Identification of distinct ChAT+ neurons and activity-dependent control of postnatal SVZ neurogenesis

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Postnatal and adult subventricular zone (SVZ) neurogenesis is believed to be primarily controlled by neural stem cell (NSC)-intrinsic mechanisms, interacting with extracellular and niche-driven cues. Although behavioral experiments and disease states have suggested possibilities for higher level inputs, it is unknown whether neural activity patterns from discrete circuits can directly regulate SVZ neurogenesis. We identified a previously unknown population of choline acetyltransferase (ChAT)+ neurons residing in the rodent SVZ neurogenic niche. These neurons showed morphological and functional differences from neighboring striatal counterparts and released acetylcholine locally in an activity-dependent fashion. Optogenetic inhibition and stimulation of subependymal ChAT+ neurons in vivo indicated that they were necessary and sufficient to control neurogenic proliferation. Furthermore, whole-cell recordings and biochemical experiments revealed direct SVZ NC responses to local acetylcholine release, synergizing with fibroblast growth factor receptor activation to increase neuroblast production. These results reveal an unknown gateway connecting SVZ neurogenesis to neuronal activity-dependent control and suggest possibilities for modulating neuroregenerative capacities in health and disease.

Robust generation of adult-born neurons, from the rodent subventricular and subependymal zone (SVZ/SEZ) neurogenic niche, is a useful experimental system for studying regenerative capacities in the mammalian brain. SVZ neurogenesis provides tractable assays to tackle molecular and cellular-level mechanisms regulating addition of new neurons into established neural circuits1–3. It also serves as a wonderful model for understanding how tissue stem cells and their progeny respond to injury and disease4–6. The consensus view currently is that postnatal and adult SVZ neurogenesis is mediated by subependymal glial fibrillary acidic protein (GFAP)+ B-type astrocytes functioning as NSCs, producing transiently amplifying Mash1+ progenitors that differentiate into doublecortin (DCX)+ neuroblasts, which then migrate to the olfactory bulb through the rostral migratory stream. Although the term SVZ neurogenesis is widely used to describe this process, neuroblasts are born in the subependymal space around the lateral brain ventricles.

As in other tissue stem cell niches, self-renewal of SVZ NSCs and production of differentiating progeny are controlled by well-conserved cell-intrinsic molecular pathways7,8. In addition, extracellular factors and cell-cell interactions in the NSC microenvironment are also critical. For example, blood vessels in the SVZ niche have been shown to regulate NSC function by acting as sources for neurogenic signals9,10. In addition, ependymal cells lining the ventricular surface can provide instructive cues to sustain new neuron production, as well as directing NSC responses to local tissue damage11,12. Together, the SVZ niche provides a rich environment for trophic factors, coordinating NSC homeostasis2,13.

Other than classical stem cell niche factors, neurotransmitters, common currencies for neural circuit activity and modulation, have also been shown to be important during adult SVZ neurogenesis14,15. Excitatory neurotransmitter glutamate can influence proliferation and differentiation of neural progenitors through mGluR activation16,17. Glutamate also enhances survival of DCX+ neuroblasts and newborn neurons through activating NMDA receptors17,18. Inhibitory neurotransmitter GABA is believed to control progenitor proliferation through GABA_A receptor19,20, activating voltage-gated calcium channels in SVZ astrocytes21. Modulatory neurotransmitter dopamine has also been shown to stimulate SVZ proliferation22 through increased epidermal growth factor (EGF) secretion23. Serotonin24,25, as well as cholinergic activation26,27, are believed to have similarly positive effects on SVZ cellular proliferation. Despite this knowledge, it remains unclear whether neuronal activity can directly regulate postnatal and adult SVZ neurogenesis, as the exact neurons capable of performing such functions have not been identified. It is currently unknown whether the SVZ niche contains resident neurons that provide local innervation.

We performed a direct comparison of neurotransmitters in vitro for their neurogenic properties. We found that acetylcholine (ACh) markedly increased DCX+ neuroblast production. As we searched for potential ACh sources in vivo, we uncovered a previously unknown population of ChAT+ neurons residing in and innervating the SVZ niche, with distinct morphological/functional properties from cholinergic neurons in the neighboring striatum. After identifying these subependymal ChAT+ neurons, we sought to determine their function.
and found an important gateway connecting postnatal and adult SVZ neurogenesis to neuronal activity-dependent modulation.

RESULTS
Neurotransmitter effect on neuroblast production in vitro
We reasoned that defining neurotransmitters with potent neurogenic capabilities may reveal the exact neurons that directly control SVZ neurogenesis. Starting with the SVZ NSC adherent culture assay, we compared the abilities of several key neurotransmitters to enhance DCX+ neuroblast production. We differentiated passage two SVZ adherent cultures in the presence of select concentrations of neurotransmitters, focusing on glutamate, GABA, serotonin, dopamine and acetylcholine (Supplementary Fig. 1a). We scored the effects of these neurotransmitters on neuroblast production after 5 d of in vitro differentiation by making protein lysates from individually treated culture plate wells and performed western blotting analyses on DCX protein levels (Supplementary Fig. 1b). This revealed a potent neurogenic effect of the modulatory neurotransmitter ACh on the production of DCX+ neuroblasts from differentiating SVZ NSC cultures (Supplementary Fig. 1a,b), which was sensitive to nicotinic or muscarinic inhibition (Supplementary Fig. 1c).

Genetic disruption of cholinergic circuit activity
Given the overall physiological importance of ACh, genetic deletions of ChAT28 or the vesicular acetylcholine transporter (VACHT)29 result in similar lethal phenotypes shortly after birth. It has been shown that disruption of Ankyrin 3 (Ank3 or AnkyrinG), a large adaptor protein that is necessary for proper axonal initial segment assembly and function, in cerebellar Purkinje neurons results in substantial defects in their abilities to initiate action potentials30. To adopt a similar strategy, we generated a conditional loxP-flanked allele for the Ank3 locus (Supplementary Fig. 2a). We crossed Ank3loxP/+ mice with Actb-cre driver mice to generate Ank3−/− mice, which were then used to generate Ank3−/− mutants. These mice died shortly after birth. Protein extracts made from whole-brain lysates confirmed a protein-null mutation for the 480- and 270-kDa isoforms of Ank3 (Supplementary Fig. 2b), which are known to localize to axon initial segments31.

To conditionally remove Ank3 from cholinergic neurons, we crossed ChatIRES-cre+; Ank3loxP/+ mice to Ank3loxP/+; R26R-tetTomatoloxPloxP mice to generate ChatIRES-cre+; Ank3+/−; R26R-tetTomatoloxPloxP (control), ChatIRES-cre+; Ank3loxP/+; R26R-tetTomatoloxPloxP (Het), and ChatIRES-cre+; Ank3loxPloxPloxP; R26R-tetTomatoloxPloxP+ (cKO) mice. The presence of Cre-dependent tdTomato reporter allowed us to visualize ChAT+ neurons for functional studies. Ank3−/− mice were born in a Mendelian ratio and showed no evidence of perinatal lethality (32 of 232 animals genotyped at postnatal day 7 (P7) were cKO mutants, 13.8% compared to expected 12.5%). We observed occasional

shaking in the movements of cKO mice, but otherwise did not detect obvious phenotypic defects. Immunohistochemical (IHC) staining of tdTomato+ cholinergic neurons from cKO mice showed the lack of Ank3 signal in their proximal axonal segments (Supplementary Fig. 2c). Similar to what had been observed in Purkinje neurons30, cholinergic neurons from the striatum of cKO mice showed noticeable defects in action potential generation to stimuli, with increasing defects to stronger inputs (Supplementary Fig. 2d). Notably, we also detected defects in Ank3−/−; cKO, and this was likely a result of differences in Ank3 expression, as Ank3−/− heterozygous mice showed lower overall Ank3 protein levels in the brain than wild-type littermates (Supplementary Fig. 2b). These functional defects are consistent with the notion that cholinergic neurons in cKO mice are unable to effectively relay inputs into action potentials and subsequent release of ACh.

When we examined DCX+ neuroblast production in these Ank3−/− mice, we saw a marked reduction in neuroblast chains along the ventricular wall (Fig. 1a). This defect in SVZ neurogenesis was noticeable at P14, becoming severe at P30 (DCX+ IHC staining coverage/ventricular area: control = 11.71 ± 0.98%, cKO = 4.09 ± 0.83% (mean ± s.e.m.), n = 5; P < 0.01, z = 2.611, Wilcoxon two-sample test; Fig. 1b,c). Ki67 IHC staining showed a corresponding decrease in SVZ cell proliferation in these animals (Fig. 1d,e), whereas caspase 3 staining showed no obvious increase in cellular apoptosis (data not shown). Consistent with this decrease in SVZ neuroblasts, we observed diminished Mash1+ transiently amplifying progenitors in the SVZ niche of P30 Ank3−/− cKO mice as compared with controls (Fig. 1d,e).

ACh exerts its function locally, as it is rapidly degraded by extracellular acetylcholinesterases. Given that striatal cholinergic neurons are anatomically adjacent to the SVZ niche, to understand whether their ACh release could be an important contributor to sustaining robustness of SVZ DCX+ neuroblast production, we analyzed a genetic
mouse model in which ACh release from striatal cholinergic neurons is largely eliminated. We performed IHC staining for DCX, K67 and Mash1 and found no obvious SVZ neurogenesis defects in P30 Nkx2.1-cre; ChatloxP/loxP mutant mice versus Nkx2.1-cre; ChatloxP/+ littermate controls (Supplementary Fig. 3).

Identification of subependymal ChAT+ neurons

We next set out to investigate potential sources for ACh in the SVZ neurogenic niche. To determine whether the SVZ received direct cholinergic innervation, we used IHC staining of SVZ whole mounts with antibody to ChAT and found extensive ChAT+ processes in the niche (Fig. 2a). Three-dimensional reconstruction of these processes showed that they reside in close proximity to the subependymal space (Fig. 2a). We did not detect ChAT expression in SVZ ependymal niche cells or B-type astrocytes and their progeny in the niche (data not shown). To determine whether these ChAT+ SVZ processes might be from ChAT+ cholinergic neurons, we performed IHC antibody staining on SVZ whole mounts from ChatIRES-cre++; R26R-tdTomato mice. We found that tdTomato+ processes in the SVZ colabeled with ChAT, as indicated by the presence of ChAT+ neurons in coronal sections from similarly prepared acute brain slices, striatal ChAT+ neurons were mainly tdTomato− (Fig. 2b), and therefore not targeted in these neurons exhibit functional variations. Whole-cell recordings revealed that the subependymal population and their striatal counterparts had similar firing rates in response to current injection (Fig. 3a–c). We found that tdTomato+ subep-ChAT+ neurons in Dnd2-cre; R26R-tdTomato mice, and Drd2-cre; ChatloxP/loxP mice showed SVZ neurogenesis defects compared with littermate controls (Fig. 3f–h).

Functional properties of subependymal ChAT+ neurons

The morphological and anatomical location differences between subependymal and striatal ChAT+ neurons raise the question of whether these neurons exhibit functional variations. Whole-cell recordings of ChAT+ neurons from P28–35 ChatIRES-cre++; R26R-tdTomato mice revealed that the subependymal population and their striatal counterparts had similar firing rates in response to current injection (Supplementary Fig. 5a). Both neuronal populations showed basal sag current, but exhibited some differences in membrane electrophysiological properties (Supplementary Fig. 5b,c). In cell-attached recording configuration from similarly prepared acute brain slices, striatal ChAT+
neurons showed characteristic patterns of spontaneous activity\(^{(34)}\) (spontaneous firing frequency = 1.786 ± 0.266 Hz (mean ± s.e.m.), \(n = 21\); Fig. 4a,b). Notably, cell-attached recordings of subependymal–striatal ChAT\(^{+}\) neurons showed little spontaneous activity (spontaneous firing frequency = 0.042 ± 0.013 Hz (mean ± s.e.m.), \(n = 53\), \(P < 0.0001\), \(t_{52} = 5.089\), Student’s \(t\) test; Fig. 4c). However, they could be induced to fire action potentials after local release of glutamate via puff pipette (Fig. 4d,e).

To further confirm these differences in evoked versus spontaneous activities between subependymal and striatal ChAT\(^{+}\) neurons, we crossed the Chat\(^{RES-loxP}\) driver line to Rosa26R-ChR2EYFP (R26R-ChR2EYFP) mice to express channelrhodopsin in ChAT\(^{+}\) neurons. Repeating the same cell-attached recording experiments, we observed a blue light–activated transient pause in spontaneous activity in striatal ChAT\(^{+}\) neurons (Fig. 4f), similar to previous observations\(^{(35)}\). Again, subependymal–striatal ChAT\(^{+}\) neurons showed little spontaneous activity, but responded robustly to blue light activation by continuously firing action potentials during the duration of stimulation (Fig. 4g). These results indicate that, not only do subependymal and striatal ChAT\(^{+}\) neurons differ morphologically, they also exhibit different activity states.

**Activity-dependent ACh release in the SVZ niche**

ChAT-expressing neurons can synthesize and release ACh. To detect ACh release from subep-ChAT\(^{+}\) neurons, we used an ACh sensor: the M1 muscarinic receptor cell-based neurotransmitter fluorescent engineered reporter (M1-CNIFER)\(^{(36)}\). These modified HEK293 cells express the Ca\(^{2+}\) indicator protein TN-XXL and the M1 muscarinic receptor. ACh binding to M1 receptor results in enhanced fluorescence resonance energy transfer (FRET) between cyan fluorescent protein (CFP) and yellow fluorescent protein (YFP) domains of TN-XXL receptor. ACh binding to M1 receptor results in enhanced fluorescence resonance energy transfer (FRET) between cyan fluorescent protein (CFP) and yellow fluorescent protein (YFP) domains of TN-XXL receptor. ACh application to adherent M1-CNIFER cells resulted in consistent and opposing fractional changes in the FRET ratio (Fig. 5a), consistent with previously published results\(^{(36)}\). Using the same imaging protocol described above on transplanted M1-CNIFER cells, we detected consistent \(\Delta F/F\) fluorescence and fractional changes in FRET ratio following ACh application (Fig. 5c).

We next repeated this SVZ transplantation experiment, using acute slices prepared from P30 Chat\(^{RES-loxP}\); R26R-ChR2EYFP mice. Instead of direct ACh application, we used 473-nm light pulses to locally excite subep-ChAT\(^{+}\) neurons and measured M1-CNIFER
cell responses (Supplementary Fig. 6c). This resulted in consistent $\Delta F/F$ fluorescence and fractional FRET changes in M1-CNiFER cells (Fig. 5d), consistent with activity-dependent release of ACh from subep-ChAT+ neurons. Focal 473-nm light stimulation of ChR2EYFP-expressing striatal ChAT+ neurons, either directly adjacent to the SVZ or deeper into the striatum, did not result in measurable SVZ M1-CNiFER cell responses (Fig. 5e). As controls, 473-nm light pulses on M1-CNiFER cells in adherent cultures or after transplantation into the SVZ of brain slices prepared from non-transgenic mice showed no $\Delta F/F$ fluorescence and FRET changes (data not shown).

**SVZ NSCs respond to local ChAT+ neuron activity**

Detection of activity-dependent ACh release led us to look for anatomical relationships between cholinergic processes and the SVZ niche. We performed transmission electron microscopy (TEM) on RFP antibody-stained, immunogold-labeled SVZ samples from ChatIRES-cre; R26R-tdTomato animals. NSCs in the SVZ niche are identified on TEM through contacts with the brain ventricular surface and their characteristic invaginated nuclei. We detected immunogold-labeled axonal projections from ChAT+ neurons, showing intracellular vesicles adjacent to SVZ NSCs (Fig. 6a). Antibody staining against nicotinic and muscarinic receptors revealed their localizations in subependymal GFAP+ astrocytes near ChAT+ neuronal processes (Supplementary Fig. 7).

To determine whether SVZ NSCs can directly respond to local ACh release, we prepared acute brain slices from P30 nestin-CreERtm4; R26R-tdTomato; Chat-ChrR2EYFP mice. P7 tamoxifen injection in these mice results in tdTomato expression in SVZ NSCs. We performed whole-cell patch recording on subependymal tdTomato NSCs (Supplementary Fig. 8a,b) while focally activating subep-ChAT+ neurons via 473-nm laser, using 10-ms pulses at 15, 30 or 50 Hz for 1 s (Fig. 6b). This resulted in consistent inward currents in SVZ

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**Figure 4** Electrophysiological properties of subependymal ChAT+ neurons. (a) tdTomato IHC antibody staining of P30 brain coronal section from ChatIRES-cre; R26R-tdTomato mice showing spatial relationships between subependymal and striatal ChAT+ neurons. (b,c) Representative traces of cell-attached recordings from striatal (b) or subependymal (c) ChAT+ neurons. (d,e) Representative traces of cell-attached recordings from striatal (d) and subependymal (e) ChAT+ neurons in response to 1 s of local application of 100 µM glutamate. Bar indicates duration of puffed drug. Peristimulus-time histogram and raster plots for 15 consecutive sweeps, as well as corresponding average spikes per second (mean ± s.e.m.) are shown below, demonstrating baseline spontaneous and glutamate-evoked frequencies. Note the robust spike frequency of subep-ChAT+ neuron during stimulation. Red dashed lines indicate start of drug application across trials. (f,g) Responses of striatal (f) or subependymal (g) ChAT+ neurons, expressing ChR2EYFP to 100 ms (top left), 10 s (top right) or 10 ms (bottom) pulses of 473-nm light. Blue bars indicate duration of light pulse. For 10-ms light pulses, peristimulus-time histogram and raster plots for 15 consecutive sweeps are shown below representative traces. Scale bar represents 20 µm (a).

**Figure 5** Detecting activity-dependent release of ACh in the SVZ niche. (a) Representative changes in M1-CNiFER fluorescent responses at 475- and 530-nm emission wavelengths, with and without ACh application. Excitation source, 920-nm laser. (b–e) Average traces of M1-CNiFER cell baseline FRET ratios and ACh- or light-induced changes in FRET ratios ($\Delta R/R$). (b) ACh applied to adherent M1-CNiFER cells in culture. Images sampled once every 2.6 s ($n = 10$). Data are presented as mean ± s.e.m. (c) ACh applied to M1-CNiFER cells transplanted into SVZ in acute brain slice preparation. Images sampled once every 5.4 s ($n = 16$). Data are presented as mean ± s.e.m. (d) M1-CNiFER cells transplanted into SVZ niche in acute slice preparation from ChatIRES-cre; R26R-ChrR2EYFP mice, followed by 473-nm light stimulation to activate subep-ChAT+ neurons (5 x 250-ms light pulses, 2 Hz). Images sampled once every 1.6 s ($n = 14$). Data are presented as mean ± s.e.m. (e) The focal light-stimulation protocol and slice preparation to image SVZ transplanted M1-CNiFER cells shown in (d) was used to activate striatal ChAT+ neurons adjacent to the SVZ (top trace). Activating striatal ChAT+ neuron at various distances from SVZ: averages of maximum $\Delta R/R$ from multiple slice imaging experiments ($n = 15$). Data are presented as mean ± s.e.m. Scale bar represents 10 µm (a).
NSCs, which could be blocked by cholinergic receptor antagonists (Fig. 6b–d). Similar results were obtained from subependymal GFP+ type B astrocytes in Gfap-GFP; ChatIRES-cre+; R26R-Chr2EYFP mice (data not shown). Although glutamatergic inhibition did not block this light-induced response in tdTomato+ SVZ NSC, it was tetrodotoxin (TTX) sensitive (Fig. 6b,d). Identical 473-nm light–stimulation experiments performed using brain slices from nestin-CreERtm4; R26R-tdTomato mice (without Chat-ChR2EYFP) did not induce measurable currents in tdTomato+ SVZ NSCs (Fig. 6b). We did not detect measurable light-induced currents in nestin-CreERtm4; R26R-tdTomato lineage-traced S100β+ SVZ ependymal niche cells or Mash1+ transiently amplifying progenitors, but we did observe light-induced cholinergic currents in DCX+ neuroblasts in the SVZ (Supplementary Fig. 8c–e).

Optogenetic modulation of SVZ cellular proliferation

Given that our in vitro data suggests that ACh has substantial neurogenic effects (Supplementary Fig. 1), we sought to examine whether activation of subep-ChAT+ neurons can result in increased cellular proliferation in vivo by implanting optical fibers targeting the lateral ventricle of P30 Chatires-cre+/R26R-Chr2EYFP mice (Fig. 7 and Supplementary Fig. 9a). Phosphorylated ribosomal protein S6 (p-rpS6) is an activity-dependent marker for cholinergic neurons in vivo.30,39. IHC staining using antibody to p-rpS6 showed that subep-ChAT+ neurons have robust p-rpS6 expression under physiological conditions (Fig. 7d). Following a light-train protocol that is effective for optogenetic activation of cholinergic neurons40 (Fig. 7a,b), p-rpS6 expression in subep-ChAT+ neurons became enhanced after 48 h of in vivo 473-nm light stimulation (Fig. 7d). Concurrently, we observed consistent increases in the numbers of Ki67+ proliferating cells in the SVZ niche as compared with fiber-implanted controls without light (Fig. 7e and Supplementary Fig. 9b). Expression of Mash1 and DCX and the numbers of Nestin+Ki67+ SVZ NSCs increased following 48 h of light stimulation versus no-light controls (Fig. 7e and Supplementary Fig. 9b).

Identical 48 h of light stimulation, in control mice lacking ChR2 expression in ChAT+ neurons, resulted in no noticeable changes to p-rpS6 expression in subep-ChAT+ neurons (data not shown) or SVZ Ki67+, Mash1+, DCX+ or Nestin+Ki67+ cell numbers (Fig. 7e).

Conversely, in P30 Chatires-cre+/R26R-ArchaerhodopsinGFP (R26R-ArchGFP) mice, we performed the same optogenetic experiment, using a 556-nm laser to silence subep-ChAT+ neurons (Fig. 7c and Supplementary Fig. 9c). This resulted in reduced p-rpS6 expression in subep-ChAT+ neurons (Fig. 7f) and corresponding decreases in the numbers of Ki67+, Mash1+, DCX+ and Nestin+Ki67+ cells in the SVZ niche (Fig. 7g and Supplementary Fig. 9d). Although p-rpS6 expression changes were robust in subep-ChAT+ neurons following light stimulation (Fig. 7d,f), we did not observe concurrent p-rpS6 changes in striatal ChAT+ neurons either adjacent to the SVZ or deeper into the striatum (Supplementary Fig. 9e,f). Similar optogenetic experiments using Chatires-cre+/R26R-Chr2EYFP or Chatires-cre+/R26R-ArchGFP mice, but with light fibers targeting the striatum instead of SVZ, resulted in no obvious changes to SVZ neurogenesis (data not shown). Implantation of optical fiber to target the lateral ventricle represents a form of injury. To understand whether gial proliferation contributed to the observed increases in SVZ Ki67+ cells following ChR2 stimulation, we performed IHC staining for Iba1, CD11b or NG2, 48 h post fiber implantation, and found no significant differences in their cell numbers or proliferation comparing light stimulation versus no-light controls (Supplementary Fig. 10a,b). Consistent with our previous findings4, we detected a delayed onset of Thbs4 protein induction in the SVZ niche 3+ d after fiber placement (Supplementary Fig. 10c). Given that the cascade of injury responses in vivo is complex, we limited our optogenetic analyses to the first 48 h after fiber placement, prior to substantial Thbs4 protein upregulation.

ACh enhances SVZ neurogenesis through the FGFR pathway

It had been reported previously that embryonic cortical progenitors proliferate to ACh stimulation through fibroblast growth factor
On FGF ligand binding, FGFR is known to activate via receptor phosphorylation on tyrosine 653/654 (ref. 42). Following removal of growth factors from primary SVZ NSC culturing media, we detected similar effects on FGFR tyrosine 653/654 phosphorylation in SVZ cultures after re-introduction of FGF (Supplementary Fig. 11a). Addition of ACh without FGF resulted in a similar increase in FGFR phosphorylation (Supplementary Fig. 11a). To determine whether this increase in ACh-mediated FGFR phosphorylation is a direct effect downstream of ACh signal transduction or an indirect pathway perhaps through increased FGF ligand production, we performed similar experiments as above with shorter incubation periods. Although we were able to detect FGF-mediated FGFR activation and phosphorylation 90 min after FGF addition to media, we did not observe noticeable increase in FGFR phosphorylation after ACh addition in the same time course (Supplementary Fig. 11b), making it less likely that ACh-mediated intracellular signals can directly activate FGFR.

To test whether this delayed increase in ACh-mediated FGFR phosphorylation may, in part, be secondary to upregulation of FGF production, we used an antibody to FGF to block FGF ligand binding to its receptor (Supplementary Fig. 11a). Although addition of ACh alone resulted in increased FGFR phosphorylation (Supplementary Fig. 11a), concurrent incubation with antibody to FGF blunted this increase (Supplementary Fig. 11a). ELISA assay to detect the presence of FGF in the culture media during in vitro differentiation revealed corresponding FGF increases in the ACh-treated conditions compared to controls (Supplementary Fig. 11c). In similar experiments, we did not detect EGF receptor (EGFR) activation following ACh addition to SVZ NSC cultures (Supplementary Fig. 11d). Furthermore, co-incubation with antibody to FGF blunted ACh-induced DCX+ neuroblast production in culture (Supplementary Fig. 11e), consistent with the notion that local release of ACh in the SVZ niche in an activity-dependent manner induces NSC production from postnatal SVZ NSCs. In search of potential sources...
for ACh in the SVZ niche, we uncovered direct cholinergic inputs from local subep-ChAT+ neurons. This previously undescribed subpopulation of cholinergic neurons showed morphological and functional differences from their neighboring striatal counterpart and could release ACh into the niche in activity-dependent fashion. In vivo optogenetic manipulation of subep-ChAT+ neurons revealed that their activity was both necessary and sufficient to modulate SVZ neurogenic proliferation. Lastly, we found that SVZ NSCs can respond to ACh release, and then act through FGFR signaling pathway to increase neuroblast production. These results will have important implications for understanding circuit-level control of postnatal and adult SVZ neurogenesis in health and disease.

Cholinergic circuit control of SVZ neurogenesis

The systemic importance of ACh is perhaps best demonstrated by the early postnatal lethality of animals lacking either ChAT28 or VACHT29. ACh can be released via both bulk (non-vesicular) and vesicular (neuronal activity dependent) mechanisms. Together they modulate wide-ranging cellular and neural circuit-level functions such as neuromuscular control and striatal gating of cortical versus thalamic inputs30. Cholinergic signaling has also been reported to influence rodent SVZ neurogenesis: in vivo infusion of nicotinic agonist can result in increased SVZ cellular proliferation, as measured by BrdU incorporation37. Given that the endogenous sources for the ACh mediating these effects were not yet identified, it was possible that indirect actions of cholinergic pharmacology may be responsible for observed phenotype.

To genetically test the importance of cholinergic circuitry on SVZ neurogenesis and to overcome early lethality associated with ChAT and VACHT deletions, we took an approach to blunt cholinergic neurons’ ability to properly scale action potential generation to the strengths of stimuli. We found that cholinergic neurons’ ability to fire precise action potentials is important to maintain the robustness of adult SVZ neurogenesis. As a neurotransmitter, ACh exerts its function locally as a result of rapid degradation by extracellular acetylcholinesterases. Given that striatal ChAT+ neurons are anatomically adjacent to the SVZ niche, we analyzed a genetic mouse model in which ACh from striatal ChAT+ neurons is largely eliminated35, but detected no obvious SVZ neurogenesis defects.

Our discovery that the SVZ niche is directly innervated by local subep-ChAT+ neurons points to exciting future directions for understanding circuit-level control of new neuron production. Subep-ChAT+ neurons highly expressed the neuronal activity-dependent marker p-rpS6 (ref. 39), indicating that these neurons are normally active in vivo. However, they did not spontaneously fire action potentials in acute slice preparations. Given that spontaneous firing of striatal cholinergic neurons is generated by intrinsic membrane properties instead of synaptic drive34, it is possible that membrane property differences contribute to the lack of spontaneous activity in subep-ChAT+ neurons. It is also possible that the functional connectivity for subep-ChAT+ neurons differs from striatal counterparts, although potential sources for excitatory and inhibitory inputs are currently unknown and will require circuit-tracing strategies to identify. Functional experiments have suggested that the rates of adult SVZ neurogenesis can be influenced by pregnancy33,44, male pheromone preference during mating45 and paternal recognition of offspring46. Although it is currently unclear whether subep-ChAT+ neuron activity patterns can be influenced by these behavioral procedures, known neural circuits involved in mediating these behaviors may serve as entry points into understanding the connectivity of subep-ChAT+ neurons.

NSC proliferation and neuroblast production

Throughout embryonic and postnatal development, NSCs self-renew and generate progeny through cell-intrinsic mechanisms that interact with microenvironmental cues. Recent results from adult hippocampal neurogenesis have shown that local neural circuits can be important for NSC proliferation and differentiation37–39. This emerging view on connections between neural circuits and stem cell biology is exciting, as it can elegantly tie together external inputs, circuit-level coding and NSC fate choices to make lasting structural changes via new neuron production. SVZ NSCs require an array of growth factors to sustain self-renewal and balance proliferation and differentiation: EGF is perhaps one of the best studied and is a key ingredient for successful culturing of NSCs in vivo, as it promotes NSC proliferation. Another important growth factor for SVZ NSCs is FGF. We found that ACh preferentially synergizes with FGFR, but not EGFR, activation, which begin to suggest cellular mechanisms for how neural modulation may feed into canonical stem-cell regulatory loops.

The inhibitory neurotransmitter GABA has been shown to enhance the maturation of newborn SVZ neuroblasts40. Although GABAs important function on neuronal inhibition is not lost in this system, as its increased level feedback onto NSCs to dampen their proliferation19,20. This elegant parallel usage of neurotransmitter for neural circuit and NSC control may ensure efficient integration of these two biological processes in the brain. Future experiments aimed to address intersections between neurotransmitters and known pathways controlling NSC proliferation and differentiation should shed further light on our understanding of circuit-level control of neurogenesis.

METHODS

Methods and any associated references are available in the online version of the paper.

Note: Any Supplementary Information and Source Data files are available in the online version of the paper.

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AUTHOR CONTRIBUTIONS

P.P.-G. designed and performed the anatomical, IHC staining and biochemical experiments. B.A. and P.P.-G. designed and performed the in vivo optogenetic experiments. B.A., E.R. and C.T.K. designed and performed the electrophysiological recordings. B.A. and C.T.K. designed and performed the imaging experiments. C.T.K. conceived the project. P.P.-G., B.A. and E.R. assembled the figures. P.P.-G., B.A. and C.T.K. wrote the paper. All of the authors discussed results and commented on the manuscript.

COMPETING FINANCIAL INTERESTS

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1. Kelsh, W., Sim, S. & Lois, C. Watching synaptogenesis in the adult brain. Neuron 33, 131–149 (2000).
2. Ihrie, R.A. & Alvarez-Buylla, A. Lake-front property: a unique germinal niche by the lateral ventricles of the adult brain. Neuron 70, 674–686 (2011).
3. Lazarini, F. & Liedo, P.M. Is adult neurogenesis essential for olfaction? *Trends Neurosci.* **34**, 569–579 (2011).

4. Aboudy, K., Capeia, A., Nazif, S. & Silver, J.H. & 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* **70**, 597–613 (2011).

5. Robel, S., Berninger, B. & Gotz, M. The stem cell potential of glia: lessons from reactive gliosis. *Nat. Rev. Neurosci.* **14**, 88–104 (2011).

6. Benner, E.J. et al. Protective astrogliosis from the SVZ niche after injury is controlled by Notch modulator Thbs4. *Nature* **497**, 369–373 (2013).

7. Kriegstein, A. & Alvarez-Buylla, A. The glial nature of embryonic and adult neural stem cells. *Annu. Rev. Neurosci.* **32**, 149–184 (2009).

8. Shu, H., Deng, W. & Gage, F.H. Signaling in adult neurogenesis. *Annu. Rev. Cell Dev. Biol.* **25**, 253–275 (2009).

9. Shen, Q. et al. Endothelial cells stimulate self-renewal and expand neurogenesis of neural stem cells. *Science* **304**, 1338–1340 (2004).

10. Lin, C.W. & Raz, J. Axonal control of the adult neural stem cell niche. *Cell Stem Cell* **10**, 76–87 (2012).

11. Liu, X., Wang, Q., Haydar, T.F. & Bordey, A. Nonsynaptic GABA signaling in postnatal neurogenesis. *Cell* **127**, 1253–1264 (2006).

12. Paez-Gonzalez, P. et al. Anki-dependent SVZ niche assembly is required for the continued production of new neurons. *Neuron* **71**, 61–75 (2011).

13. Miller, F.D. & Gauthier-Fisher, A. Home at last: neural stem cell niches defined. *Cell Stem Cell* **4**, 507–510 (2009).

14. Bovetti, S., Gribaudo, S., Puche, M.C., De Marchis, S. & Pasolli, A. From progenitors to integrated neurons: role of neurotransmitters in adult olfactory neurogenesis. *J. Chem. Neuroanat.* **34**, 304–316 (2011).

15. Young, S.Z. & Bordey, A. Neurotransmitters couple brain activity to subventricular zone neurogenesis. *Eur. J. Neurosci.* **33**, 1123–1132 (2011).

16. Brazel, C.Y., Nunez, J.L., Yang, Z. & Levison, S.W. Glutamate enhances survival and proliferation of neural progenitors derived from the subventricular zone. *Neuroscience* **131**, 55–65 (2005).

17. Platel, J.C. et al. NMDA receptors activated by subventricular zone astrocytic glutamate are critical for neuroblast survival prior to entering a synaptic network. *Neuron* **65**, 859–872 (2010).

18. Lin, C.W. et al. Genetically increased cell-intrinsic excitability enhances neuronal integration into adult brain circuits. *Neuron* **65**, 32–39 (2010).

19. Liu, X., Wang, Q., Haydar, T.F. & Bordey, A. Nonsynaptic GABA signaling in postnatal subventricular zone controls proliferation of GFAP-expressing progenitors. *Nat. Neurosci.* **8**, 1179–1187 (2005).

20. Alfonso, J., Le Maqueuse, C., Zuccotti, A., Khodosevich, K. & Monyer, H. Dizocilpine binding inhibitor promotes progenitor proliferation in the postnatal SVZ by reducing NMDA receptor activation. *J. Neurosci.* **30**, 1179–1187 (2010).

21. Young, S.Z., Platel, J.C., Nielsen, J.V., Jensen, N.A. & Bordey, A. GABA(A) increases calcium in subventricular zone astrocyte-like cells through L- and T-type voltage-gated calcium channels. *Front. Cell. Neurosci.* **4**, 8 (2010).

22. Van Kampen, J.M., Hagg, T. & Robertson, H.A. Induction of neurogenesis in the subependymal layer of the adult rat brain and enhancement of neuronal precursor cell proliferation. *Neuroscience* **145**, 470–483 (2007).

23. Misgeld, T. et al. Roles of neurotransmitter in synapse formation: development of neocortical junctions lacking choline acetyltransferase. *Neuron* **36**, 635–648 (2002).

24. de Castro, B.M. et al. The vesicular acetylcholine transporter is required for neuromuscular development and function. *Mol. Cell. Biol.* **29**, 5238–5250 (2009).

25. Zhou, D. et al. AnkyrinG is required for clustering of voltage-gated Na channels at axon initial segments and for normal action potential firing. *J. Cell Biol.* **143**, 1295–1304 (1998).

26. Kordeli, E., Lambert, S. & Bennett, V. AnkyrinG. A new ankyrin gene with neural-specific isoforms localized at the axonal initial segment and node of Ranvier. *J. Biol. Chem.* **270**, 2352–2359 (1995).

27. Patel, J.C., Rossignol, E., Rice, M.E. & Machold, R.P. Opposing regulation of dopaminergic activity and exploratory motor behavior by forebrain and brainstem cholinergic circuits. *Nat. Commun.* **3**, 1172 (2012).

28. Guzman, M.S. et al. Elimination of the vesicular acetylcholine transporter in the striatum reveals regulation of behaviour by cholinergic-glutamatergic co-transmission. *PLoS Biol.* **9**, e1001194 (2011).

29. Bennett, B.D. & Wilson, C.J. Spontaneous activity of neostriatal cholinergic interneurons in vitro. *Neurosci.** **19**, 5586–5596 (1999).

30. Ding, J.B., Guzman, J.N., Peterson, J.D., Goldberg, J.A. & Surmeier, D.J. Thalamic gating of corticostriatal signaling by cholinergic interneurons. *Neuron* **67**, 294–307 (2010).

31. Nguyen, V.T. et al. An in vivo biosensor for neurotransmitter release and in situ receptor activity. *Nat. Neurosci.* **13**, 127–132 (2010).

32. Doetsch, F., Garcia-Verdugo, J.M. & Alvarez-Buylla, A. Cellular composition and threedimensional organization of the subventricular germinal zone in the adult mammalian brain. *J. Neurosci.* **17**, 5046–5061 (1997).

33. Bertran-Gonzalez, J., Chieng, B.C., Laurent, V., Valjent, E. & Balleine, B.W. Striatal cholinergic interneurons display activity-related phosphorylation of ribosomal protein S6. *PLoS ONE* **7**, e3195 (2012).

34. Knight, Z.A. et al. Molecular profiling of activated neurons by phosphorylated ribosome capture. *Cell** **151**, 1126–1137 (2012).

35. Nagode, D.A., Tang, A.H., Karson, M.A., Klugmann, M. & Alger, B.E. Optogenetic release of ACh induces rhythmic bursts of perisomatic IPSCs in hippocampus. *PLoS ONE* **6**, e27691 (2011).

36. Ma, W. et al. Acetylcholine stimulates cortical precursor cell proliferation in vitro via muscarinic receptor activation and MAP kinase phosphorylation. *Eur. J. Neurosci.* **12**, 1227–1240 (2000).

37. Campbell, J.S., Wenderoth, M.P., Haushka, S.D. & Krebs, E.G. Differential activation of mitogen-activated protein kinase in response to basic fibroblast growth factor in skeletal muscle cells. *Proc. Natl. Acad. Sci. USA* **92**, 870–874 (1995).

38. Shingo, T. et al. Pregnancy-stimulated neurogenesis in the adult female forebrain mediated by prolactin. *Science* **299**, 117–120 (2003).

39. Wang, W. et al. Extracellular signal-regulated kinase 5 (ERK5) mediates prolatin-stimulated adult neurogenesis in the subventricular zone and olfactory bulb. *J. Biol. Chem.* **289**, 2623–2631 (2013).

40. Mak, G.K. et al. Male pheromone-stimulated neurogenesis in the adult female brain: possible role in mating behavior. *Nat. Neurosci.* **10**, 1003–1011 (2007).

41. Mak, G.K. & Weiss, S. Paternal recognition of adult offspring mediated by newly generated CNS neurons. *Nat. Neurosci.* **13**, 753–758 (2010).

42. Markwardt, S.J., Dieni, C.V., Wadiche, J.I. & Overstreet-Wadiche, L. Ivy/neurogliaform interneurons mediate neuronal circuitry-related activity in the supragranular zone of the mouse barrel cortex. *Nat. Neurosci.* **14**, 554–565 (2011).

43. Puzdrowski, B. et al. Species-typical control of the adult neural stem cell niche. *Cell Stem Cell* **10**, 500–511 (2014).

44. Cooper-Kuhn, C.M., Winkler, J. & Kuhn, H.G. Decreased neurogenesis after choline forebrain lesion in the adult rat. *J. Neurosci.* **Res.** **77**, 155–165 (2004).

45. Muzol, 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* **145**, 470–483 (2007).
Animals were anesthetized with isoflurane to target the lateral ventricle was performed as described previously. Placement of cannula under isoflurane anesthesia to target the lateral ventricle was performed as described previously (Doric), allowing free animal movement. Light-stimulation of ChR2EYFP was delivered by TTL control of 473-nm laser (IkeCool).

Optogenetic stimulation and analyses. Placement of cannula under isoflurane anesthesia to target the lateral ventricle was performed as described (Doric), using implantable mono fiber-optic fiber (200 μm, 0.22 NA, Doric). Proroducing ferule end of cannula was then connected via fiber cord to rotary coupling joint (Doric), allowing free animal movement. Light-stimulation of ChR2EYFP was delivered by TTL control (Master 8, AMPI) of 473-nm laser (IkeCool), 5-ms pulse duration bursts at 5 Hz, lasting 10 s, given once every 2 min as described. Light inhibition with ArchGFP was performed by TTL-controlled 566-nm laser (IkeCool), on for duration of experiment. For SVZ p-pS6, K67, Mash1, DCX, Nestin, Ib1, CD11b and NG2 analyses, 50-μm brain coronal sections were cut and collected serially on Leica VT1000S vibratome. Position of optical fiber tract was verified, and five coronal sections surrounding the fiber tract were selected for analyses, comparing P30 littermates with light versus no-light stimulation.

2.5 mM KCl, 1.25 mM NaH2PO4, 26 mM NaHCO3, 20 mM glucose, 2 mM CaCl2, 1.3 mM MgCl2) at 2–5 ml min−1 at 23–25 °C. Cell-attached recordings were performed using ACSF-filled micropipettes (4–6 MΩ). Signals were amplified with Multiclamp 700B (filtered at 2 kHz), digitized with Digidata 1440A (5 kHz), recorded using pClamp 10 software (Axon). Internal solutions for whole-cell recordings contained 130 mM potassium gluconate, 2 mM NaCl, 4 mM MgCl2, 20 mM HEPES, 4 mM Na2ATP, 0.4 mM NaGTP, 0.5 mM EGTA and Alexa 488 dye (Invitrogen) to visualize patched cells. Signals were amplified with Multiclamp 700B (filtered at 10 kHz), digitized with Digidata 1440A (20 kHz), recorded using pClamp 10. Pipette puff application was performed via transistor-transistor logic (TTL) control of picospritzer III (Parker). Light-activation of channelrhodopsin was delivered by TTL control of 473-nm laser (IkeCool). Tracings were analyzed using Neurounematic package (Think Random) in Igor Pro software (WaveMetrics). Voltage threshold for action potential generation was identified as point of most rapid change in membrane potential, determined by analyzing first and second derivatives in current-pulse action potential after current injection. Time zero = mean time at current pulse initiation.

DOI and DIO-labeling. Dil-labeling was performed according to previously described methods58 with the following modifications: following dissection, samples were fixed for 1 h at 23–25 °C with 4% paraformaldehyde (wt/vol) in PBS, then washed 3 × 20 min in PBS. Dil (Invitrogen, 40 μM CM-Dil in ethanol) was loaded into cells using micropipettes and 4-nA current injection for 10 min, followed by 10 min without current for equilibration. Samples were then returned to 4% paraformaldehyde in PBS at 37 °C for 24 h to allow diffusion through neuronal processes. For ventricular views, SVZ niche and striatum were dissected in whole-mount preparation59. For coronal views, brain samples were sliced into 300-μm sections with VT1000S vibratome (Leica). Neuronal processes were traced and analyzed using three-dimensional filament tracer in Imaris software (Bitplane). Dendritic fields were enveloped with three-dimensional hull surface to provide ellipsoid axis length measurements, with ‘z’ designated as the long axis in either ventricular or coronal views. Imaris filament statistics provided dendritic branch point numbers and three-dimensional Sholl analysis was run to determine filament crossings through concentric spheres centered on soma. VYbrant DIO (Invitrogen, 40 μM) was dissolved in dichloromethane (Sigma).
**SDS-PAGE and immunoblotting.** Protein extracts were prepared as described\(^{62}\), and resolved by electrophoresis through SDS-PAGE and transferred onto nitrocellulose membranes. For FACS-sorting of ChAT\(^+\) neurons, brain tissues were first dissociated with neural tissue dissociating kit (Miltenyi) according to manufacturer’s protocol, followed by sorting on BD FACS DiVa sorter via genetically labeled tdTomato fluorescence. Antibodies were diluted in PBS containing 0.2% Triton X-100 (vol/vol) and 4% non-fat dry milk (wt/vol), followed by overnight incubation at 4 °C. Detection was accomplished through secondary antibodies conjugated to horseradish peroxidase (#111-036-003, 115-036-003, 1:5,000, Jackson ImmunoResearch) and treated with enhancedchemiluminescence (Thermoscientific). We used primary antibodies to Ank3 (rabbit, 1:1,000, V. Bennett), DCX (#4604, 1:600, Cell Signaling), pFGFR1 (#06-1433, 1:2,500, Millipore), pEGFR (#2238, 1:800, Cell Signaling) and actin (#ab3280, 1:2,000, Abcam). All antibodies used were validated in our previous publications\(^{6,12}\) or by publications available on vendor website specific to each antibody.

**Statistical analysis.** No statistical methods were used to pre-determine samples sizes, but they are similar to those reported elsewhere\(^{6,11,12}\). IHC staining cell counting from animal experiments was performed blind to the experimental condition; other data collection and analyses were not performed blind to the conditions. Blocking of experimental design was assigned by animal genotype and was not randomized. Data sets were tested for normality with Igor Pro (WaveMetrics) using serial randomness test. Depending on sample size, Student’s t test (≥10) or Wilcoxon two-sample test (≥5) were used for statistical comparisons between two datasets. One-way ANOVA was performed for multivariate comparisons. In vivo optogenetic experiments were performed on sets of three littermates and compared via one-way ANOVA for correlated samples. Cell recording data was acquired first in control followed by pharmacological conditions, and compared by one-way ANOVA for correlated samples. Throughout, Tukey box-and-whisker plots were generated using BoxPlotR (http://boxplot.tyerslab.com/) to depict mean (+), median (line), low and high quartiles (boxes), range (whiskers), and outliers (o).

A Supplementary Methods Checklist is available.

51. Gong, S. et al. Targeting Cre recombinase to specific neuron populations with bacterial artificial chromosome constructs. J. Neurosci. 27, 9817–9823 (2007).

52. Gong, S. et al. A gene expression atlas of the central nervous system based on bacterial artificial chromosomes. Nature 425, 917–925 (2003).

53. Jenkins, P.M. et al. E-cadherin polarity is determined by a multifunction motif mediating lateral membrane retention through ankyrin-G and apical-lateral transcytosis through clathrin. J. Biol. Chem. 288, 14018–14031 (2013).

54. Scheffler, B. et al. Phenotypic and functional characterization of adult brain neuroepoiesis. Proc. Natl. Acad. Sci. USA 102, 9353–9358 (2005).

55. Sheikh, F., Fandrich, R.R., Kardami, E. & Cattini, P.A. Overexpression of long or short FGFR-1 results in FGF-2-mediated proliferation in neonatal cardiac myocyte cultures. Cardiovasc. Res. 42, 696–705 (1999).

56. Lim, D.A. et al. Chromatin remodeling factor Mll1 is essential for neurogenesis from postnatal neural stem cells. Nature 458, 529–533 (2009).

57. Mirzadeh, Z., Merkle, F.T., Soriano-Navarro, M., Garcia-Verdugo, J.M. & Alvarez-Buylla, A. Neural stem cells confer unique pinwheel architecture to the ventricular surface in neurogenic regions of the adult brain. Cell Stem Cell 3, 265–278 (2008).

58. Kao, Y.H. & Sterling, P. Matching neural morphology to molecular expression: single cell injection following immunostaining. J. Neurocytol. 32, 245–251 (2003).

59. Mirzadeh, Z., Doetsch, F., Sawamoto, K., Wichterle, H. & Alvarez-Buylla, A. The subventricular zone en-face: wholemount staining and ependymal flow. J. Vis. Exp. 39, 1938 (2010).

60. Mank, M. et al. A genetically encoded calcium indicator for chronic in vivo two-photon imaging. Nat. Methods 5, 805–811 (2008).

61. Beau lieu, J.M. et al. A beta-arrestin 2 signaling complex mediates lithium action on behavior. Cell 132, 125–136 (2008).

62. Abd, K.M. & Bennett, V. Adducin promotes micrometer-scale organization of beta2-spectrin in lateral membranes of bronchial epithelial cells. Mol. Biol. Cell 19, 536–545 (2008).
Erratum: Identification of distinct ChAT+ neurons and activity-dependent control of postnatal SVZ neurogenesis

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In the version of this article initially published online, the plus signs denoting the mean values in Figure 7e were displaced upward by ~0.1 units. The error has been corrected for the print, PDF and HTML versions of this article.