Front. Biol. 2015, 10(4): 310–320
DOI 10.1007/s11515-015-1362-2

REVIEW

Development of glutamatergic innervation during maturation of adult-born neurons

Cristina V. Dieni (✉), Adam J. Wieckert, Linda Overstreet-Wadiche

Department of Neurobiology and Evelyn McKnight Brain Institute, University of Alabama at Birmingham, Birmingham, AL 35294,USA

Abstract **The dentate gyrus is the entrance of the hippocampal formation and a primary target of excitatory afferents from the entorhinal cortex that carry spatial and sensory information. Mounting evidence suggests that continual adult neurogenesis contributes to appropriate processing of cortical information. The ongoing integration of adult born neurons dynamically modulates connectivity of the network, potentially contributing to dentate cognitive function. Here we review the current understanding of how glutamatergic innervation develops during the progression of adult-born neuron maturation. Summarizing the developmental stages of dentate neurogenesis, we also demonstrate that new neurons at an immature stage of maturation begin to process afferent activity from both medial and lateral entorhinal cortices.**

Keywords dentate gyrus, adult neurogenesis, glutamatergic innervation, granule cell, neuroprogenitor

Introduction

How adult generated neurons of the dentate gyrus (DG) mature and integrate into the pre-existing hippocampal network has been a subject of intense investigation over the past decade (Zhao et al., 2008; Suh et al., 2009; Deng et al., 2010; Ming and Song, 2011). The hippocampal neural network is highly plastic, able to modify connectivity by changing the strength of synaptic contacts in an activity-dependent manner and able to maintain the integrity of the existing circuitry while providing a niche to support the synaptic integration of newborn dentate granule cells (GCs). Using a variety of viral approaches and transgenic mouse models that enable selective manipulation of neurogenesis, many studies have provided functional information about the involvement of neurogenesis in learning and memory processes (Deng et al., 2010) as well as dentate specific tasks, such as pattern separation (Gilbert et al., 2001; Leutgeb et al., 2007; Deng et al., 2010; Sahay et al., 2011; Nakashiba et al., 2012). This literature indicates that ongoing neurogenesis is important for normal dentate function, but little is known about how adult-born GCs contribute to network activity. With the idea that synaptic connectivity is a crucial component of neural function, here we review current findings about the development of glutamatergic synaptic connectivity to adult-born GCs.

Received April 3, 2015; accepted April 30, 2015
Correspondence: Cristina V. Dieni
E-mail: cvdieni@gmail.com

Circuitry of the adult dentate gyrus

The dentate gyrus in rats and mice receives major afferent projections from layer II of the entorhinal cortex via the perforant pathway and minor projections from deep layers (IV-VI) (Witter and Amaral, 1991; van Groen et al., 2003). The rat entorhinal cortex projects to the ipsilateral dentate gyrus and gives rise to a crossed projection to the contralateral DG, CA3, CA1 and subiculum; this contralateral projection is more prominent in dorsal regions (Goldowitz et al., 1975; Steward, 1976; Steward and Scoville, 1976). In contrast, mice have almost no contralateral projections to the DG (Steward and Scoville, 1976; van Groen et al., 2003). Two components of the entorhinal cortex provide laminar-specific projections. Perforant path fibers originating in the lateral entorhinal cortex (LEnt) form the lateral perforant path (LPP) that terminates in the outer molecular layer (OML) of the dentate gyrus. Fibers originating from the medial entorhinal cortex (MEnt) comprise the medial perforant path (MPP) that

terminates in the middle molecular layer (MML) of the dentate gyrus (Witter, 2007). The MPP and LPP projections form glutamatergic synapses on the dendritic spines of GCs as well as on shafts of GABA-positive interneurons. Each pathway transmits information that is integrated in the DG, with the MPP providing spatial information and the LPP providing sensory information (Witter et al., 1989; Hafting et al., 2005; Hargreaves et al., 2005). Each pathway exhibits distinct release properties, with paired-pulse stimulation of the LPP producing paired-pulse facilitation and stimulation of the MPP evoking paired-pulse depression (McNaughton, 1980; Abraham and McNaughton, 1984; Petersen et al., 2013). There is also differential expression of mGluR autoreceptors at each pathway. Presynaptic mGluRs that decrease release probability have Group-III-like pharmacology at LPP synapses and mGluRs with Group-II-like pharmacology serve as autoreceptors at MPP synapses (Macek et al., 1996; Kilbride et al., 2001; Chiu and Castillo, 2008; Chancey et al., 2014).

Another important glutamatergic pathway in the DG is the associational/commissural (A/C) pathway that arises from mossy cells in the ipsi- or contralateral hilus (Amaral, 1978; Laurberg and Sørensen, 1981; Frotscher, 1991; Buckmaster et al., 1992). Mossy cells form a complementary longitudinal axon system that innervates the inner molecular layer (IML) of distant segments of the DG along the septo-temporal axis (Jinde et al., 2013; Amaral and Witter, 1989; Soltesz et al., 1993; Buckmaster et al., 1996; Sloviter and Lømo, 2012). Paired-pulse stimulation of the A/C pathway produces paired-pulse depression of glutamate release, and unlike the perforant path terminals, mossy cell axons do not have presynaptic mGluR autoreceptors but rather express presynaptic cannabinoid type 1 receptors (CB1Rs) that can reduce release probability (Chiu and Castillo, 2008; Chancey et al., 2014).

The DG also receives sparse innervation from several other brain regions. The septal nuclei have a cholinergic projection that terminate mainly onto GCs, making asymmetric excitatory contacts on dendritic spines in the inner third of the molecular layer (Mosko et al., 1973; Amaral and Kurz, 1985), while other fibers terminate on dentate interneurons and large mossy cells of the hilus (Lübke et al., 1997). Moreover, a hypothalamic projection from the supramammillary nucleus sends projections to the border of the inner molecular layer and granule cell layer (Dent et al., 1983; Maglóczky et al., 1994). These projections likely use glutamate as a primary neurotransmitter (Kiss et al., 2000), but little is known about the function of these excitatory connections. The DG also receives a minor and diffusely distributed dopaminergic projection that terminates mainly in the hilus from cells in the ventral tegmental area. Dopaminergic neurites contact progenitor cells in the subgranular zone (SGZ) (Höglinger et al., 2004) and form symmetric synapses with dendrites and perikarya of GCs (Milner and Bacon, 1989). It has been proposed that release of dopamine (DA) and the expression of DA receptors on neural precursor cells in SGZ could control aspects of neurogenesis (Diaz et al., 1997; Höglinger et al., 2004; Berg et al., 2013), although conflicting results have been reported (Domínguez-Escribà et al., 2006; Park and Enikolopov, 2010). Furthermore, DA differentionally modulates excitatory synaptic transmission from the perforant path to GCs depending on developmental stage. DA suppresses glutamate release to mature GCs via D2-like receptors while suppresses transmission to immature GCs via D1-like receptors (Mu et al., 2011). These authors also showed that dopamine modulates perforant path synaptic plasticity to immature GCs but not mature GCs. A deeper understanding of how these various brain regions exert distinct modulatory control of cortical synaptic transmission and plasticity to GCs is an important avenue for future studies.

Rate of neural maturation is variable

New neurons originate from a residential population of multiple types of adult neural stem cells (NSCs), radial and non-radial precursors, in the subgranule zone (Gage, 2000; Alvarez-Buylla and Lim, 2004; Ma et al., 2009; Lugert et al., 2010). Radial glial NSCs, also named Type 1 cells, become activated and begin asymmetric cell division to generate the intermediate neuronal progenitor cells, Type 2 cells. After ~2 rounds of symmetric division Type 2 cells exit the cell cycle and become neuroblast Type 1 cells (NB1), a majority of which do not survive. The surviving NB1 cells mature into NB2 cells also known as newborn neurons. These newborn neurons then mature into immature neurons and finally into mature GCs (Encinas et al., 2011).

It is estimated that the complete progression of maturation requires at least 7–8 weeks in a young adult mouse brain with the rate of maturation depending on many factors, including age and hippocampal region. Age-dependent maturation has been examined by comparing newborn neurons in adult and neonatal brains. Morphological maturation of spines, dendritic and axonal growths are slower in adult generated GCs compared to neonatal-generated GCs (Zhao et al., 2006). Newborn neurons in neonates reside in a network with a characteristic pattern of spontaneous depolarizing activity, absent in adults, that accelerates functional GC development (Overstreet-Wadiche and Westbrook, 2006; Overstreet-Wadiche et al., 2006). Although the rate of granule cell maturation does not differ between the suprapyramidal and infrapyramidal blades, Snyder et al. (2012) showed the timing of maturation differs along the septotemporal axis with slower maturation of new GCs occurring in the temporal DG. The regional difference in maturation along the septotemporal axis results from different levels of activity; increased network activity in the temporal DG accelerates maturation (Piatti et al., 2011).

Fundamental differences in the time course of neuronal maturation has also been demonstrated between rodents,

sheep and primates. In particular, Snyder et al. (2012) described that new GC maturation is delayed significantly in mice compared to rats, while Brus et al. (2013) showed there is a longer duration of differentiation in sheep and non-human primates. The differential timing of neuronal maturation could have a potential role in memory encoding. The long cell cycle together with long maturation of adult generated GCs results in a substrate for memory formation that may be appropriate for preservation of neural plasticity over the longer life span of primates, including humans.

In humans, direct evidence for adult neurogenesis was initially provided by a study with BrdU in postmortem tissue (Eriksson et al., 1998). In 2005, Frisen and colleagues introduced a new strategy using radiocarbon-dating for birth-dating cells from postmortem tissue (Spalding et al., 2005). Using this approach they found that neurogenesis occurs at significant levels through adulthood and until old age suggesting that neurogenesis might contribute to human brain function (Spalding et al., 2013).

Progenitor cells do not receive glutamatergic innervation

New neurons continuously generated from NSCs follow a precise developmental pathway with distinct intrinsic and synaptic characteristics compared to the surrounding mature GCs. Type 1 cells exhibit a radial process extending through the granule cell layer that branches within the IML. Type 1 cells express glial fibrillary acidic protein (GFAP), the intermediated filament protein Nestin, a radial glia marker BLBP, and the sry-related HMG-box transcription factor Sox2 (Mignone et al., 2004; Suh et al., 2007; Encinas et al., 2011). Although studies have indicated that Type 1 cells do not receive glutamateric innervation (Tozuka et al., 2005; Wang et al., 2005), recordings from patches excised from the soma and main process of Type 1 cells show functional glutamate transporters with AMPA receptors on their processes (Renzel et al., 2013). The presence of AMPARs may be important for responding to local activity-driven glutamate release and it has been proposed that glutamate transporters in Type 1 cells contribute to transmitter clearance (Regan and Smalley, 2007; Regan et al., 2007). However the functional impact of AMPARs and glutamate transporters in the absence of synaptic innervation needs further clarification since the ambient extracellular concentration of glutamate is extremely low (Herman and Jahr, 2007).

GCs receive cholinergic projections that make asymmetric excitatory contacts on dendritic spines (Leranth and Hajszan, 2007). Selective lesioning of the cholinergic system in the basal forebrain reduces proliferation of neuronal progenitor cells (Ma et al., 2000; Zhou et al., 2004; Mohapel et al., 2005). In both young and aged mice, focal application of ACh induces rapid calcium responses in Type 1 and Type 2 cells, suggesting that endogenous ACh might regulate stem cell activity by acting directly on proliferating cells (Itou et al., 2011). As mentioned above,Type 1 cells give rise to Type 2 cells that lack dendritic and axonal processes but may have short plump horizontal processes of unknown function. Wang et al. (2005) showed that the earliest signs of neuronal function could be detected in these Type 2 progenitor cell populations, such as voltage gated ion channels. Some of these cells exhibited $GABA_A$ receptor-mediated synaptic currents, suggesting that within a short period, neurons mature to a stage where they receive GABAergic synapses. Together these results suggest that the earliest neural progenitors exhibit little, if any, ionotropic glutamate receptor signaling. However, evidence for metabotropic glutamate receptor-mediated regulation of proliferation has been suggested by Nochi et al. (2012).

Initial silent synapses of newborn granule cells arise from hilar mossy cells

The post-mitotic newborn stage starts once the progenitor cells exit the cell cycle. During this period newly generated GCs develop spineless dendrites that reach the IML and receive synapses from local GABAergic interneurons (Espósito et al., 2005; Overstreet Wadiche et al., 2005; Ge et al., 2006; Markwardt et al., 2011; Song et al., 2013). Newborn GCs express functional AMPA and NMDA receptors that can be activated by exogenous agonists prior to synapse formation (Markwardt and Overstreet-Wadiche, 2008; Schmidt-Salzmann et al., 2014). During the second and third post-mitotic week adult-born neurons start to develop spines that receive glutamatergic synapses from the perforant path (Espósito et al., 2005; Ge et al., 2006; Zhao et al., 2006; Mongiat et al., 2009).

Nascent synapses on developing neurons lack AMPARs but have NMDARs that are functionally silent at normal resting potential due to Mg^{2+} block (Kullmann, 1994; Isaac et al., 1995; Liao et al., 1995; Durand et al., 1996). The conversion of these "silent synapses" to functional synapses involves activity-dependent incorporation of AMPARs ("synapse unsilencing"), a process that requires depolarization to relieve the Mg^{2+} block of NMDARs (Isaac et al., 1995; Liao et al., 1995; Durand et al., 1996; Wu et al., 1996; Isaac et al., 1997). Depolarization can be generated by AMPARs at neighboring functional synapses, or at the earliest developmental stages when immature neurons lack functional synaptic AMPARs, it was proposed that depolarization could be provided by GABAergic synaptic activity (Ben-Ari et al., 1997). Synapse unsilencing at the first synapses on adult-born neurons was recently described by Chancey et al. (2013). These authors first identified a developmental stage when adult-generated GCs have glutamatergic synaptic transmission mediated solely by NMDARs, representing the initial silent synapses before AMPAR-mediated functional transmission. This developmental stage

occurs when newborn GCs are approximately 10–12 days after mitosis and can be identified in POMC-GFP reporter mice. Synaptic GABA depolarization of newborn GCs enabled activation of NMDARs in the absence of AMPAR-mediated transmission. Synapse unsilencing could be achieved either by a conventional pairing protocol using synaptic stimulation with direct postsynaptic depolarization (Fig. 1A), or by glutamatergic and GABAergic synaptic activity *in vitro*. Brief exploration of an enriched environment also triggered synapse unsilencing *in vivo* that required GABAergic depolarization (Chancey et al., 2013). These results demonstrated that GABAergic depolarization is needed to rapidly initiate functional glutamate-mediated transmission in response to experience.

Following these results, Chancey et al. (2014) also investigated the cellular source of the initial glutamatergic synapses on newborn GCs. Newborn GCs have dendrites mainly restricted to the IML, suggesting that presynaptic glutamatergic terminals arise from hilar mossy cells (MC) that form the A/C pathway located in the IML. Since MC terminals contain presynaptic CB1Rs, the authors first showed that NMDAR-mediated exitatory postsynaptic currents (EPSCs) evoked in newborn cells were reduced by a CB1R agonist but not the Group II mGluR agonist (2S,20R,30R)-2-[20,30-dicarboxycyclopropyl] glycine (DCG-IV) (Chancey et al., 2014). This result excluded the possibility that the EPSC arises from the medial perforant path or from neighboring mature GCs (Vivar et al., 2012), since MPP terminals and mossy fiber terminals express Group II mGluRs (Manzoni and Bockaert, 1995; Manzoni et al., 1995; Kwon and Castillo, 2008).

Moreover, using optogenetics to selectively activate excitatory MC axons Chancey et al. (2014) found that the majority of newborn GCs responded to MC activation with an NMDAR EPSC (Fig. 1B). To exclude the possibility that the glutamatergic inputs arose from the perforant path, they also

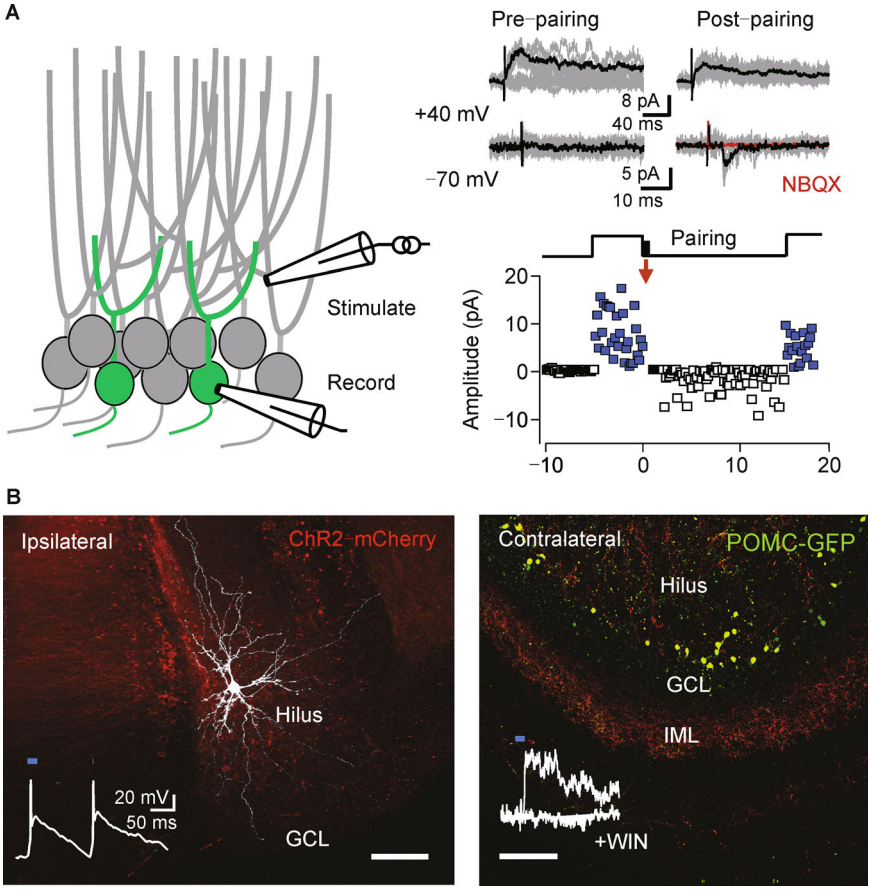

Figure 1 Initial silent synapses on adult born GCs arise from hilar mossy cells. (A) *Left*, diagram of recording from newborn GCs expressing POMC-GFP. Stimulation in the IML evokes NMDAR-only EPSCs (top right, pre-pairing) that undergo rapid incorporation of AMPARs after pairing synaptic stimulation with postsynaptic depolarization (top right, post-pairing). Ten traces overlaid (gray) with averages (black). *Right bottom*, plot of EPSC amplitude versus time at − 70 mV (black) and + 40 mV (blue). Adapted from Chancey et al., 2013. (B) *Left*, a mossy cell filled with biocytin (white) in a DG injected with ChR2-mCherry (red). *Insets*, mossy cell spiking in response to 455 nm light (blue bars). Scale bar, 100 μm. GCL, granule cell layer. *Right*, ChR2-mCherry-labeled commissural axons in the hilus and IML of the contralateral DG. *Inset*, example of light-evoked NMDAR EPSC in a POMC-GFP newborn GC that is blocked by CB1R agonist WIN 55 212-2. Scale bar, 100 μm. Adapted from Chancey et al., 2014.

expressed channelrhodopsin in the entorhinal cortex. At this early developmental stage synaptic connectivity from the entorhinal cortex is not yet established since no responses were detected in newborn GCs following light-activation of either the medial or lateral perforant path. In addition, to evoking NMDAR EPSCs, MC activation generated disynaptic GABA release onto newborn GCs, presumably resulting from recruitment of local inhibitory interneurons. Thus, MCs provide the circuitry to synchronize glutamate and GABA release for activity-dependent synapse unsilencing (Chancey et al., 2013). In addition to promoting initial synaptogenesis, MC innervation is likely to contribute to GC function in other ways. For example, MC excitation of adult-generated neurons could promote their participation in DG network activity. Early innervation from MCs that elicits prominent NMDAR-mediated signaling also suggests that MCs are important for controlling cell survival, since ablation of NMDARs promotes cell death of newly generated cells between the 2nd and 3rd postmitotic week (Tashiro et al., 2006).

Immature GCs process cortical afferent activity

As newborn GCs continue to mature, the dendrites reach the outer molecular layer, spines grow and increase in size and their axons extend through the hilus toward CA3 (Zhao et al., 2006). These cells also start to receive axosomatic, axodendritic and axospinous synapses (Zhao et al., 2006; Toni et al., 2007; Toni et al., 2008). Between 2 and 4 weeks postmitosis, the $GABA_A$ receptor reversal potential shifts in the hyperpolarizing direction, presumably in response to significant maturation of excitatory glutamatergic inputs arising from the MEnt and LEnt (Espósito et al., 2005; Ge et al., 2006; Ming and Song, 2011; Vivar et al., 2012). During this phase, developing GCs are morphologically and functionally immature displaying a high intrinsic excitability resulting from regenerative T-type Ca^{2+} currents and high input resistance (Schmidt-Hieber et al., 2004; Brunner et al., 2014). They also display a high propensity for long-term plasticity of peforant path synapses, including a low threshold for induction of long-term potentiation (LTP) and a greater magnitude of LTP than mature GCs that results from high expression of NMDA2BRs receptors (Ge et al., 2007). Moreover immature GCs integrate synaptic inputs, resulting in action potentials and release of glutamate onto target cells (Wang et al., 2000; Schmidt-Hieber et al., 2004; Ge et al., 2007; Mongiat et al., 2009; Stone et al., 2011; Gu et al., 2012; Marín-Burgin et al., 2012).

Recently van Praag and colleagues (Vivar et al., 2012, 2013) used trans-synaptic retrograde tracing to illustrate that the source of synaptic inputs to 3 week-old GCs arises from mature GCs, hilar mossy cells, a direct back projection from area CA3 pyramidal cells and sparse input from the LEnt and perirhinal cortex (PRH). The authors interpreted a lack of labeling in the MEnt to indicate that immature GCs were not innervated by MPP fibers, raising the question of when excitatory innervation from the MPP is established. Dentate GCs integrate spatial-specific information from the MEnt with sensory information from the LEnt, thus understanding the timing of innervation is important to determine when and how developing GCs participate in DG information processing, including the transformation of synaptic inputs into place cell firing and the orthogonalization of context-specific stimuli.

Abundant results from *in vitro* slice studies have shown a time-dependent increase in excitatory innervation from the perforant path that tracks the synaptic integration of adult-generated GCs into the DG circuit (Espósito et al., 2005; Ge et al., 2006; Mongiat et al., 2009; Marín-Burgin et al., 2012; Dieni et al., 2013). Using transgenic mice to identify GCs of differing postmitotic ages, we showed that focal stimulation of the MPP and LPP evoked larger synaptic currents in 4 week-old GCs compared to 3 week-old GCs, and all immature GC responses were smaller than those evoked in mature GCs (Dieni et al., 2013). Selective activation of the MPP and LPP achieved by viral-mediated expression of channelrhodopsin also illustrates the timing of perforant-path innervation of retroviral-labeled developing GCs (Kumamoto et al., 2012). A low percentage of immature GCs at 14 days post-infection (dpi) responded to MEnt and LEnt optogenetic stimulation whereas nearly all 21 and 28 dpi GCs responded to both MEnt and LEnt stimulation (Fig. 2A; Kumamoto et al., 2012). We also demonstrated that focal stimulation of the MEnt and LEnt generated EPSCs in 4 week-old GCs that showed paired pulse depression, an indicator of the MPP, and paired pulse facilitation, an indicator of the LPP, respectively (Fig. 2B). Together these results suggest that innervation of immature GCs from the MEnt and LEnt is gradually established between 2 and 3 weeks after cell birth, and that innervation of 4 week-old GCs does not achieve mature values but rather continues to increase over time.

Focal and optogenetic activation may not fully distinguish the MPP and LPP, therefore we also used a pharmacological approach to assess innervation of immature GCs by the MPP, taking advantage of the differential expression of Group II mGluR autoreceptors (Fig. 3A) (Macek et al., 1996; Kilbride et al., 2001; Chiu and Castillo, 2008; Chancey et al., 2014). To identify approximately 3 week-old immature GCs, when the absence of innervation by the MPP was proposed (Vivar et al., 2012, 2013), we used transgenic GAD67-GFP reporter mice that express GFP in GCs that are just beginning to extend dendrites through the molecular layer (Zhao et al., 2010; Dieni et al., 2013). Stimulation in the MML generated EPSCs in all mature and GAD67-labeled immature GCs while OML stimulation failed to evoke EPSCs in 53% of immature cells (Fig. 3B). We confirmed that stimulation in the MML generated EPSCs arising from MPP terminals by

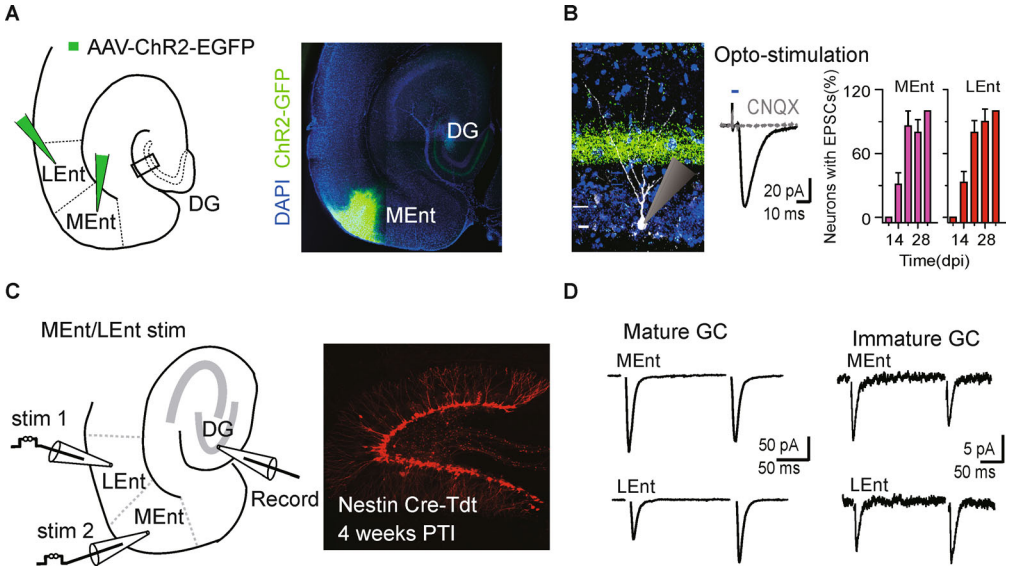

Figure 2 Immature GCs are innervated by medial and lateral enthorinal cortex. (A) *Left*, cartoon showing AAV-ChR2-EGFP injection sites in LEnt or MEnt. *Right*, the MEnt injection site results in axonal projections expressing ChR2-GFP in the MML of the DG. (B) *Left*, confocal image showing ChR2-EGFP expression in the MPP and a recorded GC (white) in acute slice. *Right*, blue light pulses evoke AMPAR-mediated EPSCs that are blocked by CNQX (gray trace). Approximately 80% of 21-day old retroviral labeled immature GCs exhibit EPSCs evoked by selective MEnt or LEnt optogenetic stimulation. Adapted from Kumamoto et al. (2012) with permission from Nature Publishing Group. (C) *Left*, focal stimulation in the MEnt and LEnt also evokes AMPAR EPSCs in dentate GCs. *Right*, confocal image of TdTomato-labeled immature GCs in Nestin-CreER mice at 4 weeks post-tamoxifen injection (PTI). (D) Example of EPSCs in mature (left) and immature (right) GCs in response to paired-pulse stimulation of the MEnt (top) or LEnt (bottom) pathways. Adapted from Dieni et al., 2013.

testing their sensitivity to the Group II mGluR agonist DCG-IV. In mature GCs, EPSCs evoked by stimulation in the MML were blocked by DCG-IV to a greater extent than EPSCs evoked by stimulation in the OML (Fig. 3C), confirming the pharmacological signature of each pathway. Importantly, EPSCs in immature GCs evoked by MML stimulation were likewise reduced by DCG-IV (Fig. 3D), confirming that EPSCs arise from MPP terminals even if there is anatomical intermingling of the two pathways. Together with prior studies, these results support a sequence of synaptic integration where MPP innervation is established upon the extension of immature dendrites within the MML with LPP innervation being established upon dendritic extension into the OML.

The balance of excitatory and inhibitory innervation

Between 4 and 6 weeks after cell birth, excitatory innervation of immature GCs continues to expand and during this period inhibitory innervation from local GABAergic interneurons is likewise increasing. GABAergic synaptogenesis is thought to follow a stereotypic pattern with dendritic-projecting interneurons that generate inhibitory postsynaptic currents (IPSCs) with slow kinetics establishing synapses prior to synaptic formation from perisomatic projecting interneurons that generate IPSCs with fast kinetics (Espósito et al., 2005; Markwardt and Overstreet-Wadiche, 2008; Markwardt et al., 2011; Marín-Burgin et al., 2012; Dieni et al., 2013). Although GABAergic signaling precedes glutamatergic synaptogenesis at the earliest stages of GC maturation, by 4 weeks immature GCs exhibit a lower ratio of inhibitory to excitatory synaptic strength compared to mature GCs (Dieni et al., 2013). The low I/E ratio and the slow rise times of inhibitory synaptic currents result in less functional inhibition of immature GCs relative to mature GCs, contributing to greater activation in response to molecular layer stimulation (Marín-Burgin et al., 2012; Li et al., 2013; Dieni et al., 2013).

Strong inhibitory circuitry is a hallmark of the DG (Coulter and Carlson, 2007). Not only are GCs subjected to strong feedforward inhibition, they are also known to recruit feedback inhibition to the granule cell layer by their innervation of inhibitory interneurons in the hilus. Interestingly, not only do immature GCs receive weak inhibitory synaptic innervation, they also generate little feedback inhibition to neighboring GCs (Temprana et al., 2015). Thus, immature GCs may function relatively independently of the strong inhibitory circuits established by interneurons of the DG. By 8 weeks after cell birth, adult born GCs appear fully mature (Laplagne et al., 2006; Mongiat et al., 2009), they have low input resistance and large dendritic arborization with prolific synaptic connections compared to the younger GCs. At this stage, excitatory function is strongly regulated by feedforward and feedback inhibition that has been

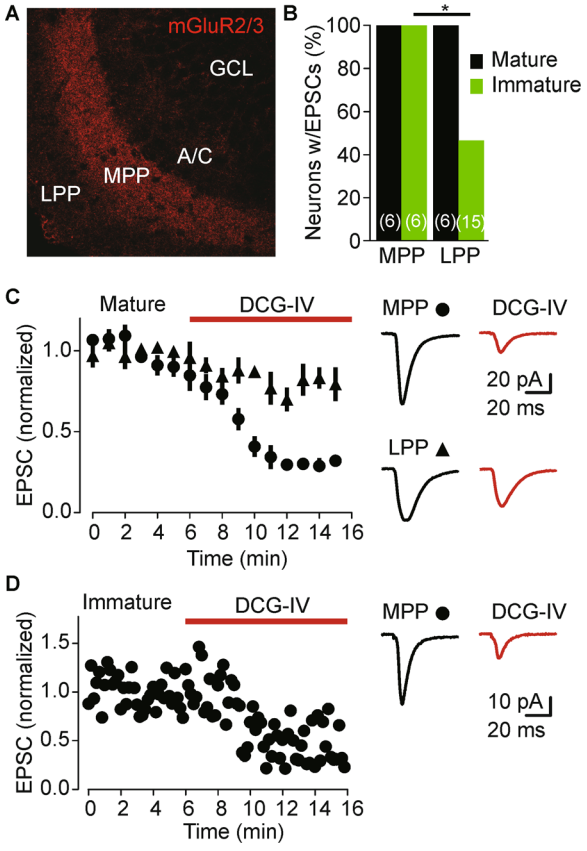

Figure 3 EPSCs in ~3 week-old GCs show pharmacology of medial perforant path. (A) Immunostaining for mGluR2/3 shows selective expression in axons of MPP. (B) Percentage of GCs exhibiting EPSCs during focal MPP and LPP stimulation (*p = 0.045 Fisher's exact test). Immature GCs were identified in GAD67-GFP reporter mice. The total number of cells recorded is indicated in the bars. (C) *Left*, DCG-IV (1μM) decreased EPSCs in mature GCs during MPP and LPP stimulation by 73% and 27% respectively (n = 6 each pathway, p = 0.0004 unpaired t-test). *Right,* examples of EPSCs recorded in mature GCs in the absence and presence of DCG-IV. (D) *Left,* DCG-IV decreased EPSCs in GAD67-GFP labeled immature GCs by 56% (n = 5, p = 0.0035, paired t-test). A representative time plot of EPSCs in an immature GC (*left*) and representative EPSCs (*right*).

suggested to be necessary in maintaining the DG's sparse patterns of neural activity (Coulter and Carlson, 2007; Ewell and Jones, 2010; Temprana et al., 2015). The eventual convergence of functional properties with neonatal GCs suggests that immature GCs make a unique contribute to DG function during the transient immature period when their distinct intrinsic and synaptic characteristics distinguish them from the much larger population of mature GCs.

Conclusions

Mapping synaptic connectivity is essential for understanding the physiological function of neural networks. Here we review how the excitatory connectivity changes during maturation of hippocampal adult-born neurons, focusing on the main glutamatergic afferents that project from the entorhinal cortex and hilar mossy cells. We showed that the first glutamatergic innervation to newborn GCs is from hilar mossy cells (Chancey et al., 2013, 2014). We also illustrated results using optogenetic, electrical stimulation and pharmacology that indicate immature GCs receive glutamatergic innervation from both the MEnt and LEnt, despite failures of trans-synaptic labeling to identify sources of innervation from the MEnt (Kumamoto et al., 2012; Dieni et al., 2013; Vivar and van Praag, 2013).

Many questions remain unanswered about synaptic function in the DG. Studies support a role of the cholinergic system in the regulation of newborn neuron maturation and survival (Kaneko et al., 2006; Campbell et al., 2010) and modulation of their excitability that may facilitate information output to the CA3 (Vogt and Regehr, 2001). Likewise studies over the last decade suggest that dopamine contributes to proliferation of neural precursor cells in the subgranular zone (Höglinger et al., 2004; Domínguez-Escribà et al., 2006; Park and Enikolopov, 2010), and modulates synaptic transmission and plasticity of GCs (Mu et al., 2011). Further investigation is necessary to understand the role of cholinergic and dopaminergic inputs during GC maturation.

Finally, the last decade has witnessed a profound increase in our understanding of spatial processing in the MEnt (Moser et al., 2014). Many unanswered questions remain about the function of the downstream EC-DG circuit. Understanding how spatial and sensory information is mapped to the DG and how GCs potentially transform disparate information into place codes will be important to address in future studies (Neunuebel and Knierim, 2012; Lisman and Jensen, 2013). Overall, the development of afferent innervation during adult-born GCs maturation suggest that immature and mature GCs may be recruited by network activity in distinct ways to provide complementary integration of new information with previous experience.

Acknowledgements

We thank Ryan Vaden and Jose Carlos Gonzalez for critical reading of this manuscript. LOW is funded by NIH (R01NS064025).

Compliance with ethics guidelines

Cristina V. Dieni, Adam J. Wieckert and Linda Overstreet-Wadiche declare that they have no conflict of interest. All animal procedures followed the Guide for the Care and Use of Laboratory Animals, US Public Health Service, and were approved by the University of Alabama at Birmingham Institutional Animal Care and Use Committee.

References

Abraham W C, McNaughton N (1984). Differences in synaptic transmission between medial and lateral components of the perforant

path. Brain Res, 303(2): 251–260

Alvarez-Buylla A, Lim D A (2004). For the long run: maintaining germinal niches in the adult brain. Neuron, 41(5): 683–686

Amaral D G (1978). A Golgi study of cell types in the hilar region of the hippocampus in the rat. J Comp Neurol, 182(4 Pt 2): 851–914

Amaral D G, Kurz J (1985). An analysis of the origins of the cholinergic and noncholinergic septal projections to the hippocampal formation of the rat. J Comp Neurol, 240(1): 37–59

Amaral D G, Witter M P (1989). The three-dimensional organization of the hippocampal formation: a review of anatomical data. Neuroscience, 31(3): 571–591

Ben-Ari Y, Khazipov R, Leinekugel X, Caillard O, Gaiarsa J L (1997). GABAA, NMDA and AMPA receptors: a developmentally regulated 'ménage à trois'. Trends Neurosci, 20(11): 523–529

Berg D A, Belnoue L, Song H, Simon A (2013). Neurotransmitter-mediated control of neurogenesis in the adult vertebrate brain. Development, 140(12): 2548–2561

Brunner J, Neubrandt M, Van-Weert S, Andrási T, Kleine Borgmann F B, Jessberger S, Szabadics J (2014). Adult-born granule cells mature through two functionally distinct states. eLife, 3: e03104

Brus M, Keller M, Lévy F (2013). Temporal features of adult neurogenesis: differences and similarities across mammalian species. Front Neurosci, 7: 135

Buckmaster P S, Strowbridge B W, Kunkel D D, Schmiege D L, Schwartzkroin P A (1992). Mossy cell axonal projections to the dentate gyrus molecular layer in the rat hippocampal slice. Hippocampus, 2(4): 349–362

Buckmaster P S, Wenzel H J, Kunkel D D, Schwartzkroin P A (1996). Axon arbors and synaptic connections of hippocampal mossy cells in the rat *in vivo*. J Comp Neurol, 366(2): 271–292

Campbell N R, Fernandes C C, Halff A W, Berg D K (2010). Endogenous signaling through alpha7-containing nicotinic receptors promotes maturation and integration of adult-born neurons in the hippocampus. J Neurosci, 30(26): 8734–8744

Chancey J H, Adlaf E W, Sapp M C, Pugh P C, Wadiche J I, Overstreet-Wadiche L S (2013). GABA depolarization is required for experience-dependent synapse unsilencing in adult-born neurons. J Neurosci, 33(15): 6614–6622

Chancey J H, Poulsen D J, Wadiche J I, Overstreet-Wadiche L (2014). Hilar mossy cells provide the first glutamatergic synapses to adult-born dentate granule cells. J Neurosci, 34(6): 2349–2354

Chiu C Q, Castillo P E (2008). Input-specific plasticity at excitatory synapses mediated by endocannabinoids in the dentate gyrus. Neuropharmacology, 54(1): 68–78

Coulter D A, Carlson G C (2007). Functional regulation of the dentate gyrus by GABA-mediated inhibition. Prog Brain Res, 163: 235–243

Deng W, Aimone J B, Gage F H (2010). New neurons and new memories: how does adult hippocampal neurogenesis affect learning and memory? Nat Rev Neurosci, 11(5): 339–350

Dent J A, Galvin N J, Stanfield B B, Cowan W M (1983). The mode of termination of the hypothalamic projection to the dentate gyrus: an EM autoradiographic study. Brain Res, 258(1): 1–10

Diaz J, Ridray S, Mignon V, Griffon N, Schwartz J C, Sokoloff P (1997). Selective expression of dopamine D3 receptor mRNA in proliferative zones during embryonic development of the rat brain. J Neurosci, 17 (11): 4282–4292

Dieni C V, Nietz A K, Panichi R, Wadiche J I, Overstreet-Wadiche L (2013). Distinct determinants of sparse activation during granule cell maturation. J Neurosci, 33(49): 19131–19142

Domínguez-Escribà L, Hernández-Rabaza V, Soriano-Navarro M, Barcia J A, Romero F J, García-Verdugo J M, Canales J J (2006). Chronic cocaine exposure impairs progenitor proliferation but spares survival and maturation of neural precursors in adult rat dentate gyrus. Eur J Neurosci, 24(2): 586–594

Durand G M, Kovalchuk Y, Konnerth A (1996). Long-term potentiation and functional synapse induction in developing hippocampus. Nature, 381(6577): 71–75

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

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

Espósito M S, Piatti V C, Laplagne D A, Morgenstern N A, Ferrari C C, Pitossi F J, Schinder A F (2005). Neuronal differentiation in the adult hippocampus recapitulates embryonic development. J Neurosci, 25 (44): 10074–10086

Ewell L A, Jones M V (2010). Frequency-tuned distribution of inhibition in the dentate gyrus. J Neurosci, 30(38): 12597–12607

Frotscher M (1991). Target cell specificity of synaptic connections in the hippocampus. Hippocampus, 1(2): 123–130

Gage F H (2000). Mammalian neural stem cells. Science, 287(5457): 1433–1438

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

Ge S, Pradhan D A, Ming G L, Song H (2007). GABA sets the tempo for activity-dependent adult neurogenesis. Trends Neurosci, 30(1): 1–8

Gilbert P E, Kesner R P, Lee I (2001). Dissociating hippocampal subregions: double dissociation between dentate gyrus and CA1. Hippocampus, 11(6): 626–636

Goldowitz D, White W F, Steward O, Lynch G, Cotman C (1975). Anatomical evidence for a projection from the entorhinal cortex to the contralateral dentate gyrus of the rat. Exp Neurol, 47(3): 433–441

Gu Y, Arruda-Carvalho M, Wang J, Janoschka S R, Josselyn S A, Frankland P W, Ge S (2012). Optical controlling reveals time-dependent roles for adult-born dentate granule cells. Nat Neurosci, 15 (12): 1700–1706

Hafting T, Fyhn M, Molden S, Moser M B, Moser E I (2005). Microstructure of a spatial map in the entorhinal cortex. Nature, 436 (7052): 801–806

Hargreaves E L, Rao G, Lee I, Knierim J J (2005). Major dissociation between medial and lateral entorhinal input to dorsal hippocampus. Science, 308(5729): 1792–1794

Herman M A, Jahr C E (2007). Extracellular glutamate concentration in hippocampal slice. J Neurosci, 27(36): 9736–9741

Höglinger G U, Rizk P, Muriel M P, Duyckaerts C, Oertel W H, Caille I, Hirsch E C (2004). Dopamine depletion impairs precursor cell proliferation in Parkinson disease. Nat Neurosci, 7(7): 726–735

Isaac J T, Crair M C, Nicoll R A, Malenka R C (1997). Silent synapses during development of thalamocortical inputs. Neuron, 18(2): 269–280

Isaac J T, Nicoll R A, Malenka R C (1995). Evidence for silent synapses:

implications for the expression of LTP. Neuron, 15(2): 427–434

Itou Y, Nochi R, Kuribayashi H, Saito Y, Hisatsune T (2011). Cholinergic activation of hippocampal neural stem cells in aged dentate gyrus. Hippocampus, 21(4): 446–459

Jinde S, Zsiros V, Nakazawa K (2013). Hilar mossy cell circuitry controlling dentate granule cell excitability. Front Neural Circuits, 7: 14

Kaneko N, Okano H, Sawamoto K (2006). Role of the cholinergic system in regulating survival of newborn neurons in the adult mouse dentate gyrus and olfactory bulb. Genes Cells, 11(10): 1145–1159

Kilbride J, Rush A M, Rowan M J, Anwyl R (2001). Presynaptic group II mGluR inhibition of short-term depression in the medial perforant path of the dentate gyrus *in vitro*. J Neurophysiol, 85(6): 2509–2515

Kiss J, Csáki A, Bokor H, Shanabrough M, Leranth C (2000). The supramammillo-hippocampal and supramammillo-septal glutamatergic/aspartatergic projections in the rat: a combined [3H]D-aspartate autoradiographic and immunohistochemical study. Neuroscience, 97 (4): 657–669

Kullmann D M (1994). Amplitude fluctuations of dual-component EPSCs in hippocampal pyramidal cells: implications for long-term potentiation. Neuron, 12(5): 1111–1120

Kumamoto N, Gu Y, Wang J, Janoschka S, Takemaru K, Levine J, Ge S (2012) A role for primary cilia in glutamatergic synaptic integration of adult-born neurons. Nat Neurosci, 15: 399–405, S391

Kwon H B, Castillo P E (2008). Role of glutamate autoreceptors at hippocampal mossy fiber synapses. Neuron, 60(6): 1082–1094

Laplagne D A, Espósito M S, Piatti V C, Morgenstern N A, Zhao C, van Praag H, Gage F H, Schinder A F (2006). Functional convergence of neurons generated in the developing and adult hippocampus. PLoS Biol, 4(12): e409

Laurberg S, Sørensen K E (1981). Associational and commissural collaterals of neurons in the hippocampal formation (hilus fasciae dentatae and subfield CA3). Brain Res, 212(2): 287–300

Leranth C, Hajszan T (2007). Extrinsic afferent systems to the dentate gyrus. Prog Brain Res, 163: 63–84

Leutgeb J K, Leutgeb S, Moser M B, Moser E I (2007). Pattern separation in the dentate gyrus and CA3 of the hippocampus. Science, 315(5814): 961–966

Li Y, Stam F J, Aimone J B, Goulding M, Callaway E M, Gage F H (2013). Molecular layer perforant path-associated cells contribute to feed-forward inhibition in the adult dentate gyrus. Proc Natl Acad Sci USA, 110(22): 9106–9111

Liao D, Hessler N A, Malinow R (1995). Activation of postsynaptically silent synapses during pairing-induced LTP in CA1 region of hippocampal slice. Nature, 375(6530): 400–404

Lisman J E, Jensen O (2013). The θ-γ neural code. Neuron, 77(6): 1002–1016

Lübke J, Deller T, Frotscher M (1997). Septal innervation of mossy cells in the hilus of the rat dentate gyrus: an anterograde tracing and intracellular labeling study. Exp Brain Res, 114(3): 423–432

Lugert S, Basak O, Knuckles P, Haussler U, Fabel K, Götz M, Haas C A, Kempermann G, Taylor V, Giachino C (2010). Quiescent and active hippocampal neural stem cells with distinct morphologies respond selectively to physiological and pathological stimuli and aging. Cell Stem Cell, 6(5): 445–456

Ma D K, Jang M H, Guo J U, Kitabatake Y, Chang M L, Pow-Anpongkul N, Flavell R A, Lu B, Ming G L, Song H (2009). Neuronal activity-induced Gadd45b promotes epigenetic DNA demethylation and adult neurogenesis. Science, 323(5917): 1074–1077

Ma W, Maric D, Li B S, Hu Q, Andreadis J D, Grant G M, Liu Q Y, Shaffer K M, Chang Y H, Zhang L, Pancrazio J J, Pant H C, Stenger D A, Barker J L (2000). Acetylcholine stimulates cortical precursor cell proliferation *in vitro* via muscarinic receptor activation and MAP kinase phosphorylation. Eur J Neurosci, 12(4): 1227–1240

Macek T A, Winder D G, Gereau R W 4th, Ladd C O, Conn P J (1996). Differential involvement of group II and group III mGluRs as autoreceptors at lateral and medial perforant path synapses. J Neurophysiol, 76(6): 3798–3806

Maglóczky Z, Acsády L, Freund T F (1994). Principal cells are the postsynaptic targets of supramammillary afferents in the hippocampus of the rat. Hippocampus, 4(3): 322–334

Manzoni O, Bockaert J (1995). Metabotropic glutamate receptors inhibiting excitatory synapses in the CA1 area of rat hippocampus. Eur J Neurosci, 7(12): 2518–2523

Manzoni O J, Castillo P E, Nicoll R A (1995). Pharmacology of metabotropic glutamate receptors at the mossy fiber synapses of the guinea pig hippocampus. Neuropharmacology, 34(8): 965–971

Marín-Burgin A, Mongiat L A, Pardi M B, Schinder A F (2012). Unique processing during a period of high excitation/inhibition balance in adult-born neurons. Science, 335(6073): 1238–1242

Markwardt S, Overstreet-Wadiche L (2008). GABAergic signalling to adult-generated neurons. J Physiol, 586(16): 3745–3749

Markwardt S J, Dieni C V, Wadiche J I, Overstreet-Wadiche L (2011). Ivy/neurogliaform interneurons coordinate activity in the neurogenic niche. Nat Neurosci, 14(11): 1407–1409

McNaughton B L (1980). Evidence for two physiologically distinct perforant pathways to the fascia dentata. Brain Res, 199(1): 1–19

Mignone J L, Kukekov V, Chiang A S, Steindler D, Enikolopov G (2004). Neural stem and progenitor cells in nestin-GFP transgenic mice. J Comp Neurol, 469(3): 311–324

Milner T A, Bacon C E (1989). Ultrastructural localization of somatostatin-like immunoreactivity in the rat dentate gyrus. J Comp Neurol, 290(4): 544–560

Ming G L, Song H (2011). Adult neurogenesis in the mammalian brain: significant answers and significant questions. Neuron, 70(4): 687–702

Mohapel P, Leanza G, Kokaia M, Lindvall O (2005). Forebrain acetylcholine regulates adult hippocampal neurogenesis and learning. Neurobiol Aging, 26(6): 939–946

Mongiat L A, Espósito M S, Lombardi G, Schinder A F (2009). Reliable activation of immature neurons in the adult hippocampus. PLoS ONE, 4(4): e5320

Moser E I, Roudi Y, Witter M P, Kentros C, Bonhoeffer T, Moser M B (2014). Grid cells and cortical representation. Nat Rev Neurosci, 15 (7): 466–481

Mosko S, Lynch G, Cotman C W (1973). The distribution of septal projections to the hippocampus of the rat. J Comp Neurol, 152(2): 163–174

Mu Y, Zhao C, Gage F H (2011). Dopaminergic modulation of cortical inputs during maturation of adult-born dentate granule cells. J Neurosci, 31(11): 4113–4123

Nakashiba T, Cushman J D, Pelkey K A, Renaudineau S, Buhl D L, McHugh T J, Rodriguez Barrera V, Chittajallu R, Iwamoto K S,

McBain C J, Fanselow M S, Tonegawa S (2012). Young dentate granule cells mediate pattern separation, whereas old granule cells facilitate pattern completion. Cell, 149(1): 188–201

Neunuebel J P, Knierim J J (2012). Spatial firing correlates of physiologically distinct cell types of the rat dentate gyrus. J Neurosci, 32(11): 3848–3858

Nochi R, Kato T, Kaneko J, Itou Y, Kuribayashi H, Fukuda S, Terazono Y, Matani A, Kanatani S, Nakajima K, Hisatsune T (2012). Involvement of metabotropic glutamate receptor 5 signaling in activity-related proliferation of adult hippocampal neural stem cells. Eur J Neurosci, 36(3): 2273–2283

Overstreet Wadiche L, Bromberg D A, Bensen A L, Westbrook G L (2005). GABAergic signaling to newborn neurons in dentate gyrus. J Neurophysiol, 94(6): 4528–4532

Overstreet-Wadiche L, Bensen A L, Westbrook G L (2006). Delayed development of adult-generated granule cells in dentate gyrus. J Neurosci, 26(8): 2326–2334

Overstreet-Wadiche L S, Westbrook G L (2006). Functional maturation of adult-generated granule cells. Hippocampus, 16(3): 208–215

Park J H, Enikolopov G (2010). Transient elevation of adult hippocampal neurogenesis after dopamine depletion. Exp Neurol, 222(2): 267–276

Petersen R P, Moradpour F, Eadie B D, Shin J D, Kannangara T S, Delaney K R, Christie B R (2013). Electrophysiological identification of medial and lateral perforant path inputs to the dentate gyrus. Neuroscience, 252: 154–168

Piatti V C, Davies-Sala M G, Espósito M S, Mongiat L A, Trinchero M F, Schinder A F (2011). The timing for neuronal maturation in the adult hippocampus is modulated by local network activity. J Neurosci, 31(21): 7715–7728

Regan J, Smalley M (2007). Prospective isolation and functional analysis of stem and differentiated cells from the mouse mammary gland. Stem Cell Rev, 3(2): 124–136

Regan M R, Huang Y H, Kim Y S, Dykes-Hoberg M I, Jin L, Watkins A M, Bergles D E, Rothstein J D (2007). Variations in promoter activity reveal a differential expression and physiology of glutamate transporters by glia in the developing and mature CNS. J Neurosci, 27(25): 6607–6619

Renzel R, Sadek A R, Chang C H, Gray W P, Seifert G, Steinhäuser C (2013). Polarized distribution of AMPA, but not GABAA, receptors in radial glia-like cells of the adult dentate gyrus. Glia, 61(7): 1146–1154

Sahay A, Wilson D A, Hen R (2011). Pattern separation: a common function for new neurons in hippocampus and olfactory bulb. Neuron, 70(4): 582–588

Schmidt-Hieber C, Jonas P, Bischofberger J (2004). Enhanced synaptic plasticity in newly generated granule cells of the adult hippocampus. Nature, 429(6988): 184–187

Schmidt-Salzmann C, Li L, Bischofberger J (2014). Functional properties of extrasynaptic AMPA and NMDA receptors during postnatal hippocampal neurogenesis. J Physiol, 592(Pt 1): 125–140

Sloviter R S, Lømo T (2012). Updating the lamellar hypothesis of hippocampal organization. Front Neural Circuits, 6: 102

Snyder J S, Ferrante S C, Cameron H A (2012). Late maturation of adult-born neurons in the temporal dentate gyrus. PLoS ONE, 7(11): e48757

Soltesz I, Bourassa J, Deschênes M (1993). The behavior of mossy cells of the rat dentate gyrus during theta oscillations *in vivo*. Neuroscience, 57(3): 555–564

Song J, Sun J, Moss J, Wen Z, Sun G J, Hsu D, Zhong C, Davoudi H, Christian K M, Toni N, Ming G L, Song H (2013). Parvalbumin interneurons mediate neuronal circuitry-neurogenesis coupling in the adult hippocampus. Nat Neurosci, 16(12): 1728–1730

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

Spalding K L, Bhardwaj R D, Buchholz B A, Druid H, Frisén J (2005). Retrospective birth dating of cells in humans. Cell, 122(1): 133–143

Steward O (1976). Topographic organization of the projections from the entorhinal area to the hippocampal formation of the rat. J Comp Neurol, 167(3): 285–314

Steward O, Scoville S A (1976). Cells of origin of entorhinal cortical afferents to the hippocampus and fascia dentata of the rat. J Comp Neurol, 169(3): 347–370

Stone S S, Teixeira C M, Zaslavsky K, Wheeler A L, Martinez-Canabal A, Wang A H, Sakaguchi M, Lozano A M, Frankland P W (2011). Functional convergence of developmentally and adult-generated granule cells in dentate gyrus circuits supporting hippocampus-dependent memory. Hippocampus, 21(12): 1348–1362

Suh H, Consiglio A, Ray J, Sawai T, D'Amour K A, Gage F H (2007). *In vivo* fate analysis reveals the multipotent and self-renewal capacities of Sox^{2+} neural stem cells in the adult hippocampus. Cell Stem Cell, 1(5): 515–528

Suh H, Deng W, Gage F H (2009). Signaling in adult neurogenesis. Annu Rev Cell Dev Biol, 25(1): 253–275

Tashiro A, Sandler V M, Toni N, Zhao C, Gage F H (2006). NMDA-receptor-mediated, cell-specific integration of new neurons in adult dentate gyrus. Nature, 442(7105): 929–933

Temprana S G, Mongiat L A, Yang S M, Trinchero M F, Alvarez D D, Kropff E, Giacomini D, Beltramone N, Lanuza G M, Schinder A F (2015). Delayed coupling to feedback inhibition during a critical period for the integration of adult-born granule cells. Neuron, 85(1): 116–130

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

Tozuka Y, Fukuda S, Namba T, Seki T, Hisatsune T (2005). GABAergic excitation promotes neuronal differentiation in adult hippocampal progenitor cells. Neuron, 47(6): 803–815

van Groen T, Miettinen P, Kadish I (2003). The entorhinal cortex of the mouse: organization of the projection to the hippocampal formation. Hippocampus, 13(1): 133–149

Vivar C, Potter M C, Choi J, Lee J Y, Stringer T P, Callaway E M, Gage F H, Suh H, van Praag H (2012). Monosynaptic inputs to new neurons in the dentate gyrus. Nat Commun, 3: 1107

Vivar C, Potter M C, van Praag H (2013). All about running: synaptic plasticity, growth factors and adult hippocampal neurogenesis. Curr Top Behav Neurosci, 15: 189–210

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

Vogt K E, Regehr W G (2001). Cholinergic modulation of excitatory synaptic transmission in the CA3 area of the hippocampus. J Neurosci, 21(1): 75–83

Wang L P, Kempermann G, Kettenmann H (2005). A subpopulation of precursor cells in the mouse dentate gyrus receives synaptic GABAergic input. Mol Cell Neurosci, 29(2): 181–189

Wang S, Scott B W, Wojtowicz J M (2000). Heterogenous properties of dentate granule neurons in the adult rat. J Neurobiol, 42(2): 248–257

Witter M P (2007). The perforant path: projections from the entorhinal cortex to the dentate gyrus. Prog Brain Res, 163: 43–61

Witter M P, Amaral D G (1991). Entorhinal cortex of the monkey: V. Projections to the dentate gyrus, hippocampus, and subicular complex. J Comp Neurol, 307(3): 437–459

Witter M P, Van Hoesen G W, Amaral D G (1989). Topographical organization of the entorhinal projection to the dentate gyrus of the monkey. J Neurosci, 9(1): 216–228

Wu G, Malinow R, Cline H T (1996). Maturation of a central glutamatergic synapse. Science, 274(5289): 972–976

Zhao C, Deng W, Gage F H (2008). Mechanisms and functional implications of adult neurogenesis. Cell, 132(4): 645–660

Zhao C, Teng E M, Summers R G Jr, Ming G L, Gage F H (2006). Distinct morphological stages of dentate granule neuron maturation in the adult mouse hippocampus. J Neurosci, 26(1): 3–11

Zhao S, Zhou Y, Gross J, Miao P, Qiu L, Wang D, Chen Q, Feng G (2010). Fluorescent labeling of newborn dentate granule cells in GAD67-GFP transgenic mice: a genetic tool for the study of adult neurogenesis. PLoS ONE, 5(9): 5

Zhou C, Wen Z X, Shi D M, Xie Z P (2004). Muscarinic acetylcholine receptors involved in the regulation of neural stem cell proliferation and differentiation *in vitro*. Cell Biol Int, 28(1): 63–67