Front. Biol. 2016, 11(3): 193–213
DOI 10.1007/s11515-016-1403-5

REVIEW

Distribution and fate of DCX/PSA-NCAM expressing cells in the adult mammalian cortex: A local reservoir for adult cortical neuroplasticity?

Richard König[1,2], Bruno Benedetti[3], Peter Rotheneichner[1,4], Anna O′ Sullivan[1,4,5], Christina Kreutzer[1,4], Maria Belles[6], Juan Nacher[6], Thomas M. Weiger[7], Ludwig Aigner (✉)[1,2], Sébastien Couillard-Després (✉)[1,4]

[1] *Spinal Cord Injury and Tissue Regeneration Center Salzburg (SCI-TReCS), Paracelsus Medical University, Salzburg, Austria*
[2] *Institute of Molecular Regenerative Medicine, Paracelsus Medical University, Salzburg, Austria*
[3] *Department of Physiology and Medical Physics, Innsbruck Medical University, Innsbruck, Austria*
[4] *Institute of Experimental Neuroregeneration, Paracelsus Medical University, Salzburg, Austria*
[5] *Department of Otorhinolaryngology, Head and Neck Surgery, Paracelsus Medical University Salzburg, Salzburg, Austria*
[6] *Neurobiology Unit, Interdisciplinary Research Structure for Biotechnology and Biomedicine Valencia, Universitat de Valencia, Comunitat Valenciana, Spain*
[7] *Division of Cellular and Molecular Neurobiology, Department of Cell Biology, University of Salzburg, Salzburg, Austria*

Abstract **The expression of early developmental markers such as doublecortin (DCX) and the polysialylated-neural cell adhesion molecule (PSA-NCAM) has been used to identify immature neurons within canonical neurogenic niches. Additionally, DCX/PSA-NCAM + immature neurons reside in cortical layer II of the paleocortex and in the paleo- and entorhinal cortex of mice and rats, respectively. These cells are also found in the neocortex of guinea pigs, rabbits, some afrotherian mammals, cats, dogs, non-human primates, and humans. The population of cortical DCX/PSA-NCAM + immature neurons is generated prenatally as conclusively demonstrated in mice, rats, and guinea pigs. Thus, the majority of these cells do not appear to be the product of adult proliferative events. The immature neurons in cortical layer II are most abundant in the cortices of young individuals, while very few DCX/PSA-NCAM + cortical neurons can be detected in aged mammals. Maturation of DCX/PSA-NCAM + cells into glutamatergic and GABAergic neurons has been proposed as an explanation for the age-dependent reduction in their population over time. In this review, we compile the recent information regarding the age-related decrease in the number of cortical DCX/PSA-NCAM + neurons. We compare the distribution and fates of DCX/PSA-NCAM + neurons among mammalian species and speculate their impact on cognitive function. To respond to the diversity of adult neurogenesis research produced over the last number of decades, we close this review by discussing the use and precision of the term "adult non-canonical neurogenesis."**

Keywords aging, cognition, doublecortin, piriform cortex, plasticity, neurogenesis

Neurogenesis in the adult mammalian brain: a controversial topic

The end of a dogma: new neurons are generated in the adult mammalian central nervous system

The subgranular zone of the dentate gyrus and the subventricular zone of the lateral ventricles, defined as the canonical neurogenic niches in the adult mammalian brain, were long believed to be the exclusive sites for the genesis of adult-born neurons. In the healthy rodent brain, adult-born neuroblasts in the subventricular zone migrate tangentially toward the olfactory bulb where they mature into two types of interneurons and integrate into pre-existing networks (Rosselli-Austin and Altman, 1979; Luskin and Boone, 1994; Betarbet et al., 1996; Zigova et al., 1996; Biebl et al., 2000; Kato et al., 2000; Petreanu and Alvarez-Buylla, 2002; Winner et al., 2002; Carleton et al., 2003; Kelsch et al., 2007). Moreover, adult-born neuroblasts in the subgranular zone of

Received January 30, 2016; accepted May 9, 2016
Correspondence: [a]Ludwig Aigner; [b]Sébastien Couillard-Després
E-mail: [a]ludwig.aigner@pmu.ac.at; [b]s.couillard-despres@pmu.ac.at

the dentate gyrus differentiate into glutamatergic granular cells and integrate into local circuits (Brown et al., 2003; Bizon et al., 2004; Bondolfi et al., 2004; Kempermann et al., 2004; Couillard-Despres et al., 2005; Encinas et al., 2011; Vivar and van Praag, 2013). In humans, adult neurogenesis was also reported in these two canonical niches, with noteworthy variability depending on the applied research methods and age of the subjects (Eriksson et al., 1998; Bédard et al., 2002; Curtis et al., 2007; Manganas et al., 2007; Sanai et al., 2011; Spalding et al., 2013; Ernst et al., 2014). However, recent findings challenge the hypothesis that these canonical neurogenic niches are the only two areas of neurogenesis in the healthy adult brain.

Current question: can adult neurogenesis occur outside the canonical neurogenic niches?

Recent studies suggest the presence of adult-born neurons or immature neurons in several regions of the adult mammalian central nervous system outside the canonical neurogenic niches, including cortical areas (Kaplan, 1981; Dayer et al., 2005; Takemura, 2005; Shapiro et al., 2007a, 2007b; Shapiro et al., 2009; de la Rosa-Prieto et al., 2010), subcortical areas (Bédard et al., 2002; Bernier et al., 2002; De Marchis et al., 2004; Vessal et al., 2007; Okuda et al., 2009; Pierce and Xu, 2010; Ehninger et al., 2011; Dirian et al., 2014; Ernst et al., 2014; Luzzati et al., 2014), and the spinal cord (Shechter et al., 2007). In humans, histological and carbon-14 dating approaches revealed that adult-born interneurons could integrate into the striatum (Ernst et al., 2014). The presence of adult-born neurons outside the canonical neurogenic niches has been reported in a variety of studies in several mammalian species, whereas their integration has been demonstrated to a lesser extent. Some of these findings could not be replicated in certain mammalian species, while others could not be reproduced since their first publication or have since been contradicted by subsequent studies. It is also possible that some of these findings are based on low-level, "incomplete" neurogenic processes that protracted from embryogenesis into the postnatal period (reviewed in Bonfanti and Peretto, 2011; Feliciano et al., 2015). Despite the controversies, these cortical and subcortical areas are currently classified as the "non-canonical neurogenic regions" of the healthy mammalian brain (Bonfanti, 2013; Peretto and Bonfanti, 2014; Feliciano et al., 2015). These putative adult neurogenic regions are illustrated in Fig. 1.

Limits and ambiguities of cell-proliferation assays used to research adult neurogenesis

The existence, manner, and origin of adult neurogenesis within non-canonical neurogenic regions have been, and continue to be, controversial topics. Besides species-specific variations (Bonfanti and Peretto, 2011), a significant limitation arises from the current labeling techniques used to detect neurogenic events. Cell-division markers, such as 5-bromo-2'-deoxyuridine (BrdU) and tritiated thymidine, only label cells in their S-phase (Nowakowski et al., 1989; Feliciano and Bordey, 2013), resulting in an underestimation of the proliferative cell number because proliferative cells in other phases of their cell cycle are potentially omitted. On the other hand, thymidine analogs can lead to an overestimation of

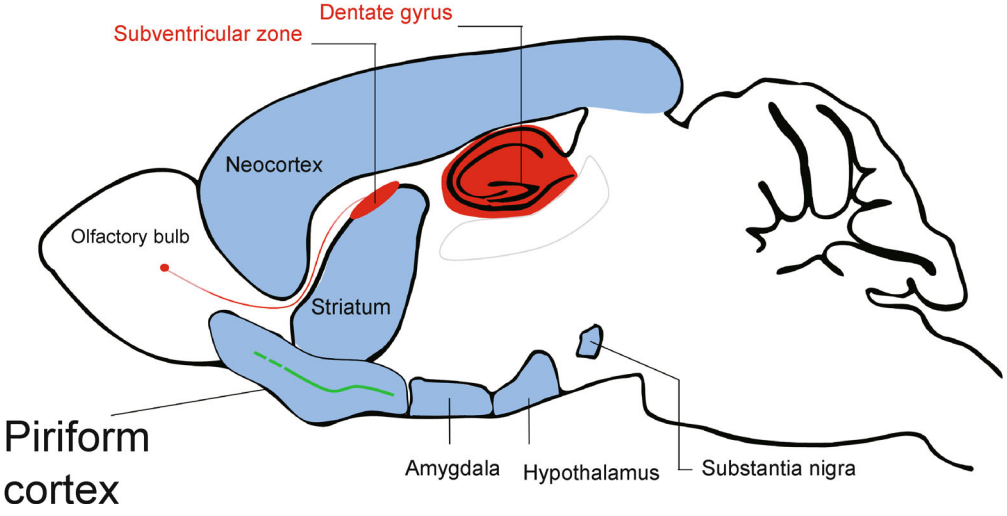


Figure 1 Schematic representation of the proliferative neurogenic sites within the adult mouse brain. Adult-born neurons generated in the subventricular zone migrate dorsally to the olfactory bulb to mature and integrate as inhibitory neurons. In the dentate gyrus, newly born neuroblasts differentiate locally into granular cells. In light blue presumptive regions supporting low-level adult neurogenesis are marked (reviewed in: (Gould, 2007)), defined as regions were proliferation markers co-occur in immunohistologically labelled neurons. The piriform cortex, which is part of the paleocortex, is highlighted in green.

proliferative cell number since these analogs can be assimilated by neurons undergoing DNA repair or non-proliferative DNA synthesis (Kunz and Kohalmi, 1991; Nowakowski and Hayes, 2000; Yang et al., 2001; Kornack and Rakic, 2001; Kuan et al., 2004; Breunig et al., 2007; Burns et al., 2007). Furthermore, thymidine analogs have been detected *in vivo* in dead neurons (Burns et al., 2006), resulting in an overestimation of proliferative cell numbers. Hence, targeting DNA-synthesis to detect neurogenesis results in a failure to detect some adult post-mitotic precursors, and the potential misclassification of labeled cells that have not undergone proliferative events (reviewed in Feliciano and Bordey, 2013). In addition, the side effects of BrdU labeling have been shown to compromise cell proliferation, cell migration, and influence the final cell position and number. BrdU is more toxic than tritiated thymidine, as demonstrated both *in vitro* in neural stem and progenitor cell cultures (Lehner et al., 2011) and *in vivo* in the rhesus monkey (Duque and Rakic, 2011).

Despite the limitations of conventional DNA-synthesis labeling techniques, if adult neurogenesis were detected independent of cell proliferation, then these labeling techniques would certainly underestimate the neurogenic potential and plasticity of the adult mammalian brain. Notably, it has been recently suggested that post-mitotic cells mature into newly formed neurons in some non-canonical neurogenic regions of the adult mammalian brain (Gómez-Climent et al., 2008; Luzzati et al., 2009; Gomez-Climent et al., 2010; Bonfanti and Peretto, 2011; Bonfanti and Nacher, 2012; Clarke et al., 2012; Rubio et al., 2015), which raises questions regarding the origin, fate, and functional relevance of these post-mitotic cells in the adult cortex.

How diverse is neurogenesis outside the canonical neurogenic niches?

Evidence for three independent cell types with neuronal fates in the healthy cortex

Adult-born neurons in canonical neurogenic niches derive from neural lineage-restricted proliferating stem and progenitor cells (Doetsch et al., 1997; Gage et al., 2008). In contrast, there is indirect evidence that even in the healthy nervous system new neurons outside the canonical niches may originate from a variety of different sources, including glial precursor cells. Innovative and meticulous studies have led to the identification of at least three different cell types that give rise to new neurons outside the canonical neurogenic niches of the (healthy) adult mammalian brain. The first was BrdU-positive neuroblasts emigrating from the subventricular zone, which are reported to give rise to a small number of new-born neurons in some ventral brain regions in the early to young postnatal period in mice and rats (De Marchis et al., 2004; Shapiro et al., 2007a; Shapiro et al., 2007b; Shapiro et al., 2009), as well as in young rabbits and monkeys, where the migratory routes of these immature neurons are well documented (Bédard et al., 2002; Bernier et al., 2002; Luzzati et al., 2003). However, since this form of neurogenesis was only observed in the very early postnatal period in these mammals, critics stated that this low-level neurogenic process was merely a protraction of an incomplete developmental neurogenesis (reviewed in Bonfanti and Peretto, 2011). Therefore, in this case, the term "adult non-canonical neurogenesis" should be used very carefully.

The second type of putative neuronal precursors is neural/glial antigen 2 (NG2)-expressing cells. NG2-expressing cells are often referred to as oligodendrocyte progenitor cells (Nishiyama et al., 2009; Nishiyama et al., 2014), or synantocytes (Butt et al., 2005) and are sometimes, perhaps erroneously, intermixed with NG2-expressing polydendrocytes (own observation). Polydendrocytes are process-bearing glial cells expressing NG2 and the platelet-derived growth factor receptor alpha (PDGFRA). We want to emphasize that while both oligodendrocyte progenitor cells and polydendrocytes express NG2, the function and fate of these cell populations may differ. Evidence suggests that polydendrocytes can give rise to oligodendrocytes under certain conditions (reviewed in Nishiyama et al., 2009), and some astroglial precursors express NG2 during murine corticogenesis (Zhu et al., 2008). Despite the ambiguity regarding their exact identity, NG2-expressing cells were reported to be present in all cortical areas investigated (Dawson et al., 2003; Nishiyama et al., 2009; Nishiyama et al., 2014) and can occasionally incorporate BrdU (Clarke et al., 2012; Psachoulia et al., 2009). In young adult mice, NG2-expressing cells were reported to constitute a local source of new neurons in the piriform cortex, motor cortex, hypothalamus, and amygdala (Rivers et al., 2008; Guo et al., 2010; Ehninger et al., 2011; Clarke et al., 2012; Robins et al., 2013). However, other studies were unable to validate a possible neurogenic fate of NG2-expressing cells (Dimou et al., 2008; Komitova et al., 2009; Kang et al., 2010; Zhu et al., 2011). Notably, technical artifacts resulting from transgenic fate mapping models have led to profound misinterpretations regarding the neurogenic potential of NG2-expressing cells *in vivo* (reviewed in Richardson et al., 2011; Nishiyama et al., 2014).

A third population of cells with a putative neuronal fate is identified by the expression of doublecortin (DCX), polysialylated-neural cell adhesion molecule (PSA-NCAM), or both molecules. These cells reside mostly in cortical layer II of the piriform cortex, the entorhinal cortex, and the neocortex of various mammals (Bonfanti et al., 1992; Seki and Arai, 1999; Nacher et al., 2001; Nacher et al., 2002; Gómez-Climent et al., 2008; Xiong et al., 2008; Cai et al., 2009; Luzzati et al., 2009; Varea et al., 2009; Varea et al., 2011; He et al., 2014; Yang et al., 2015). Moreover, comparable cells have been found in the amygdala of rats, cats, monkeys, and humans (Nacher et al., 2002; Martí-Mengual et al., 2013). Cortical cells which co-express both DCX and PSA-NCAM (DCX/PSA-NCAM cells), are likely

to constitute a population of immature and post-mitotic neurons in the adult brain, which are not tagged by short-term BrdU pulse-labeling experiments (Gómez-Climent et al., 2008; Luzzati et al., 2009; Varea et al., 2011; Bonfanti and Nacher, 2012; Rubio et al., 2015; Yang et al., 2015). Nevertheless, these cells maybe a source of new inhibitory (Xiong et al., 2008; Cai et al., 2009; Zhang et al., 2009) or excitatory neurons (Luzzati et al., 2009; Varea et al., 2011; Rubio et al., 2015). Until now, the origin, fate, and function of cortical DCX/PSA-NCAM-expressing cells in non-canonical neurogenic niches remain controversial.

The healthy murine piriform cortex might contain all three cell types with neuronal fates

The existence of one cell population with a putative neuronal fate in non-canonical regions does not exclude the presence or relevance of another. Whether or not some pyramidal neurons in the adult murine piriform cortex are derived from non-proliferating NG2-expressing cells (Guo et al., 2010; Richardson et al., 2011), does not exclude the contribution of subventricular zone-derived neuroblasts to the generation of neuron-specific nuclear protein (NeuN)-expressing cells in very young rodents (Shapiro et al., 2009; Shapiro et al., 2007a), nor does it exclude that prenatally produced DCX/PSA-NCAM-expressing cells may also be present in cortical layer II of adult mammals (Cai et al., 2009; Luzzati et al., 2009; Zhang et al., 2009; Varea et al., 2011; Rossi et al., 2014; Rubio et al., 2015). Given previous disagreements, it is possible that these three cell populations, with an assumed neuronal fate, are present in the healthy adult piriform cortex in different quantities according to age and species (e.g. Yuan et al., 2014, 2015 vs. Nacher and Bonfanti, 2015). To further complicate matters, cortical layer I has also been suggested as a fourth source of potential new neurons as an early postnatal, and perhaps young adult, neurogenic niche for cortical GABAergic interneurons under physiological conditions (Costa et al., 2007; Xiong et al., 2010). The diversity of cell populations suspected to generate or to differentiate into neurons in the piriform cortex allows this primal three-layered cortex to be one of the most suitable and interesting brain regions to study non-canonical neurogenesis in the adult mammalian brain. Deciphering origins and fates of different neuronal and non-neuronal, migrating and non-migrating, proliferating and non-proliferating progenitors or residing immature cells with delayed maturation, may lead to the elucidation and classification of the basic forms of adult cortical neurogenesis in lower and higher mammals.

The remainder of this review will focus on the alleged form of proliferation-independent maturation of neurons from cortical DCX/PSA-NCAM-expressing cells. We discuss the age-related decrease in DCX/PSA-NCAM-expressing cells, examine their distribution and plausible fates in various mammalian species, and consider their possible physiologic role in cortical circuitry. We will conclude this review by discussing the usage and precision of the term "adult non-canonical neurogenesis."

The co-expression of DCX and PSA-NCAM defines immature neurons in the adult brain

DCX and PSA-NCAM are markers of neuronal development and plasticity

The microtubule-associated protein DCX and the cell surface protein PSA-NCAM are molecules related to neuronal development and cellular plasticity (des Portes et al., 1998; Francis et al., 1999; Seki and Arai, 1999; Couillard-Despres et al., 2005; Friocourt et al., 2007). DCX is associated with dynamic instability and in turn, is crucial for axonal elongation and dendritic sprouting (Hastings and Gould, 1999; Ehninger and Kempermann, 2008). The polysialylated (PSA) form of the neuronal cell adhesion molecule (NCAM) can produce a sufficient physical hindrance between apposing membranes to attenuate intercellular adhesion (Johnson et al., 2005; Rutishauser, 2008). Thus, PSA-NCAM is involved in synaptogenesis and activity-dependent remodeling of synapses and is frequently used to identify migrating neuroblasts (Dityatev et al., 2004; Saegusa et al., 2004). Both DCX and PSA-NCAM are predominantly co-expressed in immature neurons during development. In adult mammals, the co-expression of DCX and PSA-NCAM persists in the canonical neurogenic niches and reflects neurogenic activity (Gritti et al., 2002; Couillard-Despres et al., 2005; Gould, 2007). However, the presence of either DCX or PSA-NCAM does not solely define immature neurons, since these proteins can also be detected in non-neurogenic, highly plastic cells in the adult mammalian brain (Fox et al., 2000; Nacher et al., 2001; Sairanen et al., 2007; Varea et al., 2007; Kremer et al., 2013). Therefore, in the adult brain, the co-expression of DCX and PSA-NCAM is frequently used to detect immature neurons, while the individual expression of either DCX or PSA-NCAM is used to categorize maturation stages or to indicate plasticity in mature neural cells.

Most cortical DCX/PSA-NCAM-expressing cells are immature neurons

Several lines of evidences indicate that DCX/PSA-NCAM expressing cells within the mammalian paleocortex, entorhinal cortex, and neocortex are immature neurons committed to a neuronal fate. The first evidence is that most of these cells are negative for markers of mature principal neurons and interneurons (Gómez-Climent et al., 2008; Cai et al., 2009; Luzzati et al., 2009; Varea et al., 2011; Rubio et al., 2015). Second, they are devoid of ultrastructural features that indicate synaptic inputs, as shown by transmission electron microscopy (Gómez-Climent et al., 2008). In addition, electron microscopic analysis by Shapiro and colleagueś (2007) revealed that DCX labeled cells had ultrastructural

features of immature migrating cells. Third, DCX/PSA-NCAM-expressing cells are negative for mature oligodendroglial, astroglial and microglial markers (Gómez-Climent et al., 2008; Luzzati et al., 2009; Rubio et al., 2015), and very few DCX/PSA-NCAM-expressing cells express NG2 within the piriform cortex (Gómez-Climent et al., 2008; Luzzati et al., 2009; Rubio et al., 2015). Fourth, the expression of DCX/PSA-NCAM in cortical cells overlaps with other early developmental markers typically found in immature neurons during development, such as TUC-4, TUJ-1, and CNGA3 (Gómez-Climent et al., 2008; Xiong et al., 2008; Luzzati et al., 2009). Finally, DCX/PSA-NCAM-expressing cells do not co-express cell activity markers, such as c-Fos or Arc, further emphasizing their immature state (Gómez-Climent et al., 2008). In sum, DCX/PSA-NCAM-expressing cells, at least in the piriform cortex, are considered as bona fide latent immature neurons in cortical layer II and a potential reservoir of new mature neurons upon differentiation. For this reason, we have named these cells, "cortical layer II DCX/PSA-NCAM-expressing immature neurons," abbreviated as "DCX/PSA-NCAM IN." Aside from immunohistochemistry, these cells can be identified in transgenic mice overexpressing fluorescent proteins under the control of a human DCX promoter (Couillard-Despres et al., 2006) or in genetic fate mapping in transgenic mice expressing $CreER^{T2}$ under the control of the same promoter (Zhang et al., 2010). The distribution of immature neurons targeted by the DCX promoter is shown in Fig. 2. Most DCX promoter positive cells in layer II of the murine piriform cortex co-express PSA-NCAM (own observation). It is worth mentioning that a minority of DCX/PSA-NCAM-expressing cells with morphologies comparable to semilunar-pyramidal transitional neurons also express NeuN (Gómez-Climent et al., 2008; Varea et al., 2011; Rubio et al., 2015). Ultrastructural analysis revealed some synaptic inputs to the distal dendrites of larger DCX/PSA-NCAM/NeuN-expressing semilunar-pyramidal transitional neurons (Gómez-Climent et al., 2008).

Origin, anatomical distribution, and fate of cortical layer II DCX/PSA-NCAM-expressing immature neurons (DCX/PSA-NCAM IN)

DCX/PSA-NCAM IN are post-mitotic

DCX/PSA-NCAM IN do not appear to be the product of cell division in adulthood. Instead, most of these cells arise in the piriform cortex during embryonic development: between E13.5 and E14.5 in mice according to BrdU incorporation experiments (Rubio et al., 2015), between E13.5 and E15.5 in rats (Gómez-Climent et al., 2008), between E21 and E28 in guinea pigs, and within the neocortex of guinea pigs at E35 (Yang et al., 2015). Some reports have described marginal BrdU incorporation in DCX/PSA-NCAM IN during adulthood (Shapiro et al., 2007a). However, the vast majority of DCX/PSA-NCAM IN observed in layer II of the piriform cortex and entorhinal cortex of adult mice and rats, as well as the piriform cortex, entorhinal cortex, and neocortex of guinea pigs, rabbits, and cats are post-mitotic immature resident neurons (Gómez-Climent et al., 2008; Luzzati et al., 2009; Klempin et al., 2011; Varea et al., 2011; Rubio et al., 2015). In conclusion, these studies unanimously indicate that the majority of layer II DCX/PSA-NCAM IN do not replicate their DNA in early or late adulthood, and thus, are not adult-born.

Further studies, which did not use proliferation markers, suggested that outside canonical neurogenic regions the DCX/PSA-NCAM IN are most abundant in layer II of the piriform cortex, entorhinal cortex, and neocortex of four afrotherian mammals (Patzke et al., 2014), young lupines (De Nevi et al., 2013), non-human primates (Cai et al., 2009; Zhang et al., 2009; Bloch et al., 2011), and humans (Cai et al., 2009; Mikkonen et al., 1998; Ni Dhúill et al., 1999). Although some uncertainty about the exact identity of DCX/PSA-NCAM IN in these studies resulted from the absence of BrdU administration and the variability associated with post-mortem histological analyses, the distribution and morphology of cortical cells expressing DCX and/or PSA-NCAM seemed to be comparable between these mammalian species. Therefore, we speculate that cortical DCX/PSA-NCAM IN are generated at prenatal stages not only in mice, rats, and guinea pigs, but also in other mammals, including cats, lupines, and primates. We propose that cortical DCX/PSA-NCAM IN constitute a population of resident immature neurons that are not adult-born (reviewed in Gómez-Climent et al., 2008; Nacher and Bonfanti, 2015).

The number of DCX/PSA-NCAM IN declines drastically with age

An age-dependent decrease in the number of DCX/PSA-NCAM IN was observed in the cortical areas of mice, rats, guinea pigs, rabbits, cats, dogs, and non-human primates (Abrous et al., 1997; Murphy et al., 2001; Xiong et al., 2008; Cai et al., 2009; Varea et al., 2009; Zhang et al., 2009; De Nevi et al., 2013). In cortical layer II, such a decrease could results from cell death, migration, differentiation, or maturation into adult neuronal cells. Current studies have failed to find evidence of elevated TUNEL activity in cortical layer II during aging in guinea pigs (Xiong et al., 2008) or a substantial number of pyknotic nuclei in aged rats (Gomez-Climent and Nacher, unpublished results). Maturation would imply a loss of developmental markers (DCX/PSA-NCAM) in immature cortical neurons and the progressive appearance of mature neural cell markers. Therefore, if DCX/PSA-NCAM IN constitute an immature cell population, upon maturation, these cells would be untraceable in aged mammalian brains and indistinguishable from their neighboring mature neurons. Transgenic labeling is an effective strategy to follow the fate of immature neurons and neuronal

progenitor cells, even when the expression of markers such as DCX and PSA-NCAM declines. An example of fate-mapping analysis to address the maturation hypothesis of DCX/PSA-NCAM IN using the DCX-CreER^{T2} mouse line is shown in Fig. 3 (own observation).

The "loss by maturation" hypothesis: DCX/PSA-NCAM IN mature and integrate during adulthood

Previous observations have supported the "loss by maturation" hypothesis. The first observation was that some cortical PSA-NCAM-expressing cells, with bigger somata than DCX/PSA-NCAM co-expressing cells, were endowed with synaptic spine-like protrusions and thin axon-like basal processes. These morphologies typically identify semilunar-pyramidal transitional neurons in the piriform cortex of mice, rats, and cats (Gómez-Climent et al., 2008; Varea et al., 2011; Rubio et al., 2015). Furthermore, these larger PSA-NCAM-expressing cells faintly co-expressed NeuN and the NMDA-receptor subunit NR1 in the piriform cortex of mice and the piriform cortex and entorhinal cortex of rats (Gómez-Climent et al., 2008; Rubio et al., 2015). Therefore, these cells may represent the final path or intermediate state of DCX/PSA-NCAM IN toward maturation. Due to their location, others have suggested that DCX/PSA-NCAM IN mature into semilunar, pyramidal, or semilunar-pyramidal transitional neurons in the piriform cortex. In the piriform cortex, most excitatory neurons are clustered in layer II, while GABAergic interneurons are sparsely spread across all layers, as shown in Fig. 3 (Kapur et al., 1997; Ekstrand et al., 2001; Suzuki and Bekkers, 2007, 2010a, 2010b). Among excitatory neurons, semilunar cells are particularly concentrated in the outer part of layer II, where most DCX/PSA-NCAM IN can be found in the piriform cortex of mice and rats (Gómez-Climent et al., 2008; Rubio et al., 2015). Thus, it has been suggested that semilunar cells may be the result of the maturation of DCX/PSA-NCAM IN, at least in the piriform cortex (Gómez-Climent et al., 2008; Luzzati et al., 2009; Gomez-Climent et al., 2010). While these observations support the "loss by maturation" hypothesis, the fate of DCX/PSA-NCAM IN to become either excitatory neurons and/or inhibitory neurons is still unknown.

The fate of DCX/PSA-NCAM IN: glutamatergic or GABAergic?

The fate of DCX/PSA-NCAM IN remains elusive. One laboratoryhas suggested that DCX/PSA-NCAM IN mature into inhibitory neurons in guinea pigs, cats, and primates (Xiong et al., 2008; Cai et al., 2009; Zhang et al., 2009). Others have substantiated that DCX/PSA-NCAM IN provide a post-mitotic pool of excitatory neurons in mice, rats, guinea pigs, rabbits, and cats (Gómez-Climent et al., 2008; Luzzati et al., 2009; Varea et al., 2011; Rubio et al., 2015). A detailed summary of these reports are given in Table 1, which highlights the discrepancies in expected fates, while also showing the species, locations, birth-dating methods, morphologies, and applied markers of each study. The transcription factor protein Tbr1, a marker of glutamatergic differentiation in pallium-derived neurons, has been used in studies reporting an excitatory fate for DCX/PSA-NCAM IN (Englund et al., 2005; Hevner et al., 2006). Luzzati and colleagues (2009) observed in mice, rats, guinea pigs, and rabbits that almost all DCX-expressing cortical neurons with immature morphology were also positive for Tbr1, while only a minority of post-mitotic DCX-expressing cells could be co-labeled with interneuron markers. Similarly, Rubio and colleagues (2015) found DCX/PSA-NCAM IN to be positive for Tbr1 in the paleocortical layer II of mice. Moreover, a recent study reported that small paleo-, entorhinal-, and neocortical PSA-NCAM-expressing cells seem to follow a predominantly glutamatergic fate, with virtually no overlap between PSA-NCAM and inhibitory neuronal markers in the cortices of cats (Varea et al., 2011). Some large DCX/PSA-NCAM-expressing cells co-express NeuN and have morphologies similar to common excitatory morphotypes, such as the semilunar-pyramidal transitional neurons (Gómez-Climent et al., 2008; Varea et al., 2011; Rubio et al., 2015), indicating a maturation of DCX/PSA-NCAM IN into glutamatergic cell types. In summation, of the five species examined, an excitatory fate for cortical DCX/PSA-NCAM IN is assumed in all three studies. Notably, four out of the five species are small mammals, and all researchers administered BrdU and restricted their quantitative analysis to post-mitotic immature neurons in paleocortical areas, or in the entorhinal cortex.

Other studies suggest an inhibitory neuronal fate for cortical DCX/PSA-NCAM IN. These conflicting observations are all based on studies from a single laboratory, which have found co-expression of DCX (but not PSA-NCAM) and GABAergic markers in larger mammals, such as cats, and non-human primates (Cai et al., 2009; Zhang et al., 2009), or relied on slightly ambiguous morphological criteria in dogs and humans (Cai et al., 2009; De Nevi et al., 2013). In non-human primates, Zhang and colleagues (2009) found a complete co-localization of DCX and PSA-NCAM among small cells and a co-localization NeuN or GABA with low levels of DCX (but not PSA-NCAM) in larger cortical layer II cells. These results directly contrast those of Varea et al., (2011), who observed that small unipolar and bipolar DCX-expressing cells did not exhibit NeuN or GABA reactivity in the entorhinal cortex of cats, but that larger cells with complex morphology did co-expressed DCX, NeuN, and GABA (Varea et al., 2011). In guinea pigs, an almost complete co-localization of DCX,PSA-NCAM, and Tuj-1 was found in cortical layer II cells with immature morphology, while larger cells co-expressed DCX, NeuN, and different interneuron markers (Xiong et al., 2008). Inconsistencies among these studies are likely the result of technical issues as well as species specific and regional variation in the distribution and fate of cortical DCX/PSA-

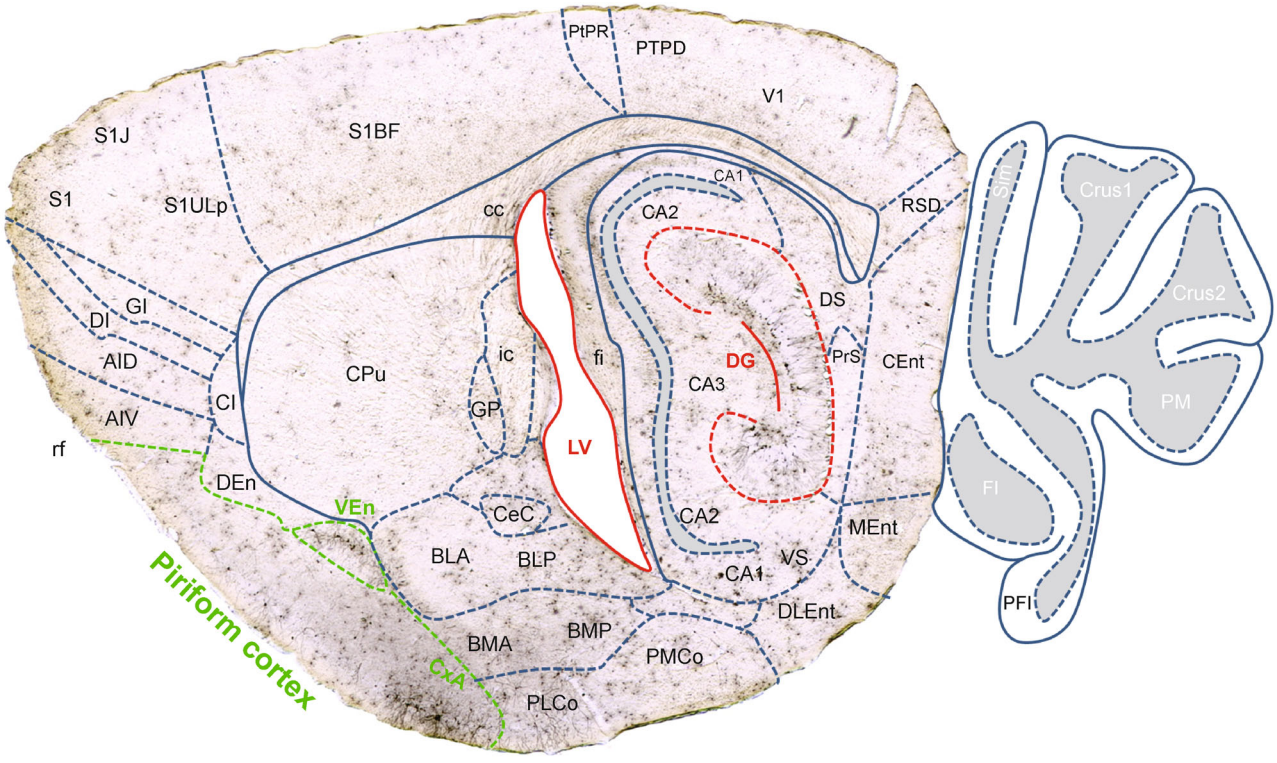


Figure 2 In the young adult mouse brain, immature neurons are most abundant in the canonical neurogenic niches and within paleocortical regions. Transgenic DCX-CreERT2 mouse line allowed for the detection of DCX/PSA-NCAM IN. In this picture, immature neurons expressing the transgene under the DCX promoter are visualized by 3,3'-Diaminobenzidine (DAB) staining. The amount of DAB positive cells is the highest within the dentate gyrus (DG), the lateral ventricular (LV) wall, and the posterior part of the piriform cortex layer II. In the piriform cortex most DAB positive cells are accumulated in the posterior part, extending into the amygdaloidal transitional area (CxA), and the ventral claustrum (VEn). It was demonstrated that most immature neurons in the layer II of the murine piriform cortex do express PSA-NCAM (Rubio et al., 2015). In contrast, the evenly distributed DAB positive cells present throughout all cortical regions, co-express the NG2 antigen, but mostly lack PSA-NCAM expression (own observation). Abbreviations: agranular insular cortex dorsal part (AID), agranular insular corte, ventral part (AIV), basolateral amygdaloid nucleus anterior part (BLA), basolateral amygdaloid nucleus posterior part (BLP), basomedial amygdaloid nucleus anterior part (BMA), basomedial amygdaloid nucleus posterior part (BMP), field cornu ammonis (CA) 1 of the hippocampus (CA1), field CA2 of the hippocampus (CA2), field CA3 of the hippocampus (CA3), corpus callosum (cc), caudomedial entorhinal cortex (CEnt), claustrum (Cl), caudate putamen (CPu), crus 1 of the ansiform lobule (Crus1), crus 2 of the ansiform lobule (Crus2), cortex-amygdala transition zone (CxA), dorsal endopiriform claustrum (DEn), dysgranular insular cortex (DI), dorsolateral entorhinal cortex (DLEnt), dorsal subiculum (DS), fimbria of the hippocampus (fi), flocculus (Fl), granular insular cortex (GI), dentate gyrus (DG), globus pallidus (GP), internal capsule (ic), lateral ventricle (LV), paraflocculus (PFl), posterolateral cortical amygdaloid area (PLCo), paramedian lobule (PM), posteriormedial cortical amygdaloid area (PMCo), presubiculum (PrS), parietal cortex, posterior area dorsalis (PtPD), parietal cortex, posterior area rostralis (PtPR), rhinal fissure (rf), primary somatosensory cortex (S1), primary somatosensory cortex, barrel field (S1BF), primary somatosensory cortex, jaw region (S1J), primary somatosensory cortex, upper lip region (S1ULp), simple lobule (Sim), primary visual cortex (V1), ventral endopiriform claustrum (VEn), ventral subiculum (VS); (Paxinos and Franklin, 2012).

NCAM IN. Since neither Xiong and colleagues (2008) nor Cai and colleagues (2009) used proliferation-markers, an intermixture of post-mitotic cortical layer II DCX/PSA-NCAM IN with proliferating precursors from cortical layer I or the subventricular zone could account for some of the reported discrepancies regarding the fate of strictly post-mitotic DCX/PSA-NCAM IN. In addition, species-specific variations in the distribution and fate of DCX/PSA-NCAM IN may reflect phylogenetic aspects of corticogenesis and cortical processing, as schematically represented in Box 1.

Before a clear line of evidence can rule out or support the hypothesis of species-specific distribution/fate/population of DCX/PSA-NCAM IN, four systematic problems have to be overcome. First, none of the studies reporting or suggesting maturation toward inhibitory neurons administered proliferation markers (Xiong et al., 2008; Cai et al., 2009; Zhang et al., 2009; De Nevi et al., 2013). Thus, studies suggesting a GABAergic fate of DCX/PSA-NCAM IN may include mitotic DCX expressing cortical cells in their quantitative analysis, as well as migrating neuroblasts of the subventricular zone, which are BrdU positive, and local proliferating progenitors. Secondly, fixation and staining protocols need to

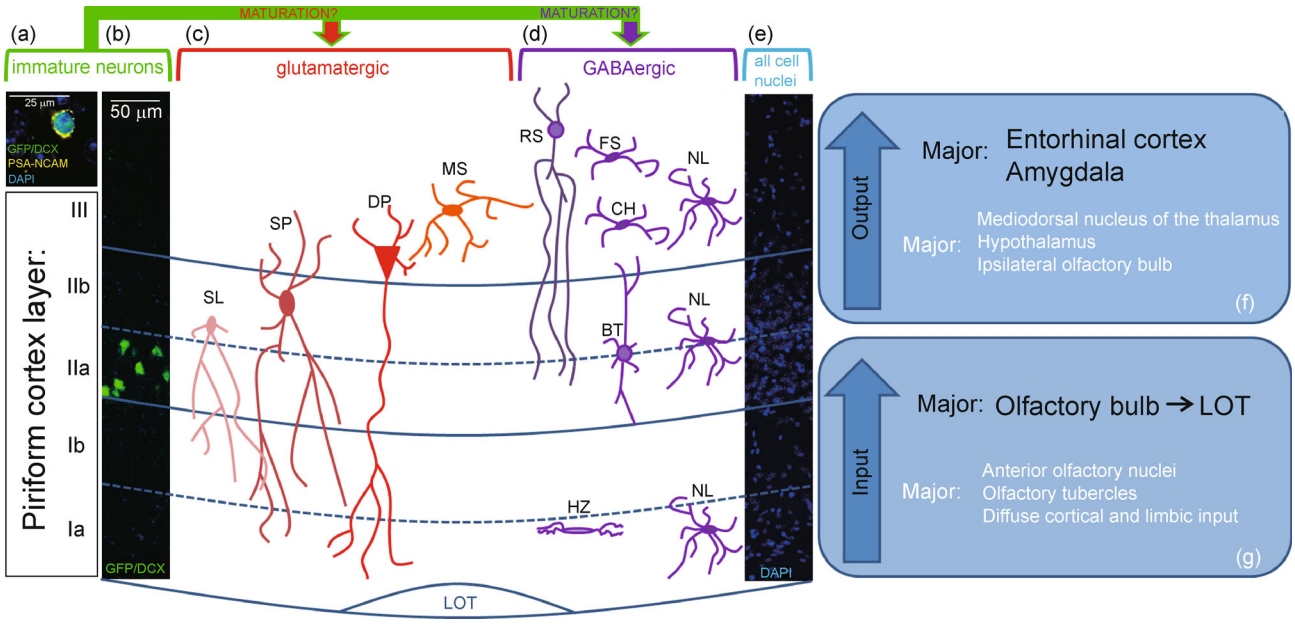


Figure 3 Schematic cytoarchitecture of the piriform cortex. (a) GFP/DCX positive cell in the piriform cortex of a mouse stained for PSA-NCAM (yellow). Most of these cells show small soma size and lack synaptic input. (b) Transgenic DCX-CreERT2 mouse line allowed for the detection of DCX/PSA-NCAM IN which predominantly resided in the layer IIa of the mouse piriform cortex. This mouse model enables fate mapping of DCX expressing cells (Zhang et al., 2010). (c) SL and SP have their somata concentrated in layers IIa and IIb, respectively. DP and MS are found at lower density in layer II. (d) GABA-releasing interneurons are distributed more sparsely and uniformly, but are less abundant in layer II. (e) Detection of the cell layer of the piriform cortex by DAPI (blue) staining. Main (f) output and (g) input regions of the piriform cortex. Abbreviations: superficial pyramidal cells (SP), semilunar cells (SL), multipolar spiny cells (MS), deep pyramidal cells (DP), bitufted cells (BT), fast spiking cells (FS), chandelier cells (CH), regular-spiking cells (RS), neuroglial-like cells (NL), lateral olfactory tract (LOT); nomenclature following (Bekkers and Suzuki, 2013).

be standardized since they currently vary among the different studies and species investigated. Thirdly, the molecular marker used to follow the fate of DCX/PSA-NCAM IN is DCX in some studies (Xiong et al., 2008; Cai et al., 2009; Zhang et al., 2009; De Nevi et al., 2013; Patzke et al., 2014; Yang et al., 2015;), but in others is PSA-NCAM (Varea et al., 2009; Varea et al., 2011). To follow the fate of DCX/PSA-NCAM IN non-ambiguously, we recommend the use of both antibodies simultaneously in combination with Tbr1 (Luzzati et al., 2009; Rubio et al., 2015). We also suggest the need for transgenic mouse models that can be utilized in proof of principle studies. Finally, to compare the fate of DCX/PSA-NCAM IN between species, it would be essential to investigate and compare the same regions across all species, e.g. allocortical areas, since the fate of DCX/PSA-NCAM IN may be region specific. For instance, small mammals, such as rodents, do not have comparable neocortical lobes to those observed in cats, dogs, and primates, and thus, the lack of neocortical DCX/PSA-NCAM IN in small mammals could bias the comparison when DCX/PSA-NCAM IN are considered across all species. Regardless of whether DCX/PSA-NCAM IN develop into glutamatergic or GABAergic neurons, the available studies, once more, unanimously suggest that recently formed neurons during adulthood arise from the maturation of layer II immature neurons.

The relevance of DCX/PSA-NCAM IN in adult cortices: facts, speculations, and definitions

DCX/PSA-NCAM IN distribution parallels cortical hierarchy: functional implications

DCX/PSA-NCAM IN are more abundant in secondary, or associative, cortical regions than in primary sensory areas. The motor cortex in most investigated species is almost completely devoid of DCX/PSA-NCAM IN (Gómez-Climent et al., 2008; Luzzati et al., 2009; Zhang et al., 2009; Rubio et al., 2015). Therefore, the distribution of DCX/PSA-NCAM IN may parallel the hierarchical levels of sensory processing. In the young adult monkey, DCX/PSA-NCAM IN are widely distributed across the entire cerebral hemisphere, denser in layer II/III with a decreasing ventrodorsal gradient (Cai et al., 2009; Zhang et al., 2009). In the neocortex of guinea pigs and rabbits, the number of DCX-expressing cells increases from primary to secondary sensory areas, reaching the highest density close to the rhinal sulcus, which marks the border between the neocortex and the limbic system (Luzzati et al., 2009). Around the rhinal sulcus, layer II DCX-expressing cells can also be found in the mouse and rat (Nacher et al., 2001; Gómez-Climent et al., 2008; Luzzati et al., 2009).

Table 1 An overview of the various reports on cortical layer II immature neurons

Mammal	Location	Expected fate	Are-related reduction?	Overlap with fate-specific or mature neural markers?	Prenatal birth dating?	Antigens	Proliferation in the adult?	Morphologies	Reference
Mouse	Piriform cortex (posterior)	Glutamatergic	n.a.	DCX expression overlapped with Tbr1. No co-expression with interneuronal markers	n.a.	Ki67; TUJ1;NeuN; DCX; PSA-NCAM; Parvalbumin; Somatostatin; Calbindin; GABA); GAD67; pan DLL; Lhx6; Tbr1; BrdU	No adult DNA synthesis	DCX/PSA-NCAM: Type 1 cells: bipolar and relatively small Type 2 cells: larger cell bodies; wide and well-developed dendritic arborisation; referred as "extraverted neurons" (Nieuwenhuys, 1994; Sanides and Sanides, 1972)	Luzzati et al., 2009
	Piriform cortex	n.a.	Reduction of DCX-DsRed cell number following bulbectomy	DCX-DsRed expression overlapped with NeuN	n.a.	BrdU; DCX; PSA-NCAM; NeuN; Iba-1; Transgenic mice expressing red fluorescent protein (DsRed) under the control of the DCX promoter [C57BL/6J-Tg (DCX-DsRed)14Qlu/J	n.a. in transgenic mice	n.a.	Rossi et al., 2014
	Piriform cortex	Glutamatergic	n.a.	PSA-NCAM expression overlapped with Tbr1 (77.7%). No co-expression with interneuronal markers	Mainly in E13.5-E14.5	BrdU; DCX; GAD67); GFAP; Ki67; NeuN; NG2; PSA-NCAM; RIP; Tbr1	Prenatal DNA-synthesis, and no adult DNA synthesis	DCX/PSA-NCAM: Small cells (6.21μm) – "Tangled Cells" Larger cells (9.64μm) – "semilunar-pyramidal transitional neurons" 10% of all cells in layer II of the piriform cortex did express PSA-NCAM	Rubio et al., 2015
Rat	Piriform cortex and entorhinal cortex (perirhinal cortex, agranular insular, ectorhinal cortices)	n.a. (Suggested by the authors: Glutamatergic fate)	n.a.	PSA-NCAM expression in larger cells overlapped with faint NeuN expression, and the expression of the NR1 subunit of the NMDA receptor. No co-expression with interneuronal markers	Mainly in E15.5	Alpha-actinin, Arc, GABA, BrdU, CaMKII, Calbindin, Calretinin, c-Fos, Cholecytstokinin; CNGA-3, DCX, GFAP, Glucocorticoid receptor, GAD-67, MAP2, NG2, Nestin, NeuN, Neuropeptide Y; NMDA receptor 1; Parvalbumin; Pax6; Phospho-CREB, RIP; PSA-NCAM; Somatostatin; TUC-4; Vasoactive intestinal peptide	Prenatal DNA-synthesis, and no adult or perinatal DNA synthesis	DCX/PSA-NCAM: Small cells (soma diameter 8.9μm) – "Tangled Cells" Small cells mostly co-expressed DCX, TUC-4, CNGA-3, which is strongly expressed by migrating neuroblasts of the rostral migratory stream (Guiterrez-Mecinas et al., 2007), and p-CREB, a molecule expressed transiently in differentiating granule neurons the adult hippocampus (Nakagawa et al., 2002) Larger cells (14.8μm) – "semilunar-pyramidal transitional neurons"	Gomez-Climent et al., 2008

(*Continued*)

Mammal	Location	Expected fate	Are-related reduction?	Overlap with fate-specific or mature neural markers?	Prenatal birth dating?	Antigens	Proliferation in the adult?	Morphologies	Reference
Rat	Piriform cortex (DCX staining mostly present in the part of the piriform cortex) Scarce DCX expressing cells in the neocortex	Glutamatergic	n.a.	DCX expression overlapped with Tbr1. No co-expression with interneuronal markers	n.a.	Ki67; TUJ1;NeuN; DCX; PSA-NCAM; Parvalbumin; Somatostatin; Calbindin; GABA); GAD67; pan DLL; Lhx6; Tbr1; BrdU	No adult DNA synthesis	DCX/PSA-NCAM: Type 1 cells: bipolar and relatively small Type 2 cells: larger cell bodies; wide and well-developed dendritic arborisation; referred as "extraverted neurons" (Nieuwenhuys, 1994; Sanides and Sanides, 1972)	Luzzati et al., 2009
	Piriform cortex	n.a.	n.a.	BrdU overlapped with NeuN, but no BrdU/NeuN positive cells 12 weeks after BrdU administration	n.a.	BrdU; NeuN; PSA-NCAM; DCX; TUC-4	Adult-born NeuN expressing cells that do not survive	Pyramidal morphology of BrdU/NeuN positive cells in layer II	Pekcec et al., 2006
	Allocortex, medial prefontal cortex, and (Amygdala)	n.a. (Suggested by the authors: Glutamatergic fate)	Age related reduction of "s cells" (small PSA-NCAM expressing cells) but not of "L cells"	n.a.	n.a.	PSA-NCAM	n.a.	PSA-NCAM:Smaller PSA-NCAM expressing cells ("s cells") were found only in the dentate gyrus, and only in the layer II of the piriform, entorhinal, perirhinal and insular cortices. "L cells" with comparatively larger soma, found in the hippocampus, the entorhinal and piriform cortices, and in all the extension of the neocortex and in different amygdaloid nucleus. The L cells were not present within the layer II of the piriform cortex. Number of "L cells" did not decrease dramatically in adulthood.	Varea et al., 2009
Afrotherian mammals (hottentot golden mole, rock hyrax, eastern rock sengi, our toed sengi)	Allocortex andneocortex	n.a.	n.a.	n.a.	n.a.	DCX; Ki-67	No Ki67 immunoreactivity of DCX expressing cells in cortical layer II	DCX:Mostly bipolar or multi-polar in shape, occasionally unipolar DCX expressing cells in cortical layer II	Patzke et al., 2014

(*Continued*)

Mammal	Location	Expected fate	Are-related reduction?	Overlap with fate-specific or mature neural markers?	Prenatal birth dating?	Antigens	Proliferation in the adult?	Morphologies	Reference
Guinea Pig	Allocortex and neocortex with a dorsal to ventral high to low gradient; (Amygdala)	n.a.	n.a.	n.a.	E21-28 in the piriform cortex, E35 in the neocortex	DCX, BrdU	Only scare DNA-synthesis in DCX expressing cortical layer II cells	DCX:Comparable to Xiong et al., 2008	Yang et al., 2015
	Paleocortex	n.a.	n.a.	n.a.	n.a.	DCX, NeuN	n.a.		He et al., 2014
	Allocortex and neocortex	GABAergic	From 3 month to 3 years of age DCX + cell number decreased about 50% for all observed areas (piriform, entorhinal, temporal, and parietal cortex)	DCX/PSA-NCAM/TuJ1 in small cells DCX/NeuN in large cells; subpopulation of DCX + cells showed weak to moderate GABA labelling; large cells with faint DCX expression showed robust GAD67, GAD65/67; some larger DCX + cells co-expressed NADPH-D or NOS	n.a.	DCX; PSA-NCAM; beta tubulin III; NeuN; GFAP; Reelin; GABA; GAD67; GAD 65/67; Calbindin; Calretinin; Neurogranin; Nitric oxidase); TuJ	n.a.	DCX: Small DCX expressing cells: (5μm soma diameter) with no/ one/two processes that extended from one or opposite poles of the somata (most abundant DCX + cell type) Medium-sized DCX expressing cells: (5-10μm) displayed moderate to heavy DCX reactivity and 2-4 processes radiating from the somata Large-sized DCX expressing cells: (10-20μm in soma diameter) had branched dendrite-like processes for several hundred micrometres	Xiong et al., 2008
	Piriform cortex, neocortex, amigdalo-pyriform transition area (Amygdala)	Glutamatergic	n.a.	DCX expression overlapped with Tbr1. No co-expression with interneuronal markers	n.a.	Ki67; TUJ1;NeuN; DCX; PSA-NCAM; Parvalbumin; Somatostatin; Calbindin; GABA); GAD67; pan DLL; Lhx6; Tbr1; BrdU	No adult DNA synthesis	DCX/PSA-NCAM: Type 1 cells: bipolar and relatively small Type 2 cells: larger cell bodies; wide and well-developed dendritic arborisation; referred as “extraverted neurons” (Nieuwenhuys, 1994; Sanides and Sanides, 1972)	Luzzati et al., 2009
Rabbit	Piriform cortex, neocortex, amygdalo-piriform transition area (Amygdala)	Glutamatergic	n.a.	DCX expression overlapped with Tbr1. No co-expression with interneuronal markers	n.a.	Ki67; TUJ1;NeuN; DCX; PSA-NCAM; Parvalbumin; Somatostatin; Calbindin; GABA); GAD67; pan DLL; Lhx6; Tbr1; BrdU	No adult DNA synthesis	DCX/PSA-NCAM: Type 1 cells: bipolar and relatively small Type 2 cells: larger cell bodies; wide and well-developed dendritic arborisation; referred as “extraverted neurons” (Nieuwenhuys, 1994; Sanides and Sanides, 1972)	Luzzati et al., 2009

(*Continued*)

Mammal	Location	Expected fate	Are-related reduction?	Overlap with fate-specific or mature neural markers?	Prenatal birth dating?	Antigens	Proliferation in the adult?	Morphologies	Reference
Cat	Allocortex and neocortex; ventrodorsal high to low gradient	Glutamatergic	n.a.	PSA-NCAM expression overlapped with Tbr1 in small cells. No co-expression with interneuronal markers	n.a.	PSA-NCAM; NeuN; GAD67; DCX; CNGA3; CaMKII; Tbr1; Calbindin; Calretinin; Parvalbumin; Cholecystokinin; Vasoactive Intestinal Peptide; Neuropeptide Y; Somatostatin; Nitric Oxide Synthase Neural	No adult DNA synthesis	PSA-NCAM:"S" cells (small) PSA-NCAM/DCX/TBR1/CNGA3 (12 μm soma size), present in layer II of various cortices "L" cells (large) PSA-NCAM/ NeuN(Gad67/CB/CR) (21 μm soma size), not present within the layer II	Varea et al., 2011
	Allocortex and neocortex; ventrodorsal high to low gradient	GABAergic	E.g. entorhinal cortex: Are-related reduction in cell number of about 60% (young = 1.5 years; old = 4.5 years)	In small cells of the layer II: DCX is coexpressed with PSA-NCAM and TuJ1; In larger cells: DCX is co-expressed with NeuN, and different interneuron markers	n.a.	DCX; PSA-NCAM; Tuj-1); NeuN; GFAP; Oligodendrocyte Protein; OX42; Neurogranin; GABA; GAD67; Calbindin; Parvalbumin; Calretinin; Somatostatin; nNOS	n.a.	DCX:Heterogenic population, ranging from small DCX expressing cells (5μm) up to larger cells (20μm)	Cai et al., 2009
Dog	Paleocortex, neocortex (mostly frontal), (and cerebellum)	n.a.	Age-related reduction (from 3 month to 5 year and 17 year old lupines) in DCX expressing cell number in the layer II of the frontal neocortex, paleocortex and piriform lobe.	n.a.	n.a.	DCX; Nucleostemin; tubulin beta III; NeuN; GFAP	n.a.	DCX:Irregular cells having a small cellular body and short extensions, but with more mature morphologies in neocortical layer II	De Nevi et al., 2013
Hesus monkey	Neocortex, entorhinal cortex, (and amygdala) ventrodorsal high to low gradient; only occasionally detected DCX expressing cells in primary motor and sensory cortical areas	GABAergic	Age-related reduction (from 12 to 21 and 31 years): 32%, and 13%	Partial co-localisation of DCX/NeuN, DCX/GABA, and DCX/TH	n.a.	DCX; PSA-NCAM; NeuN; GABA; TyrosinHydroxilase (TH)	n.a.	Most DCX expressing cells were small and bipolar, often arranged in clusters (like observed within all species investigated so far (Gomez-Climent et al., 2008; Marti-Mengual et al., 2013; Nacher et al., 2001; Varea et al., 2011); complete co-localization of DCX with PSA-NCAM A few relatively large cells with reduced DCX reactivity were present in layer II/III in temporal lobe cortical areas	Zhang et al., 2009

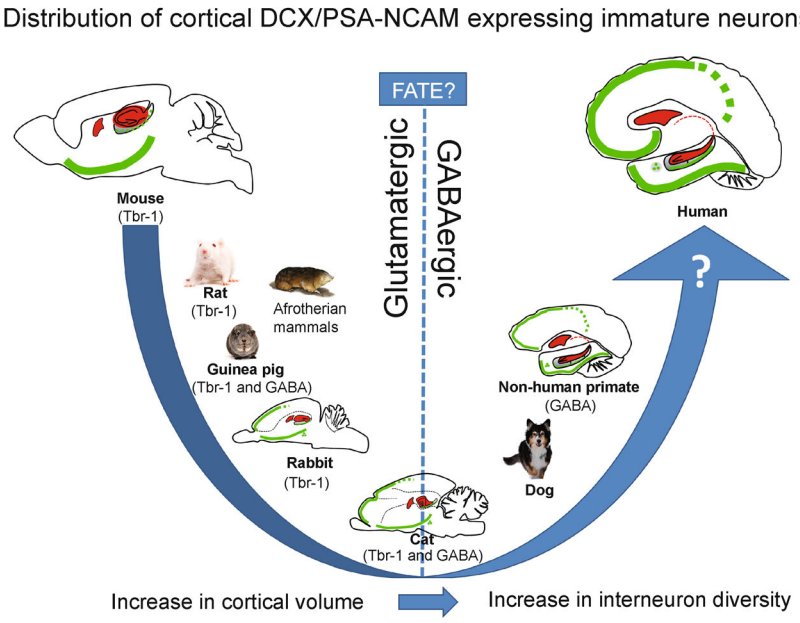
(*Continued*)

Mammal	Location	Expected fate	Are-related reduction?	Overlap with fate-specific or mature neural markers?	Prenatal birth dating?	Antigens	Proliferation in the adult?	Morphologies	Reference
Hesus monkey	Allocortex and neocortex; ventrodorsal high to low gradient	n.a. (Suggested by the authors: GABAergic fate)	n.a.	n.a.	n.a.	DCX	n.a.	DCX: Most DCX expressing cells in layer II/III were bipolar, while some appeared to be multipolar.	Cai et al., 2009
Human		n.a.	Age-related reduction (from 1 year to 3.7, and 6 years of age)	n.a.	n.a.	DCX, GAD65/67; PSA-NCAM, NeuN		Either DCX, or PSA-NCAM DCX: Small cells (8μm diameter) with mostly one/two long processes were present in layer II of the cortex in the principal sulcus, gyrus rectus and, most abundantly, around the inferior arcuate sulcus and lateral orbital sulcus. While these layer II DCX expressing cells were present in the neonatal rhesus macaque brain, and in the 1 month old brain, the density of these cells were reduced in 3.7 and 6 year old monkeys. PSA-NCAM:Heterogeneous population; co-expression of GAD65/67 in some cortical layers	Srikandarajah et al., 2009
	Entorhinal cortex (Hippocampus)	n.a.	n.a. (for the entorhinal cortex)	n.a.	n.a.	PSA-NCAM	n.a.	In the hippocampal formation, immunoreactivity was occasionally observed as a band of cells in the entorhinal cortex, in 7 month old humans.	Ni Dhuill et al., 1999
	Neocortex (temporal and frontal lopes)	n.a. (Suggested by the authors: GABAergic fate)	n.a.	n.a.	n.a.	DCX	n.a.	DCX: DCX expressing cells varied in soma size, shape, staining intensity and neuritic appearance. Anyhow, many small cells showed small bipolar and a few multipolar cells exhibiting heavy reactivity Medium-sized cells with weak to moderate DCX reactivity	Cai et al., 2009

(*Continued*)

Mammal	Location	Expected fate	Are-related reduction?	Overlap with fate-specific or mature neural markers?	Prenatal birth dating?	Antigens	Proliferation in the adult?	Morphologies	Reference
Human	Neocortex (undifferenti-ated)	n.a.	n.a.	n.a.	n.a.	BrdU (mouse igG, M0744); DCX (rabbit IgG, ab18723; goat IgG, Sc-8066; guinea pig IgG, AB2253; guinea pig IgG, AB5910); GFAP (mouse IgG1, G3893; chicken IgY, AB5541; rabbit IgG, Z0334); Nestin (rabbit IgG, AB5922; mouse IgG1, MAB377); vimentin (IgG1, M0725); NeuN, Iba-1;	n.a.	DCX: DCX was found in the cerebral cortex, highly localized at the glia limitans, layer II and layer V (non-human primate, and humans); 4.55 +/− 1.58% of total cells expressed DCX in the neocortex in nonhuman primates; DCX-positive cells with long processes were observed in the glia limitans and in the layer I; DCX-positive cells with pyramidal cell bodies were stained in layer II; DCX-positive cells with small stellar morphology were located at the limit between GM and WM Small cells, negative for Nestin, NeuN, GFAP, Iba-1, and vimentin, represented about 18% of the total DCX-positive cells in the cortex. Larger DCX-positive cells were either described to express NeuN (26%), with the shape of Cajal-Retzius cells, or to express GFAP (26%), or are positive or DCX/NeuN/GFAP (30%). DCX-positive cells in adult brain cell cultures from human brain biopsies:In-vitro 9.8% of cortical cells in-cooperated BrdU at day 35, and nearly all of the BrdU-positive cells expressed DCX/GFAP.	Bloch et al., 2011

Abbreviations: Not analysed (n.a.);
TUJ1 (Neuron-specific class III beta-tubulin), NeuN(Feminizing Locus on X-3, Fox-3, Rbfox3, or Hexaribonucleotide Binding Protein-3), DCX (Doublecortin), PSA-NCAM (polysialylated-neural cell adhesion molecule); PV (Parvalbumin), CB(Calbindin), GABA (Gamma-Aminobutyric acid), GAD67 (glutamic acid decarboxylase),Lhx6 (LIM/homeobox protein), Tbr1 (T-box, brain, 1),BrdU (Bromodeoxyuridine), Iba-1 (ionized calcium-binding adapter molecule 1), GFAP (Glial fibrillary acidic protein), NG2 (neural/glial antigen 2), RIP (Receptor-interacting protein), Alpha-actinin (1:500), Arc (activity-regulated cytoskeleton-associated protein), CaMKII (Ca^{2+}/calmodulin-dependent protein kinase II), CR(Calretinin), CNGA-3 (Cyclic nucleotide-gated ion channel subunit A3), MAP2 (Microtubule-associated protein 2), NMDA receptor 1 (N-Methyl-D-aspartic acid), Pax6 (Paired box protein),Phospho-CREB (cAMP response element-binding protein), TUC-4 (TOAD [Turned On After Division]/ Ulip/CRMP); nNOS (Nitric oxide synthases).



Box 1 Do different fates and distributions of DCX/PSA-NCAM IN reflect phylogenetic adaptations and are therefore species-specific? The fate of DCX/PSA-NCAM IN is not resolved yet, current reports are inconsistent. Species-specific variation in fate and distribution of DCX/PSA-NCAM IN may reflect phylogenetic differences in corticogenesis and/or cortical network physiology. The distribution of DCX/PSA-NCAM IN (green) and the locations of the canonical neurogenic niches (red) are schematically shown in different mammalian species. The order of the illustrated species reflects absolute cortical volume, but not the taxonomy of the listed mammals. While DCX/PSA-NCAM IN in mammals with relatively smaller cortices are mostly limited to allocortical regions, the numbers of DCX/PSA-NCAM IN and the number of regions bearing these cells in mammals with comparatively bigger cerebrums are higher. Since about 10% of all the cells in the layer II of the posterior piriform cortex in young adult mice are described as PSA-NCAM expressing cells with immature morphology (Rubio et al., 2015), it is suggested that the overall number of DCX/PSA-NCAM IN in mammals with large neocortical lobes is scaling proportional with bigger cortices and therefore the functional relevance of DCX/PSA-NCAM IN upon maturation is expected to be higher for large-brained species. Despite maturation, it is unresolved whether DCX/PSA-NCAM IN develop into GABAergic neurons in some species and glutamatergic neurons in others, or if both fates are covered in some species. We can speculate that these variances reflect species-specific assignments of DCX/PSA-NCAM IN. Alternatively, different types of neurons hypothetically originate from DCX/PSA-NCAM IN in mammals with large cerebrum, as opposed to those with small cerebrums. Nevertheless, it is also possible that the reported discrepancy in the fate of these cortical progenitors is a matter of unstandardized fixation, staining, counting, and fate-mapping methods.

However, in these two species DCX/PSA-NCAM IN are predominantly located in the posterior part of the piriform cortex (Gómez-Climent et al., 2008; Luzzati et al., 2009; Rubio et al., 2015). This brain region is an associative cortical area, thought to be responsible for higher-order olfactory processing (Neville and Haberly, 2003; Gottfried et al., 2006; Kadohisa and Wilson, 2006a, 2016b; Bekkers and Suzuki, 2013). Hence, the recurrent observation among different species is that DCX/PSA-NCAM IN are most abundant in higher-order integrative cortical areas, suggesting that these cells play strategic roles in advanced brain functions.

DCX/PSA-NCAM IN might constitute a consumable reservoir for cortical plasticity crucial for cognition

Similar to the adult-born neurons in the dentate gyrus of young rodents, which are thought to promote plasticity of hippocampal circuits and therefore cognitive function ((Gould et al., 1999; Ambrogini et al., 2000; Lemaire et al., 2000; Shors et al., 2001; Shors et al., 2002; Bizon and Gallagher, 2005), reviewed in König et al., 2016), the integration of cortical DCX/PSA-NCAM IN is predicted to provide a form of cellular plasticity for higher-order cortical circuits. The integration of neuronal precursors into existing hippocampal networks (Cameron and McKay, 1999; Hastings and Gould, 1999; Markakis and Gage, 1999; van Praag et al., 2002) leads to competition with pre-established connections (Ge et al., 2006; Toni et al., 2008; Toni et al., 2007), and provides an alternative method by which synaptic plasticity can occur (Gould et al., 1999; Toni et al., 2008; Toni et al., 2007). Similarly, maturation and integration of DCX/PSA-NCAM IN in the associative cortices and higher-order (secondary) sensory areas may facilitate remodeling and fine-tuning capacity of pre-existing cortical networks in adult mammals. Interestingly, the cortical areas containing the highest density of DCX/PSA-NCAM IN are commonly reported to be crucial for the integration of different modalities, including attention, planning, and memory storing (Purves et al., 2001). Accordingly, we propose that DCX/PSA-NCAM IN constitute a limited, post-mitotic, local

reservoir for cortical plasticity, and as such, a consumable source for cognitive plasticity during aging.

DCX/PSA-NCAM IN and the term "adult non-canonical neurogenesis"

Could the delayed maturation and integration of DCX/PSA-NCAM IN outside neurogenic niches be considered adult non-canonical neurogenesis? In the cortex, DCX/PSA-NCAM IN are generated prenatally and undergo late maturation. Thus, they are newlymatured, functional neurons but not newly born cells. To help determine how this peculiar form of cellular development can be classified as non-proliferative/non-canonical neurogenesis, we will briefly discuss the different types of mammalian neurogenesis. The first type is developmental neurogenesis: the formation of functionally mature neurons from proliferating neural stem cells during embryonic development, which can be protracted into early postnatal and adult ages in a species-specific manner (Bonfanti, 2013). The second type is adult neurogenesis: the formation of functionally mature neurons from proliferating neural stem or progenitor cells in adult canonical neurogenic niches and possibly other yet to be defined brain areas. We propose a third type of neurogenesis, which is a hybrid between developmental and adult neurogenesis. Here, cortical cells are born during embryonic development, but prematurely interrupt their maturation and can be identified as DCX/PSA-NCAM IN in the adult brain. Eventually, these cells can become newlymatured, functional neurons during adulthood. Therefore, in the beginning, DCX/PSA-NCAM IN follow normal patterns of developmental and proliferative, reflecting an incomplete neurogenesis. Later, the maturation of DCX/PSA-NCAM IN into functional neurons that integrate into pre-established networks resembles the process of adult neurogenesis without proliferation. In conclusion, this form of neurogenic or neurogenic-like process could be defined as "suspended developmental neurogenesis" or "adult non-proliferative neurogenesis." Since the neurogenesis of DCX/PSA-NCAM IN is better characterized by their adult maturation rather than their perinatal undifferentiated proliferation, the second definition is arguably the most accurate when summarizing this two-step process.

Nevertheless, we conclude that the impact of newly-matured, functional neurons on cortical networks may equal the plasticity provided byother adult-born neurons, regardless of the applied terminology. We emphasize the need for further research to validate the "loss by maturation" hypothesis using transgenic animals. Understanding the physiologic potential of DCX/PSA-NCAM IN might offer new insights into brain aging and plasticity during adulthood.

Acknowledgements

We would like to acknowledge Mr. Mark O' Sullivan for his intellectual and literary input on this paper and Mag. Roman Fuchs for the images provided.

Compliance with ethics guidelines

Richard König, Bruno Benedetti, Peter Rotheneichner, Anna O′ Sullivan, Christina Kreutzer, Maria Belles, Juan Nacher, M. Thomas Weiger, Ludwig Aigner, and Sébastien Couillard-Després declare that they have no conflicts of interest. All institutional and national guidelines for the care and use of laboratory animals were followed.

References

Abrous D N, Montaron M F, Petry K G, Rougon G, Darnaudéry M, Le Moal M, Mayo W (1997). Decrease in highly polysialylated neuronal cell adhesion molecules and in spatial learning during ageing are not correlated. Brain Res, 744(2): 285–292

Ambrogini P, Cuppini R, Cuppini C, Ciaroni S, Cecchini T, Ferri P, Sartini S, Del Grande P (2000). Spatial learning affects immature granule cell survival in adult rat dentate gyrus. Neurosci Lett, 286(1): 21–24

Bédard A, Lévesque M, Bernier P J, 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(10): 1917–1924

Bekkers J M, Suzuki N (2013). Neurons and circuits for odor processing in the piriform cortex. Trends Neurosci, 36(7): 429–438

Bernier P J, 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(17): 11464–11469

Betarbet R, Zigova T, Bakay R A, Luskin M B (1996). Dopaminergic and GABAergic interneurons of the olfactory bulb are derived from the neonatal subventricular zone. Int J Dev Neurosci, 14(7-8): 921–930

Biebl M, Cooper C M, Winkler J, Kuhn H G (2000). Analysis of neurogenesis and programmed cell death reveals a self-renewing capacity in the adult rat brain. Neurosci Lett, 291(1): 17–20

Bizon J L, Gallagher M (2005). More is less: neurogenesis and age-related cognitive decline in Long-Evans rats. Sci SAGE KE, 2005(7): re2

Bizon J L, Lee H J, Gallagher M (2004). Neurogenesis in a rat model of age-related cognitive decline. Aging Cell, 3(4): 227–234

Bloch J, Kaeser M, Sadeghi Y, Rouiller E M, Redmond D EJr, Brunet J F (2011). Doublecortin-positive cells in the adult primate cerebral cortex and possible role in brain plasticity and development. J Comp Neurol, 519(4): 775–789

Bondolfi L, Ermini F, Long J M, Ingram D K, Jucker M (2004). Impact of age and caloric restriction on neurogenesis in the dentate gyrus of C57BL/6 mice. Neurobiol Aging, 25(3): 333–340

Bonfanti L (2013).The (real) neurogenic/gliogenic potential of the postnatal and adult brain parenchyma. ISRN Neurosci, 2013: 354136

Bonfanti L, Nacher J (2012). New scenarios for neuronal structural plasticity in non-neurogenic brain parenchyma: the case of cortical layer II immature neurons. Prog Neurobiol, 98(1): 1–15

Bonfanti L, Olive S, Poulain D A, Theodosis D T (1992). Mapping of the

distribution of polysialylated neural cell adhesion molecule throughout the central nervous system of the adult rat: an immunohistochemical study. Neuroscience, 49(2): 419–436

Bonfanti L, Peretto P (2011). Adult neurogenesis in mammals—a theme with many variations. Eur J Neurosci, 34(6): 930–950

Breunig J J, Arellano J I, Macklis J D, Rakic P (2007). Everything that glitters isn't gold: a critical review of postnatal neural precursor analyses. Cell Stem Cell, 1(6): 612–627

Brown J, Cooper-Kuhn C M, Kempermann G, Van Praag H, Winkler J, Gage F H, Kuhn H G (2003). Enriched environment and physical activity stimulate hippocampal but not olfactory bulb neurogenesis. Eur J Neurosci, 17(10): 2042–2046

Burns K A, Ayoub A E, Breunig J J, Adhami F, Weng W L, Colbert M C, Rakic P, Kuan C Y (2007). Nestin-CreER mice reveal DNA synthesis by nonapoptotic neurons following cerebral ischemia hypoxia. Cereb Cortex, 17(11): 2585–2592

Burns T C, Ortiz-González X R, Gutiérrez-Pérez M, Keene C D, Sharda R, Demorest Z L, Jiang Y, Nelson-Holte M, Soriano M, Nakagawa Y, Luquin M R, Garcia-Verdugo J M, Prósper F, Low W C, Verfaillie C M (2006). Thymidine analogs are transferred from prelabeled donor to host cells in the central nervous system after transplantation: a word of caution. Stem Cells, 24(4): 1121–1127

Butt A M, Hamilton N, Hubbard P, Pugh M, Ibrahim M (2005). Synantocytes: the fifth element. J Anat, 207(6): 695–706

Cai Y, Xiong K, Chu Y, Luo D W, Luo X G, Yuan X Y, Struble R G, Clough R W, Spencer D D, Williamson A, Kordower J H, Patrylo P R, Yan X X (2009). Doublecortin expression in adult cat and primate cerebral cortex relates to immature neurons that develop into GABAergic subgroups. Exp Neurol, 216(2): 342–356

Cameron H A, McKay R D (1999). Restoring production of hippocampal neurons in old age. Nat Neurosci, 2(10): 894–897

Carleton A, Petreanu L T, Lansford R, Alvarez-Buylla A, Lledo P M (2003).Becoming a new neuron in the adult olfactory bulb. Nat Neurosci, 6(5): 507–518

Clarke L E, Young K M, Hamilton N B, Li H, Richardson W D, Attwell D (2012). Properties and fate of oligodendrocyte progenitor cells in the corpus callosum, motor cortex, and piriform cortex of the mouse. J Neurosci, 32(24): 8173–8185

Costa M R, Kessaris N, Richardson W D, Götz M, Hedin-Pereira C (2007). The marginal zone/layer I as a novel niche for neurogenesis and gliogenesis in developing cerebral cortex. J Neurosci, 27(42): 11376–11388

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(6): 1535–1545

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

Curtis M A, Eriksson P S, Faull R L (2007). Progenitor cells and adult neurogenesis in neurodegenerative diseases and injuries of the basal ganglia. Clin Exp Pharmacol Physiol, 34(5-6): 528–532

Dawson M R, Polito A, Levine J M, Reynolds R (2003). NG2-expressing glial progenitor cells: an abundant and widespread population of cycling cells in the adult rat CNS. Mol Cell Neurosci, 24(2): 476–488

Dayer A G, Cleaver K M, Abouantoun T, Cameron H A (2005). New GABAergic interneurons in the adult neocortex and striatum are generated from different precursors. J Cell Biol, 168(3): 415–427

de la Rosa-Prieto C, Saiz-Sanchez D, Ubeda-Bañon I, Argandoña-Palacios L, Garcia-Muñozguren S, Martinez-Marcos A (2010). Neurogenesis in subclasses of vomeronasal sensory neurons in adult mice. Dev Neurobiol, 70(14): 961–970

De Marchis S, Fasolo A, Puche A C (2004). Subventricular zone-derived neuronal progenitors migrate into the subcortical forebrain of postnatal mice. J Comp Neurol, 476(3): 290–300

De Nevi E, Marco-Salazar P, Fondevila D, Blasco E, Pérez L, Pumarola M (2013).Immunohistochemical study of doublecortin and nucleostemin in canine brain. Eur J Histochem, 57(1): e9

des Portes V, Pinard J M, Billuart P, Vinet M C, Koulakoff A, Carrié A, Gelot A, Dupuis E, Motte J, Berwald-Netter Y, Catala M, Kahn A, Beldjord C, Chelly J (1998). A novel CNS gene required for neuronal migration and involved in X-linked subcortical laminar heterotopia and lissencephaly syndrome. Cell, 92(1): 51–61

Dimou L, Simon C, Kirchhoff F, Takebayashi H, Götz M (2008). Progeny of Olig2-expressing progenitors in the gray and white matter of the adult mouse cerebral cortex. J Neurosci, 28(41): 10434–10442

Dirian L, Galant S, Coolen M, Chen W, Bedu S, Houart C, Bally-Cuif L, Foucher I (2014).Spatial regionalization and heterochrony in the formation of adult pallial neural stem cells. Dev Cell, 30(2): 123–136

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(42): 9372–9382

Doetsch F, García-Verdugo J M, Alvarez-Buylla A (1997).Cellular composition and three-dimensional organization of the subventricular germinal zone in the adult mammalian brain. J Neurosci, 17(13): 5046–5061

Duque A, Rakic P (2011). Different effects of bromodeoxyuridine and [3H]thymidine incorporation into DNA on cell proliferation, position, and fate. J Neurosci, 31(42): 15205–15217

Ehninger D, Kempermann G (2008). Neurogenesis in the adult hippocampus. Cell Tissue Res, 331(1): 243–250

Ehninger D, Wang L P, Klempin F, Römer B, Kettenmann H, Kempermann G (2011). Enriched environment and physical activity reduce microglia and influence the fate of NG2 cells in the amygdala of adult mice. Cell Tissue Res, 345(1): 69–86

Ekstrand J J, Domroese M E, Feig S L, Illig K R, Haberly L B (2001). Immunocytochemical analysis of basket cells in rat piriform cortex. J Comp Neurol, 434(3): 308–328

Encinas J M, Michurina T V, Peunova N, Park J H, Tordo J, Peterson D A, Fishell G, Koulakov A, Enikolopov G (2011). Division-coupled astrocytic differentiation and age-related depletion of neural stem cells in the adult hippocampus. Cell Stem Cell, 8(5): 566–579

Englund C, Fink A, Lau C, Pham D, Daza R A, Bulfone A, Kowalczyk T, Hevner R F (2005). Pax6, Tbr2, and Tbr1 are expressed sequentially by radial glia, intermediate progenitor cells, and postmitotic neurons in developing neocortex. J Neurosci, 25(1): 247–251

Eriksson P S, Perfilieva E, Björk-Eriksson T, Alborn A M, Nordborg C, Peterson D A, Gage F H (1998).Neurogenesis in the adult human hippocampus. Nat Med, 4(11): 1313–1317

Ernst A, Alkass K, Bernard S, Salehpour M, Perl S, Tisdale J, Possnert G, Druid H, Frisén J (2014). Neurogenesis in the striatum of the adult human brain. Cell, 156(5): 1072–1083

Feliciano D M, Bordey A (2013). Newborn cortical neurons: only for neonates? Trends Neurosci, 36(1): 51–61

Feliciano D M, Bordey A, Bonfanti L (2015). Noncanonical Sites of Adult Neurogenesis in the Mammalian Brain. Cold Spring Harb Perspect Biol, 7(10): a018846

Fox G B, Fichera G, Barry T, O'Connell A W, Gallagher H C, Murphy K J, Regan C M (2000). Consolidation of passive avoidance learning is associated with transient increases of polysialylated neurons in layer II of the rat medial temporal cortex. J Neurobiol, 45(3): 135–141

Francis F, Koulakoff A, Boucher D, Chafey P, Schaar B, Vinet M C, Friocourt G, McDonnell N, Reiner O, Kahn A, McConnell S K, Berwald-Netter Y, Denoulet P, Chelly J (1999). Doublecortin is a developmentally regulated, microtubule-associated protein expressed in migrating and differentiating neurons. Neuron, 23(2): 247–256

Friocourt G, Liu J S, Antypa M, Rakic S, Walsh C A, Parnavelas J G (2007). Both doublecortin and doublecortin-like kinase play a role in cortical interneuron migration. J Neurosci, 27(14): 3875–3883

Gage F H, Kempermann G, Song H (2008). Adult Neurogenesis, Vol 52. Cold Spring Harbor Laboratory Press

Ge S, Goh E L, Sailor K A, Kitabatake Y, Ming G L, Song H (2006). GABA regulates synaptic integration of newly generated neurons in the adult brain. Nature, 439(7076): 589–593

Gómez-Climent M A, Castillo-Gómez E, Varea E, Guirado R, Blasco-Ibáñez J M, Crespo C, Martínez-Guijarro F J, Nácher J (2008). A population of prenatally generated cells in the rat paleocortex maintains an immature neuronal phenotype into adulthood. Cereb Cortex, 18(10): 2229–2240

Gomez-Climent M A, Guirado R, Varea E, Nàcher J (2010). "Arrested development". Immature, but not recently generated, neurons in the adult brain. Arch Ital Biol, 148(2): 159–172

Gottfried J A, Winston J S, Dolan R J (2006). Dissociable codes of odor quality and odorant structure in human piriform cortex. Neuron, 49 (3): 467–479

Gould E (2007).How widespread is adult neurogenesis in mammals? Nat Rev Neurosci, 8(6): 481–488

Gould E, Tanapat P, Hastings N B, Shors T J (1999). Neurogenesis in adulthood: a possible role in learning. Trends Cogn Sci, 3(5): 186–192

Gritti A, Vescovi A L, Galli R (2002). Adult neural stem cells: plasticity and developmental potential. J Physiol Paris, 96(1-2): 81–90

Guo F, Maeda Y, Ma J, Xu J, Horiuchi M, Miers L, Vaccarino F, Pleasure D (2010). Pyramidal neurons are generated from oligodendroglial progenitor cells in adult piriform cortex. J Neurosci, 30(36): 12036–12049

Hastings N B, Gould E (1999). Rapid extension of axons into the CA3 region by adult-generated granule cells. J Comp Neurol, 413(1): 146–154

He X, Zhang X M, Wu J, Fu J, Mou L, Lu D H, Cai Y, Luo X G, Pan A, Yan X X (2014). Olfactory experience modulates immature neuron development in postnatal and adult guinea pig piriform cortex. Neuroscience, 259: 101–112

Hevner R F, Hodge R D, Daza R A, Englund C (2006). Transcription factors in glutamatergic neurogenesis: conserved programs in neocortex, cerebellum, and adult hippocampus. Neurosci Res, 55 (3): 223–233

Johnson C P, Fujimoto I, Rutishauser U, Leckband D E (2005). Direct evidence that neural cell adhesion molecule (NCAM) polysialylation increases intermembrane repulsion and abrogates adhesion. J Biol Chem, 280(1): 137–145

Kadohisa M, Wilson D A (2006a). Olfactory cortical adaptation facilitates detection of odors against background. J Neurophysiol, 95(3): 1888–1896

Kadohisa M, Wilson D A (2006b). Separate encoding of identity and similarity of complex familiar odors in piriform cortex. Proc Natl Acad Sci USA, 103(41): 15206–15211

Kang S H, Fukaya M, Yang J K, Rothstein J D, Bergles D E (2010). NG^{2+} CNS glial progenitors remain committed to the oligodendrocyte lineage in postnatal life and following neurodegeneration. Neuron, 68(4): 668–681

Kaplan M S (1981). Neurogenesis in the 3-month-old rat visual cortex. J Comp Neurol, 195(2): 323–338

Kapur A, Pearce R A, Lytton W W, Haberly L B (1997). GABAA-mediated IPSCs in piriform cortex have fast and slow components with different properties and locations on pyramidal cells. J Neurophysiol, 78(5): 2531–2545

Kato T, Yokouchi K, Kawagishi K, Fukushima N, Miwa T, Moriizumi T, Kato T, Yokouchi K, Kawagishi K (2000). Fate of newly formed periglomerular cells in the olfactory bulb. Acta Otolaryngol, 120(7): 876–879

Kelsch W, Mosley C P, Lin C W, Lois C (2007). Distinct mammalian precursors are committed to generate neurons with defined dendritic projection patterns. PLoS Biol, 5(11): e300

Kempermann G, Jessberger S, Steiner B, Kronenberg G (2004). Milestones of neuronal development in the adult hippocampus. Trends Neurosci, 27(8): 447–452

Klempin F, Kronenberg G, Cheung G, Kettenmann H, Kempermann G (2011). Properties of doublecortin-(DCX)-expressing cells in the piriform cortex compared to the neurogenic dentate gyrus of adult mice. PLoS ONE, 6(10): e25760

Komitova M, Zhu X, Serwanski D R, Nishiyama A (2009). NG2 cells are distinct from neurogenic cells in the postnatal mouse subventricular zone. J Comp Neurol, 512(5): 702–716

König R, Rotheneichner P, Marschallinger J, Aigner L, Couillard-Despres S (2016). Adult Neurogenesis in the Hippocampus. Elsevier, pp. 145–176

Kornack D R, Rakic P (2001). Cell proliferation without neurogenesis in adult primate neocortex. Science, 294(5549): 2127–2130

Kremer T, Jagasia R, Herrmann A, Matile H, Borroni E, Francis F, Kuhn H G, Czech C (2013). Analysis of adult neurogenesis: evidence for a prominent "non-neurogenic" DCX-protein pool in rodent brain. PLoS ONE, 8(5): e59269

Kuan C Y, Schloemer A J, Lu A, Burns K A, Weng W L, Williams M T, Strauss K I, Vorhees C V, Flavell R A, Davis R J, Sharp F R, Rakic P (2004). Hypoxia-ischemia induces DNA synthesis without cell proliferation in dying neurons in adult rodent brain. J Neurosci, 24 (47): 10763–10772

Kunz B A, Kohalmi S E (1991). Modulation of mutagenesis by deoxyribonucleotide levels. Annu Rev Genet, 25(1): 339–359

Lehner B, Sandner B, Marschallinger J, Lehner C, Furtner T, Couillard-Despres S, Rivera F J, Brockhoff G, Bauer H C, Weidner N, Aigner L (2011). The dark side of BrdU in neural stem cell biology:

detrimental effects on cell cycle, differentiation and survival. Cell Tissue Res, 345(3): 313–328

Lemaire V, Koehl M, Le Moal M, Abrous D N (2000). Prenatal stress produces learning deficits associated with an inhibition of neurogenesis in the hippocampus. Proc Natl Acad Sci USA, 97(20): 11032–11037

Luskin M B, Boone M S (1994). Rate and pattern of migration of lineally-related olfactory bulb interneurons generated postnatally in the subventricular zone of the rat. Chem Senses, 19(6): 695–714

Luzzati F, Bonfanti L, Fasolo A, Peretto P (2009). DCX and PSA-NCAM expression identifies a population of neurons preferentially distributed in associative areas of different pallial derivatives and vertebrate species. Cereb Cortex, 19(5): 1028–1041

Luzzati F, Nato G, Oboti L, Vigna E, Rolando C, Armentano M, Bonfanti L, Fasolo A, Peretto P (2014). Quiescent neuronal progenitors are activated in the juvenile guinea pig lateral striatum and give rise to transient neurons. Development, 141(21): 4065–4075

Luzzati F, Peretto P, Aimar P, Ponti G, Fasolo A, Bonfanti L (2003). Glia-independent chains of neuroblasts through the subcortical parenchyma of the adult rabbit brain. Proc Natl Acad Sci USA, 100(22): 13036–13041

Manganas L N, Zhang X, Li Y, Hazel R D, Smith S D, Wagshul M E, Henn F, Benveniste H, Djuric P M, Enikolopov G, Maletic-Savatic M (2007). Magnetic resonance spectroscopy identifies neural progenitor cells in the live human brain. Science, 318(5852): 980–985

Markakis E A, Gage F H (1999). Adult-generated neurons in the dentate gyrus send axonal projections to field CA3 and are surrounded by synaptic vesicles. J Comp Neurol, 406(4): 449–460

Martí-Mengual U, Varea E, Crespo C, Blasco-Ibáñez J M, Nacher J (2013). Cells expressing markers of immature neurons in the amygdala of adult humans. Eur J Neurosci, 37(1): 10–22

Mikkonen M, Soininen H, Kälviänen R, Tapiola T, Ylinen A, Vapalahti M, Paljärvi L, Pitkänen A (1998). Remodeling of neuronal circuitries in human temporal lobe epilepsy: increased expression of highly polysialylated neural cell adhesion molecule in the hippocampus and the entorhinal cortex. Ann Neurol, 44(6): 923–934

Murphy K J, Fox G B, Foley A G, Gallagher H C, O'Connell A, Griffin A M, Nau H, Regan C M (2001). Pentyl-4-yn-valproic acid enhances both spatial and avoidance learning, and attenuates age-related NCAM-mediated neuroplastic decline within the rat medial temporal lobe. J Neurochem, 78(4): 704–714

Nacher J, Bonfanti L (2015). New neurons from old beliefs in the adult piriform cortex? A Commentary on: "Occurrence of new neurons in the piriform cortex". Front Neuroanat, 9: 62

Nacher J, Crespo C, McEwen B S (2001). Doublecortin expression in the adult rat telencephalon. Eur J Neurosci, 14(4): 629–644

Nacher J, Lanuza E, McEwen B S (2002). Distribution of PSA-NCAM expression in the amygdala of the adult rat. Neuroscience, 113(3): 479–484

Neville K R, Haberly L B (2003). Beta and gamma oscillations in the olfactory system of the urethane-anesthetized rat. J Neurophysiol, 90 (6): 3921–3930

Ní Dhúill C M, Fox G B, Pittock S J, O'Connell A W, Murphy K J, Regan C M (1999). Polysialylated neural cell adhesion molecule expression in the dentate gyrus of the human hippocampal formation from infancy to old age. J Neurosci Res, 55(1): 99–106

Nishiyama A, Komitova M, Suzuki R, Zhu X (2009). Polydendrocytes (NG2 cells): multifunctional cells with lineage plasticity. Nat Rev Neurosci, 10(1): 9–22

Nishiyama A, Suzuki R, Zhu X (2014). NG2 cells (polydendrocytes) in brain physiology and repair. Front Neurosci, 8: 133

Nowakowski R S, Hayes N L (2000). New neurons: extraordinary evidence or extraordinary conclusion? Science, 288(5467): 771

Nowakowski R S, Lewin S B, Miller M W (1989). Bromodeoxyuridine immunohistochemical determination of the lengths of the cell cycle and the DNA-synthetic phase for an anatomically defined population. J Neurocytol, 18(3): 311–318

Okuda H, Tatsumi K, Makinodan M, Yamauchi T, Kishimoto T, Wanaka A (2009). Environmental enrichment stimulates progenitor cell proliferation in the amygdala. J Neurosci Res, 87(16): 3546–3553

Patzke N, LeRoy A, Ngubane N W, Bennett N C, Medger K, Gravett N, Kaswera-Kyamakya C, Gilissen E, Chawana R, Manger P R (2014). The distribution of doublecortin-immunopositive cells in the brains of four afrotherian mammals: the Hottentot golden mole (Amblysomus hottentotus), the rock hyrax (Procavia capensis), the eastern rock sengi (Elephantulus myurus) and the four-toed sengi (Petrodromus tetradactylus). Brain Behav Evol, 84(3): 227–241

Peretto P, Bonfanti L (2014). Major unsolved points in adult neurogenesis: doors open on a translational future? Front Neurosci, 8: 154

Petreanu L, Alvarez-Buylla A (2002). Maturation and death of adult-born olfactory bulb granule neurons: role of olfaction. J Neurosci, 22 (14): 6106–6113

Pierce A A, Xu A W (2010). De novo neurogenesis in adult hypothalamus as a compensatory mechanism to regulate energy balance. J Neurosci, 30(2): 723–730

Psachoulia K, Jamen F, Young K M, Richardson W D (2009). Cell cycle dynamics of NG2 cells in the postnatal and ageing brain. Neuron Glia Biol, 5(3-4): 57–67

Purves D, Augustine G J, Flitzpatrick D, Katz L C, LaMantia A S, McNamara J O, Williams S M (2001). Neuroscience, 2nd edition. Sunderland (MA): Sinauer Associates

Richardson W D, Young K M, Tripathi R B, McKenzie I (2011). NG2-glia as multipotent neural stem cells: fact or fantasy? Neuron, 70(4): 661–673

Rivers L E, Young K M, Rizzi M, Jamen F, Psachoulia K, Wade A, Kessaris N, Richardson W D (2008). PDGFRA/NG2 glia generate myelinating oligodendrocytes and piriform projection neurons in adult mice. Nat Neurosci, 11(12): 1392–1401

Robins S C, Trudel E, Rotondi O, Liu X, Djogo T, Kryzskaya D, Bourque C W, Kokoeva M V (2013). Evidence for NG2-glia derived, adult-born functional neurons in the hypothalamus. PLoS ONE, 8 (10): e78236

Rosselli-Austin L, Altman J (1979). The postnatal development of the main olfactory bulb of the rat. J Dev Physiol, 1(4): 295–313

Rossi S L, Mahairaki V, Zhou L, Song Y, Koliatsos V E (2014). Remodeling of the piriform cortex after lesion in adult rodents. Neuroreport, 25(13): 1006–1012

Rubio A, Belles M, Belenguer G, Vidueira S, Fariñas I, Nacher J (2015). Characterization and isolation of immature neurons of the adult mouse piriform cortex. Dev Neurobiol, doi: 10.1002/dneu.22357

Rutishauser U (2008). Polysialic acid in the plasticity of the developing

and adult vertebrate nervous system. Nat Rev Neurosci, 9(1): 26–35

Saegusa T, Mine S, Iwasa H, Murai H, Seki T, Yamaura A, Yuasa S (2004). Involvement of highly polysialylated neural cell adhesion molecule (PSA-NCAM)-positive granule cells in the amygdaloid-kindling-induced sprouting of a hippocampal mossy fiber trajectory. Neurosci Res, 48(2): 185–194

Sairanen M, O'Leary O F, Knuuttila J E, Castrén E (2007). Chronic antidepressant treatment selectively increases expression of plasticity-related proteins in the hippocampus and medial prefrontal cortex of the rat. Neuroscience, 144(1): 368–374

Sanai N, Nguyen T, Ihrie R A, Mirzadeh Z, Tsai H H, Wong M, Gupta N, Berger M S, Huang E, Garcia-Verdugo J M, Rowitch D H, Alvarez-Buylla A (2011). Corridors of migrating neurons in the human brain and their decline during infancy. Nature, 478(7369): 382–386

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(3): 503–513

Shapiro L A, Ng K, Zhou Q Y, Ribak C E (2009). Subventricular zone-derived, newly generated neurons populate several olfactory and limbic forebrain regions. Epilepsy Behav, 14(Suppl 1): 74–80

Shapiro L A, Ng K L, Kinyamu R, Whitaker-Azmitia P, Geisert E E, Blurton-Jones M, Zhou Q Y, Ribak C E (2007a). Origin, migration and fate of newly generated neurons in the adult rodent piriform cortex. Brain Struct Funct, 212(2): 133–148

Shapiro L A, Ng K L, Zhou Q Y, Ribak C E (2007b). Olfactory enrichment enhances the survival of newly born cortical neurons in adult mice. Neuroreport, 18(10): 981–985

Shechter R, Ziv Y, Schwartz M (2007). New GABAergic interneurons supported by myelin-specific T cells are formed in intact adult spinal cord. Stem Cells, 25(9): 2277–2282

Shors T J, Miesegaes G, Beylin A, Zhao M, Rydel T, Gould E (2001). Neurogenesis in the adult is involved in the formation of trace memories. Nature, 410(6826): 372–376

Shors T J, Townsend D A, Zhao M, Kozorovitskiy Y, Gould E (2002). Neurogenesis may relate to some but not all types of hippocampal-dependent learning. Hippocampus, 12(5): 578–584

Spalding K L, Bergmann O, Alkass K, Bernard S, Salehpour M, Huttner H B, Boström E, Westerlund I, Vial C, Buchholz B A, Possnert G, Mash D C, Druid H, Frisén J (2013). Dynamics of hippocampal neurogenesis in adult humans. Cell, 153(6): 1219–1227

Suzuki N, Bekkers J M (2007). Inhibitory interneurons in the piriform cortex. Clin Exp Pharmacol Physiol, 34(10): 1064–1069

Suzuki N, Bekkers J M (2010a). Distinctive classes of GABAergic interneurons provide layer-specific phasic inhibition in the anterior piriform cortex. Cereb Cortex, 20(12): 2971–2984

Suzuki N, Bekkers J M (2010b). Inhibitory neurons in the anterior piriform cortex of the mouse: classification using molecular markers. J Comp Neurol, 518(10): 1670–1687

Takemura N U (2005). Evidence for neurogenesis within the white matter beneath the temporal neocortex of the adult rat brain. Neuroscience, 134(1): 121–132

Toni N, Laplagne D A, Zhao C, Lombardi G, Ribak C E, Gage F H, Schinder A F (2008). Neurons born in the adult dentate gyrus form functional synapses with target cells. Nat Neurosci, 11(8): 901–907

Toni N, Teng E M, Bushong E A, Aimone J B, Zhao C, Consiglio A, van Praag H, Martone M E, Ellisman M H, Gage F H (2007). Synapse formation on neurons born in the adult hippocampus. Nat Neurosci, 10(6): 727–734

van Praag H, Schinder A F, Christie B R, Toni N, Palmer T D, Gage F H (2002). Functional neurogenesis in the adult hippocampus. Nature, 415(6875): 1030–1034

Varea E, Belles M, Vidueira S, Blasco-Ibáñez J M, Crespo C, Pastor A M, Nacher J (2011). PSA-NCAM is Expressed in Immature, but not Recently Generated, Neurons in the Adult Cat Cerebral Cortex Layer II. Front Neurosci, 5: 17

Varea E, Castillo-Gómez E, Gómez-Climent M A, Blasco-Ibáñez J M, Crespo C, Martínez-Guijarro F J, Nàcher J (2007).PSA-NCAM expression in the human prefrontal cortex. J Chem Neuroanat, 33(4): 202–209

Varea E, Castillo-Gómez E, Gómez-Climent M A, Guirado R, Blasco-Ibáñez J M, Crespo C, Martínez-Guijarro F J, Nácher J (2009). Differential evolution of PSA-NCAM expression during aging of the rat telencephalon. Neurobiol Aging, 30(5): 808–818

Vessal M, Aycock A, Garton M T, Ciferri M, Darian-Smith C (2007). Adult neurogenesis in primate and rodent spinal cord: comparing a cervical dorsal rhizotomy with a dorsal column transection. Eur J Neurosci, 26(10): 2777–2794

Vivar C, van Praag H (2013). Functional circuits of new neurons in the dentate gyrus. Front Neural Circuits, 7: 15

Winner B, Cooper-Kuhn C M, Aigner R, Winkler J, Kuhn H G (2002). Long-term survival and cell death of newly generated neurons in the adult rat olfactory bulb. Eur J Neurosci, 16(9): 1681–1689

Xiong K, Cai Y, Zhang X M, Huang J F, Liu Z Y, Fu G M, Feng J C, Clough R W, Patrylo P R, Luo X G, Hu C H, Yan X X (2010). Layer I as a putative neurogenic niche in young adult guinea pig cerebrum. Mol Cell Neurosci, 45(2): 180–191

Xiong K, Luo D W, Patrylo P R, Luo X G, Struble R G, Clough R W, Yan X X (2008). Doublecortin-expressing cells are present in layer II across the adult guinea pig cerebral cortex: partial colocalization with mature interneuron markers. Exp Neurol, 211(1): 271–282

Yang Y, Geldmacher D S, Herrup K (2001). DNA replication precedes neuronal cell death in Alzheimer's disease. J Neurosci, 21(8): 2661–2668

Yang Y, Xie M X, Li J M, Hu X, Patrylo P R, Luo X G, Cai Y, Li Z, Yan X X (2015). Prenatal genesis of layer II doublecortin expressing neurons in neonatal and young adult guinea pig cerebral cortex. Front Neuroanat, 9: 109

Yuan T F, Liang Y X, So K F (2014). Occurrence of new neurons in the piriform cortex. Front Neuroanat, 8: 167

Yuan T F, Liang Y X, So K F (2015). Response: New neurons from old beliefs in the adult piriform cortex? A Commentary on: "Occurrence of new neurons in the piriform cortex". Front Neuroanat, 9: 79

Zhang J, Giesert F, Kloos K, Vogt Weisenhorn D M, Aigner L, Wurst W, Couillard-Despres S (2010). A powerful transgenic tool for fate mapping and functional analysis of newly generated neurons. BMC Neurosci, 11(1): 158

Zhang X M, Cai Y, Chu Y, Chen E Y, Feng J C, Luo X G, Xiong K, Struble R G, Clough R W, Patrylo P R, Kordower J H, Yan X X (2009). Doublecortin-expressing cells persist in the associative cerebral cortex and amygdala in aged nonhuman primates. Front Neuroanat, 3: 17

Zhu X, Bergles D E, Nishiyama A (2008). NG2 cells generate both oligodendrocytes and gray matter astrocytes. Development, 135(1): 145–157

Zhu X, Hill R A, Dietrich D, Komitova M, Suzuki R, Nishiyama A (2011). Age-dependent fate and lineage restriction of single NG2 cells. Development, 138(4): 745–753

Zigova T, Betarbet R, Soteres B J, Brock S, Bakay R A, Luskin M B (1996). A comparison of the patterns of migration and the destinations of homotopically transplanted neonatal subventricular zone cells and heterotopically transplanted telencephalic ventricular zone cells. Dev Biol, 173(2): 459–474