



Neurite Development of Adult-Born Granule Cells

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Neural stem/progenitor cells (NSPCs) generate new neurons throughout life in the mammalian hippocampus. Newborn granule cells mature over several weeks to functionally integrate into the pre-existing neural circuitry. Even though an increasing number of genes that regulate neuronal polarization and neurite extension have been identified, the cellular mechanisms underlying the extension of neurites arising from newborn granule cells remain largely unknown. This is mainly because of the current lack of longitudinal observations of neurite growth within the endogenous niche. Here we used a novel slice culture system of the adult mouse hippocampal formation combined with in vivo retroviral labeling of newborn neurons and longitudinal confocal imaging to analyze the mode and velocity of neurite growth extending from immature granule cells. Using this approach we show that dendritic processes show a linear growth pattern with a speed of $2.19 \pm 0.2 \mu\text{m}$ per hour, revealing a much faster growth dynamic than expected by snapshot-based in vivo time series. Thus, we here identified the growth pattern of neurites extending from newborn neurons within their niche and describe a novel technology that will be useful to monitor neuritic growth in physiological and disease states that are associated with altered dendritic morphology, such as rodent models of epilepsy.

Adult hippocampal neurogenesis is crucial in certain forms of hippocampus-dependent learning and memory and has been associated with several neuropsychiatric diseases: among others age-dependent cognitive decline, major depression and epilepsy. In contrast to embryonic or early postnatal development, newborn granule cells in the adult

brain have to integrate into a fully mature and functioning neural circuitry: the hippocampal dentate gyrus (DG). Thus, a number of intrinsic mechanisms and extrinsic cues need to regulate the maturation and final integration of newborn neurons in the DG circuitry. After the initial division of a neural stem/progenitor cell (NSPC) the integration of a newborn granule cell requires several weeks: the extension of dendritic processes starts 2-5 days after cell birth, followed by rapid growth of the main apical dendrite, and the formation of the first glutamatergic, synaptic connections ~ 16 days after the cell is born. Dendrites and their spines continue to mature over the next 2 to 3 weeks; a time that is considered to represent a crucial period for integration and survival of new neurons and that is also characterized by heightened excitability of newborn granule cells. Approximately 6-8 weeks after a cell is born the morphological and electrophysiological maturation process appears to be finished, with newborn granule cells becoming almost indistinguishable from granule cells born during embryonic or early postnatal development.

All animal experiments were done according to the guidelines of, and were approved by, the veterinary office of the Canton of Zürich, Switzerland. For all experiments 6- to 8-week-old female C57Bl/6 mice (Janvier) or 6- to 8-week-old NestinGFP mice (NesGFP) were used.

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