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To cite this article: Brent Asrican, Patricia Paez-Gonzalez, Joshua Erb & Chay T. Kuo (2016) Cholinergic circuit control of postnatal neurogenesis, *Neurogenesis*, 3:1, e1127310, DOI: 10.1080/23262133.2015.1127310

To link to this article: <https://doi.org/10.1080/23262133.2015.1127310>



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Published online: 04 Mar 2016.



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Cholinergic circuit control of postnatal neurogenesis

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ABSTRACT

New neuron addition via continued neurogenesis in the postnatal/adult mammalian brain presents a distinct form of nervous system plasticity. During embryonic development, precise temporal and spatial patterns of neurogenesis are necessary to create the nervous system architecture. Similar between embryonic and postnatal stages, neurogenic proliferation is regulated by neural stem cell (NSC)-intrinsic mechanisms layered upon cues from their local microenvironmental niche. Following developmental assembly, it remains relatively unclear what may be the key driving forces that sustain continued production of neurons in the postnatal/adult brain. Recent experimental evidence suggests that patterned activity from specific neural circuits can also directly govern postnatal/adult neurogenesis. Here, we review experimental findings that revealed cholinergic modulation, and how patterns of neuronal activity and acetylcholine release may differentially or synergistically activate downstream signaling in NSCs. Higher-order excitatory and inhibitory inputs regulating cholinergic neuron firing, and their implications in neurogenesis control are also considered.

ARTICLE HISTORY

Received 25 June 2015
Revised 16 November 2015
Accepted 30 November 2015

KEYWORDS



acetylcholine; ACh; adult neurogenesis; ChAT neuron; cholinergic circuit; neural stem cells; neurogenic niche; SVZ; SGZ; SEZ

Introduction

Resident neural stem cells (NSCs) in the brain present exciting possibilities for tissue regeneration and remodeling.^{1,2} During embryonic development, neurogenesis proceeds like clockwork, generating a full range of neurons in correct spatial and temporal sequences, enabling proper assembly of functional neural circuits. In the postnatal and adult mammalian brain, it is now well-accepted that NSCs are retained in discrete anatomical regions including the hippocampus and the walls of the lateral ventricles. Their continuous production of newborn neurons in the rodent lateral ventricular (LV) subventricular/subependymal zone (SVZ/SEZ) niche and in the hippocampal dentate gyrus subgranular zone (SGZ) (as well as potentially in the human striatum) offers endogenous sources for tissue regeneration and neural circuit plasticity.³⁻⁶ Maintaining a tissue stem cell population requires extra energy and resources, and when they acquire oncogenic mutations these proliferative cells

can become a source for tumor formation,^{7,8} contributing harmful sequelae to the host tissue.⁹ While the need for neurogenesis during development to build the nervous system is rather clear, it remains relatively unclear what biological processes may be driving and sustaining new neuron production in specific regions in the postnatal/adult mammalian brain.

Postnatal neurogenesis in rodents provides a tractable experimental model to tackle molecular/cellular-level mechanisms regulating addition of new interneurons into established neural circuits.¹⁰⁻¹⁴ In the SGZ, astrocyte-like type 1 NSCs give rise to transient type 2 proliferative progenitors, which then produce DCX⁺ cells that mature into local dentate granule neurons.^{15,16} Ongoing SGZ neurogenesis in rodents contributes importantly to memory processing¹⁷ and neurological disease modeling,¹⁸ as well as having significant parallels in the human brain.¹⁹ LV neurogenesis is mediated by CSF-contacting GFAP⁺ glia functioning as NSCs, producing Mash1⁺

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transiently amplifying progenitors which in turn differentiate into DCX⁺ neuroblasts that migrate to and become interneurons in the olfactory bulb.^{20,21} Generation of new neurons and glia from the LV niche contributes to experience-dependent plasticity in the postnatal brain,^{22,23} tissue remodeling after injury,^{24,25} and plays a critical role in olfactory-based rodent social behaviors.²⁶⁻²⁸ In the postnatal human brain, while some groups have reported potential olfactory bulb neurogenesis into adulthood,^{29,30} there is strong evidence that LV neurogenesis generates migrating interneurons for up to 2 y after birth.³¹ It has become increasingly clear that neurodevelopmental defects can be significant contributors to various brain pathologies later in life. Thus the analogous process in rodents can help us understand experimentally how NSC production of new neurons may be influenced by sensory/neural-circuit inputs during early postnatal human brain development.

It has been well-demonstrated that self-renewal of postnatal NSCs and their differentiation into neurons are controlled by conserved, cell-intrinsic molecular pathways.³²⁻³⁴ Extracellular factors and cell-cell interactions within the neurogenic microenvironmental niche also play critical roles regulating neurogenesis.³⁵⁻³⁸ Likewise, neurotransmitters such as GABA, glutamate, dopamine, and serotonin also contribute important modulatory roles during postnatal neurogenesis³⁹⁻⁴⁴; it has been generally assumed that neurotransmitters function through bulk release/non-synaptic mechanisms in the postnatal/adult neurogenic niches. In this fashion they are cytokine or growth factor-like, controlling NSC properties as neurotransmitters are leaked from nearby neuronal synaptic contacts following activity-dependent release. GABA spill-over from local parvalbumin⁺ interneuron regulating SGZ NSC proliferation/differentiation is an excellent example.⁴⁵⁻⁴⁷

Postnatal/adult NSCs often proliferate and differentiate in close proximity to neurons firing action potentials: the LV niche is anatomically adjacent to the striatum; and the SGZ niche is an integral part of the hippocampus. Thus neuronal activity patterns are attractive modulators for NSC proliferation and differentiation. Conceptually, if NSC fate choices can be directly linked to specific instructions from neuronal activity patterns, this will have important impact on circuit-level plasticity through new neuron production, as well as in nervous system diseases. This review will focus on cholinergic circuit control of postnatal neurogenesis: due to the complexity of cholinergic receptor physiology/function,

the exact roles for acetylcholine (ACh) in this context have been difficult to define. Classical approaches including lesioning of cholinergic fibers, as well as pharmacological modulation of nicotinic and muscarinic receptors, first revealed the importance of cholinergic signaling in postnatal neurogenesis control. Recent findings incorporating optogenetic manipulation of cholinergic circuit have uncovered a direct link between neuronal activity patterns and neurogenic proliferation. In this mini-review, we will summarize these anatomical, pharmacological, and functional experimental results, and speculate on local cholinergic circuit wiring diagram and its possibilities for higher-level brain inputs that connect to behavioral paradigms/disease states.

Anatomical lesions and their effects on postnatal neurogenesis

Cholinergic neurons in the mammalian brain can be generally categorized into local interneurons such as those in the central cortex, hippocampus, and striatum; or long-range projection neurons such as those in the magnocellular basal nucleus, pontomesencephalic tegmentum, cranial nerve motor nuclei, and motor neurons in the spinal cord.⁴⁸⁻⁵² For the projection neuron subtype, motor neurons in the spinal cord and cranial nerves, parasympathetic cholinergic neurons in the spinal cord, and cholinergic neurons within the sympathetic nervous system have been well-described.⁵³ Within the brain, projecting cholinergic neurons have been organized into subgroups depending on their anatomical locations and projections (Fig. 1A).⁵⁴ The nucleus basalis group includes: the nucleus basalis of Meynert and magnocellularis (B), substantia innominate (SI), and horizontal diagonal band of Broca (HDB). The medial septal group includes the medial septal nucleus (MS) and vertical diagonal band (VDB). And the pontine cholinergic group in the upper brain stem includes cholinergic neurons in the pedunculopontine tegmental nuclei (PPT) and laterodorsal tegmental nuclei (LDT). Cholinergic neurons within the local interneuron subtype are rather diverse, and include ACh-synthesising neurons in the caudate-putamen; nucleus accumbens; striatum; main and accessory olfactory bulbs; anterior olfactory nucleus; olfactory tubercle; hippocampus; cerebral cortex; basolateral hypothalamus; and spinal cord.⁵³ Despite this anatomical diversity among cholinergic neurons, there are relatively few genetic strategies to specifically target distinct subpopulations of

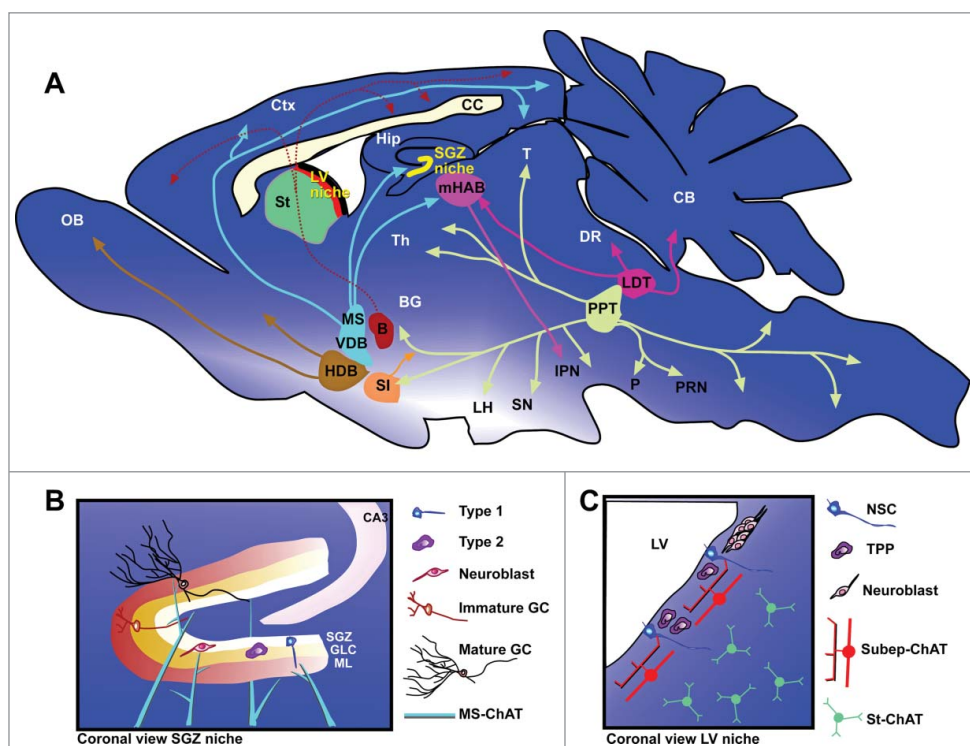


Figure 1. Cholinergic projections and neurogenic niches in the postnatal mouse brain. (A) Sagittal section view showing major cholinergic nuclei and their known projections. Nuclei of the *nucleus basalis group* include: nucleus basalis of Meynert and magnocellularis (B); horizontal diagonal band of Broca (HDB); substantia innominate (SI). Nuclei of the *medial septal group* include: medial septal nucleus (MS) and vertical diagonal band (VDB). Nuclei of the *pontine cholinergic group* include: laterodorsal tegmental nuclei (LDT) and pedunculopontine tegmental nuclei (PPT). Other notable cholinergic neuron groups are found in: medial habenular nucleus (mHAB); striatum (St); and subependymal zone (SEZ). Major cholinergic neuron/nuclei projection targets include: basal ganglia (BG); cerebellum (CB); cortex (Ctx); dorsal raphe nucleus (DR); hippocampus (Hip); interpeduncular nucleus (IPN); lateral hypothalamus (LH); olfactory bulb (OB); pons (P); pontine reticular nucleus (PRN); substantia nigra (SN); thalamus (Th); and tectum (T). Neurogenic niches (LV and SGZ) are expanded in panels below. (B) Coronal section view of the SGZ neurogenic niche in the dentate gyrus (DG). Blue fibers indicate innervating projections from medial septal cholinergic neurons. Neurogenic cell types: astrocyte-like precursor (Type 1), transiently proliferating progenitor (Type 2), neuroblast, immature granule cell (GC), and mature GC. GLC = granule cell layer; ML = molecular layer of the DG. (C) Coronal view of the LV neurogenic niche, showing subep-ChAT neurons as well as neighboring striatal cholinergic neurons (St-ChAT). Neurogenic cell types include: (NSC) neural stem cell, Mash1⁺ transiently proliferating progenitor (TPP), and neuroblasts.

cholinergic neurons. In the context of postnatal neurogenic niches, cholinergic neurons from the medial septum and the diagonal band of Broca are believed to provide most of the cholinergic innervation to the SGZ niche (Fig. 1B).^{55,56} A newly identified cholinergic neuron population residing subependymally has recently been shown to modulate LV niche neurogenesis (Fig. 1C).⁵⁷

A robust and efficient method to label all cholinergic neurons is to drive fluorescence reporter via Cre recombinase expression from *choline acetyltransferase* (*ChAT*, required for acetylcholine synthesis) gene regulatory elements,⁵⁸ although this approach does not distinguish cholinergic neuron subtypes. Primary anatomic lesion studies, while less elegant due to non-

specific side effects, do allow for regional targeting of cholinergic neurons to access their potential functions during postnatal neurogenesis. Transection of the fimbria-fornix, which disrupts basal forebrain cholinergic projections to the hippocampus,⁵⁹ has been reported to result in a concurrent decrease in dentate gyrus BrDU incorporation,⁶⁰ suggesting decreased SGZ neurogenesis. Similarly, direct injection of *N*-methyl-d-aspartate (NMDA) to the cholinergic nuclei in the medial septal region to create excitotoxic lesions also reduced SGZ neurogenesis.⁶¹ Cholinergic neurons in the basal forebrain, medial septum, nucleus basalis of Meynert, and diagonal band of Broca all express high levels of p75 neurotrophin receptor (p75NTR). This exposes them to specific cellular elimination by precise

stereotaxic injection of 192-IgG-SAP (192-Saporin), a chemical conjugate of p75NTR mouse clonal antibody to the ribosome-inactivating protein saporin. 192-Saporin-mediated removal of medial septal cholinergic neurons resulted in decreased SGZ neurogenesis,^{56,62} as well as decreased cellular proliferation in the LV niche.⁶³ Together, these anatomical studies suggested that cholinergic neurons in the brain can play important roles to control postnatal neurogenesis.

Muscarinic and nicotinic cholinergic receptor activation and signaling

In addition to anatomical lesion studies, pharmacological approaches have also implicated cholinergic signaling as an important pathway controlling postnatal neurogenesis. ACh signals through both nicotinic and muscarinic acetylcholine receptors (nAChR and mAChR, respectively), which can be specifically targeted by pharmacological agents. Muscarinic agonists such as bethanechol, pilocarpine, and oxotremorine enhanced cellular proliferation when added to NSC cultures,⁶⁴ hippocampal slices,⁶² or *in vivo*,^{65,66} while muscarinic antagonist had the opposite effect.⁶⁷ Proliferation was also enhanced in cortical precursors following mAChR activation.⁶⁸ While the effects are not as clear cut, nicotinic stimulation also appears to increase neurogenesis, as direct nicotine application *in vivo* increased LV Nestin⁺ cellular proliferation, resulting in subsequent BrdU-labeled NeuN⁺ granule neurons in the olfactory bulb.⁶⁹ In the SGZ, pharmacological activation of the $\alpha 7$ -subunit containing nAChRs have been shown to increase cellular proliferation.⁷⁰ However, high doses of nicotine delivered chronically *in vivo* have an opposite effect in decreasing SGZ neurogenesis.⁷¹ Even though characterization of ACh receptor expression in neurogenic niches has not been extensive, LV NSCs have been reported to express $\alpha 3$ - and $\alpha 4$ - subunit containing nAChRs,⁵⁷ similar to those residing in the rostral migratory stream which displayed $\alpha 3\beta 4$ nAChR activity.⁷² In contrast to NSCs and DCX⁺ neuroblasts, Mash1⁺ transiently amplifying progenitors in the LV niche did not appear to express functional ACh receptors.⁵⁷ In the SGZ, IHC and functional analyses have revealed the presence of M1 and M4 subunit mAChR expressions, as well as $\alpha 7$ and $\beta 2$ nAChR subunit expressions in

immature hippocampal neurons.^{73,74} M1 and M4 mAChRs also co-label with proliferating SGZ cells shortly following BrdU administration.⁷⁵

These results suggest that adult neural stem/progenitor cell populations are sensitive to levels and timing of acetylcholine released, and that cholinergic receptor subtypes may mediate differential effects on cellular proliferation. mAChRs are metabotropic transmembrane proteins, coupled to G proteins, and activate various intracellular signaling pathways to provide sustained cellular responses.^{76,77} Meanwhile, nAChRs are pentameric, ionotropic channels consisting of several subunits: one alpha + 4 other subunits named beta, gamma, delta, and epsilon. nAChRs mediate fast cholinergic transmission in the peripheral and central nervous system.⁷⁸ The subunit compositions of the various nAChRs determine their ionic permeability (e.g. Na⁺, K⁺, Ca²⁺), affinity for ACh, channel current kinetics, and channel desensitization.⁷⁹⁻⁸² In the brain, mAChR and nAChR types are present on neurons at both synaptic and extra-synaptic sites,⁸³⁻⁸⁵ as well as on glial cells.^{86,87}

Low-level, sustained neuronal activity patterns that result in consistently low concentrations of neurotransmitter released are termed tonic firing patterns. On the other hand, more robust, synaptic, and temporally salient neuronal activities are referred to as phasic activity, occurring on much finer time-scales.⁸⁸ Neuronal activity patterns of specific cholinergic populations can range from spontaneous low frequency spiking (tonic)⁸⁹ to those that fire very irregularly or respond strongly to specific salient stimulation (phasic).⁹⁰⁻⁹² The types of release will influence both the concentration and temporal profiles of ACh signaling, as well as the speed of ACh breakdown by acetylcholinesterases, which are particularly effective in synaptic clefts.⁹² One notable feature regarding nAChRs is their rapid desensitization following ACh-induced activation.^{80,81} This results in short bursts of ACh release having a qualitatively different effect on the receiving cell than ACh continuously present or absent (Fig. 2). Anatomically speaking, activity-dependent ACh released from synaptic sites facilitate fast, high concentration neurotransmitter access to receptors (Fig. 2A), while bulk/volume ACh release that occurs at non-synaptic sites or from nearby synaptic spillover provide low level neurotransmitter via diffusion over larger areas (Fig. 2B, C).⁹³ These principles of ACh differentially signaling through 2 receptors, with temporal dynamics on nAChR activation/desensitization, underlie key features of ACh's important functions during

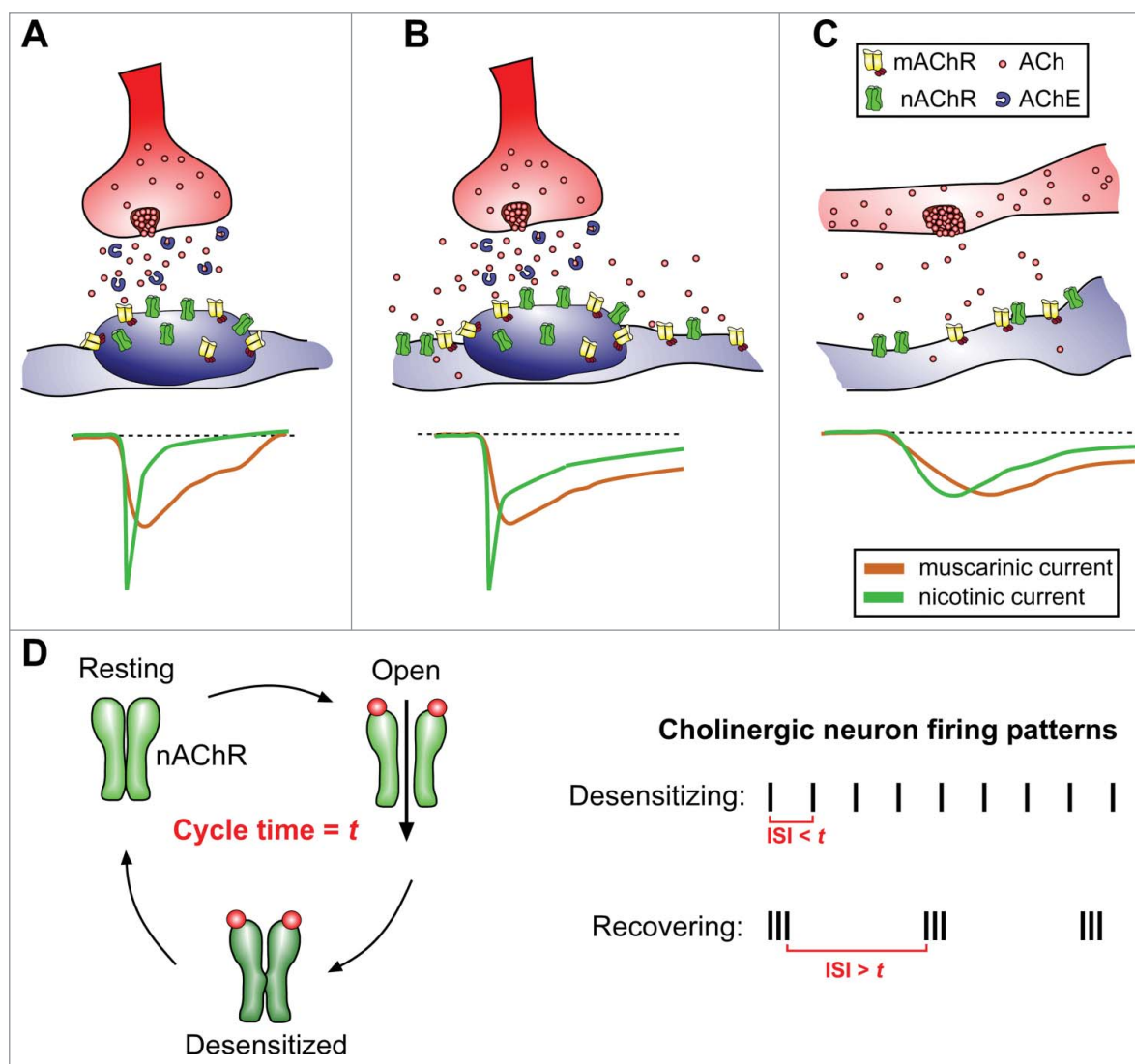


Figure 2. ACh release and receptor activation dynamics to convey neuronal activity patterns. (A) Schematic representation of ACh released directly onto a receptive zone, with a high density of nAChR and mAChR receptors. In such specialized contacts (neuronal synapse as an example), ACh upon release is quickly degraded by extracellular acetylcholinesterase. Nicotinic currents are typically rapid and fast-inactivating, while muscarinic currents are longer lasting. (B) Multiple neuronal activations can cause released ACh to spillover and activate nAChRs/mAChRs away from the immediate receptive zone. This leads to prolonged nicotinic and muscarinic currents in the responding cell. (C) Volume release of ACh stimulates larger fields of receptors at low concentrations. Cholinergic currents evoked by volume release may be small and prolonged. (D) Diagram of nAChR resting, activation, and desensitization cycle (cycle time = t , receptor subtype specific). Depending on the timing of cholinergic neuron inter-stimulus intervals (ISI), the resulting patterns of ACh release will enhance nAChR desensitization when $ISI < t$, or promote nAChR recovery for reactivation when $ISI > t$, resulting in distinct nicotinic activation dynamics in the receiving cell. AChE = acetylcholinesterase.

neural circuit modulation (Fig. 2D). In neurons, nicotinic and muscarinic signaling can often be antagonistic, inducing differential currents or polarizations, divergent calcium signaling profiles,⁹⁴ or distinct regulations of LTP.⁹⁵ However, there are also examples where mAChR and nAChR signaling can be cooperative, producing complimentary depolarizing currents and convergence onto common biological intracellular cascades.⁹⁶⁻⁹⁸ This context-dependent complexity in signaling capacity may provide a palette

richness for NSCs to functionally read-out subtle changes in local ACh availability.

Identification of resident cholinergic neurons in subependymal niche

While cholinergic pharmacology has an effect on adult NSC proliferation *in vivo*,⁶⁹ it remained unclear if these putative actions attributed to ACh are due to

neuronal activity or are in fact indirect. To determine whether activity patterns from cholinergic neurons can directly control neurogenesis, one approach is to examine outcomes upon altering intrinsic excitability of these neurons. We conditionally deleted *Ank3*, a large adapter protein known to localize to neuronal axon initial segment, specifically in cholinergic neurons (*ChAT*^{IRES-Cre/+}; *ank3*^{fllox/fllox}). Similar to previous observations in cerebellar Purkinje neurons,⁹⁹ *Ank3*-mutant *ChAT*⁺ neurons showed an inability to precisely initiate and scale action potentials to electrical stimulation in *ChAT*^{IRES-Cre/+}; *ank3*^{fllox/fllox} mutant mice.⁵⁷ There was a marked reduction in *DCX*⁺ neuroblast chains along the LV niche, becoming progressively worse in adult mice. *Ki67* and *Mash1* IHC staining revealed a corresponding decrease in LV niche cellular proliferation, while caspase 3 staining showed no obvious increase in apoptosis. These results revealed that cholinergic circuit activity and precision are required to sustain the robustness of adult LV neurogenesis.

As cholinergic innervation is widespread in the brain, disrupting its activity will likely contribute to many effects,

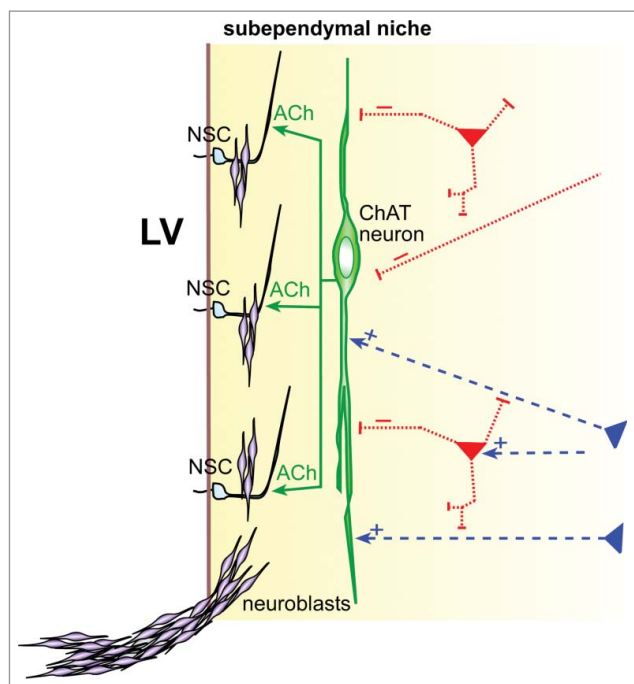


Figure 3. Subependymal cholinergic neuron bridging SEZ niche/neurogenesis to neural circuit-level control. Schematic representation of subep-ChAT neuron (green) providing ACh to modulate adult SEZ neural stem cells (NSC) production of new neuroblasts, which then migrate and assemble into neuroblast chains. Dashed lines represent putative excitatory (+, blue) or inhibitory (-, red) inputs onto subep-ChAT neuron dendrites. LV = lateral ventricle.

including modulation of non-cholinergic circuits. The observed neurogenesis defects in *ChAT*^{IRES-Cre/+}; *ank3*^{fllox/fllox} mutant mice can also be caused by decreased ACh release in the SEZ niche via direct cholinergic innervation. To examine this possibility closer, we looked for cholinergic processes along the LV wall, and detected large *ChAT*⁺ neuronal cell bodies residing subependymally within the LV niche.⁵⁷ DiI-filling of these subependymal *ChAT*⁺ (subep-ChAT) neurons revealed complex neuronal processes that were largely aspiny, and projected their axonal processes locally in the subependymal space (Fig. 3). A morphological feature for these subep-ChAT neurons, which can be located in both young and adult mice (examined up to 6 months of age), is their planar appearance paralleling the ependymal surface above. More importantly, unlike neighboring striatal cholinergic neurons which are spontaneously active, the subep-ChAT neurons did not exhibit basal-level spontaneous activity in acute brain slice preparation. In vivo optogenetic stimulation of subep-ChAT neurons in P30 *ChAT*^{IRES-Cre/+}; *Rosa26R-ChR2EYFP* mice significantly increased the numbers of *Ki67*⁺ proliferating cells and neurogenic progenitors in the LV niche. Conversely, in vivo optogenetic suppression of subep-ChAT neurons in P30 *ChAT*^{IRES-Cre/+}; *Rosa26R-ArchaeorhodopsinGFP* mice decreased the numbers of *Ki67*⁺, *Mash1*⁺, and *DCX*⁺ cells in the LV niche.

To determine whether LV NSCs can directly detect ACh release via cholinergic neuron activity, we performed whole-cell patch recording in NSCs, while simultaneously activating *ChR2*-expressing cholinergic inputs locally via 473 nm laser. This resulted in consistent frequency-dependent inward currents in NSCs, sensitive to both nicotinic and muscarinic blockade (Fig. 3).⁵⁷ These neuronal activity-dependent responses from postnatal LV NSCs appeared distinct from synaptic “spill-over” mechanisms.⁴⁶ Mechanistically, $\alpha 3\beta 4$ nAChR as well as $\alpha 7$ -subunit containing nAChRs have been reported to function during postnatal LV neurogenesis.^{70,72} Our IHC antibody staining and electrophysiological experiments revealed $\alpha 3$ - and $\alpha 4$ -subunit containing nAChR, as well as mAChR expression in *nestin-CreER* lineage-traced LV NSCs.⁵⁷ Consistent with these results: $\alpha 4\beta 2$ nAChR and M1/M4 mAChR agonists have been shown to control SGZ neurogenic proliferation and differentiation;¹⁰⁰⁻¹⁰² and $\beta 2$ -subunit nAChR mutant mice have reduced SGZ proliferation over the life of the animal.¹⁰³

These findings revealed subep-ChAT neurons as integral components of the cholinergic circuit controlling

postnatal LV neurogenesis. Beyond these subep-ChAT neurons, it remains possible that there are other cholinergic neurons whose activity contributes to LV neurogenesis control. Anatomically, the nearest such population is located in the striatum: the well-studied tonically-active striatal cholinergic neurons. While genetic deletion of ChAT (removing ACh producing ability) in the striatal cholinergic population via *Nkx2.1-Cre; ChAT^{fllox/fllox}* mutant mice revealed no obvious LV neurogenesis defects,⁵⁷ striatal cholinergic neurons may still play a role under physiological conditions that can be compensated for as needed by subep-ChAT neurons.

Circuit level control of cholinergic neuron activity

It is of great interest to understand, at the circuit level, how cholinergic neuron activity is regulated, resulting in postnatal neurogenesis control. *Ank3* deletion from ChAT⁺ neurons showed that precise cholinergic circuit activity is required to sustain the robustness of adult LV neurogenesis. While IHC staining for p-prpS6, an activity-dependent marker for cholinergic neurons,¹⁰⁴ revealed that subep-ChAT neurons are normally active in vivo, they lacked spontaneous activity in acute brain slice preparation, indicating their activity is contextually controlled by higher-level inputs. The sources for excitatory/inhibitory inputs onto subep-ChAT neurons are currently unclear, although CNS cholinergic neurons such as those found in the striatum, basal forebrain nuclei, hypothalamus, medial habenula, pontomesencephalic tegmentum, and medullary tegmentum, tend to have highly stereotyped patterns of afferent connectivity,¹⁰⁵ serving as potential blueprints for subep-ChAT neuron connectivity. First, there is rich inter-connectivity between cholinergic cell groups, which form a contiguous plexus of overlapping dendritic arbors, collectively allowing each subsystem (e.g., striatum, basal forebrain, and pontomesencephalon) to receive and integrate information from various sensory modalities.¹⁰⁶ Second, forebrain cholinergic neurons generally receive excitatory cortical inputs (Fig. 4).¹⁰⁵ This pattern of innervation has been hypothesized to provide a means for global integration of ongoing neural activity, as cholinergic cell groups are frequently implicated in the modulation of attention and arousal associated with the reticular activating system.¹⁰⁷ Furthermore, striatal cholinergic neurons adjacent to the

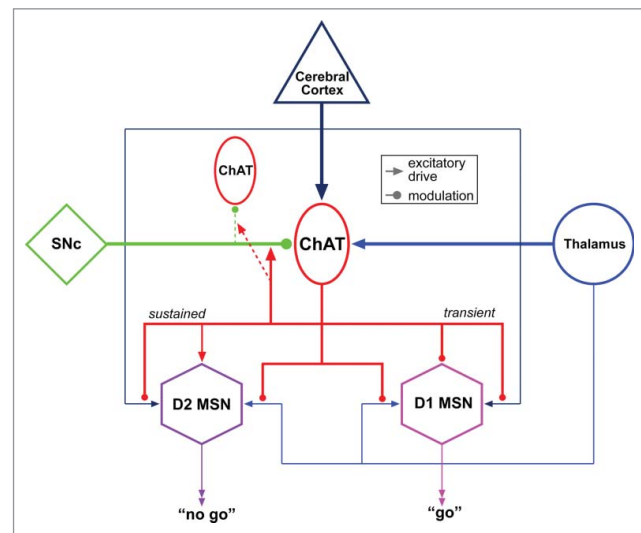


Figure 4. Example functional connectivity of a subgroup of CNS cholinergic neurons. Striatal cholinergic neurons (TANs) receive glutamatergic inputs from both cortex and intralaminar thalamus, as well as dopaminergic modulation from the substantia nigra pars compacta (SNc). Medium spiny neurons (MSNs), projection neurons in the striatum, express either type 1 or type 2 dopamine receptors (D1 or D2, respectively). Following thalamic stimulation, TANs generate a burst-pause pattern of activity that transiently and presynaptically inhibits thalamic and cortical excitation of D1 and D2 striatal MSNs through muscarinic receptor subtype M2 signaling. It also initiates a sustained, muscarinic receptor subtype M1-mediated facilitation of dendritic responsiveness in D2 MSNs: resulting in a bias of cortical and thalamic excitation toward D2 expressing, striatopallidal MSNs for the duration of the pause in TAN activity. The pause is dependent on dopaminergic signaling onto TANs. Functionally, thalamic excitation of TANs is thought to provide a window in which excitation of D2-expressing MSNs is enhanced, allowing for preferential recruitment of the striatopallidal pathway. Such wiring diagrams may serve as useful models to study subep-ChAT neuron connectivity. Distinct neuronal cell types and projection patterns are represented in different colors for clarity.

LV niche, as well as those in the nucleus basalis, receive inputs from the intralaminar thalamus, as part of the reticular activating system (Fig. 4).¹⁰⁵ All cholinergic neuron groups also receive noradrenergic input from the locus ceruleus and subceruleus.¹⁰⁸ The basal forebrain, striatal, diencephalic, and pontomesencephalic cholinergic groups also receive sparse nigral or ventral tegmental dopaminergic inputs (Fig. 3).¹⁰⁵

Within the striatum as a specific example, cholinergic neuron activity patterns are dynamically controlled via distal excitation modulating intrinsic neuronal membrane properties.^{109,110} The large, aspiny cholinergic interneurons of the striatum, referred to as tonically active neurons (TANs), represent a particularly well-studied population of spontaneously firing cholinergic neuron. In TANs, spontaneous activity is

mediated by intrinsic membrane properties, specifically a sodium current and hyperpolarization-activated cation current which together drive tonic firing.⁸⁹ Salient stimuli produce a characteristic pause in TAN firing, and TANs have long been viewed as important substrates for striatal associative and motor learning.^{111,112} In contextual recognition of salient stimuli driving action selection, the temporal, spatial, and motivational context of salient stimuli have all been shown to play a role in regulating spontaneous striatal cholinergic neuron activity.¹¹³ Similar pauses in cholinergic TAN activity are generated following stimulation of nigrostriatal afferents,¹¹⁰ and a burst-pause firing pattern is generated in response to stimulation of the intralaminar thalamus (Fig. 4).¹⁰⁹

Although subep-ChAT cells are silent under resting conditions, this is relatively rare among other cholinergic cell groups.^{90,114} It remains to be seen whether the distinct activity profiles of subep-ChAT neurons vs. neighboring striatal TANs in brain slice preparations are due to differences in intrinsic membrane properties or their inhibitory tone. Within the local microcircuitry, GABAergic inhibitory interneurons can also provide dynamic control to alter cholinergic neuron activity states.¹¹⁴ While it has been well demonstrated that GABA is an important protein for several cell types during postnatal LV neurogenesis,^{115,116} its sources in the niche remains largely unclear. Conceptually, since cholinergic neurons groups are broadly interconnected, it is an intriguing possibility that subep-ChAT neurons participate in and sample the cholinergic plexus to transform global cascades of activity within the cholinergic system into functional neurogenesis. Furthermore, if subep-ChAT neurons are involved in associative learning tasks, believed to be an important function for striatal cholinergic TANs, a similar configuration of circuit-level connectivity may allow subep-ChAT neurons to respond to environmentally salient stimuli to direct neurogenesis. The highly stereotyped connectivity patterns of other cholinergic groups provide a template and substrate for investigating the circuit wiring diagram of subep-ChAT neurons.

Conclusion

Like a computer needing hardware upgrades to run increasingly sophisticated software, it is tempting to speculate that postnatal neurogenesis endow

particular neural circuits with this capacity. The fact that neuronal firing patterns can direct NSC proliferation takes this concept one step further, and proposes that perhaps certain critical neural circuits may functionally instruct NSCs for their own neuronal additions over time. There are parallels in the glial biology field: pioneering study by Barres and Raff in 1993 showed that oligodendrocyte precursor proliferation can be dependent on neurons generating action potentials.¹¹⁷ Neuronal activity-dependent increases in myelination is now a well-accepted observation, and recent studies extended this concept to show the lack of inhibitory post-synaptic currents on oligodendrocyte precursors contributing to white matter defects following hypoxic injury,¹¹⁸ as well as glioma cellular proliferation profiting from neuronal activity.¹¹⁹ It is likely that future studies will find additional examples, perhaps in the process revealing that the control of postnatal neurogenesis by neuronal activity may be the norm, rather than an exception for cells proliferating in the brain in health and disease.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

Acknowledgments

We thank members of the Kuo laboratory, Jerry Yakel (N.I.E. H.S), and Juan Song (U.N.C. Chapel Hill) for helpful discussions.

Funding

This work was supported by N.I.H. grant R01MH105416, R01NS078192, The March of Dimes, and George & Jean Brumley Endowment (C.T.K.).

References

- [1] Aboody K, Capela A, Niazi N, Stern JH, Temple S. Translating stem cell studies to the clinic for CNS repair: current state of the art and the need for a Rosetta stone. *Neuron* 2011; 70:597-613; PMID:21609819; <http://dx.doi.org/10.1016/j.neuron.2011.05.007>
- [2] Robel S, Berninger B, Gotz M. The stem cell potential of glia: lessons from reactive gliosis. *Nat Rev Neurosci* 2011; 12:88-104; PMID:21248788; <http://dx.doi.org/10.1038/nrn2978>
- [3] Zhao C, Deng W, Gage FH. Mechanisms and functional implications of adult neurogenesis. *Cell* 2008; 132:645-60; PMID:18295581; <http://dx.doi.org/10.1016/j.cell.2008.01.033>

- [4] Ihrle RA, Alvarez-Buylla A. Lake-front property: a unique germinal niche by the lateral ventricles of the adult brain. *Neuron* 2011; 70:674-86; PMID:21609824; <http://dx.doi.org/10.1016/j.neuron.2011.05.004>
- [5] Spalding KL, Bergmann O, Alkass K, Bernard S, Salehpour M, Huttner HB, Boström E, Westerlund I, Vial C, Buchholz BA, et al. Dynamics of hippocampal neurogenesis in adult humans. *Cell* 2013; 153:1219-27; PMID:23746839; <http://dx.doi.org/10.1016/j.cell.2013.05.002>
- [6] Ernst A, Alkass K, Bernard S, Salehpour M, Perl S, Tisdale J, Possnert G, Druid H, Frisén J. Neurogenesis in the striatum of the adult human brain. *Cell* 2014; 156:1072-83; PMID:24561062; <http://dx.doi.org/10.1016/j.cell.2014.01.044>
- [7] Alcantara Llaguno S, Chen J, Kwon CH, Jackson EL, Li Y, Burns DK, Alvarez-Buylla A, Parada LF. Malignant astrocytomas originate from neural stem/progenitor cells in a somatic tumor suppressor mouse model. *Cancer Cell* 2009; 15:45-56; PMID:19111880; <http://dx.doi.org/10.1016/j.ccr.2008.12.006>
- [8] Lathia JD, Mack SC, Mulkearns-Hubert EE, Valentim CL, Rich JN. Cancer stem cells in glioblastoma. *Genes Dev* 2015; 29:1203-17; PMID:26109046; <http://dx.doi.org/10.1101/gad.261982.115>
- [9] Tomasetti C, Vogelstein B. Cancer etiology. Variation in cancer risk among tissues can be explained by the number of stem cell divisions. *Science* 2015; 347:78-81; PMID:25554788; <http://dx.doi.org/10.1126/science.1260825>
- [10] Kelsch W, Sim S, Lois C. Watching synaptogenesis in the adult brain. *Annu Rev Neurosci* 2010; 33:131-49; PMID:20572770; <http://dx.doi.org/10.1146/annurev-neuro-060909-153252>
- [11] Lazarini F, Lledo PM. Is adult neurogenesis essential for olfaction? *Trends Neurosci* 2011; 34:20-30; PMID:20980064; <http://dx.doi.org/10.1016/j.tins.2010.09.006>
- [12] Kheirbek MA, Klemenhagen KC, Sahay A, Hen R. Neurogenesis and generalization: a new approach to stratify and treat anxiety disorders. *Nat Neurosci* 2012; 15:1613-20; PMID:23187693; <http://dx.doi.org/10.1038/nn.3262>
- [13] Song J, Christian KM, Ming GL, Song H. Modification of hippocampal circuitry by adult neurogenesis. *Dev Neurobiol* 2012; 72:1032-43; PMID:22354697; <http://dx.doi.org/10.1002/dneu.22014>
- [14] Crowther AJ, Song J. Activity-dependent signaling mechanisms regulating adult hippocampal neural stem cells and their progeny. *Neurosci Bull* 2014; 30:542-56; PMID:25082534; <http://dx.doi.org/10.1007/s12264-014-1453-5>
- [15] Yu DX, Marchetto MC, Gage FH. How to make a hippocampal dentate gyrus granule neuron. *Development* 2014; 141:2366-75; PMID:24917496; <http://dx.doi.org/10.1242/dev.096776>
- [16] Kempermann G, Song H, Gage FH. Neurogenesis in the Adult Hippocampus. *Cold Spring Harb Perspect Med* 2015; 5:a018812; PMID:26351719
- [17] Kropff E, Yang SM, Schinder AF. Dynamic role of adult-born dentate granule cells in memory processing. *Curr Opin Neurobiol* 2015; 35:21-26; PMID:26100379; <http://dx.doi.org/10.1016/j.conb.2015.06.002>
- [18] Braun SM, Jessberger S. Adult neurogenesis: mechanisms and functional significance. *Development* 2014; 141:1983-6; PMID:24803647; <http://dx.doi.org/10.1242/dev.104596>
- [19] Bergmann O, Spalding KL, Frisen J. Adult Neurogenesis in Humans. *Cold Spring Harb Perspect Biol* 2015; 7:a018994; PMID:26134318; <http://dx.doi.org/10.1101/cshperspect.a018994>
- [20] Doetsch F, Caille I, Lim DA, Garcia-Verdugo JM, Alvarez-Buylla A. Subventricular zone astrocytes are neural stem cells in the adult mammalian brain. *Cell* 1999; 97:703-16; PMID:10380923
- [21] Lledo PM, Merkle FT, Alvarez-Buylla A. Origin and function of olfactory bulb interneuron diversity. *Trends Neurosci* 2008; 31:392-400; PMID:18603310; <http://dx.doi.org/10.1016/j.tins.2008.05.006>
- [22] Livneh Y, Adam Y, Mizrahi A. Odor processing by adult-born neurons. *Neuron* 2014; 81:1097-110; PMID:24508384; <http://dx.doi.org/10.1016/j.neuron.2014.01.007>
- [23] Sakamoto M, Ieki N, Miyoshi G, Mochimaru D, Miyachi H, Imura T, Yamaguchi M, Fishell G, Mori K, Kageyama R, et al. Continuous postnatal neurogenesis contributes to formation of the olfactory bulb neural circuits and flexible olfactory associative learning. *J Neurosci* 2014; 34:5788-99; PMID:24760839; <http://dx.doi.org/10.1523/JNEUROSCI.0674-14.2014>
- [24] Benner EJ, Luciano D, Jo R, Abdi K, Paez-Gonzalez P, Sheng H, Warner DS, Liu C, Eroglu C, Kuo CT. Protective astrogenesis from the SVZ niche after injury is controlled by Notch modulator Thbs4. *Nature* 2013; 497:369-73; PMID:23615612; <http://dx.doi.org/10.1038/nature12069>
- [25] Faiz M, Sachewsky N, Gascon S, Bang KW, Morshead CM, Nagy A. Adult Neural Stem Cells from the Subventricular Zone Give Rise to Reactive Astrocytes in the Cortex after Stroke. *Cell Stem Cell* 2015; 17:624-34; PMID:26456685; <http://dx.doi.org/10.1016/j.stem.2015.08.002>
- [26] Imayoshi I, Sakamoto M, Ohtsuka T, Takao K, Miyakawa T, Yamaguchi M, Mori K, Ikeda T, Itoharu S, Kageyama R. Roles of continuous neurogenesis in the structural and functional integrity of the adult forebrain. *Nat Neurosci* 2008; 11:1153-61; PMID:18758458; <http://dx.doi.org/10.1038/nn.2185>
- [27] Mak GK, Weiss S. Paternal recognition of adult offspring mediated by newly generated CNS neurons. *Nat Neurosci* 2010; 13:753-8; PMID:20453850; <http://dx.doi.org/10.1038/nn.2550>

- [28] Sakamoto M, Kageyama R, Imayoshi I. The functional significance of newly born neurons integrated into olfactory bulb circuits. *Front Neurosci* 2014; 8:121; PMID:24904263; <http://dx.doi.org/10.3389/fnins.2014.00121>
- [29] Bedard A, Parent A. Evidence of newly generated neurons in the human olfactory bulb. *Dev Brain Res* 2004; 151:159-68; PMID:15246702; <http://dx.doi.org/10.1016/j.devbrainres.2004.03.021>
- [30] Curtis MA, Kam M, Nannmark U, Anderson MF, Axell MZ, Wikkelso C, Holtas S, van Roon-Mom WM, Björk-Eriksson T, Nordborg C, et al. Human neuroblasts migrate to the olfactory bulb via a lateral ventricular extension. *Science* 2007; 315:1243-9; PMID:17303719; <http://dx.doi.org/10.1126/science.1136281>
- [31] Sanaï N, Nguyen T, Ihrle RA, Mirzadeh Z, Tsai HH, Wong M, Gupta N, Berger MS, Huang E, Garcia-Verdugo JM, et al. Corridors of migrating neurons in the human brain and their decline during infancy. *Nature* 2011; 478:382-6; PMID:21964341; <http://dx.doi.org/10.1038/nature10487>
- [32] Kriegstein A, Alvarez-Buylla A. The glial nature of embryonic and adult neural stem cells. *Annu Rev Neurosci* 2009; 32:149-84; PMID:19555289; <http://dx.doi.org/10.1146/annurev.neuro.051508.135600>
- [33] Suh H, Deng W, Gage FH. Signaling in adult neurogenesis. *Annu Rev Cell Dev Biol* 2009; 25:253-75; PMID:19575663; <http://dx.doi.org/10.1146/annurev.cellbio.042308.113256>
- [34] Urban N, Guillemot F. Neurogenesis in the embryonic and adult brain: same regulators, different roles. *Front Cell Neurosci* 2014; 8:396; PMID:25505873; <http://dx.doi.org/10.3389/fncel.2014.00396>
- [35] Tavazoie M, Van der Veken L, Silva-Vargas V, Louis-saint M, Colonna L, Zaidi B, Garcia-Verdugo JM, Doetsch F. A specialized vascular niche for adult neural stem cells. *Cell Stem Cell* 2008; 3:279-88; PMID:18786415; <http://dx.doi.org/10.1016/j.stem.2008.07.025>
- [36] Paez-Gonzalez P, Abdi K, Luciano D, Liu Y, Soriano-Navarro M, Rawlins E, Bennett V, Garcia-Verdugo JM, Kuo CT. Ank3-dependent SVZ niche assembly is required for the continued production of new neurons. *Neuron* 2011; 71:61-75; PMID:21745638; <http://dx.doi.org/10.1016/j.neuron.2011.05.029>
- [37] Ottone C, Krusche B, Whitby A, Clements M, Quadrato G, Pitulescu ME, Adams RH, Parrinello S. Direct cell-cell contact with the vascular niche maintains quiescent neural stem cells. *Nat Cell Biol* 2014; 16:1045-56; PMID:25283993; <http://dx.doi.org/10.1038/ncb3045>
- [38] Bjornsson CS, Apostolopoulou M, Tian Y, Temple S. It takes a village: constructing the neurogenic niche. *Dev Cell* 2015; 32:435-46; PMID:25710530; <http://dx.doi.org/10.1016/j.devcel.2015.01.010>
- [39] Lenington JB, Pope S, Goodheart AE, Drozdowicz L, Daniels SB, Salamone JD, Conover JC. Midbrain dopamine neurons associated with reward processing innervate the neurogenic subventricular zone. *J Neurosci* 2011; 31:13078-87; PMID:21917791; <http://dx.doi.org/10.1523/JNEUROSCI.1197-11.2011>
- [40] Young SZ, Taylor MM, Bordey A. Neurotransmitters couple brain activity to subventricular zone neurogenesis. *Eur J Neurosci* 2011; 33:1123-32; PMID:21395856; <http://dx.doi.org/10.1111/j.1460-9568.2011.07611.x>
- [41] Crowther AJ, Song J. Activity-dependent signaling mechanisms regulating adult hippocampal neural stem cells and their progeny. *Neurosci Bull* 2014; 30:542-56; PMID:25082534; <http://dx.doi.org/10.1007/s12264-014-1453-5>
- [42] Pallotto M, Deprez F. Regulation of adult neurogenesis by GABAergic transmission: signaling beyond GABA receptors. *Front Cell Neurosci* 2014; 8:166; PMID:24999317; <http://dx.doi.org/10.3389/fncel.2014.00166>
- [43] Tong CK, Chen J, Cebrian-Silla A, Mirzadeh Z, Obernier K, Guinto CD, Tecott LH, Garcia-Verdugo JM, Kriegstein A, Alvarez-Buylla A. Axonal control of the adult neural stem cell niche. *Cell Stem Cell* 2014; 14:500-11; PMID:24561083; <http://dx.doi.org/10.1016/j.stem.2014.01.014>
- [44] Alenina N, Klempin F. The role of serotonin in adult hippocampal neurogenesis. *Behav Brain Res* 2015; 277:49-57; PMID:25125239; <http://dx.doi.org/10.1016/j.bbr.2014.07.038>
- [45] Markwardt SJ, Dieni CV, Wadiche JI, Overstreet-Wadiche L. Ivy/neurogliaform interneurons coordinate activity in the neurogenic niche. *Nat Neurosci* 2011; 14:1407-9; PMID:21983681; <http://dx.doi.org/10.1038/nn.2935>
- [46] Song J, Zhong C, Bonaguidi MA, Sun GJ, Hsu D, Gu Y, Meletis K, Huang ZJ, Ge S, Enikolopov G, et al. Neuronal circuitry mechanism regulating adult quiescent neural stem-cell fate decision. *Nature* 2012; 489:150-4; PMID:22842902; <http://dx.doi.org/10.1038/nature11306>
- [47] Song J, Sun J, Moss J, Wen Z, Sun GJ, Hsu D, Zhong C, Davoudi H, Christian KM, Toni N, et al. Parvalbumin interneurons mediate neuronal circuitry-neurogenesis coupling in the adult hippocampus. *Nat Neurosci* 2013; 16:1728-30; PMID:24212671; <http://dx.doi.org/10.1038/nn.3572>
- [48] Bigl V, Woolf NJ, Butcher LL. Cholinergic projections from the basal forebrain to frontal, parietal, temporal, occipital, and cingulate cortices: a combined fluorescent tracer and acetylcholinesterase analysis. *Brain Res Bull* 1982; 8:727-49; PMID:6182962
- [49] Woolf NJ, Eckenstein F, Butcher LL. Cholinergic projections from the basal forebrain to the frontal cortex: a combined fluorescent tracer and immunohistochemical analysis in the rat. *Neurosci Lett* 1983; 40:93-8; PMID:6633976
- [50] Mesulam MM, Mufson EJ, Levey AI, Wainer BH. Atlas of cholinergic neurons in the forebrain and upper brainstem of the macaque based on monoclonal choline acetyltransferase immunohistochemistry and

- acetylcholinesterase histochemistry. *Neuroscience* 1984; 12:669-86; PMID:6382047
- [51] Nicoll RA. The septo-hippocampal projection: a model cholinergic pathway. *Trends Neurosci* 1985; 8:533-36; PMID:[http://dx.doi.org/10.1016/0166-2236\(85\)90190-0](http://dx.doi.org/10.1016/0166-2236(85)90190-0)
- [52] Smiley JF, Mesulam MM. Cholinergic neurons of the nucleus basalis of Meynert receive cholinergic, catecholaminergic and GABAergic synapses: an electron microscopic investigation in the monkey. *Neuroscience* 1999; 88:241-55; PMID:10051204
- [53] Kasa P. The cholinergic systems in brain and spinal cord. *Prog Neurobiol* 1986; 26:211-72; PMID:3523620
- [54] Mesulam MM, Mufson EJ, Wainer BH, Levey AI. Central cholinergic pathways in the rat: an overview based on an alternative nomenclature (Ch1-Ch6). *Neuroscience* 1983; 10:1185-201; PMID:6320048
- [55] Dutar P, Bassant MH, Senut MC, Lamour Y. The septo-hippocampal pathway: structure and function of a central cholinergic system. *Physiol Rev* 1995; 75:393-427; PMID:7724668
- [56] Frechette M, Rennie K, Pappas BA. Developmental forebrain cholinergic lesion and environmental enrichment: behaviour, CA1 cytoarchitecture and neurogenesis. *Brain Res* 2009; 1252:172-82; PMID:19084506; <http://dx.doi.org/10.1016/j.brainres.2008.11.082>
- [57] Paez-Gonzalez P, Asrican B, Rodriguez E, Kuo CT. Identification of distinct ChAT(+) neurons and activity-dependent control of postnatal SVZ neurogenesis. *Nat Neurosci* 2014; 17:934-42; PMID:24880216; <http://dx.doi.org/10.1038/nn.3734>
- [58] Rossi J, Balthasar N, Olson D, Scott M, Berglund E, Lee CE, Choi MJ, Lauzon D, Lowell BB, Elmquist JK. Melanocortin-4 receptors expressed by cholinergic neurons regulate energy balance and glucose homeostasis. *Cell Metab* 2011; 13:195-204; PMID:21284986; <http://dx.doi.org/10.1016/j.cmet.2011.01.010>
- [59] Rosenberg MB, Friedmann T, Robertson RC, Tuszynski M, Wolff JA, Breakefield XO, Gage FH. Grafting genetically modified cells to the damaged brain: restorative effects of NGF expression. *Science* 1988; 242:1575-8; PMID:3201248
- [60] Fontana X, Nacher J, Soriano E, del Rio JA. Cell proliferation in the adult hippocampal formation of rodents and its modulation by entorhinal and fimbria-fornix afferents. *Cereb Cortex* 2006; 16:301-12; PMID:15958781; <http://dx.doi.org/10.1093/cercor/bhi120>
- [61] Van der Borght K, Mulder J, Keijsers JN, Eggen BJ, Luiten PG, Van der Zee EA. Input from the medial septum regulates adult hippocampal neurogenesis. *Brain Res Bull* 2005; 67:117-25; PMID:16140170; <http://dx.doi.org/10.1016/j.brainresbull.2005.06.018>
- [62] Ito Y, Nochi R, Kuribayashi H, Saito Y, Hisatsune T. Cholinergic activation of hippocampal neural stem cells in aged dentate gyrus. *Hippocampus* 2011; 21:446-59; PMID:20054812; <http://dx.doi.org/10.1002/hipo.20761>
- [63] Cooper-Kuhn CM, Winkler J, Kuhn HG. Decreased neurogenesis after cholinergic forebrain lesion in the adult rat. *J Neurosci Res* 2004; 77:155-65; PMID:15211583; <http://dx.doi.org/10.1002/jnr.20116>
- [64] Calza L, Giuliani A, Fernandez M, Pirondi S, D'Intino G, Aloe L, Giardino L. Neural stem cells and cholinergic neurons: regulation by immunolesion and treatment with mitogens, retinoic acid, and nerve growth factor. *Proc Natl Acad Sci USA* 2003; 100:7325-30; PMID:12777625; <http://dx.doi.org/10.1073/pnas.1132092100>
- [65] Ma W, Li BS, Zhang L, Pant HC. Signaling cascades implicated in muscarinic regulation of proliferation of neural stem and progenitor cells. *Drug News Perspect* 2004; 17:258-66; PMID:15334175
- [66] Veena J, Srikumar BN, Mahati K, Raju TR, Shankaranarayana Rao BS. Oxotremorine treatment restores hippocampal neurogenesis and ameliorates depression-like behaviour in chronically stressed rats. *Psychopharm* 2011; 217:239-53; PMID:21494789; <http://dx.doi.org/10.1007/s00213-011-2279-3>
- [67] Kotani S, Yamauchi T, Teramoto T, Ogura H. Pharmacological evidence of cholinergic involvement in adult hippocampal neurogenesis in rats. *Neuroscience* 2006; 142:505-14; PMID:16889901; <http://dx.doi.org/10.1016/j.neuroscience.2006.06.035>
- [68] Ma W, Maric D, Li BS, Hu Q, Andreadis JD, Grant GM, Liu QY, Shaffer KM, Chang YH, Zhang L, et al. Acetylcholine stimulates cortical precursor cell proliferation in vitro via muscarinic receptor activation and MAP kinase phosphorylation. *Eur J Neurosci* 2000; 12:1227-40; PMID:10762352
- [69] Mudo G, Belluardo N, Mauro A, Fuxe K. Acute intermittent nicotine treatment induces fibroblast growth factor-2 in the subventricular zone of the adult rat brain and enhances neuronal precursor cell proliferation. *Neuroscience* 2007; 145:470-83; PMID:17241745; <http://dx.doi.org/10.1016/j.neuroscience.2006.12.012>
- [70] Narla S, Klejbor I, Birkaya B, Lee YW, Morys J, Stachowiak EK, Terranova C, Bencherif M, Stachowiak MK. alpha7 nicotinic receptor agonist reactivates neurogenesis in adult brain. *Biochem Pharmacol* 2013; 86:1099-104; PMID:23933384; <http://dx.doi.org/10.1016/j.bcp.2013.07.028>
- [71] Abrous DN, Adriani W, Montaron MF, Aurousseau C, Rougon G, Le Moal M, Piazza PV. Nicotine self-administration impairs hippocampal plasticity. *J Neurosci* 2002; 22:3656-62; PMID:11978841; <http://dx.doi.org/10.1523/JNEUROSCI.2002-02.2002>
- [72] Sharma G. The dominant functional nicotinic receptor in progenitor cells in the rostral migratory stream is the alpha3beta4 subtype. *J Neurophysiol* 2013; 109:867-72; PMID:23136348; <http://dx.doi.org/10.1152/jn.00886.2012>
- [73] Kaneko N, Okano H, Sawamoto K. Role of the cholinergic system in regulating survival of newborn neurons in the adult mouse dentate gyrus and olfactory bulb. *Genes Cells* 2006; 11:1145-59; PMID:16999735; <http://dx.doi.org/10.1111/j.1365-2443.2006.01010.x>

- [74] John D, Shelukhina I, Yanagawa Y, Deuchars J, Henderson Z. Functional alpha7 nicotinic receptors are expressed on immature granule cells of the postnatal dentate gyrus. *Brain Res* 2015; 1601:15-30; PMID:25553616; <http://dx.doi.org/10.1016/j.brainres.2014.12.041>
- [75] Mohapel P, Leanza G, Kokaia M, Lindvall O. Forebrain acetylcholine regulates adult hippocampal neurogenesis and learning. *Neurobiol Aging* 2005; 26:939-46; PMID:15718053; <http://dx.doi.org/10.1016/j.neurobiolaging.2004.07.015>
- [76] Dutar P, Nicoll RA. Classification of muscarinic responses in hippocampus in terms of receptor subtypes and second-messenger systems: electrophysiological studies in vitro. *J Neurosci* 1988; 8:4214-24; PMID:3141594
- [77] Caulfield MP. Muscarinic receptors—characterization, coupling and function. *Pharmacol Ther* 1993; 58:319-79; PMID:7504306
- [78] Dani JA. Overview of nicotinic receptors and their roles in the central nervous system. *Biol Psychiatry* 2001; 49:166-74; PMID:11230867
- [79] Le Novere N, Corringer PJ, Changeux JP. The diversity of subunit composition in nAChRs: evolutionary origins, physiologic and pharmacologic consequences. *J Neurobiol* 2002; 53:447-56; PMID:12436412; <http://dx.doi.org/10.1002/neu.10153>
- [80] Quick MW, Lester RA. Desensitization of neuronal nicotinic receptors. *J Neurobiol* 2002; 53:457-78; PMID:12436413; <http://dx.doi.org/10.1002/neu.10109>
- [81] Giniatullin R, Nistri A, Yakel JL. Desensitization of nicotinic ACh receptors: shaping cholinergic signaling. *Trends Neurosci* 2005; 28:371-8; PMID:15979501; <http://dx.doi.org/10.1016/j.tins.2005.04.009>
- [82] Smit AB, Celie PH, Kasheverov IE, Mordvintsev DY, van Nierop P, Bertrand D, Tsetlin V, Sixma TK. Acetylcholine-binding proteins: functional and structural homologs of nicotinic acetylcholine receptors. *J Mol Neurosci* 2006; 30:9-10; PMID:17192605; <http://dx.doi.org/10.1385/JMN:30:1:9>
- [83] Mrzljak L, Levey AI, Goldman-Rakic PS. Association of m1 and m2 muscarinic receptor proteins with asymmetric synapses in the primate cerebral cortex: morphological evidence for cholinergic modulation of excitatory neurotransmission. *Proc Natl Acad Sci USA* 1993; 90:5194-8; PMID:8389473
- [84] Vizi ES, Lendvai B. Modulatory role of presynaptic nicotinic receptors in synaptic and non-synaptic chemical communication in the central nervous system. *Brain Res Rev* 1999; 30:219-35; PMID:10567725
- [85] Huh KH, Fuhrer C. Clustering of nicotinic acetylcholine receptors: from the neuromuscular junction to interneuronal synapses. *Mol Neurobiol* 2002; 25:79-112; PMID:11890459; <http://dx.doi.org/10.1385/MN:25:1:079>
- [86] Loreti S, Vilaro MT, Visentin S, Rees H, Levey AI, Tata AM. Rat Schwann cells express M1-M4 muscarinic receptor subtypes. *J Neurosci Res* 2006; 84:97-105; PMID:16634060; <http://dx.doi.org/10.1002/jnr.20874>
- [87] De Angelis F, Bernardo A, Magnaghi V, Minghetti L, Tata AM. Muscarinic receptor subtypes as potential targets to modulate oligodendrocyte progenitor survival, proliferation, and differentiation. *Dev Neurobiol* 2012; 72:713-28; PMID:21913336; <http://dx.doi.org/10.1002/dneu.20976>
- [88] Parikh V, Kozak R, Martinez V, Sarter M. Prefrontal acetylcholine release controls cue detection on multiple timescales. *Neuron* 2007; 56:141-54; PMID:17920021; <http://dx.doi.org/10.1016/j.neuron.2007.08.025>
- [89] Bennett BD, Callaway JC, Wilson CJ. Intrinsic membrane properties underlying spontaneous tonic firing in neostriatal cholinergic interneurons. *J Neurosci* 2000; 20:8493-503; PMID:11069957
- [90] Manns ID, Alonso A, Jones BE. Discharge properties of juxtacellularly labeled and immunohistochemically identified cholinergic basal forebrain neurons recorded in association with the electroencephalogram in anesthetized rats. *J Neurosci* 2000; 20:1505-18; PMID:10662840
- [91] Parikh V, Sarter M. Cholinergic mediation of attention: contributions of phasic and tonic increases in prefrontal cholinergic activity. *Ann NY Acad Sci* 2008; 1129:225-35; PMID:18591483; <http://dx.doi.org/10.1196/annals.1417.021>
- [92] Unal CT, Golowasch JP, Zaborszky L. Adult mouse basal forebrain harbors two distinct cholinergic populations defined by their electrophysiology. *Front Behav Neurosci* 2012; 6:21; PMID:22586380; <http://dx.doi.org/10.3389/fnbeh.2012.00021>
- [93] Descarries L, Gisiger V, Steriade M. Diffuse transmission by acetylcholine in the CNS. *Prog Neurobiol* 1997; 53:603-25; PMID:9421837
- [94] Rathouz MM, Vijayaraghavan S, Berg DK. Acetylcholine differentially affects intracellular calcium via nicotinic and muscarinic receptors on the same population of neurons. *J Biol Chem* 1995; 270:14366-75; PMID:7782297
- [95] Zhang W, Tan YF, Atwood HL, Martin Wojtowicz J. Biphasic effects of the cholinergic agonist carbachol on long-term potentiation in the dentate gyrus of the mammalian hippocampus. *Neurosci Lett* 2010; 479:157-60; PMID:20510338; <http://dx.doi.org/10.1016/j.neulet.2010.05.056>
- [96] Furukawa K, Abe Y, Sorimachi M, Akaike N. Nicotinic and muscarinic acetylcholine responses in the embryo chick ciliary ganglion cells. *Brain Res* 1994; 657:185-90; PMID:7820617
- [97] Kanemoto Y, Ishibashi H, Doi A, Akaike N, Ito Y. An electrophysiological study of muscarinic and nicotinic receptors of rat paratracheal ganglion neurons and their inhibition by Z-338. *Br J Pharmacol* 2002; 135:1403-14; PMID:11906953; <http://dx.doi.org/10.1038/sj.bjp.0704610>
- [98] Ishibashi M, Yamazaki Y, Miledi R, Sumikawa K. Nicotinic and muscarinic agonists and acetylcholinesterase inhibitors stimulate a common pathway to enhance

- GluN2B-NMDAR responses. *Proc Natl Acad Sci USA* 2014; 111:12538-43; PMID:25114227; <http://dx.doi.org/10.1073/pnas.1408805111>
- [99] Zhou D, Lambert S, Malen PL, Carpenter S, Boland LM, Bennett V. AnkyrinG is required for clustering of voltage-gated Na channels at axon initial segments and for normal action potential firing. *J Cell Biol* 1998; 143:1295-304; PMID:9832557
- [100] Van Kampen JM, Eckman CB. Agonist-induced restoration of hippocampal neurogenesis and cognitive improvement in a model of cholinergic denervation. *Neuropharm* 2010; 58:921-9; PMID:20026137; <http://dx.doi.org/10.1016/j.neuropharm.2009.12.005>
- [101] Takarada T, Nakamichi N, Kitajima S, Fukumori R, Nakazato R, Le NQ, Kim YH, Fujikawa K, Kou M, Yoneda Y. Promoted neuronal differentiation after activation of alpha4/beta2 nicotinic acetylcholine receptors in undifferentiated neural progenitors. *PLoS One* 2012; 7:e46177; PMID:23056257; <http://dx.doi.org/10.1371/journal.pone.0046177>
- [102] He N, Wang Z, Wang Y, Shen H, Yin M. ZY-1, a novel nicotinic analog, promotes proliferation and migration of adult hippocampal neural stem/progenitor cells. *Cell Mol Neurobiol* 2013; 33:1149-57; PMID:24057433; <http://dx.doi.org/10.1007/s10571-013-9981-0>
- [103] Harrist A, Beech RD, King SL, Zanardi A, Cleary MA, Caldarone BJ, Eisch A, Zoli M, Picciotto MR. Alteration of hippocampal cell proliferation in mice lacking the β 2 subunit of the neuronal nicotinic acetylcholine receptor. *Synapse* 2004; 54:200-6; PMID:15472930; <http://dx.doi.org/10.1002/syn.20081>
- [104] Knight ZA, Tan K, Birsoy K, Schmidt S, Garrison JL, Wysocki RW, Emiliano A, Ekstrand MI, Friedman JM. Molecular profiling of activated neurons by phosphorylated ribosome capture. *Cell* 2012; 151:1126-37; PMID:23178128; <http://dx.doi.org/10.1016/j.cell.2012.10.039>
- [105] Woolf NJ. Cholinergic systems in mammalian brain and spinal cord. *Prog Neurobiol* 1991; 37:475-524; PMID:1763188
- [106] Schafer MK, Eiden LE, Weihe E. Cholinergic neurons and terminal fields revealed by immunohistochemistry for the vesicular acetylcholine transporter. I. Central nervous system. *Neuroscience* 1998; 84:331-59; PMID:9539209
- [107] Everitt BJ, Robbins TW. Central cholinergic systems and cognition. *Annu Rev Psychol* 1997; 48:649-84; PMID:9046571; <http://dx.doi.org/10.1146/annurev.psych.48.1.649>
- [108] Sarter M, Bruno JP. Cortical cholinergic inputs mediating arousal, attentional processing and dreaming: differential afferent regulation of the basal forebrain by telencephalic and brainstem afferents. *Neuroscience* 2000; 95:933-52; PMID:10682701
- [109] Ding JB, Guzman JN, Peterson JD, Goldberg JA, Surmeier DJ. Thalamic gating of corticostriatal signaling by cholinergic interneurons. *Neuron* 2010; 67:294-307; PMID:20670836; <http://dx.doi.org/10.1016/j.neuron.2010.06.017>
- [110] Straub C, Tritsch NX, Hagan NA, Gu C, Sabatini BL. Multiphasic modulation of cholinergic interneurons by nigrostriatal afferents. *J Neurosci* 2014; 34:8557-69; PMID:24948810; <http://dx.doi.org/10.1523/JNEUROSCI.0589-14.2014>
- [111] Kimura M, Rajkowski J, Evarts E. Tonicly discharging putamen neurons exhibit set-dependent responses. *Proc Natl Acad Sci USA* 1984; 81:4998-5001; PMID:6589643
- [112] Zhou FM, Wilson CJ, Dani JA. Cholinergic interneuron characteristics and nicotinic properties in the striatum. *J Neurobiol* 2002; 53:590-605; PMID:12436423; <http://dx.doi.org/10.1002/neu.10150>
- [113] Apicella P. Leading tonically active neurons of the striatum from reward detection to context recognition. *Trends Neurosci* 2007; 30:299-306; PMID:17420057; <http://dx.doi.org/10.1016/j.tins.2007.03.011>
- [114] Boucetta S, Jones BE. Activity profiles of cholinergic and intermingled GABAergic and putative glutamatergic neurons in the pontomesencephalic tegmentum of urethane-anesthetized rats. *J Neurosci* 2009; 29:4664-74; PMID:19357291; <http://dx.doi.org/10.1523/JNEUROSCI.5502-08.2009>
- [115] Liu X, Wang Q, Haydar TF, Bordey A. Nonsynaptic GABA signaling in postnatal subventricular zone controls proliferation of GFAP-expressing progenitors. *Nat Neurosci* 2005; 8:1179-87; PMID:16116450; <http://dx.doi.org/10.1038/nn1522>
- [116] Young SZ, Lafourcade CA, Platel JC, Lin TV, Bordey A. GABAergic striatal neurons project dendrites and axons into the postnatal subventricular zone leading to calcium activity. *Front Cell Neurosci* 2014; 8:10; PMID:24478632; <http://dx.doi.org/10.3389/fncel.2014.00010>
- [117] Barres BA, Raff MC. Proliferation of oligodendrocyte precursor cells depends on electrical activity in axons. *Nature* 1993; 361:258-60; PMID:8093806; <http://dx.doi.org/10.1038/361258a0>
- [118] Zonouzi M, Scafidi J, Li P, McEllin B, Edwards J, Dupree JL, Harvey L, Sun D, Hübner CA, Cull-Candy SG, et al. GABAergic regulation of cerebellar NG2 cell development is altered in perinatal white matter injury. *Nat Neurosci* 2015; 18:674-82; PMID:25821912; <http://dx.doi.org/10.1038/nn.3990>
- [119] Venkatesh HS, Johung TB, Caretti V, Gibson EM, Mount CW, Polepalli J, Mitra SS, Woo PJ, Malenka RC, Vogel H, et al. Neuronal Activity Promotes Glioma Growth through Neuroligin-3 Secretion. *Cell* 2015; 161:803-16; PMID:25913192; <http://dx.doi.org/10.1016/j.cell.2015.04.012>