The Timing of Neuronal Development in Adult Hippocampal Neurogenesis

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The granule cell layer (GCL) of the adult dentate gyrus (DG) is a heterogeneous structure formed by neurons of different ages because a significant proportion of neurons continues to be generated throughout life. The subgranular zone of the DG contains neural progenitor cells (NPCs) that divide, differentiate, and migrate to produce functional dentate granule cells (DGCs) that become incorporated into the existing hippocampal circuitry. New available tools to identify adult-born neurons in live and fixed brain sections have allowed the transition from NPC to functional neuron to be characterized in great detail. Maturation of the neuronal phenotype includes changes in membrane excitability and morphology as well as the establishment of appropriate connectivity within the existing circuits, a process that lasts several weeks. The events leading to neuronal maturation share many of the features of the developing brain, and electrical activity is emerging as a key modulator of neuronal development in the adult DG. The underlying mechanisms are now beginning to be understood. NEUROSCIENTIST 12(6):463–468, 2006. DOI: 10.1177/1073858406293538

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Heterogeneous Properties of Dentate Granule Cells

In most areas of the adult brain, neurons are born at specific periods of embryonic development. In contrast, dentate granule cells (DGCs) are generated throughout developmental and adult life. Morphogenesis of the granule cell layer (GCL) begins during late embryonic development as neuroblasts migrating from the secondary dentate matrix align to form the outer shell of the suprapyramidal (upper) blade (Fig. 1; Altman and Bayer 1990). The GCL continues to be formed during early postnatal life, when the infrapyramidal (lower) blade is originated and the tertiary dentate matrix is established in the prospective hilar region. Neural progenitor cells (NPCs) migrate radially from the tertiary dentate matrix to the inner part of the GCL. Subsequently, proliferative NPCs establish at the innermost layer, the subgranular zone, and will continue to generate DGCs with limited radial migration (Kempermann and others 2003; Espósito and others 2005; Zhao and others 2006). Thus, the combination of tangential and radial migratory waves generates a 2-dimensional gradient of neuronal ages: 1) Neurons located in the upper blade of the GCL are older than neurons from the lower blade and 2) neurons of the outer GCL are older than neurons of the inner GCL.

Early studies on the functional properties of DGCs were aimed at revealing whether neuronal populations with different properties were found in the inner GCL, the putative site of adult-born young neurons. To that end, Wojtowicz and colleagues (Wang and others 2000) used whole-cell recordings to compare the plasticity of glutamatergic postsynaptic responses from DGCs located in the inner and outer GCL. Synaptic responses from outer DGCs displayed long-term potentiation (LTP) in response to high-frequency stimulation of the medial perforant path only when GABAergic inhibition was blocked. In contrast, DGCs of the inner GCL displayed LTP of glutamatergic currents even when GABAergic inhibition was left intact. These observations suggested that individual neurons of the inner GCL might have either a lower threshold for LTP induction and/or reduced GABAergic inhibition compared to outer DGCs. In a follow-up study, the overall contribution of inner DGCs to the global LTP was analyzed by measurements of field potentials (Snyder and others 2001). Two types of LTP could be pharmacologically identified: 1) a form of LTP independent of GABAergic transmission and sensitive to ifenprodil, a specific antagonist of the NMDA receptor subunit NR2B, and 2) a form of LTP that was evidenced only after blockade of GABAergic inhibition, sensitive to the N-methyl-D-aspartate receptor antagonist APV-5 but not to ifenprodil. These observations suggested the existence of DGCs with distinct functional properties within the dentate gyrus (DG). Indeed, Snyder and others (2001) went a step further, showing that GABA-independent LTP was specifically eliminated when cell proliferation in the brain was halted by γ-irradiation 3 weeks prior to electrophysiological recordings. These observations strongly suggest that

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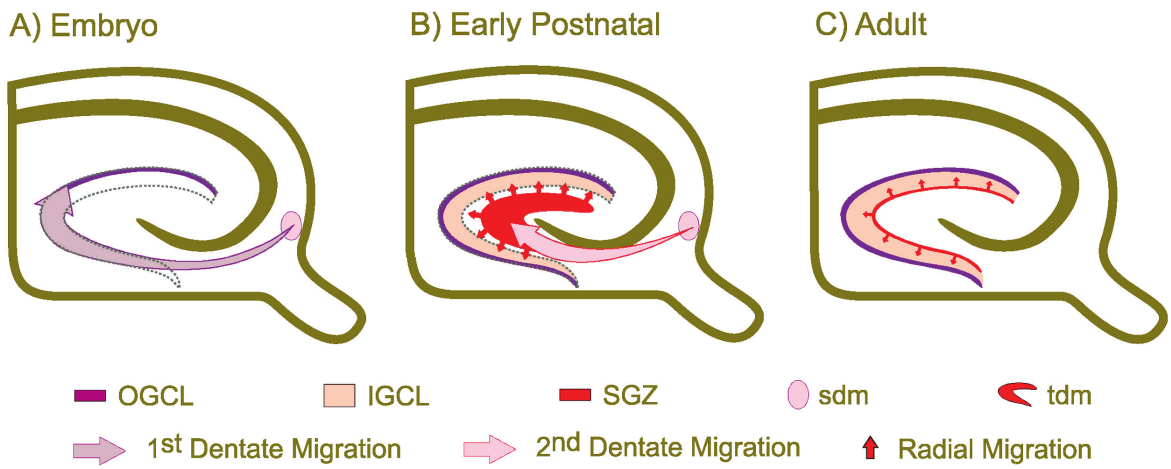

Fig. 1. Migration and distribution of dentate granule cells during embryonic and postnatal development. *A*, In mice and rats, development of the dentate gyrus begins with NPCs migrating from the primary dentate neuroepithelium to generate the secondary dentate matrix (sdm). By approximately E19, NPCs arising from the first dentate migration form the outer shell of the granule cell layer (OGCL). *B*, After birth, the second dentate migration gives rise to the tertiary dentate matrix (tdm). NPCs from this matrix migrate radially to generate the inner granule cell layer (IGCL). *C*, After P20, proliferative cells accumulate in the subgranular zone (SGZ), becoming the source of granule cells during adulthood. Adapted from Altman and Bayer (1990).

young adult-born neurons (less than 3 weeks old) were the substrate of such forms of synaptic modification. More recently, immature DGCs of the inner GCL identified by their high input resistance (>1.5 GΩ), expression of the polysialylated form of the neural cell adhesion molecule, and dendritic tree morphology were compared to mature DGCs (Schmidt-Hieber and others 2004). Immature neurons showed a lower threshold for the induction of LTP in the presence of $GABA_AR$ antagonists, probably because of the presence of Ca^{2+} spikes that can boost Na^+-dependent action potentials increasing membrane excitability. Taken together, these studies have demonstrated that young neurons of the adult DG are a distinctive functional population with enhanced synaptic plasticity and can be readily distinguished from mature DGCs. Therefore, the adult DG should not be viewed as static and homogeneous but rather as a highly dynamic structure with a significant proportion of young DGCs bearing immature neuronal properties.

Neuronal Development in the Adult Hippocampus

At the cellular level, adult neurogenesis can be viewed as a process by which an NPC undergoes discrete developmental stages that include proliferation, differentiation, survival, migration, and maturation before they finally become fully functional neurons in the hippocampal circuitry (van Praag and others 2002; Abrous and others 2005; Ming and Song 2005; Lledo and others 2006; Overstreet-Wadiche and Westbrook 2006). Work by several laboratories using various techniques to identify adult-born neurons has recently demonstrated that NPCs of the adult DG follow a precise pathway through neuronal maturation and functional integration. Genetic marking with retroviruses was used to express enhanced green fluorescent protein (GFP) in the neuronal progeny of dividing NPCs, allowing accurate dating, electrophysiological recordings, and morphological analysis of adult-born neurons (van Praag and others 2002; Espósito and others 2005; Ge and others 2006; Zhao and others 2006). An alternative method exploits the capacity of the pro-opiomelanocortin (POMC) promoter to drive the transient expression of GFP in young neurons of the DG, allowing the morphological and functional characterization of approximately 2-week-old neurons (Overstreet Wadiche and others 2005).

NPCs of the adult DG follow a precise sequence for the maturation of neuronal function and connectivity that requires about 4 weeks and exhibits a striking similarity to the events observed during hippocampal development (Fig. 2). The neuronal phenotype is acquired within the first few days. Those early immature neurons show small action potentials, express immature neuronal markers, and are spatially restricted to the subgranular zone. They lack afferent synaptic contacts and display a high membrane resistance that reflects a low density of ion channels but show tonic activation of $GABA_A$ receptors. One week later, neurons are localized in the inner GCL, exhibit spineless dendrite trees that reach the inner molecular layer, and receive depolarizing GABAergic inputs of dendritic origin (Ambrogini and others 2004; Espósito and others 2005; Overstreet-Wadiche and others 2005; Wang and others 2005; Ge and others 2006; Karten and others 2006). By the third week, newborn neurons begin to receive functional glutamatergic afferents and display repetitive action potentials with high-frequency adaptation. Detailed morphological analysis of retrovirally labeled neurons resulted in a starting point for dendritic spine formation of 16 days, indicative

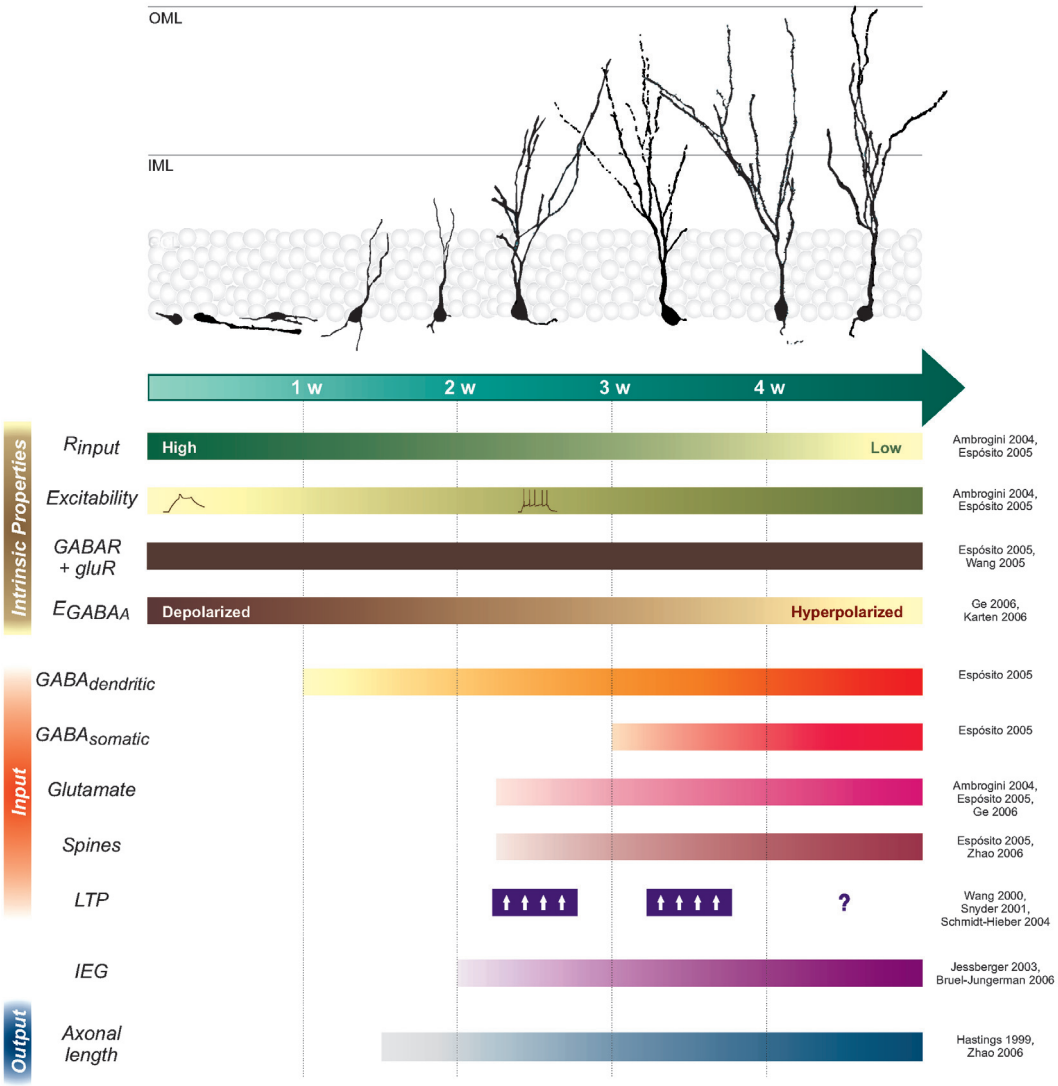

Fig. 2. The timing of functional integration of adult-born DGCs. *Top*, Morphological maturation of adult-born DGCs identified by retroviral green fluorescent protein (GFP) labeling (adapted from Espósito and others 2005). *Bottom*, Schematic representation of the sequence of functional maturation. Bars overlap with the interval at which properties were detected. Bar color intensity symbolizes the number or strength of each parameter. OML = outer molecular layer; IML = inner molecular layer; R_{input} = input resistance; E_{GABAA} = GABA reversal potential; IEG = immediate early gene expression stimulated by increased electrical activity of hippocampal circuits.

of glutamatergic synaptogenesis (Zhao and others 2006). Consistent with these observations, kainate-induced seizures elicited activity-dependent expression of the immediate early genes c-fos, zif268, and Homer1A in BrdU-labeled neurons of greater than 15 days of age (Jessberger and Kempermann 2003). In a similar study, expression of zif268 was observed in 2-week-old neurons after tetanic stimulation of the perforant path in vivo (Bruel-Jungerman and others 2006). Neuronal maturation becomes complete by the fourth week with the onset of perisomatic GABAergic contacts and the presence of spiny dendrites reaching the outer molecular layer (Espósito and others 2005).

In contrast to the detailed descriptions of afferent connectivity, the functional output of adult-born DGCs remains to be investigated (i.e., what neurons are their targets, when do they connect, can they excite CA3 pyramidal cells?). Yet development of axonal projections of adult-born DGCs has been characterized anatomically. In early studies, retrograde tracers delivered to CA3 were used to identify $BrdU^+$ cells whose axons have reached their main target region. Few retrogradely labeled newborn cells were found 10 days after BrdU injection, whereas maximal colocalization was observed by 17 days (Hastings and Gould 1999). Recently, the same question was addressed by studying axonal projections of retrovirally

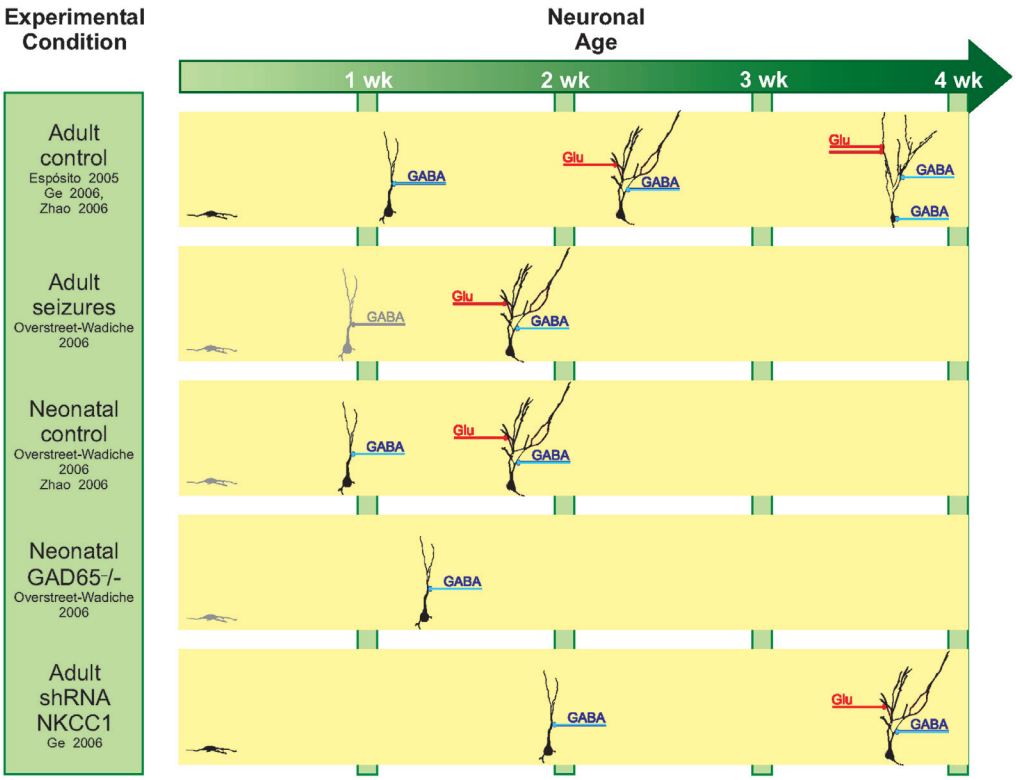

Fig. 3. Experimental conditions that regulate the rate of neuronal maturation. Each condition accelerates or delays neuronal maturation, represented by the hallmarks shown in the upper panel. The onset of GABAergic and glutamatergic inputs is depicted by blue and red terminals. Multiple terminals denote increased connection strength. Diagrams in gray color indicate hypothetical stages.

labeled neurons expressing GFP (Zhao and others 2006). In accordance with the previous study, GFP^+ axons reaching CA3 were first observed 10 days after viral injection, and their length increased progressively to reach the maximal distance by days 16 to 56. Therefore, axons appear to be fully developed before the first dendritic spines are formed on newborn neurons, similarly to what happens in the developing hippocampus (Jones and others 2003). Although electrophysiological recordings of synaptic transmission from adult-born DGCs onto postsynaptic target cells have not yet been done, a recent study has suggested that excitatory granule cells are perhaps not the only neurons generated in the adult DG. Newborn GABAergic interneurons identified by means of coexpression of BrdU and parvalbumin, a marker of Basket cells and axo-axonic interneurons, were found to release GABA onto mature DGCs in paired whole-cell recordings and might represent ~14% of adult-born neurons (Liu and others 2003). This study provided the first evidence of functional outputs recorded from adult-born neurons.

Role of the Activity of Surrounding Circuits on Functional Maturation

The process outlined above can be interpreted as a sequence of precise transitions that take the developing neuron through the discrete steps of maturation and functional integration in the hippocampal network (Fig. 2). Although the mechanisms underlying those transitions have not yet been addressed, increasing evidence points to a critical role for the electrical activity of surrounding circuits as either permissive or instructive signals. Labeling immature neurons of the adult DG using POMC-GFP transgenic mice, Overstreet-Wadiche, Bromberg, and others (2006) have recently shown that neuronal maturation can be dramatically accelerated by epileptogenic activity. They observed that 2-week-old neurons of mice with pilocarpine-induced seizures display a complex dendritic tree with spines and functional glutamatergic afferents, which, under normal conditions, would appear about 1 or 2 weeks later (Fig. 3). Notably, these pathological conditions also induced aberrant connectivity such as basal dendrites, mossy fiber sprouting, and recurrent polysynaptic inputs in newly generated neurons. The transgenic POMC-GFP mice were also used to demonstrate that neuronal maturation is faster during early postnatal development compared to the adult DG (Overstreet-Wadiche, Bensen, and others 2006). POMC-GFP neurons of the developing and adult DG showed similar morphological and functional properties. However, DGCs in the neonatal brain expressed GFP at a younger age and for a shorter period, suggesting a shortened life

span for the immature neuronal stages. Consistent with this notion, spine formation, dendritic development, and axonal growth occurred at a faster rate in retrovirally labeled DGCs of the neonatal versus the adult brain (Zhao and others 2006). Neuronal maturation in the neonatal hippocampus was delayed by reduction of GABAergic activity by genetic deletion of glutamic acid decarboxylase 65, a GABA-synthesizing enzyme (Overstreet-Wadiche, Bensen, and others 2006). In addition, analysis of the expression of activity-induced transcription factors such as NeuroD and p-CREB suggested that the neonatal hippocampus represents an environment with higher levels of excitatory activity that might increase the speed of neuronal maturation. These observations suggested that high levels of depolarizing GABAergic spontaneous activity characteristic of the neonatal brain could be involved in the accelerated maturation (Ben Ari 2002).

Fast GABAergic neurotransmission is exerted through the $GABA_A$ receptor, an anion-selective channel. The postsynaptic effect of GABA is set by the intracellular Cl^- concentration ($[Cl^-]_i$), which is controlled by the concerted action of the cation-chloride cotransporters NKCC1 (Cl^- uptake) and KCC2 (Cl^- extrusion; Payne and others 2003). In the developing brain, the high expression of NKCC1 in immature neurons imposes an elevated $[Cl^-]_i$, which renders GABA depolarizing. As neurons mature, KCC2 expression becomes predominant, $[Cl^-]_i$ decreases, and GABA becomes inhibitory. The developmental GABA switch from depolarizing to hyperpolarizing was also described in adult-born DGCs identified by in vivo retroviral labeling (Ge and others 2006). Moreover, a key role for depolarizing GABAergic activity in neuronal maturation in the adult DG was demonstrated. Retroviral expression of specific short hairpin RNAs (shRNAs) against NKCC1 was used to render endogenous $GABA_A$-mediated signaling hyperpolarizing in immature neurons. Young neurons expressing the shRNA were never depolarized by GABA, and the consequence was a remarkable delay in GABAergic and glutamatergic synaptogenesis as well as in dendritic development of adult-born DGCs. These observations highlight a modulatory rather than an instructive role for GABAergic depolarizing activity because functional maturation was delayed but finally occurred.

Depolarization elicited by tonic activation of $GABA_A$ receptors induced neuronal differentiation of NPCs by elevation of intracellular Ca^{2+} levels followed by the concomitant expression of the neurogenic transcription factor neuroD (Tozuka and others 2005). In agreement, K^+-induced depolarization of NPCs in vitro was shown to induce neuronal differentiation, probably because of the decreased expression of glial fate genes Hes1 and Id2 and the increased expression of NeuroD (Deisseroth and others 2004). It is likely that tonic depolarizing stimulus in the latter study could mimic the steady depolarization induced by ambient levels of GABA in vivo. Thus, neurotransmitter receptors in NPCs could be acting as sensors for the overall activity of surrounding networks to regulate their fate.

Impact of Young Neurons in Hippocampal Function

The presence of a distinctive population of young DGCs with a higher plasticity in the adult DG opens the question of whether this property is relevant to their own maturation process and/or to the function of the whole hippocampus (Schinder and Gage 2004). As new neurons incorporate into the existing networks, an enhanced sensitivity to activity-dependent synaptic modification might serve as a mechanism for the selection of presynaptic and postsynaptic partners (Muller and Nikonenko 2003). The establishment of persistent connectivity of incoming neurons could be tailored by the activity of surrounding circuits. In fact, a recent study has demonstrated that survival of adult-born DGCs depends on the activation of NMDA receptors at young neuronal stages and the degree of receptor activation compared to that of surrounding cells (Tashiro and others forthcoming). Immature neurons with enhanced plasticity could also participate in information processing, provided they were capable of neurotransmitter release onto a target cell (a question that has not yet been addressed neither for immature nor for mature adult-born DGCs). In fact, a recent exciting hypothesis proposes that immature neurons of the DG may be instrumental in the association of new memories that occurred within a restricted temporal window (Aimone and others 2006). Whether adult-born neurons exert their primary function at immature stages or after reaching maturity remains to be elucidated.

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