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GABA sets the tempo for activity-dependent adult neurogenesis

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GABA, a major inhibitory neurotransmitter in the adult brain, activates synaptic and extrasynaptic $GABA_A$ receptors, causing hyperpolarization of mature neurons. As in the embryonic nervous system, GABA depolarizes neural progenitors and immature neurons in the adult brain. Several recent studies have suggested that GABA has crucial roles in regulating different steps of adult neurogenesis, including proliferation of neural progenitors, migration and differentiation of neuroblasts, and synaptic integration of newborn neurons. Here, we review recent findings on how GABA regulates adult neurogenesis in the subventricular zone of the lateral ventricles and in the dentate gyrus of the hippocampus. We also discuss an emerging view that GABA serves as a key mediator of neuronal activity in setting the tempo of adult neurogenesis.

Introduction

Activity-dependent structural reorganization is a fundamental mechanism of adult neural plasticity [1]. A striking example of such anatomical change is the functional integration of newly generated neurons in the adult central nervous system (CNS). Since the pioneering work of Altman and colleagues [2], numerous studies have repeatedly demonstrated that active neurogenesis, a process that generates functional neurons from neural progenitor and/or stem cells (NPCs), occurs throughout life in discrete brain regions of all mammals, including humans [3,4]. Adult neurogenesis recapitulates the complete process of neuronal development in a mature CNS environment, from proliferation and neuronal fate specification of NPCs, through differentiation, migration and targeting of neurons, to synaptic integration and survival of new neurons [3,4]. In the olfactory system (Figure 1), NPCs located within the subventricular zone (SVZ) give rise to neuroblasts and immature neurons that migrate significant distances – first tangentially via the rostral migratory stream (RMS) and then radially into the olfactory bulb. These new neurons eventually differentiate into two types of interneuron: granule cells and periglomerular cells. In the dentate gyrus of the hippocampus (Figure 2), NPCs located within the subgranular zone (SGZ), at the border between the granule cell layer and hilar region, generate neuroblasts that migrate into the inner granule cell layer and differentiate into new granule cells. These new neurons send axonal projections through the hilus to the CA3 region, and receive glutamatergic and GABAergic synaptic inputs from the entorhinal cortex and local interneurons (Figure 2b).

Accumulating evidence suggests that different steps of adult neurogenesis are differentially regulated by physiological and pathological stimuli, including an enriched environment, stress, learning and seizures [3]. Although these studies also indicate that neuronal activity is essential for this adult form of structural plasticity [5], how electrical activity directly regulates NPCs and their progeny is largely unknown. Surprisingly GABA, a major inhibitory neurotransmitter in the adult brain, has emerged as a key player regulating multiple steps of adult neurogenesis. Here, we review how GABA regulates the development of NPCs and their neuronal progeny in the adult brain, and discuss a potential role for GABA as a sensor of neuronal activity and as a key regulator of the speed and extent of adult mammalian neurogenesis.

Modes of activation and classic roles of GABA in the adult brain

GABA was identified as the first clear example of an inhibitory neurotransmitter in the mammalian brain during the 1950s [6]. Three types of GABA receptor were subsequently identified [7] (Figure 3). $GABA_A$ receptors are bicuculline-sensitive ionotropic receptors that carry primarily Cl^- and, less efficiently, HCO_3^-. $GABA_C$ receptors are related Cl^--selective ionotropic receptors that are insensitive to bicuculline. $GABA_B$ receptors are G-protein-coupled metabotropic receptors that mediate presynaptic and postsynaptic inhibition by reducing Ca^{2+} currents and activating K^+ currents, respectively.

GABA-induced activation of $GABA_A$ receptors can lead to either depolarization or hyperpolarization of the target cell, largely depending on the Cl^- gradient across the cell membrane (Box 1). Because most mature CNS neurons have low concentrations of intracellular Cl^- ($[Cl^-]_i$) [7], release of GABA from synaptic vesicles locally activates synaptic $GABA_A$ receptors, leading to an influx of Cl^- and subsequent hyperpolarization of the target neurons (Figure 3). In addition, ambient GABA, which accumulates owing to diffusion from GABAergic synapses and non-synaptic release by neurons and astrocytes, also persistently activates the extrasynaptic $GABA_A$ receptors to generate tonic inhibitory currents in specific populations of mature neurons (Figure 3). These two modes of GABA action have been termed phasic and tonic activation, respectively [8,9].

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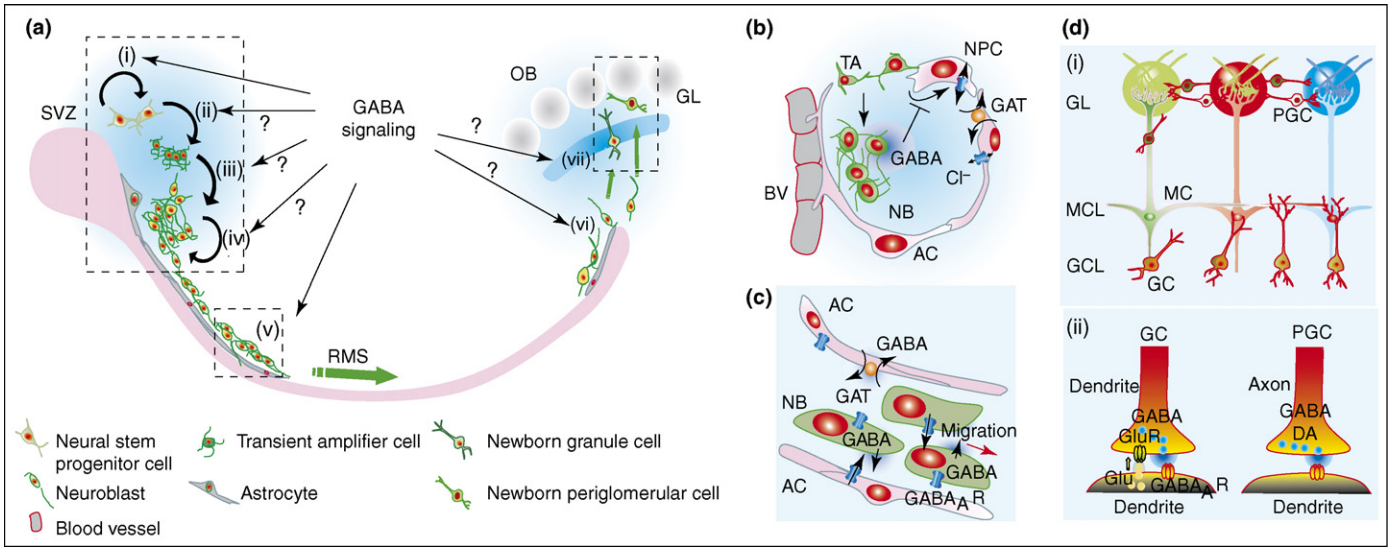

Figure 1. Generation of new olfactory interneurons during adult neurogenesis. **(a)** Sequential steps involved in generating functional and integrated olfactory bulb interneurons from neural progenitor and stem cells (NPCs) in the adult subventricular zone (SVZ). (i) Proliferation of NPCs, which exhibit some properties of astrocytes. (ii,iii) Differentiation of NPCs into neuroblasts, via the stage of transient amplifying cells. (iv) Proliferation of neuroblasts. (v) Tangential migration of neuroblasts in a chain through a tube formed by astrocytes within the rostral migratory stream (RMS). (vi) Radial migration of immature neurons into the olfactory bulb (OB). (vii) Synaptic integration of new interneurons into the existing olfactory bulb circuitry. GABA signaling might regulate multiple steps, some of which remain to be demonstrated experimentally (indicated by question marks). The boxes regions outlined are shown in more detail in (b–d). Additional abbreviation: GL, glomerular layer. **(b)** Cell–cell interactions in the adult SVZ. GABA released from neuroblasts (NB) negatively regulates the proliferation of NPCs. Reuptake of GABA is controlled by a GABA transporter (GAT) expressed in NPCs. Additional abbreviations: BV, blood vessel; TA, transient amplifying cell. **(c)** Cell–cell interactions in the RMS. Neuroblasts migrate in tight association as a chain ensheathed by astrocytes (AC). GABA released by neuroblasts in a soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor (SNARE)-independent fashion negatively regulates the speed of neuroblast migration. Ensheathing astrocytes express GAT and so control the ambient level of GABA through GABA reuptake. **(d)** Cellular organization in the olfactory bulb. (i) Mitral cells (MC) and tufted cells (not shown) are output neurons in the olfactory bulb that are innervated by two types of interneuron: periglomerular cells (PGC) and granule cells (GC). Once new neurons reach the olfactory bulb, they detach from the migratory chains and invade the overlaying layers, where they differentiate into granule cells [in the deep layer of the olfactory bulb, the granule cell layer (GCL)] or periglomerular cells [in the most superficial layer, the glomerular layer (GL)]. Additional abbreviation: MCL, mitral cell layer. (ii) Granule cells in the OB are unusual interneurons that lack an axon and instead release GABA from dendritic spines to act on $GABA_A$ receptors ($GABA_AR$) on dendrites of mitral or tufted cells. These are specialized reciprocal synapses, in that glutamate (Glu) is also released from the mitral or tufted cell dendrites to stimulate ionotropic glutamate receptors (GluR) on the granule cells. Some periglomerular neurons release dopamine (DA) in addition to GABA.

Pioneering studies by Ben-Ari, Kriegstein and others have shown that GABA is also a developmental signal during stages of embryonic neurogenesis, including progenitor proliferation, neuronal migration and neurite outgrowth [7,10,11]. This is largely attributed to the depolarizing effect of GABA on NPCs and immature neurons because of their high $[Cl^-]_i$ [7] (Box 1). Recent studies have shown that GABA is also excitatory for some mature neurons in established brain networks, owing to changes in Cl^- homeostasis caused by neuronal activation or under pathological conditions [12].

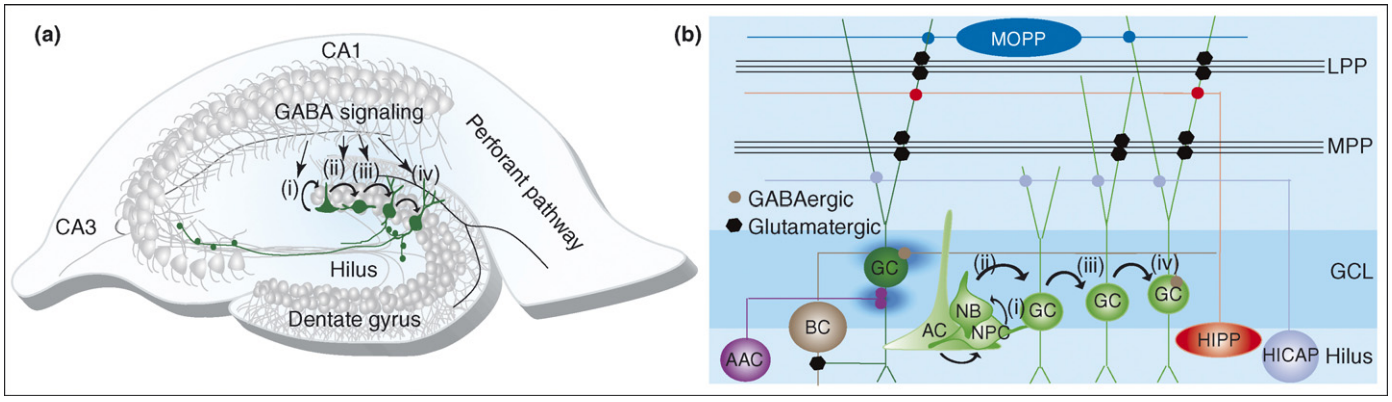

Figure 2. Adult neurogenesis in the dentate gyrus of the hippocampus. **(a)** Sequential steps involved in generating functional and integrated new granule cells from neural progenitor and stem cells (NPCs) in the adult hippocampus. (i) Proliferation of NPCs localized in the subgranular zone (SGZ). (ii) Differentiation of NPCs into neuroblasts and immature neurons. (iii) Migration and axonal and dendritic development of new granule cells. (iv) Synaptic integration of new granule cells into the existing hippocampal circuitry. GABA can potentially regulate all of these steps. **(b)** Interaction between NPCs, immature neurons and local interneurons through GABA-mediated signaling. Cellular organization and synaptic connections within the dentate gyrus are shown. Newborn granule cells are sequentially integrated into the existing neuronal circuitry. (i) Radial-glia-like astrocytes (AC), NPCs and their neuroblast progeny (NB) are tonically activated by ambient GABA released from local interneurons. (ii) Immature granule cells (GC) receive GABAergic synaptic inputs at their dendrites in the inner molecular layer from hilar cells with axonal projections to the associational pathway (e.g. HICAP cells). (iii) New granule cells receive glutamatergic synaptic inputs from the medial and lateral perforant pathways (LPP and MPP) and GABAergic synaptic inputs in the outer molecular layer from hilar and local interneurons (HIPP cells and MOPP cells, respectively). (iv) Mature new granule cells receive somatic GABAergic synaptic inputs from basket cells (BC) and axoaxonic cells (AAC). Axoaxonic cells, which are also known as chandelier cells, are located within the granule cell layer (GCL) or SGZ and their axons form rows of 2–30 boutons on axon initiation segment of granule cells.

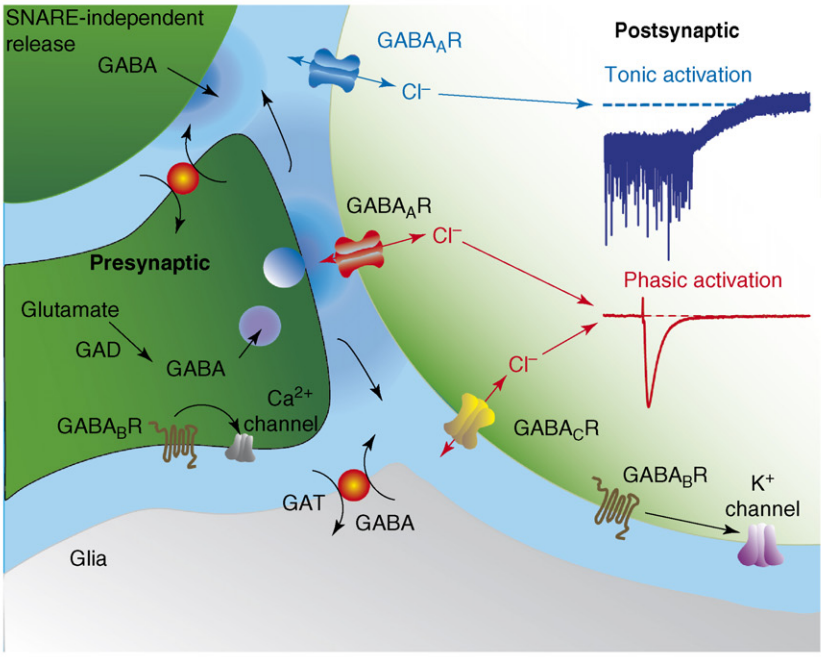

Figure 3. Sources of GABA and modes of GABA receptor activation. GABA is synthesized in GABAergic neurons from glutamate by glutamic acid decarboxylase (GAD) and released from the synaptic vesicles in a SNARE-dependent fashion. GABA is also released through non-vesicular means from neurons and glia in a SNARE-independent fashion. Reuptake of GABA occurs through GABA transporters (GAT) in both neurons and glia. GABA receptors are localized at both presynaptic and postsynaptic sites. $GABA_A$ receptors ($GABA_AR$) are bicuculline-sensitive ionotropic receptors that conduct primarily Cl^-; $GABA_C$ receptors ($GABA_CR$) are related Cl^--selective ionotropic receptors that are insensitive to bicuculline. $GABA_B$ receptors ($GABA_BR$) are G-protein-coupled metabotropic receptors that cause presynaptic inhibition by suppressing Ca^{2+} influx and reducing transmitter release, and that cause postsynaptic inhibition by activating K^+ channels and membrane hyperpolarization. Synaptic release of GABA leads to activation of $GABA_A$ receptors that are clustered in the membrane immediately beneath the release site, resulting in a transient synaptic current (phasic activation, pathways shown in red). A low concentration of ambient GABA, which is accumulated by diffusion from synaptic sites and by non-vesicular release from neurons and glia, also activates high-affinity extrasynaptic $GABA_A$ receptors (tonic activation, pathways shown in blue). $GABA_A$ receptor activation can lead to either depolarization via efflux of Cl^- or hyperpolarization via influx of Cl^-, depending on the Cl^- gradient across the membrane.

Regulation of adult neurogenesis by GABA signaling

Adult neurogenesis seems to recapitulate the neuronal developmental processes of embryonic stages, despite significant differences in the local environment [13–16]. Recent technical advances in using retroviruses and developmentally regulated promoters to drive green fluorescent protein (GFP) expression have enabled visualization of newborn cells in living preparations for physiological characterization [17]. Electrophysiological analysis has revealed the expression of functional GABA receptors in NPCs and their neuronal progeny in both juvenile and adult animals [14,15,18–20]. Furthermore, GABA initially depolarizes these immature cells in the adult brain during the first 2–3 weeks of their neuronal development [13–15,21,22] (Figure 4b; Box 1). Adult-born neurons go through the same sequence of developmental milestones as occurs in the embryonic CNS: initial activation by ambient GABA, then activation by GABAergic synaptic inputs and, finally, activation by glutamatergic inputs [15,18,21–23]. This stereotypic developmental sequence sets the stage for GABA to be a key signal for regulating adult neurogenesis in response to neuronal activity.

Box 1. How GABA excites immature neurons

$GABA_A$ receptors carry primarily Cl^-, and the direction of flow across the membrane largely determines whether GABA depolarizes or hyperpolarizes target cells. Previous studies have identified two major Cl^- transporters that control Cl^- homeostasis in neurons, the Na^+–K^+–Cl^- co-transporter NKCC1 (a Cl^- accumulator) and the K^+–Cl^- co-transporter KCC2 (a Cl^- exporter) [7,10]. During embryonic development, NKCC1 is expressed in NPCs and immature neurons, whereas little KCC2 is expressed, resulting in high neuronal $[Cl^-]_i$. During embryonic development and maturation, neurons downregulate NKCC1 expression and upregulate KCC2 expression, resulting in low $[Cl^-]_i$ in most mature neurons. The same expression pattern of NKCC1 and KCC2 occurs during adult neurogenesis [15] (Figure 4a). Electrophysiological studies [15] have further demonstrated that the reversal potential for GABA-induced currents (E_{GABA}) in newborn granule cells in the adult brain gradually decreases during maturation (Figure 4b), indicating a developmentally regulated decrease of $[Cl^-]_i$ (Figure 4c). However, the resting membrane potential (V_{rest}) decreases only slightly over this period (Figure 4b). Because V_{rest} is significantly more negative than E_{GABA} during the first two weeks after a granule cell is born, $GABA_A$ receptor activation of these cells leads to Cl^- efflux and membrane depolarization. However, synaptically integrated newborn granule cells and mature neurons have a low $[Cl^-]_i$; therefore, $GABA_A$ receptor activation leads to Cl^- influx and membrane hyperpolarization.

Progenitor proliferation

Adult NPCs are localized within specialized microenvironments or niches [24,25] that consist of multiple cell types, including endothelial cells of blood vessels [26], astrocytes [25] and immature progeny of NPCs [27]. A decision to self-renew, differentiate or be quiescent requires NPCs to decipher multiple signals within the niche.

Within the adult SVZ, some glial fibrillary acidic protein (GFAP)-expressing cells exhibit NPC properties and generate neuroblasts [24] (Figure 1b). Using electrophysiological recordings from acute slices, Bordey and colleagues

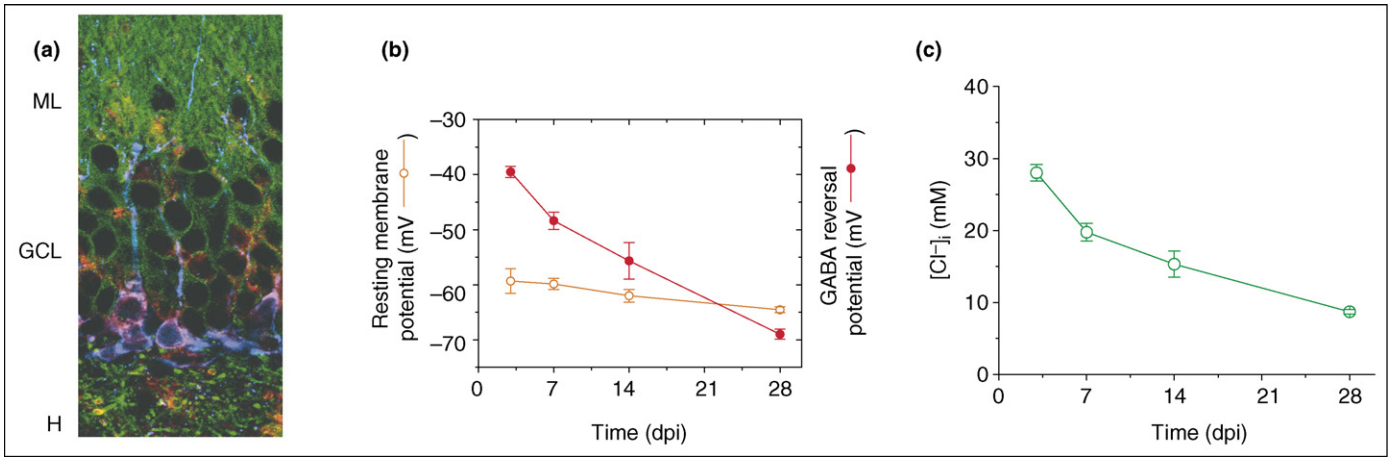

Figure 4. Effects of GABA on newborn neurons during adult neurogenesis. **(a)** Expression of a Na^+–K^+–Cl^- co-transporter (NKCC1) and a K^+–Cl^- co-transporter (KCC2) in the dentate gyrus of adult mouse brain. Confocal images of immunostaining for NKCC1 (red), KCC2 (green) and doublecortin (DCX, a marker of immature neurons; blue). Abbreviations: GCL, granule cell layer; H, hilus; ML, molecular layer. **(b)** Time course of the $GABA_A$-receptor equilibrium potential and resting membrane potential of newborn granule cells during their development in the adult brain. Data are derived from electrophysiological recordings of newborn dentate granule cells at different days post infection (dpi) with retroviruses to express enhanced green fluorescent protein (EGFP). **(c)** Calculated $[Cl^-]_i$ of newborn granule neurons during their maturation in the adult brain. Modified, with permission, from Ref. [15].

have demonstrated that functional $GABA_A$ receptors are expressed in NPCs of the SVZ in juvenile animal brains [27]. It is known that GABA depolarizes NPCs and inhibits their proliferation during embryonic development [28]. Whether GABA also depolarizes postnatal NPCs in the SVZ remains to be demonstrated. Interestingly, $GABA_A$ receptors in NPCs are tonically activated by ambient GABA released from neuroblasts in a non-synaptic, non-vesicular fashion [27]. Furthermore, GABA signaling seems to limit the progression of NPCs through the cell cycle, because pharmacological inhibition of $GABA_A$ receptors increases DNA synthesis in these NPCs in a slice-culture preparation [27]. Thus, GABA released from neuroblasts could provide a negative-feedback signal to control the proliferation of NPCs through tonic activation of their $GABA_A$ receptors (Figure 1b). GABA might also regulate the proliferation of neuroblasts within the postnatal SVZ. It was shown that GABA negatively regulates proliferation of neuroblasts in the postnatal striatum [29]. In addition, GABA transporters (GATs) are expressed in NPCs in the adult SVZ; such expression could regulate the local ambient GABA levels and, thus, control NPC proliferation through a negative-feedback loop (Figure 1b,c). Taken together, GABA could be a negative signal for proliferation, to ensure that the proper numbers of new NPCs and their progeny are generated.

Within the adult SGZ, radial-glia-like astrocytes also serve as NPCs and give rise to neuroblasts [24] (Figure 2b). Electrophysiological analysis of retrovirally labeled NPCs and immature neurons in the SGZ have shown that, within 2–3 weeks of their birth, these cells have a resting membrane potential around −60 mV and are depolarized by GABA because of their high $[Cl^-]_i$ [15] (Figure 4b,c). Interestingly, these immature cells also exhibit a depolarizing tonic response to GABA [15]. In this case, the ambient GABA could come from local interneurons (Figure 2b), because stimulation of interneurons leads to an increase in tonic activation [15]. Two independent studies have further shown that SGZ NPCs that expressed GFP under control of the nestin promoter in adult transgenic mice exhibited both spontaneous and evoked GABAergic synaptic transmission [14,20]. Whether tonic and/or phasic GABA activation also regulates the proliferation of the NPCs and neuroblasts in the SGZ remains to be determined.

Fate specification and differentiation

Adult neurogenesis begins with NPCs in the SVZ or SGZ and results in new granule cells in the dentate gyrus or new interneurons in olfactory bulb, respectively [3,4]. Kempermann and colleagues have defined stages of hippocampal neurogenesis using a series of immunohistological markers [30,31]. This characterization has provided a blueprint for determining how different intrinsic and extrinsic cues affect specific phases of adult neurogenesis. Currently, it is unknown whether GABA regulates neuronal or glial fate choice of adult NPCs in the SVZ and SGZ. Interestingly, application of GABA to acute slices of brain increases the expression of the transcription factor NeuroD in nestin-expressing NPCs within the SGZ [14]. NeuroD is a positive regulator of neuronal differentiation that mediates the terminal differentiation of granule cells in the dentate gyrus [14]. Further *in vivo* studies using $GABA_A$ receptor agonists [32] support the notion that excitatory activity of GABA increases the tempo and extent of neuronal differentiation during adult hippocampal neurogenesis.

Neuronal migration

Immature neurons generated from SVZ NPCs migrate significant distances: first tangentially via the RMS and then radially into the olfactory bulb (Figure 1a). In the RMS, which is tightly encapsulated by astrocytes, neuroblasts undergo a specialized type of migration in a chain formation (Figure 1b). Electrophysiological analysis has demonstrated that tangentially migrating neuroblasts from the SVZ, although still lacking synaptic input, already express functional $GABA_A$ receptors [18]. Furthermore, these neuroblasts have a resting membrane potential of around −59 mV and are depolarized by application of GABA [13,19]. Interestingly, increasing ambient GABA

concentrations, by enhancing non-vesicular GABA release from migrating neuroblasts or by blocking GABA uptake into ensheathing astrocytes, reduces the migration rate of the SVZ neuroblasts in acute slices from juvenile animals [33]. These findings suggest that, among migrating neuroblasts and ensheathing astrocytes, GABA is a signal that regulates the speed of neuronal migration during adult SVZ neurogenesis. Because SVZ neuroblasts also migrate to injury sites after stroke and in degenerative neurological diseases [3], it would be interesting to determine whether GABA also has similar role in pathological conditions.

In the dentate gyrus, immature neurons migrate only a short distance into the inner granule cell layer (Figure 2b). Neuroblasts and immature neurons seem to migrate along the radial fibers of radial glia-like NPCs that extend through the granule cell layer [34]. In slice cultures of embryonic brains, activation of $GABA_C$ and $GABA_B$ receptors promotes radial migration of postmitotic neurons out of the ventricular zone and intermediate zone, whereas activation of $GABA_A$ receptors serves as a stop signal once cells have reached the cortical plate [35]. Whether GABA also regulates neuronal migration during adult hippocampal neurogenesis has yet to be determined. It will also be interesting to determine whether GABA signaling is involved in the aberrant migration of new granule cells into the hilus after seizures induced by kainic acid or pilocarpine in animal models [36], because extracellular GABA levels are elevated after epilepsy [37–40].

Synaptic integration

New neurons become integrated into the existing adult neuronal circuitry in the olfactory bulb [18,41] and dentate gyrus [15,23,42] within several weeks of birth. Recent *in vivo* experiments suggest that GABA-mediated activation is essential for the dendritic development and synaptic integration of newborn granule cells in the adult hippocampus [15]. Using retrovirus-mediated expression of short-hairpin RNA (shRNA) in adult-born neurons to knock down endogenous expression of the Na^+–K^+–$2Cl^-$ cotransporter NKCC1 (which accumulates Cl^- within cells; Box 1), it is possible to reduce $[Cl^-]_i$ significantly, such that GABA causes hyperpolarization instead of depolarization of these newborn cells [15]. Confocal imaging and electrophysiological analysis have shown that, in the absence of GABA-induced depolarization, newborn neurons exhibit significant defects in their dendritic development with delayed formation of GABAergic and glutamatergic synapses in the adult brain [15].

Using pro-opiomelanocortin (POMC)-GFP mice, in which a specific population of newborn neurons is labeled [43], Westbrook and colleagues have shown that development of newborn granule cells takes much longer in the adult brain than in neonates [44]. It was suggested that the prolonged development is partially due to the reduced depolarizing GABA-mediated network activity in the adult brain. Interestingly, pilocarpine-induced seizures greatly accelerate the dendritic development and synaptic integration of newborn granule cells in adult mice [45]. Because seizures also increase the local ambient GABA levels [37–40], it would be interesting to determine the role of GABA-mediated activation in these pathological conditions.

GABA signaling as a sensor of neuronal activity during adult neurogenesis

Neurogenesis is classically thought to be regulated by neuronal activity [46], and many *in vivo* manipulations that influence electrical activity also affect adult neurogenesis [5]. For example, activation of the mossy-fiber pathway [47] increases hippocampal neurogenesis, whereas partial septohippocampal denervation [48] or lesions of the fimbria and fornix [49] have an opposite effect. Electroconvulsive shock [50,51] and seizures [52] increase NPC proliferation in the SGZ, and prolonged seizures increase neuroblast proliferation in the SVZ [53]. Seizures also lead to accelerated dendritic development and integration of newborn granule neurons in the adult brain [45]. In addition, an enriched environment and some learning paradigms regulate NPC proliferation and/or survival of newborn neurons during adult olfactory bulb and hippocampal neurogenesis [4,54,55].

As we have already discussed, GABA might enable NPCs and their neuronal progeny to respond to activity and so might control the tempo and extent of adult neurogenesis at stages from proliferation of NPCs, through migration and differentiation of neuroblasts, to synaptic integration of new neurons. Ambient GABA activates NPCs and immature neurons when they have not yet received any synaptic input, and is likely to continue to have a pivotal role even beyond the initial formation of GABAergic synapses [15]. Electrophysiological analysis has determined that the total charge carried by hyperpolarizing action of tonic GABA in mature granule cells is several times higher than that attributed to phasic activation by GABA [56]. Tonic activation by GABA is also likely to be the principal cause of depolarization when GABAergic synaptic inputs are relatively weak [15].

The amount of ambient GABA in the adult SVZ is regulated by the activity of neuroblasts and controls the rate of NPC proliferation and neuroblast migration [27,33]. In the adult SGZ, the ambient GABA level is controlled by the activity of local interneurons and regulates the differentiation and synaptic integration of newborn neurons [14,15]. Because of extensive recurrent connections between principal neurons and interneurons within the dentate gyrus [57] (Figure 2b), the ambient GABA levels might reflect the general activity of local neuronal networks and could therefore serve as a general indicator of the dynamic neuronal network activity. Once GABAergic synaptic transmission becomes stronger, synaptic release of GABA could act as an activity sensor in an input-specific manner to regulate neuronal development. It is interesting that in adult-born neurons somatic GABAergic synapses are formed later than glutamatergic synapses are [23] (Figure 2b), suggesting additional roles for GABA after initial synaptic integration of new neurons.

GABA activation can be modulated during both its tonic and phasic modes of action. For example, the major targets of cholinergic inputs in the dentate gyrus are interneurons [58] and it is possible that ACh promotes neurogenesis [59] through GABA release from interneurons receiving cholinergic inputs. 5-Hydroxytryptamine (5-HT or serotonin) inputs also target interneurons in the dentate gyrus [60] and regulate adult hippocampal neurogenesis [31].

GABA-mediated activation can also be regulated by aberrant activity in the adult brain, as during seizures [37–40], and by neurosteroids through non-synaptic pathways [8,61]. In addition, modulation of local astrocyte properties, by changing their uptake and/or release of GABA, could also potentially regulate adult neurogenesis.

Potential mechanisms that underlie GABA-mediated regulation of adult neurogenesis

Little is known about how GABA-mediated stimulation regulates adult neurogenesis. For example, the molecular compositions of the receptors and the downstream signaling pathways have yet to be determined.

Receptor activation

The expression profiles of different GABA receptors during adult neurogenesis have not been well characterized. In particular, $GABA_A$ receptors can be assembled from a large number of diverse subunits [7]. It is generally believed that GABA activation by the phasic or tonic mode is mediated by different $GABA_A$ receptors that have different subunit compositions [8]. Phasic GABA-mediated transmission is produced by short lived (<1 ms) high concentrations of GABA (0.3–1.0 mM) in the synaptic cleft, where $GABA_A$ receptors usually contain γ subunits, in particular γ2 subunits. By contrast, tonic activation is sustained and involves much lower concentrations of GABA; thus, subunits of the $GABA_A$ receptor that are responsible for tonic activation are likely to have high affinity for GABA and low desensitization probability [8,9]. $GABA_A$ receptors that contain δ or α5 subunits seem to have major roles in mediating tonic hyperpolarization of mature granule cells [61,62] and CA1 pyramidal neurons [62,63] in the adult hippocampus, respectively. However, tonic inhibition of cerebellar granule neurons is mediated by a high-affinity $GABA_A$ receptor that contains the α6 subunit [64]. It is unknown whether these subunits are also involved in tonic activation of newborn neurons in the adult brain. Identification of the specific subunit compositions would provide a basis for genetic manipulations to interfere specifically with either tonic or phasic activation by GABA, thus enabling the unique roles of these activation types to be examined during different steps of adult neurogenesis.

Downstream signaling

How does activation of GABA receptors lead to different behaviors of NPCs and their neuronal progeny? Ca^{2+}, a major second messenger of neurotransmitter receptor activation, is likely to have a significant role (Figure 5). Ca^{2+}-imaging analysis of adult SGZ nestin-expressing progenitors has shown that bath application of GABA induces elevation of Ca^{2+} levels in these cells [14]. Nickel ions, which block Ca^{2+}-channels, inhibit both the GABA-induced Ca^{2+} rise and expression of NeuroD in these progenitors [14]. Interfering with GABA-induced internal Ca^{2+} signaling also alters the speed of neuroblast migration in the SVZ [13].

At the early phase of adult neurogenesis, tonic activation by GABA leads to only a small change in the membrane potential [13,15] and is, thus, unlikely to activate most of

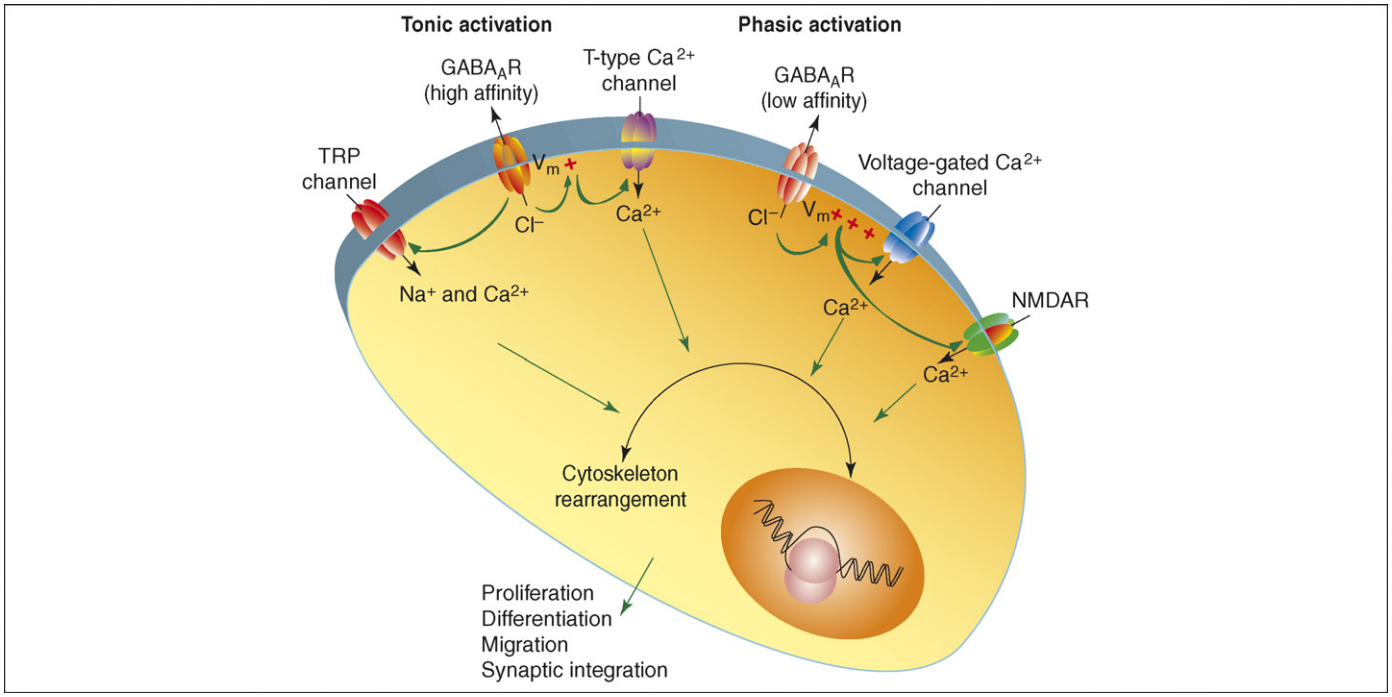

Figure 5. Potential mechanisms underlying $GABA_A$-receptor-mediated regulation of adult neurogenesis. There are two modes of GABA-mediated activation through $GABA_A$ receptors in NPCs and immature neurons. In the tonic activation mode, ambient GABA at low concentrations activates high-affinity extrasynaptic $GABA_A$ receptors, leading to Cl^- efflux. The resulting small depolarization can potentially stimulate low-voltage-activated T-type Ca^{2+} channels, causing Ca^{2+} influx. Other types of Ca^{2+}-permeable channel, such as transient receptor potential (TRP) channels, might also be activated in a voltage-independent fashion. In the phasic activation mode, synaptic release of GABA at high concentrations activates synaptic $GABA_A$ receptors, leading to Cl^- efflux and sufficient membrane depolarization to activate voltage-gated Ca^{2+} channels (e.g. L-type Ca^{2+} channels) and NMDA receptors (NMDAR). The resulting rise of intracellular Ca^{2+} activates downstream signaling cascades, leading to cytoskeleton rearrangement and activation of transcription, which are important for proliferation, differentiation, migration, development of axons and dendrites and synapse formation.

the voltage-gated Ca^{2+}-permeable channels. Interestingly, newborn neurons in the dentate gyrus express high levels of low-voltage-activated T-type Ca^{2+} channels [65]. These Ca^{2+} channels can be activated at below –57 mV [65], a value close to the resting membrane potential of immature neurons [15] (Figure 4b). Therefore, tonic activation by GABA could lead to sufficient membrane depolarization to activate these T-type Ca^{2+} channels (Figure 5). Stimulation of GABA receptors might also lead to the activation of other types of Ca^{2+}-permeable channels (e.g. transient potential channels) through different mechanisms (Figure 5), such as osmotic tension [66]. In addition to the influx of extracellular Ca^{2+}, tonic GABA-induced release of Ca^{2+} from internal stores can also be involved [33]. Future studies using, for example, shRNA-mediated knockdown or genetically modified mice, will directly test these possibilities.

Once neurons receive GABAergic synaptic inputs, the depolarization induced by synaptically released GABA could then cause sufficient membrane depolarization to activate other voltage-gated Ca^{2+} channels, such as L-type Ca^{2+} channels (Figure 5). *In vitro* studies have shown that activation of L-type Ca^{2+} channels in NPCs promotes their neuronal differentiation [67]. In addition, synaptic GABA-mediated activation can facilitate the opening of NMDA-receptor channels in response to glutamate and the subsequent Ca^{2+} influx – a role taken over by AMPA receptors later in development [68] (Figure 5).

During the past decade, we have gained significant knowledge about how Ca^{2+} signaling mediates neuronal development and plasticity [69,70]. Ca^{2+}-activated cascades can lead to both acute effects through cytoskeleton rearrangement and long-term effects by activation of transcriptional programs. Future studies are needed to determine how these pathways are differentially activated to regulate multiple steps of adult neurogenesis.

Concluding remarks

A traditional view of adult neural plasticity involves only postmitotic neuronal modifications and limited turnover of non-neuronal cells. The discovery of active adult neurogenesis across various mammalian species suggests a greater capacity for plasticity in the mature CNS than was previously imagined. The prospect of stimulating endogenous neurogenesis and integrating transplanted neurons also raises hope for new cell-replacement therapies to repair the mature CNS after injury or neurological diseases.

Adult neurogenesis is a complex process that is regulated by neuronal activity. GABA is emerging as a key signal within the neurogenic niche, enabling NPCs and their neuronal progeny to sense neuronal activity and regulate their development. There are many unanswered questions regarding GABA-mediated regulation of adult neurogenesis. Which temporal subunit compositions of $GABA_A$ receptors regulate different steps of adult neurogenesis? Is GABA-mediated activation also involved in the pathological stimulation of adult neurogenesis, in aberrant migration in the SGZ and SVZ, or even in neurogenesis outside these two regions? In addition, the roles of $GABA_B$ and $GABA_C$ receptors in adult neurogenesis remain largely unexplored. GABA might have important roles in other aspects of adult neurogenesis, such as neuronal fate specification of NPCs, axonal development of newborn neurons, and the formation of synapses between newborn neurons and their target neurons. Studies of GABA signaling in adult neurogenesis have so far served as an entry point for exploring the basic principles of neuronal development in the mature CNS. Future attempts to elucidate mechanisms that couple network activity to cellular differentiation face the challenge of developing and employing new methods to track and control circuit activity *in vivo*, and of determining how progenitor cells proliferate, differentiate and integrate in response to different known levels of physiological network activity. A better understanding of endogenous adult neurogenesis will bring great benefits in applying stem cell therapy to brain disorders.

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References

1 Kempermann, G. *et al.* (2000) Activity-dependent regulation of neuronal plasticity and self repair. *Prog. Brain Res.* 127, 35–48
2 Altman, J. and Das, G.D. (1965) Autoradiographic and histological evidence of postnatal hippocampal neurogenesis in rats. *J. Comp. Neurol.* 124, 319–335
3 Ming, G.L. and Song, H. (2005) Adult neurogenesis in the mammalian central nervous system. *Annu. Rev. Neurosci.* 28, 223–250
4 Lledo, P.M. *et al.* (2006) Adult neurogenesis and functional plasticity in neuronal circuits. *Nat. Rev. Neurosci.* 7, 179–193
5 Lehmann, K. *et al.* (2005) Offer and demand: proliferation and survival of neurons in the dentate gyrus. *Eur. J. Neurosci.* 21, 3205–3216
6 Roberts, E.A. (1988) *GABA and Benzodiazepine Receptors* (Squires, R., ed.), pp. 1–21, CRC
7 Owens, D.F. and Kriegstein, A.R. (2002) Is there more to GABA than synaptic inhibition? *Nat. Rev. Neurosci.* 3, 715–727
8 Farrant, M. and Nusser, Z. (2005) Variations on an inhibitory theme: phasic and tonic activation of $GABA_A$ receptors. *Nat. Rev. Neurosci.* 6, 215–229
9 Mody, I. and Pearce, R.A. (2004) Diversity of inhibitory neurotransmission through $GABA_A$ receptors. *Trends Neurosci.* 27, 569–575
10 Ben-Ari, Y. (2002) Excitatory actions of GABA during development: the nature of the nurture. *Nat. Rev. Neurosci.* 3, 728–739
11 Barker, J.L. *et al.* (1998) GABAergic cells and signals in CNS development. *Perspect. Dev. Neurobiol.* 5, 305–322
12 Marty, A. and Llano, I. (2005) Excitatory effects of GABA in established brain networks. *Trends Neurosci.* 28, 284–289
13 Wang, D.D. *et al.* (2003) GABA depolarizes neuronal progenitors of the postnatal subventricular zone via $GABA_A$ receptor activation. *J. Physiol.* 550, 785–800
14 Tozuka, Y. *et al.* (2005) GABAergic excitation promotes neuronal differentiation in adult hippocampal progenitor cells. *Neuron* 47, 803–815
15 Ge, S. *et al.* (2006) GABA regulates synaptic integration of newly generated neurons in the adult brain. *Nature* 439, 589–593
16 Bordey, A. (2006) Adult neurogenesis: basic concepts of signaling. *Cell Cycle* 5, 722–728
17 Song, H. *et al.* (2005) New neurons in the adult mammalian brain: synaptogenesis and functional integration. *J. Neurosci.* 25, 10366–10368
18 Carleton, A. *et al.* (2003) Becoming a new neuron in the adult olfactory bulb. *Nat. Neurosci.* 6, 507–518
19 Wang, D.D. *et al.* (2003) Biophysical properties and ionic signature of neuronal progenitors of the postnatal subventricular zone *in situ*. *J. Neurophysiol.* 90, 2291–2302

20 Wang, L.P. *et al.* (2005) A subpopulation of precursor cells in the mouse dentate gyrus receives synaptic GABAergic input. *Mol. Cell. Neurosci.* 29, 181–189
21 Overstreet Wadiche, L. *et al.* (2005) GABAergic signaling to newborn neurons in dentate gyrus. *J. Neurophysiol.* 94, 4528–4532
22 Karten, Y.J. *et al.* (2006) GABAergic signaling in young granule cells in the adult rat and mouse dentate gyrus. *Hippocampus* 16, 312–320
23 Esposito, M.S. *et al.* (2005) Neuronal differentiation in the adult hippocampus recapitulates embryonic development. *J. Neurosci.* 25, 10074–10086
24 Alvarez-Buylla, A. and Lim, D.A. (2004) For the long run: maintaining germinal niches in the adult brain. *Neuron* 41, 683–686
25 Ma, D.K. *et al.* (2005) Glial influences on neural stem cell development: cellular niches for adult neurogenesis. *Curr. Opin. Neurobiol.* 15, 514–520
26 Palmer, T.D. *et al.* (2000) Vascular niche for adult hippocampal neurogenesis. *J. Comp. Neurol.* 425, 479–494
27 Liu, X. *et al.* (2005) Nonsynaptic GABA signaling in postnatal subventricular zone controls proliferation of GFAP-expressing progenitors. *Nat. Neurosci.* 8, 1179–1187
28 LoTurco, J.J. *et al.* (1995) GABA and glutamate depolarize cortical progenitor cells and inhibit DNA synthesis. *Neuron* 15, 1287–1298
29 Nguyen, L. *et al.* (2003) Autocrine/paracrine activation of the $GABA_A$ receptor inhibits the proliferation of neurogenic polysialylated neural cell adhesion molecule-positive (PSA-NCAM+) precursor cells from postnatal striatum. *J. Neurosci.* 23, 3278–3294
30 Kempermann, G. *et al.* (2004) Milestones of neuronal development in the adult hippocampus. *Trends Neurosci.* 27, 447–452
31 Encinas, J.M. *et al.* (2006) Fluoxetine targets early progenitor cells in the adult brain. *Proc. Natl. Acad. Sci. U. S. A.* 103, 8233–8238
32 Li, G. and Pleasure, S.J. (2005) Morphogenesis of the dentate gyrus: what we are learning from mouse mutants. *Dev. Neurosci.* 27, 93–99
33 Bolteus, A.J. and Bordey, A. (2004) GABA release and uptake regulate neuronal precursor migration in the postnatal subventricular zone. *J. Neurosci.* 24, 7623–7631
34 Seri, B. *et al.* (2004) Cell types, lineage, and architecture of the germinal zone in the adult dentate gyrus. *J. Comp. Neurol.* 478, 359–378
35 Behar, T.N. *et al.* (2000) GABA receptor antagonists modulate postmitotic cell migration in slice cultures of embryonic rat cortex. *Cereb. Cortex* 10, 899–909
36 Parent, J.M. (2003) Injury-induced neurogenesis in the adult mammalian brain. *Neuroscientist* 9, 261–272
37 Ueda, Y. and Tsuru, N. (1995) Simultaneous monitoring of the seizure-related changes in extracellular glutamate and γ-aminobutyric acid concentration in bilateral hippocampi following development of amygdaloid kindling. *Epilepsy Res.* 20, 213–219
38 Cossart, R. *et al.* (2005) Multiple facets of GABAergic neurons and synapses: multiple fates of GABA signalling in epilepsies. *Trends Neurosci.* 28, 108–115
39 Naylor, D.E. *et al.* (2005) Trafficking of $GABA_A$ receptors, loss of inhibition, and a mechanism for pharmacoresistance in status epilepticus. *J. Neurosci.* 25, 7724–7733
40 Patrylo, P.R. *et al.* (2001) GABA uptake and heterotransport are impaired in the dentate gyrus of epileptic rats and humans with temporal lobe sclerosis. *J. Neurophysiol.* 85, 1533–1542
41 Belluzzi, O. *et al.* (2003) Electrophysiological differentiation of new neurons in the olfactory bulb. *J. Neurosci.* 23, 10411–10418
42 van Praag, H. *et al.* (2002) Functional neurogenesis in the adult hippocampus. *Nature* 415, 1030–1034
43 Overstreet, L.S. *et al.* (2004) A transgenic marker for newly born granule cells in dentate gyrus. *J. Neurosci.* 24, 3251–3259
44 Overstreet-Wadiche, L.S. *et al.* (2006) Delayed development of adult-generated granule cells in dentate gyrus. *J. Neurosci.* 26, 2326–2334
45 Overstreet-Wadiche, L.S. *et al.* (2006) Seizures accelerate functional integration of adult-generated granule cells. *J. Neurosci.* 26, 4095–4103
46 Linden, R. (1994) The survival of developing neurons: a review of afferent control. *Neuroscience* 58, 671–682
47 Derrick, B.E. *et al.* (2000) Increased granule cell neurogenesis in the adult dentate gyrus following mossy fiber stimulation sufficient to induce long-term potentiation. *Brain Res.* 857, 300–307
48 Van der Borght, K. *et al.* (2005) Input from the medial septum regulates adult hippocampal neurogenesis. *Brain Res. Bull.* 67, 117–125
49 Fontana, X. *et al.* (2006) Cell proliferation in the adult hippocampal formation of rodents and its modulation by entorhinal and fimbria-fornix afferents. *Cereb. Cortex* 16, 301–312
50 Madsen, T.M. *et al.* (2000) Increased neurogenesis in a model of electroconvulsive therapy. *Biol. Psychiatry* 47, 1043–1049
51 Scott, B.W. *et al.* (2000) Neurogenesis in the dentate gyrus of the rat following electroconvulsive shock seizures. *Exp. Neurol.* 165, 231–236
52 Parent, J.M. *et al.* (1997) Dentate granule cell neurogenesis is increased by seizures and contributes to aberrant network reorganization in the adult rat hippocampus. *J. Neurosci.* 17, 3727–3738
53 Parent, J.M. *et al.* (2002) Prolonged seizures increase proliferating neuroblasts in the adult rat subventricular zone-olfactory bulb pathway. *J. Neurosci.* 22, 3174–3188
54 van Praag, H. *et al.* (2000) Neural consequences of environmental enrichment. *Nat. Rev. Neurosci.* 1, 191–198
55 Leuner, B. *et al.* (2006) Is there a link between adult neurogenesis and learning? *Hippocampus* 16, 216–224
56 Nusser, Z. and Mody, I. (2002) Selective modulation of tonic and phasic inhibitions in dentate gyrus granule cells. *J. Neurophysiol.* 87, 2624–2628
57 Freund, T.F. and Buzsaki, G. (1996) Interneurons of the hippocampus. *Hippocampus* 6, 347–470
58 Ji, D. and Dani, J.A. (2000) Inhibition and disinhibition of pyramidal neurons by activation of nicotinic receptors on hippocampal interneurons. *J. Neurophysiol.* 83, 2682–2690
59 Mohapel, P. *et al.* (2005) Forebrain acetylcholine regulates adult hippocampal neurogenesis and learning. *Neurobiol. Aging* 26, 939–946
60 McMahon, L.L. and Kauer, J.A. (1997) Hippocampal interneurons are excited via serotonin-gated ion channels. *J. Neurophysiol.* 78, 2493–2502
61 Stell, B.M. *et al.* (2003) Neuroactive steroids reduce neuronal excitability by selectively enhancing tonic inhibition mediated by δ subunit-containing $GABA_A$ receptors. *Proc. Natl. Acad. Sci. U. S. A.* 100, 14439–14444
62 Scimemi, A. *et al.* (2005) Multiple and plastic receptors mediate tonic $GABA_A$ receptor currents in the hippocampus. *J. Neurosci.* 25, 10016–10024
63 Caraiscos, V.B. *et al.* (2004) Tonic inhibition in mouse hippocampal CA1 pyramidal neurons is mediated by α5 subunit-containing γ-aminobutyric acid type A receptors. *Proc. Natl. Acad. Sci. U. S. A.* 101, 3662–3667
64 Brickley, S.G. *et al.* (2001) Adaptive regulation of neuronal excitability by a voltage-independent potassium conductance. *Nature* 409, 88–92
65 Schmidt-Hieber, C. *et al.* (2004) Enhanced synaptic plasticity in newly generated granule cells of the adult hippocampus. *Nature* 429, 184–187
66 Chavas, J. *et al.* (2004) Osmotic tension as a possible link between $GABA_A$ receptor activation and intracellular calcium elevation. *Neuron* 44, 701–713
67 Deisseroth, K. *et al.* (2004) Excitation-neurogenesis coupling in adult neural stem/progenitor cells. *Neuron* 42, 535–552
68 Ben-Ari, Y. *et al.* (1997) $GABA_A$, NMDA and AMPA receptors: a developmentally regulated 'menage a trois'. *Trends Neurosci.* 20, 523–529
69 West, A.E. *et al.* (2001) Calcium regulation of neuronal gene expression. *Proc. Natl. Acad. Sci. U. S. A.* 98, 11024–11031
70 Konur, S. and Ghosh, A. (2005) Calcium signaling and the control of dendritic development. *Neuron* 46, 401–405