Published in final edited form as:

Brain Res Rev. 2007 October ; 55(2): 390–394.

Dendritic development of newly generated neurons in the adult brain

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Abstract

Ramon y Cajal described the fundamental morphology of the dendritic and axonal growth cones of neurons during development. However, technical limitations at the time prevented him from describing such growth cones from newborn neurons in the adult brain. The phenomenon of adult neurogenesis is briefly reviewed, and the structural description of dendritic and axonal outgrowth for these newly generated neurons in the adult brain is discussed. Axonal outgrowth into the hilus and CA3 region of the hippocampus occurs later than the outgrowth of dendrites into the molecular layer, and the ultrastructural analysis of axonal outgrowth has yet to be completed. In contrast, growth cones on dendrites from newborn neurons in the adult dentate gyrus have been described and this observation suggests that dendrites in adult brains grow in a similar way to those found in immature brains. However, dendrites in adult brains have to navigate through a denser neuropil and a more complex cell layer. Therefore, some aspects of dendritic outgrowth of neurons born in the adult dentate gyrus are different as compared to that found in development. These differences include the radial process of radial glial cells acting as a lattice to guide apical dendritic growth through the granule cell layer and a much thinner dendrite to grow through the neuropil of the molecular layer. Therefore, similarities and differences exist for dendritic outgrowth from newborn neurons in the developing and adult brain.

Introduction

It is now a well-established phenomenon that neurogenesis occurs not only during development of the mammalian central nervous system but also in the adult (Altman and Bayer, 1965, 1990; Altman and Das, 1965; Bayer et al., 1982; Cameron and McKay, 2001; Eriksson et al., 1998; Kaplan and Hinds, 1977; Kempermann et al., 2003; Kornack and Rakic, 1999; Palmer et al., 2000; Van Praag et al., 2002). Ramon y Cajal was the first to study process outgrowth from newly generated neurons in the fetal brain and spinal cord (Ramon y Cajal, 1960). His Golgi preparations revealed details of the growing tips of axons in the spinal cord of chicks, including aspects of the growth cone with its thin appendages (filopodia) and wider ones (lamellipodia). In fact, his description of process outgrowth has been confirmed in many other neural systems and brain regions. This description as well as his other work led him to postulate the Neuron Theory and argue against the Reticular Theory. Thus, Cajal described the neuron, a cell like no other in the body, as consisting of multiple processes including dendrites (short processes) and axons (long processes for some neurons). Through his developmental studies on neuronal process outgrowth, Cajal has provided an important basis for understanding the pattern of process outgrowth for newborn neurons in the adult brain.

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In this review, it will be shown that process outgrowth for newborn neurons in the adult brain is similar to that in the developing brain. However, because of the complex architecture of the adult brain, some aspects of neuronal development are different. For example, the adult brain has a more elaborate, mature and denser neuropil with fully-grown dendrites and axons. Furthermore, the cell layers of some cortical structures appear to have somata closely apposed with very little room for a young neuron to squeeze its process through. Thus, a newborn neuron in the adult brain faces unique challenges for it to successfully migrate to reach its adult destination and to establish its dendritic connections and arborizations. Data from several studies provide a structural basis for understanding the development of dendritic processes for newly generated neurons in the adult brain. These studies will be highlighted in the following sections.

A comparison between developmental and adult dendritic outgrowth in the rat dentate gyrus

During rat development, cells from a secondary dentate matrix (a derivative of the primary dentate neuroepithelium) stream in a subpial position towards the granule cell layer of the dorsal blade (Altman and Bayer, 1990). A thicker inner shell of the granule cell layer, formed during infancy and the juvenile period derives from an intrinsic tertiary germinal matrix. Schlessinger et al. (1975) showed that the dorsal blade of the granule cell layer forms before the ventral blade. Also, they showed that in all regions of the dentate gyrus, the more superficial neurons in the granule cell layer are formed earlier than the deeper granule cells. In the early postnatal dentate gyrus, Jones et al. (2003) have described a multipolar morphology for the developing dendrites of granule cells. Thus, they showed that from the superficial granule cells, several dendrites, including apical and basal dendrites, grow into the surrounding neuropil, and that these dendrites have the classical appearance of growing dendrites, including lamellipodia and filopodia as described previously (Seress and Pokorny, 1981; Lubbers and Frotscher, 1988). It was also shown that the basal dendrites regress because they are a transient feature of granule cells during their development and adult rodent granule cells never display basal dendrites (Jones et al., 2003). Consistent with previous developmental studies, dendritic arbors become pruned to form only a few apical dendrites that branch in regular ways in the molecular layer (Claiborne et al., 1990; Desmond and Levy, 1984; Green and Juraska, 1985; Rihn and Claiborne, 1990).

The pattern of process outgrowth for granule cells born in the adult brain is different from that found in the developing brain (Shapiro et al., 2005; Zhao et al., 2006). In the adult brain, neurons are generated in the subgranular zone from progenitor cells, which include radial glial cells and proliferative neuronal cells (Seri et al., 2001, 2004; Kempermann et al., 2003). This niche for adult neurogenesis is situated close to blood vessels (Palmer et al., 2000; Shapiro et al., 2005; Shapiro and Ribak, 2006). Consistent with these data, newborn neurons that are double-labeled for BrdU and doublecortin (DCX) can be found in the subgranular zone as early as 4 hours after a single BrdU injection, and such cells display only a rudimentary process (Shapiro et al., 2006).

In order for the newborn neurons to integrate and survive, its rudimentary process must find its way through the granule cell layer to arborize in the molecular layer where it is targeted for synaptogenesis. Moreover, the newborn neuron must migrate from the subgranular zone to the granule cell layer. Once such cells reach the granule cell layer, they exhibit unipolar or bipolar morphology in that some display a basal dendrite and/or an apical dendrite (Ribak et al., 2004; Rao and Shetty, 2004). As will be detailed below, the apical dendrites of newborn neurons at this stage of development grow along a radial glial process. Thus, it is possible that the newborn neuron migrates into the granule cell layer by locomotive migration along this radial glial process. An interesting contrast to the migration of these cells is a population of CCK-

positive interneurons that migrate in the opposite direction in the early postnatal rat brain without the physical guidance of radial glial cells (Morozov et al., 2006).

Initial observations using DCX-immunolabeling showed that some of the thicker processes from DCX-labeled cell bodies were clearly dendrites while the thinner processes had features of growth cones with filopodia and lamellipodia (Ribak et al., 2004). Some of the DCX-labeled cells had bifurcating and trifurcating filopodia spreading through the granule cell layer (Fig. 1), and some of these filopodia may become an apical dendrite. Therefore, the newborn neurons of the adult brain have a different developmental profile compared to the multipolar morphology of those described during early postnatal development (cf. Fig. 2C of Jones et al., 2003).

The exact rate of growth of the apical dendrite through the granule cell layer was recently described (Shapiro et al., 2006). Following a single BrdU injection, the dendrites from BrdU/DCX double-labeled cells showed dendritic growth cones with lamellipodia and filopodia 24–48 hr after BrdU injections. These growth cones were previously described for DCX-positive cells in the dentate gyrus (Ribak et al., 2004). At later timepoints after BrdU (72–96h), one or two apical dendrites were apparent traversing the granule cell layer and then branching in the molecular layer (Fig. 2). Thus, dendritic growth is scaled down from development (Jones et al., 2003; Seress and Pokorny, 1981) where pruning of a robust dendritic tree occurs because process outgrowth is more or less limited to one direction in the adult.

How does the DCX-labeled apical dendrite navigate through the granule cell layer?

Light and electron microscopy of DCX-labeled cells have provided evidence to support working hypotheses that address this issue. Shapiro et al. (2005) showed that DCX-labeled cell bodies in the subgranular zone are mostly enveloped by the non-radial processes of radial glial cells. This and a subsequent study (Shapiro et al., 2006) showed that soon after newborn neurons are generated a process is observed to emanate from the pole of their cell body that is opposite the pole apposed to the cell body of the radial glial cell (Fig. 3). DCX-labeled cells that are found at the base of the granule cell layer show a thin DCX-labeled process apposed to the radial process of a radial glial cell (Shapiro et al., 2005). These growing dendrites have many mitochondria in their tips, a characteristic previously described for growth cones (Sotelo and Palay, 1968). It is interesting to note that light microscopic preparations double-labeled for DCX and GFAP also showed that DCX-labeled dendrites are apposed to the radial processes of radial glial cells in the granule cell layer (Shapiro et al., 2005). Seki and Arai (1999) using another marker for immature neurons, PSA-NCAM, showed the same close relationship between the growing apical dendrite of newborn granule cells and the radial processes of radial glial cells in the granule cell layer. These observations are consistent with the hypothesis that the apical dendrite of newborn granule cells grows along radial glial processes that guide it to reach the molecular layer.

DCX-labeled dendrites were also examined in the granule cell and molecular layers to determine their diameter (Shapiro et al., 2005, 2006). These dendrites were much thinner than the unlabeled mature dendrites (0.25 μm in diameter vs. 1 μm for the mature dendrites) and were aspiny (Shapiro et al., 2006). It is hypothesized that this thinner dendrite is necessary to allow it to navigate through the dense neuropil of the molecular layer after losing its association with the radial glial process that it followed through the granule cell layer.

The presence of synapses was also described for DCX-labeled apical dendrites from newborn granule cells. In the molecular layer, the DCX-labeled apical dendrites had synapses formed by small axon terminals (Shapiro et al., 2006). These developing axo-dendritic shaft synapses

featured only a few synaptic vesicles in the axon terminals indicating their relative immaturity (Shapiro et al., 2006). However, there were no synapses on DCX-labeled apical dendrites in the granule cell layer (Shapiro et al., 2005). The lack of synapses on the segment of apical dendrite in the granule cell layer is probably due to the radial processes of radial glial cells apposing the surfaces of DCX-labeled apical dendrites in this layer, thereby preventing an axon terminal from targeting it. It remains to be shown whether the developing synapses on DCX-labeled dendrites are transient or if they persist as axospinous synapses as these newborn cells differentiate into mature granule cells with spiny dendrites.

Basal dendrites: a transient feature of newborn neurons in the dentate gyrus

Previous studies have shown that basal dendrites are a transient feature of newborn neurons during development (Seress and Pokorny, 1981; Ribak and Seress, 1990). Recent studies of DCX-labeled cells in the adult dentate gyrus also indicate that basal dendrites are a transient feature of newborn granule cells (Ribak et al., 2004; Rao and Shetty, 2004). Recently, Shapiro and Ribak (2006) examined basal dendrites for synapses. They hypothesized that no synapses would be present on these basal dendrites because they do not persist in the normal adult brain. The fact that no synapses were found on basal dendrites from control rats but were found on persistent basal dendrites from epileptic rats supported this hypothesis (Shapiro and Ribak, 2006). Thus, the basal dendrite in the normal rat may have another function, such as migration or for sensing molecular cues.

What is known about axon outgrowth from newborn granule cells?

Two early studies showed that axons of newborn dentate granule cells grow into CA3 (Hastings and Gould, 1999; Markakis and Gage, 1999). In both studies, retrograde markers were combined with BrdU labeling to demonstrate that axons from newborn neurons were projecting to CA3. In addition, Hastings and Gould (1999) analyzed the temporal outgrowth of axons from newborn granule cells and described their presence in CA3 of adult rats by 10 days after being born. More recently, Zhao et al. (2006) using retrovirus to label the newborn granule cells in mice have analyzed the daily progression of axon growth into stratum lucidum of CA3. They showed that axons were not found in CA3a by 10 days after injection although thin fibers were seen in the hilus at this timepoint. Between 11 and 16 days after injection, the labeled axonal fibers projected farther and farther into the CA3 region (Zhao et al., 2006). Note that the projection of the axon into CA3 occurs prior to the appearance of dendritic spines that occurs 16 days after birth. These details provide important information about axonal outgrowth from newborn neurons in rats and mice.

Some important issues of axonal outgrowth still remain to be addressed. First, the axons of granule cells form large mossy fiber synapses with the shafts and complex spines of CA3 pyramidal cells, but no studies have yet determined when mossy fibers from newborn granule cells form these synapses. Second, the axons of newborn granule cells must traverse the neuropil of the hilus to reach stratum lucidum. It would be interesting to know if there is a lattice or tract that guides the outgrowing axon. Because the axons of granule cells form bundles and are unmyelinated, their membranes come into direct contact with each other in the hilus and stratum lucidum of CA3 (Laatsch and Cowan, 1966). It is hypothesized that these axons may express specific recognition proteins that help them to appose each other. Based on these data, it is hypothesized that the growing axons of newborn granule cells use existing granule cell axons to grow along. If this were the case, then the predicted position of DCX-labeled axons would be apposed to the outside of unlabeled, unmyelinated axon bundles in the hilus and stratum lucidum of CA3. Future studies will be performed to test this hypothesis.

Conclusions

These descriptions of process outgrowth for newborn neurons in the adult brain demonstrate that despite a well-developed neuropil and cytoarchitecture, the manner in which dendrites grow is similar to that in the developing brain. The appearance of growth cones on newborn neurons in the adult brain is consistent with the description of growth cones in the developing brain by Ramon y Cajal. Understanding all of the differences by which these newborn neurons in the adult migrate and integrate into circuitry will be a vital step toward successful implementation of stem cell therapies for neurodegenerative diseases that involve the dentate gyrus and hippocampus.

Acknowledgements

We would like to thank Ms. Lulu Wang for helpful suggestions to improve previous drafts of this manuscript. We also acknowledge the support from NIH grant R01-NS38331 (to C.E.R.) and NIH training grant T32-NS45540 (for L.A.S.).

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Fig 1.

Light micrograph of DCX-immunolabeled cells in the granule cell layer (GL) at the border with the hilus (H). Two labeled cells are shown with their apical dendrites (arrowheads). They display both lamellipodia (asterisks) and filipodia (arrows). Note that the cell on the right has a branching apical dendrite, and one of its branches has trifurcating filipodia (top three arrows). Scale bar = 8 μ m. (Reprinted with permission from Ribak et al., 2004)

Fig 2. Confocal micrograph of BrdU-immunolabeled and DCX/BrdU double-labeled cells at 72 h after a single BrdU injection from the adult dentate gyrus. Three BrdU-labeled cells (asterisks) are shown and two of them are double-labeled for DCX (arrows) while the third cell only has a nearby DCX-labeled process. Note that the two double-labeled cells have apical dendrites (arrowheads) that extend through the granule cell layer and enter the molecular layer (ML). Scale bar = 15 μ m. (Reprinted with permission from Shapiro et al., 2006)

Fig 3.

Electron micrograph of a DCX-labeled newborn neuron (NN) and an astrocyte cell body (GC) at the border (dashed line) between the granule cell layer (GL) and subgranular zone (SGZ). The DCX-labeled NN has immunoreaction product within its thin shell of perikaryal cytoplasm (arrowheads) and its rudimentary apical dendrite (arrows). Note that the dendrite is directed into the GL and arises from the pole of the DCX-labeled cell that is opposite to the pole apposed by the astrocyte cell body. Mature granule cells are found on each side of the DCX-labeled cell. Scale bar = 2 μm. (Reprinted with permission from Shapiro et al., 2005)