

"Arrested development". Immature, but not recently generated, neurons in the adult brain

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ABSTRACT

After the division of neuronal precursors, many of the newly generated cells become immature neurons, which migrate to their final destination in the nervous system, extend neurites and make appropriate connections. For most neurons these events occur in a narrow time window and, once in their definitive location, they immediately start the final stages of their differentiation program, remaining immature only for a short time. The main objective of this review is to present and discuss recent data on a peculiar population of cells in the adult brain, which retain an immature neuronal phenotype for an unusually prolonged time. We review and discuss recent evidence on the temporal and spatial origin of these cells, their distribution in rodents and other mammals, their structure and neurochemical phenotype, and their putative fate and function. The review is mainly focused on the population of immature neurons located in the layer II of certain cortical regions, but we will also describe similar populations found in other regions of the peripheral and central nervous systems.

Key words

Adult neurogenesis • Doublecortin • Entorhinal cortex • Piriform cortex • PSA-NCAM

Finding immature neurons in the mature brain. The case of "standby mode" neurons in cortical layer II

During the last years the view of the adult CNS as a stable structure in terms of neuronal numbers has been challenged by many studies, which have shown that neuronal production and/or incorporation are common phenomena in certain regions (Kempermann, 2005). Consequently, the search for immature/recently-generated neurons in the adult brain has become a major objective for the Neuroscience field in the last two decades. During these years, different experimental approaches have been used to identify these immature neurons, to determine their time of origin and to characterize their phenotype and fate. These approaches include

the analysis of immature and mature neuronal markers, cell birth dating, structural and ultrastructural studies and functional analyses.

Markers for immature neurons in the mature brain

After being generated from a progenitor cell, new neurons start a differentiation program, which involves the transient expression of different "neurodevelopmental" genes. These genes encode molecules implicated in the migration, neurite extension and synaptogenesis of the recently generated neurons. Consistent with their role in neuronal development, most of these molecules are highly expressed in the developing CNS and their expression is dramatically downregulated during adulthood. Obviously, this expression persists intensely

in the recently generated neurons of adult neurogenic regions, but it can also be detected in other cerebral regions. None of these genes is exclusively expressed by immature neurons: Individually, each of them can also be found in certain mature neurons or glial cells, in which they are probably involved in different plastic events. However, the simultaneous expression of many of these genes in a given cell, and the lack of expression of mature neuronal markers, can be considered a solid evidence of an immature neuronal phenotype.

Among the markers for immature neurons, one of the most widely used is the polysialylated form of the neural cell adhesion molecule (PSA-NCAM), because of its involvement in neurodevelopmental processes such as migration, neurite extension or synaptogenesis (Bonfanti, 2006; Gascon et al., 2007; Rutishauser, 2008). In adult mammals, PSA-NCAM is abundantly expressed by immature neurons in the typical neurogenic niches: the subventricular zone/rostral migratory stream/olfactory bulb (Rousselot et al., 1995) and the subgranular zone (SGZ) of the dentate gyrus (Seki and Arai, 1991b), as well as in cells of the layer II of certain cortical regions (Seki and Arai, 1991a; Bonfanti et al., 1992; Nacher et al., 2002a) (Fig. 1). However, it has to be noted that PSA-NCAM expression is also found in a subpopulation of mature cortical interneurons (Nacher et al., 2002b; Varea et al., 2005) and in mature neurons and glial cells in subcortical areas (Bonfanti, 2006). Another of the proteins that has been widely used as a marker for immature neurons is doublecortin (DCX), a microtubule associated protein, which is intensely expressed in recently generated neurons and plays a critical role in neuronal migration and neurite outgrowth (Francis et al., 1999; Gleeson et al., 1999; Friocourt et al., 2003). DCX is expressed in new neurons in the adult neurogenic regions (Nacher et al., 2001; Brown et al., 2003) and it is also co-expressed in most, if not all, PSA-NCAM expressing cells in the cerebral cortex layer II in all the species studied to date (Nacher et al., 2001; Cai et al., 2009; Gomez-Climent et al., 2008; Xiong et al., 2008) (Fig. 1). Although DCX was thought to be exclusively expressed by certain neuronal progenitor cells and cells committed to the neuronal lineage (Brown et al., 2003; Dayer et al., 2005; Walker et al., 2007), a recent report has described its expression in a subpopulation of human cortical astrocytes (Verwer et al., 2007).

Some other proteins are also expressed transiently in recently generated neurons during development and adulthood, such as TUC4, a protein of the TOAD/Ulip/CRMP family of axonal guidance proteins (Minturn et al., 1995), the cyclic nucleotide-gated ion channel 3 (CNGA-3) (Gutierrez-Mecinas et al., 2007), the phosphorylated cAMP response element-binding protein (p-CREB) (Nakagawa et al., 2002), the class III beta-tubulin (TuJ1) (Menezes and Luskin, 1994) or the antiapoptotic protein Bcl-2 (Bernier and Parent, 1998a). All these proteins are also expressed in cells of the cerebral cortex layer II (Bernier and Parent, 1998a; Nacher et al., 2000; Nacher et al., 2001; Bernier et al., 2002; Gomez-Climent et al., 2008; Xiong et al., 2008) (Fig. 1). However, most of these molecules can also be found in mature neurons and/or glial cells in the adult CNS (Bernier and Parent, 1998b; Nacher et al., 2000; Thome et al., 2000; Gutierrez-Mecinas et al., 2008).

Distribution of cells expressing markers of immature neurons in the adult mammalian cerebral cortex

Using the markers of immature neurons described above, especially DCX and PSA-NCAM, different laboratories have elaborated comprehensive mappings of the distribution of these cells in the adult mammalian cerebral cortex. Logically, many of these immature neurons are located in the hippocampal SGZ (Kempermann, 2005), but a numerous population can also be found in the layer II of several cortical regions in different mammalian species. The expression of these markers and the presence of immature neurons in extracortical regions will be discussed in the last chapter of this review.

A large population of PSA-NCAM and DCX expressing cells can be found in the layer II of the piriform and lateral entorhinal cortices of adult rats. Scattered cells also populate the layer II of the perirhinal and the most ventral region of the agranular insular and entorhinal cortices (Seki and Arai, 1991a; Bonfanti et al., 1992; Nacher et al., 2001; Nacher et al., 2002a; Gomez-Climent et al., 2008) (Figs. 2 and 3D). A similar distribution was found in mice (Phillips et al., 2006; Shapiro et al., 2007a) (Fig. 3A). By contrast, in mammals with larger cerebral cortices there is a wider distribution of these cells, which can be found also in various neocortical areas, being specially enriched in spe-

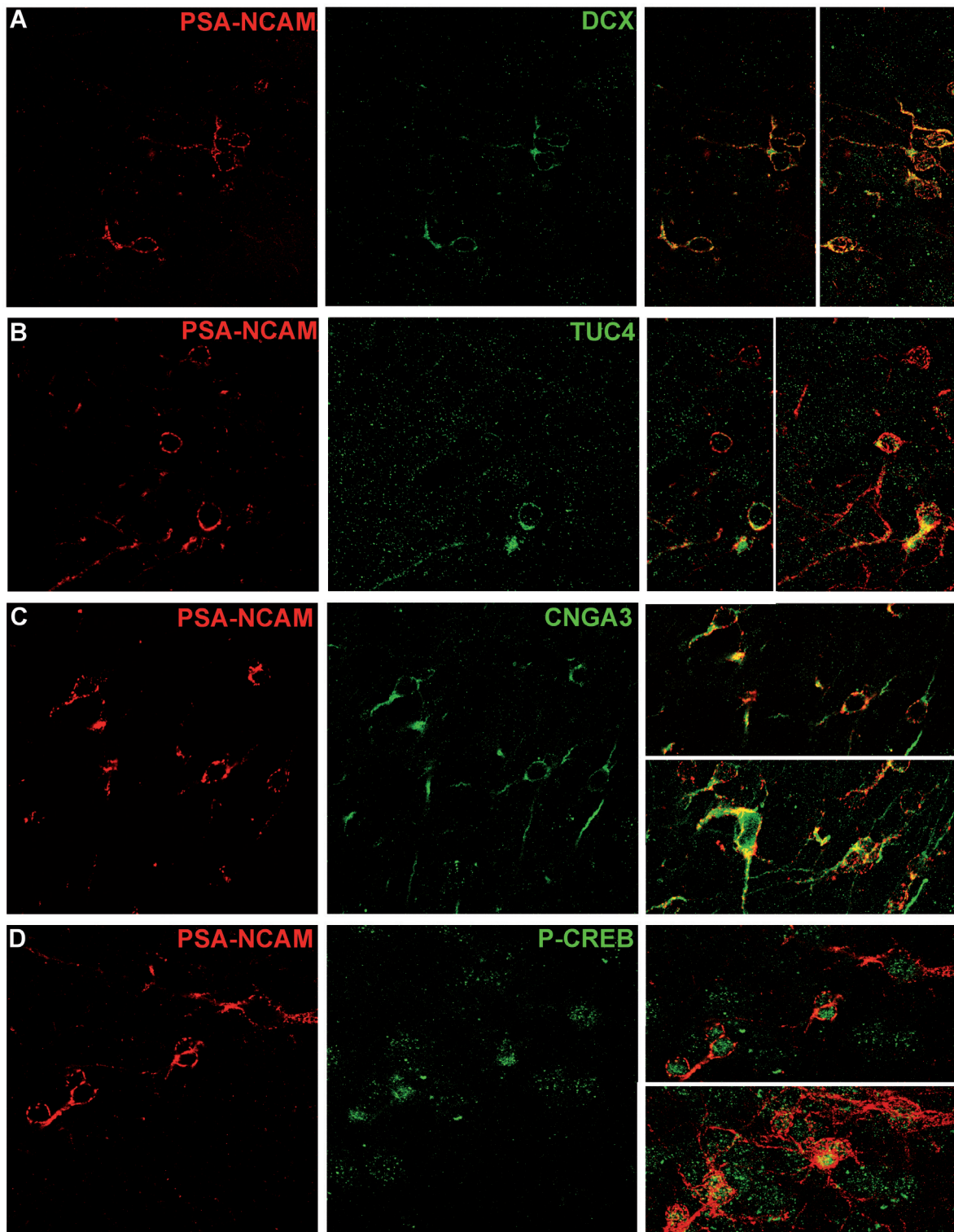


Fig. 1. - Confocal microscopic analysis of immature neuronal markers in the paleocortex layer II of adult rats. (A) Cells coexpressing PSA-NCAM and DCX. (B) PSA-NCAM/TUC-4 expressing cells. (C) Cells coexpressing PSA-NCAM and CNGA-3. (D) PSA-NCAM expressing cells displaying p-CREB expression in their nuclei. Scale bar: 25 μ m. Insets are 2D projection of 9(A), 8(B), 9(C) or 13(D) consecutive focal planes located 1 μ m apart. DCX: doublecortin; TUC-4: TOAD64/Ulip/CRMP4; CNGA3: cyclic nucleotide-gated cation channel; p-CREB: phosphorylated cAMP response element-binding protein. Images in this figure have been taken from the material generated in Gomez-Climent et al. (2008) study.

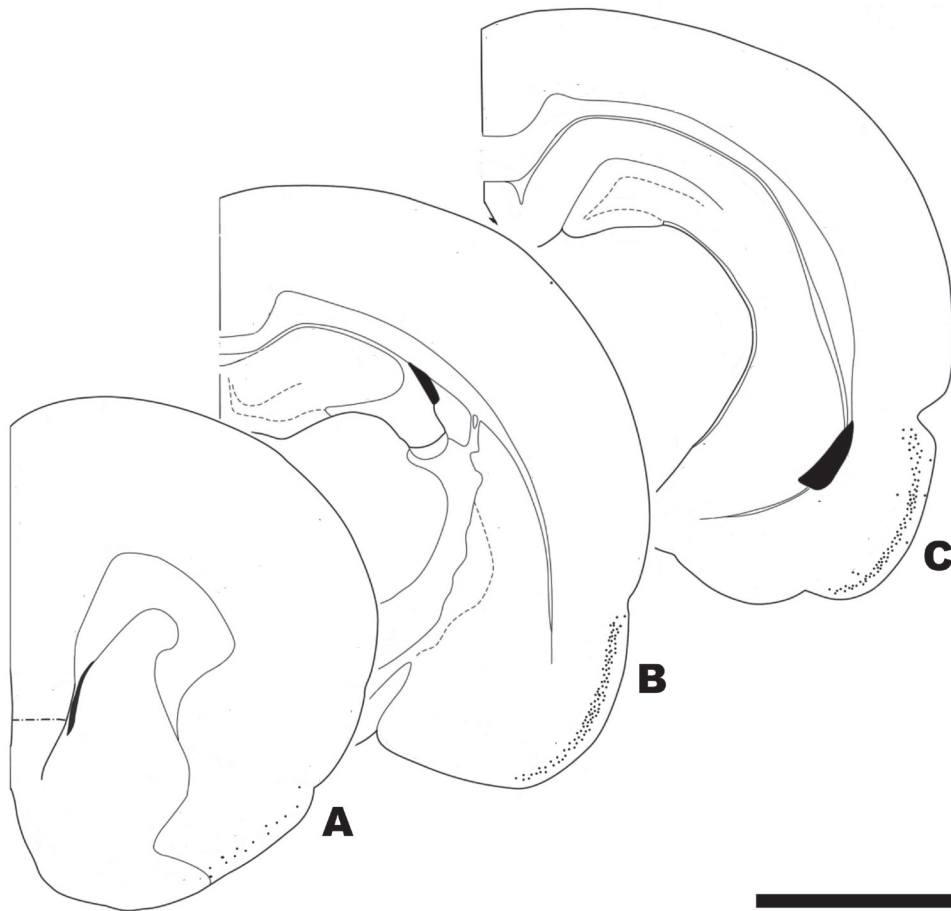


Fig. 2. - Distribution of PSA-NCAM immunoreactive cells in the paleocortex layer II of the adult rat. The dots represent individual cell somata in layer II of piriform (A & B) and lateral entorhinal cortices (C). Scale bar: 3.4 mm.

cific associative regions. In rabbits (Fig. 3B) and guinea pigs DCX/PSA-NCAM expressing cells can be found in layers II and upper III of the piriform, perirhinal and entorhinal cortices, as well as in the amygdaloid-piriform transitional region. In the neocortex of these animals these cells can be found in the somatosensory cortex and different regions of the insula, among others (Bonfanti, 2006; Luzzati et al., 2009; Xiong et al., 2008). In adult cats, DCX and PSA-NCAM expressing cells in layers II and upper III, can be found more widely dispersed in the cerebral cortex, being specially abundant in the entorhinal cortex and in ventral portions of the frontal and temporoparietal lobes, but relatively scarce in dorsal regions, such as the primary visual areas (Cai et al., 2009) (Fig. 3C). A similar widespread distribution

has been described in non-human primates, where these cells are also more common in associative rather than in primary cortical regions (Kornack et al., 2005; Cai et al., 2009; Zhang et al., 2009). The presence of a band of PSA-NCAM expressing cells similar to that found in layer II in rodents can be observed at least in the entorhinal cortex of human infants (Ni Dhuill et al., 1999) and a recent report has described the presence of DCX expressing cells in the upper border of cortical layer II of humans of different ages (Cai et al., 2009).

A discrete population of small monopolar and bipolar PSA-NCAM/DCX expressing cells, with short processes oriented vertically, can also be observed in the piriform cortex layer III, the endopiriform nucleus and deep layers of the entorhinal cortex

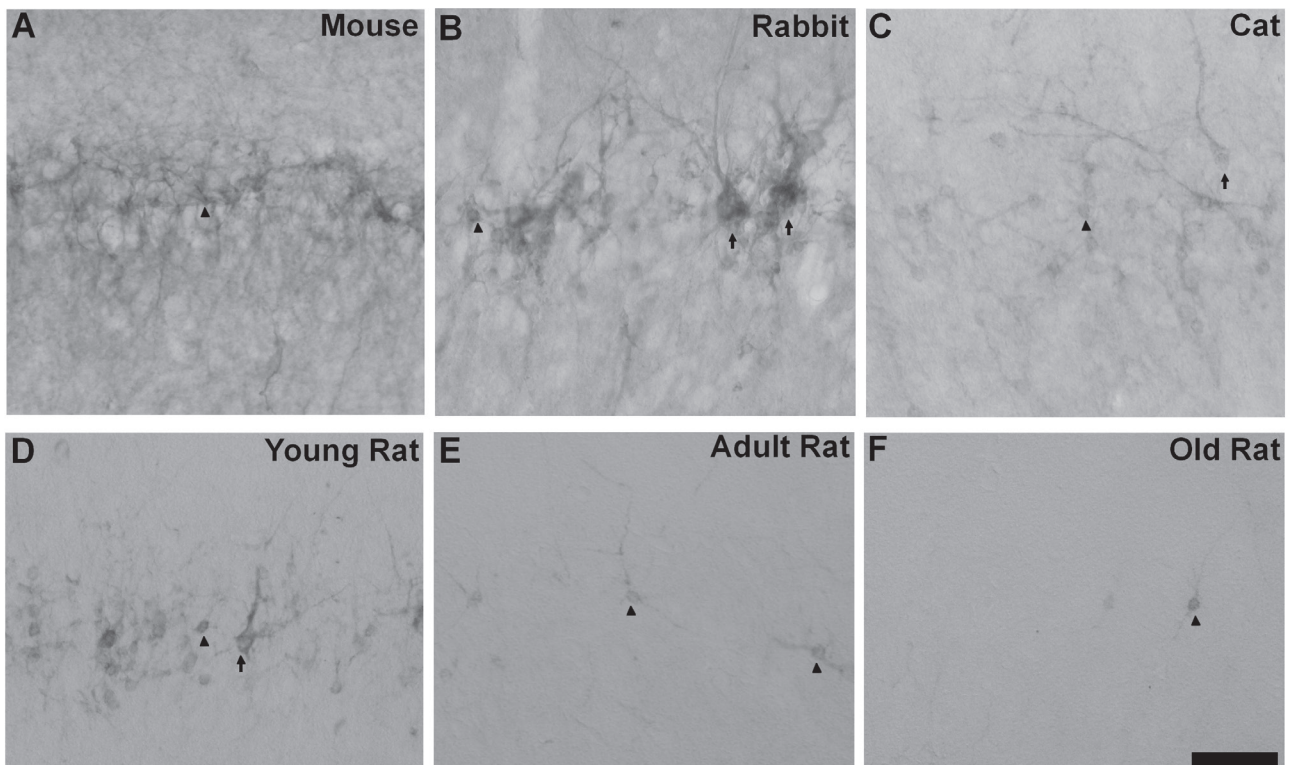


Fig. 3. - PSA-NCAM expressing cells in the paleocortex layer II of different young-adult mammals: mouse (A), rabbit (B) cat (C) and rat (D). Figures D-F show changes in the number of PSA-NCAM expressing cells in piriform cortex layer II during aging in rats. (D) PSA-NCAM expressing cells in young-adult rat (3 months old). (E) PSA-NCAM expressing cells in adult rat (1 year old). (F) PSA-NCAM expressing cells in old rat (2 years old). Arrows indicate semilunar-pyramidal transitional neurons and arrowheads indicate tangled cells. Scale bar: 100 μ m. Images of rat paleocortex in this figure have been taken from the material generated in Varea et al. (2009) study.

of adult rodents. Some of them can also be found forming aggregates at the ventral end of the external capsule of the corpus callosum. Frequently, these small cells in deep paleocortical layers are located adjacent to long PSA-NCAM immunoreactive vertical processes (Nacher et al., 2001; Nacher et al., 2002a; Nacher et al., 2010).

Structural and ultrastructural features of immature neurons

Immature neurons in the adult CNS can also be identified by their morphology under the light and the electron microscope. Recently generated neurons have less complex dendritic trees and reduced spine density when compared with mature neurons of the same type. Additionally, it is frequent that these immature neurons show transiently abnormal morphological features, such as the basal dendritic arborizations or aberrant dendritic trajectories found in many immature hippocampal granule neurons (Nacher et al., 2001; Ribak et al., 2004).

Two main cell populations co-expressing DCX and PSA-NCAM can be distinguished on basis of their size and morphology in the cerebral cortex layer II (Fig. 3). This classification has been used in rats, guinea pigs and rabbits; similar cells have been described in cats and non-human primates, although in these species a more heterogeneous morphology appears to exist (Cai et al., 2009; Zhang et al., 2009). In rats, the majority of PSA-NCAM/DCX expressing cells are small (around 9 μ m soma diameter) and show processes with highly irregular trajectories, usually restricted to layer II, although some of these processes with vertical trajectories can also be found in layer I. These immature neurons have been recently classified as tangled (Gomez-Climent et al., 2008) or type I cells (Luzzati et al., 2009). The population of larger cells (around 15 μ m soma diameter) usually displays one or two long dendrites expanding into layer I, which show occasional bead-like swellings, protrusions resembling thin dendritic spines or even typical spines (Gomez-Climent et al.,

2008). These cells also show thin basal processes (Nacher et al., 2001; Gomez-Climent et al., 2008), which resemble the transient basal dendrites of newly generated granule neurons in the adult hippocampus (Nacher et al., 2001; Ribak et al., 2004). Most of these DCX/PSA-NCAM expressing cells correspond to those described in the opossum as pyramidal-semilunar transitional neurons (Haberly, 1983). However, some cells with the morphology of semilunar, pyramidal or fusiform neurons can also be found (Gomez-Climent et al., 2008; Nacher et al., 2002b); Luzzati et al. (2009) have chosen to denominate these larger cells type II. In any case, cells with characteristics of both tangled cells and semilunar-pyramidal transitional neurons can be found in the cerebral cortex layer II, which may indicate a transition between these two cell types.

Under the transmission electron microscope, early immature neurons, such as those found in the adult SGZ (Seri et al., 2004), are usually characterized by a reduced cytoplasm, restricted to a small perinuclear rim, a complete absence of axosomatic or axodendritic synapses and abundant heterochromatin clumps in their nuclei. Tangled and semilunar-pyramidal transitional cells in the rat paleocortex layer II have these immature ultrastructural features and are also characterized by the presence of astrocyte lamellae and swellings of the extracellular space in close apposition to their plasma membrane (Shapiro et al., 2007a; Gomez-Climent et al., 2008). Interestingly, asymmetric synapses can be observed, very occasionally, in layer I, contacting some apical dendrites of semilunar-pyramidal transitional neurons (Gomez-Climent et al., 2008).

Immature neurons lack expression of mature neuronal markers

We can obtain further evidence of the immature neuronal phenotype of a given cell by analyzing the (lack of) expression of certain proteins exclusively expressed by mature neurons. Probably the most frequently used of these mature neuronal markers is NeuN, a nuclear protein of unknown function, which is exclusively expressed by mature neurons (Mullen et al., 1992). However, it has to be noted that some mature neuronal populations in the adult CNS, such as Purkinje cells, lack the expression of this nuclear protein. NeuN is absent from progenitor cells and early immature neurons in the typical adult

neurogenic regions and starts to be expressed faintly during the last stages of neuronal differentiation (Kempermann, 2005). In the cerebral cortex layer II of rodents, cats, guinea pigs and primates, NeuN expression is absent from the nuclei of most tangled cells, although it is faintly expressed in many of the larger cells (Fig. 4A) (Nacher et al., 2001; Nacher et al., 2002a; Cai et al., 2009; Gomez-Climent et al., 2008; Xiong et al., 2008; Zhang et al., 2009). A similar pattern of expression has been found for MAP-2, a microtubule associated protein intensely expressed in mature neurons (Gomez-Climent et al., 2008). Other mature neuronal markers commonly employed are Hu (Barami et al., 1995) or the neuronal specific enolase (Cameron et al., 1993), although, to our knowledge, they have not been used on the population of immature neurons in cortical layer II. The immature characteristics of these cells are also evident when the expression of cell activity markers, such as c-fos or Arc is analyzed (Fig. 4B) (Gomez-Climent et al., 2008).

Oligodendroglial, astroglial and microglial markers are also commonly used in order to exclude the possibility that the cells under study were glial cells. These glial markers are absent from the immature neurons in the cerebral cortex layer II, in rats, guinea pigs and cats (Cai et al., 2009; Gomez-Climent et al., 2008; Xiong et al., 2008).

Origin of immature neurons in the cerebral cortex layer II

The expression of molecules related to neuronal development in a population of cells of the adult rat paleocortex layer II obviously lead different research groups to explore whether these cells were recently generated. Although an initial study did not find evidence of neuronal incorporation in adult rats (Nacher et al., 2002a), subsequent 5'BrU labeling studies in the piriform cortex of rats (Shapiro et al., 2007a), mice (Pekcec et al., 2006; Shapiro et al., 2007b) and *Old/New world* primates (Bernier et al., 2002) found that at least a discrete number of neurons in this region had been generated during adulthood. However, we have recently demonstrated that the majority of immature neurons in the layer II of the piriform and entorhinal cortices of adult rats have been generated during embryonic develop-

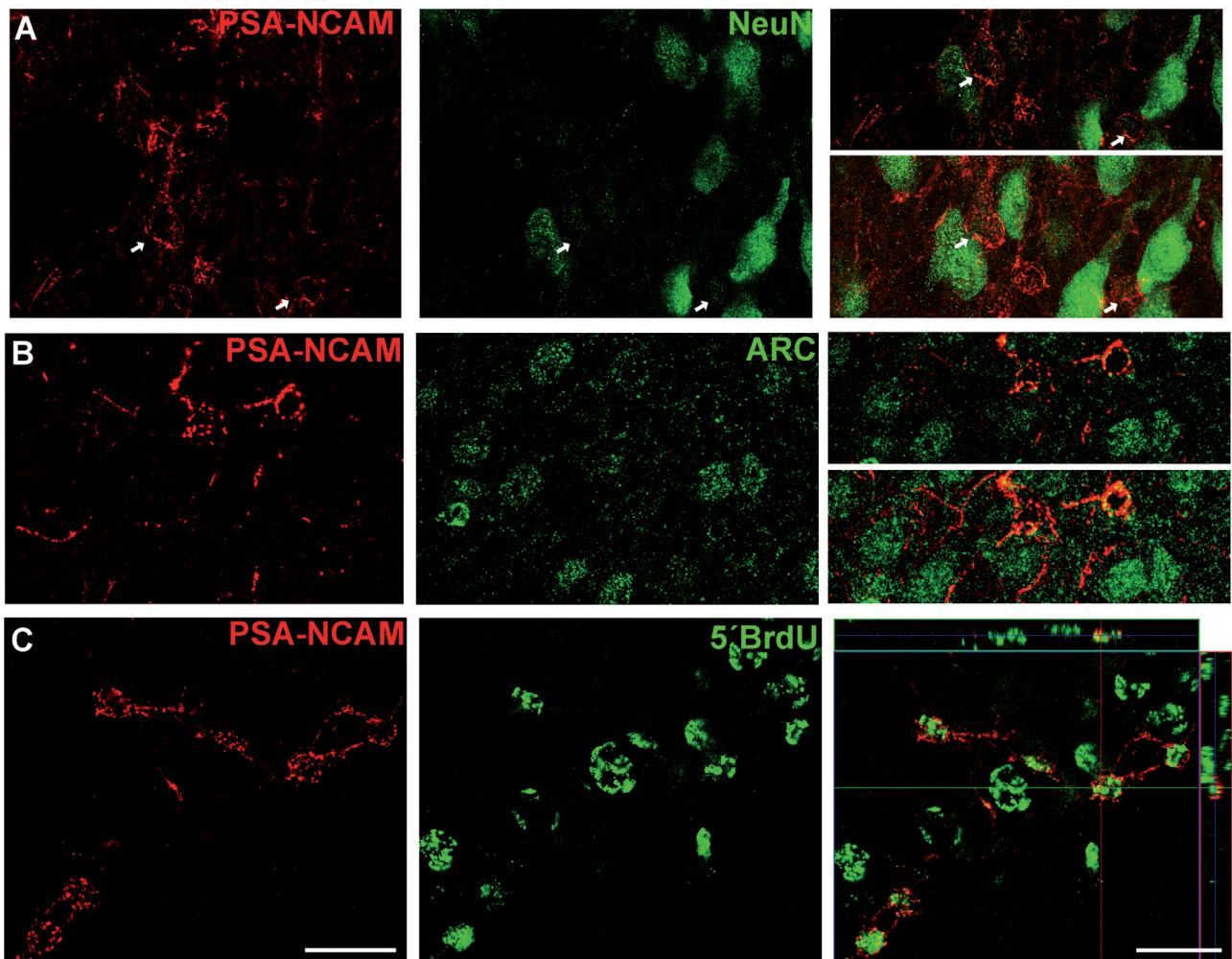


Fig. 4. - Confocal microscopic analysis of PSA-NCAM immunoreactive cells in the rat paleocortex layer II. (A) Most PSA-NCAM immunoreactive cells lack NeuN expression in their nuclei. However, some of them, specially large cells, display faint NeuN expression (arrow). (B) PSA-NCAM expressing cells lacking Arc expression in their nuclei. (C) PSA-NCAM expressing cells containing 5'BrdU immunoreactive nuclei in the piriform cortex of an adult rat injected with 5'BrdU at E15.5. The photograph on the right is an orthogonal projection of the PSA-NCAM/5'BrdU double-labeled cell located in the center. Scale bar: 25 μ m. Insets are 2D projections of 7(A) or 5(B) consecutive focal plane located 1 μ m apart. NeuN: neuronal nuclear antigen; 5'BrdU: 5'bromodeoxyuridine. Images in this figure have been taken from the material generated in Gomez-Climent et al. (2008) study.

ment, mainly in E15.5 (Fig. 4C) (Gomez-Climent et al., 2008). Moreover, in contrast with the studies describing the presence of neurons generated during adulthood in the piriform cortex of adult mammals, some other reports have failed to find evidence that DCX, TUC4 or PSA-NCAM expressing neurons in the paleocortex layer II of adult rats and mice (Nacher et al., 2000; Nacher et al., 2002a; Fontana et al., 2005; Gomez-Climent et al., 2008; Luzzati et al., 2009), rabbits (Luzzati et al., 2003; Luzzati et al., 2009), guinea pigs (Luzzati et al., 2009) or primates (Kornack et al., 2005) were recently generated. One possible explanation for this discrepancy may be

that the number of newly generated neurons in the adult cerebral cortex layer II is very low. In fact, this is particularly evident when comparing the numbers of DCX or PSA-NCAM cells present in the piriform cortex of rodents (Nacher et al., 2002a) with those of recently generated 5'BrdU labeled cells (Pekcec et al., 2006; Shapiro et al., 2007a; Shapiro et al., 2007b). Another, non-excluding, possibility is that most of the neurons generated during adulthood may become incorporated to layer III, where a certain number of cells expressing immature neuronal markers can also be found (Nacher et al., 2001; Nacher et al., 2002a), and not to layer II.

Fate of immature neurons in the cerebral cortex layer II

The mysterious disappearance of immature neurons in the cortical layer II

Probably one of the most intriguing features of the population of immature neurons in the cerebral cortex layer II is its almost complete disappearance during aging. Different studies have demonstrated that the number of PSA-NCAM expressing cells in the rat paleocortex layer II dramatically declines during aging (Figs. 3D-F) (Abrous et al., 1997; Murphy et al., 2001; Varea et al., 2009) and this reduction is also observed when analyzing DCX or TUC4 expression (Nacher et al., unpublished observations). Similar results have been observed in the cerebral cortex of guinea pigs (Xiong et al., 2008), cats (Cai et al., 2009) and primates (Zhang et al., 2009). This decrease is already found in 6 month old rats, persists in 1 year and 2 year old animals (Varea et al., 2009) (Figs. 3D-F), and parallels that observed in the number of recently generated granule neurons in the hippocampus (Seki and Arai, 1995). The fate of these immature neurons in adult/middle aged and aged brains is not known yet. One possibility is that these cells may have only a transient existence and progressively die during the progression from youth to adulthood. However, Xiong et al. (2008) did not find evidence of elevated TUNEL activity in the cortical layer II of guinea pigs and we have failed to find substantial numbers of pyknotic nuclei in rats, cats or mice (Nacher et al., unpublished observations). The other, more likely, possibility is that these cells may be progressively differentiating into mature neurons, which would lack these immature markers and thus would be no longer detectable. It may be hypothesized that tangled cells may mature eventually into some of the larger immature neurons (semilunar-pyramidal transitional neurons etc.) and then into mature neurons. This is supported by the fact that intermediate cell types between tangled and larger cells are habitually found in layer II and that the later appear more mature than the former. Then, immature neurons in cortical layer II may constitute a “reservoir”, which in different circumstances may complete its differentiation program. It is important to consider here that the number of these immature neurons is particularly high: For instance, in young adult rats a stereological estimation of the total num-

ber of PSA-NCAM expressing neurons in layer II revealed more than 55000 cells, only considering the piriform cortex and not the entorhinal cortex and the rest of cortical regions, where many of these cells can also be found (Nacher et al., 2002a). The numbers of these immature neurons in young mammals with larger cerebral cortices, including humans, must be impressive because of their similar density and their much wider distribution.

Fate and phenotype.

Interneurons vs. principal cells

If we assume that most of these immature neurons are differentiating with age, what do they become, interneurons or principal cells? Unfortunately, to date this is still an open question. While the studies by Luzzati et al. (2009) and our own (Gomez-Climent et al., 2008) support the hypothesis of a differentiation principally towards excitatory neurons, other studies suggest a major interneuronal fate for immature neurons in cortical layer II (Cai et al., 2009; Xiong et al., 2008; Zhang et al., 2009).

First of all, it is important to note that large DCX or PSA-NCAM expressing cells in the cerebral cortex layer II do not show morphological characteristic of interneurons: most of these cells have been classified as semilunar-pyramidal transitional, semilunar or pyramidal neurons, which are common excitatory neural cell types in the layer II of the cerebral cortex. Moreover, the morphology of inhibitory neurons in this region does not clearly coincide with that of DCX/PSA-NCAM expressing cells (Suzuki and Bekkers, 2007).

The fate of immature neurons in the cortical layer II has been also studied with the analysis of the expression of different transcription factors, which can discriminate the site of origin of different cortical cell populations because they continue to be expressed in adult animals; this site of origin is indicative of the final phenotype that these neurons will display. This study strongly indicates that the majority of immature neurons in the cortical layer II of mice, rats, rabbits and guinea pigs do not come from the subpallium (a region where most cortical interneurons originate), because almost none of them expresses Lhx6 or pan distalless (DLL) (Luzzati et al., 2009). Moreover, most of these immature neurons express a transcription factor (Tbr1) specific for pallium-derived principal neurons (Luzzati et

al., 2009). However, it has to be noted that, although very rarely, some elements displaying faint DCX and DLL expression have been found in the cortex layer II of different mammals (Luzzati et al., 2009). We can also obtain clues about the final phenotype of an immature neuron by studying the expression of different molecules typical of either interneurons or principal cells. Our laboratory found, using confocal microscopy, that markers of mature interneurons, such as GABA, GAD67, calbindin, parvalbumin, calretinin, somatostatin, neuropeptide Y, cholecystokinin or VIP (Fig. 5) were never expressed by DCX/PSA-NCAM expressing cells in the paleocortex layer II of adult rats (Gomez-Climent et al., 2008). These results on parvalbumin, calbindin and somatostatin expression have been replicated using the same technique by Luzzati et al. (2009), not only in rats, but also in mice, guinea pigs and rabbits. However, they acknowledged the scarce presence of some elements faintly labeled for both GABA and DCX. Despite this absent or reduced co-localization of PSA-NCAM/DCX and interneuronal markers, we also failed to find colocalization with Ca(2+)/CaM-dependent protein kinase II (CAMKII), a marker of excitatory neurons (Fig. 5A) (Gomez-Climent et al., 2008).

In contrast with these results, different reports from Dr. Yan's laboratory, based on the analysis of DCX expressing neurons, have provided data suggesting a major interneuronal fate for immature neurons in the cortical layer II of adult mammals. These colocalization studies have found that, in guinea pigs, cats and non human primates, GABA expression was absent from DCX expressing tangled cells, but that the larger cells (especially those expressing low levels of DCX) were faintly labeled with anti-GABA or anti GAD antibodies (Cai et al., 2009; Xiong et al., 2008; Zhang et al., 2009). In guinea pigs, these DCX-low expressing cells lacked expression of calcium binding proteins, but they were labeled with nitrinergic interneuron markers (Xiong et al., 2008). In adult cats faint DCX immunoreactive large cells co-expressed parvalbumin, calbindin, somatostatin and nitrinergic markers, but not calretinin (Cai et al., 2009). Of note, these authors found many of these DCX-low expressing cells with a putative interneuronal phenotype in deep cortical layers. In contrast with these results, we have never found similar large DCX expressing cells in these layers, neither in rats (Nacher et al., 2001; Gomez-Climent et al., 2008) nor in mice (Nacher

et al., 2010), cats or primates (Nacher et al., unpublished). Although Cai et al. (2009) erroneously indicated that we had found DCX/somatostatin expressing cells in deep cortical layers, what we described was a population of cells expressing exclusively PSA-NCAM, but never DCX, which have been classified as mature interneurons (Varea et al., 2005; Varea et al., 2007). Cai et al (2009) also studied the expression of markers of excitatory neurons using neurogranin immunohistochemistry in cats, finding also negative results (Cai et al., 2009).

In summary, further experimental work is needed to clarify the fate of immature neurons in cortical layer II. It is possible that the mature neuronal markers used until now only start to be expressed once the neurons have already lost the expression of the immature markers. Consequently, new excitatory/inhibitory markers, expressed earlier in neuronal development, should be assayed, or new experimental approaches should be discovered to follow the progress of these immature neurons in real time. Alternatively, the use of stereological estimations of the total number of mature neurons, principal neurons and interneurons during aging or after different experimental manipulations may also shed light on this subject. Even when this incognita is solved, there will be more questions still open, such as what molecules, and in which circumstances, regulate the differentiation of these immature neurons and their incorporation to the circuitry. In fact, we already know that different intrinsic or extrinsic factors affect these cells: Different types of learning paradigms (O'Connell et al., 1997; Fox et al., 2000), chronic stress and chronic corticosterone treatment (Nacher et al., 2004) transient cerebral ischemia (Hayashi et al., 2001) and different pharmacological treatments, including NMDA receptor antagonists (Nacher et al., 2002a), valproic acid (Murphy et al., 2001) or antidepressant treatment (Sairanen et al., 2007). Given the abundant presence of immature neurons in regions that receive a major input from the olfactory bulb, an extremely plastic region of the CNS, it is tempting to speculate that the putative maturation of these cells may be a cellular mechanism to adapt to the plasticity of olfactory bulb connections. However, it has to be noted that in cats and primates these cells are widely dispersed in the cerebral cortex, in regions where no direct olfactory input is found.

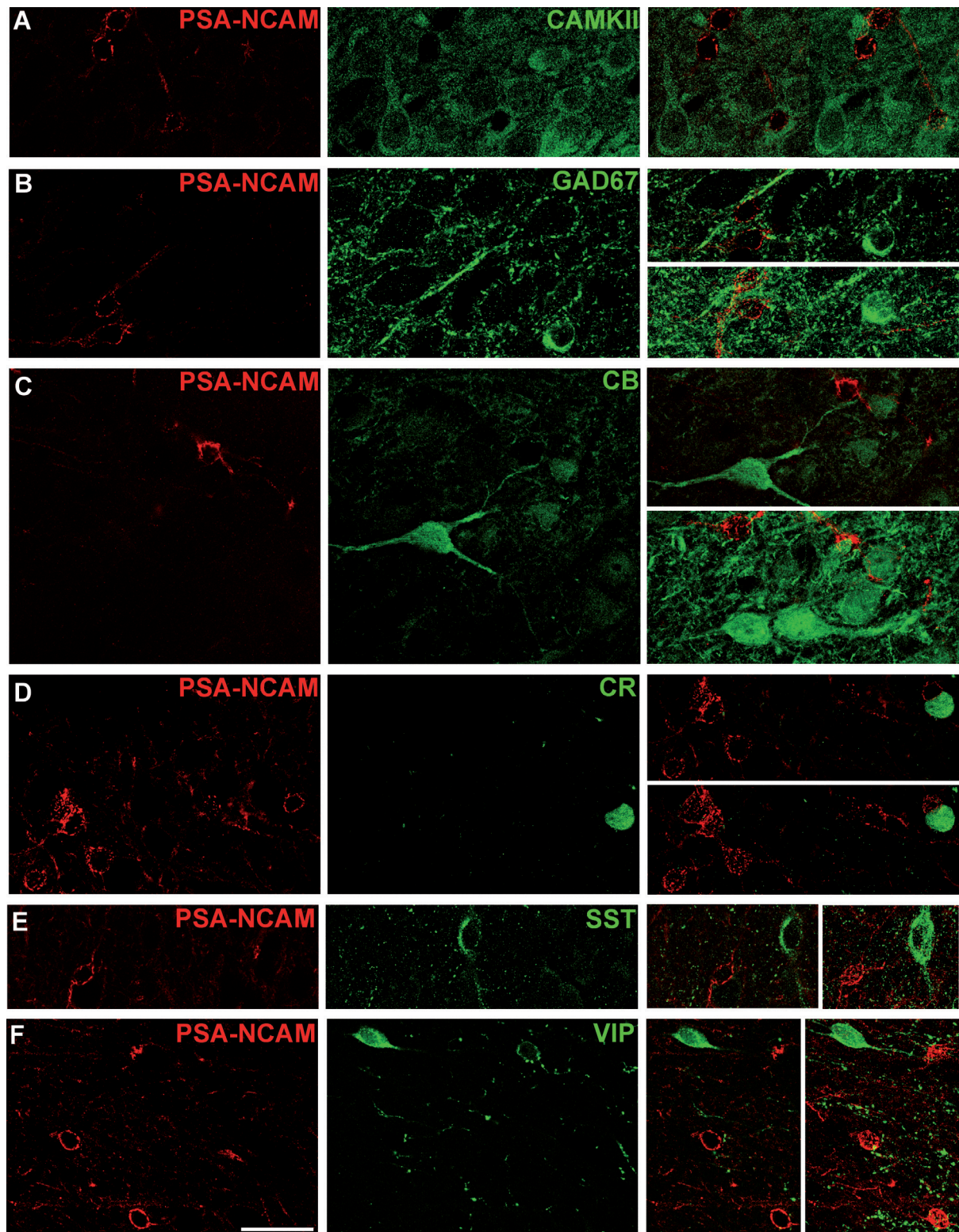


Fig. 5. - Confocal microscopic analysis of PSA-NCAM immunoreactive cells in the paleocortex layer II. PSA-NCAM expressing cells lacking CAMKII (A) GAD67 (B), CB (C), CR (D), SST (E), or VIP (F) expression. Scale bar: 25 μ m. Insets are 2D projection of 3(A), 5(B) 4(C) or 3(D), 5(E), or 7(F) consecutive focal plane located 1 μ m apart. CAMKII: Ca²⁺/calmodulin dependent protein kinase II; GAD67: Glutamic acid decarboxylase-67; CB: Calbindin; CR: Calretinin; SST: Somatostatin; CCK: Cholecystokinin; VIP: Vaso-intestinal peptide. Images in this figure have been taken from the material generated in Gomez-Climent et al. (2008) study.

"Standby mode" neurons in other cerebral regions

Immature neurons not recently generated, similar to those found in the cerebral cortex layer II, have also been found in the sensory ganglia of adult animals. DCX and PSA-NCAM are expressed by cells in the adult trigeminal ganglion (TG) (Lagares et al., 2007; Quartu et al., 2008) and DCX, but not PSA-NCAM, expressing cells can also be found in adult dorsal root ganglia (DRG) (Dellarole and Grilli, 2008). As it occurs in the cerebral cortex layer II, many of these cells fail to express mature neuronal markers (Lagares et al., 2007; Dellarole and Grilli, 2008), confirming their immature phenotype. These immature cells appear to become progressively incorporated as mature ganglion neurons, since recent stereological studies have confirmed an increase in the number of neurons in the TG and the DRG of adult rats (Cecchini et al., 1993; Farel, 2002; Lagares et al., 2007). Studies using tritiated thymidine and 5'BrdU have discarded the possibility that these cells were generated during adulthood (Ciaroni et al., 2000; Geuna et al., 2000; Lagares et al., 2007). The suprachiasmatic nucleus of the hypothalamus is another region of the adult nervous system that maintains neurons with an immature phenotype. These cells express DCX (Geoghegan and Carter, 2008) and PSA-NCAM (Glass et al., 1994) and usually display low levels of NeuN expression (Geoghegan and Carter, 2008). To our knowledge there are neither evidences of neuronal incorporation during adulthood, nor of an embryonic or perinatal origin of the immature cells in this hypothalamic region. Cells coexpressing PSA-NCAM and DCX have also been found contacting the central canal of the spinal cord in neonatal rats (Marichal et al., 2009). As immature neurons in cerebral cortex layer II, these spinal cord cells have been generated during embryonic development and retain an immature phenotype during postnatal life. They lack mature neuronal markers and show ultrastructural features of immature neurons, which have been further confirmed by electrophysiological studies (Marichal et al., 2009).

Summary

Marichal et al., (2009) denominated the immature neurons in the perinatal spinal cord "standby mode"

neurons. This term reflects very well the nature of these cells, as well as that of the other neurons that retain an immature phenotype for an unusually prolonged time. This review has focused mainly in the immature neurons in the cerebral cortex layer II, but cell populations with these characteristics can be found in other areas of the central and peripheral nervous systems, such as the suprachiasmatic nucleus and the trigeminal and dorsal root ganglia. These cells are generated during embryonic development and remain immature until adulthood, when they progressively cease to express immature markers. However, we are still far from understanding what may be their fate when they exit the "standby mode" or whether they may play an important role after this exit: An exciting research line is open for us to enjoy it in the near future.

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