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REVIEW

Immunohistological markers for proliferative events, gliogenesis, and neurogenesis within the adult hippocampus

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Abstract Biologists long believed that, once development is completed, no new neurons are produced in the forebrain. However, as is now firmly established, new neurons can be produced at least in two specific forebrain areas: the subventricular zone (SVZ) and the dentate gyrus (DG) of the hippocampal formation. Neurogenesis within the adult DG occurs constitutively throughout postnatal life, and the rate of neurogenesis within the DG can be altered under various physiological and pathophysiological conditions. The process of adult neurogenesis within the DG is a multi-step process (proliferation, differentiation, migration, targeting, and synaptic integration) that ends with the formation of a post-mitotic functionally integrated new neuron. Various markers are expressed during specific stages of adult neurogenesis. The availability of such markers allows the time-course and fate of newly born cells to be followed within the DG in a detailed and precise fashion. Several of the available markers (e.g., PCNA, Ki-67, PH3, MCM2) are markers for proliferative events, whereas others are more specific for early phases of neurogenesis and gliogenesis within the adult DG (e.g., nestin, GFAP, Sox2, Pax6). In addition, markers are available allowing events to be distinguished that are related to later steps of gliogenesis (e.g., vimentin, BLBP, S100beta) or neurogenesis (e.g., NeuroD, PSA-NCAM, DCX).

Keywords BrdU · Progenitor · Precursor · Stem cell · Learning

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Introduction

Biologists long believed that neurogenesis is restricted to embryonic brain development. In the 1960s, Altman and Das (1965, 1967) provided the first evidence that new cells can indeed be generated in the postnatal hippocampus. About 30 years later, these results were confirmed and extended by the use of the thymidine analog, bromodeoxyuridine (BrdU), which labels DNA during the S-phase of the cell cycle. The use of BrdU as a marker for cell division has enabled scientists to demonstrate that, in the adult hippocampus, neuronal progenitor cells can divide at the interface between the hilus and the granule cell layer, and that the rate of neurogenesis can be altered under various physiological and pathophysiological conditions, at least in rodents (Kuhn et al. 1996; Parent et al. 1997; Kempermann et al. 1997; Scott et al. 1998). Several years later, neurogenesis in the adult brain was also demonstrated for monkeys (Gould and Rakic 1981; Kornack and Rakic 1999; Gould et al. 1999a). Finally, Eriksson and coworkers (1998) demonstrated that neurogenesis can also be monitored in the adult human hippocampus.

Neurogenesis in the hippocampus is restricted to a relatively limited area, the subgranular zone (SGZ) of the dentate gyrus (DG; Fig. 1). The newly formed cells integrate into the granular layer of the DG and start to extend their axons and dendrites (Fig. 2) into their target areas (von Bohlen und Halbach 2007).

The hippocampus is a brain region capable of structural reorganization. Pre-existing neural circuits within the adult hippocampus can undergo modifications not only in cell numbers within the DG (neurogenesis), but also in dendritic spine numbers in all hippocampal subfields. These morphological alterations can induce long-lasting changes in hippocampal neuronal plasticity. Experience-induced changes both in the dendritic spines (von Bohlen und

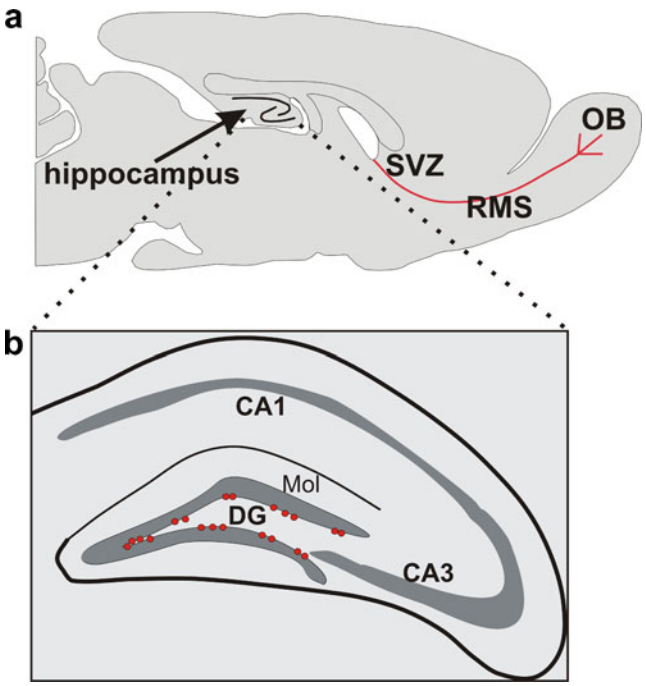

Fig. 1 Adult neurogenesis. **a** Neurogenesis in the adult brain is mainly seen in the subventricular zone (*SVZ*) and in the hippocampus. Newly generated cells from the SVZ migrate through the rostral migratory stream (*RMS*) to reach their final destination in the olfactory bulb (*OB*). Representation of a sagittal section. **b** Within the adult hippocampus, the progenitor cells (*red*) are located in the subgranular zone of the dentate gyrus (*DG*). These cells proliferate and migrate into the granular layer of the DG and later extend their dendrites toward the molecular (*Mol*) layer of the DG (*CA1*, *CA3* areas 1 and 3 of the hippocampus). Representation of a coronal section

Halbach 2009) and in hippocampal neurogenesis can be observed, and a variety of factors, e.g., neurotrophins, are capable of modulating spine densities and neurogenesis. Thus, for example, the lack of trkB, the cognate receptor for brain-derived neurotrophic factor, has been demonstrated to result in reduced spine densities (von Bohlen und Halbach et al. 2006, 2008), reductions in neurogenesis (Bergami et al. 2008), and altered neuronal plasticity (Minichiello et al. 1999). Hence, both morphological alterations can be observed in the context of neuronal plasticity. However, whether and how these various plastic changes depend on each other remain to be clarified.

Aside from the DG, a further prominent region, in which ongoing adult neurogenesis can be observed, is the subventricular zone (SVZ). The newly born cells of the SVZ migrate and differentiate into neurons of the olfactory bulb (OB; Luskin 1993, 1994). Specifically, the newly generated neuronal cells in the SVZ migrate over a long distance to the OBs through the rostral migratory stream (RMS) and differentiate into interneurons at their final destination (Fig. 1). These newly generated neuronal cells in the OB establish synaptic contacts and functional connections with neighboring cells (Carlen et al. 2002; Belluzzi et al. 2003).

In addition to these regions, some reports have indicated that neurogenesis can also occur in other brain areas of adult mammals, including the neocortex (Gould et al. 1999b; Takemura 2005), the striatum (Van Kampen et al. 2004; Bedard et al. 2006), the amygdala (Bernier et al. 2002), the subcallosal zone (Seri et al. 2006), and the substantia nigra (Zhao et al. 2003; Yoshimi et al. 2005). However, neurogenesis in these areas seems to occur at substantially lower levels or might be induced under non-physiological conditions.

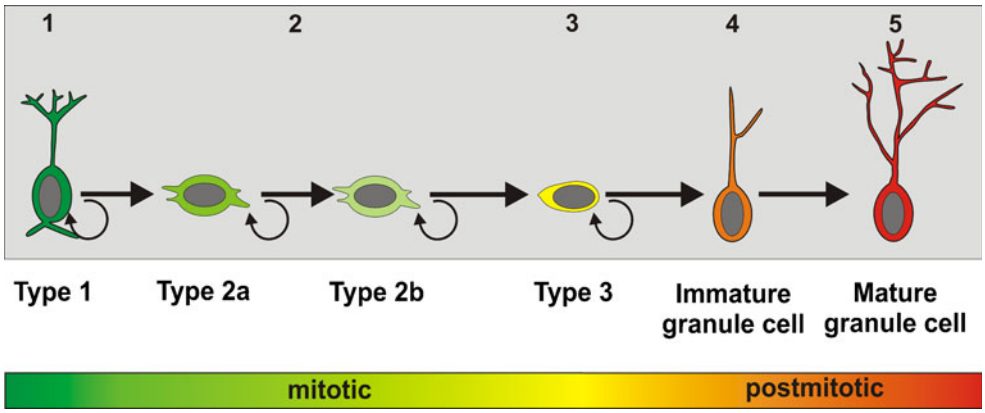

Fig. 2 Stages of neurogenesis in the dentate gyrus. The generation of new neurons within the granular layer of the DG can be subdivided into five stages. Adult neurogenesis originates from a putative stem cell (*Type 1* cell; *stage 1*) that has radial glial and astrocytic properties. Based on the expression of various markers, three different types of putative, transiently amplifying progenitors can be identified (*Type-2a*, *Type-2b*, *Type-3*) after the initial first stage. During this phase (*stages 2–3*), the proliferating cells differentiate and start to express neuronal markers and migrate over a short distance to reach the granular layer of the DG. The newly formed cells become postmitotic and transiently start to express calretinin (*stage 4*). The new cells extend their axons in the direction of area CA3 and send their dendrites toward the molecular layer and become functionally integrated into the hippocampal network (*stage 5*), receiving inputs from the entorhinal cortex and sending outputs to hippocampal area CA3 and the hilus

Stages of adult neurogenesis in the dentate gyrus

Neurogenesis within the DG occurs constitutively throughout postnatal life and is influenced by environment, behavior (Kempermann et al. 1997; Young et al. 1999; Ra et al. 2002; Kim et al. 2002; Uda et al. 2006), and aging (Zechel et al. 2010; von Bohlen und Halbach 2010). The hippocampal formation is involved in episodic and spatial memory (Rolls 2000), and an increased rate of hippocampal neurogenesis has been observed after hippocampal-dependent learning tasks (van Praag et al. 1999; Drapeau et al. 2003). However, other groups have not observed a correlation between hippocampal cell genesis and spatial learning ability (Merrill et al. 2003; Van der Borght et al. 2005). These differences might be attributed to neurogenesis being relate to some, but not all, types of hippocampal-dependent learning (Shors et al. 2002).

Currently, adult hippocampal neurogenesis is thought to consist of several developmental stages (Kempermann et al. 2004; Ming and Song 2005) that are characterized by morphological distinct cells. Based on this, the time-course of adult neurogenesis can be divided into various stages (Fig. 2). Since the precursor cells of the SVZ do not appear to be identical to the cells found in the SGZ of the DG (Seaberg and van der Kooy 2002), an alternative nomenclature has been proposed for the neurogenic cells located in the DG; this scheme is based on numbers (Kempermann et al. 2004), instead of the letters that had been originally used to describe the neurogenic cells within the SVZ (Doetsch et al. 1997).

Type 1 cells

In restricted zones of the brain, radial glia cells not only give rise to astrocytes, but may also transform into astroglial stem cells (Merkle et al. 2004) or progenitor cells. Adult hippocampal neurogenesis originates from a cell with morphological and functional characteristics of a glial cell. Type 1 cells are thought to constitute the resident early precursor population. The somata of these cells are triangular-shaped and located in the SGZ. They extend an apical process toward the molecular layer of the DG and sometimes contact blood vessels. In addition, they can also extend shorter tangentially oriented processes at the base of the SGZ (Seri et al. 2001; Fukuda et al. 2003; Filippov et al. 2003). Cells belonging to type 1 are relatively abundant within the SGZ, but these cells are thought to divide rarely. The type 1 cells express glial fibrillary acidic protein (GFAP) and the intermediate filament nestin but are negative for the calcium-binding protein S-100beta, which is, for example, expressed in a distinct postmitotic astrocyte population (Seri et al. 2001; Ehninger and Kempermann 2008). Moreover, the type 1 cells express the radial glia marker brain lipid-binding protein (BLBP) and SRY-related HMG-box gene2 (Sox2; Steiner et al. 2006; Ehninger and Kempermann 2008). Notably, the SGZ also contains horizontally oriented astrocytes that lack a radial process (Filippov et al. 2003; Seri et al. 2004). Whether these astrocytes can also act as progenitors for other cells is still unclear (Ihrie and Alvarez-Buylla 2008).

Type 2a and 2b cells

Type 1 cells give rise to fast-proliferating intermediate precursors. Most of the expansion of the pool of newly generated cells occurs during the stage of type 2 cells. These cells are characterized by a small soma, irregularly shaped nucleus, and short and horizontally oriented processes (a strong apical process is missing). The type 2 cells show an overlap in the expression of several glial and neuronal markers. Early type 2 cells express the stem-cell marker Sox2 (Steiner et al. 2006). Type 2 cells can be divided in two subpopulations, both nestin-positive, one being negative and one being positive for the immature neuronal marker doublecortin (DCX), and are therefore named type-2a and type-2b, respectively (Kempermann et al. 2004).

Type 3 cells

The type 3 stage is a transition phase from the slowly proliferating “neuroblasts” to the postmitotic immature neuron. Under normal conditions, type 3 cells show little proliferative activity, but under pathophysiological conditions, such as in the case of seizures, they can dramatically increase their proliferative activity (Jessberger et al. 2005). Type 3 cells express markers of the neuronal lineage, but no markers of the glial lineage. They migrate over a short distance into the granular layer. The morphology of the type 3 cells is highly variable, reflecting their developmental transition: the orientation of the processes changes from horizontal to vertical and the processes vary in length and complexity. Exit from the cell cycle occurs at this stage and coincides with the transient expression of the calcium-binding protein calretinin.

The various early progenitor cells might also be combined into a first stage of neurogenesis that is characterized by proliferative events. Thus, during stage 1 (proliferative stage), the newly generated cells express the markers GFAP and nestin (Fukuda et al. 2003; Filippov et al. 2003). These precursors share many characteristics with embryonic radial glia cells (Levitt and Rakic 1980; Eckenhoff and Rakic 1984; Cameron et al. 1993), which act as neuronal progenitors during embryonic development (Hartfuss et al. 2001). In the next stage (stage 2: differentiation phase), the transient amplifying cells differentiate into immature neurons in the

SGZ. The early stage 2 cells are nestin-positive but GFAP-negative and highly proliferative (Kronenberg et al. 2003). During this phase the cells are also thought to commit to a neuronal lineage. At later timepoints, the stage 2 cells transiently stop expressing nestin and start to express DCX (Kronenberg et al. 2003; Fukuda et al. 2003). After this stage, a short migration phase (stage 3) can be observed, during which immature neurons migrate a short distance into the granule cell layer of the DG.

Thereafter, the cells start to express calbindin and the development and elongation of the dendritic trees toward the molecular layer of the DG, and axon elongation toward area CA3 occurs (Hastings and Gould 1999; Ehninger and Kempermann 2008). The immature neurons still express DCX. In addition, the early postmitotic neurons (at least in mice) transiently express the calcium-binding protein calretinin (Brandt et al. 2003; Llorens-Martin et al. 2006) and start to express the postmitotic neuronal marker, neuron-specific nuclear protein (NeuN; Brandt et al. 2003), the most widely used indicator for "mature neurons" (Kempermann et al. 2004). Thus, during this stage (stage 4; Fig. 2), the newly generated neurons become postmitotic.

In the next stage (stage 5; Fig. 2), the newly formed granule neurons establish their synaptic contacts for receiving inputs from the entorhinal cortex and for sending outputs to the CA3 and hilar regions. Approximately 2–3 weeks after the newly generated cells have become postmitotic, calretinin is exchanged for calbindin in mature granule cells (Brandt et al. 2003; Kempermann et al. 2004). Calbindin is present in all mature granule cells (Rami et al. 1987; Baimbridge 1992), and the newly formed cells that express calbindin become functionally integrated into the hippocampus (van Praag et al. 2002). These neurons also express the postmitotic neuronal marker NeuN (Kuhn et al. 1996).

The concept of dividing neurogenesis into different stages allows the monitoring of hippocampal neurogenesis in more detail, since the various developmental stages correlate with the expression of different markers (Fig. 3).

Markers for proliferative events in adult dentate gyrus

5-Bromo-2'-deoxyuridine

A breakthrough in the identification of newly born cells in the brain was made on the development of 5-bromo-2'-deoxyuridine (BrdU) immunohistochemistry (Miller and Nowakowski 1988). Detection of newborn granule cells is mainly based on the assumption that BrdU is an S-phase-specific marker, and thus, the incorporation of BrdU into the DNA allows the detection of newly formed cells.

BrdU application is mainly conducted by intraperitoneal injection. Depending on the application mode (duration, concentration of applied BrdU, and survival times after

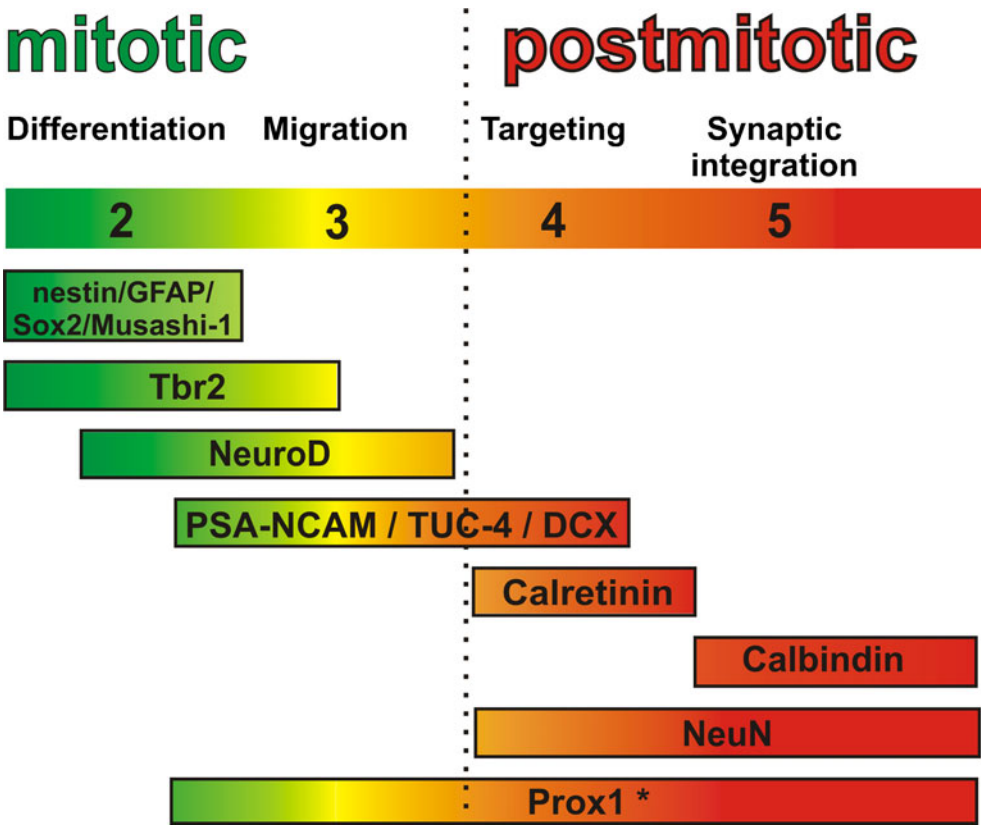

Fig. 3 Markers for staging adult hippocampal neurogenesis. At various stages of adult neurogenesis in the DG, different specific molecules are expressed by the newly formed cells; these markers not only can serve as general markers of adult neurogenesis in the DG, but can also help to monitor the different stages of neurogenesis in more detail. Prospero-related homeobox gene 1 (Prox1; *star*) is a marker for postmitotic young neurons in the DG; however, Prox1 expression has recently been reported to begin in type 2b cells (Steiner et al. 2008), indicating that Prox1 can also be used as a marker for the neuronal lineage

BrdU injection), the numbers of labeled cells can vary within the brain (Cameron and McKay 2001; Dayer et al. 2003). For example, high concentrations of BrdU (about 300 mg per kg body weight) are needed to label all S-phase cells in the adult DG, because BrdU has to cross the blood-brain barrier (Cameron and McKay 2001). Treatments that disturb or disrupt the function of the blood-brain barrier, e.g., kainate lesions, epilepsy, or ischemia (Pardridge et al. 1975; Cornford and Oldendorf 1986; Bolton and Perry 1998), might therefore induce increases in the numbers of BrdU-labeled cells. This increase, however, might be independent of changes in proliferation, since the effects are attributable to altered BrdU availability in the brain. Moreover, BrdU turns out not to be an S-phase-specific marker, but, as a thymidine analog, a marker of DNA synthesis (Taupin 2007). Therefore, the study of neurogenesis with BrdU requires that cell proliferation and neurogenesis can be distinguished from other events involving DNA synthesis, such as DNA repair, abortive cell cycle re-entry, and gene duplication (Nowakowski and Hayes 2000; Bauer and Patterson 2005; Taupin 2007). However, several sets of experimental data suggest that the concentration at which BrdU is commonly applied is insufficient to detect cells undergoing DNA repair (Cooper-Kuhn and Kuhn 2002; Bauer and Patterson 2005). Further detailed information concerning the use of BrdU immunohistochemistry for studying adult neurogenesis can be found in the excellent review by Phillip Taupin (2007).

Proliferating cell nuclear antigen

Proliferating cell nuclear antigen (PCNA) is a subunit of DNA polymerase-delta and is essential for both DNA replication and the repair of DNA errors (Zacchetti et al. 2003). PCNA has its highest expression during G_1 and S-phases, and its expression decreases in G_2 and M-phases (Linden et al. 1992). This marker is also present in the early G_0 phase because of its long half-live of 8–20 h (Zacchetti et al. 2003). Since PCNA is involved in DNA replication, PCNA can be used as a proliferation marker for adult hippocampal neurogenesis (Jin et al. 2001; Limke et al. 2003). Both PCNA and Ki-67 (see below) can label dividing cells. PCNA is expressed in all phases of the cell cycle including those not expressing Ki-67; however, use of an optical disector has revealed no significant difference in the number of PCNA- or Ki-67-positive cells within the hippocampus (Jinno 2011).

Ki-67

The name Ki-67 is derived from the city of origin (Kiel, Germany) and the number of the original clone in a 96-well plate (Gerdes et al. 1983). Ki-67 is expressed in all phases of the cell cycle except the resting phase and at the beginning of the G_1 phase (Zacchetti et al. 2003). Because of its short half-life of about 1 h, it is rarely detectable in cells in the G_O phase (Zacchetti et al. 2003).

Ki-67 is not detectable during DNA repair processes and is mainly absent in quiescent cells (Zacchetti et al. 2003). The number of Ki-67-positive cells is about 50% higher than that of BrdU-labeled cells in the DG, since BrdU can be incorporated into DNA only during the S-phase of the mitotic process, whereas Ki-67 is expressed throughout its duration (Kee et al. 2002). Since no major differences have been found in cell numbers expressing PCNA or Ki-67 within the DG (Jinno 2011), and as Ki-67 is thought to represent a more reliable marker for identifying cells that re-enter the cell cycle than PCNA (Kee et al. 2002), Ki-67 might be preferred as a marker. In addition, side effects that might accompany BrdU application (stress during application and mutagenesis following incorporation) are not applicable, since Ki-67 is intrinsically expressed (Kee et al. 2002).

Phosphohistone H3

Histone H3 is a part of the histone octamer. The phosphorylated form of histone H3 (phosphohistone H3; PH3) is present during the late G2 phase and in the M phase of cell division (Hendzel et al. 1997; Taupin 2007). PH3 is widely used to identify proliferating and mitotic cells in the hippocampus. Hypoxia-ischemia has recently been shown to induce DNA synthesis without cell proliferation in dying neurons in adult rodent brain. In this context, the proliferative cell marker Ki-67 has been demonstrated to be induced by hypoxia–ischemia in the affected hippocampus and is restricted to the pyknotic neuronal nuclei, whereas PH3 immunoreactivity has not been detected in pyknotic nuclei after hypoxia–ischemia (Kuan et al. 2004).

Minichromosome maintenance protein 2

Minichromosome maintenance protein 2 (MCM2) is involved in the control of DNA replication. The expression of MCM2 starts in early G1 and is maintained throughout the cell cycle. MCM2 is also expressed in cells that proliferate without actually synthesizing DNA and is thus present in higher numbers than the short-lived proliferation marker Ki-67 (Lucassen et al. 2010). MCM2 has been shown to be a suitable marker for cell proliferation, since it is not induced by apoptosis (Kodani et al. 2001; Osaki et al. 2002). Moreover, MCM2 has been reported to represent a better marker for cell proliferation than Ki-67 (Hanna-Morris et al. 2009). Thus, MCM2 has been successfully introduced as a proliferation marker in adult hippocampal neurogenesis (Amrein et al. 2007; Sivilia et al. 2008; Knoth et al. 2010; Lucassen et al. 2010).

Limitations of cell cycle markers

The use of cell cycle markers for studying adult neurogenesis within the hippocampus is however limited by the temporal expression of cell cycle proteins; thus, these markers were unable to identify (after exit from the cell cycle) newly born cells. Moreover, cell cycle markers do not allow us to distinguish whether the new cells belong to the glial or neuronal lineage or to other cell populations that are capable of cell division within the mature brain (Table 1). To determine whether changes in proliferation are indeed related to altered neurogenesis, these labels have to be combined with other immunohistological markers that identify newly formed neurons at later stages during the time-course of neurogenesis.

Markers for early stages of adult gliogenesis and neurogenesis

Glial fibrillary acidic protein

In the adult brain, GFAP is widely known as a marker for mature astrocytes. However, a large proportion of the newborn cells in the SGZ of the hippocampal region are also GFAP-immunopositive (Eckenhoff and Rakic 1988; Maslov et al. 2004). Since cell genesis in the adult brain can give rise to neurons and glial cells, the GFAP-positive new cells might represent cells that are generated during gliogenesis (Eckenhoff and Rakic 1988; Steiner et al. 2004). However, during adult neurogenesis, new neurons are reported to originate from a cell that has astrocytic properties and expresses GFAP (Doetsch et al. 1997). The use of transgenic animals has convincingly demonstrated that GFAP-expressing progenitors are the principal source of constitutive neurogenesis in the adult mouse forebrain (Garcia et al. 2004): targeted ablation of dividing GFAP-expressing cells in the adult mouse SGZ abolishes the generation of neuroblasts and new neurons in the DG. Moreover, transgenically targeted cell-fate mapping has shown that essentially all neuroblasts and neurons that are newly generated in the adult mouse forebrain in vivo are derived from progenitors that express GFAP. GFAP-expressing progenitors are thought to represent the predominant sources of constitutive adult neurogenesis (von Bohlen und Halbach 2010). However, the use of GFAP as a marker for neurogenesis is hampered because the glial cell lineage and mature astrocytes are also GFAP-positive (Table 1).

Nestin

In 1990, nestin was discovered to be an intermediate filament expressed in many, if not all, neural precursor cells (Lendahl et al. 1990). Two years later, proliferating cells in the adult rodent brain were shown initially to express the neural-specific intermediate filament nestin and subsequently to develop the morphology and antigenic properties of neurons and astrocytes (Reynolds and Weiss 1992). During brain development, nestin is expressed by astrocytes and radial glia cells, and nestin expression starts to disappear around postnatal day 11 (P11) in the rat cortex (Kalman and Ajtai 2001).

Based on these data, nestin might provide an ideal marker to examine neurogenesis within the adult brain. Cells immunoreactive for nestin are thought to be involved in neurogenesis and to differentiate into neurons (Doyle et al. 2001; Cao et al. 2006; Yue et al. 2006). Thus, during the first stage of neurogenesis, the newly generated cells express nestin and GFAP (Fukuda et al. 2003; Filippov et al. 2003). During the second stage, in which the transient amplifying cells differentiate into immature neurons in the SGZ, the early stage 2 cells are nestin-positive but negative for GFAP (Kronenberg et al. 2003).

However, limitations also exist in the use of nestin in brain sections, since a variety of factors, such as cerebral ischemia (Duggal et al. 1997), traumatic brain injury (Sahin et al. 1999), de-afferentiation of the DG (Brook et al. 1999), or neurotoxicity (Yoo et al. 2005) can induce nestin re-expression in glial cells (Table 1). Furthermore, experiments involving organotypic slice cultures must be carefully interpreted, since the persistent expression of nestin in glial cells has been demonstrated in organotypic slice culture of the rat cortex (Schmidt-Kastner and Humpel 2002).

SRY-related HMG-box gene 2

The high-mobility group transcription factor Sox2 is expressed by stem cells and precursor cells during development and by neural stem cells (NSC), and therefore, Sox2 is likely to be involved in self-renewal and precursor differentiation (Episkopou 2005; Jiang et al. 2008). Deletion of Sox2 in mice has been found only to induce minor brain defects at birth; however, shortly afterwards, neurogenesis has been found to be completely lost in the hippocampus, leading to DG hypoplasia (Favaro et al. 2009).

Sox2 is expressed in the adult brain in proliferating precursor cells (as marked by the incorporation of BrdU) and in glial-like cells that are believed to represent stem cells (Ferri et al. 2004). Since Sox2 is known to be expressed within neural progenitors throughout adulthood (Brazel et al. 2005), Sox2 has been established as a stem cell marker in adult neurogenesis. Sox2 is expressed mainly by type 1 and type 2a cells but is rarely observed to be expressed by type 2b or type 3 cells (Steiner et al. 2006).

Musashi-1

In the developing murine central nervous system (CNS), Musashi-1 protein is highly enriched in the CNS stem cells

Table 1 Specificity of markers for adult neurogenesis within the dentate gyrus (*DG*). Several markers for monitoring adult neurogenesis within the DG are not exclusively expressed in the DG but can also be observed during adult neurogenesis arising in the subventricular zone (*SVZ*) and enabling the addition of new neurons in the olfactory bulb via the rostral migratory stream (*RMS*). Moreover, several of these markers label further cell populations in the adult hippocampus and those of other areas within the adult brain. Data have been obtained from various sources (Sloviter 1989; Schmidt-Kastner et al. 1990; Jacobowitz and Winsky 1991; Miettinen et al. 1992; Gulyas et al. 1992; Stoykova and Gruss 1994; Young et al. 1996; Stringer 1996; Duggal et al. 1997; Jankovski et al. 1998; Sahin et al. 1999; Krum and Rosenstein 1999; Plachez et al. 2000; Liu et al. 2000; Nacher et al. 2001, 2002, 2005; Bedard et al. 2002; Pham et al. 2003; Seri et al. 2004; Chen et al. 2004; Sundholm-Peters et al. 2004; Poulsen et al. 2005; Crespel et al. 2005; Navarro-Quiroga et al. 2006; Verwer et al. 2007; Liu et al. 2008; Osumi et al. 2008; Steiner et al. 2008; Shen et al. 2008; Roybon et al. 2009; Bramanti et al. 2010; Modi and Kanungo 2010; Gomez-Climent et al. 2011)

Marker (explanation of abbreviation)	Cell type	DG	Other hippocampal areas	SVZ/RMS	Other brain areas	Re-expression in the hippocampus
PCNA (proliferating cell nuclear antigen)	Proliferating cells	+	+	+	+	
Ki-67 (Kiel 67)	Proliferating cells	+	+	+	+	
PH3 (phosphohistone H3)	Proliferating cells	+	+	+	+	
MCM2 (minichromosome maintenance protein 2)	Proliferating cells	+	+	+	+	
GFAP (glial fibrillary acidic protein)	Progenitors, astrocytes	+	+	+	+	Reactive astrocytes
Nestin	Progenitors	+		+		Reactive astrocytes
Sox2 (SRY-related HMG-box gene2)	Progenitors	+		+	Possibly in aged oligodendrocytes in the corpus callosum	
Musashi-1	Progenitors	+		+		Ectopic expression in CA1 after seizures
Pax6 (paired box gene 6)	Progenitors	+	Subpopulation of astrocytes	+	+ e.g., Olfactory bulbs, septal nuclei, basolateral amygdala	
Vimentin	Glial lineage	+		+	+	Reactive astrocytes
BLBP (brain lipid-binding protein)	Glial lineage	+		+	+	
S100beta	Astrocytes	+	+	+	+	
EAAT1 (also called glutamate-aspartate transporter) and EAAT2 (also called glutamate transporter)	Astrocytes	+	EAAT2: subpopulation of CA1-CA3 neurons	+	+	EAAT1: reactive astrocytes
Tbr2 (T-box brain gene 2)	Neuronal lineage	+		SVZ-RMS axis		
NeuroD (neurogenic differentiation)	Neuronal lineage	+		SVZ-RMS axis		
PSA-NCAM (polysialylated embryonic form of the neural cell adhesion molecule)	Neuronal lineage	+	Non-pyramidal layers of areas CA1-CA3	SVZ-RMS axis	+ e.g., Interneurons in the cerebral cortex	
TUC-4 (turned on after division/UNC-33-like protein/collapsin response-mediator protein 4)	Neuronal lineage	+	Weakly in hilus and CA1	RMS		
DCX (doublecortin)	Neuronal lineage	+		SVZ-RMS axis	Striatum, some cortical areas (possibly astrocytes)	
Tuj-1 (neuron-specific class III beta-tubulin)	Neuronal lineage	+	Possibly basket cells in the DG	RMS		

Table 1 (continued)

Marker (explanation of abbreviation)	Cell type	DG	Other hippocampal areas	SVZ/RMS	Other brain areas	Re-expression in the hippocampus
Calretinin	Neuronal lineage	+	Mainly interneurons (CA1-CA3 and DG)	Some cells in the RMS	Interneurons, e.g., olfactory bulb, cortex, hippocampus, …	
Calbindin	Mature DG granule cells	+	Subpopulation of CA-neurons		+ Interneurons, e.g., olfactory bulb; cerebellum	
NeuN (neuron-specific nuclear protein)	Mature DG neurons	+	+	RMS	+	
Prox1 (Prospero-related homeobox gene 1)	Neuronal cells and neurons in the DG	+			Cerebellum	

(Sakakibara et al. 1996). Single-cell culture experiments indicate that Musashi-1 expression is associated with neural precursor cells that are capable of generating neurons and glia, whereas in fully differentiated neuronal and glial cells, Musashi-1 expression is lost (Sakakibara et al. 1996). Musashi-1 has subsequently been shown to be expressed by cells that are mainly positive for nestin, rarely positive for DCX or polysialylated embryonic form of the neural cell adhesion molecule (PSA-NCAM), and negative for markers of mature neurons or astrocytes (Crespel et al. 2005). Thus, Musashi-1 might represent a further marker for identifying neuronal progenitor cells within the DG.

Paired box gene 6

Paired box gene 6 (Pax6) was first identified as a paired box (Pax) family member that was expressed during CNS development and was cloned on the basis of its homology to the *Drosophila* gene for paired (Walther and Gruss 1991). Shortly after the identification of Pax6, mutations in the PAX6 gene were shown to be associated with aniridia (Ton et al. 1991; Jordan et al. 1992; Hanson et al. 1993; Davis and Cowell 1993). The transcription factor Pax6 is expressed in precursor cells during embryonic CNS development and plays an important role in the regulation of cell proliferation and neuronal fate determination (Götz et al. 1998; Heins et al. 2002; Englund et al. 2005).

Pax6-expressing cells are also present in the adult DG and SGZ (Nacher et al. 2005). In the SGZ, Pax6 is expressed in early progenitor cells that show radial glia-like morphology and are positive for GFAP and nestin (Maekawa et al. 2005; Nacher et al. 2005; Hevner et al. 2006; Osumi et al. 2008), whereas a smaller population of the Pax6-positive cells exhibits PSA-NCAM or DCX immunoreactivity (Maekawa et al. 2005; Nacher et al. 2005). Moreover, Pax6 immunoreactivity is also found in neurogenic differentiation (NeuroD)-immunopositive cells (Nacher et al. 2005). Thus, Pax6 might represent a suitable marker for newly generated cells in the DG during differentiation. However, we should take into account that a small subpopulation of hilar mature neurons and certain astrocytes of the adult hippocampus also express Pax6 (Nacher et al. 2005). Concerning the expression of Pax6 in astrocytes (Table 1), Pax6 has been demonstrated to be not only a key transcription factor that controls neurogenesis, but also a regulator of proliferation, differentiation, and migration of astrocytes in the CNS (Sakurai and Osumi 2008).

Markers for the glial lineage

Based on their orientation, two different types of astrocytes can be distinguished in the DG (Seri et al. 2004): radial astrocytes (displaying a large cell body with a major radial

process that penetrates the granular layer) and horizontal astrocytes (without a radial process, but with extended branched processes parallel to the subgranular layer and short thin secondary branches into the hilus and the granular layer).

Glial fibrillary acidic protein

GFAP is widely known as a marker for mature astrocytes in the adult brain. However, a large proportion of the newborn cells in the SGZ of the DG are also GFAP-immunopositive. Thus, GFAP is not an exclusive marker for mature astrocytes but is also a marker for newly born cells (see above). With regard to the astrocytes located in the DG, GFAP stains both horizontal and radial glia cells (Seri et al. 2004)

Vimentin

The two major intermediate filament proteins of glial cells are vimentin and GFAP. Vimentin can form intermediate filaments with either nestin or GFAP as obligatory partners, whereas GFAP can form filaments on its own (Eliasson et al. 1999).

Early during brain development, radial glia and immature astrocytes express mainly vimentin. Toward the end of gestation, a switch occurs whereby vimentin is progressively replaced by GFAP in differentiated astroglial cells. Thus, during the development of the human hippocampus, the first glial cells that appear are vimentin-positive radial glial cells. At week 8, a gradual transition from vimentin to GFAP reactivity in the radial glial cells occurs, and these GFAP-positive radial glial cells transform into astrocytes from week 14 onward (Stagaard Janas et al. 1991).

In normal adult CNS, vimentin is not expressed in astrocytes but only in some specialized glial cells such as those of the Bergmann glia and radial glia, and ependymal cells (Bramanti et al. 2010). However, vimentin has been reported to be expressed by both horizontal and radial glia cells in the adult DG (Seri et al. 2004).

Brain lipid-binding protein

BLBP is a small nucleocytoplasmic protein expressed by radial glia cells during brain development and by adult radial glia cells (Pinto and Gotz 2007). It is expressed by radial glia cells in various brain regions during development but has also been observed to be expressed by type 1 cells in the DG (Brunne et al. 2010). Moreover, BLBP is expressed in type-2 cells but labels only a small percentage of the proliferating cells (Steiner et al. 2006). BLBP is thought to represent an radial-glia-like progenitor marker, since it is co-expressed neither with the mature astrocytic marker S100beta nor with the markers of the neuronal lineage, e.g., DCX or NeuN (Brunne et al. 2010). BLBP-positive radial glia cells can divide and thus are positive for Ki-67 (Hartfuss et al. 2001). However, PCNA (used as a proliferation marker) is only expressed by less than 10% of the BLBP-immunopositive cells within the DG (Jinno 2011). BLBP represents a marker for radial glia cells and can be used to monitor gliogenesis within the DG; however, under specific circumstances, BLBP can also be expressed by astrocytes (Pinto and Gotz 2007), e.g., by Gomori-positive astrocytes (Young et al. 1996).

S100beta

S100beta is a member of the S100 family. This family of proteins was termed “S100” because it was soluble in 100% saturated ammonium sulfate solution. The calcium-binding protein S100beta is, for example, expressed in a distinct postmitotic astrocyte population (Seri et al. 2001; Ehninger and Kempermann 2008) and in Schwann cells of the peripheral nervous system. S100beta is thought to represent one of the most specific and reliable markers for astrocytes (Savchenko et al. 2000). At least in the SVZ, S100beta expression defines a state in which GFAP-expressing cells lose their neural stem cell potential and acquire a more mature developmental stage (Raponi et al. 2007); thus, GFAP and nestin co-expressing progenitors have been shown to be negative for S100beta (Filippov et al. 2003). Within the DG, horizontal but not radial astrocytes can be stained with S100beta (Seri et al. 2004).

EAAT1 and EAAT2

EAAT1 (also known as glutamate-aspartate transporter; GLAST) and EAAT2 (also known as glutamate transporter; GLT1) represent markers of the glial lineage. Thus, GLT1 and GLAST proteins are demonstrable in astrocytes (Kugler and Schleyer 2004). Concerning the DG, a majority of the proliferating cells in the hilar region that express S100beta has been described as also being immunopositive for GLAST (Namba et al. 2005). GLAST is a marker for glial differentiation; it is expressed from embryonic days 13/14 (E13/14) in mice and persists into adulthood (Barry et al. 2008). However, by using hippocampal cultures, GLT1 protein (Brooks-Kayal et al. 1998) or both GLAST and GLT (Plachez et al. 2000) have been found to be expressed in neuronal subpopulations. With regard to this issue, we should note that, following the original cloning of GLT1 (Pines et al. 1992), GLT1 protein was thought to be localized exclusively in astrocytes in the normal mature brain. GLT1 and GLAST are typically present as alternately spliced forms (Lee and Pow 2010). Currently, GLT1 is known to occur in diverse variants. With respect to the

GLT1a isoform, its mRNA is strongly expressed by hippocampal neurons, especially in area CA3 (Chen et al. 2004), and mRNA for the GLT1b isoform can also be detected in neurons of area CA3 (Chen et al. 2004). Thus, GLAST can be used in brain sections to identify astrocytes within the hippocampal formation. As far as GLT is concerned, we should keep in mind that, at least in the hippocampal areas CA1-CA3, it is also expressed by neuronal populations (Table 1).

Markers for the neuronal lineage

T-box brain gene 2

In 1999, a new member of the mammalian brain-specific T-box gene family, Tbr2, was identified and characterized and shown to be expressed in several regions of the developing brain (Kimura et al. 1999). Interestingly, Tbr2 mRNA expression was detected in the hippocampus only from E18.5 onward; whereas Tbr2 expression disappeared in most parts of the mature adult brain, it remained detectable in the hippocampus and OB (Kimura et al. 1999), raising the idea that Tbr2 might be involved in adult neurogenesis. Indeed, Tbr2 protein expression has been found to be restricted to the SGZ of the adult DG. Tbr2 does not co-localize with the glial marker S-100beta, but with a small fraction of Sox2-positive and Pax6-positive cells (Hevner et al. 2006; Hodge et al. 2008). Furthermore, Tbr2 expression overlaps with NeuroD expression in some cells (Hevner et al. 2006), and a small fraction (about 25%) of cells positive for DCX and PSA-NCAM (Hodge et al. 2008) and for Prospero-related homeobox gene 1 (Prox1; Lavado et al. 2010) have been found to be immunopositive for Tbr2, suggesting that Tbr2 is progressively down-regulated as the cells become committed to the neuronal lineage and exit the mitotic cycle (Hodge et al. 2008). Consistent with this, Tbr2 does not co-localize with calretinin, calbindin, or NeuN, which are expressed either in immature or mature granule cells (Hodge et al. 2008). Thus, Tbr2 is thought to be expressed mainly by type 2 and by a smaller fraction of type 3 progenitors and that Tbr2-positive progenitors are committed to the neuronal but not glial lineage (Hevner et al. 2006; Hodge et al. 2008).

NeuroD

The basic helix-loop-helix protein NeuroD has been identified as a differentiation factor for neurogenesis in diverse species, ranging from *Xenopus* to humans (Lee et al. 1995; Tamimi et al. 1996). NeuroD represents a transcription factor expressed at later stages of neuronal commitment (Lee et al. 1995) and might act as a neuronal determination gene (Tamimi et al. 1996). Moreover, NeuroD has been shown to be important for the proper development of the DG (Miyata et al. 1999; Liu et al. 2000).

NeuroD is expressed during neurogenesis in the adult DG (Kawai et al. 2004). NeuroD-positive cells can be found in the SGZ and inner granule cell layer (Seki 2002a; Hevner et al. 2006), and about 50% of Pax6-positive cells co-express NeuroD (Nacher et al. 2005). Furthermore, NeuroD expression is found in PSA-NCAM-positive cells within the DG (Seki 2002a, 2002b; Seri et al. 2004), but NeuroD expression precedes that of PSA-NCAM (Seki 2002b). Thus, NeuroD is a marker for the early cells of the neuronal lineage and thus can be used to identify early mitotic active neuronal cells in the DG.

PSA-NCAM

The polysialylated embryonic form of the neural cell adhesion molecule (NCAM), abbreviated to PSA-NCAM, is highly expressed during brain development. PSA-NCAM immunoreactivity has been found in cells that seem to be progenitor cells related to neural stem cells (Ben-Hur et al. 1998).

With regard to the adult brain, newly generated and developing granule cells in the adult DG highly express PSA-NCAM (Seki and Arai 1991), and some days after BrdU-injection, BrdU-positive/PSA-NCAM-positive cells can be observed within the DG (Seki 2002b).

PSA-NCAM-positive cells do not express GFAP (Seki and Arai 1999), but most PSA-NCAM-expressing cells are positive for NeuroD and DCX or for the mature neuronal marker NeuN (Seki 2002a). Thus, PSA-NCAM seems to be expressed at a later stage of neurogenesis, and PSA-NCAM expression seems to persist in young postmitotic neurons.

Within the hippocampus, non-granular PSA-NCAM-positive neurons can also be observed (Table 1), most of which are located in the non-pyramidal layers of hippocampal areas CA1-CA3 (Nacher et al. 2002). PSA-NCAM has been shown to be up-regulated in the hippocampus during hippocampal-dependent learning tasks (Venero et al. 2006) and seems also to play a role in promoting synaptogenesis and activity-dependent remodeling of synapses (Dityatev et al. 2004), indicating that PSA-NCAM is an important regulator of hippocampal plasticity (Cremer et al. 2000). Thus, changes in hippocampal PSA-NCAM immunoreactivity might not exclusively be correlated with changes in hippocampal neurogenesis.

Various forms of stress have been reported to decrease neurogenesis in the adult hippocampus (Luo et al. 2005; Warner-Schmidt and Duman 2006; Mitra et al. 2006). Chronically stressed rats have been reported to express reduced amounts of NCAM but increased levels of polysialylation (Sandi et al. 2001). Along this line, treatment with

3 weeks of chronic restraint stress, on one hand, suppresses proliferation within the DG by nearly 25% but, on the other hand, increases PSA-NCAM expression by 40% (Pham et al. 2003). Thus, under certain experimental conditions, the results obtained by using PSA-NCAM as an immunohistological marker of ongoing neurogenesis within the hippocampus might have to be interpreted with caution.

TOAD/Ulip/CRMP 4

The TUC (TOAD [turned on after division]/Ulip [UNC-33-like protein]/CRMP [collapsin response-mediator protein]) protein family members are thought to be involved in growth cone collapse (Minturn et al. 1995a). During brain development, TUC-4 is not expressed by progenitor cells but is expressed by postmitotic neurons as they begin their migration (Minturn et al. 1995b). TUC-4 expression reaches its highest levels in all neurons during the peak of axonal growth and is down-regulated afterward but can be re-expressed during adulthood, e.g., by axotomy (Quinn et al. 1999).

TUC-4 can be used as a marker for early postmitotic neurons (Fernandez et al. 2002) but seems also to be expressed in mitotic cells during neurogenesis. Thus, in vitro experiments investigating the neurogenesis of stem cells derived from marrow stromal cells have shown that TUC-4 is expressed in mitotic and postmitotic presumptive neurons (Munoz-Elias et al. 2003). The time-window of TUC-4 expression during neurogenesis resembles that of PSA-NCAM and DCX (Cecchini et al. 2003). Thus, TUC-4 is co-expressed with PSA-NCAM in young rats but seems to be expressed for a longer period than PSA-NCAM (Seki 2002a). TUC-4 stains not only the cell bodies, but also their processes, which often extend through the granule cell layer into the molecular layer. TUC-4 immunostaining results in an intense labeling of new neurons located in the DG, and in the hilus and CA1, a few cells also display faint TUC-4 immunoreactivity (Poulsen et al. 2005). Thus, TUC-4 can be used as a marker for various stages of adult neurogenesis in the DG, since TUC-4 is expressed by mitotic cells and by early immature neurons.

Doublecortin

DCX is a brain-specific microtubule-associated protein whose exact function is still not fully understood. It is thought to act as a microtubule stabilizer. Mutations in the human X-linked gene DCX cause, in females, defects in the cortical layering, known as "double cortex" syndrome (Gleeson et al. 1998, 1999b). DCX is a protein that promotes microtubule polymerization and is present in migrating neuroblasts and young neurons (Francis et al. 1999; Gleeson et al. 1999a). DCX is also present in the tips of neurites of non-migratory immature neurons, suggesting its role in the growth of neuronal processes, downstream of directional or guidance signals (Friocourt et al. 2003). Since DCX is present not only in the soma, but also in the processes of newly generated neurons, this marker can be used in combination with others, for example, with tract-tracing dyes such as dextran amines (von Bohlen und Halbach and Albrecht 1998), to investigate the morphology of these neurons in detail.

A central phase of neurogenesis is associated with the expression of DCX. This phase ranges from the progenitor stage to the calretinin-positive stage, during which the newly generated cells extend their dendrites and axons to establish functional connections (Knoth et al. 2010). Thus, DCX expression is thought to be specific for newly generated neurons, since nearly all DCX-positive cells express early neuronal antigens but lack antigens specific for glial cells, undifferentiated cells, or apoptotic cells (Rao and Shetty 2004).

Concerning DCX expression at early stages of adult neurogenesis, no overlap with the expression of nestin has been shown, and upon neuronal specification, the expression of nestin is thought to be abruptly terminated (Couillard-Despres et al. 2005). However, we should mention that, by using transgenic mice expressing green fluorescent protein (GFP) under the nestin promoter, a brief overlap of DCX expression with nestin expression has been found (Kronenberg et al. 2003; Steiner et al. 2006). Temporally, the expression of DCX is largely in-frame with the expression of PSA-NCAM; therefore, double-labeling with these markers does not help in grouping the differently marked cells to different stages of neurogenesis. However, by combining DCX-labeling with the labeling of markers specific for postmitotic neurons, a differentiation between mitotic and postmitotic neurons can be made. Thus, transient co-expression of DCX and NeuN has been observed (Brown et al. 2003; Couillard-Despres et al. 2006), and the down-regulation of DCX expression coincides with the induction of NeuN expression (Brown et al. 2003; Couillard-Despres et al. 2006). Therefore, DCX can be used as a marker for new neurons within the granule layer of the DG, but outside this structure, DCX has been observed in brain regions that have not been linked to adult neurogenesis (Table 1), such as in some cortical areas and within the striatum (Nacher et al. 2001; Liu et al. 2008). In addition, at least in the adult human neocortex, DCX has also been found to be expressed by mature astrocytes (Verwer et al. 2007).

Neuron-specific class III beta-tubulin

Antibodies against the neuron-specific class III beta-tubulin (Tuj-1) were initially used to study the distribution and

morphology of immature neurons in the developing mouse telencephalon (Easter et al. 1993; Menezes and Luskin 1994). Expression of Tuj-1 starts as early as E8.5 in mice (Easter et al. 1993) and can be detected throughout brain development (Menezes and Luskin 1994). Tuj-1 has been found to label newly generated immature postmitotic neurons (Menezes and Luskin 1994). With regard to adult neurogenesis, Tuj-1 is used as a neuron-specific marker of newly generated cells (Parent et al. 1997; Doetsch et al. 1997; Gould et al. 2001). Tuj-1 is expressed in early postmitotic and differentiated neurons and in some mitotically active neuronal precursors; based on this, Tuj-1 immunoreactivity has been shown in DCX-immunoreactive (Yang et al. 2004) and PSA-NCA-immunopositive (Ambrogini et al. 2004) neurons. The expression of mRNA for Tuj-1 persists in neurons that display a high complexity in dendritic trees and electrophysiological properties that resemble those of mature DG cells. These neurons also show immunoreactivity for NeuN (Ambrogini et al. 2004) and therefore represent postmitotic neurons. In contrast to DCX immunostaining, that for Tuj-1 staining is weaker, and Tuj-1 immunoreactivity does not extend into neurites (Kempermann et al. 2003). Furthermore, evidence has been presented that Tuj-1 might also be expressed by basket cells in the DG (Seri et al. 2004).

Calretinin

Calretinin is known as a marker for specific non-pyramidal gamma-aminobutyric acid (GABA)ergic neurons within the adult hippocampus. Calretinin-positive neurons, mainly interneurons, can be found in all layers of all hippocampal fields, including areas CA1-CA3 and the DG (Jacobowitz and Winsky 1991; Miettinen et al. 1992; Gulyas et al. 1992). In 1996, a subpopulation of calretinin-positive neurons in the DG was described that was localized at the interface with the hilus. These cells were not positive for interneuron markers such as GABA but were immunoreactive for PSA-NCAM (Liu et al. 1996) and were therefore considered to represent newly generated postmitotic neurons.

We now know that the transient expression of calretinin can be observed during neurogenesis in the murine DG. Calretinin is not expressed by early progenitor cells, since calretinin-expressing cells are negative for Ki-67 (Brandt et al. 2003). At late phases of neurogenesis, new neurons express calretinin together with DCX (Brandt et al. 2003; Jinno 2011) or NeuN but do not express GABA (Brandt et al. 2003). At later time-points, the newly generated neurons stop expressing calretinin and start to express calbindin, a marker of mature DG cells (Brandt et al. 2003). Thus, calretinin expression within the DG is restricted to a short postmitotic time-window in which axonal and dendritic targeting is supposed to take place (Kempermann et al. 2004; Ming and Song 2005).

Calretinin was believed to represent a marker for the late stage of adult neurogenesis only in the murine DG. However, as recently reported, calretinin is expressed by newly formed neurons (that are also immuno-positive for DCX) in the human DG (Knoth et al. 2010).

Calbindin

Calbindin mRNA is highly expressed by cerebellar Purkinje cells and in granule cells of the DG (Sequier et al. 1988). Calbindin protein is present in granule cells of the DG, in a large proportion of CA1 and CA2 pyramidal neurons, and in a distinct population of local circuit neurons (Seress et al. 1991, 1992). During development, calbindin immunoreactivity has been shown to occur postnatally, and the expression of calretinin correlates with the onset of synaptogenesis in the hippocampus (Rami et al. 1987). Calbindin is used as a marker for mature DG granule cells (Rami et al. 1987; Eriksson et al. 1998; Liu et al. 1998; Nilsson et al. 1999; Dominguez et al. 2003), since it is expressed in mature neurons together with NeuN (Scharfman et al. 2005) but is not co-expressed with PSA-NCAM (Dominguez et al. 2003) or with calretinin, the marker for immature postmitotic neurons (Nacher et al. 2002; Brandt et al. 2003).

Neuron-specific nuclear protein

The expression of NeuN is observed in most neuronal cell types throughout the nervous system, with the exception of some neuronal populations such as cerebellar Purkinje cells and OB mitral cells (Mullen et al. 1992) and cells located in the glomerular layer of the OB (Winner et al. 2002). However, NeuN is not expressed by non-neuronal cells (Wolf et al. 1996).

NeuN is a soluble nuclear protein (Mullen et al. 1992) that has been localized to the cell nucleus and to the cytoplasm of postmitotic neurons (Lind et al. 2005). Within the hippocampus, NeuN can be used as a marker of postmitotic cells and labels both “normal” postmitotic neurons and newly generated postmitotic neurons. Markers such as PSA-NCAM or DCX are expressed during neurogenesis by mitotic and early postmitotic neurons. Thus, double-labeling with one of these markers together with NeuN allows us to distinguish between early mitotic and late postmitotic neurons.

Prospero-related homeobox gene 1

The homeobox gene Prox1 is expressed in the DG during embryonic development and adult neurogenesis. Prox1 is important for the maintenance of intermediate progenitors during adult neurogenesis (Lavado et al. 2010). Prox1 is required for the maturation of granule cells within the DG,

since conditional inactivation of Prox1 results in an absence of progenitors in the SGZ, and adult Nestin-Cre-Prox1$^{F/F}$ mice display nearly a complete loss of granule cells in the DG (Lavado et al. 2010).

Prox1 is not expressed in nestin- or Sox2-positive cells but is expressed in DCX-positive new cells, and Prox1 expression can also be observed in calretinin-positive cells in the DG and in adult granule cells (Lavado et al. 2010). Thus, Prox1 can be used as a marker for postmitotic young neuronal cells in the DG (Liu et al. 2000; Navarro-Quiroga et al. 2006); however, Prox1 expression has also been reported to start in type 2b cells (Steiner et al. 2008), indicating that Prox1 can be used as a marker for the neuronal lineage. Since Prox1 is also expressed by NeuN-positive granule cells in the DG (Steiner et al. 2008), the use of Prox1 as a single marker does not allow us to distinguish between newly formed neurons and mature granule cells.

Perspectives

Neurogenesis within the adult hippocampus is not a simple switch from a dividing precursor to a functional mature neuron but consists of a series of developmental events that occur in a specific sequence. Currently, whether hippocampal neurogenesis starts with a stem cell that is located within or outside the hippocampus is still not clear. Progenitor cells are thought to be located in the SGZ of the DG, where they proliferate and differentiate and can give rise to new neurons.

The establishment of the BrdU technique has been a breakthrough in monitoring adult neurogenesis. However, newly formed progenitor cells in the brain need not necessarily provide new neurons, since the population of early dividing cells is heterogeneous, and since neurogenesis is interspersed with gliogenesis (Seri et al. 2001; Kempermann et al. 2004; Steiner et al. 2004).

Markers that can be used to stain proliferative events (such as Ki-67 and other markers) might not, per se, label cells that give rise to new neurons (or glia cells). Markers such as Sox2 or nestin are helpful for identifying cells that might give rise to new glia cells or neurons in the adult hippocampus. The availability of diverse markers, specific for either gliogenesis or adult neurogenesis, not only allows us to distinguish between these two distinct processes, but also enables us to monitor the time course and fate of the newly generated cells in detail. Since most of the available markers have various advantages and disadvantages, the careful combination of the different markers can help to elucidate more precisely the roles and functions of adult gliogenesis and neurogenesis under a variety of conditions, e.g., in relation to learning and memory or in the context of neurological disorders. During the last few years, new markers to monitor gliogenesis and neurogenesis in the adult DG have been discovered and successfully introduced. A major problem in studies of adult neurogenesis is currently the lack of a unique marker defining adult neural stem cells (Landgren and Curtis 2011). Stem cells express many genes that are also expressed by astrocytes (Doetsch 2003; Garcia et al. 2004). Nevertheless, absolutely reliable markers that allow neuronal and glial precursors to be distinguished at early stages of adult gliogenesis and neurogenesis are currently not available.

References

Altman J, Das GD (1965) Autoradiographic and histological evidence of postnatal hippocampal neurogenesis in rats. J Comp Neurol 124:319–335

Altman J, Das GD (1967) Postnatal neurogenesis in the guinea-pig. Nature 214:1098–1101

Ambrogini P, Lattanzi D, Ciuffoli S, Agostini D, Bertini L, Stocchi V, Santi S, Cuppini R (2004) Morpho-functional characterization of neuronal cells at different stages of maturation in granule cell layer of adult rat dentate gyrus. Brain Res 1017:21–31

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:e455

Baimbridge KG (1992) Calcium-binding proteins in the dentate gyrus. Epilepsy Res Suppl 7:211–220

Barry G, Piper M, Lindwall C, Moldrich R, Mason S, Little E, Sarkar A, Tole S, Gronostajski RM, Richards LJ (2008) Specific glial populations regulate hippocampal morphogenesis. J Neurosci 28:12328–12340

Bauer S, Patterson PH (2005) The cell cycle-apoptosis connection revisited in the adult brain. J Cell Biol 171:641–650

Bedard A, Levesque M, Bernier PJ, Parent A (2002) The rostral migratory stream in adult squirrel monkeys: contribution of new neurons to the olfactory tubercle and involvement of the antiapoptotic protein Bcl-2. Eur J Neurosci 16:1917–1924

Bedard A, Gravel C, Parent A (2006) Chemical characterization of newly generated neurons in the striatum of adult primates. Exp Brain Res 170:501–512

Belluzzi O, Benedusi M, Ackman J, LoTurco JJ (2003) Electrophysiological differentiation of new neurons in the olfactory bulb. J Neurosci 23:10411–10418

Ben-Hur T, Rogister B, Murray K, Rougon G, Dubois-Dalcq M (1998) Growth and fate of PSA-NCAM+ precursors of the postnatal brain. J Neurosci 18:5777–5788

Bergami M, Rimondini R, Santi S, Blum R, Gotz M, Canossa M (2008) Deletion of TrkB in adult progenitors alters newborn neuron integration into hippocampal circuits and increases anxiety-like behavior. Proc Natl Acad Sci USA 105:15570–15575

Bernier PJ, Bedard A, Vinet J, Levesque M, Parent A (2002) Newly generated neurons in the amygdala and adjoining cortex of adult primates. Proc Natl Acad Sci USA 99:11464–11469

Bolton SJ, Perry VH (1998) Differential blood-brain barrier breakdown and leucocyte recruitment following excitotoxic lesions in juvenile and adult rats. Exp Neurol 154:231–240

Bramanti V, Tomassoni D, Avitabile M, Amenta F, Avola R (2010) Biomarkers of glial cell proliferation and differentiation in culture. Front Biosci (Schol Ed) 2:558–570

Brandt MD, Jessberger S, Steiner B, Kronenberg G, Reuter K, Bick-Sander A, Behrens W von der, Kempermann G (2003)

Transient calretinin expression defines early postmitotic step of neuronal differentiation in adult hippocampal neurogenesis of mice. Mol Cell Neurosci 24:603–613

Brazel CY, Limke TL, Osborne JK, Miura T, Cai J, Pevny L, Rao MS (2005) Sox2 expression defines a heterogeneous population of neurosphere-forming cells in the adult murine brain. Aging Cell 4:197–207

Brook GA, Perez-Bouza A, Noth J, Nacimiento W (1999) Astrocytes re-express nestin in deafferented target territories of the adult rat hippocampus. Neuroreport 10:1007–1011

Brooks-Kayal AR, Munir M, Jin H, Robinson MB (1998) The glutamate transporter, GLT-1, is expressed in cultured hippocampal neurons. Neurochem Int 33:95–100

Brown JP, Couillard-Despres S, Cooper-Kuhn CM, Winkler J, Aigner L, Kuhn HG (2003) Transient expression of doublecortin during adult neurogenesis. J Comp Neurol 467:1–10

Brunne B, Zhao S, Derouiche A, Herz J, May P, Frotscher M, Bock HH (2010) Origin, maturation, and astroglial transformation of secondary radial glial cells in the developing dentate gyrus. Glia 58:1553–1569

Cameron HA, McKay RD (2001) Adult neurogenesis produces a large pool of new granule cells in the dentate gyrus. J Comp Neurol 435:406–417

Cameron HA, Woolley CS, McEwen BS, Gould E (1993) Differentiation of newly born neurons and glia in the dentate gyrus of the adult rat. Neuroscience 56:337–344

Cao F, Hata R, Zhu P, Ma YJ, Tanaka J, Hanakawa Y, Hashimoto K, Niinobe M, Yoshikawa K, Sakanaka M (2006) Overexpression of SOCS3 inhibits astrogliogenesis and promotes maintenance of neural stem cells. J Neurochem 98:459–470

Carlen M, Cassidy RM, Brismar H, Smith GA, Enquist LW, Frisen J (2002) Functional integration of adult-born neurons. Curr Biol 12:606–608

Cecchini T, Ciaroni S, Ferri P, Ambrogini P, Cuppini R, Santi S, Del Grande P (2003) Alpha-tocopherol, an exogenous factor of adult hippocampal neurogenesis regulation. J Neurosci Res 73:447–455

Chen W, Mahadomrongkul V, Berger UV, Bassan M, DeSilva T, Tanaka K, Irwin N, Aoki C, Rosenberg PA (2004) The glutamate transporter GLT1a is expressed in excitatory axon terminals of mature hippocampal neurons. J Neurosci 24:1136–1148

Cooper-Kuhn CM, Kuhn HG (2002) Is it all DNA repair? Methodological considerations for detecting neurogenesis in the adult brain. Brain Res Dev Brain Res 134:13–21

Cornford EM, Oldendorf WH (1986) Epilepsy and the blood-brain barrier. Adv Neurol 44:787–812

Couillard-Despres S, Winner B, Schaubeck S, Aigner R, Vroemen M, Weidner N, Bogdahn U, Winkler J, Kuhn HG, Aigner L (2005) Doublecortin expression levels in adult brain reflect neurogenesis. Eur J Neurosci 21:1–14

Couillard-Despres S, Winner B, Karl C, Lindemann G, Schmid P, Aigner R, Laemke J, Bogdahn U, Winkler J, Bischofberger J, Aigner L (2006) Targeted transgene expression in neuronal precursors: watching young neurons in the old brain. Eur J Neurosci 24:1535–1545

Cremer H, Chazal G, Lledo PM, Rougon G, Montaron MF, Mayo W, Le Moal M, Abrous DN (2000) PSA-NCAM: an important regulator of hippocampal plasticity. Int J Dev Neurosci 18:213–220

Crespel A, Rigau V, Coubes P, Rousset MC, Bock F de, Okano H, Baldy-Moulinier M, Bockaert J, Lerner-Natoli M (2005) Increased number of neural progenitors in human temporal lobe epilepsy. Neurobiol Dis 19:436–450

Davis A, Cowell JK (1993) Mutations in the PAX6 gene in patients with hereditary aniridia. Hum Mol Genet 2:2093–2097

Dayer AG, Ford AA, Cleaver KM, Yassaee M, Cameron HA (2003) Short-term and long-term survival of new neurons in the rat dentate gyrus. J Comp Neurol 460:563–572

Dityatev A, Dityateva G, Sytnyk V, Delling M, Toni N, Nikonenko I, Muller D, Schachner M (2004) Polysialylated neural cell adhesion molecule promotes remodeling and formation of hippocampal synapses. J Neurosci 24:9372–9382

Doetsch F (2003) The glial identity of neural stem cells. Nat Neurosci 6:1127–1134

Doetsch F, Garcia-Verdugo JM, Avarez-Buylla A (1997) Cellular composition and three-dimensional organization of the subventricular germinal zone in the adult mammalian brain. J Neurosci 17:5046–5061

Dominguez MI, Blasco-Ibanez JM, Crespo C, Marques-Mari AI, Martinez-Guijarro FJ (2003) Calretinin/PSA-NCAM immunoreactive granule cells after hippocampal damage produced by kainic acid and DEDTC treatment in mouse. Brain Res 966:206–217

Doyle KL, Khan M, Cunningham AM (2001) Expression of the intermediate filament protein nestin by sustentacular cells in mature olfactory neuroepithelium. J Comp Neurol 437:186–195

Drapeau E, Mayo W, Aurousseau C, Le Moal M, Piazza PV, Abrous DN (2003) Spatial memory performances of aged rats in the water maze predict levels of hippocampal neurogenesis. Proc Natl Acad Sci USA 100:14385–14390

Duggal N, Schmidt-Kastner R, Hakim AM (1997) Nestin expression in reactive astrocytes following focal cerebral ischemia in rats. Brain Res 768:1–9

Easter SS Jr, Ross LS, Frankfurter A (1993) Initial tract formation in the mouse brain. J Neurosci 13:285–299

Eckenhoff MF, Rakic P (1984) Radial organization of the hippocampal dentate gyrus: a Golgi, ultrastructural, and immunocytochemical analysis in the developing rhesus monkey. J Comp Neurol 223:1–21

Eckenhoff MF, Rakic P (1988) Nature and fate of proliferative cells in the hippocampal dentate gyrus during the life span of the rhesus monkey. J Neurosci 8:2729–2747

Ehninger D, Kempermann G (2008) Neurogenesis in the adult hippocampus. Cell Tissue Res 331:243–250

Eliasson C, Sahlgren C, Berthold CH, Stakeberg J, Celis JE, Betsholtz C, Eriksson JE, Pekny M (1999) Intermediate filament protein partnership in astrocytes. J Biol Chem 274:23996–24006

Englund C, Fink A, Lau C, Pham D, Daza RA, Bulfone A, Kowalczyk T, Hevner RF (2005) Pax6, Tbr2, and Tbr1 are expressed sequentially by radial glia, intermediate progenitor cells, and postmitotic neurons in developing neocortex. J Neurosci 25:247–251

Episkopou V (2005) SOX2 functions in adult neural stem cells. Trends Neurosci 28:219–221

Eriksson PS, Perfilieva E, Bjork-Eriksson T, Alborn AM, Nordborg C, Peterson DA, Gage FH (1998) Neurogenesis in the adult human hippocampus. Nat Med 4:1313–1317

Favaro R, Valotta M, Ferri AL, Latorre E, Mariani J, Giachino C, Lancini C, Tosetti V, Ottolenghi S, Taylor V, Nicolis SK (2009) Hippocampal development and neural stem cell maintenance require Sox2-dependent regulation of Shh. Nat Neurosci 12:1248–1256

Fernandez A, Radmilovich M, Trujillo-Cenoz O (2002) Neurogenesis and gliogenesis in the spinal cord of turtles. J Comp Neurol 453:131–144

Ferri AL, Cavallaro M, Braida D, Di Cristofano A, Canta A, Vezzani A, Ottolenghi S, Pandolfi PP, Sala M, DeBiasi S, Nicolis SK (2004) Sox2 deficiency causes neurodegeneration and impaired neurogenesis in the adult mouse brain. Development 131:3805–3819

Filippov V, Kronenberg G, Pivneva T, Reuter K, Steiner B, Wang LP, Yamaguchi M, Kettenmann H, Kempermann G (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

Francis F, Koulakoff A, Boucher D, Chafey P, Schaar B, Vinet MC, Friocourt G, McDonnell N, Reiner O, Kahn A, McConnell SK,

Berwald-Netter Y, Denoulet P, Chelly J (1999) Doublecortin is a developmentally regulated, microtubule-associated protein expressed in migrating and differentiating neurons. Neuron 23:247–256

Friocourt G, Koulakoff A, Chafey P, Boucher D, Fauchereau F, Chelly J, Francis F (2003) Doublecortin functions at the extremities of growing neuronal processes. Cereb Cortex 13:620–626

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

Garcia AD, Doan NB, Imura T, Bush TG, Sofroniew MV (2004) GFAP-expressing progenitors are the principal source of constitutive neurogenesis in adult mouse forebrain. Nat Neurosci 7:1233–1241

Gerdes J, Schwab U, Lemke H, Stein H (1983) Production of a mouse monoclonal antibody reactive with a human nuclear antigen associated with cell proliferation. Int J Cancer 31:13–20

Gleeson JG, Allen KM, Fox JW, Lamperti ED, Berkovic S, Scheffer I, Cooper EC, Dobyns WB, Minnerath SR, Ross ME, Walsh CA (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

Gleeson JG, Lin PT, Flanagan LA, Walsh CA (1999a) Doublecortin is a microtubule-associated protein and is expressed widely by migrating neurons. Neuron 23:257–271

Gleeson JG, Minnerath SR, Fox JW, Allen KM, Luo RF, Hong SE, Berg MJ, Kuzniecky R, Reitnauer PJ, Borgatti R, Mira AP, Guerrini R, Holmes GL, Rooney CM, Berkovic S, Scheffer I, Cooper EC, Ricci S, Cusmai R, Crawford TO, Leroy R, Andermann E, Wheless JW, Dobyns WB, Walsh CA (1999b) Characterization of mutations in the gene doublecortin in patients with double cortex syndrome. Ann Neurol 45:146–153

Gomez-Climent MA, Guirado R, Castillo-Gomez E, Varea E, Gutierrez-Mecinas M, Gilabert-Juan J, Garcia-Mompo C, Vidueira S, Sanchez-Mataredona D, Hernandez S, Blasco-Ibanez JM, Crespo C, Rutishauser U, Schachner M, Nacher J (2011) The polysialylated form of the neural cell adhesion molecule (PSA-NCAM) is expressed in a subpopulation of mature cortical interneurons characterized by reduced structural features and connectivity. Cereb Cortex 21:1028–1041

Götz M, Stoykova A, Gruss P (1998) Pax6 controls radial glia differentiation in the cerebral cortex. Neuron 21:1031–1044

Gould BB, Rakic P (1981) The total number, time or origin and kinetics of proliferation of neurons comprising the deep cerebellar nuclei in the rhesus monkey. Exp Brain Res 44:195–206

Gould E, Reeves AJ, Fallah M, Tanapat P, Gross CG, Fuchs E (1999a) Hippocampal neurogenesis in adult Old World primates. Proc Natl Acad Sci USA 96:5263–5267

Gould E, Reeves AJ, Graziano MS, Gross CG (1999b) Neurogenesis in the neocortex of adult primates. Science 286:548–552

Gould E, Vail N, Wagers M, Gross CG (2001) Adult-generated hippocampal and neocortical neurons in macaques have a transient existence. Proc Natl Acad Sci USA 98:10910–10917

Gulyas AI, Miettinen R, Jacobowitz DM, Freund TF (1992) Calretinin is present in non-pyramidal cells of the rat hippocampus–I. A new type of neuron specifically associated with the mossy fibre system. Neuroscience 48:1–27

Hanna-Morris A, Badvie S, Cohen P, McCullough T, Andreyev HJ, Allen-Mersh TG (2009) Minichromosome maintenance protein 2 (MCM2) is a stronger discriminator of increased proliferation in mucosa adjacent to colorectal cancer than Ki-67. J Clin Pathol 62:325–330

Hanson IM, Seawright A, Hardman K, Hodgson S, Zaletayev D, Fekete G, Heyningen V van (1993) PAX6 mutations in aniridia. Hum Mol Genet 2:915–920

Hartfuss E, Galli R, Heins N, Gotz M (2001) Characterization of CNS precursor subtypes and radial glia. Dev Biol 229:15–30

Hastings NB, Gould E (1999) Rapid extension of axons into the CA3 region by adult-generated granule cells. J Comp Neurol 413:146–154

Heins N, Malatesta P, Cecconi F, Nakafuku M, Tucker KL, Hack MA, Chapouton P, Barde YA, Gotz M (2002) Glial cells generate neurons: the role of the transcription factor Pax6. Nat Neurosci 5:308–315

Hendzel MJ, Wei Y, Mancini MA, Van Hooser A, Ranalli T, Brinkley BR, Bazett-Jones DP, Allis CD (1997) Mitosis-specific phosphorylation of histone H3 initiates primarily within pericentromeric heterochromatin during G2 and spreads in an ordered fashion coincident with mitotic chromosome condensation. Chromosoma 106:348–360

Hevner RF, Hodge RD, Daza RA, Englund C (2006) Transcription factors in glutamatergic neurogenesis: conserved programs in neocortex, cerebellum, and adult hippocampus. Neurosci Res 55:223–233

Hodge RD, Kowalczyk TD, Wolf SA, Encinas JM, Rippey C, Enikolopov G, Kempermann G, Hevner RF (2008) Intermediate progenitors in adult hippocampal neurogenesis: Tbr2 expression and coordinate regulation of neuronal output. J Neurosci 28:3707–3717

Ihrie RA, Alvarez-Buylla A (2008) Cells in the astroglial lineage are neural stem cells. Cell Tissue Res 331:179–191

Jacobowitz DM, Winsky L (1991) Immunocytochemical localization of calretinin in the forebrain of the rat. J Comp Neurol 304:198–218

Jankovski A, Garcia C, Soriano E, Sotelo C (1998) Proliferation, migration and differentiation of neuronal progenitor cells in the adult mouse subventricular zone surgically separated from its olfactory bulb. Eur J Neurosci 10:3853–3868

Jessberger S, Romer B, Babu H, Kempermann G (2005) Seizures induce proliferation and dispersion of doublecortin-positive hippocampal progenitor cells. Exp Neurol 196:342–351

Jiang J, Chan YS, Loh YH, Cai J, Tong GQ, Lim CA, Robson P, Zhong S, Ng HH (2008) A core Klf circuitry regulates self-renewal of embryonic stem cells. Nat Cell Biol 10:353–360

Jin K, Minami M, Lan JQ, Mao XO, Batteur S, Simon RP, Greenberg DA (2001) Neurogenesis in dentate subgranular zone and rostral subventricular zone after focal cerebral ischemia in the rat. Proc Natl Acad Sci USA 98:4710–4715

Jinno S (2011) Topographic differences in adult neurogenesis in the mouse hippocampus: a stereology-based study using endogenous markers. Hippocampus 21:467–480

Jordan T, Hanson I, Zaletayev D, Hodgson S, Prosser J, Seawright A, Hastie N, Heyningen V van (1992) The human PAX6 gene is mutated in two patients with aniridia. Nat Genet 1:328–332

Kalman M, Ajtai BM (2001) A comparison of intermediate filament markers for presumptive astroglia in the developing rat neocortex: immunostaining against nestin reveals more detail, than GFAP or vimentin. Int J Dev Neurosci 19:101–108

Kawai T, Takagi N, Miyake-Takagi K, Okuyama N, Mochizuki N, Takeo S (2004) Characterization of BrdU-positive neurons induced by transient global ischemia in adult hippocampus. J Cereb Blood Flow Metab 24:548–555

Kee N, Sivalingam S, Boonstra R, Wojtowicz JM (2002) The utility of Ki-67 and BrdU as proliferative markers of adult neurogenesis. J Neurosci Methods 115:97–105

Kempermann G, Kuhn HG, Gage FH (1997) More hippocampal neurons in adult mice living in an enriched environment. Nature 386:493–495

Kempermann G, Gast D, Kronenberg G, Yamaguchi M, Gage FH (2003) Early determination and long-term persistence of adult-generated new neurons in the hippocampus of mice. Development 130:391–399

Kempermann G, Jessberger S, Steiner B, Kronenberg G (2004) Milestones of neuronal development in the adult hippocampus. Trends Neurosci 27:447–452

Kim SH, Kim HB, Jang MH, Lim BV, Kim YJ, Kim YP, Kim SS, Kim EH, Kim CJ (2002) Treadmill exercise increases cell proliferation without altering of apoptosis in dentate gyrus of Sprague-Dawley rats. Life Sci 71:1331–1340

Kimura N, Nakashima K, Ueno M, Kiyama H, Taga T (1999) A novel mammalian T-box-containing gene, Tbr2, expressed in mouse developing brain. Brain Res Dev Brain Res 115:183–193

Knoth R, Singec I, Ditter M, Pantazis G, Capetian P, Meyer RP, Horvat V, Volk B, Kempermann G (2010) Murine features of neurogenesis in the human hippocampus across the lifespan from 0 to 100 years. PLoS ONE 5:e8809

Kodani I, Shomori K, Osaki M, Kuratate I, Ryoke K, Ito H (2001) Expression of minichromosome maintenance 2 (MCM2), Ki-67, and cell-cycle-related molecules, and apoptosis in the normal-dysplasia-carcinoma sequence of the oral mucosa. Pathobiology 69:150–158

Kornack DR, Rakic P (1999) Continuation of neurogenesis in the hippocampus of the adult macaque monkey. Proc Natl Acad Sci USA 96:5768–5773

Kronenberg G, Reuter K, Steiner B, Brandt MD, Jessberger S, Yamaguchi M, Kempermann G (2003) Subpopulations of proliferating cells of the adult hippocampus respond differently to physiologic neurogenic stimuli. J Comp Neurol 467:455–463

Krum JM, Rosenstein JM (1999) Transient coexpression of nestin, GFAP, and vascular endothelial growth factor in mature reactive astroglia following neural grafting or brain wounds. Exp Neurol 160:348–360

Kuan CY, Schloemer AJ, Lu A, Burns KA, Weng WL, Williams MT, Strauss KI, Vorhees CV, Flavell RA, Davis RJ, Sharp FR, Rakic P (2004) Hypoxia-ischemia induces DNA synthesis without cell proliferation in dying neurons in adult rodent brain. J Neurosci 24:10763–10772

Kugler P, Schleyer V (2004) Developmental expression of glutamate transporters and glutamate dehydrogenase in astrocytes of the postnatal rat hippocampus. Hippocampus 14:975–985

Kuhn HG, Dickinson-Anson 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

Landgren H, Curtis MA (2011) Locating and labeling neural stem cells in the brain. J Cell Physiol 226:1–7

Lavado A, Lagutin OV, Chow LM, Baker SJ, Oliver G (2010) Prox1 is required for granule cell maturation and intermediate progenitor maintenance during brain neurogenesis. PLoS Biol 8:e1000460

Lee A, Pow DV (2010) Astrocytes: glutamate transport and alternate splicing of transporters. Int J Biochem Cell Biol 42:1901–1906

Lee JE, Hollenberg SM, Snider L, Turner DL, Lipnick N, Weintraub H (1995) Conversion of *Xenopus* ectoderm into neurons by NeuroD, a basic helix-loop-helix protein. Science 268:836–844

Lendahl U, Zimmerman LB, McKay RD (1990) CNS stem cells express a new class of intermediate filament protein. Cell 60:585–595

Levitt P, Rakic P (1980) Immunoperoxidase localization of glial fibrillary acidic protein in radial glial cells and astrocytes of the developing rhesus monkey brain. J Comp Neurol 193:815–840

Limke TL, Cai J, Miura T, Rao MS, Mattson MP (2003) Distinguishing features of progenitor cells in the late embryonic and adult hippocampus. Dev Neurosci 25:257–272

Lind D, Franken S, Kappler J, Jankowski J, Schilling K (2005) Characterization of the neuronal marker NeuN as a multiply phosphorylated antigen with discrete subcellular localization. J Neurosci Res 79:295–302

Linden MD, Torres FX, Kubus J, Zarbo RJ (1992) Clinical application of morphologic and immunocytochemical assessments of cell proliferation. Am J Clin Pathol 97:S4–S13

Liu J, Solway K, Messing RO, Sharp FR (1998) Increased neurogenesis in the dentate gyrus after transient global ischemia in gerbils. J Neurosci 18:7768–7778

Liu M, Pleasure SJ, Collins AE, Noebels JL, Naya FJ, Tsai MJ, Lowenstein DH (2000) Loss of BETA2/NeuroD leads to malformation of the dentate gyrus and epilepsy. Proc Natl Acad Sci USA 97:865–870

Liu Y, Fujise N, Kosaka T (1996) Distribution of calretinin immunoreactivity in the mouse dentate gyrus. I. General description. Exp Brain Res 108:389–403

Liu YW, Curtis MA, Gibbons HM, Mee EW, Bergin PS, Teoh HH, Connor B, Dragunow M, Faull RL (2008) Doublecortin expression in the normal and epileptic adult human brain. Eur J Neurosci 28:2254–2265

Llorens-Martin M, Torres-Aleman I, Trejo JL (2006) Pronounced individual variation in the response to the stimulatory action of exercise on immature hippocampal neurons. Hippocampus 16:480–490

Lucassen PJ, Stumpel MW, Wang Q, Aronica E (2010) Decreased numbers of progenitor cells but no response to antidepressant drugs in the hippocampus of elderly depressed patients. Neuropharmacology 58:940–949

Luo C, Xu H, Li XM (2005) Quetiapine reverses the suppression of hippocampal neurogenesis caused by repeated restraint stress. Brain Res 1063:32–39

Luskin MB (1993) Restricted proliferation and migration of postnatally generated neurons derived from the forebrain subventricular zone. Neuron 11:173–189

Luskin MB (1994) Neuronal cell lineage in the vertebrate central nervous system. FASEB J 8:722–730

Maekawa M, Takashima N, Arai Y, Nomura T, Inokuchi K, Yuasa S, Osumi N (2005) Pax6 is required for production and maintenance of progenitor cells in postnatal hippocampal neurogenesis. Genes Cells 10:1001–1014

Maslov AY, Barone TA, Plunkett RJ, Pruitt SC (2004) Neural stem cell detection, characterization, and age-related changes in the subventricular zone of mice. J Neurosci 24:1726–1733

Menezes JR, Luskin MB (1994) Expression of neuron-specific tubulin defines a novel population in the proliferative layers of the developing telencephalon. J Neurosci 14:5399–5416

Merkle FT, Tramontin AD, Garcia-Verdugo JM, Alvarez-Buylla A (2004) Radial glia give rise to adult neural stem cells in the subventricular zone. Proc Natl Acad Sci USA 101:17528–17532

Merrill DA, Karim R, Darraq M, Chiba AA, Tuszynski MH (2003) Hippocampal cell genesis does not correlate with spatial learning ability in aged rats. J Comp Neurol 459:201–207

Miettinen R, Gulyas AI, Baimbridge KG, Jacobowitz DM, Freund TF (1992) Calretinin is present in non-pyramidal cells of the rat hippocampus. II. Co-existence with other calcium binding proteins and GABA. Neuroscience 48:29–43

Miller MW, Nowakowski RS (1988) Use of bromodeoxyuridine-immunohistochemistry to examine the proliferation, migration and time of origin of cells in the central nervous system. Brain Res 457:44–52

Ming GL, Song H (2005) Adult neurogenesis in the mammalian central nervous system. Annu Rev Neurosci 28:223–250

Minichiello L, Korte M, Wolfer D, Kuhn R, Unsicker K, Cestari V, Rossi-Arnaud C, Lipp HP, Bonhoeffer T, Klein R (1999) Essential role for TrkB receptors in hippocampus-mediated learning. Neuron 24:401–414

Minturn JE, Fryer HJ, Geschwind DH, Hockfield S (1995a) TOAD-64, a gene expressed early in neuronal differentiation in the rat, is related to unc-33, a *C. elegans* gene involved in axon outgrowth. J Neurosci 15:6757–6766

Minturn JE, Geschwind DH, Fryer HJ, Hockfield S (1995b) Early postmitotic neurons transiently express TOAD-64, a neural specific protein. J Comp Neurol 355:369–379

Mitra R, Sundlass K, Parker KJ, Schatzberg AF, Lyons DM (2006) Social stress-related behavior affects hippocampal cell proliferation in mice. Physiol Behav 89:123–127

Miyata T, Maeda T, Lee JE (1999) NeuroD is required for differentiation of the granule cells in the cerebellum and hippocampus. Genes Dev 13:1647–1652

Modi PK, Kanungo MS (2010) Age-dependent expression of S100beta in the brain of mice. Cell Mol Neurobiol 30:709–716

Mullen RJ, Buck CR, Smith AM (1992) NeuN, a neuronal specific nuclear protein in vertebrates. Development 116:201–211

Munoz-Elias G, Woodbury D, Black IB (2003) Marrow stromal cells, mitosis, and neuronal differentiation: stem cell and precursor functions. Stem Cells 21:437–448

Nacher J, Crespo C, McEwen BS (2001) Doublecortin expression in the adult rat telencephalon. Eur J Neurosci 14:629–644

Nacher J, Blasco-Ibanez JM, McEwen BS (2002) Non-granule PSA-NCAM immunoreactive neurons in the rat hippocampus. Brain Res 930:1–11

Nacher J, Varea E, Blasco-Ibanez JM, Castillo-Gomez E, Crespo C, Martinez-Guijarro FJ, McEwen BS (2005) Expression of the transcription factor Pax 6 in the adult rat dentate gyrus. J Neurosci Res 81:753–761

Namba T, Mochizuki H, Onodera M, Mizuno Y, Namiki H, Seki T (2005) The fate of neural progenitor cells expressing astrocytic and radial glial markers in the postnatal rat dentate gyrus.Eur J Neurosci 22:1928–1941

Navarro-Quiroga I, Hernandez-Valdes M, Lin SL, Naegele JR (2006) Postnatal cellular contributions of the hippocampus subventricular zone to the dentate gyrus, corpus callosum, fimbria, and cerebral cortex. J Comp Neurol 497:833–845

Nilsson M, Perfilieva E, Johansson U, Orwar O, Eriksson PS (1999) Enriched environment increases neurogenesis in the adult rat dentate gyrus and improves spatial memory. J Neurobiol 39:569–578

Nowakowski RS, Hayes NL (2000) New neurons: extraordinary evidence or extraordinary conclusion? Science 288:771

Osaki M, Osaki M, Yamashita H, Shomori K, Yoshida H, Ito H (2002) Expression of minichromosome maintenance-2 in human malignant fibrous histiocytomas: correlations with Ki-67 and P53 expression, and apoptosis. Int J Mol Med 10:161–168

Osumi N, Shinohara H, Numayama-Tsuruta K, Maekawa M (2008) Concise review: Pax6 transcription factor contributes to both embryonic and adult neurogenesis as a multifunctional regulator. Stem Cells 26:1663–1672

Pardridge WM, Connor JD, Crawford IL (1975) Permeability changes in the blood-brain barrier: causes and consequences. CRC Crit Rev Toxicol 3:159–199

Parent JM, Yu TW, Leibowitz RT, Geschwind DH, Sloviter RS, Lowenstein DH (1997) Dentate granule cell neurogenesis is increased by seizures and contributes to aberrant network reorganization in the adult rat hippocampus. J Neurosci 17:3727–3738

Pham K, Nacher J, Hof PR, McEwen BS (2003) Repeated restraint stress suppresses neurogenesis and induces biphasic PSA-NCAM expression in the adult rat dentate gyrus. Eur J Neurosci 17:879–886

Pines G, Danbolt NC, Bjoras M, Zhang Y, Bendahan A, Eide L, Koepsell H, Storm-Mathisen J, Seeberg E, Kanner BI (1992) Cloning and expression of a rat brain L-glutamate transporter. Nature 360:464–467

Pinto L, Gotz M (2007) Radial glial cell heterogeneity—the source of diverse progeny in the CNS. Prog Neurobiol 83:2–23

Plachez C, Danbolt NC, Recasens M (2000) Transient expression of the glial glutamate transporters GLAST and GLT in hippocampal neurons in primary culture. J Neurosci Res 59:587–593

Poulsen FR, Blaabjerg M, Montero M, Zimmer J (2005) Glutamate receptor antagonists and growth factors modulate dentate granule cell neurogenesis in organotypic, rat hippocampal slice cultures. Brain Res 1051:35–49

Quinn CC, Gray GE, Hockfield S (1999) A family of proteins implicated in axon guidance and outgrowth. J Neurobiol 41:158–164

Ra SM, Kim H, Jang MH, Shin MC, Lee TH, Lim BV, Kim CJ, Kim EH, Kim KM, Kim SS (2002) Treadmill running and swimming increase cell proliferation in the hippocampal dentate gyrus of rats. Neurosci Lett 333:123–126

Rami A, Brehier A, Thomasset M, Rabie A (1987) Cholecalcin (28-kDa calcium-binding protein) in the rat hippocampus: development in normal animals and in altered thyroid states. An immunocytochemical study. Dev Biol 124:228–238

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

Raponi E, Agenes F, Delphin C, Assard N, Baudier J, Legraverend C, Deloulme JC (2007) S100B expression defines a state in which GFAP-expressing cells lose their neural stem cell potential and acquire a more mature developmental stage. Glia 55:165–177

Reynolds BA, Weiss S (1992) Generation of neurons and astrocytes from isolated cells of the adult mammalian central nervous system. Science 255:1707–1710

Rolls ET (2000) Memory systems in the brain. Annu Rev Psychol 51:599–630

Roybon L, Deierborg T, Brundin P, Li JY (2009) Involvement of Ngn2, Tbr and NeuroD proteins during postnatal olfactory bulb neurogenesis. Eur J Neurosci 29:232–243

Sahin KS, Mahmood A, Li Y, Yavuz E, Chopp M (1999) Expression of nestin after traumatic brain injury in rat brain. Brain Res 840:153–157

Sakakibara S, Imai T, Hamaguchi K, Okabe M, Aruga J, Nakajima K, Yasutomi D, Nagata T, Kurihara Y, Uesugi S, Miyata T, Ogawa M, Mikoshiba K, Okano H (1996) Mouse-Musashi-1, a neural RNA-binding protein highly enriched in the mammalian CNS stem cell. Dev Biol 176:230–242

Sakurai K, Osumi N (2008) The neurogenesis-controlling factor, Pax6, inhibits proliferation and promotes maturation in murine astrocytes. J Neurosci 28:4604–4612

Sandi C, Merino JJ, Cordero MI, Touyarot K, Venero C (2001) Effects of chronic stress on contextual fear conditioning and the hippocampal expression of the neural cell adhesion molecule, its polysialylation, and L1. Neuroscience 102:329–339

Savchenko VL, McKanna JA, Nikonenko IR, Skibo GG (2000) Microglia and astrocytes in the adult rat brain: comparative immunocytochemical analysis demonstrates the efficacy of lipocortin 1 immunoreactivity. Neuroscience 96:195–203

Scharfman H, Goodman J, Macleod A, Phani S, Antonelli C, Croll S (2005) Increased neurogenesis and the ectopic granule cells after intrahippocampal BDNF infusion in adult rats. Exp Neurol 192:348–356

Schmidt-Kastner R, Humpel C (2002) Nestin expression persists in astrocytes of organotypic slice cultures from rat cortex. Int J Dev Neurosci 20:29–38

Schmidt-Kastner R, Szymas J, Hossmann KA (1990) Immunohistochemical study of glial reaction and serum-protein extravasation in relation to neuronal damage in rat hippocampus after ischemia. Neuroscience 38:527–540

Scott BW, Wang S, Burnham WM, De BU, Wojtowicz JM (1998) Kindling-induced neurogenesis in the dentate gyrus of the rat. Neurosci Lett 248:73–76

Seaberg RM, van der Kooy D (2002) Adult rodent neurogenic regions: the ventricular subependyma contains neural stem cells, but the dentate gyrus contains restricted progenitors. J Neurosci 22:1784–1793

Seki T (2002a) 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

Seki T (2002b) Hippocampal adult neurogenesis occurs in a microenvironment provided by PSA-NCAM-expressing immature neurons. J Neurosci Res 69:772–783

Seki T, Arai Y (1991) The persistent expression of a highly polysialylated NCAM in the dentate gyrus of the adult rat. Neurosci Res 12:503–513

Seki T, Arai Y (1999) Temporal and spacial relationships between PSA-NCAM-expressing, newly generated granule cells, and radial glia-like cells in the adult dentate gyrus. J Comp Neurol 410:503–513

Sequier JM, Hunziker W, Richards G (1988) Localization of calbindin D28 mRNA in rat tissues by in situ hybridization. Neurosci Lett 86:155–160

Seress L, Gulyas AI, Freund TF (1991) Parvalbumin- and calbindin D28k-immunoreactive neurons in the hippocampal formation of the macaque monkey. J Comp Neurol 313:162–177

Seress L, Gulyas AI, Freund TF (1992) Pyramidal neurons are immunoreactive for calbindin D28k in the CA1 subfield of the human hippocampus. Neurosci Lett 138:257–260

Seri B, Garcia-Verdugo JM, McEwen BS, Alvarez-Buylla A (2001) Astrocytes give rise to new neurons in the adult mammalian hippocampus. J Neurosci 21:7153–7160

Seri B, Garcia-Verdugo JM, Collado-Morente L, McEwen BS, Alvarez-Buylla A (2004) Cell types, lineage, and architecture of the germinal zone in the adult dentate gyrus. J Comp Neurol 478:359–378

Seri B, Herrera DG, Gritti A, Ferron S, Collado L, Vescovi A, Garcia-Verdugo JM, Alvarez-Buylla A (2006) Composition and organization of the SCZ: a large germinal layer containing neural stem cells in the adult mammalian brain. Cereb Cortex 16 (Suppl 1):i103–i111

Shen S, Liu A, Li J, Wolubah C, Casaccia-Bonnefil P (2008) Epigenetic memory loss in aging oligodendrocytes in the corpus callosum. Neurobiol Aging 29:452–463

Shors TJ, Townsend DA, Zhao M, Kozorovitskiy Y, Gould E (2002) Neurogenesis may relate to some but not all types of hippocampal-dependent learning. Hippocampus 12:578–584

Sivilia S, Giuliani A, Del Vecchio G, Giardino L, Calza L (2008) Age-dependent impairment of hippocampal neurogenesis in chronic cerebral hypoperfusion. Neuropathol Appl Neurobiol 34:52–61

Sloviter RS (1989) Calcium-binding protein (calbindin-D28k) and parvalbumin immunocytochemistry: localization in the rat hippocampus with specific reference to the selective vulnerability of hippocampal neurons to seizure activity. J Comp Neurol 280:183–196

Stagaard Janas M, Nowakowski RS, Mollgard K (1991) Glial cell differentiation in neuron-free and neuron-rich regions. II. Early appearance of S-100 protein positive astrocytes in human fetal hippocampus. Anat Embryol (Berl) 184:559–569

Steiner B, Kronenberg G, Jessberger S, Brandt MD, Reuter K, Kempermann G (2004) Differential regulation of gliogenesis in the context of adult hippocampal neurogenesis in mice. Glia 46:41–52

Steiner B, Klempin F, Wang L, Kott M, Kettenmann H, Kempermann G (2006) Type-2 cells as link between glial and neuronal lineage in adult hippocampal neurogenesis. Glia 54:805–814

Steiner B, Zurborg S, Horster H, Fabel K, Kempermann G (2008) Differential 24 h responsiveness of Prox1-expressing precursor cells in adult hippocampal neurogenesis to physical activity, environmental enrichment, and kainic acid-induced seizures. Neuroscience 154:521–529

Stoykova A, Gruss P (1994) Roles of Pax-genes in developing and adult brain as suggested by expression patterns. J Neurosci 14:1395–1412

Stringer JL (1996) Repeated seizures increase GFAP and vimentin in the hippocampus. Brain Res 717:147–153

Sundholm-Peters NL, Yang HK, Goings GE, Walker AS, Szele FG (2004) Radial glia-like cells at the base of the lateral ventricles in adult mice. J Neurocytol 33:153–164

Takemura NU (2005) Evidence for neurogenesis within the white matter beneath the temporal neocortex of the adult rat brain. Neuroscience 134:121–132

Tamimi R, Steingrimsson E, Copeland NG, Dyer-Montgomery K, Lee JE, Hernandez R, Jenkins NA, Tapscott SJ (1996) The NEUROD gene maps to human chromosome 2q32 and mouse chromosome 2. Genomics 34:418–421

Taupin P (2007) BrdU immunohistochemistry for studying adult neurogenesis: paradigms, pitfalls, limitations, and validation. Brain Res Rev 53:198–214

Ton CC, Hirvonen H, Miwa H, Weil MM, Monaghan P, Jordan T, Heyningen V van, Hastie ND, Meijers-Heijboer H, Drechsler M (1991) Positional cloning and characterization of a paired box- and homeobox-containing gene from the aniridia region. Cell 67:1059–1074

Uda M, Ishido M, Kami K, Masuhara M (2006) Effects of chronic treadmill running on neurogenesis in the dentate gyrus of the hippocampus of adult rat. Brain Res 1104:64–72

Van der Borght K, Wallinga AE, Luiten PG, Eggen BJ, van der Zee AE (2005) Morris water maze learning in two rat strains increases the expression of the polysialylated form of the neural cell adhesion molecule in the dentate gyrus but has no effect on hippocampal neurogenesis. Behav Neurosci 119:926–932

Van Kampen JM, Hagg T, Robertson HA (2004) Induction of neurogenesis in the adult rat subventricular zone and neostriatum following dopamine D receptor stimulation. Eur J Neurosci 19:2377–2387

van Praag H, Christie BR, Sejnowski TJ, Gage FH (1999) Running enhances neurogenesis, learning, and long-term potentiation in mice. Proc Natl Acad Sci USA 96:13427–13431

van Praag H, Schinder AF, Christie BR, Toni N, Palmer TD, Gage FH (2002) Functional neurogenesis in the adult hippocampus. Nature 415:1030–1034

Venero C, Herrero AI, Touyarot K, Cambon K, Lopez-Fernandez MA, Berezin V, Bock E, Sandi C (2006) Hippocampal up-regulation of NCAM expression and polysialylation plays a key role on spatial memory. Eur J Neurosci 23:1585–1595

Verwer RW, Sluiter AA, Balesar RA, Baayen JC, Noske DP, Dirven CM, Wouda J, van Dam AM, Lucassen PJ, Swaab DF (2007) Mature astrocytes in the adult human neocortex express the early neuronal marker doublecortin. Brain 130:3321–3335

von Bohlen und Halbach O (2007) Immunohistological markers for staging neurogenesis in adult hippocampus. Cell Tissue Res 329:409–420

von Bohlen und Halbach O (2009) Structure and function of dendritic spines within the hippocampus. Ann Anat 191:518–531

von Bohlen und Halbach O (2010) Involvement of BDNF in age-dependent alterations in the hippocampus. Front Aging Neurosci 2:1–11

von Bohlen und Halbach O, Albrecht D (1998) Tracing of axonal connectivities in a combined slice preparation of rat brains—a study by rhodamine-dextran-amine-application in the lateral nucleus of the amygdala. J Neurosci Methods 81:169–175

von Bohlen und Halbach O, Krause S, Medina D, Sciarretta C, Minichiello L, Unsicker K (2006) Regional- and age-dependent reduction in trkB receptor expression in the hippocampus is associated with altered spine morphologies. Biol Psychiatry 59:793–800

von Bohlen und Halbach O, Minichiello L, Unsicker K (2008) TrkB but not trkC receptors are necessary for postnatal maintenance of hippocampal spines. Neurobiol Aging 29:1247–1255

Walther C, Gruss P (1991) Pax-6, a murine paired box gene, is expressed in the developing CNS. Development 113:1435–1449

Warner-Schmidt JL, Duman RS (2006) Hippocampal neurogenesis: opposing effects of stress and antidepressant treatment. Hippocampus 16:239–249

Winner B, Cooper-Kuhn CM, Aigner R, Winkler J, Kuhn HG (2002) Long-term survival and cell death of newly generated neurons in the adult rat olfactory bulb. Eur J Neurosci 16:1681–1689

Wolf HK, Buslei R, Schmidt-Kastner R, Schmidt-Kastner PK, Pietsch T, Wiestler OD, Blumcke I (1996) NeuN: a useful neuronal marker for diagnostic histopathology. J Histochem Cytochem 44:1167–1171

Yang HK, Sundholm-Peters NL, Goings GE, Walker AS, Hyland K, Szele FG (2004) Distribution of doublecortin expressing cells near the lateral ventricles in the adult mouse brain. J Neurosci Res 76:282–295

Yoo YM, Lee U, Kim YJ (2005) Apoptosis and nestin expression in the cortex and cultured astrocytes following 6-OHDA administration. Neurosci Lett 382:88–92

Yoshimi K, Ren YR, Seki T, Yamada M, Ooizumi H, Onodera M, Saito Y, Murayama S, Okano H, Mizuno Y, Mochizuki H (2005) Possibility for neurogenesis in substantia nigra of Parkinsonian brain. Ann Neurol 58:31–40

Young JK, Baker JH, Muller T (1996) Immunoreactivity for brain-fatty acid binding protein in Gomori-positive astrocytes. Glia 16:218–226

Young D, Lawlor PA, Leone P, Dragunow M, During MJ (1999) Environmental enrichment inhibits spontaneous apoptosis, prevents seizures and is neuroprotective. Nat Med 5:448–453

Yue F, Chen B, Wu D, Dong K, Zeng SE, Zhang Y (2006) Biological properties of neural progenitor cells isolated from the hippocampus of adult cynomolgus monkeys. Chin Med J (Engl) 119:110–116

Zacchetti A, Garderen E van, Teske E, Nederbragt H, Dierendonck JH, Rutteman GR (2003) Validation of the use of proliferation markers in canine neoplastic and non-neoplastic tissues: comparison of KI-67 and proliferating cell nuclear antigen (PCNA) expression versus in vivo bromodeoxyuridine labelling by immunohistochemistry. APMIS 111:430–438

Zechel S, Werner S, Unsicker K, von Bohlen und Halbach O (2010) Expression and functions of fibroblast growth factor 2 (FGF-2) in hippocampal formation. Neuroscientist 16:357–373

Zhao M, Momma S, Delfani K, Carlen M, Cassidy RM, Johansson CB, Brismar H, Shupliakov O, Frisen J, Janson AM (2003) Evidence for neurogenesis in the adult mammalian substantia nigra. Proc Natl Acad Sci USA 100:7925–7930