Abstract

Strong age-related declines in conjunction with comparatively easy experimental manipulations of adult hippocampal neurogenesis have generated considerable public and scientific interest in the prospect of “new neurons for old brains”. Only few studies addressed the time course of the natural changes, which are the substrate for interventions that may realize this prospect. We provide a monthly or bimonthly account of cell proliferation, neurogenesis and cell death during the first 9 months of the life of C57Bl/6J mice. Ki67- and DCX-positive cell numbers declined exponentially without an intermittent plateau (∼40% per month). Cell death in relation to cell proliferation was lowest at 1 month, increased at 2 months to remain constant until 4 months, and decreased again at 5 months to remain stable until 9 months. Granule cell number did not change with age. Our results suggest that manipulations of proliferation and neurogenesis may, at any time, interact with strong natural changes of these processes. Mediators of their age-related decline may be studied over periods much shorter than those typically used.

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1. Introduction

Adult hippocampal neurogenesis (AhN) consists of neural stem cell proliferation in the subgranular zone of the dentate gyrus, neuronal differentiation, and the maturation into fully functional and integrated neurons in the granule cell layer (reviewed in Abrous et al., 2005). It occurs constitutively throughout adulthood in many mammalian species, from rodents to primates and including humans (Altman and Das, 1965; Eriksson et al., 1998). Natural factors regulating AhN include physical activity (Van Praag et al., 1999), environmental complexity (Kempermann et al., 1997b), hormones and growth factors (Cameron and Gould, 1994; Cameron and Gould, 1994; Cameron et al., 1998). Although hippocampal neurogenesis persists throughout adulthood in many species, the formation of new granule cells peaks very early during postnatal life and decreases thereafter, through adulthood. Numerous studies in rodents have identified age as one of the strongest and most consistent modulators of AhN (Seki and Arai, 1995; Kuhn et al., 1996; Eriksson et al., 1998; Kempermann et al., 1998; Cameron and McKay, 1999; Lemaire et al., 2000; Bizon and Gallagher, 2003; Gallagher et al., 2003; Rao et al., 2006). The first reports documenting age-related changes in cell proliferation and neurogenesis in rats suggested a prominent decrease of both aspects between the ages of 2 and 18 months (Seki and Arai, 1995) or 6 and 27 months (Kuhn et al., 1996). In adult mice, age-related changes in AhN were found between the ages of 6 and 18 months (Kempermann et al., 1998), between 3, 7–10 and 22–24 months (Harrist et al., 2004), and at around 9 months of age (Kempermann et al., 1998; Kronenberg et al., 2006).

Most studies of age-related changes in AhN have used two or three ages, typically consisting of 1–3 months, 6–12 months, or 12–24 months old subjects. Which of these groups is considered young, adult, and aged may vary with the study at hand (Coleman, 2004). The underlying sentiment for these groupings seems to be an assumption of relatively...
static parameters of AhN during these life history periods. We recently reported a decrease in AhN between the ages of 3 and 4 months in a mouse model of cranial X-irradiation (Ben Abdallah et al., 2007), and the expectation of stasis was clearly brought out by, in part, our own surprise and by some incredulity this finding was met with. A systematic study of early changes in neurogenesis similar to those performed in rats (Seki and Arai, 1995; Rao et al., 2006) is, to our knowledge, not available for the mouse. It is unclear when AhN changes by how much or if there are distinct periods in the life of a mouse that share some or all aspects of AhN. Here, we provide data on proliferation, neurogenesis, and cell death at seven time points during the first 9 months of life of C57Bl/6J mice, which are widely used in current research of both aging and AhN. An endogenous marker, Ki67, was used to show proliferating cells (Scholzen and Gerdes, 2000; Kee et al., 2002) and doublecortin (DCX) to show cycling precursors of neuronal lineage and maturing young granule cells (Couillard-Despres et al., 2005). Cell survival was assessed by relating proliferation to neuronal differentiation and to cell death. Total granule cell number was estimated to address the issue of replacement versus addition of new cells to the granule cell layer.

2. Materials and methods

2.1. Animals

We used C57Bl/6J mice at the ages of 1–5, 7 and 9 months, with three males and three females in each group, except in the 9 months-group where only males were available. Mice were bred in our laboratory (breeders from Jackson Laboratories, Bar Harbor, ME), and were kept in groups of four to six sex-matched littersmates in a light, temperature, and humidity-controlled room with free access to laboratory animal diet and water. All experimental procedures were conducted in accordance with the Swiss animal welfare guidelines and approved by the cantonal veterinarian office of Zürich, Switzerland.

2.2. Tissue processing

Mice were anesthetized with sodium pentobarbital (50 mg/kg intraperitoneally) and perfused transcardially with ice-cold phosphate buffered saline (PBS; pH 7.3), followed by a phosphate-buffered 0.6% sodium sulfide solution and 4% paraformaldehyde with 15% saturated picric acid in PBS. Brains were removed, postfixed for 8 h in 4% paraformaldehyde with picric acid in PBS, weighed and divided into hemispheres. The left hemisphere was cryoprotected in 30% sucrose. Forty-µm thick horizontal sections were cut on a freezing microtome and kept at −20 °C in cryoprotectant until further processing. The right hemisphere was embedded in glycolmethacrylate (Technovit 7100, Kulzer, Wehrheim, Germany) as described previously (West et al., 1991).

2.3. Immunohistochemistry

To demonstrate Ki67 and DCX we used a primary rabbit polyclonal anti-Ki67-antibody (1:5000; NCL-Ki67p, Novocastra, Newcastle upon Tyne, UK) and a primary goat polyclonal anti-DCX-antibody (Doublecortin; 1:1000; sc-8066, Santa Cruz Biotechnology, Santa Cruz, CA). Every fifth section of each animal was processed free-floating. Rinses were performed between all steps using 0.05% Triton in Tris-buffered saline (Tris–Triton), pH 7.4, or only Tris-buffered saline (TBS, pH 7.4), before or after the primary antibody incubation, respectively.

For epitope retrieval, sections processed for Ki67-immunohistochemistry were incubated in citrate buffer (ChemMate, DAKO, Glostrup, Denmark) in distilled water, for 40 min at 95 °C. For DCX-immunohistochemistry, sections were incubated in 0.6% H2O2 in Tris-Triton for 30 min at room temperature to block endogenous peroxidase activity. Thereafter, sections were preincubated in 2% normal serum (depending on the species in which the secondary antibody was raised) in 0.2% Triton in TBS for 1 h at room temperature and, subsequently, in primary antibody overnight at 4 °C. Sections were rinsed and incubated with biotinylated secondary antibodies (goat anti-rabbit IgG for Ki67; rabbit anti-goat IgG for DCX; both diluted 1:300, Vectastain Elite ABC kit, Vector Laboratories, Burlingame, CA). Finally, sections were incubated in avidin–biotin complex (Vectastain Elite ABC kit) for 30 min at room temperature, and stained using diaminobenzidine as chromogen. Sections stained for DCX were counterstained with hematoxylin solution (51275 Fluka, Buchs, Switzerland).

2.4. Giemsa staining

Twenty-µm thick glycolmethacrylate horizontal sections were cut, every fifth section was mounted, and slides were dried at 70 °C for 1 h. Sections were incubated in Giemsa stock solution (Merck, Darmstadt, Germany) diluted 1:5 in 67 mmol KH2PO4 for 40 min (Iniguez et al., 1985), differentiated in 1% acetic acid and 96% ethanol, dehydrated in 99% ethanol, cleared and cover slipped.

2.5. Quantitative procedures

Ki67-immunoreactive (Fig. 1a and b) and pyknotic cells (Fig. 1d and e) were counted in the subgranular zone of the dentate gyrus, using a 40× oil-immersion objective (Zeiss Plan-Neofluar with a numerical aperture of 1.3). Cells in the uppermost focal plane of the section were excluded. Total cell number was calculated by multiplying the number of cells counted by the inverse of the section sampling fraction, i.e. five. Pyknotic cells were counted in glycolmethacrylate embedded sections and identified by their strongly and homogeneously stained nuclei, in which chromatin condensation and fragmentation of the nucleus are major characteristics (Fig. 1d and e). We have previously shown that the number...
of pyknotic cells correlates well with the number of TUNEL labeled cells in the same brain (Amrein et al., 2004a).

Total numbers of granule and DCX-positive cells were estimated using the optical fractionator (West et al., 1991; StereoInvestigator, Microbrightfield Inc., Williston, VT, USA) with a 100× oil-immersion objective. Granule cell number was estimated in glycolmethacrylate-embedded sections using a 10 μm × 10 μm counting frame placed at 190 μm intervals along the x- and y-axes, and a dissector height of 10 μm. For DCX-positive cells, counting frames sized 45 μm × 35 μm were placed over the dentate gyrus at intervals of 135 μm along the x-axis and 105 μm along the y-axis. DCX-positive neurons, i.e. hematoxylin stained nuclei surrounded by a DCX-positive cytoplasm (Fig. 1c), were counted throughout the section thickness but excluding cells in the uppermost focal plane. Cell numbers (N) were estimated by multiplying the number of cells counted with the inverse of the sampling fraction, i.e. N = sum of the cells counted × (1/thickness sampling fraction) × (1/area sampling fraction) × (1/section sampling fraction). All cell number estimates were obtained from a known fraction of the tissue and are therefore unbiased by possible age-related changes in the volume of the granule cell layer or subgranular zone.

2.6. Statistical analysis

Statistical analysis and presentation follow the guidelines of the American Physiological Society (Curran-Everett and Benos, 2004). In all statistical comparisons, one- or two-way ANOVA was performed. Differences between age groups or sexes were considered significant when the p-value was ≤0.05. Further comparisons between groups used Fisher’s protected least significant difference (PLSD) with the significance level at p ≤ 0.05. For the sake of clarity, only significant differences between subsequent age groups or, if an age group did not differ from the preceding one, for the next group along the timeline that did, are mentioned in the text and were added to the illustrations.

Changes of the numbers of Ki67-positive and DCX-positive cells with age were best fitted to an exponential model. Changes of the number of pyknotic cells were best fitted to a gamma probability density function. Analyses were performed using StatView 5TM and Matlab (R2007a).

Coefficients of error (CE) of individual cell number estimates were calculated using the approach detailed in Gundersen et al. (1999), with a smoothness constant (m) of 0 (Sloianka and West, 2005).
2.7. Photography

For illustrations, we selected sections from animals close to their group means and from comparable septotemporal levels of the dentate gyrus. Brightness and contrast were adjusted to resemble the appearance of the sections under the microscope. No local image modifications were performed.

3. Results

3.1. Absence of gender differences

We did not observe gender differences or gender × age interactions for the numbers of pyknotic (gender: \( p = 0.91 \) and gender × age: \( p = 0.37 \)), Ki67- (\( p = 0.59 \) and \( p = 0.86 \)), DCX-positive cells (\( p = 0.44 \) and \( p = 0.97 \)) or total granule cell number (\( p = 0.88 \) and \( p = 0.20 \)). The non-significant percent differences of females relative to males of each age group averaged across all age groups (excluding the 9 months old animals which only consisted of males) were −9% for pyknotic cells, −2% for Ki67-positive cells, 10% for DCX-positive cells, and −9% for total granule cell number. All further comparisons are based on data pooled across genders.

3.2. Ki67-immunoreactive cells

The number of Ki67-positive cells (Figs. 1a, b, 2 and 3) changed significantly with age (\( p < 0.0001 \)). Changes in the number of proliferating cells in the subgranular zone best fitted a negative exponential curve (\( Y = 1051 \times e^{(-0.475X)} \), with a correlation coefficient \( r^2 = 0.95 \)). Cell proliferation was highest in the 1-month-old mice (6538, S.D. 736), but decreased by ∼40% in mice aged 2 months (4137, S.D. 565; \( p < 0.0001 \)). Subsequently, proliferation decreased by ∼40% between the ages of 2 and 3 months (2649, S.D. 705) and by ∼55% between the ages of 3 and 4 months (1230, S.D. 288; \( p < 0.0001 \)). At 4 months, proliferation amounted to only ∼20% of that observed at 1 month. No difference in the number of Ki67-immunoreactive cells was observed between the ages of 4 and 5 months (1006, S.D. 214; \( p = 0.131; p = 0.41 \)). Cell number estimates continued to decline at the ages of 7 months (559, S.D. 132; comparison 4–7 months: \( p = 0.01 \); comparison 5–7 months: \( p = 0.11 \)) and 9 months (416, S.D. 288; comparison 4–9 months: \( p = 0.005 \); comparison 5–9 months: \( p = 0.03 \)). No difference in the number of Ki67-immunoreactive cells was observed between the 7-months- and 9-months-groups (\( p = 0.60 \)).

The coefficient of error of the age groups ranged between 0.08 and 0.17. Methodologically introduced variance generally contributed little to the observed group variance. The ratio \( CE^2/CV^2 \) was less then 0.5 for all groups except for the 1-month-old animals. In the case in which the \( CE^2/CV^2 \) ratio exceeded 0.5 this was not due to a large methodologically introduced variance (1 month: \( CE = 0.11 \)) but due to small group variance. Similar observations were made for the CEs and \( CE^2/CV^2 \) ratio of DCX-positive and pyknotic cells numbers.

3.3. Doublecortin-immunoreactive cells

Age-related changes between groups in the number of DCX-positive cells (Figs 1c, 4 and 5; \( p < 0.0001 \)) were similar to those observed in cell proliferation, and were best described by a negative exponential curve (\( Y = 33,110 \times e^{(-0.389X)} \), \( r^2 = 0.91 \)). The number of DCX-positive cells decreased significantly by ∼35% between the
Fig. 3. Age-related decrease in Ki67-positive cell numbers was significant \((p<0.0001)\). As compared to 1-month-old animals, the number of proliferating cells declines by \(\sim 40\%\) already in 2 months old mice. Proliferation continues to decrease at or close to this rate also in the subsequent intervals following a negative exponential mode. Grey bars and circle represent standard deviations and mean; black circles represent individual data points. \(*p<0.05; **p<0.01; ***p<0.001.\)

ages of 1 month (22,385, S.D. 4863) and 2 months (14,947, S.D. 2931; \(p<0.0001\)). Similar decreases \((\sim 25\%)\) were also present between the 2 and 3 months-groups (11,362, S.D. 1685; \(p=0.008\)). The decrease in the number of DCX-positive neurons was also significant between the 3 and 4 months-groups (6202, S.D. 1098; \(p=0.0003\), corresponding to \(\sim 45\%). The number of DCX-positive cells did not change significantly between the ages of 4 and 5 months (4725, S.D. 1206; \(p=0.25\), but decreased again between the ages of 5 and 7 months (1875, S.D. 530; \(p=0.03\). No difference in the number of DCX-positive cells was observed between the 7 and 9 months-groups (1342, S.D. 703; \(p=0.68\)). However, decreases of DCX immuno-reactivity was significant at later ages when comparing 4 and 7 months-groups \((p=0.001), 4\) and 9 months-groups \((p=0.0006),\) and 5 and 9 months-groups \((p=0.01).\)

One-way ANOVA did not show age related changes for the ratio of Ki67-positive to DCX-positive cells (Fig. 6; \(p=0.27).\)

3.4. Pyknotic cells

Age significantly affected \((p<0.0001)\) the number of pyknotic cells in the dentate gyrus (Figs. 1 d, e and 7), in a fashion best described with a gamma probability density function \((Y=985 \times x^{3.55} e^{-1.92 x}; r^2=0.78)\). Their number increased by \(\sim 80\%\) between the ages of 1 month (142, S.D. 31) and 2 months (258, S.D. 74; \(p=0.0001\). This increase was followed by a significant decrease between the ages of 2 and 3 months (139, S.D. 78; \(p<0.0001\) corresponding to \(\sim 50\%). Additionally, the number of pyknotic cells decreased of \(\sim 45\%\) between the ages of 3 and 4 months (77, S.D. 43; \(p=0.02\)). The number of pyknotic cell decreased by a further \(\sim 65\%\) in mice aged 5 months (27, S.D. 24; \(p=0.08\). Although number estimates decreased further in the 7 months (8, S.D. 7.5) and 9 months-groups (12, S.D. 10), group comparisons did not show any further significant changes (5–7 months: \(p=0.48\); and 7–9 months: \(p=0.88\).)

As an indicator of cell survival, we calculated the ratio between pyknotic cells and Ki67-immunoreactive cells. The ratio differed significantly between age groups (Fig. 8; \(p=0.03). When compared to the 1 month-group (0.023, S.D. 0.006), the ratio of pyknotic cells to the number of Ki67-positive cells increased about three fold in the 2 months- (0.065, S.D. 0.025; \(p=0.04\), 3 months- (0.06, S.D. 0.044; \(p=0.07\), and 4 months-groups (0.065, S.D. 0.042; \(p=0.04\). In comparison with the 4 months old animals, the ratio decreased in the 5 months (0.025, S.D. 0.021; \(p=0.04\) and 7 months (0.015, S.D. 0.015; \(p=0.0001\).)
Fig. 5. DCX-positive cell numbers in the dentate gyrus decreased significantly with age ($p < 0.0001$). The number of DCX-positive cells decreases exponentially by $\sim 35\%$ between age groups. Grey bars and circle represent standard deviations and mean; black circles represent individual data points. **$p < 0.01$; ***$p < 0.0001$.

Fig. 6. Ratios between Ki67-positive and DCX-positive cells suggest a stable rate of neuronal differentiation across all age groups. Bars represent standard deviations.

Fig. 7. Total numbers of pyknotic cells. The rate of cell death fitted a gamma probability density function. Cell death was modest in 1-month-old animals, highest in the 2 months old animals after which it decreases continuously with age. Grey bars and circle represent standard deviations and mean; black circles represent individual data points. *$p < 0.05$; **$p < 0.01$; ***$p < 0.0001$.

Fig. 8. Ratios between pyknotic cells and Ki67-positive as an indicator of the survival of the newly generated cells. Note the high values at 1 month, when proliferation and neurogenesis are also highest, and at ages older than 4 months, when proliferation and neurogenesis are lowest. Bars represent standard deviations.

**$p < 0.01$; ***$p < 0.0001$.

Fig. 9. Total granule cell number in the dentate gyrus. We did not observe any changes between different age groups. Bars represent standard deviations.

3.5. Total number of granule cells

The total number of granule cells (Fig. 9) did not differ between animals of different ages ($p = 0.11$). The mean values in millions were 0.44 (S.D. 0.34) at 1 month, 0.54 (S.D. 0.54) at 2 months, 0.59 (S.D. 0.46) at 3 months, 0.50 (S.D. 0.36) at 4 months, 0.58 (S.D. 0.50) at 5 months, 0.47 (S.D. 0.35) at 7 months, and 0.55 (S.D. 0.35) at 9 months.

4. Discussion

In most adult mammals, new cells are continuously added to the hippocampal granule cell layer throughout life (Altman and Das, 1965; Kaplan and Hinds, 1977; Eriksson et al., 1998). Several studies reported an age-related decrease in adult neurogenesis (Seki and Arai, 1995; Kuhn et al., 1996; Kempermann et al., 1998; Ben Abdallah et al., 2007; Leuner et al., 2007; Andres-Mach et al., 2008). Most previous reports, however, investigated few time points, leaving it an open question when...
the changes occur that are observed in the aged animals.

Briefly, our findings indicate that there is no period during the first 9 months of the life of C57Bl/6J mice during which proliferation and neurogenesis are stable over extended periods of time, and they cannot define life history periods corresponding to ‘young’, ‘adult’ or ‘aged’. Both the number of Ki67-positive proliferating cells and DCX-positive cells of neuronal lineage decline exponentially, without signs of an intermittent plateau, by 30–40% each month. In contrast, the ratio between pyknotic and proliferating cell numbers, does define three periods during this period. This ratio is lowest at one month of age, suggesting a high cell survival. It increases sharply at the age of 2 months to remain constant until the age of 4 months, and, finally, decreases again at the age of 5 months to remain constant until the last time point investigated. Changes in survival are not reflected in changes in neurogenesis. Also, granule cell number remains constant between the ages of 1 and 9 months.

Changes between the groups aged 3 and 4 months very closely replicate the results we obtained earlier (Ben Abdallah et al., 2007).

4.1. Age, cell proliferation, and cell death

Ki67 is a nuclear marker for proliferating cells involved in cell cycle progression and expressed from the late G1 phase onwards (Scholzen and Gerdes, 2000; Kee et al., 2002; Eisch and Mandyam, 2007). We observed a ∼40% decrease between the ages of one and 2 months. A similar monthly decline in proliferation persisted up to 4 months, when it corresponded to only 20% of 1 month’s rate. Further decreases were observed between subsequent age intervals, although longer periods were required to reach significance.

A study of wild mice indicated that cell proliferation changes with age in a species-specific manner (Amrein et al., 2004b). We will therefore focus the comparison of our results to data obtained in laboratory mice. A ∼50% and ∼70% decrease in the number of proliferating cells have been reported between the ages of 6 weeks and 9 months and 6 and 18 months in C57 mice (Kempermann et al., 1998; Kronenberg et al., 2006). In transgenic mice with a C57Bl/6J background, cell proliferation decreased by ∼70% between the ages of 3 and 7–10 months (Harrist et al., 2004), while in B6D2F2 mice proliferation decreased over 75% between 2 and 12 months of age (Donovan et al., 2006). Reductions in cell proliferation have also been reported to exceed 90% between the ages of 2 and 12 months in C57Bl/6J mice (Bondolfi et al., 2004). These figures agree with the decreases we observed between the ages of 4 and 9 months (67%), and the ages of 3 and 9 months (85%). Differences in exact numbers may relate to differences between Ki67 and the more commonly used BrdU (Eisch and Mandyam, 2007) and possible genetic differences in proliferation (Kempermann et al., 1997a, 2006).

Ongoing neurogenesis in the adult dentate gyrus is physiologically regulated by programmed cell death (Biebl et al., 2000). We addressed cell death by means of pyknotic cells, most of which were found in the subgranular zone, which is consistent with previous reports (Cooper-Kuhn and Kuhn, 2002; Dayer et al., 2003). Changes in cell death were best described with a gamma distribution, peaking at the age of 2 months, and decreasing continuously thereafter until the age of 5 months, after which pyknotic cells were barely detectable. The ratio of pyknotic to Ki67-positive cells was relatively low at 1 month of age, most likely marking the tail-end of the early postnatal generation of the majority of granule cells (Schlessinger et al., 1975) and a lower cell death rate that allows the addition of neurons during this period, whereas AhN in most studies of laboratory rodents does no longer significantly affect total granule cell number (see Section 4.3). The ratio of pyknotic to Ki67-positive cells is stable between the ages of 2–4 months in comparison with the 1 month-group. Between the ages of 5 and 9 months, the ratio of pyknotic to Ki67-positive cells significantly decreased at a time when cell proliferation and neuronal differentiation are significantly reduced. This decrease in the ratio of pyknotic to proliferating cells might reflect a strategy for coping with the decrease of proliferation and/or for maintaining the newly generated cells (Amrein et al., 2004a). Cell survival has been previously addressed by comparing the number of cells labeled with BrdU 12 days or 4 weeks post-injection to that of cells labeled shortly after the BrdU injection. Although age did not significantly alter the survival of cells in C57 mice, a non-significant increase from 42% at 6 months to 60% at 18 months (Kempermann et al., 1998) and from 27% at 2 months to 46% and 55% at 12 and 24 months (Bondolfi et al., 2004) were reported. In F344 rats, cell survival corresponded to 67% at 4 months and 74% at 12 months (Rao et al., 2006).

4.2. Age and neuronal differentiation

In the adult dentate gyrus, DCX expression starts in type-2b cells of neuronal lineage (Kronenberg et al., 2003; Ehninger and Kempermann, 2008), when stem cell markers (Sox2 and Nestin) are down-regulated (Steiner et al., 2006). The expression of DCX ceases with the appearance of mature neuronal markers (Francis et al., 1999; Brown et al., 2003; Couillard-Despres et al., 2005). In adult mice, ∼25% of the dividing cells are DCX-positive (Kronenberg et al., 2003). A remarkable decrease was qualitatively observed in CD1 mice between the ages of 3–20 months (Jin et al., 2003). Decreases in DCX expression were also observed in rats between the ages of 3, 12, and 24 months (Driscoll et al., 2006), and between the ages of 7.5 and 9 (41%), and 9 and 12 months (>60%, Rao et al., 2006). In F344 rats, Rao et al. reported that the fraction of newborn cells that differentiate into neurons is similar in rats aged 4, 12 and 24 months (Rao et al., 2005). These results agree with the changes in the number of DCX-positive cells with age and with the unchanged ratio
between DCX- and Ki67-positive cells we report here. An unchanged fraction of BrdU labeled cells colocalizing NeuN was also reported in C57 mice between the ages of 12 and 24 months (Bondolfi et al., 2004), indicating that, like in the rat, the fraction of newborn cells differentiating into neurons remains constant throughout the life of rats and mice.

4.3. Effect of aging on the total number of granule cells

Similar to the results obtained in this study, no significant changes in the number of granule cells were found in mice (C57, Calhoun et al., 1998; B6D2F2, Donovan et al., 2006); rats (Long-Evans, Rapp et al., 1996; Fischer 344, Merrill et al., 2003), dogs (Siwak-Tapp et al., 2008), rhesus monkeys (Keuker et al., 2003) and humans (West, 1993; Simic et al., 1997). The daily generation of cells amounts to only 0.2–0.3% of the resident granule cell population in rodents (Cameron and McKay, 2001; Amrein et al., 2004a), and even less in primates (0.004%; Kornack and Rakic, 1999). However, ~50% of these cells, of which more than 80% express neuronal marker, survive for 5 months or more in rats (Kempermann et al., 1998; Bondolfi et al., 2004; Rao et al., 2005). Over the lifespan of the animals, they would add significantly to the granule cell population if cell generation were not balanced by cell death. Comparing data from two studies in C57 mice (Kempermann et al., 1997a, 1998), it was found that the number of granule cells increased by ~40,000 cells between the ages of 2 and 6 months. Notably, this difference (~10%) is smaller than differences between the groups of mice of the same age in this and our previous study (~20% at both 3 and 4 months), which were processed and analyzed in identical ways.

4.4. Absence of gender effects

We did not observe any gender difference within age groups or gender effects on age-related changes. Although the number of animals is rather small in the present study, this observation generally agrees with our previous study (Ben Abdallah et al., 2007) in which we used slightly larger group sizes. Here, we could not replicate a gender difference in the number of DCX-positive cells at the age of 3 months. Also, no gender differences and no differences related to the estrous cycle were found in a recent study of C57Bl/6j mice (Lagace et al., 2007). This is in contrast to observations in voles (Galea and McEwen, 1999; Ormerod and Galea, 2003), while data obtained in rats are equivocal, with both reports supporting gender differences (Perfilieva et al., 2001) or the lack thereof (Falconer and Galea, 2003; Greaves et al., 2005).

4.5. Mechanisms and consequences of age-related changes in AhN

Changes in progenitor cell number or changes in cell cycle length may both affect proliferation and neurogenesis. An age-dependent decrease in cell proliferation was not accompanied by a significant change in cell cycle length, which was ~39 h at 10 weeks and ~30 h in 10 months old rats (Olariu et al., 2007). Also, the number of Vimentin-GFAP-positive cells (putative stem cells) was reported to decrease significantly in the dentate gyrus between 2–3 and 12–14 months old rats (Alonso, 2001). In contrast, an age-related lengthening of the cell cycle was suggested by an increased fraction of Sox2-positive putative stem cells which did not express proliferation markers or were labeled by BrdU in 12 and 24 months old F344 rats when compared to 4 months old animals (Hattiangady and Shetty, 2008), while the number of SOX2-positive cells remained stable.

Multiple factors have been held responsible for the age-related decline of AhN, many of which implicate an adverse microenvironment for the precursor cells. The vascular environment, which is affected by aging (Farkas and Luiten, 2001), is an important regulator of cell proliferation (Palmer et al., 2000). Cellular and molecular mediators of inflammation have been reported to increase in the aging brain (Krabbe et al., 2004; for review see Conde and Streit, 2006) and were linked to changes in AhN subsequent to irradiation experiments (Monje and Palmer, 2003; Monje et al., 2003). However, micro- and astroglial cell numbers did not differ in animals aged 4–5, 13–14, and 27–28 months (Long et al., 1998). Aging is also associated with elevated basal corticosterone levels (Landfield et al., 1978; Sapolsky, 1985; Lupien et al., 1998; Montaron et al., 1999, 2006) which decreases AhN (Mirescu and Gould, 2006), while removal of adrenal steroids is associated with a restoration of AhN in aged rats (Cameron and McKay, 1999; Montaron et al., 1999, 2006). The age-related decrease of AhN can be reversed by central administration of growth and neurotrophic factors, some of which have been found to decrease with advancing age (Lichtenwalner et al., 2001; Cheng et al., 2002; Jin et al., 2003; Hattiangady et al., 2005; Shetty et al., 2005).

Studies of behavior after the ablation of AhN have found effects on specific aspects of hippocampus-dependent tasks (Seigers et al., 2008; Zhang et al., 2008), which supports the suggested involvement of AhN in hippocampal function based on correlated changes of AhN and behavior after indirect experimental manipulations (see Leuner et al., 2006). With the consolidation of a role of AhN in hippocampal function, correlated changes in AhN and behavior with age (Van Praag et al., 2005) have become more likely to be causally related, and AhN is a promising target for experimental manipulation that could remedy specific aspects of age-related cognitive dysfunctions.

4.6. Conclusion

We show that large differences in proliferation and neurogenesis can be observed over much shorter intervals than used previously, which often range between 6 and 9 months (Kempermann et al., 1998; Harrist et al., 2004; Kronenberg et al., 2006). Experimental manipulations of adult neurogenesis, which extend over periods that would...
have been considered relatively brief, will have to cope with a background of large, natural changes of proliferation and neurogenesis and possible interactions with the factors governing these changes. Life history periods cannot be defined by changes in the rate of proliferation or the number of cells of neuronal lineage alone.

We found changes in the ratio between dying and proliferating cells between the ages of 1 and 2 months and, again, between 4 and 5 months. The first interval contains the time point at which mice enter their reproductive period of life (Berry and Bronson, 1992). The second change is positioned slightly beyond the average life expectancy of mice that entered the adult population in natural, non-commensal habitats, which may be as short as 100 days (Berry and Bronson, 1992). To consider mice at the age of 5 months ‘old’ may be premature, although we observed a corresponding change in the ratio between dying and proliferating cell numbers only in very old yellow-necked wood mice (Amrein et al., 2004a).

However one may classify these periods, changes in this ratio at specific time points may be possible confounders in experiments that aim at altering cell survival, but, again, may also allow the study of the natural mediators of these changes.

Conflict of interest

None.

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References


