Striatal neurogenesis in adult humans

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Neurons are added throughout life in the hippocampus and olfactory bulb in most mammals, although humans represent an exception without detectable olfactory bulb neurogenesis. Nevertheless, neuroblasts are generated in the lateral ventricle wall in humans, the neurogenic niche for olfactory bulb neurons in other mammals. We show that, in humans, new neurons integrate adjacent to this neurogenic niche, in the striatum. The neuronal turnover in the striatum appears restricted to interneurons and we show that postnatally generated striatal neurons are preferentially depleted in Huntington's disease. This demonstrates a unique pattern of neurogenesis in the adult human brain.

HIGHLIGHTS

• Nuclear-bomb-test-derived ^{14}C in human striatal neurons reveals adult neurogenesis

• Striatal neuronal turnover occurs within the interneuron fraction

• Annual turnover rate is 2.7% within the renewing fraction in adult humans

• Postnatally generated striatal neurons are depleted in Huntington's disease

INTRODUCTION

The generation of new neurons in the adult brain serves to maintain a pool of neurons with unique properties, present for a limited time after their birth, which enable specific types of neural processing (Ge et al., 2007; Schmidt-Hieber et al., 2004). Adult neurogenesis is important for pattern separation in memory formation and odor discrimination in rodents (Clelland et al., 2009; Nakashiba et al., 2012; Sahay et al., 2011), and alterations in adult neurogenesis are implicated in psychiatric disease in humans (Eisch and Petrik, 2012; Kheirbek et al., 2012).

Whether adult neurogenesis has decreased with evolution has long been a topic of debate (Kempermann, 2012; Rakic, 1985). Humans appear unique among mammals, in that there is no detectable adult olfactory bulb neurogenesis (Bergmann et al., 2012; Sanai et al., 2011). However, there is substantial hippocampal neurogenesis, with comparable neuronal turnover rates in middle-aged humans and mice (Eriksson et al., 1998b; Spalding et al., 2013). A larger fraction of hippocampal neurons are subject to exchange in humans than in mice and adult hippocampal neurogenesis shows a much less dramatic decline with aging in humans compared to mice (Spalding et al., 2013).

It may appear intuitive that hippocampal neurogenesis has been retained during human evolution, to provide cognitive adaptability, and that olfactory bulb

neurogenesis has decreased with the reduced dependence on olfaction in humans compared to our predecessors. However, neuronal precursor cells (neuroblasts) are generated not only in the hippocampus, but also in the lateral ventricle wall in adult humans, the site of origin of olfactory bulb neurons in other mammals. The extent and dynamics of neuroblast generation in humans in these two regions is remarkably similar, with a dramatic decline during the first postnatal months, followed by sustained generation, decreasing slowly with age (Göritz and Frisén, 2012; Knoth et al., 2010; Sanai et al., 2011). The difference between humans and other mammals is thus not the pattern of neuroblast generation, but that the neuroblasts generated in the lateral ventricle wall neurogenic niche do not migrate to the olfactory bulb. The fate of neuroblasts born in the human lateral ventricle wall has been unknown.

We here report that neuroblasts are not restricted to the lateral ventricle wall in humans, but that they are also present in the adjacent striatum. Retrospective birth dating revealed continuous generation of striatal interneurons in humans. In Huntington's disease, a neurodegenerative disease affecting striatal neurons (Walker, 2007; Zuccato et al., 2010), we find that postnatally generated neurons are absent in advanced stages of the disease. This identifies a unique pattern of adult neurogenesis in humans.

RESULTS

Neuroblasts in the adult human striatum

The finding that neurons are not added in the olfactory bulb of adult humans (Bergmann et al., 2012), in spite of the generation of neuronal precursors in the subventricular zone (Sanai et al., 2011; Wang et al., 2011a), posed the question whether neuroblasts may migrate to another location close to the ventricle. Analysis of transcriptome data available from a large number of developing and adult human brains (Kang et al., 2011), demonstrated that the expression of *doublecortin (DCX)*, a commonly used neuroblast marker, was at least as high in the adult human striatum as in the hippocampus (Figure 1A, Table S1). Only background levels were detected in the non-neurogenic adult cerebellum (Figure 1A, Table S1). When comparing *DCX* expression levels in the striatum with other brain regions close to the lateral ventricle, high *DCX* mRNA levels were specific to the striatum in the data from Kang et al. (2011) as well as in an additional human transcriptome dataset (Figure S1F). *DCX* transcript levels in the human hippocampus correlate closely with the number of neuroblasts at different ages (Kang et al., 2011) and the number of DCX-positive cells in the hippocampus in turn correlates with the number of newly generated neurons (Spalding et al., 2013).

Western blot analysis of the human postmortem hippocampus, striatum and cerebellum from subjects aged 21 to 68 years showed that DCX and polysialylated neural cell adhesion molecule (PSA-NCAM), another marker associated with neuroblasts, were as abundant in the striatum as in the hippocampus, and low to undetectable in the adult cerebellum (Figure 1B). This is in line with a previous study

demonstrating DCX and PSA-NCAM by Western blot in the adult human striatum, with similar levels in the caudate nucleus and putamen (Tong et al., 2011).

Immunohistochemistry revealed DCX-positive putative neuroblasts in the adult human dentate gyrus of the hippocampus and in the subventricular zone of the lateral wall of the lateral ventricle (Figure S1), in line with previous studies (Knoth et al., 2010; Sanai et al., 2011; Wang et al., 2011b). DCX-positive cells were also present in the caudate nucleus and the putamen of striatum (Figure 1C-F). The morphology of the DCX-positive cells in the striatum varied from rounded without processes, elongated with few processes to highly branched (Figure 1C-F), similar to the neuroblasts in the subventricular zone and hippocampus (Knoth et al., 2010; Sanai et al., 2011; Wang et al., 2011b). The DCX-positive cells in the striatum were most often found as single isolated cells, and there were no apparent streams of potentially migratory cells from the subventricular zone. Most of the DCX-positive cells in the dentate gyrus, subventricular zone and striatum were also positive for PSA-NCAM and the neuronal marker NeuN, but they were invariably negative for the astrocytic marker GFAP (Figure 1C and S1, Table S2). The presence of the mature neuronal marker NeuN in many DCX-positive neurons does not necessarily imply that they are not newborn; in non-human primates, adult born neurons in the dentate gyrus maintain DCX for at least six months and co-express NeuN (Kohler et al., 2011), suggesting that they may retain a juvenile state for a substantial period of time.

The five main neuronal subtypes in the striatum can be identified by the markers dopamine- and cAMP-regulated neuronal phosphoprotein (DARPP32), calretinin, choline acetyltransferase, parvalbumin and neuropeptide Y. DCX most commonly co-localized with calretinin and more rarely with neuropeptide Y (Figure 1D-E, Table S2), whereas we did not find any co-localization with markers for the other subtypes.

We did not detect DCX-positive cells with the apoptotic marker activated caspase-3 in the subventricular zone or striatum. In contrast, cleaved caspase-3 was detected in some DCX-positive cells in the subventricular zone of subjects with Huntington's disease (Figure S1 B-E).

New neurons in the adult human striatum

Over time, cells accumulate the age pigment lipofuscin, consisting of autofluorescent matrix and lipid droplets (Sulzer et al., 2008). There are no definitive molecular markers for neuroblasts, and some non-renewing neurons have been reported to express DCX (Klempin et al., 2011; Plumpe et al., 2006) or PSA-NCAM (Nacher et al., 2002; Seki, 2002). However, most DCX-positive cells in the striatum were devoid of lipofuscin (Figure 1F), suggesting that they are indeed newly generated cells, and those that did have lipofuscin contained only a few granules. The vast majority of the hippocampal neurons of the granular zone of the dentate gyrus also lack lipofuscin in adult humans (Table S3).

We examined lipofuscin accumulation in the five major neuronal subtypes of the human striatum. We found that 4.7% of the calretinin expressing interneurons were devoid of lipofuscin and a larger fraction (16.8%) had low lipofuscin content in adult humans (Figure 2A and B). A similar fraction of NPY-positive neurons had a low lipofuscin content (19.5%), but only very few (0.6%) were completely devoid of lipofuscin. The other neuronal subclasses were dominated by cells with high lipofuscin content, and we failed to find any such cells devoid of lipofuscin. It is not possible to deduce the age of a cell based on its lipofuscin content. However, we did not find any lipofuscin in striatal neurons in a 9 months old subject, whereas >96% of

striatal neurons contained lipofuscin in a 2.8 and a 6 year old individual (as well as in all older studied subjects), suggesting that it may take a few years for newborn striatal neurons to accumulate lipofuscin (Table S3).

Thymidine analogs incorporated into the DNA of dividing cells can be detected in their progeny, allowing for the identification of newly generated cells. We analyzed postmortem tissue from the striatum, hippocampus and cortex from cancer patients who received iododeoxyuridine (IdU) for radiosensitization (see Table S4 for information on the patients). In patients receiving the lowest IdU concentrations, we failed to detect any IdU-labeled cells, neuronal or nonneuronal, in the brain. However, in all individuals in whom we found IdU-labeled nonneuronal cells (n=4 subjects, age 20-71), we also detected IdU-labeled cells in the striatum co-expressing the neuronal markers NeuN, MAP2 and/or calretinin (Figure 2C-E). IdU-labeled neurons were found in both the caudate nucleus and the putamen. The nuclei of IdU-positive cells were uniformly labeled, and not in a punctuate pattern, the later being associated with DNA repair. IdU-positive neurons were also detected in the dentate gyrus of the hippocampus in the same subjects (Figure S2), but not in the cerebral cortex, in line with previous studies (Bhardwaj et al., 2006; Eriksson et al., 1998a).

Retrospective birth dating of striatal cells

It is difficult to estimate the dynamics of neurogenesis based on the incorporation of labeled nucleotides. This is especially true in humans, as the access to tissue is very limited and it is inevitable that the subjects receive different doses at different times prior to their death. In order to explore turnover dynamics of cells in humans, we have developed a method to retrospectively birth date cells, which is based on the

integration of nuclear bomb test-derived ^{14}C in DNA of proliferating cells (Figure 3A) (Spalding et al., 2005a).

We isolated cell nuclei from the human postmortem lateral wall of the lateral ventricle and from the striatum by gradient centrifugation. Analysis of the ^{14}C concentration in their genomic DNA by accelerator mass spectrometry revealed levels corresponding to time points after the birth of the individuals, showing that there is postnatal cell turnover in the human striatum and in the lateral wall of the lateral ventricle (Figure 3B and Table S5). There was no significant difference in cell turnover in the striatum and in the lateral ventricle wall ($p=0.71$, two-tailed Mann-Whitney test). In line with a population comprising several cell types, subpopulation dynamics analysis pointed to a heterogeneous group of cells, with some subpopulations having high turnover rates, others low ones and a large proportion of cells not being exchanged at all postnatally (Figure 3C).

Adult striatal neurogenesis in humans

To specifically birth date different cell types in the lateral ventricle wall and striatum, nuclei were incubated with antibodies against the neuron-specific nuclear epitope NeuN and the oligodendrocyte lineage marker SOX10. Neuronal, oligodendrocyte lineage and nonneuronal/non-oligodendrocyte lineage nuclei were isolated by flow cytometry (Figure 4 and Figure S3) (Bergmann et al., 2012; Spalding et al., 2013). Reanalysis by flow cytometry and analysis of mRNA expression indicated the specificity of the isolation (Figure S3). The ^{14}C concentration in genomic DNA from neurons (n=30 individuals), oligodendrocyte lineage cells (n=28) and

nonneuronal/non-oligodendrocyte lineage cells (n=26) was measured in subjects from 3 to 79 years of age (^{14}C data are given in Table S5).

First, we analyzed the ^{14}C concentration in neuronal genomic DNA in the lateral wall of the lateral ventricle and in the striatum (Figure 4C). For the majority of the analyzed subjects, the ^{14}C concentration in neuronal genomic DNA corresponded to the concentration in the atmosphere after the birth of the individual, showing the postnatal generation of striatal neurons. In contrast, cortical, cerebellar and olfactory bulb neurons are not renewed postnatally to a detectable level in humans, and ^{14}C levels in their DNA correspond to the time around the birth of the individual (Figure S3) (Bergmann et al., 2012; Bhardwaj et al., 2006; Spalding et al., 2005b). Individuals born before the nuclear bomb tests had lower ^{14}C levels in striatal neuron DNA than at any time after 1955, establishing that although some neurons are generated postnatally, a large majority of striatal neurons is not exchanged after birth. Mathematical modeling of ^{14}C data allows a comprehensive analysis of cell turnover (Bergmann et al., 2009; Bergmann et al., 2012; Spalding et al., 2013). By fitting several mathematical models to the data, the cell renewal rate and the fraction of cells showing turnover were estimated (see Table S6 and Extended Experimental Procedures). The best model was a scenario in which a subpopulation of the neurons is renewing whereas the majority is not. The size of the cycling neuronal population was 25% (95% confidence interval: 6-51%). Individual estimates of turnover rates showed a modest decline in turnover over age within the cycling population (Figure 4D). The median turnover rate of neurons within the renewing fraction was 2.7% per year in adulthood, which is not significantly different ($p=0.7$, two-tailed Mann-Whitney test) compared to the turnover rate of neurons within the renewing fraction in the adult human hippocampus (Spalding et al., 2013).

Turnover of striatal interneurons

Given that a large proportion of striatal neurons are not renewed postnatally, we next wanted to identify the subset of striatal neurons that is subject to exchange. The medium spiny projection neurons make up 75-80% of the striatal neurons, and the four subtypes of interneurons together represent 20-25% of the striatal neurons (Cicchetti et al., 2000). However, it is not feasible to separately carbon date each of these five neuronal populations with the current sensitivity of accelerator mass spectrometry. For this reason, we aimed at carbon dating independently medium spiny neurons and interneurons. It is challenging to isolate these populations at high purity and therefore not possible to obtain robust quantitative estimates; however, it can point to which neuronal fraction the turnover is in. Striatal nuclei were incubated with antibodies against the neuron-specific nuclear epitope NeuN and the medium spiny neuron marker DARPP32. Nuclei of medium spiny neurons (NeuN+/DARPP32+), interneurons (NeuN+/DARPP32-) and nonneuronal cells (NeuN-) were isolated by flow cytometry (Figure 5 and Figure S4). The ^{14}C concentration in genomic DNA from medium spiny neurons (n=26 individuals), interneurons (n=11) and nonneuronal cells (n=18) was measured by accelerator mass spectrometry (^{14}C data are given in Table S5).

^{14}C levels in medium spiny neuron DNA corresponded to the time around the birth for most of the individuals (Figure 5C), showing that this subtype of striatal neurons is probably not renewed postnatally to a significant level. This finding is in line with the presence of the age pigment lipofuscin in all medium spiny neurons as well as the lack of co-localization of DARPP32 with neuroblast markers or IdU (Figures 1 and

2). In contrast, the ^{14}C concentration in genomic DNA from interneurons corresponded to time points after the birth of the individuals for the majority of the analyzed subjects (Figure 5D), demonstrating postnatal generation of striatal interneurons. Modeling indicates that the striatal neuronal turnover occurs within the interneuron fraction (Table S6).

Turnover of nonneuronal cells

Next, we assessed the turnover dynamics of nonneuronal cells (NeuN-), oligodendrocyte lineage cells (SOX10+) and nonneuronal/non-oligodendrocyte lineage cells (NeuN-/SOX10-) in the lateral wall of the lateral ventricle and in the striatum (Figures 6 and S5). The ^{14}C concentration in genomic DNA of all of these cell populations corresponded to time points after the birth of the individuals (Figure 6 A-C), establishing turnover of nonneuronal cells.

The most accurate model for oligodendrocyte lineage cells was a scenario with a high initial turnover rate and lower replacement rate of older cells, suggesting the existence of a subset of short-lived cells (Table S6). This fraction of short-lived cells may correspond to immature oligodendrocyte progenitor cells, since approximately 23% of the SOX10+ nuclei were positive for the mature oligodendrocyte marker APC, indicating that the majority of the isolated oligodendrocyte lineage cells were progenitors. For the nonneuronal/non-oligodendrocyte lineage cells (NeuN-/SOX10-), as for the subset containing all nonneuronal cells (NeuN-), models that allowed one compartment turning over constantly and one non-renewing population, fitted the data best (Table S6). Individual turnover estimates suggested a decline in striatal nonneuronal cell turnover during aging (Figure 6 D-F).

Adult generated neurons are depleted in Huntington's disease

Huntington's disease is a neurodegenerative disorder that primarily affects the striatum. A previous study reported increased cell proliferation in the subventricular zone of Huntington's disease patients (Curtis et al., 2003). Therefore, we wanted to determine whether cell turnover dynamics might be affected in the striatum of Huntington's disease patients. Neuronal, oligodendrocyte lineage and nonneuronal/non-oligodendrocyte lineage cell nuclei were isolated from the postmortem striatum of Huntington's disease patients by flow cytometry. The ^{14}C concentration in genomic DNA from neurons (n=11 individuals), oligodendrocyte lineage (n=9) and nonneuronal/non-oligodendrocyte lineage cells (n=8) was measured (^{14}C data are given in Table S5). The ^{14}C concentration in genomic DNA of oligodendrocyte lineage cells corresponded to time points after the birth of the individuals (Figure 7A), but oligodendrocyte lineage cells had significantly lower turnover rates compared to those of healthy age-matched subjects (Figure 7B).

Interestingly, the ^{14}C levels in genomic DNA of neurons from grade 2 and 3 Huntington's disease patients corresponded to the time before the onset of the nuclear bomb tests in 1955 (white dots in Figure 7C), indicating an absence of postnatally generated neurons. Two subjects with grade 1 Huntington's disease (grey dots in Figure 7C), showed slightly elevated ^{14}C levels relative to their time of birth. Striatal neurons of patients with Huntington's disease had significantly lower turnover rates compared to non-affected age-matched subjects born in the same time frame (Figure 7D and Table S5). In line with this, we found only 1 out of 786 analyzed striatal neurons devoid of lipofuscin in subjects with Huntington's disease (Table S7).

Individual turnover rates from nonneuronal/non-oligodendrocyte lineage cells were not significantly different between Huntington's disease patients and non-affected subjects (Figure 7E and 7F), showing that the depletion of adult-born cells was specific to neurons and oligodendrocyte lineage cells in Huntington's disease.

DISCUSSION

New neurons are continuously added in the olfactory bulb and hippocampus in most mammals. Humans show substantial hippocampal neurogenesis (Spalding et al., 2013), but are unique in that there is no detectable addition of neurons in the olfactory bulb. However, the density of neuroblasts is very similar in the subventricular zone and the dentate gyrus of the hippocampus. We report that cells expressing the neuroblast markers DCX and PSA-NCAM are present not only in the adult human subventricular zone, but also in the adjacent striatum. IdU in striatal interneurons indicate the generation of this cell type in adult humans and retrospective birth dating of striatal neurons confirms the generation of interneurons. We furthermore report that adult-generated striatal neurons are preferentially depleted in Huntington's disease. The identification of continuous generation of striatal interneurons identifies a unique pattern of adult neurogenesis in humans.

It is important to consider alternative interpretations of our results. Both the integration of IdU and ^{14}C monitor DNA synthesis during cell proliferation. However, chromosomal damage and repair can result in DNA synthesis. Since DNA damage and repair occur almost exclusively during the cell cycle, this would not affect the outcome of our analysis. DNA repair is thought to be very limited in postmitotic cells, and well below the detection limit of carbon dating (Spalding et al., 2005a). We have not been able to detect any incorporation of ^{14}C in DNA over several decades in cerebellar, cortical or olfactory bulb neurons in humans (Bergmann et al., 2012; Bhardwaj et al., 2006). Even ^{14}C concentrations in genomic DNA from cortical neurons after ischemic stroke, which induces massive DNA damage, are not significantly different from that present in the atmosphere at the time of birth of the individuals (our unpublished data). Mathematical modeling established that the

integration of ^{14}C in DNA is limited to a subpopulation of neurons, and if the DNA synthesis would be due to DNA damage and repair, these neurons would have to have exchanged their entire genomes, which appears highly unlikely. The finding that neurons in a distinct striatal subpopulation express neuroblast markers, lack age pigment and are labeled with IdU and ^{14}C, together lend strong support to the conclusion that striatal neurons are generated in adulthood in humans.

The vast majority of neurons generated in the subventricular zone in rodents integrate in the olfactory bulb. There are, however, reports suggesting the generation of small numbers of striatal neurons in non-human primates (Bedard et al., 2002a; Bedard et al., 2002b; Tonchev et al., 2005) and of calretinin-positive striatal interneurons in rats and rabbits (Dayer et al., 2005; Luzzati et al., 2006). It is well documented that substantial numbers of striatal neurons are generated from the subventricular zone in both rodents and monkeys in response to stroke (Arvidsson et al., 2002; Hou et al., 2008; Tonchev et al., 2005; Tonchev et al., 2003; Wei et al., 2011). Thus, although the olfactory bulb is the principal destination of subventricular zone neuroblasts in most mammals, there may be limited striatal neurogenesis under homeostatic conditions and more abundant in response to stroke. It appears likely that the neuroblasts and new neurons in the adult human striatum derive from the subventricular zone, although we cannot exclude other origins. The difference in subventricular zone neurogenesis between rodents and humans may thus mainly be the direction of neuroblast migration from the subventricular zone. Altered expression of a single axon guidance molecule is sufficient to redirect growing axons and develop new circuitry in experimental animals, suggesting that rather small genetic changes can underlie brain evolution (Feldheim et al., 1998). It is conceivable that differential expression of a limited number of guidance molecules may result in

rerouting of neuroblasts and integration of neurons in two different and functionally distinct brain regions.

Calretinin expressing interneurons are much more rare in rodents, constituting less than 1% of striatal neurons, compared to humans, in whom 10% of striatal interneurons are calretinin positive. The function of this neuronal subclass is essentially unknown, to a large extent due to its paucity in animals amenable to experimental manipulation. Similar to their human counterpart, rodent calretinin-positive interneurons are of medium size and aspiny. However, only a very limited description of their axonal arborization is available and their electrophysiological profile remains unknown (Tepper and Bolam, 2004).

The depletion of adult born neurons in Huntington's disease may be due to reduced generation of neurons and/or preferential degeneration of adult born neurons. Huntington's disease is thought to be the result of both loss of function of the normal Huntingtin protein and toxic effects of the modified protein (Zuccato et al., 2010). Huntingtin has been implicated in both embryonic and adult neurogenesis (Ben M'Barek et al., 2013; Godin et al., 2010), and it is possible that failing striatal neurogenesis contributes to the depletion of adult generated neurons. However, since the subjects with early stage disease showed a more modest depletion of adult-born neurons than at the advanced stages (Figure 7), adult born neurons may be lost with progression of the disease, although the number of subjects available for analysis was limited.

We can currently only speculate about the potential function of continuous striatal neurogenesis in humans, and the functional integration of the new neurons into existing neuronal circuits as well as their effects remain to be investigated. The

striatum was first associated with motor control, but it is today well-established that this region also is important for many cognitive functions. Huntington's disease results in motor, cognitive and psychiatric symptoms, with the motor symptoms often being preceded by cognitive impairment. The selective depletion of adult generated neurons in Huntington's disease indicates that the effect of losing such neurons may be found within the symptoms that these patients display. However, medium spiny neurons account for most of the neuronal loss, and most of the symptomatology is likely explained by this.

The finding that neurons are continuously added in the adult human striatum poses the question whether this process can be utilized therapeutically in neurological disease. The presence of increased numbers of putative neuroblasts in the human striatum after stroke may indicate increased neurogenesis in this situation (Macas et al., 2006), similarly to what is seen in other mammals. It will be important to study if such neuroblasts give rise to mature neurons. Increasing the generation or promoting the survival of new neurons is an interesting option in stroke. Moreover, studies in rodents have suggested that promoting cell proliferation in the subventricular zone can have a positive effect in models of Parkinson's disease, likely mediated by the new cells having a neurotrophic effect on the nigro-striatal system (Androutsellis-Theotokis et al., 2009; Zachrisson et al., 2011). Investigating how striatal neurogenesis is potentially affected in pathological situations and identifying factors that promote the renewal of striatal neurons may facilitate the development of therapeutic strategies to improve functional recovery after injury or to tackle neurodegenerative disorders.

EXPERIMENTAL PROCEDURES

Tissue Collection

After informed consent from the relatives was given, tissues were obtained from cases admitted between 1991 and 2013 to the Swedish National Department of Forensic Medicine for autopsy, the NICHD Brain and Tissue Bank from the University of Maryland (US) and the Cambridge Brain Bank (UK). Ethical permission for this study was granted by Regional Ethics Committee of Sweden (No 02-418, 2005/185, 2006/1029-31/2, 2006/189-31, 2010-313/31-3), the institutional review boards of the University of Maryland and from Cambridge University. Lateral wall of the lateral ventricle or striatum (caudate nucleus including the lateral wall of the lateral ventricle, putamen and internal capsule) were dissected. Cerebellum and occipital cortex samples served as controls. Brain tissue was frozen and stored at -80°C until analysis. Formalin-fixed and paraffin-embedded sections of the striatum, hippocampus and cortex were obtained from cancer patients who received IdU infusion as a radiosensitizer for therapeutic purposes at the National Heart, Lung and Blood Institute, National Institutes of Health, Bethesda, US. Additionally, olfactory bulbs from donors who did not receive IdU were examined.

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, 1 mM DTT). Homogenized samples were suspended in 20 ml of sucrose solution (1.8 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 x g for 2.4 hr at 4°C. The isolated nuclei were resuspended in nuclei storage buffer (10mMTris [pH = 7.2], 2mM $MgCl_2$, 70mM KCl, 15% sucrose) for immunostaining and flow cytometry analysis.

FACS Sorting and Analysis

Isolated nuclei were stained sequentially with mouse NeuN (A-60) (Millipore, 1:800) and either rabbit DARPP32 (Cell signaling, 1:200) or goat SOX10 (R&D, 1:300). NeuN (A-60) antibody was directly conjugated to Alexa 647 (Alexa Fluor 647 Antibody Labeling Kit, Invitrogen) and DARPP32 antibody was directly conjugated to Alexa 488 (Cell signaling). Secondary antibody conjugated to Alexa 488 (Invitrogen, 1:500) was used to detect SOX10. Flow cytometry analyses and sorting were performed with a BD FACS Influx sorter (BD Biosciences). The FACS gating strategies for sorts are shown in Figures 4 and 5.

DNA Purification

DNA purification was carried out in a clean room (ISO8) to avoid any carbon contamination of the samples. All glassware was prebaked at 450°C for more than 4 hr. DNA isolation was performed according to a modified protocol (Miller et al., 1988). Briefly, 500 µl 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 was added (Ambion) and samples were incubated at 65°C for 1 hr. Half of the existing volume of 5 M NaCl

solution was added, and the mixture was 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. Then, 3 volumes of absolute ethanol were 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 0.1 M NaCl) and transferred to 500 µl DNase- and RNase-free water (Gibco). The DNA was quantified, and DNA purity was verified by UV spectroscopy (NanoDrop).

Accelerator Mass Spectrometry (AMS)

All AMS analyses were performed blind to the identity of the sample. Purified DNA samples suspended in water were lyophilized to dryness. To convert the DNA samples into graphite, excess CuO was added to each dry sample, and the tubes were evacuated and sealed with a high-temperature torch. Tubes were placed in a furnace set at 900°C for 3.5 hr 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 at 550°C for 6 hr. Graphite targets were measured at the Department of Physics and Astronomy, Ion Physics, of Uppsala University (Salehpour et al., 2013a; Salehpour et al., 2013b). Large CO_2 samples (>100 µg) were split, and $\delta^{13}C$ was measured by stable isotope ratio mass spectrometry, which established the $\delta^{13}C$ correction to –22.3‰ ± 0.5‰ (1 SD), which was applied for all samples. The measurement error was determined for each sample and ranged between ±4‰ and ±12‰ (1 SD) $\Delta^{14}C$ for the large and small samples (10 µg C), respectively. All ^{14}C data are reported as decay-corrected $\Delta^{14}C$ or fraction modern.

Summary of the Mathematical Modelling

Mathematical modelling was based on birth-and-death processes representing different lifetime scenarios, as described (Bergmann et al., 2009; Spalding et al., 2013) and as outlined in detail in the Extended Experimental Procedures. These models were integrated along the atmospheric ^{14}C curve to yield a predicted DNA ^{14}C concentration. Nonlinear least-square and Monte Carlo Markov Chain methods were used to estimate the best global parameters for each scenario, for the neuronal and the non-neuronal cell samples. The small-sample-corrected Akaike Information Criterion (AICc) was used to rank the different scenarios. Individual turnover rates were estimated from Scenarios A and 2POP, for all the samples. Scenario A provided a direct turnover rate estimate for each sample. However, for Scenario 2POP, fixed fractions of renewing were set based on the best fit for the global parameter estimates.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Extended Experimental Procedures, five figures and seven tables.

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FIGURE LEGENDS

Figure 1. Neuroblasts in the adult human striatum

(A) *DCX* expression in the striatum (green), hippocampus (red) and cerebellum (blue) across the human lifespan. Data from Kang et al, 2011. The striatal area used for the transcriptome analysis comprises the caudate nucleus (lateral ventricle wall included), the nucleus accumbens and the putamen.

(B) Western blot analysis of DCX, PSA-NCAM and β-actin in the hippocampus (H), striatum (S) and cerebellum (C) of human subjects of different ages.

(C-E) Confocal microscopy of DCX-positive cells in the striatum. The majority of the DCX-positive cells express the mature neuronal marker NeuN but all of them lack expression of the astrocytic marker GFAP (C). Most of the DCX-positive cells also express PSA-NCAM (D). DCX occasionally co-localizes with neuronal markers calretinin (CR) (D) and NPY (E). Most DCX-positive cells have little or no lipofuscin (arrow) whereas the majority of DCX-negative cells contain lipofuscin pigments (stars) (F). Autofluorescent lipofuscin pigments are not visible in all channels because an anti-autofluorescence treatment was applied. Scale bars indicate 20 μm for panels C-E and 10 μm for panel F. Cell nuclei are labeled with DAPI and appear blue. See also Figure S1 and Table S2.

Figure 2. New neurons in the adult human striatum

(A) Lipofuscin quantification by confocal microscopy in the five main subtypes of striatal neurons. DARPP32, dopamine- and cAMP-regulated neuronal

phosphoprotein; CR, calretinin; ChAT, Choline acetyltransferase; PV, parvalbumin; NPY, neuropeptide Y. Data are shown as mean values for three donors aged between 21 and 26. Error bars represent standard deviation. A minimum of 200 neurons per subtype and per subject were analyzed.

(B) Confocal images with orthogonal views demonstrate the absence of lipofuscin granules in a calretinin-positive neuron (arrow) while other cells have autofluorescent lipofuscin pigments in their cytoplasm, visible in all channels (star).

(C-E) Newly generated cells can be detected in the adult human striatum in patients previously receiving the thymidine analog IdU. Confocal images with orthogonal projections showing IdU-labeled neuronal nuclei in the striatum of two subjects aged 20 and 41. Neurons are identified by the expression of NeuN, MAP2 and/or calretinin (CR). Scale bars represent 20 μm. See also Figure S2, Table S3 and Table S4.

Figure 3. Postnatal cell turnover in the human lateral ventricle wall and in the striatum

(A) Schematic illustration of ^{14}C concentration measurements in genomic DNA. The black line shows the atmospheric ^{14}C concentration over time. Individually measured ^{14}C concentrations in human genomic DNA are plotted at the time of the subject's birth (vertical lines), before (blue symbols) or after the ^{14}C bomb spike (red symbols). Data points above the bomb curve for subjects born before the bomb peak and below the bomb curve for subjects born after the nuclear tests indicate cell turnover. Data points on the atmospheric curve show the absence of turnover. Data from Bhardwaj et al 2006, Bergmann et al 2012 and Spalding et al 2013. (B) The ^{14}C concentrations in

genomic DNA from cell nuclei isolated from the lateral wall of the lateral ventricle (black dots) and from the striatum (white dots) demonstrate postnatal cell turnover in subjects born before and after the bomb spike. (C) In line with a tissue composed of many different cell types, subpopulation dynamics analysis indicates that striatal cells form a heterogeneous group, some fractions having high turnover rates and some having very low ones. The grey area represents the range of acceptable values. The resolution of this type of analysis does not allow differentiating between turnover rates above 10%.

Figure 4. Neuronal turnover dynamics in the lateral ventricle wall and in the striatum

(A, B) Isolation of nuclei from neurons, oligodendrocyte lineage and nonneuronal/non-oligodendrocyte lineage cells from the lateral ventricle wall and from the striatum. Cell nuclei were isolated from human postmortem tissue and left unstained (A) or incubated with antibodies against the neuron-specific epitope (NeuN) and the oligodendrocyte lineage marker SOX10 (B). The neuronal (NeuN+), oligodendrocyte lineage (SOX10+) and nonneuronal/non-oligodendrocyte lineage populations (NeuN-/SOX10-) were isolated by flow cytometry. The sorting gates are indicated. (C) ^{14}C concentrations in the lateral ventricle wall and striatal neuron genomic DNA correspond to a time after the date of birth of the individual for the majority of the analyzed subjects, demonstrating neurogenesis throughout life. Error bars indicate two standard deviations in ^{14}C concentration in the respective DNA sample. (D) Individual turnover rates for neuronal cells computed on the basis of individual data fitting according to the 2POP scenario (see Extended Experimental

Procedures). Individual turnover rates are sensitive to deviations in measured ^{14}C, especially for young individuals due to the shallow slope of the ^{14}C curve, and values <0.001 (n=3) or >1.00 (n=8) were excluded from the plot, but the full data are given in Table S4. The individual turnover rates for adult subjects were not significantly different between neurons from the lateral ventricle wall and from the striatum ($p=0.9$, Mann-Whitney test) but significantly higher than the turnover rates observed in the cortex or cerebellum ($p<0.05$, Mann-Whitney test). See also Figure S3 and Table S6.

Figure 5. Turnover of striatal interneurons

Cell nuclei were isolated from the human postmortem striatum and left unstained (A) or incubated with antibodies against NeuN and against the medium spiny neuron marker DARPP32 (B). The interneuron (NeuN+/DARPP32-), medium spiny neuron (NeuN+/DARPP32+) and nonneuronal populations (NeuN-) were isolated by flow cytometry. The sorting gates are indicated. (C) ^{14}C concentrations in genomic DNA from striatal medium spiny neuron nuclei (NeuN+/DARPP32+) are not significantly different from atmospheric ^{14}C concentrations at birth for the vast majority of the analyzed individuals. (D) The ^{14}C concentration in genomic DNA from striatal interneurons corresponds to time points after the birth of most individuals, demonstrating interneuron renewal throughout life. Error bars indicate two standard deviations in ^{14}C concentration in the respective DNA sample. See also Figure S4 and Table S6.

Figure 6. Turnover of nonneuronal cells in the striatum

The ^{14}C concentration in genomic DNA from oligodendrocyte lineage cells (SOX10+) (A), from nonneuronal/non-oligodendrocyte lineage cells (NeuN-/SOX10-) (B), and from all nonneuronal cells (NeuN-) (C), corresponds to time points well after the birth of each individual in the lateral ventricle wall (black dots) and in the striatum (white dots). (D-F) Individual turnover rates for oligodendrocyte lineage cells (D), nonneuronal/ non-oligodendrocyte lineage cells (E) and all nonneuronal cells (F) computed on the basis of individual data fitting. Individual turnover rates are sensitive to deviations in measured ^{14}C and values <0.001 (n=2) were excluded from the plots, but the full data are given in Table S2. All individual turnover rates estimates are based on the 2POP scenario. See also Figure S5 and Table S6.

Figure 7. Cell turnover in the striatum of Huntington's disease patients

Cell turnover in the striatum of patients affected by Huntington's disease. Grey dots indicate individually measured ^{14}C concentrations in the genomic DNA of nuclei isolated from the striatum of individuals with grade 1 Huntington's disease. White dots show ^{14}C concentrations in the genomic DNA of striatal nuclei from individuals with grade 2 or grade 3 Huntington's disease. The ^{14}C concentration in genomic DNA from oligodendrocyte lineage cells, defined by SOX10 expression, corresponds to time points after the birth of each individual (A). The individual turnover rates from oligodendrocyte lineage cells are significantly lower in individuals with Huntington's disease (HD) compared to age-matched non-affected subjects (B). ^{14}C concentrations in genomic DNA from neuronal nuclei isolated from the striatum of individuals with grade 1 Huntington's disease are slightly above atmospheric ^{14}C concentrations at

birth, whereas ^{14}C concentrations in genomic DNA from neuronal nuclei isolated from the striatum of individuals with grade 2 or 3 Huntington's disease are not significantly different from atmospheric ^{14}C concentrations at birth (C). The individual turnover rates from striatal neurons are significantly lower in individuals with Huntington's disease compared to non-affected age-matched controls (D). The ^{14}C concentrations of genomic DNA from nonneuronal/non-oligodendrocyte lineage cells, defined by the absence of NeuN and SOX10 labeling, demonstrate postnatal cell turnover in the striatum of patients affected by Huntington's disease (E). The individual turnover rates from nonneuronal/non-oligodendrocyte lineage cells do not significantly differ between individuals with Huntington's disease and non-affected subjects (F). *$p < 0.05$, Mann-Whitney test for equal medians. Individual turnover rate estimations are based on scenario A (see Extended Experimental procedures) to allow comparing healthy subjects with Huntington's disease patients in spite of the difference in the respective renewing fractions. This results in an underestimation of the turnover rate in the healthy donors compared to the turnover rate in the renewing fraction (scenario 2POP). See also Table S6.