein is sufficient to prevent pathogenesis.

Huntington disease is a progressive and fatal neurodegenerative disorder characterized by involuntary movements resulting from the loss of medium spiny neurons in the striatum. The disease is caused by an expansion of a trinucleotide CAG repeat that encodes a polyglutamine tract in the widely expressed huntingtin (htt) protein, yet the precise molecular mechanisms causing the polyglutamine-expanded htt toxicity are not fully understood. A recent paper in *Cell* by Graham and colleagues[1] demonstrates that a critical cleavage site in the htt protein is necessary for behavioral impairment and neurodegeneration in a mouse model of Huntington disease.

The expansion of CAG repeats causes several neurodegenerative diseases, including spinal and bulbar muscular atrophy, dentatorubral-pallidoluysian atrophy and some spinocerebellar ataxias[2]. Because the disease-causing proteins are seemingly unrelated, early research in this field focused on the common polyglutamine tract itself. Overexpression of isolated polyglutamine tracts alone is toxic and can cause neurodegeneration both *in vitro* and *in vivo*[3]. However, regions outside the polyglutamine tract dramatically alter pathogenesis by affecting phosphorylation, sumoylation, protein-protein interactions or cleavage[4–7].

Truncated fragments of the htt protein are found in brain extracts isolated from transgenic mice and in Huntington disease postmortem tissue[8]. Caspase-3, caspase-6, aspartyl endopeptidases and calpain are all implicated in the cleavage of htt, but the pathological relevance of these fragments *in vivo* has been unknown. To determine if the caspase cleavage sites or the resulting fragments are critical for pathogenesis, Graham *et al.* generated a series of four YAC transgenic mouse lines that expressed polyglutamine-expanded htt as the wild type or with mutations in the following sites: (i) three caspase-3 cleavage sites, (ii) one caspase-6 cleavage site or (iii) both caspase-3 and caspase-6 cleavage sites.

The phenotype in htt YAC transgenic mice is determined by expression level[9]. Thus the authors created several transgenic lines and rigorously proved that the lines that they compared had equivalent mRNA and protein expression. They next verified that their caspase mutant htt proteins were indeed resistant to cleavage by caspase-3 or caspase-6. Incubation of recombinant caspase-3 and caspase-6 with brain lysates from YAC transgenic mice expressing polyglutamine-expanded htt unmutated at caspase cleavage sites generated the predicted htt fragments. As expected, polyglutamine-expanded htt transgenic mice with mutated caspase-3 cleavage sites were resistant to caspase-3 cleavage. Likewise, polyglutamine-expanded htt transgenic mice with mutated caspase-6 cleavage sites were resistant to caspase-6 cleavage.

The key test of the importance of these cleavage sites came from evaluating the motor phenotypes (rotarod and open field) and neuropathology (brain weight, striatal volume and nuclear htt accumulation) of polyglutamine-expanded htt transgenic mice with mutated cleavage sites. Similar behavioral phenotypes and neurodegeneration were seen in YAC transgenic mice expressing polyglutamine-expanded htt with unmutated caspase cleavage sites and in mice expressing polyglutamine-expanded htt with mutations at three caspase-3 cleavage sites. (This form of polyglutamine-expanded htt is still cleavable at the caspase-6 site.) In contrast, transgenic mice expressing polyglutamine-expanded htt with a mutated caspase-6 cleavage site did not manifest behavioral deficits or neurodegeneration, even when the expression level of htt exceeded that in mice expressing polyglutamine-expanded htt.

To investigate why htt with a mutant caspase-6 cleavage site was resistant to neurodegeneration, the authors evaluated the subcellular localization of htt and found that htt mutated at caspase-6 (but not caspase-3) cleavage sites had a significant delay in its

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