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The Age of Human Cerebral Cortex Neurons

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Abstract

The traditional static view of the adult mammalian brain has been challenged by the realization of continuous generation of neurons from stem cells. Based mainly on studies in experimental animals, adult neurogenesis may contribute to recovery after brain insults and decreased neurogenesis has been implicated in the pathogenesis of neurological and psychiatric diseases in man. The extent of neurogenesis in the adult human brain has, however, been difficult to establish. We have taken advantage of the integration of ^{14}C, generated by nuclear bomb tests during the Cold War, in DNA to establish the age of neurons in the major areas of the human cerebral cortex. Together with the analysis of the cortex from patients who received BrdU, which integrates in the DNA of dividing cells, our results demonstrate that whereas non-neuronal cells turn over, neurons in the human cerebral cortex are not generated postnataly at detectable levels, but are as old as the individual.

Introduction

New neurons derived from endogenous stem or progenitor cells are continuously added to the adult mammalian brain. This may be important for processes requiring plasticity, such as memory formation (1), and new neurons have been suggested to replace lost cells after stroke and other insults (2, 3). Furthermore, neurogenesis has been implicated in the pathogenesis of human neurological and psychiatric diseases (4-7).

There is a considerable degree of plasticity in the cortex, enabling for example memory formation (8), and there is evidence of structural alterations resulting in detectable changes in volumes in distinct areas in the adult human cortex with age and in response to certain conditions (9, 10). Much of the plasticity can be accounted for by modulating pre-existing cells and their connections, but it is important to determine if neuronal turnover may contribute to cortical plasticity in humans.

It has remained controversial whether neurons are added to the cerebral cortex in adult mammals. Some studies have suggested that neurogenesis persists in the adult rodent (11, 12) and monkey cortex (13-15), whereas other studies have failed to detect neurogenesis (16-18) or only in response to an insult (19, 20).

The most common way to detect neurogenesis is by the integration of labeled nucleotides, such as BrdU, but there are inherent risks of both false positive and negative results, making room for controversy (21, 22). Moreover, there are difficulties in performing these types of studies in humans, and there is little BrdU labeled material available for analysis. We have recently developed a new method to retrospectively determine the age of cells in humans by measuring ^{14}C in DNA (23). The entry of cosmic rays into the atmosphere results in de novo generation of ^{14}C, which is matched by

radioactive decay ($t_{1/2}$=5730 years), resulting in stable steady-state atmospheric levels. A striking exception was caused by above ground nuclear bomb tests during the Cold War, which produced an approximate doubling of ^{14}C levels in the atmosphere from 1955 to 1963 that rapidly distributed around the globe (24, 25). After the 1963 Test Ban Treaty there have been no significant above ground high yield nuclear detonations and the ^{14}C levels have decreased nearly exponentially (26), not because of radioactive decay, but due to equilibration with the oceans and uptake in the biotope. ^{14}C in the atmosphere reacts with oxygen to form CO_2 and is taken up by plants in photosynthesis. Our consumption of plants and animals that live off plants, results in the ^{14}C levels in the human body mirroring those in the atmosphere at any given time (23, 27-29). Since DNA is stable after a cell has gone through its last cell division, the ^{14}C level in DNA serves as a date mark for when a cell was born and can be used to retrospectively birth date cells in humans (23).

Here we present a systematic analysis of cell turnover in the major areas of the human cortex. We have retrospectively birth dated neurons by measuring the level of ^{14}C and analyzed the brains of individuals that received BrdU. We failed to detect BrdU labeled neurons and report that cortical neurons have ^{14}C levels corresponding to the atmospheric levels at the time of birth of the individual.

Results

We have measured the ^{14}C concentration in DNA of cells in the major areas of the human cerebral cortex by accelerator mass spectrometry. DNA was extracted from neurons and non-neuronal cells, respectively, after flow cytometric sorting of nuclei incubated with an antibody against the neuron specific nuclear epitope NeuN (Figure 1A). Flow cytometric gates were set to ensure the inclusion of all nuclei irrespective of size in the different populations, because adult born cortical neurons in the rodent have been reported to be small (12). By comparing the measured ^{14}C level in DNA to atmospheric concentrations at different times, we can establish the average year of birth for the cell populations (Figure 1B) (23).

^{14}C levels in DNA of cerbral cortex cells from all lobes were analyzed, and the specific areas are indicated in Figure 2A. Both prefrontal and premotor cortices were analyzed in the frontal lobe. We first studied individuals born after the Cold War and the Test Ban Treaty, since the decline in nuclear bomb test derived ^{14}C in the atmosphere during this period provides resolution as to when cells were born. Non-neuronal NeuN-negative cells always had ^{14}C levels that were lower than those in the atmosphere at the time of birth of the individual, demonstrating cell turnover within this population (Figure 2B and Supplementary Information). These cells were in average born 5.5±1.3 years (mean±SEM, n=5 measurements) after the birth of the individual. In contrast, the ^{14}C levels in every analyzed sample of NeuN-positive neuronal nuclei from all individuals and all cortical regions showed ^{14}C levels corresponding to the time of birth of the individual (Figure 2B and Supplementary Information). The cortical neurons were born

0.3±0.2 years (mean±SEM, n=5 measurements) before the birth date of the individual, i.e. during the fetal period.

The strategy to birth date cells builds on the steep slope of ^{14}C decline in the atmosphere after the nuclear bomb tests. The resolution in time before the bomb tests is very poor. However, the low levels of ^{14}C before the bomb-pulse makes the detection of a small population of cells born during or after the bomb tests especially sensitive, and a population constituting as little as 1% of the total cell population over the lifespan can be detected (23). ^{14}C levels in DNA from NeuN-negative non-neuronal cells were invariably higher than the pre-bomb levels in individuals born before the nuclear tests, again demonstrating turnover within this population (Figure 2C and Supplementary Information). However, as these ^{14}C levels correspond to levels both at the time of increasing and decreasing ^{14}C levels, the turnover rate of these populations cannot be inferred from these data alone. The ^{14}C levels in DNA of NeuN-positive neurons, in contrast, corresponded to the atmospheric levels prior to the nuclear bomb tests in all samples from all cortical regions in all individuals born prior to 1955 (Figure 2C and Supplementary Information). Thus, if there is any generation of stably integrating cortical neurons during adulthood, they amount to less than 1% of the neuronal population up to the age of 72 years (the oldest individual included in the ^{14}C analysis).

The ^{14}C analysis provides cumulative information about the age of cells, and about potential cell turnover, over the life span of the individual. The analysis is therefore sensitive for the detection of very low-grade continuous generation of new cells that stably integrate and survive long term. However, if transitory cells are produced that are not maintained, they would go unnoticed by this method if they constituted <1% of the

cells at any given time. It has been suggested that neurons are generated in the adult monkey cortex, but that they have a short life span and are transient (15). We next analyzed the cortex of cancer patients that had received an injection of BrdU for diagnostic purposes (30). The time of death ranged between 4.2 months and 4.3 years after BrdU administration. As a positive control for the detection of adult born neurons, we analyzed sections from the hippocampus from the same patients and were able to detect cells double-labeled with BrdU and NeuN in the granular layer of the dentate gyrus (30). In the negative control brains from individuals who did not receive a BrdU injection, we were unable to detect any BrdU labeling (data not shown).

BrdU-positive cells were disproportionately distributed through the depth of the motor cortex; 46% of the BrdU-positive cells were located in the white matter, and <1% to 17% in the specific lamina (Figure 3A). We analyzed the identity of labeled cells in the frontal and motor cortex by immunohistochemistry using antibodies against cell type specific markers. Less than 1% of the BrdU-positive cells were glia-like satellite cells, and a small subpopulation constituted GFAP-immunoreactive astrocytes (Figure 3B). Most importantly, none of the BrdU labeled cells had neuronal morphology or was immunoreactive to the neuronal markers NeuN or neurofilament. In cases where a BrdU-positive nucleus was located in close proximity to a NeuN- or neurofilament-immunorective neuron (n=515), three-dimensional confocal reconstruction was performed to establish whether the labels co-existed in the same cell, but this was never the case (see examples in Figure 3C and D). Thus, we conclude that neurons are not generated in the adult human brain at levels detectable with the employed methods, and if transient neurons are generated, they have a life span of less than 4.2 months.

Discussion

Our analysis revealed that neurons in the adult human cerebral cortex have ^{14}C levels in their genomic DNA corresponding to atmospheric levels at the time when the individual was born and we failed to detect BrdU labeled neurons, arguing against postnatal cortical neurogenesis in man.

It is important to underscore that both of our approaches to detect cell turnover in the adult cortex have detection limits, and that we cannot exclude neurogenesis below this level. Retrospective ^{14}C birth dating gives a cumulative measure that provides a high sensitivity to detect a low grade continuous generation of new cells, even if these cells would only account for 1% of the neurons over the entire life span in the analyzed area (23). However, this requires that the newborn cells integrate stably and are maintained. It has been suggested that newborn neurons in the monkey cortex have a short life span and are not maintained long term (15). If at any given time such neurons account for less than 1% of the neurons in the analyzed area, they would not be detectable by retrospective ^{14}C birth dating with the current sensitivity.

In this context, BrdU labeling has the advantage that it labels new born cells at a given point in time, and it would be easy to detect very much less than 1% of neurons being labeled at the time of analysis. The time period between BrdU administration and death of the individuals we analyzed ranged between 4.2 months and 4.3 years. Our results thus indicate that there can only be little (<1%), if any, stable integration of cortical neurons in the adult human brain, and if there is a production of transitory neurons, they have a life span of less than 4.2 months.

Several studies have demonstrated the presence of cells with *in vitro* neural stem cell potential in the human cortex, including in subcortical white matter (31). Our results do not exclude the possibility that cortical neurogenesis may occur in certain pathologies or that it may be possible to induce it, as has been suggested in the rodent cortex (19, 20, 32). There is no or minimal neurogenesis in the rodent striatum under normal conditions, but large numbers of neurons are generated in response to growth factor administration or stroke (2, 3, 33, 34). Although our current study indicates that cortical neurogenesis does not take place in man under normal conditions, it will be important to analyze whether there is a latent potential that results in neurogenesis in pathological situations.

There are clear species differences with regards to the extent of adult neurogenesis in vertebrates. Large numbers of neurons may be added throughout life in fish (35). However, fish often continue to grow throughout life, which could be viewed as a continuation of development. Substantial numbers of new neurons, including both interneurons and projection neurons, are added to several regions in birds such as zebra finches and canaries (36). In rodents, interneurons are added to the dentate gyrus of the hippocampus and to the olfactory bulb in mature animals (1). There are many reports indicating more low-grade neurogenesis in other areas of the rodent brain, but many of these studies await confirmation. The number of neurons that are added in the rodent hippocampus and olfactory bulb decrease substantially with age, although neurogenesis continues at low levels throughout life. One study demonstrated neurogenesis in the adult human dentate gyrus (30), but it remains controversial whether neurons are added to the adult human olfactory bulb (37, 38). Thus the distribution of adult neurogenesis appears to have been gradually more restricted with evolution, although there is still limited

information available regarding the extent and distribution of neurogenesis in the adult human brain.

Plasticity is an important aspect of cortical function, and is necessary for example for the integration of new memories. It is also easy to see the importance of stability for the maintenance of memories, for example. There must be a delicate balance between plasticity and stability, and the lack of human cortical neurogenesis suggests that cellular stability has been favored, whereas other aspects of plasticity may have increased.

Materials and Methods

Tissue collection

Tissues for ^{14}C analysis were procured from cases admitted for autopsy during 2003 and 2004 to the Department of Forensic Medicine in Stockholm, with the consent of relatives. Ethical permission for this study was granted by the Karolinska Institute Ethical Committee. Tissue from seven individuals born between 1933 and 1973 (five individuals born before and two born after the nuclear bomb tests), was analyzed for ^{14}C content in this study. The cause of death was chest trauma (n=1), hanging (n=4), electrocution (n=1) or myocardial infarction (n=1). Tissues were frozen in 1 g samples and stored at –80°C until further analysis.

Bromodeoxyuridine (250 mg in saline) was administered i.v. to assess the proliferative nature of tumor cells in patients diagnosed with squamous cell carcinoma at the base of the tongue, in the pharynx or larynx. Metastatic spread of the carcinomas was not seen in the brain in any of these patients and no anti-cancer therapy was administered before, during or shortly after BrdU administration.

Flow cytometry of nuclei

Nuclear isolation and flow cytometry were performed as described (23). NeuN antibodies (39) were directly conjugated with Zenon mouse IgG labelling reagent (Alexa 488, Molecular Probes). To ensure that only single nuclei were sorted, an aliquot of nuclei was stained with DRAQ5 and the singlet population was plotted as a function of forward scatter width (FSC-W) versus forward scatter height (FCS-H). Using these parameters it is easy to discriminate single nuclei from doublets, triplets and potential higher order

aggregates as well as background noise (40). Nuclei were sorted based on purity, and the purity of all sorts was confirmed by re-analyzing the sorted populations. $\Delta^{14}C$ levels were corrected when the purity was less than 100%. All FACS analysis and sorting was performed using a FACS Vantage DIVA (BD). Nuclei pellets were collected by centrifugation and stored at -80°C until extraction with NaI as described (23). DNA purity for all samples was analyzed by spectrophotometry and HPLC.

Accelerator mass spectrometry

All accelerator mass spectrometry analyses were performed blind to age and origin of the sample. Purified DNA samples suspended in water were transferred to quartz combustion tubes and evaporated to dryness in a convection oven maintained at 90-95°C. To covert the DNA sample into graphite, excess CuO and silver wire were added to each dry sample, the tubes were evacuated and sealed with a H_2/O_2 torch. Tubes were placed in a furnace set at 900°C for 3.5 h to combust all carbon to CO_2. The evolved CO_2 was purified, trapped and reduced to graphite in the presence of iron catalyst in individual reactors (41). Graphite targets were measured at the Center for Accelerator Mass Spectrometry at Lawrence-Livermore National Laboratory and at the ANTARES AMS Facility at the Australian Nuclear Science and Technology Organisation (42, 43).

Large CO_2 samples (> 500 μg) were split and $\delta^{13}C$ was measured by stable isotope ratio mass spectrometry, which established the $\delta^{13}C$ correction to –23 +/-2, which was applied for all samples. Corrections for background contamination introduced during sample preparation were made following the procedures of Brown and Southon (44). The measurement error was determined for each sample and ranged between ±2-10‰ (1 SD)

$\Delta^{14}C$. All ^{14}C data are reported as decay corrected $\Delta^{14}C$ following the dominant convention of Stuiver and Polach (1977).

Detection of BrdU and phenotypic markers

The brains from patients that received BrdU were removed and the cortex and hippocampi were dissected, post-fixed in 4% paraformaldehyde for 24 h and then incubated in 30% sucrose until equilibrated. Sections were cut on a freezing sledge microtome in the coronal plane and the sections were stored in cryoprotective buffer containing 25% ethylene glycol, 25% glycerin and 0.05 M phosphate buffer. Free-floating sections were washed, incubated with HCl to denature DNA (45) and blocked 1 hour in 3% human and 3% horse serum. The sections were incubated with antibodies against BrdU (1:200, Accurate), NeuN (1:50, Chemicon), neurofilament 200 (1:200, Sigma) or GFAP (1:5000, Dako) for 48 h, then washed and incubated for 12 h in goat anti-mouse alexa 488 and goat anti-rat alexa 594 (1:200 molecular probes). The sections were then washed and mounted onto glass slides and coverslipped using Dako mounting medium.

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Figure legends

Figure 1 Determination of the age of cortical neurons

Neuronal (NeuN-positive) and non-neuronal (NeuN-negative) cell nuclei from the adult human cerebral cortex were separated and isolated by flow cytometry (A). The levels of ^{14}C in the atmosphere have been stable over long time periods, with the exception of a large addition of ^{14}C in 1955-1963 as a result of nuclear weapons tests (blue line in B, data from(46), making it possible to infer the time of birth of cell populations by relating the level of ^{14}C in DNA to that in the atmosphere (horizontal arrows) and reading the age off the x-axis (vertical arrows). The average age of all cells in the prefrontal cortex is younger than the individual (black arrows), indicating cell turnover. Dating of non-neuronal cells demonstrates that they are younger, whereas neurons are approximately as old as the individual. The vertical bar indicates the year of birth of the individual. ^{14}C levels from modern samples are by convention given in relation to a universal standard and corrected for radioactive decay, giving the $\Delta^{14}C$ value (47).

Figure 2 Cortical neurons are as old as the individual

(A) The cerebral lobes are outlined and the cortical area analyzed within each lobe is color coded. Both prefrontal (blue) and premotor (light blue) areas were analyzed in the frontal lobe. The analysis of occipital cortex was reported in (23). (B) A representative example of values obtained from one individual born after the nuclear weapons tests plotted on to the curve of atmospheric ^{14}C levels indicates that non-neuronal cells turn over while the cortical neurons were generated close to the time of birth. (C) A representative example of the analysis of an individual born before the nuclear tests,

indicating no measurable cortical neurogenesis. The ^{14}C level in the non-neuronal cells demonstrates that there is turnover within this population, but there are several possible interpretations of these data and the age of this population cannot be concluded from this material alone. The coloring of symbols in panels B and C corresponds to the regions in A. Vertical bars in B and C indicate the birth date of the individual.

Figure 3 BrdU incorporation in the adult human cerebral cortex

(A) Distribution of BrdU labeled cells in the adult human motor cortex. A subset of BrdU labeled cells are immunoreactive to the astrocyte marker GFAP (B). None of the BrdU labeled cells are immunoreactive to the neuronal markers NeuN (C) or neurofilament (D). The scale bars are 70 μm in B and 100 μm in C and D.

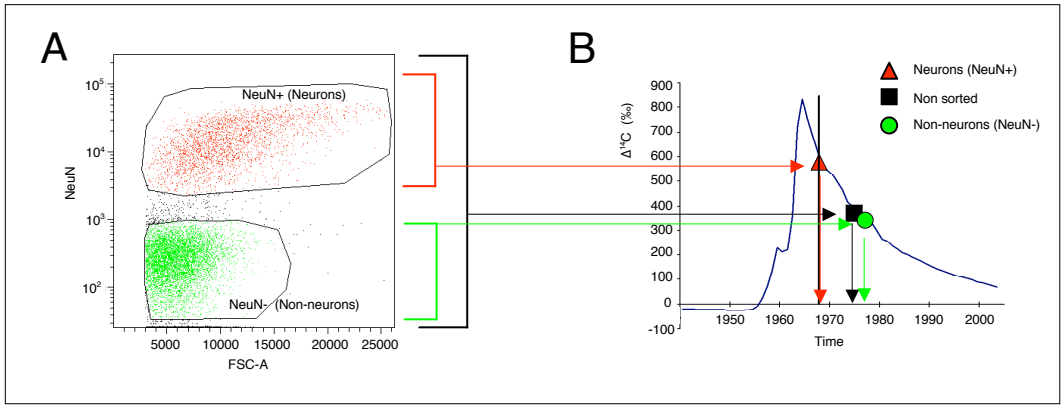
A
B
NeuN+ (Neurons)
NeuN- (Non-neurons)
NeuN
FSC-A
Neurons (NeuN+)
Non sorted
Non-neurons (NeuN-)
Time

Figure 1

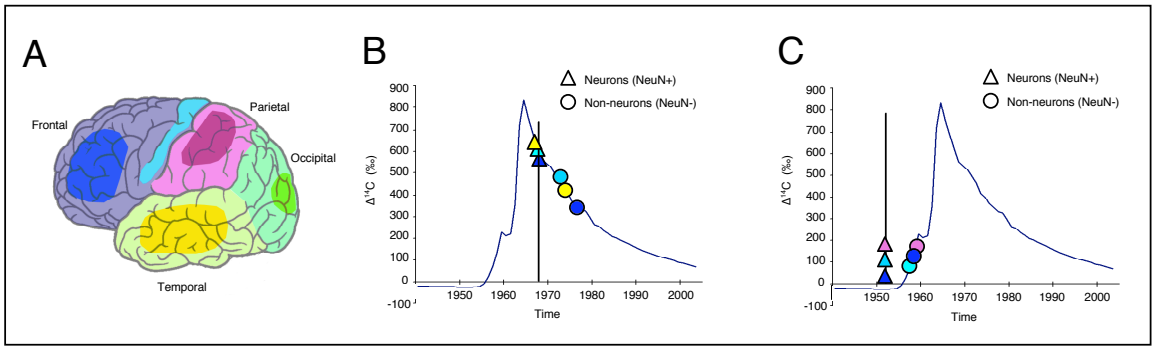
A
Frontal
Parietal
Occipital
Temporal
B
Neurons (NeuN+)
Non-neurons (NeuN-)
Δ14C (‰)
900
800
700
600
500
400
300
200
100
0
-100
1950
1960
1970
1980
1990
2000
Time
C
Neurons (NeuN+)
Non-neurons (NeuN-)
Δ14C (‰)
Time

Figure 2

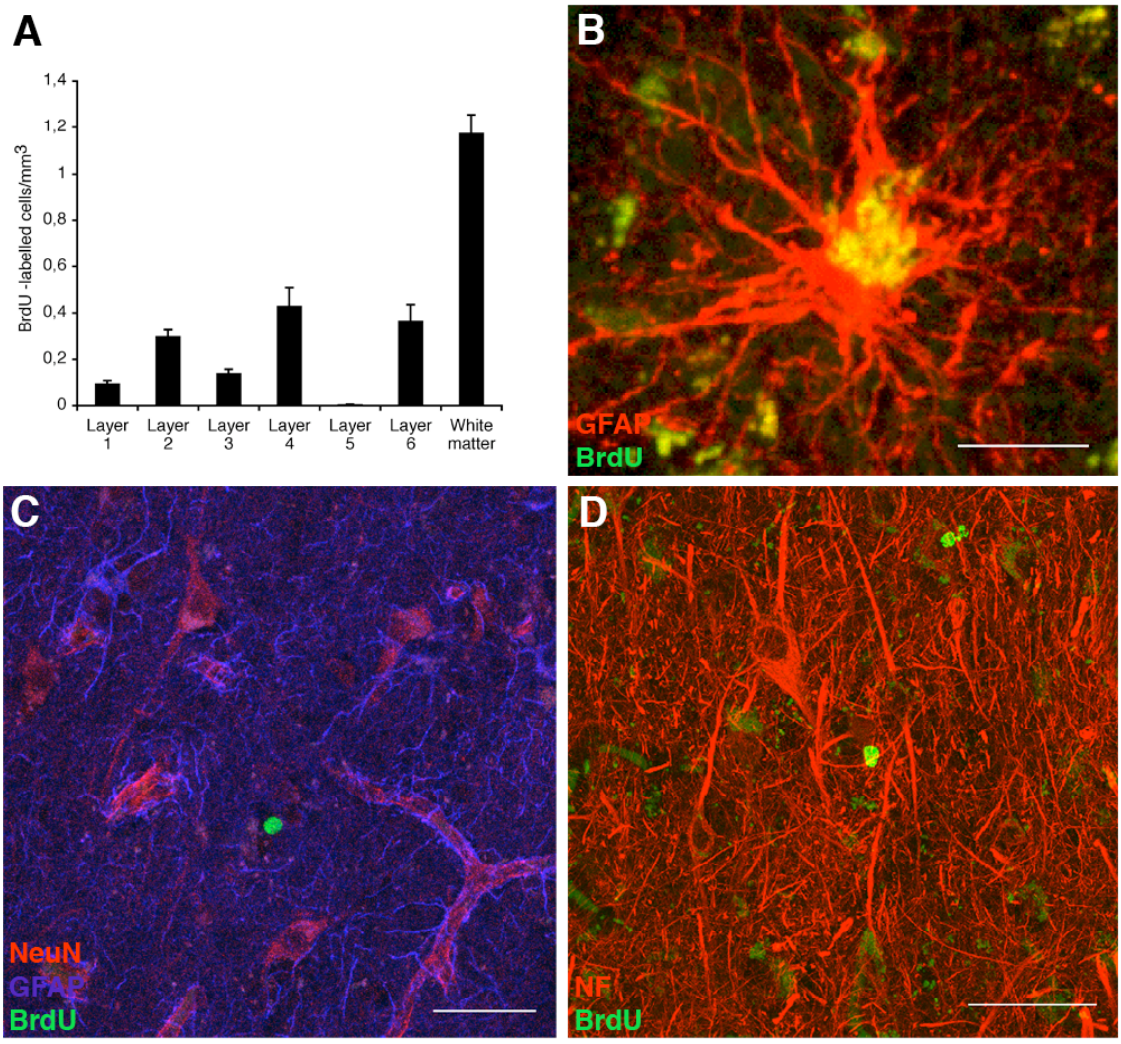
A
BrdU -labelled cells/mm3
1,4
1,2
1
0,8
0,6
0,4
0,2
0
Layer 1
Layer 2
Layer 3
Layer 4
Layer 5
Layer 6
White matter
B
GFAP
BrdU
C
NeuN
GFAP
BrdU
D
NF
BrdU

Figure 3

Supplementary information

The direct data obtained from AMS came in the form of percent modern carbon, or pMC values (as in column 5 from Table1). All data were determined using an isotopic fractionation correction of –23 for DNA in human brain tissue.

For ease of presentation in graphical form, we derived the corresponding $\Delta^{14}C$ value from the experimental pMC data. The term $F^{14}C$ refers to fraction of modern carbon (defined in equation 2 from PJ Reimer, Radiocarbon, 46(3),1299-1304, 2004), and is equivalent to pMC/100.

$\Delta^{14}C$ was calculated using the following formula:

$\Delta^{14}C = 1000 * \{F^{14}C * \exp[\lambda*(1950 - y)] - 1\}$ where $\lambda = 1/8267\ yr^{-1}$

To accurately represent these $\Delta^{14}C$ data on the Levin data set graph, this value was decay corrected. This was done to compensate for the small amount of radioactive decay of ^{14}C that will occur during the time of formation to measurement. This was done according to the following equation (from Paula Reimer, personal communication):

Age corrected $\Delta^{14}C = [e^{(2005\text{-date of creation})/\ 8267} * (\text{AMS } \Delta^{14}C \text{ value } +1000)] - 1000$

The year of formation data was determined based on using the AMS values, and applying them to the software provided on the freely accessible Calibomb website (http://calib.qub.ac.uk/CALIBomb/frameset.html). All values are given in the table below.

A further important consideration regarding data analysis, is based on the purity obtained from the flow cytometry stage. Given the fact, that the purity per sample is not always 100%, this step aids in increasing interpretation accuracy. In the case where a NeuN + and NeuN – sample are both available:

Solve for x (delta ^{14}C NeuN+ population) and y (delta ^{14}C NeuN- population):

(1) AMS value for delta ^{14}C NeuN+ = x (% FACS purity for NeuN+)/100 + y(1 – % FACS purity for NeuN+)/100

(2) AMS value for delta ^{14}C NeuN- = y (% FACS purity for NeuN-)/100 + x(1 – % FACS purity for NeuN-)/100

In the case where one has the non sorted and either the NeuN + or NeuN- values, then the third point can be determined by taking into account the percentage of the NeuN+ population within that given sort. The NeuN- population will be the remaining percentage (ie. 28% NeuN + within a given sort implies 72% NeuN- purity). Since, the non sorted delta ^{14}C value is known, the missing data point can be calculated.

Cortical Area	SAMPLE ID#	Patient #	Population	Percent Modern Carbon		Delta 14C		FACS Purity Correction	Yr of Birth	Yr 14C
				pMC	Error	Delta 14C (delta13C =-23)	ERROR	Delta 14C		corresponds to
PREFRONTAL										
	121400	18	Non Sort	108,76	0,50	80,25	5,020	80,25	1953,4	1956,0
	121401	18	NeuN Pos	98,18	0,52	-24,79	5,225	-29,30	1953,4	1953,4
		18	NeuN Neg					129,68	1953,4	1957,0
		17	Non Sort					33,38	1952,0	1956,0
	OZI344	17	NeuN Pos	98,07	0,73	-25,8	7,279	-26,90	1952,0	1952,0
	OZI345	17	NeuN Neg	104,47	0,85	37,75	8,473	37,75	1952,0	1956,0
	OZI397	14	Non Sort	139,32	0,84	383,9	8,311	383,90	1968,3	1974,7
	OZI398	14	NeuN Pos	155,84	1,14	548,1	11,277	553,20	1968,3	1968,3
	OZI399	14	NeuN Neg	138,06	1,12	371,4	11,167	365,10	1968,3	1975,5
	OZI373	9	Non Sort	135,64	0,86	347,4	8,574	347,4	1973,3	1976,8
	OZI374	9	NeuN Pos	143,37	1,01	424,2	9,999	427,70	1973,3	1973,1
	OZI375	9	NeuN Neg	137,44	1,00	365,3	9,962	362,43	1973,3	1975,5
FRONTAL										
	OZI340	17	Non Sort	107,28	0,54	65,7	5,4	65,7	1952,0	1956,0
	OZI341	17	NeuN Pos	96,40	0,49	-42,4	4,9	-43,59	1952,0	1952,0
	OZI342	17	NeuN Neg	105,32	0,57	46,2	5,7	48,03	1952,0	1956,0
		22	Non Sort					26,32	1946,5	1955,0
	121398	22	NeuN Pos	97,94	0,51	-27,3	5,1	-29,63	1946,5	1946,5
	121399	22	NeuN Neg	105,45	0,79	47,3	7,9	48,08	1946,5	1957,0
	120950	14	Non Sort	142,33	0,97	413,9	9,7	413,9	1968,3	1973,6
	120951	14	NeuN Pos	157,31	1,16	562,6	11,6	566,6	1968,3	1968,3
	120952	14	NeuN Neg	151,19	3,47	501,8	34,7	496,5	1968,3	1971,3
	OZI361	27	Non Sort	104,20	0,63	35,1	6,2	35,1	1933,5	1956,0
	OZI362	27	NeuN Pos	97,71	0,67	-29,4	6,6	-33,2	1933,5	1933,5
	OZI363	27	NeuN Neg	103,64	0,69	29,5	6,9	29,5	1933,5	1955,0
PARIETAL										
	OZI388	17	Non Sort	108,03	0,62	73,1	6,2	73,1	1952,0	1956,0
	OZI389	17	NeuN Pos	96,44	0,98	-42,0	9,7	-44,39	1952,0	1952,0
		17	NeuN Neg					115,2	1952,0	1957,0
	OZI383	27	Non Sort	102,31	0,73	16,4	7,3	16,40	1933,5	1955,0
	OZI384	27	NeuN Pos	97,42	0,67	-32,2	6,7	-38,09	1933,5	1933,5
	OZI385	27	NeuN Neg	107,30	0,91	65,9	9,1	77,45	1933,5	1956,0
	OZI377	22	Non Sort	108,22	0,72	75,0	7,2	75,00	1946,5	1956,0
	OZI378	22	NeuN Pos	98,24	0,64	-24,1	6,3	-33,87	1946,5	1946,5
		22	NeuN Neg					102,95	1946,5	1956,0
	OZI332	23	Non Sort	100,19	0,48	-4,7	4,8	-4,70	1945,8	1954,0
	OZI333	23	NeuN Pos	96,69	0,61	-39,5	6,1	-39,50	1945,8	1945,8
		23	NeuN Neg					5,12	1945,8	1955,0
TEMPORAL										
	120956	14	Non Sort	139,78	0,75	388,5	7,5	388,50	1968,3	1975,5
	120957	14	NeuN Pos	164,70	1,95	636,1	19,5	644,66	1968,3	1967,2
	120958	14	NeuN Neg	147,46	2,73	464,8	27,3	417,09	1968,3	1973,6
	121408	22	Non Sort	105,80	0,43	50,8	4,3	50,80	1946,5	1956,0
	121409	22	NeuN Pos	99,23	0,46	-14,4	4,6	-21,80	1946,5	1946,5
	121410	22	NeuN Neg	106,08	0,49	53,6	4,9	53,70	1946,5	1956,0
	OZI380	27	Non Sort	104,65	0,78	39,5	7,8	39,50	1933,5	1956,0
	OZI381	27	NeuN Pos	99,26	0,86	-14,0	8,6	-17,40	1933,5	1933,5
		27	NeuN Neg					61,62	1933,5	1956,0
	OZI364	9	Non Sort	125,91	0,82	250,8	8,2	250,80	1973,3	1981,3
	OZI365	9	NeuN Pos	141,16	0,99	402,2	9,8	420	1973,3	1973,2
	OZI366	9	NeuN Neg	126,11	1,36	252,7	13,5	242	1973,3	1982,6
	OZI349	23	Non Sort	102,76	0,37	20,8	3,7	20,8	1945,8	1955,0
	OZI350	23	NeuN Pos	95,74	0,42	-49,0	4,2	-54,13	1945,8	1945,8
		23	NeuN Neg					48,5	1945,8	1956,0
	OZI370	18	Non Sort	109,47	0,64	87,5	6,4	87,5	1953,4	1956,0
	OZI371	18	NeuN Pos	101,45	0,70	7,8	7,0	-1,29	1953,4	1953,4
	OZI372	18	NeuN Neg	115,02	0,85	142,5	8,4	147,72	1953,4	1957,0