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Optogenetic stimulation of Glutamatergic Neuronal Activity in the Striatum Enhances Neurogenesis in the Subventricular Zone of Normal and Stroke Mice

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Abstract

Neurogenesis in the subventricular zone (SVZ) of the adult brain may contribute to tissue repair after brain injuries. Whether SVZ neurogenesis can be upregulated by specific neuronal activity in vivo and promote functional recovery after stroke is largely unknown. Using the spatial and cell type specific optogenetic technique combined with multiple approaches of *in vitro*, *ex vivo* and *in vivo* examinations, we tested the hypothesis that glutamatergic activation in the striatum could upregulate SVZ neurogenesis in the normal and ischemic brain. In transgenic mice expressing the light-gated channelrhodopsin-2 (ChR2) channel in glutamatergic neurons, optogenetic stimulation of the glutamatergic activity in the striatum triggered glutamate release into SVZ region, evoked membrane currents, Ca²⁺ influx and increased proliferation of SVZ neuroblasts, mediated by AMPA receptor activation. In ChR2 transgenic mice subjected to focal ischemic stroke, optogenetic stimuli to the striatum started 5 days after stroke for 8 days not only promoted cell proliferation but also the migration of SVZ neuroblasts into the peri-infarct cortex with increased neuronal differentiation and improved long-term functional recovery. These data provide the first morphological and functional evidence showing a unique striatum-SVZ neuronal regulation via a semi-phasic synaptic mechanism that can boost neurogenic cascades and stroke recovery. The benefits from stimulating endogenous glutamatergic activity suggest a novel regenerative strategy after ischemic stroke and other brain injuries.

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Conflict of Interests

The authors declare no conflict of interests related to this research.

Keywords

SVZ neurogenesis; Striatum stimulation; Optogenetics; Glutamatergic neurons; Ischemic stroke; Semi-phasic synaptic regulation; Functional recovery

Introduction

In the adult brain, the subventricular zone (SVZ) of the lateral ventricle and the subgranular zone (SGZ) in the hippocampus are primary neurogenic niches where neural stem cells (NSCs) and neuroblasts are generated. Among the two regenerative regions, most investigations have focused on the regulation of SGZ neurogenesis, likely due to its important role in neurodegenerative disorders such as Alzheimer's disease (Kempermann et al., 2015). SVZ neurogenesis, on the other hand, is responsive to brain injuries such as stroke and traumatic brain injury (Ohab and Carmichael, 2008; Robin et al., 2006). Although SVZ has drawn some heated attention due to recent reports on its contribution to astrogenesis in the injured brain (Benner et al., 2013; Faiz et al., 2015), SVZ neurogenesis and its regulation remain to be better understood. Normally, SVZ neuroblasts migrate to the olfactory bulb via the rostral migratory stream (RMS) and become interneurons (Doetsch and Alvarez-Buylla, 1996; Doetsch et al., 1999). After stroke, migration of SVZ neuroblasts is redirected towards the lesion site, where expression of the chemoattractant factor stromal cell-derived factor-1 (SDF-1) is greatly up-regulated (Robin et al., 2006; Wang et al., 2014). This post-stroke SVZ neurogenesis has been regarded as a potential regenerative mechanism of damaged brain tissues (Ohab et al., 2006; Robin et al., 2006). Unfortunately, the SVZ typically does not generate enough cells for tissue repair; moreover, few of these cells can reach to and survive in the lesion site. To utilize SVZ neurogenesis for tissue repair after brain injuries, a better understanding of regulatory mechanisms that can promote the endogenous regenerative activities is essential and critically important.

Neural progenitor cells (NPCs)/neuroblasts express γ -aminobutyric acid (GABA) and glutamate receptors, suggesting they can receive neuronal inputs (Nakamichi et al., 2009). GABA and glutamate may act as paracrine factors to affect cell proliferation in the SVZ (Vicini, 2008; Young et al., 2011). GABA predominately exerts a tonic inhibitory action on SVZ cell proliferation (Berg et al., 2013; Liu et al., 2005), while glutamate enhances the survival and proliferation of cultured SVZ cells (Brazel et al., 2005; Whitney et al., 2008). The source of those neurotransmitters in the in vivo regulation of SVZ neurogenesis, however, has been poorly defined. It was shown that GABA and glutamate are released from SVZ NPCs and astrocytes via a non-vesicular mechanism (Bolteus and Bordey, 2004; Liu et al., 2005; Platel et al., 2010b). This mechanism, however, cannot explain why neuronal activities and environmental factors under normal and disease states can have long-range effects on SVZ neurogenesis.

The striatum region is anatomically adjacent to the SVZ and presumably in a suitable location to affect cellular activities in the SVZ. A recent investigation showed that GABAergic neurons in the striatum send axonal processes into the SVZ, release GABA and regulate SVZ cellular activities via activation of the GABA_A receptor (Young et al., 2014).

We here examined the possibility that SVZ neurogenesis could be upregulated by stimulating glutamatergic activities in the striatum and this strategy could be utilized as a regenerative therapy. A transgenic mouse expressing the light-gated channelrhodopsin-2 (ChR2) in glutamatergic neurons (Jasnow et al., 2013; Wang et al., 2007) was tested under normal and stroke conditions. Our data demonstrate that selective stimulation of glutamatergic neurons/axons in the striatum enhances a cascade of SVZ cellular responses with increased regenerative activities and functional recovery after stroke in a focal cerebral ischemic model of mice.

Materials and Methods

Animals

All animal experimental procedures were approved by IACUC (Institutional Animal Care and Use Committee) at Emory University and conformed to the guidelines of the National Institute of Health Guide for the Care and Use of Laboratory Animals. Male adult (3 months) channelrhodopsin-2 (ChR2) transgenic mice (B6.Cg-Tg(Thy1-COP4/EYFP)18Gfng/J) and C57/B6L mice were purchased from the Jackson Laboratory and bred at Emory University animal facilities. Animals were allowed free access to food and water. The animal protocol for this study was approval by the Emory Animal Care and Use Committee (IACUC); all studies were conducted in accordance with the United States Public Health Service's Policy on Use of Laboratory Animals.

Focal ischemic stroke surgical procedures

Animals were subjected to anesthesia by 3% isoflurane in a mixture of 30% O₂ and 70% N₂O. After induction of anesthesia, 1.5% isoflurane was maintained with mechanical ventilation. Body temperature was maintained at 37 ± 0.5 °C using a heating pad regulated by a rectal temperature probe. Focal ischemic stroke was induced in the right somatosensory cortex of mice (Li et al., 2008; Song et al., 2013). In brief, distal branches of the right middle cerebral artery (MCA) that supplies blood flow to the somatosensory cortex was permanently ligated combined with bilateral common carotid arteries (CCA) ligations for 7 minutes. Sham control mice only received surgery lesions on the skull surface.

Partial reperfusion due to incomplete (spontaneous and postthrombolytic) recanalization after an ischemic attack occurs in 30% and up to 70% of stroke patients at different times after the onset of ischemia (Barber et al., 1998; Hakim et al., 1987; Jorgensen et al., 1994; Neumann-Haefelin et al., 2004). For example, in a CT/SPECT scan study on 354 stroke patients, the incidence of spontaneous reperfusion was 77% in patients with cortical infarcts (Jorgensen et al., 1994). In this regard, the release of CCAs ligation while MCA was permanently occluded in our cortical stroke model resemble to a great extent a partial reperfusion condition seen in many clinical stroke cases.

Optical fiber implantation and laser light stimulation

Four days after stroke induction, mice were anesthetized by isoflurane and mounted on a stereotaxic frame (Narishige, Tokyo, Japan). After shaving the hair and exposing the skull, a small hole was made using a dental drill. Optical fibers (with 0.25 mm diameter, Doric

lenses, QC, Canada) were implanted into the right striatum (0.75 mm anterior to bregma, 1.2 mm lateral of the midline to the right, 2.4 mm below the dura) and mounted with a cannula and bone cement on the skull (see Fig. 5A). Stroke control mice also received optical fiber implantation into the striatum but without laser light stimulation. The 473 nm blue laser light was generated by a DPSS laser system equipped with a mono fiber patch cord that can be connected to the optical cannula (Doric lenses). The laser light stimulation started at day 5 after stroke. Animals received a 15-min stimulation session per day. The session had 15 bursts and each burst contained 120 pulses (5 ms width of each, firing at 10 Hz), thus the inter-burst interval was 48 seconds. The 10-Hz frequency stimulation is considered within physiological range and has been applied for stroke treatment (Cheng et al., 2014). In addition, we previously studied stimulation-based stroke rehabilitation in which whisker stimulation of 15 min per day showed promoting effects on sensorimotor functional recovery after the focal ischemic stroke (Li et al., 2008). The power of the blue laser light typically ranged from 0.35 to 1.02 mW pre-measured by a Laser Power and Energy Meter (Thorlabs Inc., Newton, NJ). This light power is similar to that used by other experimental studies to activate ChR2 and conduct optogenetic brain stimulation (Arenkiel et al., 2007; Zhang et al., 2006). When non-stroke mice were studied, laser light stimulation was conducted for 4 days. According previous characterization, laser power transmitted by the fiber will vary with fiber type (multimode or single-mode) and the spread of light from the fiber is partially determined by the numerical aperture (NA), with low NA corresponding to a narrow angle and high NA corresponding to a larger angle (Cardin et al., 2010). Power also decreases with distance from the light source. As a principle for blue light transmission through brain tissue, the total transmitted light power is reduced by 50% after passing through 100- μ m tissue (Aravanis et al., 2007). We thus estimated that the tissue of approximate 100 to a few hundred μ m around the fiber tip was effectively stimulated.

To detect the glutamate increases in the brain triggered by the 15-min laser light stimulation, the brain region of interest was taken out under ice cold conditions, prepared and measured by following the instructions of a commercial glutamate assay kit (Abcam, Cambridge, MA).

Coronal brain slices, extracellular and whole-cell patch clamp recordings and Ca²⁺ imaging

The ChR2 transgenic mice were anaesthetized with isoflurane, decapitated, and the brain was dropped in ice-cold artificial cerebrospinal fluid (aCSF) containing: 124 mM NaCl, 3 mM KCl, 1.25 mM NaH₂PO₄, 6.0 mM MgCl₂, 26 mM NaHCO₃, 2.0 mM CaCl₂, and 10 mM glucose. aCSF was saturated with 95% O₂ and 5% CO₂, at pH 7.4. The brain was cut into 400- μ m thick transverse slices with a vibratome (Vibratome 1000) Plus Sectioning System, St Louis, MO). After incubation at room temperature (23–24°C) in aCSF containing 1 mM MgCl₂ for 60–90 min, slices were placed in a recording chamber (RC-22C, Warner Instruments, Hamden, CT) on stage of an up-right microscope (Nikon's Eclipse FN1; Nikon Instruments Inc., NY, NY) and perfused at a rate of 3 ml per min with aCSF containing 1 mM MgCl₂ at 33–35°C. The optical fiber with 0.4 mm diameter (Doric Lenses) was positioned to the striatum region. In extracellular recordings of evoked field potentials, the recording electrode was filled with aCSF. Whole-cell patch clamp recording was performed

on SVZ cells using a borosilicate glass microelectrode (Sutter Instrument, Novato, CA) of 5–8 MΩ tip resistance filled with an intracellular solution containing (in mM): 140 KCl, 2 MgCl₂, 1 CaCl₂, 2 Na₂ATP, 8 EGTA, and 10 HEPES at pH of 7.2. Recordings and data acquisition were controlled by an EPC-9 amplifier and the PULSE software (HEKA Elektronik, Lambrecht, Germany). The pulse of blue laser (473 nm) stimulation was controlled by the trigger function of the EPC-9 amplifier. Data were filtered at 3 kHz and digitized at sampling rates of 20 kHz.

For Ca²⁺ imaging, brain slices were put in a staining chamber and perfused under interface-mode with aCSF at 35°C. The calcium dye Fura-2 AM (50 μM dissolved in DMSO) was pipetted directly over the SVZ region of interest and incubated for 40 min. The slices were transferred back to the recording chamber. Selected cells were illuminated by excitation light at 340 and 380 nm, and the ratio of F340 to F380 (F340/F380) was regarded as the amount of intracellular Ca²⁺ ([Ca²⁺]_i). Ca²⁺ images were acquired every 3 sec and recorded with a digital CCD camera (HAMAMATSU ORCA-ER, Hamamatsu Photonics K.K., Hamamatsu City, Japan) and analyzed with the software Slidebook 4.1 (SciTech Pty Ltd., Preston, Victoria, Australia).

In Vivo axonal tracer labeling

Striatum injection of axon labeling was performed using a protocol modified from previous method (Zhao et al., 2003). Briefly, 1, 1-Dioctadecyl-6, 6-di (4-sulfophenyl)-3, 3, 3-tetramethylindocarbocyanine (DiI; Molecular Probes, Inc., Eugene, OR) was dissolved in dimethyl sulfoxide (DMSO; 0.2% wt/vol final concentration) and delivered using intracerebral stereotaxic injection of 1 μL into the left striatum (Bregma +0.7 mm, ML 2.0 mm, DV –3.4 mm). The brain was dissected 14 days after injection and fixed by immersion with 4% PFA post-fixed overnight at 4°C. Coronal brain sections were prepared for immunofluorescent imaging under a fluorescent microscope (Olympus FV1000-IX81 laser scanning confocal microscope, Olympus, Tokyo, Japan).

Culture of SVZ neuroblasts and treatment

Neural neuroblasts cells were isolated and cultured as previously described (Brazel et al., 2005). Briefly, cell suspensions were isolated from the SVZ of neonatal ChR2 mouse brain and plated in proliferation medium, containing DMEM and Ham's F12 medium (DMEM/F12, 1:1) (Invitrogen, Carlsbad, CA) supplemented with 2% B27 (Invitrogen), 20 ng/mL epidermal growth factor (EGF) and 20 ng/mL basic fibroblast growth factor (bFGF; Invitrogen). Cell suspensions were cultured at 37°C in an incubator with a humidified atmosphere containing 5% CO₂/95% air. In this floating system, neural progenitors proliferated and formed neurospheres. After passage 2–3, neurospheres were incubated in trypsin for 15 min and mechanically disassociated into single cells. Disassociated cells were plated onto poly-D-lysine and laminin-coated cell culture dishes, cultured for 4–5 days and identified as neuroblasts by staining of DCX.

Adhesive dot removal test

The adhesive dot removal behavioral test was employed to monitor the sensorimotor function of mice by placing a small adhesive sticker onto the forepaw of the legs as

previously described (Bouet et al., 2009; Freret et al., 2009; Ogle et al., 2012). In mice of focal stroke to the right somatosensory cortex, their left paws were expected to develop functional deficits. We recorded the time in seconds it took the mice to touch and remove the stickers. Animals were numbered and trained 5 times per day for 3 days before stroke. Animals were randomly placed into different groups and treated with laser light stimulation or set as stroke controls. The touch latency and removal time was recorded at 3, 10, 17, 24, and 31 days post-stroke by a blinded experimenter.

5-bromo-2'-deoxyuridine (BrdU) labeling and administration of other drugs

To label newborn cell, each animal received a daily injection of bromodeoxyuridine (BrdU; Sigma-Aldrich, St. Louis, MO) at 50 mg/kg, intraperitoneally. BrdU was dissolved in 0.9% sterile saline, injected 2 hours before laser light stimulation, and administrated throughout the 4- or 8-day treatment period. To prevent AMPA receptor activation in the brain, 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo[f]quinoxaline-2,3-dione (NBQX) disodium salt (25 mg/kg; Abcam) was intraperitoneally injected into mice 50 min before laser light stimulation. To suppress cell proliferation of neuroblasts or their migration, Cytosine arabinoside (AraC, 10 mg/kg in saline) or AMD3100 (2 mg/kg in saline) were delivered once daily into the mouse brain intranasally along with 8-day laser stimulation. Animals received 100 U hyaluronidase (Sigma) dissolved in sterile saline 30 min prior to intranasal delivery of drugs. Hyaluronidase was used to disrupt the barrier function of the nasopharyngeal mucosa and facilitate drug entry to the brain.

Immunofluorescent staining and cell counting

At different time points as shown in the experimental time lines, mice were deeply anesthetized and perfused with 4% paraformaldehyde (PFA) in phosphate-buffered saline (PBS) through the heart. The entire brain was immersed in 4% PFA for 24 hrs and cut into coronal or sagittal sections with 10 μ m thickness using a cryostat microtome (Leica CM 1950, Germany) and stored at -80°C until being stained. Immunofluorescence staining of DCX and BrdU was performed as previously described (Song et al., 2013).

Immunofluorescence staining was performed with the primary antibodies against VGLUT1 (1:400; UC Davis/NIH NeuroMab Facility), VGLUT2 (1:400; Alomone Labs, Israel.) CaMKII α (1:400; Millipore), NeuN and Nestin (1:500; Millipore), GluR1 and GluR2 (1:300; Millipore), NR1 and NR2B (1:300; Abcam), Iba1 (1:300; Abcam) and Cleaved Caspase-3 (1:400; Cell Signaling, Danvers, MA). Nissl staining was performed by fixing the sections in a 1:1 mixture of formalin and acetic acid for 10 min and then washing with distilled water. Sections were then placed in a working solution of Cresyl violet, rinsed with 70% ethanol and finally dried overnight.

Cell counting was performed following a modification of the principles of design based stereology. BrdU⁺ and DCX⁺ cells were counted in the SVZ, corpus callosum, and along the peri-infarct region distributed on 6 serial sections (10 μ m thicknesses) per brain spaced 150 μ m apart and spanning the lesion. Sections from different animals represent the same area in the anterior-posterior direction. Slides were examined with a fluorescence microscope (BX61, Olympus) and digitized images were taken by Slidebook 4.2 software (Intelligent

Imaging Innovations). The high resolution z-stack images were taken by using the Olympus FV1000-IX81 laser scanning confocal microscope and Olympus Fluoview v1.7 software (Olympus, Tokyo, Japan).

Statistical data analysis

Data from cell counting and behavioral tests were analyzed by two-way ANOVA and followed by post hoc Tukey's test. Simple two group comparison was performed by Student's t-test as indicated in the text. Changes were identified as significant when $P < 0.05$. All data were expressed as Mean $\pm$ SEM. Data was graphed using GraphPad Prism, version 4 (GraphPad Software, Inc., San Diego, CA).

Results

ChR2-YFP expression in glutamatergic neurons/axons in the cortex and striatum of the ChR2 transgenic mouse

The optogenetic technique allows for selective modulation of specific cells in the living brain (Welberg, 2013; Yizhar et al., 2011). Previous studies on the ChR2 transgenic mouse (B6.Cg-Tg(Thy1-COP4/EYFP)18Gfng/J) confirmed that the ChR2-YFP protein is selectively expressed in the excitatory glutamatergic neurons (Jasnow et al., 2013; Wang et al., 2007). Laser light stimulation of ChR2-expressing cells depolarizes the membrane and elicit the glutamatergic activity in a spatially selected brain region (Zhang et al., 2006). We observed in immunohistochemical imaging that ChR2-YFP fluorescence co-localized with glutamatergic neuronal markers vesicular glutamate transporter 1 (VGLUT1), VGLUT2 and Ca^{2+} /calmodulin-dependent protein kinase II α (CaMKII α) (Fig. 1). In the striatum, the characteristic patch-matrix organization was seen (Fig. 1A), which is consistent with the classical anatomical feature of axons and neurons in the striatum (Gerfen, 1989; Nisenbaum et al., 1998). As expected, we observed that all ChR2-YFP expressing neurons and axons expressed VGLUT1/VGLUT2 and CaMKII α in the cortex and striatum, although not all VGLUT1/VGLUT2- and CaMKII α -positive cells expressed ChR2-YFP (Fig. 1B-1D). The existence of glutamatergic neurons in the striatum was detected in brain sections triple-stained for neurofilament (NF), VGLUT2 and DAPI (Fig. 2A). Compared to the dominate population of GABAergic cells in the striatum, we estimated these glutamatergic neurons represented a previously ignored small subpopulation (~0.5%) of total DAPI-positive striatal cells.

Glutamatergic axons projected and/or passing through from the striatum to SVZ

In order to specifically examine whether glutamatergic axons projected from the striatum to SVZ, we prepared sagittal brain sections from wild-type (WT) adult C57/B6L mice. The striatum region was examined using VGLUT1 and VGLUT2 along with two neuronal specific markers NF and Synapsin-1. Some NF-labeled axonal fibers were co-labeled with VGLUT2 and extended toward the SVZ neuroblasts marked with microtubule-associated protein doublecortin (DCX) (Fig. 2B, 2C). As a pre-synaptic protein associated with the vesicular membrane, Synapsin-1 is a specific marker for nerve terminals (De Camilli et al., 1983). Glutamatergic terminals, illustrated by the co-localization of VGLUT1 and Synapsin-1, were markedly dense in and around the border region between the striatum and

SVZ (Fig. 2D). There were cells in the SVZ showing overlapped staining of DCX and Synapsin-1, while most of staining markers was not overlapped (Fig. 2D). This is consistent with the general idea that NPCs and neuroblasts are progenitor cells that are not mature enough to form synapses.

To confirm axonal projections from the striatum to SVZ, the fluorescent dye DiI was injected into the striatum. Fourteen days later, scattered but noticeable DiI distribution was seen in the SVZ and the bordering region next to the striatum (Fig. 2E). This result supports that axonal projections exist between the striatum and SVZ and, in conjunction with the glutamatergic markers data, that at least some of those projections are glutamatergic axons originated from the striatum and other brain regions such as the cortex and thalamus (Wilson, 1987).

Expression of glutamate receptors in SVZ neuroblasts

Others and our data showed that extracellular glutamate enhances proliferation of cultured neuroblasts isolated from the SVZ (Fig. 3A, 3B)(Brazel et al., 2005; Whitney et al., 2008). We thus predicted that glutamate receptors are expressed in SVZ neuroblasts. The α -Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor subunits GluR1 and GluR2 were identified in cultured neuroblasts (Fig. 3C) as well as in the dorsal SVZ in brain sections, co-localized with DCX staining (Fig. 4A). The expression of N-Methyl-D-aspartic acid (NMDA) subunits NR1 and NR2B was absent or at low levels in the SVZ (Fig. 4B), which was in line with previous reports (Platel et al., 2010a).

Glutamatergic activation in the striatum triggered glutamate release and cellular responses in the SVZ

In ChR2 transgenic mice, the ChR2-YFP expression is neuron specific (Jasnow et al., 2013; Wang et al., 2007). The SVZ region showed the absence of the fluorescent green color of YFP, indicating no ChR2-YFP expression in naïve SVZ cells (Fig. 4 and Fig. 5D). Given the finding that the striatum extended axonal projections toward the SVZ and AMPA receptors were identified in SVZ cells, we predicted that glutamatergic activation in the striatum might affect SVZ cells through glutamate release. To examine this hypothesis, a Laser probe was inserted into the right striatum of ChR2 mice (Fig. 5A)(see Methods for the probe stereotactic positioning), blue laser light stimuli of 473 nm wavelength were applied for 15 min (15 bursts of 10 Hz pulses of 5 ms duration). The brain tissue contained the SVZ region was dissected out immediately after laser stimulation for glutamate measurements. Glutamate level in the stimulated samples was significantly higher than that in the sham control tissue; there was no difference between the non-stimulated samples and sham controls (Fig. 5B and 5C).

To verify a neuronal functional connection from the striatum to SVZ, brain slices were prepared from adult ChR2 transgenic mice. Recordings were performed on slices where the cortex and thalamus were removed to eliminate off-site neuronal activation in these two regions. An extracellular microelectrode was placed in the SVZ and an optical probe was positioned on the adjacent striatum (~80–100 μ m away from the SVZ) for the selective stimulation of glutamatergic neurons/axons within the striatum (Fig. 5D and 5E).

Neuroblasts were electrophysiologically identified based on their biophysical properties that are distinct from other types of SVZ cells (Lacar et al., 2010; Lai et al., 2010; Wang et al., 2003). Normally, these cells had high input resistances ($4.6 \pm 0.7 \text{ G}\Omega$; $n=12$) and a mean resting membrane potential of $-56.5 \pm 4.9 \text{ mV}$ ($n=12$). They were not able to generate fast action potentials, yet they expressed low levels of the TTX sensitive inward Na^+ current (Fig. 5F and 5G). The membrane response was recorded in the presence of the GABA receptor antagonist bicuculline ($50 \mu\text{M}$). Laser light elicited excitatory field potentials were detected in these cells, which increased with higher laser powers and were suppressed by the AMPA receptor antagonist NBQX ($20 \mu\text{M}$) but not affected by the NMDA receptor antagonist AP-5 ($200 \mu\text{M}$) (Fig. 5H and 5I). All these features agreed with their identity as being SVZ neuroblasts. Similar results were obtained from brain slices without removal of the cortex and thalamus (data not shown).

In next experiment, Ca^{2+} imaging using the membrane permeable Ca^{2+} dye Fura-2-AM was performed on brain slices perfused with artificial cerebrospinal fluid (aCSF) containing bicuculline ($50 \mu\text{M}$). Laser light stimulation to the striatum evoked single or multiple Ca^{2+} responses with slow time courses in SVZ cells. The Ca^{2+} response was mediated by the AMPA receptor, blocked by the bath application of NBQX ($20 \mu\text{M}$) (Fig. 5J), which is consistent to the AMPA receptor expression in neuroblasts.

Glutamatergic inputs from the striatum enhanced proliferation of neuroblasts in the SVZ of the normal brain

To investigate the putative glutamatergic regulation of SVZ neurogenesis *in vivo*, the right striatum of ChR2 transgenic mice was subjected to 15-min stimulation of 15 bursts of 10 Hz pulses each day for 4 days. The sham control mice had optical fiber similarly implanted but without light stimulation. BrdU was injected daily (50 mg/kg , i.p.) to label newborn cells. Brain sections were collected at day 7 for neurogenesis assays. In the brain of light-stimulated mice, there were marked increases in BrdU-positive (BrdU^+), DCX^+ and DCX/BrdU double positive cells in the ipsilateral SVZ (Fig. 6A - 6C). The AMPA receptor antagonist NBQX (25 mg/kg , i.p.) abrogated this effect (Fig. 6B and 6C). In the contralateral SVZ, the numbers of BrdU^+ , DCX^+ and DCX/BrdU^+ cells were not significantly different among all groups (Fig. 6D). No DCX^+ cells were observed in the striatum (data not shown; see (Yamashita et al., 2006)).

Optogenetic stimulation of glutamatergic activity in the striatum after stroke increased SVZ neuroblasts proliferation and migration to the peri-infarct cortex

Adult ChR2 mice were subjected to a focal cerebral ischemia targeting the right sensorimotor cortex (Li et al., 2008; Song et al., 2013). Laser light stimulation of the right striatum was applied at day 5 after stroke, 15 min per day at 10 Hz for 8 days. Daily BrdU injection (50 mg/kg , i.p.) was undertaken to label newborn cells. In the ipsilesional hemisphere 14 days after stroke, DCX^+ cells were significantly increased in the SVZ (Fig. 7). DCX not only is a marker for neuronal precursor cells, it has been used to identify migrating neuroblasts after stroke (Li et al., 2010; Zhang et al., 2007). In the post-stroke ipsilateral side, DCX^+ cells formed a migration stream originated from the SVZ, passing through the corpus callosum and reached the peri-infarct region of the ischemic cortex (Fig.

7A). DCX and BrdU double-labeled (DCX⁺/BrdU⁺) cells were found in the peri-infarct region (Fig. 7A-7C). This migration activity was more striking in light-stimulated stroke animals than in stroke control mice (Fig. 7D). Stroke mice received light stimuli showed significantly more DCX⁺, BrdU⁺ and DCX⁺/BrdU⁺ cells along the migration path, including the SVZ, corpus callosum and the peri-infarct region (Fig. 7E).

The infarct volume in stroke control and stroke plus laser stimulation groups was about the same (Fig. 7F and 7G), which was expected since the light stimulation was started 5 days after stroke when infarction was already well developed (Xu et al., 2006). As a comparison, the number of BrdU⁺ and/or DCX⁺ cells in the contralesional hemisphere was unaffected by the optogenetic stimulation (Fig. 7G). Considering the striatal optogenetic stimulation could be a potential therapeutic treatment, we examined whether the laser probe insertion and light stimuli could elicit cell damage and inflammation in the striatum. In immunohistochemical assays, no immunoreactivity of cleaved caspase-3 was found in the striatum of all groups (Fig. 8A and 8D), although caspase-3 activation was seen in the ischemic cortex (Fig. 8C). Iba1⁺ microglia cells were found within the striatum, but the number of Iba1⁺ cells was similar among all groups (Fig. 8B). In the ischemic brain, Iba1⁺ cells increased to similar levels in stroke control and stroke plus light stimulation groups, suggesting comparable inflammatory response in these two groups (Fig. 8D and 8E).

Survival and neuronal differentiation of recruited neuroblasts in the peri-infarct region of the ischemic brain

DCX⁺ cells arrived at the lesion site could contribute to cell replacement and tissue repair if they could survive and differentiate into neurons. To track the fate of DCX⁺ cells in the peri-infarct region, animals were sacrificed 31 days after stroke (19 days after the end of 8-day light stimulation) for immunohistochemical examinations. At this delayed time point, the numbers of DCX⁺ BrdU⁺, and DCX⁺/BrdU⁺ cells in the dorsal SVZ no longer showed difference between stroke controls and light-stimulated mice (Fig. 9A). In the corpus callosum and peri-infarct cortex, the numbers of DCX⁺ and DCX⁺/BrdU⁺ cells were still higher in the light-stimulated group (Fig. 9A). Additionally, we found more newly formed mature neurons (NeuN⁺/BrdU⁺ cells) within the peri-infarct region of light-stimulated mice (Fig. 10A), suggesting that more DCX⁺ cells could have survived in the peri-infarct region and differentiated into neurons. Although that, in addition to proliferating cells, BrdU might label injured cells, it was highly unlikely because significant cell death does not occur at this very delayed time point.

The number of DCX/BrdU double-labeled cells in the peri-infarct region dropped from 14 to 31 days after stroke. The decreased DCX⁺ cells were likely a result from neuronal maturation of neuroblasts and disappearance of the DCX expression during the differentiation process. Therefore, the actual number of neuroblast-originated cells that survived in the peri-infarct region could be much greater than the counted DCX⁺ cells.

We also expected that, neuroblasts undergoing neuronal differentiation/maturation began to express the glutamatergic neuron marker YFP in this transgenic mouse before the complete disappearance of DCX. Supporting this idea, there were indeed some BrdU⁺/DCX⁺ cells that exhibited green YFP color in the peri-infarct region (Fig. 9B). BrdU, DCX and YFP

triple-labeled cells were significantly more in light-stimulated stroke mice than that in stroke controls (17.4 ± 2.2 vs 7.3 ± 2.3 /field, $P < 0.01$, $n=8$; two-way ANOVA). Since the green ChR2-YFP-expressing cells in the ChR2 mouse represented a population of glutamatergic neurons, the results suggested these newborn neuronal precursors turned into mature glutamatergic neurons. Supporting this conclusion, glutamatergic neuronal markers, VGLUT1 and the NMDA receptor subunit NR1 were identified in these newborn neurons. There were NeuN⁺/BrdU⁺, NR1⁺/DCX⁺ and VGLUT1⁺/BrdU⁺ cells in the peri-infarct region and their numbers were significantly higher in laser stimulated stroke mice (Fig. 10A-10C). AMPA receptors were not measured in this assessment because they are not neuron specific; for example GluR-1,-2,-3,-4 subunits are detected in rat cerebral astroglial cultures (Conadorelli et al., 1993).

Striatal stimulation of glutamatergic neurons/axons improved functional recovery after stroke

The sensorimotor function of sham control, stroke control and laser-stimulated stroke mice was assessed using the adhesive dot removal test at 3 to 31 days after stroke (Fig. 11A and 11B). At 3 days after stroke, mice in stroke group spent much longer time than the pre-stroke baseline to touch and remove the sticky dots on the left forepaws, indicating a functional deficit correlated to the damage in the right sensorimotor cortex. Afterwards, the dysfunction underwent a trend of spontaneous recovery. Laser-stimulated stroke animals recovered significantly faster than the stroke control mice and eventually spent shorter time in detecting and removing the sticky dots at 24 and/or 31 days after stroke (Fig. 11A and 11B).

In order to verify the possible link between enhanced neurogenesis and improved functional recovery, the DNA synthesis inhibitor, cytosine arabinoside (AraC) that has been used in brain regeneration mechanism research (Faiz et al., 2015) and the SDF-1 receptor CXCR4 blocker, AMD3100, were applied to block cell proliferation and directional cell migration, respectively (Faiz et al., 2015; Saha et al., 2013; Yasuda et al., 2011). AraC (10 mg/kg) or AMD3100 (2 mg/kg) was delivered once daily into the ChR2 mouse brain via the intranasal route before the 15-min light stimuli. This intranasal route is a well established non-invasive method of brain delivery that helps avoid non specific peripheral actions of drugs (Guyot et al., 2001). In immunohistochemical assays on brain sections, laser stimulation of the striatum significantly increased the number of newborn neuroblasts (DCX⁺/BrdU⁺ cells) in the ipsilesional SVZ at 14 days after stroke. As expected, AraC but not AMD3100 suppressed this increased cell proliferation (Fig. 11C). As shown above, laser stimulation markedly enhanced the number of DCX⁺/BrdU⁺ cells recruited into the peri-infarct region 31 days after stroke. Both AraC and AMD3100 treatments prevented this increase (Fig. 11D). The adhesive dot removal test showed that AraC and AMD3100 treatments abrogated laser stimulation-induced behavioral gains in stroke mice (Fig. 11E and 11F).

Discussion

Although it is widely accepted that neurogenesis takes place in the SVZ and SGZ of the adult brain, there has been no clinical regenerative treatment that can utilize endogenous

neurogenesis for tissue repair and functional recovery after brain injuries. The present investigation focuses on promoting SVZ neurogenesis by stimulating glutamatergic activation in the striatum and demonstrates improved functional recovery after ischemic stroke. Electrical stimulation of the striatum was previously tested to improve behavioral recovery in a rat stroke model (Morimoto et al., 2011). Electric stimuli, however, could not dissect the specific cellular mechanism in the regulation. In neurogenesis research, the effect of neurotransmitters on neurogenesis has been mostly elucidated in cultured cells, while the in vivo or ex vivo examinations are primarily performed in the hippocampus (Berg et al., 2013; Bernabeu and Sharp, 2000). Here, we conducted the first ex vivo and in vivo study that gives insight information on a glutamatergic control of SVZ neurogenesis. In our experiments, the 10 Hz neuronal firing elicited by the laser light stimulation mimics the physiological activity frequency. The short duration of 15 min stimuli per day is a procedure resembling a physical therapy episode. The ChR2 transgenic mouse and optogenetic method allowed us to selectively activate glutamatergic neurons and axons where ChR2 is expressed.

In this study, optogenetic stimulation directly activates glutamatergic projection system in the striatum and trigger release of glutamate that acts on SVZ neuroblasts and enhances their neurogenic activity. Glutamate may modulate the excitability of striatal GABAergic neurons through binding glutamate/AMPA receptors that may express at low levels in these cells (Racca et al., 1996). This possibility, if existed, did not abrogate the promoting effect by glutamatergic activation. The optogenetic stimulation to the striatum may activate not only striatal glutamatergic neurons but also glutamatergic axons passing through the striatum and originated from other brain regions including the cortex and thalamus (Wilson, 1987). However, we show here that targeting the striatum is a highly effective approach to enhance SVZ neurogenesis, due to the merging location of glutamatergic inputs in the striatum and its close proximity to the SVZ. It is thus suggested that the striatum can be a therapeutic target for promoting SVZ neurogenesis. Clinically, this is achievable using deep brain stimulation (DBS) and brain-implanted devices of closed-loop technology (Krook-Magnuson et al., 2015; Mikell et al., 2016; Velasques et al., 2014). Other novel methods including optogenetics and pharmacogenetic may also have clinical potential of specific stimulation (Little and Brown, 2014; Peled, 2011).

We show that the glutamatergic terminals extended from the striatum contain pre-synaptic vesicular protein Synapsin-1, suggesting the existence of a glutamate vesicular pool in these terminals. Supporting this idea, glutamatergic activities in the striatum trigger glutamate release into the SVZ region and prompt local cellular responses including AMPA receptor activation and enduring changes in the Ca^{2+} homeostasis in SVZ cells. The time courses of these cellular responses are slower than that normally seen with synaptic neurotransmission, which agrees with the absence of synapse formation between neuroblasts (Platel et al., 2010a). It is now well-recognized that neurotransmitter communications happens not only between neurons, it can also occur between neurons and non-neuronal cells or even between non-neuronal cells (Berg et al., 2013). In addition to the neurotransmitter signaling at the synapse (i.e. phasic activation), targeted cells can be activated by neurotransmitters such as GABA that diffuse away from the synapse or by non-synaptic secretion (i.e. tonic activation) (Farrant and Nusser, 2005). We propose that the striatal control of SVZ cells is achieved by a unique mechanism different from phasic and tonic transmitter activities. In this semi-phasic

mechanism, glutamate is released from terminal vesicles upon neuronal activation and diffuses to nearby cells in the absence of post-synaptic structures (Fig. 12). Consistently, the slow Ca^{2+} response (in seconds) was likely due to combinatorial factors including the time needed for glutamate vesicular release, diffusion from the “pre-synaptic” terminal, as well as accumulation near SVZ cells, followed by cellular/subcellular events such as AMPA receptor activation and Ca^{2+} -dependent Ca^{2+} release from intracellular pools. This regulatory cascade, although slower than a typical synaptic transmission, is suitable and sufficient for the purpose of regulating cell proliferation and other cellular cascades in SVZ neurogenesis. This model coincidentally agrees with the prediction by Berg et al. that “the control of neurogenesis, i.e. the progression of neural stem cells into functionally integrated mature neurons, may be a function of neurotransmitters that is as significant as, but evolutionarily primordial to, their role in synaptic transmission” (Berg et al., 2013).

Cerebral ischemia-induced excessive glutamate release leads to excitotoxicity; more recent data suggest that appropriate glutamate release can trigger and enhance neurogenic response in the brain (Bernabeu and Sharp, 2000). In these studies, early blockade of AMPA and NMDA glutamate receptors resulted in the inhibition of ischemia-induced neurogenesis. In rodent stroke models, the elevation of extracellular glutamate returns to basal level quickly (Goyagi et al., 2011). Therefore, its benefit on neurogenesis is transient and limited. In our experiments, laser-stimulation to the striatum was started 5 days after stroke and conducted for 8 days. This design allows for elevated glutamate levels in the local environment of SVZ for increased and sustained neurogenesis and cellular reactions. The amplified neurogenesis favors stroke recovery by driving more neuroblasts to migrate to and survive in the peri-infarct cortex, differentiating into glutamatergic neurons and significantly improving functional recovery after stroke. Detailed molecular mechanisms during this process remain to be elucidated.

Previous studies reported that VGLUT1 is expressed in the axon terminals of cortical neurons, whereas VGLUT2 is primarily expressed in projections from the thalamus (Doig et al., 2010; Lei et al., 2013). This is consistent with the notion that, in addition to the projections from striatal glutamatergic neurons, many axons projected to the SVZ could originate from cortical and thalamic neurons. To eliminate a possible off-site effect of the optogenetic stimulation in our electrophysiological and Ca^{2+} imaging studies, the cortex and thalamus regions were removed from the brain slices. Our data indicate that the evoked responses in the SVZ were similar with and without the removal of the remote brain regions. Although the number of glutamatergic neurons residing in the striatum is small, their regulatory roles on SVZ neurogenesis may not be ignorable and should not be underestimated. It is understandable that the functional significance of a neuronal population cannot be judged merely by their numbers but should include other criteria such as location and resultant cellular/molecular consequences. We also recognized that the striatal stimulation may directly or indirectly affect astrogenesis in the SVZ. The possibility of a neuronal regulation on astrogenesis should be explored. Moreover, regulations on the progression from B, C to A cells in the SVZ may be examined using specific markers.

In the ischemic brain, we and many others have demonstrated a migrating path of newborn DCX^{+} neuroblasts from the SVZ to the peri-infarct region (Li et al., 2008; Moraga et al.,

2014; Ohab et al., 2006; Saha et al., 2013; Zhang et al., 2007). This cell migration is deemed as a self-repair mechanism after stroke and is closely correlated with the improved stroke recovery (Yamashita et al., 2006; Zhang et al., 2007). In the present study, suppressing the proliferation of neuroblasts or their migration significantly abrogates laser stimulation-induced increases in cells arriving at the peri-infarct cortex and the behavioral gain. The DNA synthesis inhibitor, AraC, is not cell type specific; nevertheless, AraC has been used as an effective tool for blocking SVZ cell proliferation in regeneration research (Faiz et al., 2015). Moreover, the “off target” action of AraC on other cells cannot explain its effect on neuroblasts proliferation after striatum stimulation. AMD3100 was applied to selectively block the SDF-1 receptor CXCR4. The SDF-1/CXCR4 signaling is the most prominent chemoattractant axis for the directional migration of neuroblasts toward the lesion site in the ischemic brain (Saha et al., 2013). Therefore, these data provide supporting information for the roles of increased proliferation of neuroblasts and homing of these cells to the peri-infarct cortex in observed functional improvements.

A previous study suggested that recruited DCX⁺ neuroblasts have the potential of differentiating into glutamatergic neurons (Brill et al., 2009). A consistent finding in the present study is that neuroblasts arriving at the peri-infarct region can survive and begin to express YFP in this transgenic mouse, indicating differentiating into mature glutamatergic neurons. Two recent high impact papers reported that SVZ NSCs differentiate into astrocytes and contribute to scar formation but not neurogenesis in the injured cortex (Benner et al., 2013; Faiz et al., 2015). It should be pointed out that SVZ cells investigated in these papers were most likely different subpopulations from cells in our investigation. Benner et al. (Benner et al., 2013) focused on SVZ-generated Thbs4^{hi} positive cells that showed GFAP expression but not labeled with DCX. On the other hand, Faiz et al. (Faiz et al., 2015) showed neurosphere formation and astrogenesis from SVZ derived Nestin⁺ NSCs. Those Nestin⁺ NSCs are not necessarily DCX⁺ neuroblasts. For example, in acutely isolated NSCs only 20% or none of Nestin⁺ NSCs co-labeled with DCX (Walker et al., 2007). It is known that endogenous neurogenesis is sensitive to the type and degree of brain injuries (Kernie and Parent, 2010; Parent, 2003). For instance, the SVZ neurogenic activity is highly dependent on the ischemic infarct size (Moraga et al., 2014). We noticed that, in above two investigations, stroke or to be more accurate restricted brain injury was generated by endothelin-1 injection, Pial vessel disruption, and the photothrombosis-induced cortical injury; none of them are typical ischemic stroke models. The insult, injury mechanism and resultant damage are different in multiple aspects from common ischemic stroke models of MCA occlusion. In MCA occlusion/ligation stroke models, many different groups have demonstrated a marked upregulation of SDF-1 expression in the ischemic region and significant numbers of DCX⁺ neuroblasts expressing the SDF-1 receptor CXCR4 can be attracted to the peri-infarct region and differentiate into neurons (Christie and Turnley, 2012; Li et al., 2008; Li et al., 2010; Ohab et al., 2006; Robin et al., 2006; Wei et al., 2012). Thus, the results of regenerative activities reported in above two papers may not be comparable to observations in typical stroke models, and their observations do not rule out the post-stroke migration of DCX⁺ neuroblasts from the SVZ to the ischemic cortex and their contribution to neurogenesis. It is likely that neurogenesis and astrogenesis from different subpopulations of SVZ cells, or similar cells at different post-stroke stages, are controlled by distinct

regulatory mechanisms. The regulation of and interaction between these regenerative activities are very appealing and potentially important but remain to be examined in basic and translational regeneration research.

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References

- Aravanis AM, et al. An optical neural interface: in vivo control of rodent motor cortex with integrated fiberoptic and optogenetic technology. *J Neural Eng.* 2007; 4:S143–S156. [PubMed: 17873414]
- Arenkiel BR, et al. In vivo light-induced activation of neural circuitry in transgenic mice expressing channelrhodopsin-2. *Neuron.* 2007; 54:205–218. [PubMed: 17442243]
- Barber PA, et al. Spontaneous reperfusion after ischemic stroke is associated with improved outcome. *Stroke.* 1998; 29:2522–2528. [PubMed: 9836763]
- Benner EJ, et al. Protective astrogenesis from the SVZ niche after injury is controlled by Notch modulator Thbs4. *Nature.* 2013; 497:369–373. [PubMed: 23615612]
- Berg DA, et al. Neurotransmitter-mediated control of neurogenesis in the adult vertebrate brain. *Development.* 2013; 140:2548–2561. [PubMed: 23715548]
- Bernabeu R, Sharp FR. NMDA and AMPA/kainate glutamate receptors modulate dentate neurogenesis and CA3 synapsin-I in normal and ischemic hippocampus. *J Cereb Blood Flow Metab.* 2000; 20:1669–1680. [PubMed: 11129783]
- Bolteus AJ, Bordey A. GABA release and uptake regulate neuronal precursor migration in the postnatal subventricular zone. *J Neurosci.* 2004; 24:7623–7631. [PubMed: 15342728]
- Bouet V, et al. The adhesive removal test: a sensitive method to assess sensorimotor deficits in mice. *Nat Protoc.* 2009; 4:1560–1564. [PubMed: 19798088]
- Brazel CY, et al. Glutamate enhances survival and proliferation of neural progenitors derived from the subventricular zone. *Neuroscience.* 2005; 131:55–65. [PubMed: 15680691]
- Brill MS, et al. Adult generation of glutamatergic olfactory bulb interneurons. *Nat Neurosci.* 2009; 12:1524–1533. [PubMed: 19881504]
- Cardin JA, et al. Targeted optogenetic stimulation and recording of neurons in vivo using cell-type-specific expression of Channelrhodopsin-2. *Nat Protoc.* 2010; 5:247–254. [PubMed: 20134425]
- Cheng MY, et al. Optogenetic neuronal stimulation promotes functional recovery after stroke. *Proc Natl Acad Sci U S A.* 2014; 111:12913–12918. [PubMed: 25136109]
- Christie KJ, Turnley AM. Regulation of endogenous neural stem/progenitor cells for neural repair-factors that promote neurogenesis and gliogenesis in the normal and damaged brain. *Front Cell Neurosci.* 2012; 6:70. [PubMed: 23346046]
- Condorelli DF, et al. AMPA-selective glutamate receptor subunits in astroglial cultures. *J Neurosci Res.* 1993; 36:344–356. [PubMed: 8271313]
- De Camilli P, et al. Synapsin I (Protein I), a nerve terminal-specific phosphoprotein. II. Its specific association with synaptic vesicles demonstrated by immunocytochemistry in agarose-embedded synaptosomes. *J Cell Biol.* 1983; 96:1355–1373. [PubMed: 6404911]
- Doetsch F, Alvarez-Buylla A. Network of tangential pathways for neuronal migration in adult mammalian brain. *Proc Natl Acad Sci U S A.* 1996; 93:14895–14900. [PubMed: 8962152]
- Doetsch F, et al. Subventricular zone astrocytes are neural stem cells in the adult mammalian brain. *Cell.* 1999; 97:703–716. [PubMed: 10380923]
- Doig NM, et al. Cortical and thalamic innervation of direct and indirect pathway medium-sized spiny neurons in mouse striatum. *J Neurosci.* 2010; 30:14610–14618. [PubMed: 21048118]

- Faiz M, et al. Adult Neural Stem Cells from the Subventricular Zone Give Rise to Reactive Astrocytes in the Cortex after Stroke. *Cell Stem Cell*. 2015; 17:624–634. [PubMed: 26456685]
- Farrant M, Nusser Z. Variations on an inhibitory theme: phasic and tonic activation of GABA(A) receptors. *Nat Rev Neurosci*. 2005; 6:215–229. [PubMed: 15738957]
- Freret T, et al. Behavioral deficits after distal focal cerebral ischemia in mice: Usefulness of adhesive removal test. *Behav Neurosci*. 2009; 123:224–230. [PubMed: 19170448]
- Gerfen CR. The neostriatal mosaic: striatal patch-matrix organization is related to cortical lamination. *Science*. 1989; 246:385–388. [PubMed: 2799392]
- Goyagi T, et al. Neuroprotective effects and suppression of ischemia-induced glutamate elevation by beta1-adrenoreceptor antagonists administered before transient focal ischemia in rats. *J Neurosurg Anesthesiol*. 2011; 23:131–137. [PubMed: 21150456]
- Guyot LL, et al. Real-time measurement of glutamate release from the ischemic penumbra of the rat cerebral cortex using a focal middle cerebral artery occlusion model. *Neurosci Lett*. 2001; 299:37–40. [PubMed: 11166932]
- Hakim AM, et al. The effect of spontaneous reperfusion on metabolic function in early human cerebral infarcts. *Ann Neurol*. 1987; 21:279–289. [PubMed: 3496844]
- Jasnow AM, et al. Thy1-expressing neurons in the basolateral amygdala may mediate fear inhibition. *J Neurosci*. 2013; 33:10396–10404. [PubMed: 23785152]
- Jorgensen HS, et al. Spontaneous reperfusion of cerebral infarcts in patients with acute stroke. Incidence, time course, and clinical outcome in the Copenhagen Stroke Study. *Arch Neurol*. 1994; 51:865–873. [PubMed: 8080386]
- Kempermann G, et al. Neurogenesis in the Adult Hippocampus. *Cold Spring Harb Perspect Biol*. 2015; 7:a018812. [PubMed: 26330519]
- Kernie SG, Parent JM. Forebrain neurogenesis after focal Ischemic and traumatic brain injury. *Neurobiol Dis*. 2010; 37:267–274. [PubMed: 19909815]
- Krook-Magnuson E, et al. Neuroelectronics and Biooptics: Closed-Loop Technologies in Neurological Disorders. *JAMA Neurol*. 2015; 72:823–829. [PubMed: 25961887]
- Lacar B, et al. Imaging and recording subventricular zone progenitor cells in live tissue of postnatal mice. *Front Neurosci*. 2010; 4
- Lai B, et al. Electrophysiological properties of subventricular zone cells in adult mouse brain. *Brain Res*. 2010; 1340:96–105. [PubMed: 20434436]
- Lei W, et al. Confocal laser scanning microscopy and ultrastructural study of VGLUT2 thalamic input to striatal projection neurons in rats. *J Comp Neurol*. 2013; 521:1354–1377. [PubMed: 23047588]
- Li WL, et al. Enhanced neurogenesis and cell migration following focal ischemia and peripheral stimulation in mice. *Dev Neurobiol*. 2008; 68:1474–1486. [PubMed: 18777565]
- Li Y, et al. Sublethal transient global ischemia stimulates migration of neuroblasts and neurogenesis in mice. *Transl Stroke Res*. 2010; 1:184–196. [PubMed: 21792374]
- Little S, Brown P. Focusing brain therapeutic interventions in space and time for Parkinson's disease. *Curr Biol*. 2014; 24:R898–R909. [PubMed: 25247369]
- Liu X, et al. Nonsynaptic GABA signaling in postnatal subventricular zone controls proliferation of GFAP-expressing progenitors. *Nat Neurosci*. 2005; 8:1179–1187. [PubMed: 16116450]
- Mikell CB, et al. Neurosurgery for schizophrenia: an update on pathophysiology and a novel therapeutic target. *J Neurosurg*. 2016; 124:917–928. [PubMed: 26517767]
- Moraga A, et al. Toll-like receptor 4 modulates cell migration and cortical neurogenesis after focal cerebral ischemia. *FASEB J*. 2014; 28:4710–4718. [PubMed: 25063846]
- Morimoto T, et al. Striatal stimulation nurtures endogenous neurogenesis and angiogenesis in chronic-phase ischemic stroke rats. *Cell Transplant*. 2011; 20:1049–1064. [PubMed: 21092409]
- Nakamichi N, et al. Neurogenesis mediated by gamma-aminobutyric acid and glutamate signaling. *J Pharmacol Sci*. 2009; 110:133–149. [PubMed: 19483378]
- Neumann-Haefelin T, et al. Effect of incomplete (spontaneous and postthrombotic) recanalization after middle cerebral artery occlusion: a magnetic resonance imaging study. *Stroke*. 2004; 35:109–114. [PubMed: 14671246]

- Nisenbaum LK, et al. Early patterning of prelimbic cortical axons to the striatal patch compartment in the neonatal mouse. *Dev Neurosci*. 1998; 20:113–124. [PubMed: 9691187]
- Ogle ME, et al. Inhibition of prolyl hydroxylases by dimethyloxaloylglycine after stroke reduces ischemic brain injury and requires hypoxia inducible factor-1 alpha. *Neurobiology of Disease*. 2012; 45:733–742. [PubMed: 22061780]
- Ohab JJ, Carmichael ST. Poststroke neurogenesis: emerging principles of migration and localization of immature neurons. *Neuroscientist*. 2008; 14:369–380. [PubMed: 18024854]
- Ohab JJ, et al. A neurovascular niche for neurogenesis after stroke. *J Neurosci*. 2006; 26:13007–13016. [PubMed: 17167090]
- Parent JM. Injury-induced neurogenesis in the adult mammalian brain. *Neuroscientist*. 2003; 9:261–272. [PubMed: 12934709]
- Peled A. Optogenetic neuronal control in schizophrenia. *Med Hypotheses*. 2011; 76:914–921. [PubMed: 21482453]
- Platel JC, et al. NMDA receptors activated by subventricular zone astrocytic glutamate are critical for neuroblast survival prior to entering a synaptic network. *Neuron*. 2010a; 65:859–872. [PubMed: 20346761]
- Platel JC, et al. Neurotransmitter signaling in postnatal neurogenesis: The first leg. *Brain Res Rev*. 2010b; 63:60–71. [PubMed: 20188124]
- Racca C, et al. Expression of AMPA-glutamate receptor B subunit in rat hippocampal GABAergic neurons. *Eur J Neurosci*. 1996; 8:1580–1590. [PubMed: 8921250]
- Robin AM, et al. Stromal cell-derived factor 1alpha mediates neural progenitor cell motility after focal cerebral ischemia. *J Cereb Blood Flow Metab*. 2006; 26:125–134. [PubMed: 15959456]
- Saha B, et al. Cortical lesion stimulates adult subventricular zone neural progenitor cell proliferation and migration to the site of injury. *Stem Cell Res*. 2013; 11:965–977. [PubMed: 23900166]
- Song M, et al. Restoration of intracortical and thalamocortical circuits after transplantation of bone marrow mesenchymal stem cells into the ischemic brain of mice. *Cell Transplant*. 2013; 22:2001–2015. [PubMed: 23069268]
- Velasques B, et al. Deep brain stimulation: a new treatment in mood and anxiety disorders. *CNS Neurol Disord Drug Targets*. 2014; 13:961–971. [PubMed: 24923335]
- Vicini S. The role of GABA and glutamate on adult neurogenesis. *J Physiol*. 2008; 586:3737–3738. [PubMed: 18708493]
- Walker TL, et al. The doublecortin-expressing population in the developing and adult brain contains multipotential precursors in addition to neuronal-lineage cells. *J Neurosci*. 2007; 27:3734–3742. [PubMed: 17409237]
- Wang DD, et al. Biophysical properties and ionic signature of neuronal progenitors of the postnatal subventricular zone in situ. *J Neurophysiol*. 2003; 90:2291–2302. [PubMed: 12801891]
- Wang H, et al. High-speed mapping of synaptic connectivity using photostimulation in Channelrhodopsin-2 transgenic mice. *Proceedings of the National Academy of Sciences of the United States of America*. 2007; 104:8143–8148. [PubMed: 17483470]
- Wang LL, et al. Mobilization of endogenous bone marrow derived endothelial progenitor cells and therapeutic potential of parathyroid hormone after ischemic stroke in mice. *PLoS One*. 2014; 9:e87284. [PubMed: 24503654]
- Wei L, et al. Transplantation of hypoxia preconditioned bone marrow mesenchymal stem cells enhances angiogenesis and neurogenesis after cerebral ischemia in rats. *Neurobiol Dis*. 2012; 46:635–645. [PubMed: 22426403]
- Welberg L. Techniques: Optogenetics takes more control. *Nat Rev Neurosci*. 2013; 14:587. [PubMed: 23942468]
- Whitney NP, et al. Calcium-permeable AMPA receptors containing Q/R-unedited GluR2 direct human neural progenitor cell differentiation to neurons. *FASEB J*. 2008; 22:2888–2900. [PubMed: 18403631]
- Wilson CJ. Morphology and synaptic connections of crossed corticostriatal neurons in the rat. *J Comp Neurol*. 1987; 263:567–580. [PubMed: 2822779]

- Xu XH, et al. Development of cerebral infarction, apoptotic cell death and expression of X-chromosome-linked inhibitor of apoptosis protein following focal cerebral ischemia in rats. *Life Sci.* 2006; 78:704–712. [PubMed: 16139848]
- Yamashita T, et al. Subventricular zone-derived neuroblasts migrate and differentiate into mature neurons in the post-stroke adult striatum. *Journal of Neuroscience.* 2006; 26:6627–6636. [PubMed: 16775151]
- Yasuda M, et al. Multiple forms of activity-dependent competition refine hippocampal circuits in vivo. *Neuron.* 2011; 70:1128–1142. [PubMed: 21689599]
- Yizhar O, et al. Optogenetics in neural systems. *Neuron.* 2011; 71:9–34. [PubMed: 21745635]
- Young SZ, et al. GABAergic striatal neurons project dendrites and axons into the postnatal subventricular zone leading to calcium activity. *Front Cell Neurosci.* 2014; 8:10. [PubMed: 24478632]
- Young SZ, et al. Neurotransmitters couple brain activity to subventricular zone neurogenesis. *Eur J Neurosci.* 2011; 33:1123–1132. [PubMed: 21395856]
- Zhang F, et al. Channelrhodopsin-2 and optical control of excitable cells. *Nat Methods.* 2006; 3:785–792. [PubMed: 16990810]
- Zhang RL, et al. Neuroblast division during migration toward the ischemic striatum: a study of dynamic migratory and proliferative characteristics of neuroblasts from the subventricular zone. *J Neurosci.* 2007; 27:3157–3162. [PubMed: 17376977]
- Zhao M, et al. Evidence for neurogenesis in the adult mammalian substantia nigra. *Proc Natl Acad Sci USA.* 2003; 100:7925–7930. [PubMed: 12792021]

Highlights

- Glutamatergic neurons and axons in the striatum extend projections to the SVZ.
- Optogenetic stimulation of glutamatergic activity in the striatum releases glutamate into SVZ.
- The glutamate release increased proliferation of SVZ neuroblasts, mediated by AMPAR activation.
- Stimulation of striatal glutamatergic activity augments neuroblasts proliferation, survival, and migration after stroke.
- The striatum promoted neurogenesis improves functional recovery after stroke.

Figure 1. ChR2-YFP expression in the glutamatergic axons and neurons in the cortex and striatum

A. Immunofluorescence images from brain sections show expression of ChR2-YFP (green) in the cortex and striatum of the ChR2 transgenic mouse. The characteristic patch-matrix structure was seen in the striatum. **B to D.** In brain sections from ChR2-YFP transgenic mice, immunofluorescence staining shows co-localization of ChR2-YFP with the excitatory glutamatergic neuron marker VGLUT1 (B), VGLUT2 (C) and CaMKIIα (D),

demonstrating that ChR2-YFP-expressing neurons (arrows) and fibers have glutamatergic phenotype residing within the cortex and striatum.

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Figure 2. Glutamatergic neurons and axonal terminals from the striatum and glutamate release in the SVZ

A. Confocal imaging of immunostaining was performed in brain sections from wild type C57/B6L mice. The overlapped neurofilament (NF) and VGLUT2 markers and their co-expression with DAPI identified a glutamatergic neuron in the striatum. **B** and **C.** Sagittal brain striatum sections show VGLUT2 and NF expressing axons extending to the adjacent SVZ. Images in **C** show the box area in panel **B**. The enlarged images show co-localization of VGLUT2 and neurofilament (NF) in the striatum (arrow head), and extension of these nerve fibers toward the SVZ. **D.** Immunostaining for DCX, the glutamatergic marker VGLUT1 and the presynapse marker Synapsin-1 in the SVZ, and striatum. The merged

image revealed dense distribution of the pre-synaptic protein Synapsin-1 in the striatum/SVZ bordering area. The enlarged image from the box area illustrates a few possible synapses co-labeled with Synapsin-1 and DCX⁺ cells (arrows); most of Synapsin-1 staining, however, located in close proximity of blue colored neuroblasts. **E.** Axon tracker DiI imaging 14 days after injection into the striatum. The DiI (red) distribution (arrows) due to axonal trafficking was seen along the SVZ and the border region between SVZ and striatum. Representative of brain sections from 5 WT animals.

Figure 3. Glutamate enhanced proliferation of cultured DCX⁺ cells

Neuroblasts were isolated from the SVZ of ChR2 transgenic mice and cultured for 4–5 days.

A and **B**. Cell proliferation was assessed by the quantification of BrdU incorporation into the cell DNA. The cultures were treated with L-glutamate, glutamate + NBQX and NBQX alone for 48 hrs, then fixed and stained for BrdU, DCX and Nestin. In panel B, the percentage of DCX⁺/BrdU⁺ cells was quantified with and without the addition of different concentrations of L-glutamate and 20 μ M NBQX. DCX⁺/BrdU⁺ cells significantly increased after a 48-hr treatment with 5 and 20 μ M L-glutamate. NBQX blocked the proliferation effect of

glutamate. *. $P < 0.05$ and **. $P < 0.01$ vs. glutamate-free controls; #. $P < 0.05$, 20 μM glutamate vs glutamate + NBQX; . $P < 0.05$, 5 μM glutamate vs glutamate + NBQX; Two-way ANOVA, $n=5$ independent assays. **C.** Images show DCX⁺ cells and the expression of AMPA receptor subunits GluR1 and GluR2.

Figure 4. Expression of AMPA and NMDA receptors in the SVZ neuroblasts of the ChR2 mouse
 Immunohistochemical staining was performed on the SVZ region of brain sections. **A.** In the dorsal SVZ, the AMPA receptor subunits GluR1 and GluR2 are co-localized with the neuroblast marker DCX. LV: lateral ventricle. **B.** Staining of DCX and the NMDA receptor subunit NR1 and NR2B shows the low level or lack of overlapping of these two markers. In 4B, a few DCX+ cells appeared to show green color suggested of expression of ChR2-YFP. These neural progenitor cells appeared on the way of migrating out from the neurogenic

niche and in the process of neuronal differentiation. See Figure 9B for further evidence for the expression of YFP in differentiating cells. Representatives of 5–7 animal assays.

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Figure 5. Optogenetic stimulation of the striatum elicits glutamatergic responses in the SVZ neuroblasts

A. Nissl staining of brain sections (~0.8 mm anterior bregma) 3 days after stroke induction and the ischemic damage in the right cortex (infarct). The schematic symbol shows the optical fiber implanted in the striatum. **B.** The glutamate content measured in the ipsilateral SVZ tissue isolated from sham control and stimulated hemisphere after a 15-min laser light stimulation. ****.** $P < 0.01$, paired student-t test; $n = 7$ per group. **C.** The glutamate content in the SVZ isolated from non-stimulated hemispheres. $N = 7$ per group. **D.** The immunofluorescence

image shows striatal ChR-YFP-expressing neuronal axons (green) extending into the adjacent SVZ. Blue color is the staining of doublecortin (DCX). See Figure 2 for more data of striatal innervations into the SVZ. LV: lateral ventricle. **E.** The brain striatum slice was illuminated by 473 nm blue laser light through an optical fiber. A glass microelectrode was placed in the SVZ to record synaptic responses in neuroblasts. **F.** Whole cell patch-clamp recording on neuroblasts. The voltage traces in current clamp mode show membrane responses to current injections from -50 to 130 pA. No action potential was evoked by depolarizing steps. **G.** In the same cell as in F, the tetrodotoxin (TTX) sensitive inward Na^+ current was detected. The electrophysiological property was consistent with SVZ neuroblasts. **H** and **I.** In slice extracellular recordings, the striatum region was illuminated by 473 nm blue laser conducted through the optical fiber connected to the laser source (E). Traces in H show membrane excitatory potentials responding to laser light stimulation at different power levels. The Laser light-induced response was completely suppressed by AMPA receptor antagonist NBQX but not affected by NMDA receptor antagonist AP-5 (I). Note the first wave in each panel pointed by the Stim/arrow marker was the stimulation artifact but not the receptor-mediated response. **(J)** Laser light induced intracellular Ca^{2+} responses in neuroblasts were measured using Fura-2-AM. Either a single response or multiples waves was seen following the striatum glutamatergic activation. The Ca^{2+} increases were completely suppressed by NBQX ($20\text{ }\mu\text{M}$). Traces in the figure are representative of 10–20 cells. Application of laser light (laser pulse of 5 ms, at 14.1 mW/mm^2) is indicated by the arrow. Stim: laser stimulation. The electrophysiological and Ca^{2+} imaging were conducted on acute brain slices in the presence bicuculline ($50\text{ }\mu\text{M}$) in the aCSF.

Figure 6. Optogenetic stimulation of the striatum enhances proliferation of neuroblasts in the adjacent dorsal SVZ of the ChR2 mouse

A. Newborn DCX⁺ neuroblasts in the SVZ are illustrated by double immunofluorescence labeling of DCX and BrdU. The confocal z-stack image shows co-localized DCX (red) and BrdU (blue) staining. The images were from box A in the second row of **B**. **B.** In brain sections with ChR2-YFP (green) expression, DCX⁺ (red) and BrdU⁺ (blue) cells were counted. Images show the dorsal SVZ of ipsilateral (laser probe implantation side) and contralateral brain sections of the sham control slices, laser-stimulated and laser-stimulated

plus pre-treated with NBQX (25 mg/kg, i.p.). Stim: stimulation; LV: lateral ventricle. **C.** Quantified data from experiments in B. Laser stimulation significantly increases the numbers of BrdU⁺, DCX⁺ and DCX⁺/BrdU⁺ cells in the ipsilateral SVZ, which was blocked by NBQX. **D.** The cell numbers in contralateral SVZ are similar among all groups. NBQX did not show effect on the basal proliferation of neuroblasts, likely indicated that some other mechanisms involved in the baseline activity of proliferation. **. $P < 0.01$, laser stimulation vs either sham control or laser stimulation + NBQX group; Two-way ANOVA, n=6 per group.

Figure 7. Optogenetic stimulation of the striatum increases migration of neuroblasts to the peri-infarct cortex

Immunofluorescence images show migrating neuroblasts from the SVZ to the ischemic cortex 14 days after stroke. (A) In stroke control mice, DCX⁺ neuroblasts form a migration chain originating from the SVZ, passing through the corpus callosum with some cells reaching the peri-infarct region of the ischemic cortex. LV: lateral ventricle. (B) The enlarged box area in panel A. (C) The enlarged box area in panel B, showing a confocal image of co-localized DCX (red) and BrdU (blue) in the area. (D) Enhanced migration chain

of neuroblasts in laser-stimulated stroke mice. **(E)** Quantified summaries of the experiments in A to D. The numbers of BrdU⁺, DCX⁺ and DCX⁺/BrdU⁺ cells in three brain regions of stroke control and laser-stimulated stroke mice. *. $P < 0.05$, stroke control *vs* laser-stimulated stroke mice; Two-way ANOVA, n=6 per group. Stim: laser stimulation. **F.** The focal ischemia-induced infarction was measured using Nissl-staining 14 days after stroke. The bar graph of summarized measurements indicates similar infarctions between two groups (determined by the formula: infarct ratio = [total contralesional area – (total ipsilesional area – infarct area)]/(total contralesional area). **G.** The numbers of BrdU⁺, DCX⁺ and DCX⁺/BrdU⁺ cells were counted 14 days after stroke in the contralateral SVZ of stroke control and light-stimulated stroke mice. N=7 each group.

Figure 8. Immunofluorescence staining of cleaved Caspase-3 and microglia marker Iba1
Apoptotic cell death and inflammatory microglial cells were examined using the specific antibodies in striatum and cortex areas of normal and stroke animals. **A.** In the brain regions from normal Chr2-YFP mice, no positive immunoreaction of Caspase-3 (Cas-3) was detected in the striatum that received optical fiber implantation, 4-day laser stimulation or stimulation plus NBQX treatment. Iba1 positive activated microglial cells were found within the striatum of each group. **B.** The number of Iba1⁺ cells was normalized against the total number of cells (by counting nuclei in DAPI) in the striatum of fiber insertion control,

stimulation and stimulation plus NBQX groups. There was no difference between the three groups. **C.** Positive immunoreaction of Cas-3 was found in the peri-infarct cortex 14 days after stroke. **D.** Immunostaining of striatum of stroke mice. No caspase-3 cleavage was seen in the striatum while iba1 positive microglia existed there at 14 days after stroke. **E.** The number of Iba1⁺ cells was normalized against the total cells in the striatum of sham control and stroke groups. Stroke increased microglia but light stimulation did not impose any additional effect on this inflammatory assay. *. $P < 0.05$; One-way ANOVA, n=6 per group.

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Figure 9. Cell migration and differentiation of recruited neuroblasts/NPCs in the peri-infarct region after ischemic stroke

Neuroblasts/NPCs along the migration path of the Chr2 mouse brain were identified by DCX and other markers 31 days after stroke. **A.** The numbers of BrdU⁺, DCX⁺ and DCX⁺/BrdU⁺ cells in the SVZ, corpus callosum and peri-infarct area were compared between stroke and stroke plus laser stimulation. At this delayed time point, more migrating new cells were seen in the corpus callosum and peri-infarct regions of laser-stimulated mice. *, $P < 0.05$ vs. stroke control; Two-way ANOVA, $n = 8$ per group. **B.** Immunofluorescence staining of

DCX and BrdU in the peri-infarct region of the brain. These cells migrated closer to the ischemic core in light stimulated mice. The box areas were enlarged and confocal 3-D images are shown in bottom rows. Arrows indicate cells triple-labeled with DCX (red), BrdU (blue) and YFP (green). The green color (YFP) indicates newborn neuroblasts differentiating into glutamatergic neurons.

Figure 10. Identification of differentiated glutamatergic neurons in the peri-infarct region 31 days after stroke

A. The NeuN and BrdU double labeled cell (arrow) in the peri-infarct region of stroke mice. The bar graph shows the number of NeuN⁺/BrdU⁺ cells in each group. Green is YFP. **B.** The DCX and NR1 double labeled cell (arrow) in the peri-infarct region of stroke mice. The bar graph shows the number of DCX⁺/NR1⁺ cells in each group. **C.** The VGLUT1 positive cell incorporated BrdU (arrows) in the peri-infarct region of stroke mice. The bar graph shows the number of VGLUT1⁺/BrdU⁺ cells in each group. *, $P < 0.05$; Two-way ANOVA, $n = 6$ per group.

Figure 11. Functional recovery after focal ischemic stroke and optogenetic stimulation

The adhesive dot removal test was performed at 1 day before stroke, and 3, 10, 17, 24, and 31 days post-stroke. **A** and **B**. The time for mice to touch (A) and remove (B) the sticker attached to their left forepaw was compared between stroke control and stroke + stimulation groups. *, $P < 0.05$ and **, $P < 0.01$ vs. stroke controls; Two-way ANOVA, $n = 12$ per group. Stim: laser stimulation. **C** and **D**. The adhesive dot removal tests were repeated with cell proliferation blocker AraC (10 mg/kg) or the SDF-1/CXCR4 chemo-attractant blocker AMD3100 (2 mg/kg), given daily during the 8 day light stimulation. At 31 days after stroke,

AraC or AMD3100 prevented laser stimulation-induced behavioral gain. *, $P<0.05$, stroke control vs. laser-stimulated stroke mice; Two-way ANOVA, $n=7$ per group. **E.** The numbers of DCX⁺/BrdU⁺ cells in the ipsilateral SVZ at 14 days after stroke. AraC significantly inhibited cell proliferation. **F.** DCX⁺/BrdU⁺ cells were counted in the peri-infarct cortex 31 days after stroke. Both AraC and AMD3100 antagonized the light stimulation effect, the number of neuroblasts migration to the cortex region remained at the stroke control level. *, $P<0.05$ vs. stroke control, #, $P<0.05$ vs. stroke + stim. Two-way ANOVA; $n=6$ per group.

Figure 12. Models of neurotransmitter actions

Working models for synaptic and paracrine actions of neurotransmitters after releasing from transmitter pools. The neuronal activity evoked transmitter release from vesicles and their fast binding to the post-synaptic sites represent the classical and typical synaptic transmission. On the other hand, non-neuronal cells may secrete certain transmitter molecules that can diffuse into the surrounding area and show tonic regulation on cellular activities of other cells. For the striatum regulation of SVZ neurogenesis, we propose the hypothetical model of semi-phasic regulation, by which neurotransmitter such as glutamate can be released from vesicles in the nerve terminal upon neuronal activation. The released transmitter then diffuses to nearby cells for a relatively slow and persistent regulation of cellular events such as proliferation, differentiation and migration.