Murine Features of Neurogenesis in the Human Hippocampus across the Lifespan from 0 to 100 Years

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Abstract

Background: Essentially all knowledge about adult hippocampal neurogenesis in humans still comes from one seminal study by Eriksson et al. in 1998, although several others have provided suggestive findings. But only little information has been available in how far the situation in animal models would reflect the conditions in the adult and aging human brain. We therefore here mapped numerous features associated with adult neurogenesis in rodents in samples from human hippocampus across the entire lifespan. Such data would not offer proof of adult neurogenesis in humans, because it is based on the assumption that humans and rodents share marker expression patterns in adult neurogenesis. Nevertheless, together the data provide valuable information at least about the presence of markers, for which a link to adult neurogenesis might more reasonably be assumed than for others, in the adult human brain and their change with increasing age.

Methods and Findings: In rodents, doublecortin (DCX) is transiently expressed during adult neurogenesis and within the neurogenic niche of the dentate gyrus can serve as a valuable marker. We validated DCX as marker of granule cell development in fetal human tissue and used DCX expression as seed to examine the dentate gyrus for additional neurogenesis-associated features across the lifespan. We studied 54 individuals and detected DCX expression between birth and 100 years of age. Caveats for post-mortem analyses of human tissues apply but all samples were free of signs of ischemia and activated caspase-3. Fourteen markers related to adult hippocampal neurogenesis in rodents were assessed in DCX-positive cells. Total numbers of DCX expressing cells declined exponentially with increasing age, and co-expression of DCX with the other markers decreased. This argued against a non-specific re-appearance of immature markers in specimen from old brains. Early postnatally all 14 markers were co-expressed in DCX-positive cells. Until 30 to 40 years of age, for example, an overlap of DCX with Ki67, Mcm2, Sox2, Nestin, Prox1, PSA-NCAM, Calretinin, NeuN, and others was detected, and some key markers (Nestin, Sox2, Prox1) remained co-expressed into oldest age.

Conclusions: Our data suggest that in the adult human hippocampus neurogenesis-associated features that have been identified in rodents show patterns, as well as qualitative and quantitative age-related changes, that are similar to the course of adult hippocampal neurogenesis in rodents. Consequently, although further validation as well as the application of independent methodology (e.g. electron microscopy and cell culture work) is desirable, our data will help to devise the framework for specific research on cellular plasticity in the aging human hippocampus.

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Introduction

Adult hippocampal neurogenesis, i.e. the production of new granule cell neurons in the adult hippocampus, has captured the imagination of a wide audience and is beginning to influence hypotheses for clinical medicine. Adult neurogenesis is conserved in all mammalian species studied so far including non-human primates [1,2,3,4], curiously except for most bat species [5]. Detection of newborn granule cells is generally based on the stable incorporation of S-phase marker bromodeoxyuridine (BrdU) into the DNA of a dividing precursor cell and the later immunohistochemical visualization of BrdU in a neuron [6]. Whereas this method is applicable in animal experiments, the detailed description of adult neurogenesis in humans has been limited by the fact that experiments with humans are impossible. The Eriksson study [7] relied on the opportunity that patients had received BrdU for tumor staging purposes within a treatment study. Some of these patients consented to have their brains examined after their death. This rare situation allowed to study adult neurogenesis in humans with the methods established for animals. BrdU incorporation was found in hippocampal granule cells in human individuals as old as 72 years. The Eriksson study
was complemented by the discovery of neural precursor cells in surgical specimens from adult human hippocampus [8,9,10,11]. Because of the immense medical implications of adult neurogenesis in humans we intended to find additional information about neuronal development in the adult human dentate gyrus (DG) despite the prevailing limitations and also extended the analysis to the entire lifespan. Several studies have confirmed that adult neurogenesis is present even in the old rodent brain [6,12,13] but decreases strongly in early adulthood and remains on a low level thereafter [1,14,15,16].

Adult hippocampal neurogenesis in mice has been described in considerable detail, and distinct developmental stages have been identified [17]. A central phase during this development is associated with the expression of doublecortin (DCX) [18,19,20]. This phase ranges from a progenitor cell stage to the calretinin-positive period, during which dendrites and axons of the new cells establish functional connections [21].

DCX is a brain-specific microtubule-associated protein whose exact function is not yet known. It appears to act as microtubule stabilizer in a way that is particularly pertinent to migration [22,23]. Its presence in the tips of neurites of apparently non-migratory yet immature neurons suggest an additional role in neurite development [24]. Mutation in the human DCX gene causes a characteristic defect in cortical layering, the name-giving doublecortex [25,26].

DCX can serve as a marker for new neurons in the adult rodent hippocampus as long as the transient nature of its expression is taken into account. In addition, DCX is expressed elsewhere in the rodent (and human) brain, where no link to adult neurogenesis can be made [27,28,29]. DCX alone is not specific for adult neurogenesis. Nevertheless, DCX has already been used to detect neurogenesis in the human brain [30,31] but in those studies, the validity of the marker was only inferred from rodent data and has been questioned [27,32]. While it is undisputed that true validation for the human hippocampus would require exactly the methods used by Eriksson et al., we reasoned that DCX expression in the DG across the lifespan might be used to generate important information about the combined presence of those parameters in the human dentate gyrus that are known to relate to adult neurogenesis in mice and rats. The underlying assumption is that if the patterns of such features in humans and rodents were highly similar this would also be indicative of a common function. Such functionality can obviously not be proven with the present methodology but even the descriptive part that we can deliver is much more than has been obviously not be proven with the present methodology but even the descriptive part that we can deliver is much more than has been.
also similar to the depiction in the Alan Brain Atlas for the murine hippocampus (www.brain-map.org, image series 70946414).

We focused on DCX expression in the DG. DCX-positive (DCX+) cells in the DG could be found in samples across the entire lifespan between 1 day and 100 years of age (exemplary in Fig. 4 and Fig. S3; list of subjects in Table S2). The hilar border of the granule cell layer did not appear as sharp as it is in rodents (see Fig. 4, G-K for comparative images from the mouse brain). No clear subgranular zone (SGZ) could be distinguished (Fig. 4C, D). In young individuals many DCX+ cells showed the dendritic features of immature neurons (Fig. 5), which were not as readily identifiable at older age. However, in both species DCX expressing cells with the morphology of maturing granule cells can be found even at old age, further supporting that no old neurons re-express DCX (Fig. 4D and K). We found co-labeled DCX-positive cells in both male and female specimens but our sample size was too low to address the question of potential gender differences.

Decreasing Evidence of Neuronal Development in the Aging Human Hippocampus

We next established whether DCX expression in the human DG could be linked to other markers that in the rodent are associated with adult hippocampal neurogenesis (Complete list in Table S4). The findings are summarized in Fig. 6, examples are shown in Figs. 7 and 8.

We first examined markers for cell proliferation. PCNA expression in DCX+ cells could be detected at all ages (Fig. 7B). In contrast, we did not detect Ki67 immunoreactivity later than 38 years (Fig. 7A) and Mcm2 later than 65 years (Figs. 6, 7L, 8M). This implies that with advanced age, the level of evidence for ongoing proliferation of DCX positive cells is lower. But in contrast to PCNA (Fig. S1), Mcm2 and Ki67 have not been connected with false-positive signals due to apoptotic cell death [35,36]. Hence, especially the detection of DCX+/Mcm2+ cells is a relatively strong indicator of persistent proliferation of DCX+ cells (Fig. 8M) — however, in our samples, not beyond the age of 65.

Based on our previous studies in rodents and reports from the literature we next focused on nestin [37,38], PSA-NCAM [39], NeuroD, Sox2 [40], Prox1 [41], Tuc4 [39], β-III-tubulin, NeuN, and calretinin [21]. These markers could be found in combination with DCX, as predicted from the rodent data [40]. As with the proliferation markers we found that with increasing age fewer of the additional markers showed unambiguous immunoreactivity.
Sox2 is a precursor cell marker that in rodents can be detected in a proportion of hippocampal progenitor cells [42], including a low percentage of DCX+ type-2b and type-3 cells [40]. We found examples of Sox2 in DCX+ cells as late as 79 years of age (Fig. 7E).

Prox1 expression, a transcription factor closely related to granule cell development in rodents [43,44], was found in DCX+ cells across the entire lifespan (Figs. 6 and 7A, F, H). Calretinin, which shows a partial overlap with DCX in the later phase of neuronal development [21] was detected up to the age of 100 years (Fig. 7H). An overlap between DCX and NeuN, characterizing early postmitotic neurons, was found up to 85 years (Figs. 6, 7G, 8N).

Although being glutamatergic neurons, granule cells can co-express GABA and GABA synthesizing enzymes. We detected GAD67/DCX co-localization up to 89 years of age (Fig. 6). GAD65 is primarily expressed in axonal terminals [45]. The fact that the developing new neurons receive early synaptic GABAergic input [46] prompted us to search for evidence of GAD65-positive terminals on DCX+ cells. We found examples such as those presented in Fig. 7K.

Age-Related Decrease in the Number of DCX-Positive Cells

Finally, we attempted a semi-quantitative assessment of neurogenesis in the human DG (including the hilar polymorphic layer) across the lifespan. We found that the density of DCX+ cells showed a log-log linear course of the regression curve over the period of life (Fig. 9). Although we found DCX-PCNA double positive cells even at the oldest age (Figs. 6, 7B), about 20% of the PCNA-positive cells showed a co-localization with GFAP (Fig. 8O, Table S3). The nature of the remaining cells could not finally be determined because cell type specific markers are rarely expressed.
Expression Patterns of Neurogenesis-Associated Markers

Adult hippocampal neurogenesis in mice and rats shows a sequence of marker expressions that has been described in increasing detail [17,40,44,48,49]. Figure 10 summarizes this pattern for the scope of markers used in this study.

Presence of stem-like cells was suggested by the detection of Sox2 [50], but no radial glia-like cells, the presumed stem cells in the DG of rodents [37,51] were found. Most studies find that these cells are lacking in the DG of old mice as well, in which neurogenesis is unambiguously detectable. We have postulated that cells with radial glia-like properties but without radial morphology (type-2a), some of which are Sox2+, might act as stem cells in the DG [40]. Again, this remains to be proven, notably for the human brain. But in our sample Sox2 (as well as nestin and Prox1) remained expressed in some DCX-positive cells up to oldest age. Sox2 is expressed in neural stem cells but is not specific to them. Sox2 is also expressed in some astrocytic populations without any known or plausible function in neurogenesis [40].

Because precursor cells are by definition proliferating cells markers of cell division help to substantiate the nature of the DCX-positive cells. Reif et al., who based their conclusions on adult human neurogenesis on immunohistochemistry for proliferation marker Ki67 and studied 60 samples between 25 and 68 years, did not detect positive cells in all cases and found Ki67 up to 62 years ([52] and personal communication). In our sample, Ki67 was not detectable in DCX+ cells beyond the age of 38 and Mcm2 after age 65. On the other hand, the number of dividing DCX+ cells was also extremely low in the aging mouse hippocampus [53], despite ongoing neurogenesis as demonstrated in several studies [54].

In rodents, the phase of DCX expression is also the phase during which cells are eliminated by cell death [55,56,57]. There is no indication, however, that DCX itself could be linked to the control of cell death. Quite contrary, the function of DCX is generally seen in maturation processes responding to extrinsic stimuli [23], most notably cell migration and neurite extension, all of which we find in the DCX phase of adult neurogenesis in rodents.

DCX is associated with neurogenesis in the dentate gyrus of adult rodents but expression of DCX is not limited to the context of adult neurogenesis. The overlap with other markers corroborates the idea that the link might exist in the human dentate gyrus as well but in humans as in rodents, DCX is not a neurogenesis marker with high specificity. In the rodent hippocampus its sensitivity is high.

In the murine dentate gyrus DCX shows a nearly complete overlap with the expression of the polysialilated form of the Neural Cell Adhesion Molecule (PSA-NCAM). [19,39]. We here could confirm that PSA-NCAM can be found immunohistochemically with DAB as chromogen in progenitor cells in the human dentate gyrus (Fig. S3), but PSA-NCAM specific immunofluorescence is considerably more problematic than for DCX. There is, however, a body of literature on PSA-NCAM expression in the adult human brain related to pathology or plasticity [30,58,59,60]. To relate these observations directly to adult neurogenesis is as problematic as in the case of relying on DCX alone. The extensive study by Ni Dhuill et al. covering 13 samples from 7 months to 82 years also suggest that besides PSA-NCAM expression in “granule-like cells and their mossy fiber axons” prevailing in young age, other expression sites in the hippocampus become more prominent with increasing age [61]. This raises an important caveat also pertinent to our study, which we have attempted to overcome by co-applying a panel of altogether 24 markers.

Of particular interest are the round-shaped DCX-positive cells, which from their morphology and co-marker expression might relate to the DCX-positive progenitor cells, type-2b and -3 that have been described for the rodent brain. This extrapolation is speculative because further information about the exact course of adult neurogenesis would be needed. Our data might provide at first indication. More detailed analysis is hindered by the fact, however, that these cells are only a subset of all DCX-positive cells and in any given section only few markers can be tested. To describe the course of adult neurogenesis and its dynamics a study based on a cohort of cells that can be followed through the stages of development would be needed. This, however, requires the BrdU-method (or a virus-based approach), which is not readily applicable to humans.
If we take the BrdU-method as the Gold standard (which appears to be generally accepted in the field), there is currently no evidence of adult hippocampal neurogenesis in humans older than 72 years, the oldest sample in the Eriksson study. Because a new...
BrdU-based study in even older subjects is unlikely to be presented anytime soon, surrogate markers and cumulative supportive evidence gains weight for this age bracket. Single markers will generally not suffice to prove or disprove adult neurogenesis in a particular human sample but contextual information as offered here might help to judge these cases. The use of markers is generally affected by the many caveats associated to investigating human post mortem samples. Combination of markers will decrease the likelihood of false-positive results, but the most serious concern is that with increasing age, markers that in young age are indeed associated with neurogenesis might be increasingly indicative of degeneration and cell death. DNA-synthesis [62] and other cell-cycle-related events have been brought into connection with neuronal cell death in hypoxia and, for example, Alzheimer disease [63]. Also, degenerating cells, more frequently in older brains, might (re-) express immature markers and falsely suggest developmental events. We thus took great care to avoid the detection of false-positive signals due to hypoxic damage or other alterations in relation to the post-mortem interval.

In any case, however, and contrary to the apprehension that with increasing age the amount of non-specific labeling and false-positive marker expression might generally increase, we found a reduction in DCX expression and a reduction in marker overlap. In no case, overlaps not present at a younger age were found in old age. To our knowledge ours is the first study to undertake the analysis of so many histological markers in human samples and this marker loss by itself constitutes an interesting result. Most published studies have been concerned with the age-dependent appearance of histological features, most notably neurofibrillary tangles, senile plaques, deposition of age-pigment, or the increased proliferation of macro- and microglial cells.

Still, the subjects in our series were not healthy but died for extracerebral reasons listed in Table S2. One might reasonably postulate that the data obtained from our samples might rather reflect an underestimate of neurogenesis-associated features. The suggestive presence of DCX-positive cells co-expressing progenitor cell markers even at oldest age might thus indicate that these cells are actually fairly robust against adverse events and the problems associated with the analysis of postmortem tissue.

PCNA is co-factor of DNA polymerases and is expressed during G1 and S phase of the cell cycle. PCNA is critically involved in DNA replication [64] and has thus been established and is widely used as a proliferation marker. Unexpectedly, our semi-quantitative data on PCNA expression did not reveal a substantial change across the age span (but note that the assessment was semi-quantitative and did not obey stereological rules). These data cannot serve as more than an invitation for further studies. They might indicate the increase in astrocytic proliferation in aged brains, although we did not see a substantial overlap with GFAP. PCNA as “proliferation marker” is also problematic in that it might stay on in postmitotic cells [65] and also plays a role in DNA repair [66,67]. Despite these issues and in the absence of a clear interpretation we did not want to withhold these data.

**Decrease in Neurogenesis-Associated Features with Increasing Age**

We also assessed the temporal change in the number of DCX+ cells with age and found an exponential decrease, again similar to the decrease in neurogenesis in rodents [1,14,16] and non-human primates [68]. The exponential decrease found in our analysis of DCX-positive cells across the lifespan draws a suggestively similar picture than the related rodent studies. In those, the decrease in adult hippocampal neurogenesis is steepest during the first 3 to 6 months of age and reaches very low and relatively constant levels thereafter [14,16]. Extrapolated to the human condition and scaled to the human lifespan we might expect that the detectability of neurogenesis-associated features should be highest during adolescence and young adulthood.

In fact we found in the present study that the panel of overlapping markers was exhaustive up to 30 to 40 years (Fig. 6). At that time the overlap comprised Ki67, Mcm2, Sox2, nestin, Prox1, PSA-NCAM, calretinin, and NeuN, essentially the key markers (besides BrdU incorporation), on which most rodent studies are based. This age also coincides with the age suggested by...
a study using MR spectroscopy to assess adult neurogenesis in the adult human brain [69].

Stereological studies of the human DG, covering samples up to 101 years of age, determined that the number of granule cells in humans is very stable across the lifespan but varies greatly [70,71]. Given the age-related decline in hippocampal functioning in the absence of dementia, our demonstration of a loss of markers that at least in rodents are related to particular aspects of brain plasticity deserves further analysis. We and others have observed a similar marker loss in the aging rodent hippocampus, although the set of data is far from being conclusive and more studies are needed.

The reduced presence of neurogenesis-associated features in the aged human hippocampus suggests that, as in rodents, neurogenesis is likely to occur on a very low scale, at least if compared to younger age. One of the intriguing questions is thus, how so few new neurons might be functionally beneficial at all. The alternative position to this approach is the theory that because in the course of evolution the ability to produce neurons in adulthood became increasingly restricted, humans are different from animals in that their hippocampus does not rely on this option to alter its neuronal network structure any longer [72].

We have proposed models that help to explain why already very few neurons might make relevant functional contributions in the particular network situations of the hippocampus and we believe that such models also explain why postnatal and young-adult neurogenesis is necessary but increasingly dispensable with increasing age [73,74]. Also, even absence in oldest age would not argue against a functional role at younger age.

Conclusions

Overall, our results suggest a pattern of adult neurogenesis in the aging human dentate gyrus that shows large similarities to rodents. Our data alone cannot prove or disprove the true presence or absence of neurogenesis at any age and the consideration of isolated samples might be misleading. Our key results lie in the fact that we see an expression pattern for key markers for adult neurogenesis also in the human brain and that this expression pattern changes with time both qualitatively and quantitatively.

Material and Methods

Full description of the experimental procedures can be found in the supplemental material (Text S1).

Specimens

Autopsy cases were selected from the archive at the Department of Neuropathology, University Hospital, Freiburg, Germany, according to age, sex, postmortem interval and lack of clinical or postmortem evidence of neuropathology. Paraffin sections from 3 fetal brain tissue samples (GW11, 20 and 40), hippocampal pieces from a patient suffering from temporal lobe epilepsy and from 51 deceased persons without central nervous damage aged from 1 day to 100 years were analyzed (Table S2).

Western Blotting and In Situ Hybridization

Western blotting and in situ hybridization were performed as described previously [75]. To ascertain the specificity of doublecortin antibody and cRNA anti sense probe in juvenile and adult human hippocampal tissues we included human fetal (GW 11, 20) brain tissue as positive control and reference for western blotting (Fig. 1), in situ hybridization (Figs. 2), and RT-PCR (Fig S5).

Immunochemistry

Standard protocols were followed. The detailed description is found in the Supplementary Material. Antibodies are listed Table S5. DCX-immunohistochemistry on mouse tissue was performed as described previously [40]. Negative controls were performed by omitting primary antibodies (see Fig. S2C). They did not show any fluorescence signals. DCX-immunohistochemistry on mouse tissue was performed as described previously [57].

Supporting Information

Text S1 Supplemental material and methods. Found at: doi:10.1371/journal.pone.0008809.s001 (0.06 MB DOC)

Figure S1 Test of juvenile and adult human GCL specimens for potential perimortal hypoxia. A, No co-expression of DCX with the Heat Shock Protein 27 (HSP27) was detected and no signs of microglia activation (CD68) were found in the samples. B, Similarly, microglial marker Glut1 and Hypoxia-Induced Factor 1α (HIF-1α) were not increased. C, Matrix MetalloProteinases (MMP) are important for migration of young cells within established neural networks. Here, MMP9 shows strong co-labeling with DCX, whereas the same cell is not stimulated to express Vascular Endothelial Growth Factor A (VEGFA), which would be indicative of a hypoxic milieu. D, Proliferation markers like PCNA might show expression in cells undergoing apoptosis. Here, a DCX+ cell in a 2 month-old GCL presents signs of programmed cell death by activated-Caspase-3 (Casp-3). E, Whereas apoptosis is very physiologic in neonatal age and crucial for neuronal network consolidation, the DG of a 58-years-old subject revealed no signs of degradation. The lack of PCNA- and activated-caspase-3 labeling in the presented cell argues against the interpretation that DCX expression in higher age is induced by stress factors. Scale bars, 5 μm and 10 μm as indicated. Found at: doi:10.1371/journal.pone.0008809.s002 (0.33 MB JPG)

Figure S2 A, B, Proof of reactivities of anti activated-caspase-3 (A) and anti heat-shock protein HSP27 antibodies (B) in a glioblastoma multiforme specimen. Positive cells are devoid of DCX and PCNA immunoreactions, resp. C, Technical control incubation: Omitting primary antibodies, the incubation of serial sections with secondary antibodies alone failed to generate any specific fluorescence signal. D, E, Search for DCX expressing neuronal cells outside the hippocampus. Bipolar DCX+ cells could be detected in the parietal neocortical parenchyma (LII) (D) as well as in the temporal migratory stream (TMS) of the piriform Cortex/Amygdala (E). There are neither co-labelings with the microglial marker CD68 and the heat-shock-protein HSP27 nor with the cell type specific markers NeuN and GFAP.

Figure S3 PSA-NCAM expression. PSA-NCAM is expressed in a small hippocampal SGZ cell of a 68 years-old male. DAB staining. Found at: doi:10.1371/journal.pone.0008809.s003 (56.30 MB TIFF)

Figure S4 Estimation of PCNA+ cell densities in the DG across the entire human lifespan. Comprising data from 25 patients, aged between 1 day and 100 years (see Table S1), the number of labeled cells in the GCL were plotted against the age of the individual. The regression curve enables a log-log-linear interpretation. PCNA+ cell densities increase slightly with age (PPC: 0.154; p = 0.463).
Figure S5 Doublecortin mRNA. Generation, purification and cloning of human DCX-specific cDNA targets aimed to generate DIG-labeled cRNA probes for northern blotting and in situ hybridization. A, After bulk separation in a preparative 1.5% agarose gel, the fetal brain DNA DCX-PCR product (580 bp, lane 2) was recovered and in B, ligated into a pSP19 vector (3.104 bp, lane 1), cloned and amplified for correct insertion by digestion with EcoRI and HindIII resulting in a linearized vector (3.104 bp) and the DCX insert (lane 2) designed for the cRNA-DIG probe generation by in vitro transcription. C, Separation of total RNA from fetal brain in an ethidium bromide stained band of DCX mRNA was visible at the correct position of 9.54 kb.

References
1. Altman J, Das GD (1965) Autoradiographic and histological evidence of postnatal hippocampal neurogenesis in rats. J Comp Neurol 124: 519–333.
2. Kornack DR, Rakic P (1999) Continuation of neurogenesis in the hippocampus of the macaque monkey. Proc Natl Acad Sci USA 96: 5768–5773.
3. Gould E, Reeves AJ, Fialah M, Tanapat P, Gross CG, et al. (1999) Hippocampal neurogenesis in adult old world primates. Proc Natl Acad Sci USA 96: 5263–5267.
4. Kempermann G, Kuhn HG, Gage FH (1997) More hippocampal neurons in adult mice living in an enriched environment. Nature 386: 493–495.
5. Amrein I, Dechmann DK, Winter Y, Lipp HP (2007) Absent or low rate of adult neurogenesis in the hippocampus of bats (Chiroptera). PLoS ONE 2: e2455.
6. Kuhn HG, Dickman-Anon H, Gage FH (1996) Neurogenesis in the dentate gyrus of the adult rat: age-related decrease of neuronal progenitor proliferation. J Neurosci 16: 2027–2033.
7. Eriksson PS, Perfilieva E, Bjork-Eriksson T, Alborn AM, Nordberg G, et al. (1998) Neurogenesis in the adult human hippocampus. Nat Med 4: 1313–1317.
8. Roy NS, Wang S, Jiang L, Kang J, Benraiss A, et al. (2000) In vitro neurogenesis by progenitor cells isolated from the adult human hippocampus. Nat Med 6: 271–277.
9. Hermann A, Maiel M, Liebho S, Gerlach M, Klegger A, et al. (2006) Mesodermal cell types induce neurogenesis from adult human hippocampal progenitor cells. J Neurochem 98: 629–640.
10. Palmer TD, Schwartz PH, Taupin P, Kaspar B, Stein SA, et al. (2001) Cell culture. Progenitor cells from human brain after death. Nature 411: 42–45.
11. McE, Vershese M, Danilow AI, Westerlund U, Ramm-Petersen J, et al. (2005) Multipotent progenitor cells from the adult human brain: neurophysiological differentiation to mature neurons. Brain 128: 2189–2199.
12. Cameron HA, McKay RD (1999) Restoring production of hippocampal neurons in old age. Nat Neurosci 2: 894–897.
13. Montaron MF, Petry KG, Rodriguez JJ, Marinelli M, Aurousseau C, et al. (1998) Adrenalecromy increases neurogenesis but not PSA-NCAM expression in aged dentate gyrus. Eur J Neurosci 7: 1479–1485.
14. Ben Abdulmalik S, Slomianka L, Vyssotski AL, Kuhn HG (2008) Early age-related changes in hippocampal neurogenesis in C57 mice. Neurobiol Aging; doi:10.1016/j.neurobiolaging.2008.1001.1002.
15. McDowall H, Wojtowicz JM (2005) Dynamics of neurogenesis in the dentate gyrus of adult rats. Neurosci Lett 350: 70–75.
16. Kronenberg G, Bick-Sander A, Bunk E, Wolf C, Elnminger D, et al. (2006) Physical exercise prevents age-related decline in precursor cell activity in the mouse dentate gyrus. Neurobiol Aging 27: 1505–1513.
17. Kempermann G, Jesberger S, Steiner B, Kronenberg G (2004) Milestones of neuronal development in the adult hippocampus. Trends Neurosci 27: 447–452.
18. Couillard-Despres S, Winner B, Schaubeck S, Aguirre G, Vroemen M, et al. (2005) Doublecortin expression levels in adult brain reflect neurogenesis. Eur J Neurosci 21: 1–14.
19. Nacher J, Crespo C, McEwen BS (2001) Doublecortin expression in the adult rat telencephalon. Eur J Neurosci 14: 629–644.
20. Rao MS, Shetty AK (2004) Efficacy of doublecortin as a marker to analyse the absolute number and dendritic growth of newly generated neurons in the adult dentate gyrus. Eur J Neurosci 19: 234–246.
21. Brandt MD, Jesberger S, Steiner B, Kronenberg G, Reuter K, et al. (2003) Transient calretinin expression defines early postmitotic step of neuronal differentiation in adult hippocampal neurogenesis of mice. Mol Cell Neurosci 20: 603–613.
22. Gleeson JG, Lim PT, Flanagan LA, Walsh CA (1999) Doublecortin is a microtubule-associated protein and is expressed widely by migrating neurons. Neuroreport 23: 257–271.
23. Weiner JM, Antoun ES (2006) Doubling up on microtubule stabilizers: synergistic functions of doublecortin-like kinase and doublecortin in the developing cerebral cortex. Neuron 49: 3–4.
24. Friocourt G, Koukaloff A, Chafey P, Boucher D, Fauchereau F, et al. (2003) Doublecortin functions at the extremities of growing neuronal processes. Cereb Cortex 13: 620–626.
25. Gleeson JG, Allen KM, Fox JW, Lamperth EI, Berkovic S, et al. (1998) Doublecortin, a brain-specific gene mutated in human X-linked lissencephaly and double cortex syndrome, encodes a putative signaling protein. Cell 92: 63–72.
26. Gleeson JG, Minnerath SR, Fox JW, Allen KM, Luo RF, et al. (1999) Characterization of mutations in the gene doublecortin in patients with double cortex syndrome. Ann Neurol 45: 146–153.
27. Verwer RW, Shifter AA, Bialek RA, Baeyen JC, Nuske DP, et al. (2007) Mature astrocytes in the adult human neocortex express the early neuronal marker doublecortin. Brain 130: 3321–3335.
28. Nacher J, Alonso-Llosa G, Rosell DR, McEwen BS (2003) NMDA receptor antagonist treatment increases the production of new neurons in the aged rat hippocampus. Neurobiol Aging 24: 273–284.
29. Liu YW, Curtis MA, Gibbons HM, Mee EW, Bergin PS, et al. (2008) Doublecortin expression in the normal and epileptic adult human brain. Eur J Neurosci 26: 2254–2265.
30. Jin K, Peel AL, Mao XO, Xie L, Cotrell BA, et al. (2004) Increased hippocampal neurogenesis in Alzheimer’s disease. Proc Natl Acad Sci U S A 101: 343–347.
31. Fahrner A, Kaun G, Flubacher A, Heinrich C, Freiman TM, et al. (2007) Granule cell dispersion is not accompanied by enhanced neurogenesis in temporal lobe epilepsy patients. Exp Neurol 203: 320–332.

32. Boekhoff K, Joels M, Lucassen PJ (2006) Increased proliferation reflects glial and vascular-associated changes, but not neurogenesis in the prion-like Alzheimer hippocampus. Neuro報 24: 1–14.

33. Seki T, Arai Y (1995) Age-related production of new granule cells in the adult dentate gyrus. Neuroreport 6: 2479–2482.

34. Ratt J, Pallini C, Cova I, Fantozzi R, Calarossa G, et al. (2006) A role for the ELAV RNA-binding proteins in neural stem cells: stabilization of Msi1 mRNA. J Cell Sci 119: 1442–1452.

35. Lindner K, Gergen J, Montgomery S, Krayevsky SE (2002) Essential role of MCM proteins in premeiotic DNA replication. Mol Biol Cell 13: 435–444.

36. Osaki M, Osaki M, Yamashita H, Shomori K, Yoshida H, et al. (2006) Expression of minichromosome maintenance-2 in human malignant fibrous histiocytomas: Correlations with Ki-67 and F53 expression, and apoptosis. Int J Mol Med 10: 161–168.

37. Filippov V, Kronenberg G, Pinueva T, Reuter K, Steiner B, et al. (2003) Subpopulation of nestin-expressing progenitor cells in the adult murine hippocampus shows electrophysiological and morphological characteristics of astrocytes. Mol Cell Neurosci 23: 373–382.

38. Fukuda S, Kato F, Tozuka Y, Yamaguchi M, Miyamoto Y, et al. (2003) Two distinct subpopulations of nestin-positive cells in adult mouse dentate gyrus. J Neurosci 23: 9357–9366.

39. Seki T (2002) Expression patterns of immature neuronal markers PSA-NCAM, CRMP-4 and NeuRoD in the hippocampus of young adult and aged rodents. J Neurosci Res 70: 327–334.

40. Steiner B, Klempp F, Wang L, Kott M, Kettemann H, et al. (2006) Type-2 cells as link between glial and neuronal lineage in adult hippocampal neurogenesis. Glia 54: 801–814.

41. Pleasure SJ, Collins AE, Lowenstein DH (2000) Unique expression patterns of cell fate molecules delineate sequential stages of dentate gyrus development. J Neurosci 20: 6095–6105.

42. Komitova M, Eriksson PS (2004) Sox-2 is expressed by neural progenitors and astroglia in the adult rat brain. Neurosci Lett 369: 24–27.

43. Pleasure SJ, Anderson S, Hevner R, Bagri A, Marin O, et al. (2000) Cell migration from the ganglionic eminences is required for the development of hippocampal GABAergic interneurons. Neuron 28: 727–740.

44. Steiner B, Zurborg S, Horster H, Fabel K, Kempermann G (2008) Differential 24 h responsiveness of Prox1-expressing precursor cells in adult hippocampal neurogenesis. J Neurosci Res 79: 1076–1085.

45. Pleasure SJ, Collins AE, Lowenstein DH (2000) Unique expression patterns of cell fate molecules delineate sequential stages of dentate gyrus development. J Neurosci 20: 6095–6105.

46. Komitova M, Eriksson PS (2004) Sox-2 is expressed by neural progenitors and astroglia in the adult rat brain. Neurosci Lett 369: 24–27.

47. Pleasure SJ, Anderson S, Hevner R, Bagri A, Marin O, et al. (2000) Cell migration from the ganglionic eminences is required for the development of hippocampal GABAergic interneurons. Neuron 28: 727–740.

48. Steiner B, Zurborg S, Horster H, Fabel K, Kempermann G (2008) Differential 24 h responsiveness of Prox1-expressing precursor cells in adult hippocampal neurogenesis. J Neurosci Res 79: 1076–1085.

49. Pleasure SJ, Anderson S, Hevner R, Bagri A, Marin O, et al. (2000) Cell migration from the ganglionic eminences is required for the development of hippocampal GABAergic interneurons. Neuron 28: 727–740.

50. Steiner B, Zurborg S, Horster H, Fabel K, Kempermann G (2008) Differential 24 h responsiveness of Prox1-expressing precursor cells in adult hippocampal neurogenesis. J Neurosci Res 79: 1076–1085.

51. Seri B, Garcia-Verdugo JM, McEwen BS, Alvarez-Buylla A (2001) Astrocytes undergo terminal differentiation and give rise to new neurons in the adult mammalian hippocampus. J Neurosci 21: 514–522.

52. Reif A, Fritzen S, Finger M, Strobel A, Lauer M, et al. (2006) Neural stem cell proliferation is decreased in schizophrenia, but not in depression. Mol Psychiatry 11: 514–522.

53. Garcia A, Steiner B, Kronenberg G, Bick-Sander A, Kempermann G (2004) Age-dependent expression of glucocorticoid- and mineralocorticoid receptors on neural precursor cell populations in the adult murine hippocampus. Aging Cell 3: 363–371.

54. Kempermann G, Kuhn HG, Gage FH (1998) Experience-induced neurogenesis in the senescent dentate gyrus. J Neurosci 18: 3206–3212.

55. Biedl M, Cooper CM, Winkler J, Kuhn HG (2000) Analysis of neurogenesis and programmed cell death reveals a self-renewing capacity in the adult rat brain. Glia 31: 17–20.

56. Kuhn HG, Biebl M, Wilhelm D, Li M, Friedlander RM, et al. (2005) Increased generation of granule cells in adult Bel-2-overexpressing mice: a role for cell death during continued hippocampal neurogenesis. Eur J Neurosci 22: 1816–1817.

57. Plump T, Ehninger D, Steiner B, Klempp F, Jesberger S, et al. (2006) Variability of doublecortin-associated dendrite maturation in adult hippocampal neurogenesis is independent of the regulation of precursor cell proliferation. Eur J Neurosci 7: 77–77.

58. Webster M, Edelmman S, Schipper P, Trauer H, Franke H, et al. (2006) Increased polyglutamic acid neural cell adhesion molecule expression in human hippocampus of heroin addicts. Neuroscience 136: 1215–1223.

59. Crespel A, Rigaux V, Coubes P, Roussel MC, de Beek F, et al. (2003) Increased number of neural progenitors in human temporal lobe epilepsy. Neurobiol Dis 19: 436–450.

60. Barbeau D, Liang JJ, Robitaille Y, Quinron R, Sreeratava LK (1995) Decreased expression of the embryonic form of the neural cell adhesion molecule in schizophrenia brains. Proc Natl Acad Sci U S A 92: 2785–2789.

61. Ni Dhaill CM, Fox GB, Pittock SJ, O’Connell AW, Murphy KJ, et al. (1999) Polysialylated neural cell adhesion molecule expression in the dentate gyrus of the human hippocampal formation from infancy to old age. J Neurosci Res 55: 99–106.

62. Kuan CY, Schloemer AJ, Lu A, Burns KA, Weng WL, et al. (2004) Hypoxia-ischemia induces DNA synthesis without cell proliferation in dying neurons in adult rodent brain. J Neurosci 24: 10763–10772.

63. Nagy Z (2000) Cell cycle regulatory failure in neurons: causes and consequences. Neurobiol Aging 21: 761–769.

64. Moldovan GL, Pfander B, Jeut Sch S (2007) PCNA, the maestro of the replication fork. Cell 129: 663–679.

65. Manthey CD, Harburg GC, Eisich AJ (2007) Determination of key aspects of precursor cell proliferation, cell cycle length and kinetics in the adult mouse subgranular zone. Neuroscience 146: 108–122.

66. Prive G, Gottfried V (2008) The p21 and PCNA partnership: a new twist for an old plot. Cell Cycle 7: 3840–3846.

67. Lee KY, Myung K (2008) PCNA modifications for regulation of post-replication repair pathways. Mol Cells 26: 5–11.

68. Leuner B, Kozorovitcky Y, Gross CG, Gould E (2007) Diminished adult neurogenesis in the marmoset brain precedes old age. Proc Natl Acad Sci U S A 104: 17169–17173.

69. Manganas LN, Zhang X, Li Y, Hazel RD, Smith SD, et al. (2007) Magnetic resonance spectroscopy identifies neural progenitor cells in the live human brain. Science 318: 900–905.

70. West MJ, Coleman PD, Flood DG, Troncoso JC (1994) Differences in the pattern of hippocampal neuronal loss in normal ageing and Alzheimer’s disease. Lancet 344: 769–772.

71. Sriniv V, Kostovic I, Wimbald B, Boglanovic N (1995) Volume and number of neurons of the human hippocampal formation in normal aging and Alzheimer’s disease. J Comp Neurol 370: 492–494.

72. Rakic P (1985) Limits of Neurogenesis in Primates. Science 227: 1054–1056.

73. Winkler I, Rasch MJ, Kempermann G (2010) A functional hypothesis for adult hippocampal neurogenesis: avoidance of catastrophic interference in the dentate gyrus. Hippocampus 16: 329–334.

74. Kempermann G (2008) The neurogenic reserve hypothesis: what is adult hippocampal neurogenesis good for? Trends Neurosci 31: 163–169.

75. Singe I, Knooth R, Ditter M, Froscher M, Volk B (2003) Neurogranin expression by cerebellar neurons in rodents and non-human primates. J Comp Neurol 458: 278–289.