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Report

The Age of Olfactory Bulb Neurons in Humans

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SUMMARY

Continuous turnover of neurons in the olfactory bulb is implicated in several key aspects of olfaction. There is a dramatic decline postnatally in the number of migratory neuroblasts en route to the olfactory bulb in humans, and it has been unclear to what extent the small number of neuroblasts at later stages contributes new neurons to the olfactory bulb. We have assessed the age of olfactory bulb neurons in humans by measuring the levels of nuclear bomb test-derived ^{14}C in genomic DNA. We report that ^{14}C concentrations correspond to the atmospheric levels at the time of birth of the individuals, establishing that there is very limited, if any, postnatal neurogenesis in the human olfactory bulb. This identifies a fundamental difference in the plasticity of the human brain compared to other mammals.

INTRODUCTION

Neural stem cells residing in the walls of the lateral ventricles of the brain give rise to neuroblasts that migrate to the olfactory bulb throughout life (Lois et al., 1996; Ming and Song, 2011). The new neurons integrate into the synaptic circuitry and are implicated in complex processes such as olfactory memory formation, odorant discrimination, and social interactions (Carlén et al., 2002; Lazarini and Lledo, 2011). Olfactory bulb neurogenesis is well characterized in rodents and has been shown to persist in adult monkeys (Kornack and Rakic, 2001), but the extent and potential role of postnatal olfactory bulb neurogenesis in humans is unclear. Anosmia is a common and early symptom in neurodegenerative diseases such as Alzheimer's and Parkinson's disease, and it has been suggested that this may be due to reduced adult olfactory bulb neurogenesis (Höglinger et al., 2004; Winner et al., 2011).

There are neural stem cells lining the lateral ventricles in the adult human brain (Johansson et al., 1999; Sanai et al., 2004), but it was controversial to what extent they give rise to neuroblasts that migrate to the olfactory bulb (Curtis et al., 2007; Sanai et al., 2004). Recently, two studies demonstrated a dramatic decline in the number of cells with a marker profile and morphology of migratory neuroblasts after birth in humans (Sanai et al., 2011; Wang et al., 2011). However, both studies found neuroblasts also in adult subjects, albeit the cells did not form a distinct migratory stream but appeared as individual cells and at a very much lower frequency than in the perinatal period (Sanai et al., 2011; Wang et al., 2011).

It is difficult to infer the extent of neurogenesis from the number of neuroblasts, as it is not possible to know whether the neuroblasts differentiate to mature neurons and integrate stably in the circuitry. Even in a situation in which a very small number of neuroblasts are present at any given time, the neuroblasts could potentially give rise to a substantial proportion of olfactory bulb neurons if they would integrate efficiently and if this process would be continuous over a long time. Moreover, there are also neural stem cells present in the adult rodent and human olfactory bulb, and new neurons may not only derive from the ventricle wall but may be generated locally in the olfactory bulb (Gritti et al., 2002; Pagano et al., 2000).

Due to the important role of adult olfactory bulb neurogenesis in experimental animals and the suggested alteration of this process underlying common symptoms of neurodegenerative diseases, we set out to establish to what extent this process is operational in humans. We report that there is a continuous turnover of nonneuronal cells throughout life but that there is minimal, if any, addition of new neurons after the perinatal period in humans.

RESULTS AND DISCUSSION

Cell Turnover in the Human Olfactory Bulb

We have determined the age of olfactory bulb cells by measuring the concentration of nuclear bomb test-derived ^{14}C in genomic DNA (Spalding et al., 2005a). Atmospheric ^{14}C levels were stable until nuclear bomb tests conducted during the Cold War resulted in a dramatic increase (De Vries, 1958; Nydal and Lövseth, 1965). There have been no major above ground nuclear tests after the International Test Ban Treaty in 1963, and the atmospheric ^{14}C levels have since declined due to uptake by the biotope and diffusion from the atmosphere (Levin and Kromer, 2004; Levin et al., 2010). ^{14}C in the atmosphere reacts with oxygen to form

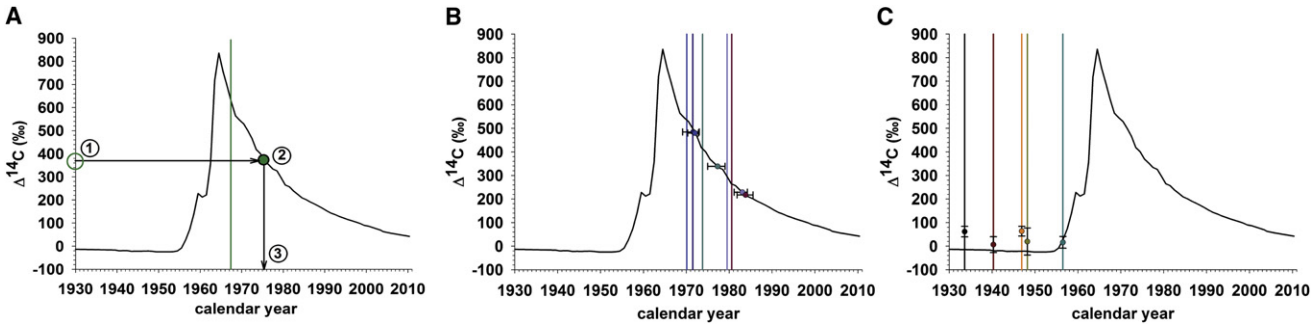

Figure 1. Postnatal Cell Turnover in the Human Olfactory Bulb

(A) Schematic illustration of the strategy to establish cell age by ^{14}C dating. The black curve shows the excess of the atmospheric ^{14}C concentrations over the natural level ($\Delta^{14}C = 0$) (data from Levin and Kromer, 2004; Levin et al., 2010), and the vertical line indicates the date of birth of the studied individual in all figures. The measured genomic ^{14}C concentration (1) is related to the atmospheric ^{14}C concentration at or after the person was born (2). The birth date of a cell population is then read off the x axis (3). (B and C) The ^{14}C concentration in genomic DNA from human olfactory bulb cells in subjects born after (B) or before (C) the nuclear bomb tests corresponds to time points after the time of birth, demonstrating postnatal cell turnover. A vertical line and a dot of the same color indicate the date of birth and ^{14}C data, respectively, for each individual. Error bars for subjects born after the nuclear bomb tests are given in years by calibrating to atmospheric ^{14}C concentrations (see Supplemental Experimental Procedures). Analysis of individuals born before the increase in the atmospheric ^{14}C concentration provides high sensitivity to detect whether any cell renewal occurs later in life, but it is not possible to directly infer the age of the cell population, and therefore the error bars indicate the measurement error in ^{14}C concentration in the respective DNA sample.

$^{14}CO_2$ and enters the food chain through plant photosynthesis. By eating plants and animals that live off plants, the ^{14}C concentration in the human body closely parallels that in the atmosphere at any given time (Harkness, 1972; Libby et al., 1964; Spalding et al., 2005b). When cells undergo mitosis and duplicate their DNA, they integrate ^{14}C with a concentration corresponding to that in the atmosphere, resulting in a stable date mark. By measuring ^{14}C in genomic DNA and determining when the corresponding ^{14}C concentration was present in the atmosphere, it is possible to establish the birth date of cells (Figure 1A) and their turnover dynamics (Bergmann et al., 2009; Bhardwaj et al., 2006; Spalding et al., 2005a, 2008). Changes in DNA methylation can alter the ^{14}C content of DNA, but not to a degree that can influence the analysis of cell turnover (Spalding et al., 2005a). ^{14}C abundance can be measured by accelerator mass spectrometry, and we developed a protocol to enable analysis with increased sensitivity (see Supplemental Experimental Procedures available online).

Analysis of the ^{14}C concentration in postmortem olfactory bulb genomic DNA from adult humans revealed levels corresponding to time points after the birth of the individual, establishing that there is significant postnatal cell turnover in the human olfactory bulb ($p < 0.02$; Figures 1B and 1C; Table S1 and Supplemental Information). The oldest studied individual, born more than 20 years before the onset of the increase in atmospheric ^{14}C levels, had a ^{14}C concentration significantly higher than that present in the period up to 1955, establishing that there is substantial cell turnover at least up to early adulthood in humans (Figure 1C). However, several of the individuals born before 1950 had genomic ^{14}C concentrations lower than at any time after the onset of the nuclear bomb tests, indicating that there must be very long-lived cells in the olfactory bulb that have remained for more than 50 years. The human olfactory bulb contains approximately equal numbers of neurons and nonneuronal cells, and it is not possible to conclude from this data whether all cell types are exchanged or if cell turnover is restricted to one of these populations.

Isolation of Neuronal and Nonneuronal Nuclei

In order to specifically establish the age and turnover of neurons and nonneuronal cells, respectively, we isolated neuronal nuclei labeled with an antibody to NeuN (Fox3) by flow cytometry (Figures 2A and 2B) (Bhardwaj et al., 2006; Spalding et al., 2005a). NeuN has been extensively validated as a marker for most neuronal subsets, but mitral cells and some glomerular layer neurons in the olfactory bulb are not immunoreactive to NeuN in rodents (Mullen et al., 1992). Histological analysis revealed that there is a small subset of neurons also in the human olfactory bulb that are NeuN− (Figure 2C). We therefore wanted to develop an additional strategy to isolate neuronal nuclei from the human olfactory bulb, which would not exclude any neuronal subtype. We used antibodies to the RNA binding protein HuD, which is specific to postmitotic neurons (Barami et al., 1995), to isolate nuclei from the adult human olfactory bulb (Figures 2D and 2E). Histological analysis confirmed that HuD antibodies label all cells with neuronal characteristics in the adult human olfactory bulb (Figure 2F and Figure S1). However, we found that HuD antibodies, in addition to neurons, also labeled a subset of nonneuronal cells (Figure 2F). Histology and flow cytometry revealed that the nonneuronal population labeled with HuD antibodies had oligodendrocyte morphology and coexpressed the oligodendrocyte lineage markers Sox10 and CNPase (Figures 2E and 2F and Figures S2 and S3). Thus, by isolating cell nuclei that were HuD+ and Sox10−, we were able to specifically isolate neuronal nuclei (Figure 2E). All NeuN+ nuclei were within the HuD+/Sox10− population and 93.5% ± 3.6% (mean ± SD) of HuD+/Sox10− nuclei were NeuN+, in line with only a small subpopulation of neurons being NeuN− in the adult human olfactory bulb. We used both these isolation strategies to birth date neurons and nonneuronal cells.

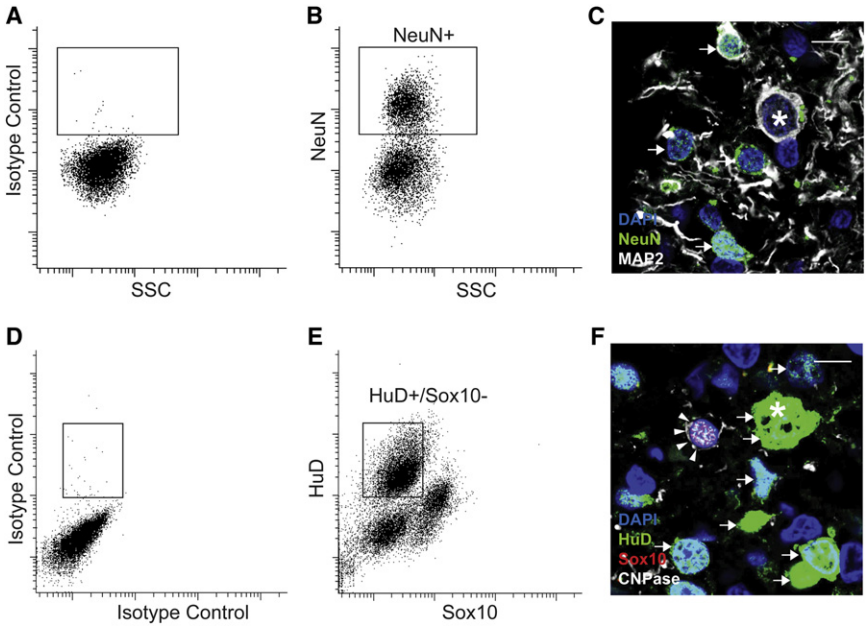

Figure 2. Identification and Isolation of Neuronal Nuclei in the Human Olfactory Bulb

(A and B) Nuclei were labeled with isotype control antibody (A) or with antibodies to NeuN/Fox3 (B). (C) Antibodies to NeuN label most neurons (arrows), but not large neurons in the mitral/granular layer. (D and E) Nuclei were labeled with isotype control antibody (D) and neuronal nuclei were identified by the presence of HuD and the absence of Sox10 (E). (F) HuD antibodies label mitral cells (asterisk), other neurons (arrows), and a subset of oligodendrocyte lineage cells identified by Sox10 and CNPase expression (arrowheads). Arrows indicate NeuN+ (C) or HuD+/Sox10− (F) neuronal nuclei. The sorting gates for neuronal nuclei are indicated in (B) and (E). SSC, side scatter. Scale bars indicate 10 μm.

New Nonneuronal Cells in the Adult Human Olfactory Bulb

Analysis of the ^{14}C concentration in genomic DNA from isolated nonneuronal nuclei from the adult human olfactory bulb revealed levels corresponding to concentrations well after the birth of the individual in all cases, establishing substantial turnover of nonneuronal cells ($p = 0.0002$; Figure 3; see Supplemental Information). Integrating data from several individuals born at different times in relation to the nuclear bomb tests allows estimating the turnover dynamics of a cell population (Bergmann et al., 2009; Spalding et al., 2008). This indicated an annual turnover rate of 2.0%–3.4% in the nonneuronal cell population (see Supplemental Information). This represents an average for all cells negative for the respective neuronal marker profile, and it is likely that the turnover dynamics vary between specific nonneuronal cell types.

Olfactory Bulb Neurons Are as Old as the Person

We next assessed the ^{14}C concentration in genomic DNA from NeuN+ or HuD+/Sox10− neuronal nuclei. In all cases ($n = 15$), the ^{14}C concentration in neuronal genomic DNA was very close to that present in the atmosphere at the time of birth of each individual (Figure 4) and not significantly different from what one would see if there was no postnatal generation of olfactory bulb neurons ($p = 0.91$; see Supplemental Information). We cannot exclude that there may be low-grade turnover of neurons, but at a constant rate, the annual turnover would be 0.008% ± 0.08% (mean ± SE; see Supplemental Information). That corresponds to <1% of neurons being exchanged after 100 years. It has been estimated that up to 50% of olfactory bulb neurons are exchanged annually in rodents (Imayoshi et al., 2008), and if there is any postnatal olfactory bulb neurogenesis in humans, its extent is orders of magnitude lower.

Neurodegenerative and psychiatric diseases and substance abuse have been suggested to reduce olfactory bulb neurogenesis (Hansson et al., 2010; Höglinger et al., 2004; Negoias et al., 2010; Turetsky et al., 2000; Winner et al., 2011). Some individuals in our study were diagnosed with one or more of these conditions (Table S2). However, as all studied individuals had neuronal ^{14}C concentrations corresponding to the time around birth, we did not find any apparent correlation between these conditions and postnatal olfactory bulb neurogenesis in humans. Anosmia is a common and early symptom in several neurodegenerative diseases, and it has been suggested to be related to reduced adult olfactory bulb neurogenesis (Höglinger et al., 2004; Winner et al., 2011), but this appears unlikely.

Functional studies in rodents have implicated adult neurogenesis in olfactory memory formation, odorant discrimination, and social interactions (Lazarini and Lledo, 2011). The lack of comparable adult olfactory bulb neurogenesis in humans poses the question whether these functions are mediated by conceptually different mechanisms in humans, or whether the more limited dependence on olfaction in humans compared to rodents in part may be due to the lack of one type of plasticity, adult neurogenesis.

EXPERIMENTAL PROCEDURES

Tissue Collection

Tissues were procured from cases admitted during 2005 and 2011 to the Department of Forensic Medicine in Stockholm for autopsy, after informed consent from relatives. Ethical permission for this study was granted by the Regional Ethical Committee in Stockholm. Whole olfactory bulbs from both hemispheres were analyzed. Cerebellar cortex samples from the same subjects served as controls. Brain tissue was frozen and stored at −80°C until further analysis.

Nuclei Isolation

Tissue samples were thawed and Dounce homogenized in 10 ml lysis buffer (0.32 M sucrose, 5 mM $CaCl_2$, 3 mM magnesium acetate, 0.1 mM EDTA, 10 mM Tris-HCl [pH 8.0], 0.1% Triton X-100, and 1 mM DTT). Homogenized samples were suspended in 20 ml of sucrose solution (1.7 M sucrose, 3 mM magnesium acetate, 1 mM DTT, and 10 mM Tris-HCl [pH 8.0]), layered onto a cushion of 10 ml sucrose solution, and centrifuged at 36,500 × *g* for 2.4 hr

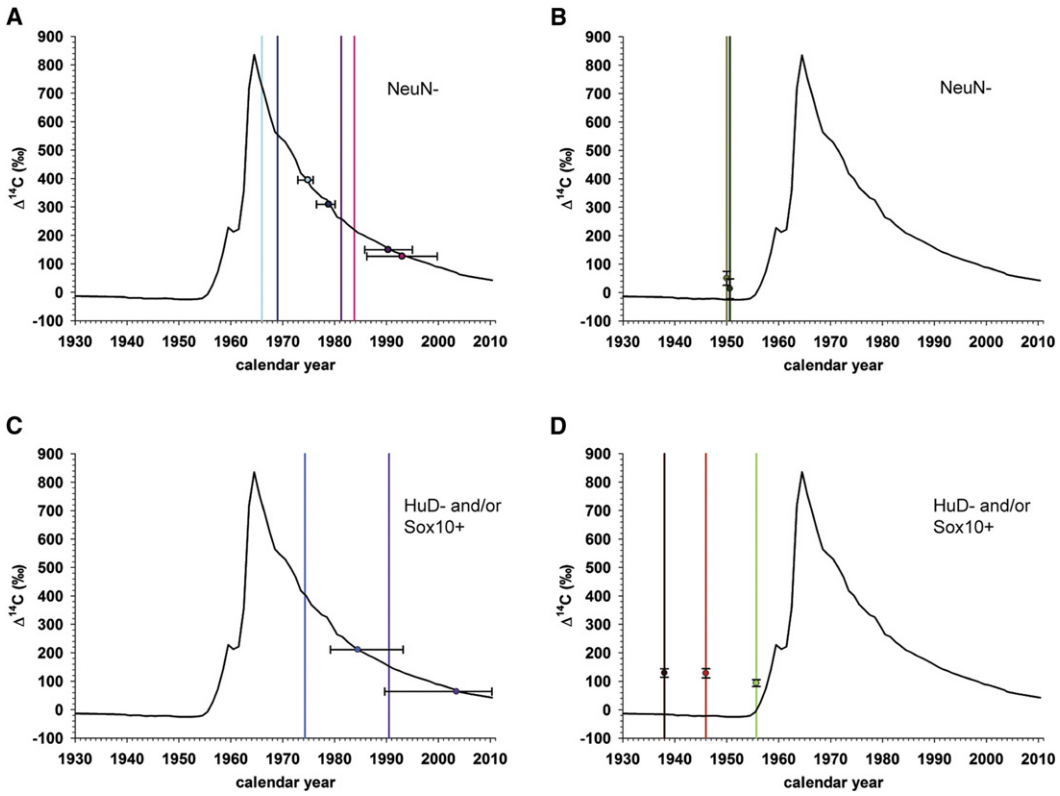

Figure 3. Turnover of Nonneuronal Cells

The ^{14}C concentration in genomic DNA from nonneuronal cells, defined by the absence of NeuN labeling (A and B) or not being HuD+/Sox10− (C and D), corresponds to time points well after the birth of each individual. The vertical bar indicates the year of birth of the individual, with the correspondingly colored data point indicating the ^{14}C concentration. Error bars indicate two standard deviations in ^{14}C concentration in the respective DNA sample.

at 4°C. The isolated nuclei were resuspended in nuclei storage buffer (NSB) (10 mM Tris [pH 7.2], 2 mM $MgCl_2$, 70 mM KCl, and 15% sucrose) for consecutive immunostaining and flow cytometry analysis.

FACS Sorting and Analysis

Isolated nuclei were stained with mouse NeuN (A-60) (Millipore, 1:1,000), rabbit Fox3 (Atlas Antibody, 1:300), mouse HuD (E-1) (Santa Cruz, 1:100), mouse HuD/HuC 16A11-biotin (Invitrogen, 1:300), or goat Sox10 (R&D, 1:300). NeuN (A-60) antibody was directly conjugated to Alexa 647 (Invitrogen Antibody Labeling Kit Alexa 647). All other primary antibodies were visualized with appropriate secondary antibodies conjugated to Alexa 488 (1:500), Alexa 647 (1:500) (Invitrogen), or R-phycoerythrin (PE) (Santa Cruz, 1:100). Flow cytometry sorting was performed with a BD FACS Diva and flow cytometry analysis was performed with a BD FACS Aria instrument.

Immunohistochemistry

Olfactory bulbs were fixed in 4% formaldehyde buffered in PBS for 24 hr and embedded in low-melting paraffin (52°C–54°C), according to standard procedures. Olfactory bulbs were sectioned (5 μm) longitudinally and orthogonally according to their long axis. For immunohistochemistry, sections were deparaffinized in xylene and rehydrated in a descending ethanol series. Antigen retrieval was performed in citraconic acid solution (pH = 7.4; 0.05% citraconic acid) for 20 min in a domestic steamer (Namimatsu et al., 2005). The sections were allowed to cool down for 20 min before immunostaining was started. Sections were incubated with the respective primary antibody overnight at 4°C: mouse NeuN (Millipore A-60 clone; 1:100), rabbit Fox3 (Atlas Antibody, 1:300), goat Sox10 (R&D, 1:100), rabbit calbindin (Abcam, 1:200), chicken MAP-2 (Abcam, 1:1,000), rabbit calretinin (Abcam, 1:200), rabbit parvalbumin (Abcam, 1:1,000), rabbit tyrosine hydroxylase (TH) (Millipore, 1:1,000), rabbit GAD65/67 (Millipore, 1:500), rabbit CNPase (Atlas Antibody, 1:400), mouse GFAP (Sigma Aldrich, 1:1,000), rabbit Iba1 (Wako, 1:1,000), mouse HuD (E-1) (Santa Cruz, 1:100), and mouse HuD/HuC 16A11-biotin (Invitrogen, 1:100), and visualized with the matching secondary antibody and streptavidin conjugated to Alexa 488, 546, or 647 (1:1,000, Invitrogen).

DNA Purification

All experiments were carried out in a clean room (ISO8) to prevent any carbon contamination of the samples. All glassware was prebaked at 450°C for 4 hr. DNA isolation was performed according to a modified protocol from Miller et al. (1988). Five hundred microliters of DNA lysis buffer (100 mM Tris [pH 8.0], 200 mM NaCl, 1% SDS, and 5 mM EDTA) and 6 μl Proteinase K (20 mg/ml) were added to the collected nuclei and incubated overnight at 65°C. RNase cocktail (Ambion) was added and incubated at 65°C for 1 hr. Half of the existing volume of 5 M NaCl solution was added and agitated for 15 s. The solution was spun down at 13,000 rpm for 3 min. The supernatant containing the DNA was transferred to a 12 ml glass vial. Three times the volume of absolute ethanol was added, and the glass vial was inverted several times to precipitate the DNA. The DNA precipitate was washed three times in DNA washing solution (70% Ethanol [v/v] and 0.5 M NaCl) and transferred to 500 μl DNase/RNAase free water (GIBCO/Invitrogen). The DNA was quantified and DNA purity verified by UV spectroscopy (NanoDrop).

Accelerator Mass Spectrometry

^{14}C accelerator mass spectrometry (AMS) measurements were performed on graphitized samples. DNA in aqueous solution was freeze dried, combusted to CO_2, and reduced to graphite according to the procedures described in

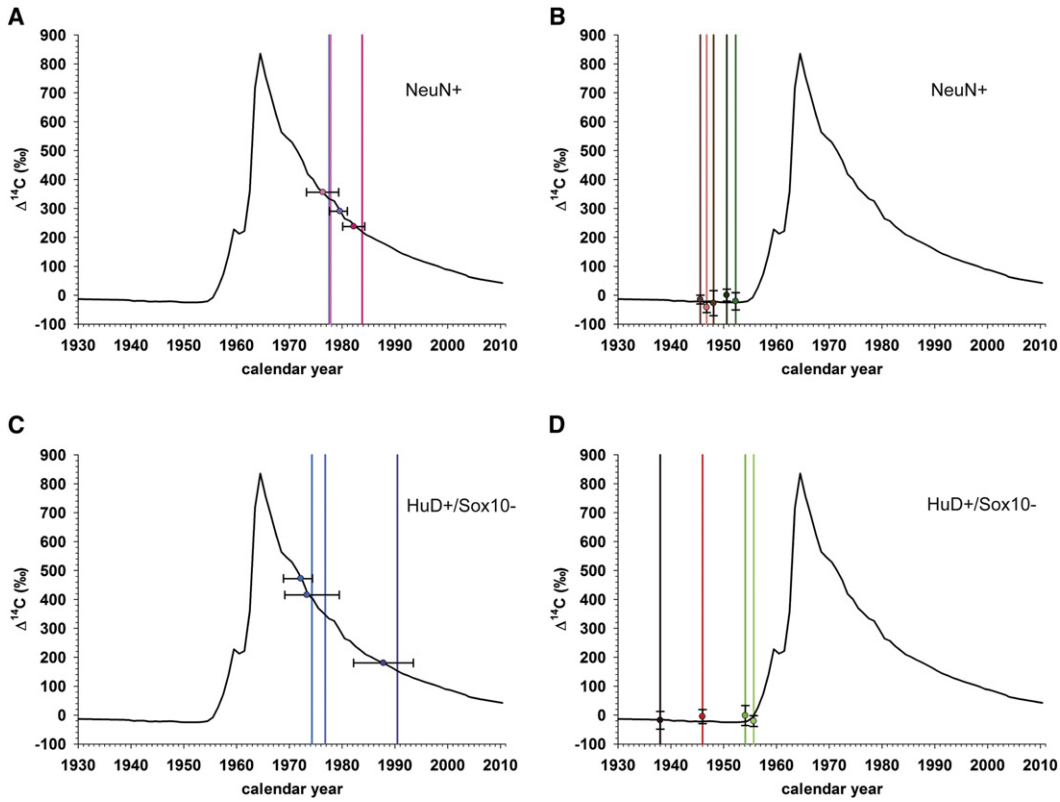

Figure 4. Limited Neurogenesis in the Adult Human Olfactory Bulb

^{14}C concentrations in genomic DNA from neuronal nuclei isolated with antibodies against NeuN (A and B) or by the marker combination HuD+/Sox10− (C and D) were not significantly different from atmospheric ^{14}C concentrations at birth. The vertical bars indicate the year of birth of each individual, with the correspondingly colored data point indicating the ^{14}C concentration. Error bars indicate two standard deviations in ^{14}C concentration in the respective DNA sample.

Liebl et al. (2010). ^{14}C AMS measurements of graphitized samples were carried out at the Vienna Environmental Research Accelerator (VERA) of the University of Vienna, a 3 MV Pelletron tandem AMS system (Priller et al., 1997; Rom et al., 1998; Steier et al., 2004). The setup of VERA for heavy isotopes was described earlier (Vockenhuber et al., 2003). ^{14}C measurement results are reported as $F^{14}C$ according to the recommendation of Reimer et al. (2004). Age calibration of ^{14}C concentrations was performed using the software CALIbomb (http://calib.qub.ac.uk/CALIBomb) with the following parameters: smoothing in years, 1 year; resolution, 0.2; ^{14}C calibration, two sigma.

For details related to accelerator mass spectrometry measurements and correction for FACS impurities, see Supplemental Experimental Procedures and Figure S4.

SUPPLEMENTAL INFORMATION

Supplemental Information includes six figures, Supplemental Experimental Procedures, and four tables and can be found with this article online at doi:10.1016/j.neuron.2012.03.030.

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REFERENCES

Barami, K., Iversen, K., Furneaux, H., and Goldman, S.A. (1995). Hu protein as an early marker of neuronal phenotypic differentiation by subependymal zone cells of the adult songbird forebrain. J. Neurobiol. *28*, 82–101.

Bergmann, O., Bhardwaj, R.D., Bernard, S., Zdunek, S., Barnabé-Heider, F., Walsh, S., Zupicich, J., Alkass, K., Buchholz, B.A., Druid, H., et al. (2009). Evidence for cardiomyocyte renewal in humans. Science *324*, 98–102.

Bhardwaj, R.D., Curtis, M.A., Spalding, K.L., Buchholz, B.A., Fink, D., Björk-Eriksson, T., Nordborg, C., Gage, F.H., Druid, H., Eriksson, P.S., and Frisén, J. (2006). Neocortical neurogenesis in humans is restricted to development. Proc. Natl. Acad. Sci. USA *103*, 12564–12568.

Carlén, M., Cassidy, R.M., Brismar, H., Smith, G.A., Enquist, L.W., and Frisén, J. (2002). Functional integration of adult-born neurons. Curr. Biol. *12*, 606–608.

Curtis, M.A., Kam, M., Nannmark, U., Anderson, M.F., Axell, M.Z., Wikkelso, C., Holtås, S., van Roon-Mom, W.M., Björk-Eriksson, T., Nordborg, C., et al. (2007). Human neuroblasts migrate to the olfactory bulb via a lateral ventricular extension. Science *315*, 1243–1249.

De Vries, H. (1958). Atomic bomb effect: variation of radiocarbon in plants, shells, and snails in the past 4 years. Science *128*, 250–251.

Gritti, A., Bonfanti, L., Doetsch, F., Caille, I., Alvarez-Buylla, A., Lim, D.A., Galli, R., Verdugo, J.M., Herrera, D.G., and Vescovi, A.L. (2002). Multipotent neural stem cells reside into the rostral extension and olfactory bulb of adult rodents. J. Neurosci. *22*, 437–445.

Hansson, A.C., Nixon, K., Rimondini, R., Damadzic, R., Sommer, W.H., Eskay, R., Crews, F.T., and Heilig, M. (2010). Long-term suppression of forebrain neurogenesis and loss of neuronal progenitor cells following prolonged alcohol dependence in rats. Int. J. Neuropsychopharmacol. *13*, 583–593.

Harkness, D.D. (1972). Further investigations of the transfer of bomb ^{14}C to man. Nature *240*, 302–303.

Höglinger, G.U., Rizk, P., Muriel, M.P., Duyckaerts, C., Oertel, W.H., Caille, I., and Hirsch, E.C. (2004). Dopamine depletion impairs precursor cell proliferation in Parkinson disease. Nat. Neurosci. *7*, 726–735.

Imayoshi, I., Sakamoto, M., Ohtsuka, T., Takao, K., Miyakawa, T., Yamaguchi, M., Mori, K., Ikeda, T., Itohara, S., and Kageyama, R. (2008). Roles of continuous neurogenesis in the structural and functional integrity of the adult forebrain. Nat. Neurosci. *11*, 1153–1161.

Johansson, C.B., Svensson, M., Wallstedt, L., Janson, A.M., and Frisén, J. (1999). Neural stem cells in the adult human brain. Exp. Cell Res. *253*, 733–736.

Kornack, D.R., and Rakic, P. (2001). The generation, migration, and differentiation of olfactory neurons in the adult primate brain. Proc. Natl. Acad. Sci. USA *98*, 4752–4757.

Lazarini, F., and Lledo, P.M. (2011). Is adult neurogenesis essential for olfaction? Trends Neurosci. *34*, 20–30.

Levin, I., and Kromer, B. (2004). The tropospheric $^{14}CO_2$ level in mid latitudes of the northern hemisphere (1959-2003). Radiocarbon *46*, 1261–1272.

Levin, I., Naegler, T., Kromer, B., Diehl, M., Francey, R.J., Gomez-Pelaez, A.J., Steele, L.P., Wagenbach, D., Weller, R., and Worthy, D.E. (2010). Observations and modelling of the global distribution and long-term trend of atmospheric $^{14}CO_2$. Tellus *62*, 26–46.

Libby, W.F., Berger, R., Mead, J.F., Alexander, G.V., and Ross, J.F. (1964). Replacement Rates for Human Tissue from Atmospheric Radiocarbon. Science *146*, 1170–1172.

Liebl, J., Avalos Ortiz, R., Golser, R., Handle, F., Kutschera, W., Steier, P., and Wild, E.M. (2010). Studies on the preparation of small ^{14}C samples with an RGA and ^{13}C-enriched material. Radiocarbon *52*, 1394–1404.

Lois, C., García-Verdugo, J.-M., and Alvarez-Buylla, A. (1996). Chain migration of neuronal precursors. Science *271*, 978–981.

Miller, S.A., Dykes, D.D., and Polesky, H.F. (1988). A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res. *16*, 1215.

Ming, G.L., and Song, H. (2011). Adult neurogenesis in the mammalian brain: significant answers and significant questions. Neuron *70*, 687–702.

Mullen, R.J., Buck, C.R., and Smith, A.M. (1992). NeuN, a neuronal specific nuclear protein in vertebrates. Development *116*, 201–211.

Namimatsu, S., Ghazizadeh, M., and Sugisaki, Y. (2005). Reversing the effects of formalin fixation with citraconic anhydride and heat: a universal antigen retrieval method. J. Histochem. Cytochem. *53*, 3–11.

Negoias, S., Croy, I., Gerber, J., Puschmann, S., Petrowski, K., Joraschky, P., and Hummel, T. (2010). Reduced olfactory bulb volume and olfactory sensitivity in patients with acute major depression. Neuroscience *169*, 415–421.

Nydal, R., and Lövseth, K. (1965). Distribution of radiocarbon from nuclear tests. Nature *206*, 1029–1031.

Pagano, S.F., Impagnatiello, F., Girelli, M., Cova, L., Grioni, E., Onofri, M., Cavallaro, M., Etteri, S., Vitello, F., Giombini, S., et al. (2000). Isolation and characterization of neural stem cells from the adult human olfactory bulb. Stem Cells *18*, 295–300.

Priller, A., Golser, R., Hille, P., Kutschera, W., Rom, W., Steier, P., Wallner, A., and Wild, E.M. (1997). First performance tests of VERA. Nucl. Instrum. Methods Phys. Res. B *123*, 193–198.

Reimer, P.J., Brown, T.A., and Reimer, R.W. (2004). Discussion: reporting and calibration of post-bomb ^{14}C data. Radiocarbon *46*, 1299–1304.

Rom, W., Golser, R., Kutschera, W., Priller, A., Steier, P., and Wild, E.M. (1998). Systematic investigations of ^{14}C measurements at the Vienna Environmental Research Accelerator. Radiocarbon *40*, 255–263.

Sanai, N., Tramontin, A.D., Quiñones-Hinojosa, A., Barbaro, N.M., Gupta, N., Kunwar, S., Lawton, M.T., McDermott, M.W., Parsa, A.T., Manuel-García Verdugo, J., et al. (2004). Unique astrocyte ribbon in adult human brain contains neural stem cells but lacks chain migration. Nature *427*, 740–744.

Sanai, N., Nguyen, T., Ihrie, R.A., Mirzadeh, Z., Tsai, H.H., Wong, M., Gupta, N., Berger, M.S., Huang, E., Garcia-Verdugo, J.M., et al. (2011). Corridors of migrating neurons in the human brain and their decline during infancy. Nature *478*, 382–386.

Spalding, K.L., Bhardwaj, R.D., Buchholz, B.A., Druid, H., and Frisén, J. (2005a). Retrospective birth dating of cells in humans. Cell *122*, 133–143.

Spalding, K.L., Buchholz, B.A., Bergman, L.-E., Druid, H., and Frisén, J. (2005b). Forensics: age written in teeth by nuclear tests. Nature *437*, 333–334.

Spalding, K.L., Arner, E., Westermark, P.O., Bernard, S., Buchholz, B.A., Bergmann, O., Blomqvist, L., Hoffstedt, J., Näslund, E., Britton, T., et al. (2008). Dynamics of fat cell turnover in humans. Nature *453*, 783–787.

Steier, P., Dellinger, F., Kutschera, W., Priller, A., Rom, W., and Wild, E.M. (2004). Pushing the precision limit of ^{14}C AMS. Radiocarbon *46*, 5–16.

Turetsky, B.I., Moberg, P.J., Yousem, D.M., Doty, R.L., Arnold, S.E., and Gur, R.E. (2000). Reduced olfactory bulb volume in patients with schizophrenia. Am. J. Psychiatry *157*, 828–830.

Vockenhuber, C., Ahmad, I., Golser, R., Kutschera, W., Liechenstein, V., Priller, A., Steier, P., and Winkler, S. (2003). Accelerator mass spectrometry of heavy long-lived radionuclides. Int. J. Mass Spectrom. *223-224*, 713–732.

Wang, C., Liu, F., Liu, Y.-Y., Zhao, C.-H., You, Y., Wang, L., Zhang, J., Wei, B., Ma, T., Zhang, Q., et al. (2011). Identification and characterization of neuroblasts in the subventricular zone and rostral migratory stream of the adult human brain. Cell Res. *21*, 1534–1550.

Winner, B., Kohl, Z., and Gage, F.H. (2011). Neurodegenerative disease and adult neurogenesis. Eur. J. Neurosci. *33*, 1139–1151.