國立台灣師範大學生命科學系碩士論文

台灣三種蝙蝠之出生後神經元新生

Postnatal Neurogenesis in Three Species of Bats in Taiwan

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中華民國 103 年七月

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摘要

在哺乳類動物大腦中終其一生都會有神經元新生。出生後的神經元新生發生在大腦的兩個區域，一為嗅球（OB）和另一為海馬迴的齒狀迴（DG）。位於側腦室旁的神經幹細胞會產生嗅球的神經元，而和齒狀迴內側的神經幹細胞則會產生齒狀迴神經元。新生的神經元要建立突觸連接，融入原有的神經迴路才會存活下來並且發揮它的功能。到目前為止，大部分出生後神經元新生的研究都集中在實驗室飼養的嚙齒類動物，至於在野生動物大腦裡的出生後神經元新生並未完全闡明。蝙蝠是唯一會飛行且比嚙齒類動物較為長壽的哺乳類動物。除此之外，蝙蝠是靠回聲定位系統辨別方向、辨認障礙物的大小材質以及捕抓獵物等。由於蝙蝠有特殊的空間辨識能力，加上台灣蝙蝠大腦內的出生後神經元新生到目前為止並沒有被研究過，因此本研究主要想探討台灣蝙蝠腦內的出生後神經元新生。我們使用胸苷類似物 BrdU 來標定新生的細胞和可以辨認與神經發育相關特定細胞群的抗體來研究台灣蝙蝠的出生後神經元新生的狀況。根據實驗結果，我們發現高頭蝠有與老鼠相似的嗅球神經元新生，但缺乏海馬迴神經元新生。而在摺翅蝠和葉鼻蝠則都有嗅球和海馬迴神經元新生。我們也使用了 Sox2 抗體標定在側腦室旁和齒狀迴內側的神經幹細胞。在這三種類的蝙蝠

中都有神經幹細胞存在在這兩個腦區域。除此之外，我們也利用 PSA-NCAM 和 DCX 抗體來標定神經母細胞。除了高頭蝠的齒狀迴之外，在側腦室旁和齒狀迴都可以偵測到神經母細胞的存在。另外，我們也使用了 Ki67 抗體標定在側腦室旁和齒狀迴正在細胞週期內的細胞。除了高頭蝠的齒狀迴以外，存在有神經幹細胞的腦區內都有發現正在進行細胞分裂的細胞。整體而言，蝙蝠的大腦神經元新生是一種種間特異的生理現象。

關鍵字：出生後神經元新生、嗅球、海馬迴、齒狀迴、高頭蝠、東亞摺翅蝠、台灣葉鼻蝠

Abstract

Postnatal neurogenesis occurs in mammals throughout life. Neural stem cells (NSCs) in the subventricular zone (SVZ) of the lateral ventricle and in the subgranular zone (SGZ) of the dentate gyrus (DG) give rise to new neurons in the olfactory bulb (OB) and DG, respectively. Newborn neurons can establish synaptic connections and integrate into the neural circuit. Most of the studies focus on molecular mechanisms of postnatal neurogenesis in captive animals, especially lab rodents. However, whether it also persists in wild mammals is not fully elucidated. Bats, the long-lived flying mammals, can use echolocation to navigate. The pattern of postnatal neurogenesis in various bat species in Taiwan has never been reported. We used the thymidine analog, bromodeoxyuridine (BrdU), and cell type-specific markers to study postnatal neurogenesis in bats in Taiwan. We found that postnatal neurogenesis persisted in the OB of Chestnut bats (*Scotophilus kuhliias*). However, postnatal hippocampal neurogenesis was completely absent in these bats. Postnatal neurogenesis in the OB and DG was found in Japanese long-winged bats (*Miniopterus schreibersii fuliginosus*) and Formosan leaf-nosed bats (*Hipposideros armiger terasensis*). $Sox2^+$ NSCs were present in the SVZ and SGZ of these three species of bats. Furthermore, there were neuroblasts present in the SVZ and SGZ of these three species of bats except in the SGZ of Chestnut bats. Lastly, we found that proliferative precursor cells existed in the SVZ-OB pathway in these three species of bats. In the SGZ, proliferative precursor cells were only found in Japanese long-winged

bats and Formosan leaf-nosed bats, but absent in Chestnut bats. Overall, there is species-specific pattern of postnatal neurogenesis in these species of bats.

Key word: postnatal neurogenesis, olfactory bulb, hippocampus, dentate gyrus, Chestnut bats, Japanese long-winged bats, Formosan leaf-nosed bats, *Scotophilus kuhliias*, *Miniopterus schreibersii fuliginosus*, *Hipposideros armiger terasensis*

Introduction

Postnatal neurogenesis

Over the past decades, the classic idea that no new neurons are born in the adult endothermic vertebrate brain has completely been rebutted in the field of neuroscience. In past decades, evidences show new neurons continue to be added to HVC related to birdsong throughout life[1-3]. Postnatal neurogenesis is a spatially restricted biological event that occurs in the mammalian brain throughout life. Neural stem cells (NSCs) are present in two brain regions, the subventricular zone (SVZ) of the lateral ventricle and the subgranular zone (SGZ) in the dentate gyrus (DG) of the hippocampus[4,5]. Postnatal neurogenesis can be studied from four perspectives, including the proliferation of NSCs or progenitors, migration of neuroblasts, differentiation and fate determination of progenitor cells and the survival, maturation, and integration of newborn neurons[4]. Postnatal neurogenesis is a dynamic and finely tuned process and can be regulated by various stimuli including physiological, pathological and pharmacological stimuli[4,5]. This finding leads to the acceptance that postnatal neurogenesis is present in higher vertebrates, such as rodents, non-human primates and humans[6-9].

Postnatalbulbar neurogenesis

NSCs in the postnatal SVZ are a population of slowly proliferating cells, lying adjacent to the ependymal layer of the lateral ventricle and capable to give rise to neurons and glia[10,11]. These multi-potent NSCs can produce clusters of rapidly dividing immature precursors, transit-amplifying cells,

which produce neuroblasts[12,13]. Neuroblasts born in the postnatal SVZ migrate tangentially over a great distance through the rostral migratory stream (RMS) to the olfactory bulb (OB). When neuroblasts arrive in the subependymal zone (SEZ) in the core of the OB, they begin radial migration toward the granule cell layer (GCL) and the periglomerular layer (PGL) of the OB. Those migrating to the GCL will become granule interneurons, whereas those toward the PGL differentiate intoperiglomerular interneurons[4,5,11,14-16]. 75% of neuronal precursor cells from the SVZ differentiate into granule interneurons and the rest 25% become periglomerular interneurons[14]. The survival of newborn neurons in the OB depends on sensory inputs. After going through morphological, physiological, and electrophysiological development, newborn neurons integrate into the existing neural circuit. Newborn neurons that integrate into the bulbar network will survive whereas those failing to integrate will undergo programmed cell death[17].

Newborn neurons in the OB experience several stages of development. Previous studies have shown that it takes approximately 1 week for newborn cells from the SVZ to reach the OB (4 to 10 days, in rodents) and 2 weeks for them to migrate into the GCL or PGL of the OB. Newborn cells differentiate into mature neurons about 3 weeks after their birth in rodents[14,18].

Postnatal bulbar neurogenesis is regulated by the olfactory experiences. Previous studies reveal that olfactory learning enhance postnatal bulbar neurogenesis. This leads to the hypothesis that postnatal bulbar neurogenesis plays an important role in olfactory circuitry[18].

In the OB, postnatal neurogenesis is required for the maintenance and reorganization of the entire interneuron system, and contributes to odor learning, discrimination and adaptive behaviors[4,5,16,18].

Postnatal hippocampal neurogenesis

Besides its well-known role in spatial learning and memory, the hippocampus is involved in the regulation of emotional, motivational and stress responses. In particular, the dorsal part of the hippocampus is involved in spatial learning and memory processes, whereas the ventral hippocampus is responsible for affective behaviors.

Postnatal neurogenesis occurs in the DG of the hippocampus throughout life. In the SGZ, proliferating NSCs (radial glia-like cells) give rise to intermediate progenitor cells, which in turn generate neuroblasts. Neuroblasts then migrate into the inner granule cell layer and differentiate into dentate granule neurons. Newborn neurons follow a series of stereotypic processes for synaptic integration into the existing hippocampal neuronal network[4,5].

Newborn neurons in the DG go through several developmental stages. Newborn granule cells initially become depolarized in response to GABA from ambient neurons due to high intracellular concentration of chloride ions[4]. The response to GABA switches from depolarization to hyperpolarization 2-4 weeks after birth. At the same time, the dendritic spines grow and glutamatergic responses take place. New neurons already form synapses with hilar and CA3 targets 2 weeks after birth. The complexity of these efferent synapses increase as neurons mature.

Newborn neurons in the DG display typical features of mature granule neurons 4 weeks after birth. The density of mushroom spines continues to increase 8 weeks after birth. Compared to mature granule neurons, newborn neurons exhibit hyperexcitability and enhance synaptic plasticity during specific developmental stages[4,5].

Postnatal hippocampal neurogenesis plays important roles in synaptic plasticity, memory and mood regulation. Chronic stress and major depression disorder negatively regulate postnatal hippocampal neurogenesis. However, chronic antidepressant treatment, physical exercise or enriched environment all promote postnatal hippocampal neurogenesis in rodents[4,5].

Bats

Echolocating bats are long-live mammals, which produce short ultrasonic calls through either mouth or nostrils and use the returning echoes for orientation within complex environment and foraging for food at the night[19]. Furthermore, the sonar system of bats is used for evaluation the properties of the target, including the size, velocity and shape of the target[20].

According to the spectral structure, ultrasonic calls are classified into frequency modulated (FM) calls, constant-frequency (CF) calls and ultrasonic clicks. FM calls, with durations of 0.5-20 msec, often contain harmonics. Bats that use these calls are defined as “FM bats”, and they are the majority of echolocating bat species. “CF-FM bats” produce a CF call that usually start and end with a short FM component. CF calls can

last tens of milliseconds and the Doppler shift of the echo allows the bats to access the motion of targets. The ultrasonic clicks, as short as 40-50 μsec, are similar to sonar clicks that produced by marine mammals, such as whales and dolphins. The clicks are produced via the tongue, whereas CF-FM and FM calls are from the larynx[20].

In 2007, Amrein and her colleagues examined the DG of 12 tropical bats species by using Ki67, minichromosome maintenance complex component 2 (MCM2) and doublecortin (DCX) antibodies to detect proliferating cells and neuroblasts. Their data reveal that 9 out of 12 species of bats (*Glossophaga soricina, Carollia perspicillata, Phyllostomus discolor, Nycteris macrotis, Nycteris thebaica, Hipposideros cyclops, Neoromicia rendalli, Pipistrellus guineensis, and Scotophilus leucogaster*) show a completely absence of postnatal hippocampal neurogenesis while the rest (*Chaerephon pumila, Mops condylurus and Hipposideros caffer*) with low rate of postnatal hippocampal neurogenesis[21]. Another study reports postnatal neurogenesis in bats by using megachiroptera/megabats. Eight species of bats (*Casinycteris argynnis, Eidolon helvum, Epomops franqueti, Hypsignathus monstrosus, Megaloglossus woermanni, Rousettus aegyptiacus, Scotonycteris zenkeri and E. wahlbergi*) are examined by using Ki67 and DCX. Their results demonstrate that there is high density of $Ki67^+$ and DCX^+ cells found in the RMS and SVZ. In the SGZ, the cell densities varied from high to low among this eight species of bats evaluated[22]. Although these two studies show that adult neurogenesis may persist in bats by identifying the presence of neural progenitor cells in

NSC niches, they did not examine the presence of newborn neurons directly. In our study, we used a more stringent method to define postnatal neurogenesis and intended to find out in which process during the development of a newborn neurons leads to the result observed in previous study. We used the thymidine analog, bromodeoxyuridine (BrdU), and co-staining with a mature neuron marker, NeuN to exam postnatal neurogenesis in those bat species. In addition, we used cell type-specific markers, sex determining region Y-box 2 (Sox2) to exam the existence of NSCs, polysialylated-neural cell adhesion molecule (PSA-NCAM) and DCX to detect neuroblasts, and Ki67 to exam proliferating cells.

Taiwan is known for its eminent mountain features that span from the north to the south of the island. Therefore, Taiwan provides a diversified ecological environment for wildlife habitats. Due to its subtropical climate, furthermore, Taiwan has some rare species of mammals, such as Formosan Rock Macaque and Formosan black bears. There are around 80 species of mammals found in this island, and more than 30 of them are bats[23]. Taiwan is one of the countries with the highest diversity of bats in the world, including CF-FM and FM bats. Postnatal neurogenesis is a type of plasticity for the brain to adapt to the complex environment. Although more and more studies show that this form of plasticity is required for olfactory behaviors and learning and memory, they are from captive animals, especially rodents. It is important to investigate whether postnatal neurogenesis also happens in bats with complex behaviors and to elucidate the relationship between the patterns of postnatal

neurogenesis with the echolocation call types that they produce. Therefore, we took Formosan leaf-nosed bat (*Hipposideros armiger terasensis*) as our experimental animals in CF-FM bats category and the Chestnut bat (*Scotophiluskuhliias*) and Japanese long-winged bat (*Miniopterusschreibersiifuliginosus*) as our experimental animals in FM bats category in this study.

Chestnut bats

Chestnut bats are nocturnal and insectivorous animals. They are commonly found in urban areas of Taiwan and Kinmen. They often rest at the base of the palm tree leaves, but are also found in buildings. They are migratory bat. If their environment is not interrupted, they repeatedly migrate to fixed habitat in April. Their echolocation is frequency-modulated and ranged from 35 to 115 kHz. The call structures include one fundamental frequency and a harmonics. The call peak frequency ranges from 35 to 85 kHz. They fly fast and forages in open habitat and at the edges of cluttered environments[23].

Japanese long-winged bats

Japanese long-winged bats are nocturnal and insectivorous animals. They roost in natural caves, abandoned mines, bomb shelters, unused tunnels and underground channels. This species is widely distributed in the whole country from low elevation caves to middle to high elevation forests. They often share a same cave with Formosan lesser horseshoe bats and Formosan leaf-nosed bats for roosting but in different areas.Their

echolocation is frequency-modulated and ranges from 40 to 160 kHz. The call structures include one fundamental frequency and a harmonics. The call peak frequency ranges from 50 to 110 kHz. They forage in a variety of open and semi-open natural and artificial habitats, including suburban areas. They stay close to each other at the habitat during daytime. The clustering number can be up to thousands or even tens of thousands[23].

Formosan leaf-nosed bats

Formosan leaf-nosed bats are nocturnal and insectivorous animals. They are generally distributed in Taiwan at low to middle elevations. They are commonly found in natural caves, tunnels, abandoned buildings, and other man-made structures, usually near broadleaf forests with a stream. Their echolocation is constant-frequency frequency-modulated and ranges from 20 to 135 kHz. The call structures includes one fundamental frequency and two to three harmonics. The call peak frequency ranges from 50 to 75 kHz. They forage in open natural habitats. Their mating season is from September to October.The pregnancy season is from March to April and reproductive period is from May to August[23].

Therefore, we carried out series of experiments to study the pattern of postnatal neurogenesis in wild bats in Taiwan. We found that postnatal neurogenesis persisted in the OB of Chestnut bats. However, postnatal hippocampal neurogenesis was completely absent in them. Postnatal neurogenesis in the OB and DG was found in Japanese long-winged bats with DG neurogenesis in a lower rate. In addition, postnatal neurogenesis

in these two regions was low in Formosan leaf-nosed bats. Taken together, there is a species-specific pattern of postnatal neurogenesis in bats in Taiwan.

Materials and methods

Experimental animals

Formosan leaf-nosed bats, Chestnut bats and Japanese long-winged bats were trapped in suburbs in Taipei, Taiwan by using standard bat trapping techniques. All animals were caught from late spring to autumn, which are their breeding and reproductive season. All animals were juvenile or adult, but their exact ages and genders were neglected. None of the captured species were threatened or protected. Animals were housed in plastics cages and maintained in a 12 hours light/dark cycle with free access of food and water ad libitum or by feeding. All animals were trained to adapt in a new environment before the experiment start. All animals were kept in isolation for the duration of all experiments.

BrdU administrations

To label mitotic cells, animals were intraperitoneally injected with bromodeoxyuridine (BrdU) twice a day, 6 hours apart (100 mg/kg body weight; Sigma). Animals were then sacrificed one, two or three weeks after BrdU injections (Fig.1).

Fixation and sectioning

Following anesthesia with Avertin (0.025 g 2,2,2-tribromoethanol and 0.025 mL of 22-methyl-2-butanol or amylene hydrate in 0.975 mL of distilled water; 17 mL/kg body weight; i.p.), animals were transcardially perfused with 0.9% (w/v) saline followed by ice-cold 4%

paraformaldehyde (Sigma) in PBS. The brain was post-fixed overnight in 4% paraformaldehyde at 4℃ followed by 20% sucrose in PBS with 0.02% sodium azide for another 24 hours for dehydration purpose. Dehydrated brains were then frozen by using dry ice and stored in -80℃ refrigerator[24].

Coronal brain sections (40 μm) were serially cut using a microtome (Leica SM 2010R) and stored at 4℃ refrigerator until processed for immunohistochemistry[24].

Immunohistochemistry

To visualize NSCs, neuroblasts and neuronal fate of proliferating cells, three serial brain sections with equivalent interval from the anterior to posterior part of RMS, SVZ and DG (240 μm apart from each section) were rinsed with Tris-buffered saline (TBS; pH 7.4) for three times and incubated in blocking buffer for 1 hour at room temperature before the overnight incubation of primary antibodies at 4℃. After 16-18 hours of primary antibody incubation, brain sections were rinsed again with TBS for three times before the incubation of secondary antibodies for 2 hours at room temperature. Sections were rinsed with TBS before counterstained with DAPI (4,6-diamidino-2-phenylindole) for 30 min at room temperature. Sections were then rinsed with TBS, mounted on slides, coverslipped with anti-fade reagent (ProLong Gold, Invitrogen) and stored at 4℃.

To visualize newborn neurons, six serial brain sections with

equivalent interval from anterior to posterior part of OB and DG (160 μm apart from each section) were rinsed with Tris-buffered saline (TBS; pH 7.4) for three times, incubated with 2 N hydrochloric acid (HCl) at 37℃ for 30 min to denature DNA and then incubated in 0.1 M sodium borate for 10 min at room temperature for neutralization. Sections were rinsed again with TBS for three times followed by the incubation of blocking buffer for 1 hour at room temperature. The sections were then incubated with primary antibodies at 4℃ overnight. After 16-18 hours of primary antibody incubation, brain sections were rinsed again with TBS for three times before the incubation of secondary antibodies for 2 hours at room temperature. Sections were rinsed with TBS, mounted on slides, coverslipped with anti-fade reagent (ProLong Gold; Invitrogen) and stored at 4℃.

Primary antibodies used for immunostaining included rat polyclonal of BrdU (1:500; Accurate Chemcial&Scientefic Co.) to label newborn cells, mouse polyclonal antibody of NeuN (1:1000; Millipore Co.) to label neurons, rabbit polyclonal antibody of Ki67 (1:250; NovocastraLaboratones Ltd, UK) to detect proliferating cells, rabbit polyclonal antibody of Sox2 (1:1000, Millipore Co.) to detect NSCs and mouse polyclonal antibody of PSA-NCAM (1:200; Hybridoma bank) to detect neuroblasts in Chestnut bats and Japanese long winged bats while guinea pig polyclonal antibody of DCX (1:5000; Millipore) to label neuroblasts in Formosan leaf-nosed bats. Secondary antibodies used include Dylight 488-conjugated goat anti-rat IgG, Dylight 550-conjugated

goat anti-rabbit IgG, anti-mouse IgM and Dylight 594-conjugated donkey anti-guinea pig IgG (1:500; Jackson ImmunoResearch Laboratories).

Confocal and statistics analysis

Images were obtained with a confocal microscope (Leica TCS SP2 Confocal Spectra Microscope Imaging System). Images of immunostaining were processed using Photoshop CS4 software (Adobe). The numbers of labeled cells were counted in 2 μm confocal sections with standard counting frames and normalized by frame area (mm^2). Statistical analyses were performed by SPSS. Multiple comparisons were by one-way ANOVA with Tukey-HSD *post hoc* test. For the other cell type experiments, the cell density differences were obtained by comparing the cell density in evaluated brain regions with the ventral part of the SVZ (vSVZ). All data were presented as mean ± standard error of the mean (SEM), and significant level is $p < 0.05$.

Result

As mentioned previously, it takes around 7 days for newborn cells to reach the OB, 14 days to migrate into the granule cell layer and 21 days to differentiate into mature granule cells in rodents[4,5]. Similarly, it takes 2 weeks for newborn cells to migrate to the GCL in the DG and 3 weeks for them to differentiate into DG granule neurons[4,5]. To study the pattern of postnatal neurogenesis in FM and CF-FM bats in Taiwan, BrdU, a thymidine analog, was administrated to all animals to label newborn cells. To examine neuronal differentiation of these newborn cells, animals were sacrificed one, two or three weeks after BrdU injections (Fig.1).

Postnatal neurogenesis in Chestnut bats

To investigate the pattern of postnatal neurogenesis in FM bats, we first studied it in Chestnut bats. We examined BrdU and NeuN, a mature neuron marker, staining in the OB (Fig.2A-C) and DG (Fig.2D-F). The number of newborn cells ($BrdU^+$) and newborn neurons ($BrdU^+NeuN^+$) in the OB (Fig.2G) and DG (Fig.2H) were quantified.

We found that newborn cells arrived in the SEZ of the OB 1 week after BrdU injections. There was a trend increase of newborn cells in the OB 1 to 3 weeks after BrdU injections (Fig.2A-C, G; Table 1). 1 weeks after BrdU administration, newborn cells migrated into the GCL of the OB (Fig.2A-C, G; table 1). 3 weeks after BrdU injections, we observed some $BrdU^+NeuN^+$ newborn neurons in the GCL of the OB (Fig.2C, G; Table 1). Therefore, this result suggests that the pattern of postnatal

bulbar neurogenesis in Chestnut bats is very similar with the pattern found in rodents.

We found that there were no new neurons in the DG of Chestnut bats even though there were some newborn cells observed in the SGZ and the GCL of the DG 1 week after BrdU injections (Fig.2D, H; Table 2). This result suggests that there is no postnatal hippocampal neurogenesis in Chestnut bats.

Postnatal neurogenesis in Japanese long-winged bats

To examine the pattern of postnatal neurogenesis in another species of FM bats, we studied Japanese long-winged bats. We first analyzed the number of newborn cells ($BrdU^+$) and newborn neurons ($BrdU^+NeuN^+$) in the OB (Fig.3A-C) and DG (Fig.3D-F). There was a trend decrease of newborn cells in the SEZ of the OB 1 to 3 weeks after BrdU injections (Fig.3A-C, G; Table 3). In the SGZ of the DG, there was no significant difference in the cell density of newborn cells 1 to 3 weeks after BrdU injection (Fig.3C-F, H; Table 4). Furthermore, we found that newborn cells migrate into the GCL of the OB and DG 1 week after BrdU injections (Fig.3; Table 3, 4). This result suggests that there is postnatal bulbar and hippocampal neurogenesis in Japanese long-winged bats. From the result, we found that newborn cells differentiate into new neurons in the OB and DG 1 week after BrdU injections. This result suggests that the migration of newborn cells into the OB and DG seems to be faster in Japanese long-winged bats compared with that in lab rodents.

Taken together, our finding shows that the pattern of postnatal neurogenesis is quite differentin these two species of FM bats, indicating that there is species-specific pattern of postnatal neurogenesis in bats.

Postnatal neurogenesis in Formosan leaf-nosed bats

To investigate the pattern of postnatal neurogenesis in CF-FM bats, we chose Formosan leaf-nosed bats as our animal model in this study. By examining the number of newborn cells and newborn neurons in the OB (Fig.4A-C, G) and DG (Fig.4D-F, H), we found that the pattern of postnatal neurogenesis was quite similar with that in Japanese long-winged bats. Newborn cells arrived in the SEZ of the OB 1 week after BrdU injections and there was no significant difference in the cell density of newborn cells 1 to 3 weeks after BrdU injections (Fig.4 A-C, G; Table 5). Newborn cells were found in the SGZ of the DG as early as one week after BrdU injections and newborn cells migrated into the GCL of the DG 1 week after BrdU injections (Fig.4 D-F, H; Table 6). As early as 1 week after BrdU administration, there were some newborn neurons detected in the GCL of the OB and DG (Fig.4; Table 5, 6).

Our result suggests that there is postnatal bulbar and hippocampal neurogenesis in Formosan leaf-nosed bats. The rate of postnatal hippocampal neurogenesis is low. The migration and differentiation time course seems to be faster than those in rodents.

Taken together, the pattern of postnatal neurogenesis is a species-specific pattern in bats in Taiwan.

Neural stem cells confirmed with Sox2

From our finding, postnatal neurogenesis persists in the OB and DG of Japanese long-winged bats and Formosan leaf-nosed bats. However, it is only found in the OB but not in the DG of Chestnut bats. It could be due to lack of NSCs in the SGZ of Chestnut bats. Therefore, we decided to examine the existence of NSCs in the SVZ and SGZ of these bats. Previous studies show that the NSCs exist in postnatal mammalian nervous system and their multi-potency enables NSCs to self-renew and to generate neurons and glia[25]. Furthermore, evidence shows that NSCs are more commonly found in the ventral part of the SVZ in rodents. To examine the existence of NSCs in bats, we used Sox2 antibody to label NSCs in these two brain regions. We examined the dorsal part of the SVZ (dSVZ), ventral part of the SVZ (vSVZ) and SGZ.

In Chestnut bats, we found that $Sox2^+$ cells were present in the SVZ and SGZ of these species (Fig.5A-D). There were no differences in $Sox2^+$ cell densities in the dSVZ and vSVZ (Fig.5 A-B, D; Table 7). There were $Sox2^+$ cells present in the SGZ, and the $Sox2^+$ cell density was significantly lower in the SGZ than that in the ventral part of the SVZ (Fig.5C-D; Table 7).

In Japanese long-winged bats, there were $Sox2^+$ cells found in the dorsal and ventral part of the SVZ and SGZ (Fig.6A-D). There were no differences in $Sox2^+$ cell densities in this species of bats (Fig.6D; Table 7).

In Formosan leaf-nosed bats, $Sox2^+$ cells were present in the dorsal and ventral part of the SVZ and SGZ (Fig.7A-D; Table 7). Moreover, the

$Sox2^+$ cell density in the SGZ was significantly lower than that in the ventral part of the SVZ (Fig.7D).

Taken together, we found that $Sox2^+$ cells were present in the SVZ and SGZ of these three species of bats. In our finding, there was no significant difference in $Sox2^+$ cell densities between the dorsal and ventral part of the SVZ in these three species of bats. In Chestnut bats, lower NSC cell densities may explain why there was no postnatal neurogenesis found in the DG of Chestnut bats.

Neuroblasts confirmed with PSA-NCAM or DCX

From our finding, NSCs exist and distribute in the SVZ and SGZ of three species of bats. In the SVZ, NSCs give rise to transit-amplifying cells that produce neurablasts migrating along the RMS to the OB. In the SGZ, NSCs give rise to neuroblasts in the DG. Therefore, we examined whether there were neuroblasts in the RMS, dSVZ, vSVZ and SGZ. We used the neuroblast-specific marker, polysialylated neuronal cell-adhesion molecule (PSA-NCAM; Fig.8-9A-D) and doublecortin (DCX; Fig.10A-D) antibodies to label neuroblasts in these brain regions.

In Chestnut bats, we found that there were PSA-$NCAM^+$ neuroblasts in the RMS, the dorsal and ventral part of the SVZ, but not in the SGZ (Fig.8A-E). PSA-$NCAM^+$ cells were more concentrated in the dorsal part of the SVZ than those in the ventral part of the SVZ (Fig.8E; Table 8).

There were PSA-$NCAM^+$ neuroblasts present in the RMS, the

dorsal and ventral part of the SVZ and in the SGZ of Japanese long-winged bats (Fig.9A-E). There was no significant difference in PSA-NCAM$^+$ cell densities in the dorsal and ventral part of the SVZ, but less PSA-NCAM$^+$ neuroblasts were found in the SGZ than those in the ventral part of the SVZ (Fig.9E; Table 8).

In Formosan leaf-nosed bats, we detected DCX$^+$ neuroblasts in the RMS, the dorsal and ventral part of the SVZ and in the SGZ (Fig.10A-E). DCX$^+$ neuroblasts were more present in the ventral part of the SVZ when compared with the dorsal part of the SVZ. We found less DCX$^+$ neuroblasts in the SGZ than those in the ventral part of the SVZ (Fig.10E; Table 8).

In conclusion, there are neuroblasts present in the SVZ and SGZ of Japanese long-winged bats and Formosan leaf-nosed bats and in the SVZ of Chestnut bats. The absence of neuroblasts in the SGZ of Chestnut bats may explain why no postnatal neurogenesis is found in the DG of these bats.

Proliferating cells confirmed with Ki67

NSCs, transit-amplifying cells and neuroblasts, are all proliferative. Therefore, we also examined the level of proliferation in the SVZ-OB pathway and SGZ. We used Ki67 antibody to label proliferating cells in the RMS, dSVZ, vSVZ and SGZ.

In Chestnut bats, Ki67$^+$ cells were presented in the RMS, dorsal and ventral part of the SVZ, but not in the SGZ (Fig.11A-E). There was

no difference of proliferative cell densities in the dorsal and ventral part of the SVZ (Fig.11E; Table 9).

In Japanese long-winged bats, we found that $Ki67^+$ cells existed in these brain regions (Fig.12A-E). There was no difference in $Ki67^+$ cell densities in the dorsal and ventral part of the SVZ. We found that there were less $Ki67^+$proliferative cells in the SGZ than those in the ventral part of the SVZ (Fig.12E; Table 9).

There were $Ki67^+$ proliferative cells detected in the RMS, dorsal and ventral part of the SVZ and SGZ of Formosan leaf-nosed bats (Fig.13A-E). Proliferative cells of Formosan leaf-nosed bats were found mostly in the ventral part of the SVZ than in the dorsal part of the SVZ. Similar with the result shown in Japanese long-winged bats, there were less $Ki67^+$ proliferative cells in the SGZ than those in the ventral part of the SVZ (Fig.13E; Table 9).

Our finding demonstrates that proliferative precursor cells exist in the SVZ-OB pathway in these three species of bats. In the SGZ, proliferative precursor cells are only found in Japanese long-winged bats and Formosan leaf-nosed bats. No $Ki67^+$ proliferative cells found in the SGZ of Chestnut bats may be caused by the quiescence of NSCs in the SGZ. It further explains why there is no postnatal hippocampal neurogenesis found in the DG of this species of bats. Moreover, less $Ki67^+$ proliferative cells found in the SGZ is consistent with lower rate of postnatal hippocampal neurogenesis in the DG of Japanese long-winged bats and Formosan leaf-nosed bats.

Discussions

Here we report for the first time on the pattern of postnatal neurogenesis in echolocating bats in Taiwan. This allows a direct comparison with the previous findings in rodents and primates, which opens the door to a broad cross-species understanding of postnatal neurogenesis. The SVZ-OB pathway is the main postnatal neurogenesis site in mammals, and this statement is once again verified by our studies of wild bats. Interestingly, postnatal DG neurogenesis is only found in Japanese long-winged bats and Formosan leaf-nosed bats, but not in Chestnut bats, suggesting that postnatal DG neurogenesis is not a universal phenomenon in mammals.

The current study reveals that the presence of postnatal bulbar neurogenesis in all examined bat species but postnatal hippocampal neurogenesis is absent in one of these three bat species. In Chestnut bats, we found no newborn neurons in the granule cell layer of the DG (Fig.2D-F, H). The absence is not due to a methodological error since there is postnatal bulbar neurogenesis detected in the same animal evaluated (Fig.2A-C, G). Furthermore, the absence of neuroblasts (Fig.8 D, E) and proliferating cells (Fig.11D, E) in the SGZ of this bat species may be the reason. Since we detected NSCs in the SGZ of Chestnut bats, the absence of neuroblasts and proliferating cells may be due to the quiescence of NSCs. Moreover, we found that the amount of NSCs in the SVZ was higher than that in the SGZ (Fig. 5). This may further explains the absence of postnatal hippocampal neurogenesis in Chestnut bats. Besides, NSCs have the ability to differentiate into astrocytes and

oligodendrocytes[4]. The existence of NSCs in Chestnut bats may be for that purpose.

In Japanese long-winged bats and Formosan leaf-nosed bats, we found that there was postnatal bulbar and hippocampal neurogenesis. The migration and differentiation of newborn cells seems to be faster compared with that in lab rodents. Intrinsic differences in the program of neuronal differentiation may be a factor.

There are only two species of CF-FM bats found in Taiwan, Formosan lesser horseshoe bats and Formosan leaf-nosed bats. Formosan lesser horseshoe bats are harder to maintain in the laboratory due to their tiny figure. Consequently, we took Formosan leaf-nosed bats as our animal model in CF-FM bats. In Formosan leaf-nosed bats, our finding indicates that there are postnatal bulbar and hippocampal neurogenesis, but with larger variation compared to those in other species of bats. This species of bats are harder to maintain in the laboratory compared to Chestnut bats and Japanese long-winged bats. They respond poorly, such as vomiting, anorexia and even death after BrdU injections. Moreover, a study has indicated that BrdU has detrimental effects on cell cycle, differentiation and survival *in vitro*[26]. The side effects of BrdU may lead to a more chronic response in Formosan leaf-nosed bats, but less in Chestnut bats, Japanese long-winged bats and rodents.

There is an obvious concern regarding to the immunohistochemistry techniques applied in this comparative studies. The absence of species-specific antibodies may provide false negative data. However, this is not the case here. Ki67 is evolutionarily conserved

protein that can be found in other vertebrate species investigated[27]. Moreover, the same immunohistochemistry procedure and antibodies used can visualize immunopositive cells in other neurogenic region in the brain of the same animal investigated.

Postnatal hippocampal neurogenesis is a prominent biological event in rodents. We found that hippocampal neurogenesis was absent in Chestnut bats and low in Japanese long-winged bats and Formosan leaf-nosed bats. Previous studies indicate that species with longer life expectancies have less abundant or complete absence of newborn neurons in the postnatal DG[21,27,28]. Long-lived and short-lived mammals have different major life history stage, including the age of maturity and aging. Therefore, life-span may be the critical factor regulating postnatal hippocampal neurogenesis. Bats are long-lived flying mammals. Therefore, bats is another example showing the inverted correlation between life-span and postnatal hippocampal neurogenesis.

Aging is one of the causes leading to the decline of postnatal hippocampal neurogenesis in rodents or even in primates[29-31]. Due to the difficulty of accurate age determination in postnatal bats, our samples probably contain young animals as well as older animals. However, Chestnut bats in this study are all juvenile and they have no postnatal hippocampal neurogenesis. Hence, aging may not be the main factor contributing to the absence of postnatal hippocampal neurogenesis in Chestnut bats. In Japanese long-winged bats and Formosan leaf-nosed bats, they do not show any individual differences in terms of neurogenic activity in the OB and DG. Therefore, factors other than aging may

contribute to the difference of postnatal neurogenesis found in different species.

Activity-dependent regulation of postnatal hippocampal neurogenesis has been reported in previous studies[4]. For instance, physical exercise such as voluntary running appears to affects postnatal hippocampal neurogenesis by enhancing the proliferation of precursor cells[4]. Flying is a voluntary physical exercise that happened in their daily activity. However, we deprived their physical exercise by locking them up in the insect cages. In this case, we may underestimate the level of postnatal neurogenesis of these captive bats.

Postnatal hippocampal neurogenesis has also been demonstrated to play an important role in mood regulation, another hippocampal-associated brain function[4,32]. Abrupt change in the environment and lifestyle may cause stress in wild bats. In previous studies, stress can down-regulate the postnatal hippocampal neurogenesis[4,32]. This may also be the reason for the lower rate of postnatal hippocampal neurogenesis found in our study.

Postnatal hippocampal neurogenesis is a complex biological event that is regulated by existing neuronal circuit. The neural network is highly dynamic, in which synaptic connections have the capability to modify the existing neuronal network. Granule cells in the DG, and pyramidal neurons in the CA1 and CA3 are synaptically connected to form the closed circuit named as “trisynaptic circuit”[4,33]. Previous studies show that newborn neurons are involve in the formation of memories by establishing new synaptic connections or replacing the older connections

in the trisynaptic circuit[33]. In a recent study reported by the Akers et al., the increase in hippocampal neurogenesis during infancy reduces the hippocampal-dependent memories[34]. Therefore, the lack of or lower rate of hippocampal neurogenesis in bats may maintain their memory.

From our findings, we cannot conclude a relationship between postnatal hippocampal neurogenesis with their echolocation call types. From our results and the features of those bat species, we speculate that habitats may contribute to the pattern of postnatal hippocampal neurogenesis. Postnatal hippocampal neurogenesis is found in Japanese long-winged bats and Formosan leaf-nosed bats, but not in Chestnut bats. According to Kaohsiung City biodiversity database, Chestnut bats are mainly found at low altitudes and they prefer resting at the base of the palm tree leaves. They are also found in buildings. Japanese long-winged bats are widely distributed from low elevation caves to middle to high elevation forests. Meanwhile, Formosan leaf-nosed bats are generally distributed in natural caves at middle elevation, tunnels, abandoned buildings and broadleaf forests with stream. Moreover, Formosan leaf-nosed bats often share a same cave with Japanese long-winged bats for roosting but in different areas[23]. The structural complexity of broadleaf forest increases the heterogeneity of the environment, and therefore increases the niche diversity. In previous studies, enriched environment plays a part in the regulation of postnatal hippocampal neurogenesis by increasing the survival of newborn neuron in rodents[4,33]. Japanese long-winged bats and Formosan leaf-nosed bats seem to have a quite complex roost habitat relative to Chestnut bats. Collectively, the

complexity of a roost habitat may positively regulate postnatal hippocampal neurogenesis.

Bats use a variety of landscapes or habitats throughout their lifetime to feed, roost and travel. The habitats that bats go through in their lifetime include roosting habitats, foraging habitats and commuting habitats[23]. Roosting habitats may regulate the pattern of postnatal hippocampal neurogenesis. To further elucidate this, we have to exam the pattern of postnatal hippocampal neurogenesis of more bat species with similar roosting habitats. For example, we can examine the pattern of postnatal hippocampal neurogenesis of Taiwanese woolly bats (*Kerivoula* sp.) and Taiwanese tube-nosed bats (*Murinaputa*), as they roost in tree leaves similar to Chestnut bats[23].

Bats show a completely absence to low rate of postnatal hippocampal neurogenesis in the study of Amreinet. al., in 2007[21]. Another evidence showed that there is high density of $Ki67^+$ and DCX^+ cells found in the RMS and SVZ while the cell densities varied from high to low in the SGZ among those eight species of megabats evaluated[22]. Our result show that there is absent-to-low postnatal hippocampal neurogenesis and high density of postnatal bulbar neurogensis in 3 species of microchiroptera/microbats examined by using BrdU and NeuN, to define postnatal neurogenesis. Collectively, our observations are consistent with these previous reports that postnatal neurogenesis can be found in both megachiroptera and microchiroptera bat species.

In rodents, olfaction is a chemosensory for some essential functions such as food selection, danger detection and conspecific

interactions. Therefore, the olfactory system has to detect and discriminate between odorants from environment and then turns the information into olfaction memory[16]. Many vertebrates, including most mammals, have two distinct olfactory system, the main olfactory system and accessory olfactory system. The main olfactory bulb (MOB) in main olfactory system senses volatile airborne stimuli while the accessory olfactory bulb (AOB) in accessory olfactory system detects fluid-phase stimuli, probably pheromones[11,24,35,36]. Previous study indicates that pregnancy can induces neurogenesis in the MOB mediated by prolactin[37]. Moreover, odor enrichment can also increase neurogenesis in the MOB[24]. Furthermore, previous studies indicated that OB neurogenesis is involved in odor discrimination and establishing olfaction memory. Previous studies show that odor discrimination and social odor discrimination require OB neurogenesis[38,39]. While other groups suggest that olfaction memory requires OB neurogenesis[40,41]. In bats, the olfaction system may be involved in foraging, communicating and reproduction.In our finding, postnatal bulbar neurogenesis persists in all three examined bat species. These results indicate that bats may also require OB neurogenesis for odor discrimination and olfaction memory. This may indicate that postnatal bulbar neurogenesis is a conservative biological event in mammals.

Taken together, the pattern of postnatal hippocampal neurogenesis can be considered to be species-specific. It further indicates that postnatal hippocampal neurogenesis may not be required for spatial learning and memory in higher mammals.

Figures

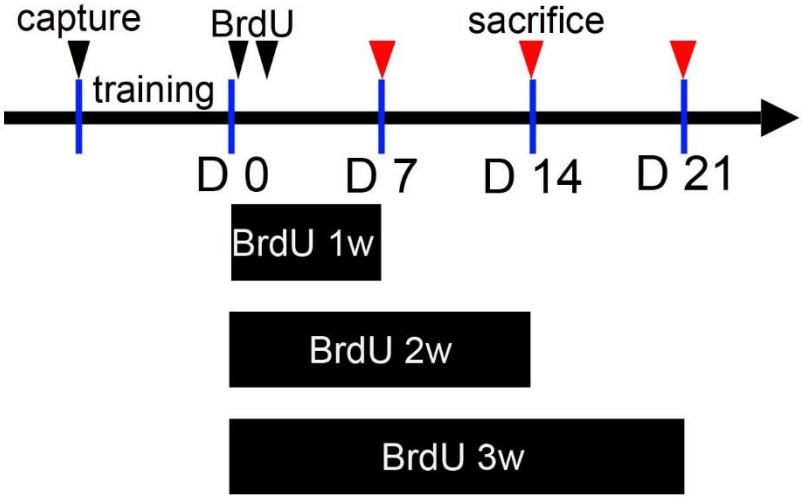

Figure 1. Experimental timeline. To study the pattern of postnatal neurogenesis in FM and CF-FM bats in Taiwan, BrdU was administrated twice a day and animals were sacrificed 1 (BrdU 1w), 2 (BrdU 2w), or 3 (BrdU 3w) weeks after BrdU administration.

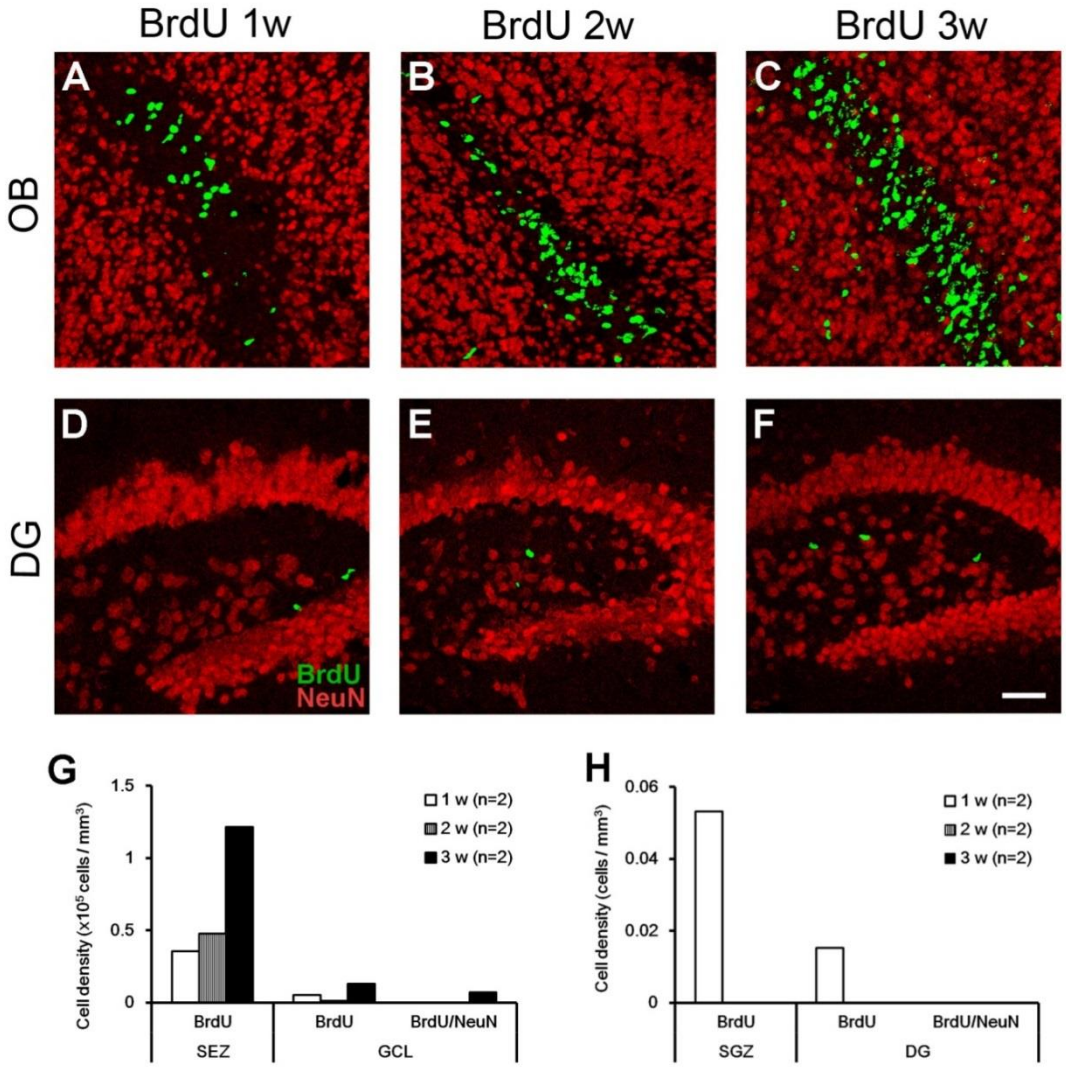

Figure 2. Postnatal bulbar but not hippocampal neurogenesis persists in Chestnut bats. Newborn cells were labeled with BrdU (green) and mature neurons were labeled with NeuN (red) in the OB (A-C) and DG (D-F). There was a trend increase of newborn cells in the OB 1 to 3 weeks after BrdU injections (G). Newborn cells were detected in the SGZ and DG 1 week after BrdU injections. However, none of them differentiate into neurons at any time point (H). Data are presented as means ± SEM. Scale bar: 40 μm.

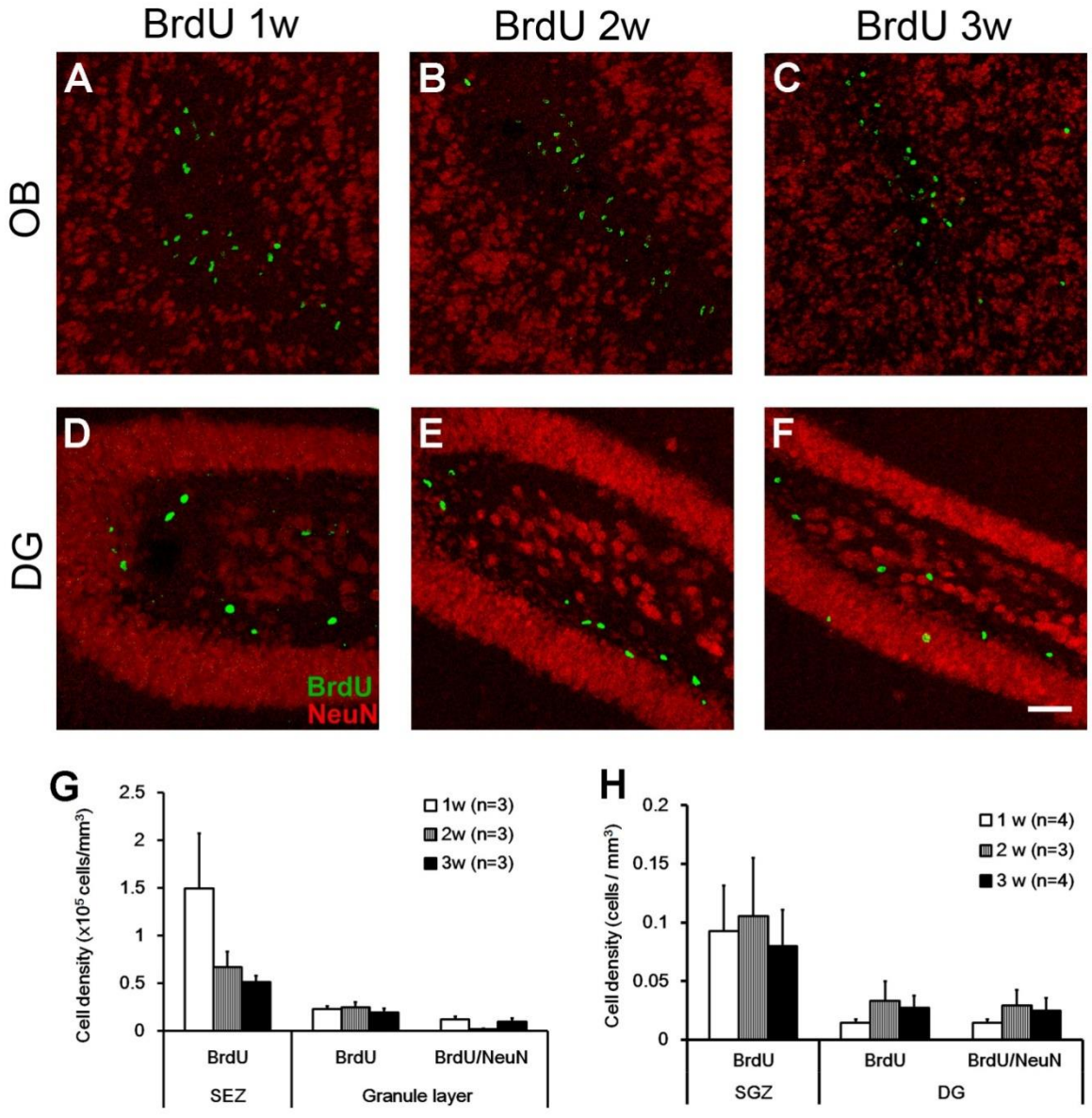
BrdU 1w
BrdU 2w
BrdU 3w
A
B
C
OB
D
E
F
DG
BrdU
NeuN
G
Cell density (x10^5 cells/mm^3)
2.5
2
1.5
1
0.5
0
1w (n=3)
2w (n=3)
3w (n=3)
BrdU
BrdU
BrdU/NeuN
SEZ
Granule layer
H
Cell density (cells / mm^3)
0.2
0.15
0.1
0.05
0
1 w (n=4)
2 w (n=3)
3 w (n=4)
BrdU
BrdU
BrdU/NeuN
SGZ
DG

Figure 3. Postnatal bulbar and hippocampal neurogenesis occur in Japanese long-winged bats. Newborn cells were labeled with BrdU (green) and mature neurons were labeled with NeuN (red) in the OB (A-C) and DG (D-F). There was a trend decrease of newborn cells in the SEZ of the OB 1 to 3 weeks after BrdU injections, but new neurons appeared in the GCL as early as 1 week after BrdU injections (G). There was no significant difference in the cell density of newborn cells and newborn neurons found in the SGZ and GCL of the DG 1 to 3 weeks after BrdU injections. Newborn cells migrated into the GCL of the DG and differentiate into neurons 1 week after BrdU injections. Data are presented as means ± SEM. Scale bar: 40 μm.

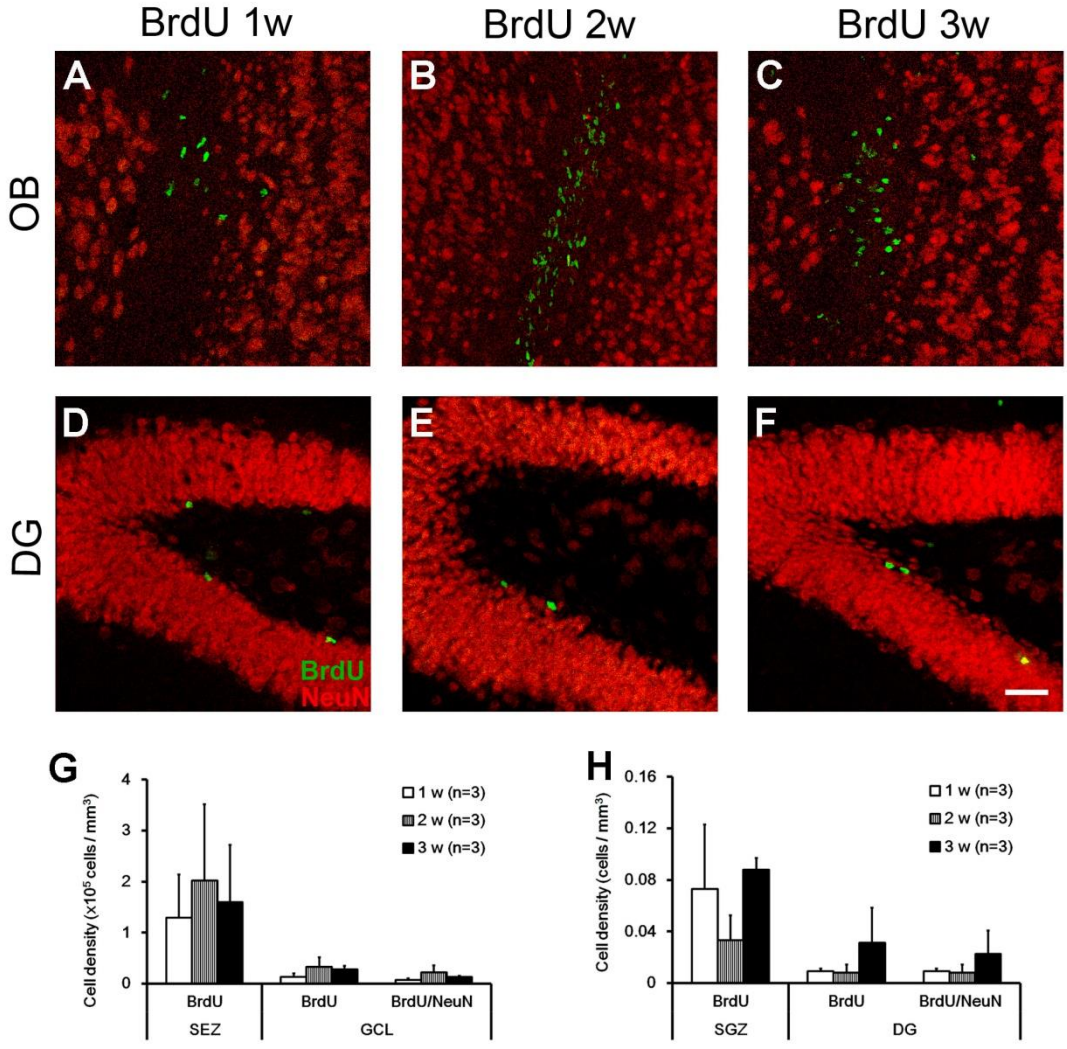
BrdU 1w
BrdU 2w
BrdU 3w
A
B
C
OB
D
E
F
DG
BrdU
NeuN
G
Cell density (x10^5 cells / mm^3)
4
3
2
1
0
1 w (n=3)
2 w (n=3)
3 w (n=3)
BrdU
BrdU
BrdU/NeuN
SEZ
GCL
H
Cell density (cells / mm^3)
0.16
0.12
0.08
0.04
0
1 w (n=3)
2 w (n=3)
3 w (n=3)
BrdU
BrdU
BrdU/NeuN
SGZ
DG

Figure 4. Postnatal bulbar and hippocampal neurogenesis occur in Formosan leaf-nosed bats. Newborn cells were labeled with BrdU (green) and mature neurons were labeled with NeuN (red) in the OB (A-C) and DG (D-F). Newborn cells arrived in the SEZ of the OB 1 week after BrdU injections. There were some newborn neurons found in the GCL of the OB as early as 1 week after BrdU injections (G). Newborn cells migrated into the GCL of the DG and differentiate into neurons 1 week after BrdU injections (H). Data are presented as means ± SEM. Scale bar: 40 μm.

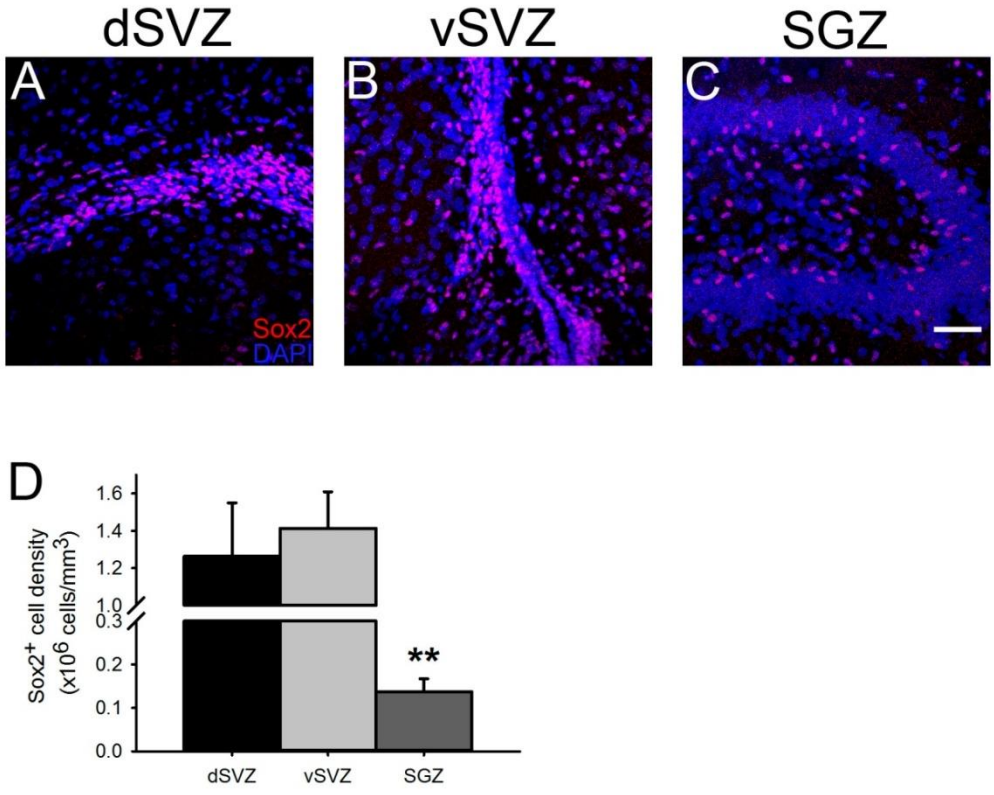

Figure 5. NSCs are present in the SVZ and SGZ of Chestnut bats. NSCs were labeled with Sox2 (red) and nuclei were labeled with DAPI (blue) in the dorsal part of the SVZ (dSVZ: A), ventral part of the SVZ (vSVZ: B), and SGZ (SGZ: C). $Sox2^+$ cell densities were quantified in the dSVZ, vSVZ and SGZ in Chestnut bats (D). There was no difference in cell densities in the dorsal and ventral part of the SVZ. The $Sox2^+$ cell density was significantly lower in the SGZ than that in the ventral of the SVZ. Scale bar: 40 μm. Data are presented as mean ± SEM. *, compared to vSVZ. ** $p < 0.01$.

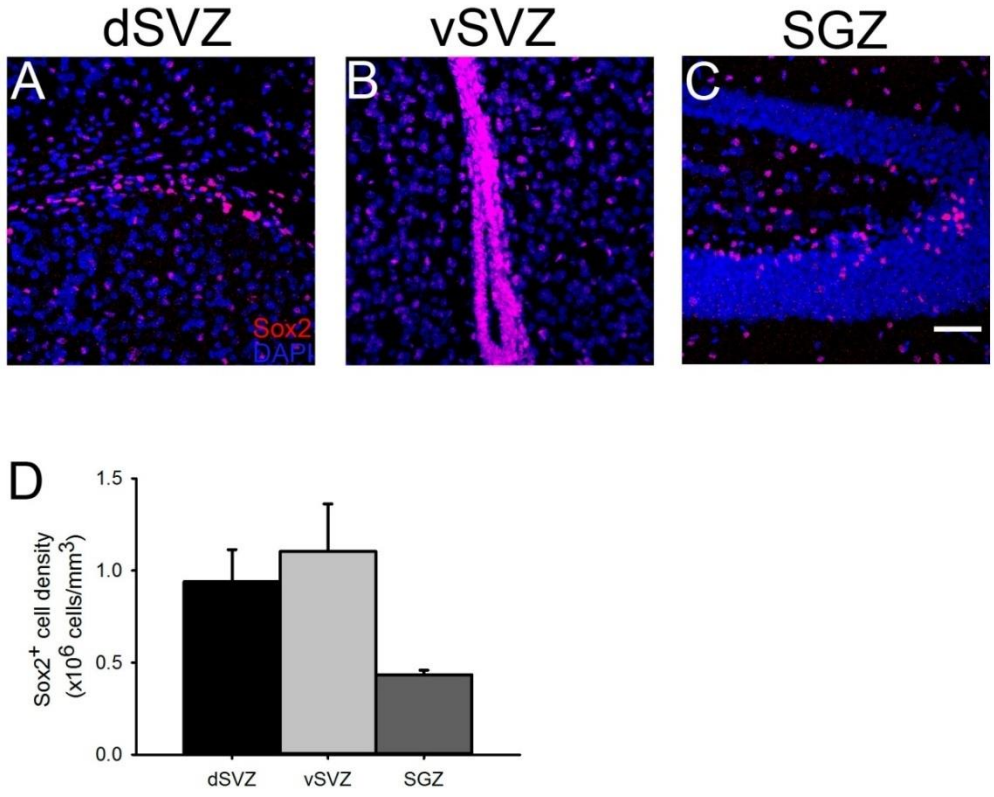

Figure 6. NSCs are present in the SVZ and SGZ of Japanese long-winged bats. NSCs were labeled with Sox2 (red) and nuclei were labeled with DAPI (blue) in the dorsal part of the SVZ (dSVZ: A), ventral part of the SVZ (vSVZ: B), and SGZ (SGZ: C). $Sox2^+$ cell densities were quantified in the dSVZ, vSVZ and SGZ in Japanese long-winged bats (D). $Sox2^+$ cells were presented in these brain regions. There was no difference in cell densities in these areas. Scale bar: 40 μm. Data are presented as mean ± SEM.

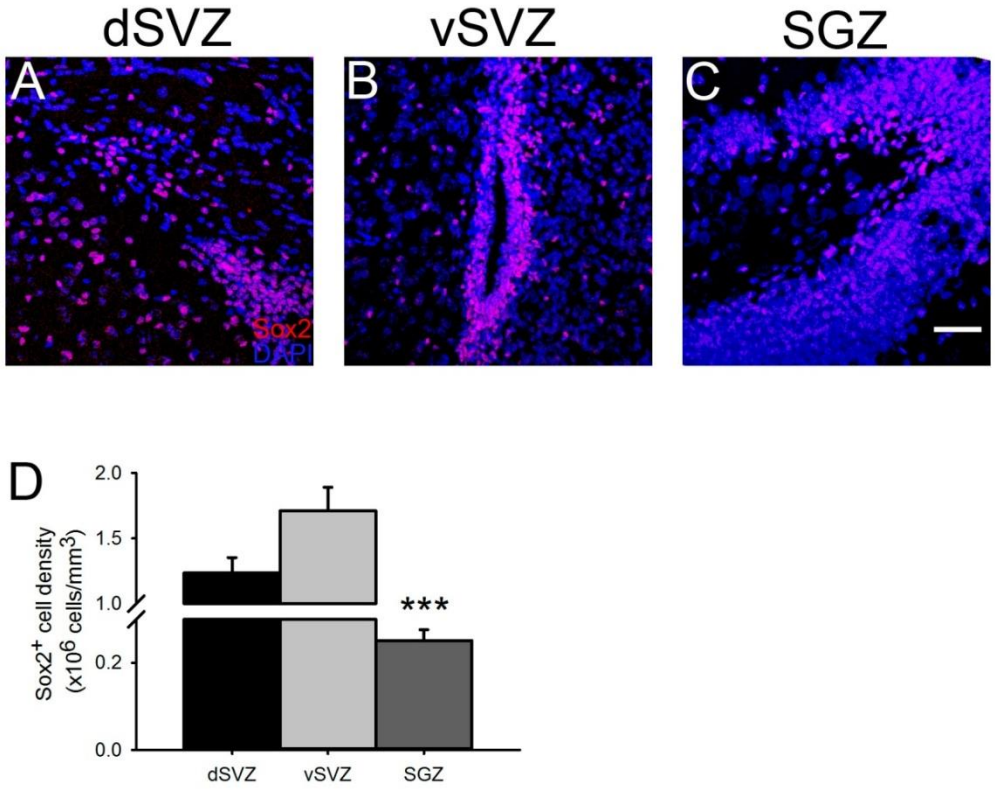

Figure 7. NSCs are present in the SVZ and SGZ of Formosan leaf-nosed bats. NSCs were labeled with Sox2 (red) and nuclei were labeled with DAPI (blue) in the dorsal part of the SVZ (dSVZ: A), ventral part of the SVZ (vSVZ: B), and SGZ (SGZ: C). $Sox2^+$ cell densities were quantified in the dSVZ, vSVZ and SGZ in Formosan leaf-nosed bats (D). $Sox2^+$ cells were detected in these brain regions. There was no difference in cell densities in the dorsal and ventral part of the SVZ. The $Sox2^+$ cell density in the SGZ was significantly lower than that in the ventral part of the SVZ. Scale bar: 40 μm. Data are presented as mean ± SEM. *, compared to vSVZ. *** $p< 0.001$.

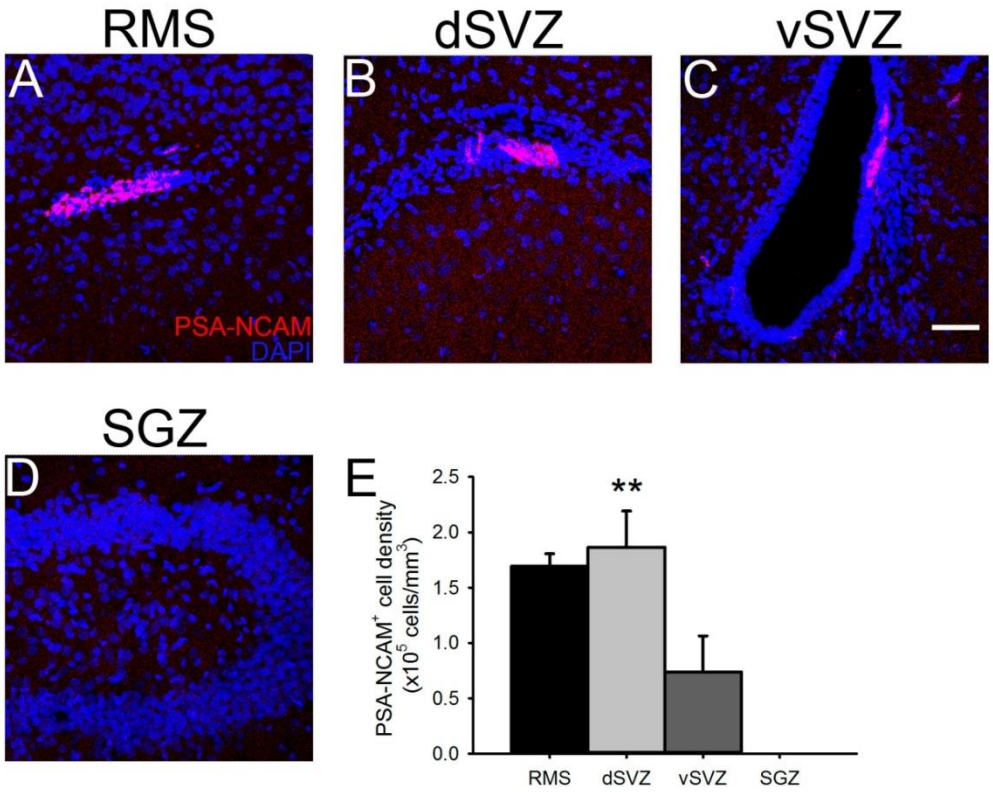

Figure 8. Neuroblasts are present in the SVZ but not in the SGZof Chestnut bats. Neuroblasts were labeled with PSA-NCAM (red) and nuclei were labeled with DAPI (blue) in the RMS (RMS: A), dorsal part of the SVZ (dSVZ: B), ventral part of the SVZ (vSVZ: C), and SGZ (SGZ: D). Densities of neuroblasts were quantified in these brain regions (E). Neuroblasts were existed in the RMS, dSVZ and vSVZ but not in the SGZ. In the SVZ, neuroblasts were mostly concentrated at the dorsal part of the SVZ in Chestnut bats. Scale bar: 40 μm. Data are presented as mean ± SEM. *, compared to vSVZ. ***p*<0.01.

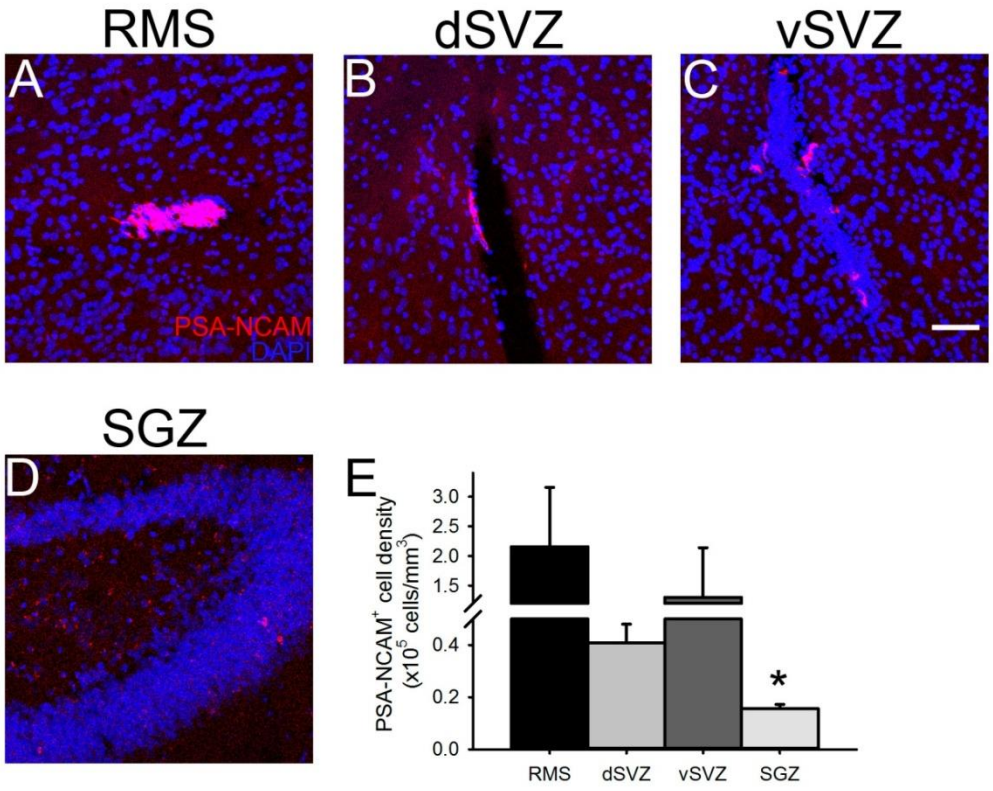

Figure 9. Neuroblasts are present in the SVZ and SGZof Japanese long-winged bats. Neuroblasts were labeled with PSA-NCAM (red) and nuclei were labeled with DAPI (blue) in the RMS (RMS: A), dorsal part of the SVZ (dSVZ: B), ventral part of the SVZ (vSVZ: C), and SGZ (SGZ: D). Densities of neuroblasts were quantified in these brain regions (E). Neuroblasts were found in these brain regions. There was no difference in PSA-NCAM$^+$ cell densities in the dorsal and ventral SVZ of this species of bats. There were less PSA-NCAM$^+$ neuroblasts in the SGZ than those in the ventral part of the SVZ. Scale bar: 40 μm. Data are presented as mean ± SEM.*, compared to vSVZ. * $p< 0.05$.

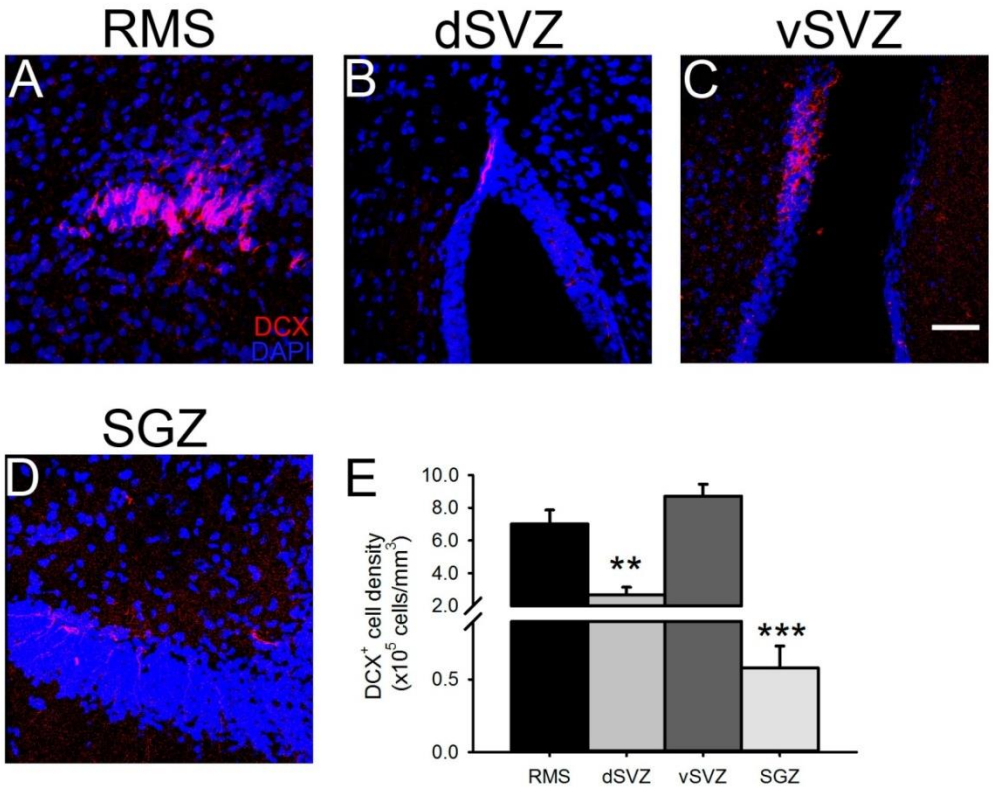

Figure 10.Neuroblasts are present in the SVZ and SGZ of Formosan leaf-nosed bats. Neuroblasts were labeled with DCX (red) and nuclei were labeled with DAPI (blue) in the RMS (RMS: A), dorsal part of the SVZ (dSVZ: B), ventral part of the SVZ (vSVZ: C), and SGZ (SGZ: D). Densities of neuroblasts were quantified in these brain regions (E). Neuroblasts were found in the RMS, dSVZ, vSVZ and SGZ. In the SVZ, neuroblasts were mostly concentrated at the ventral part of the SVZ in this species of bats. There were less DCX^+ neuroblasts in the SGZ than those in the ventral part of the SVZ. Scale bar: 40 μm. Data are presented as mean ± SEM. *, compared to vSVZ. **p<0.01; ***p<0.001.

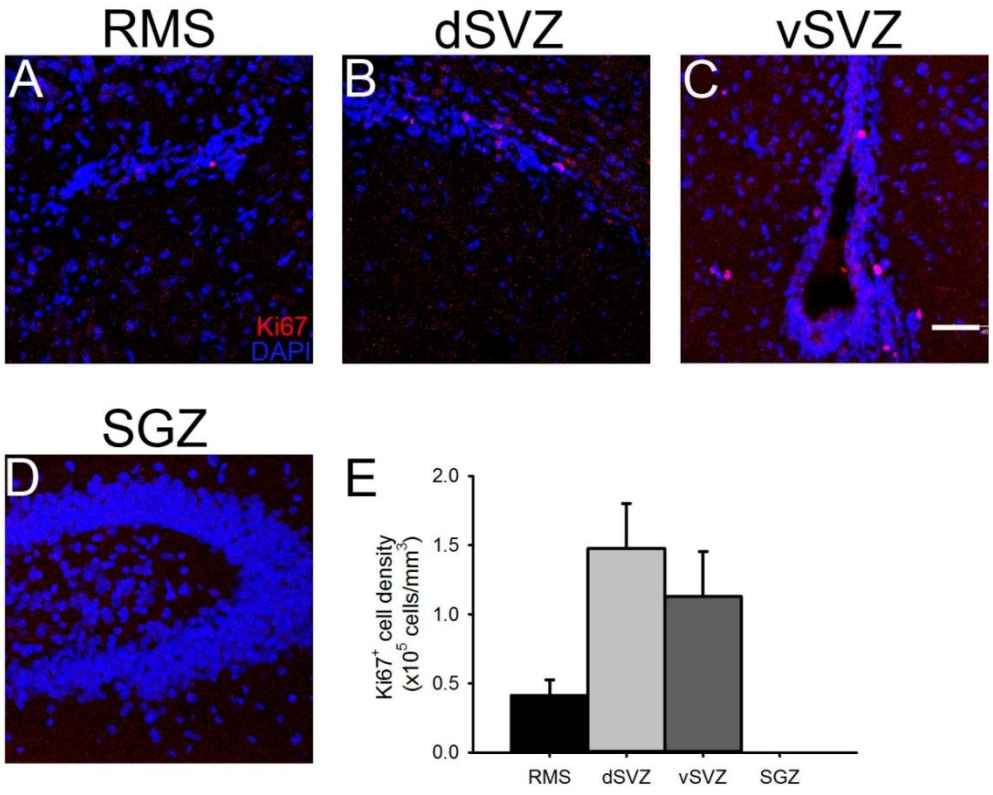

Figure 11. Proliferating cells are present in the SVZ but not in the SGZ of Chestnut bats. Proliferating cells were labeled with Ki67 (red) and nuclei were labeled with DAPI (blue) in the RMS (RMS: A), dorsal part of the SVZ (dSVZ: B), ventral part of the SVZ (vSVZ: C), and SGZ (SGZ: D). $Ki67^+$ cell densities were quantified in the RMS, dSVZ, vSVZ and SGZ in Chestnut bats (E). $Ki67^+$ cells were found in the RMS, the dorsal and ventral part of the SVZ but not in the SGZ. There was no difference in the $Ki67^+$ proliferative cells in the RMS and dorsal part of the SVZ compared with those in the ventral part of the SVZ. Scale bar: 40 μm. Data are presented as mean ± SEM.

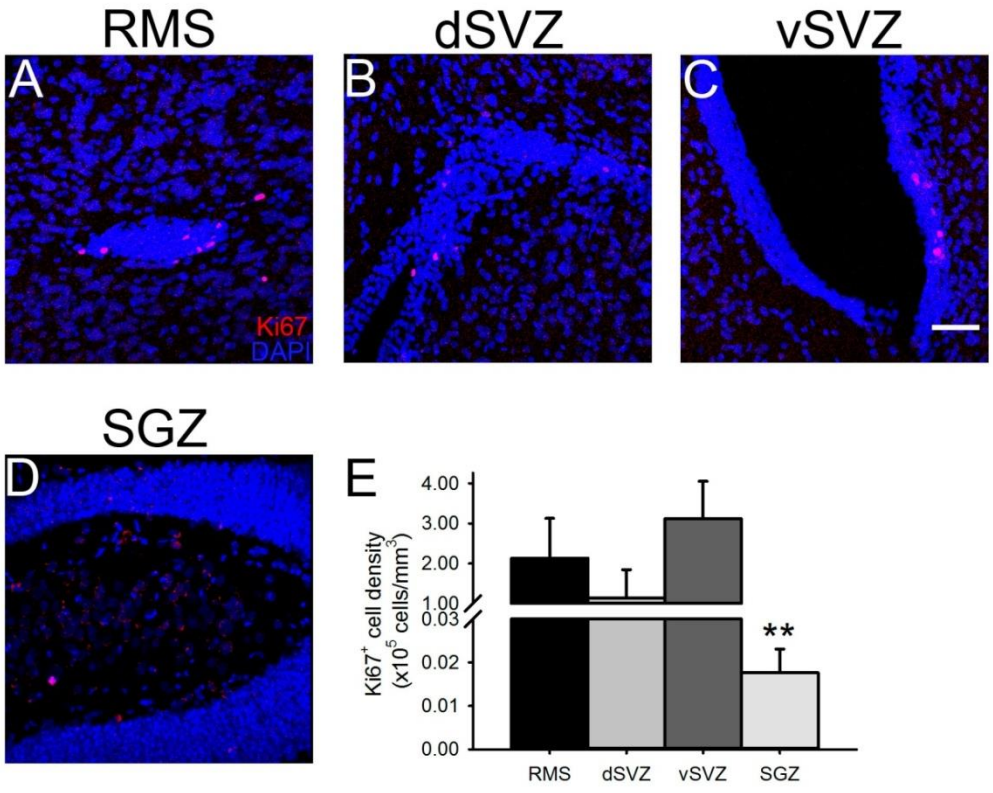

Figure 12. Proliferating cells are present in the SVZ and SGZ of Japanese long-winged bats. Proliferating cells were labeled with Ki67 (red) and nuclei were labeled with DAPI (blue) in the RMS (RMS: A), dorsal part of the SVZ (dSVZ: B), ventral part of the SVZ (vSVZ: C), and SGZ (SGZ: D). $Ki67^+$ cell densities were quantified in the RMS, dSVZ, vSVZ and SGZ in Japanese long-winged bats (E). There was no difference in $Ki67^+$ proliferative cells in the dorsal and ventral part of the SVZ. There were less $Ki67^+$ proliferative cells in the SGZ than those in the ventral part of the SVZ. Scale bar: 40 μm. Data are presented as mean ± SEM. *, compared to vSVZ. ***p*<0.01.

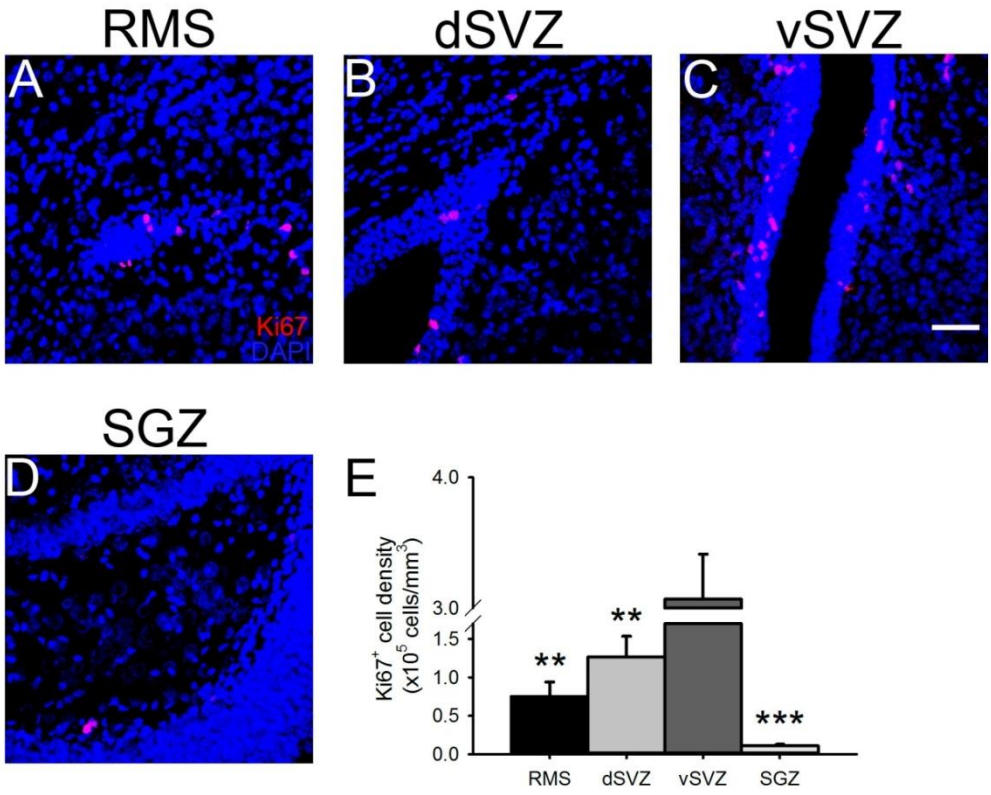

Figure 13. Proliferating cells are present in the SVZ and SGZ of Formosan leaf-nosed bats. Proliferating cells were labeled with Ki67 (red) and nuclei were labeled with DAPI (blue) in the RMS (RMS: A), dorsal part of the SVZ (dSVZ: B), ventral part of the SVZ (vSVZ: C), and SGZ (SGZ: D). $Ki67^+$ cell densities were quantified in the RMS, dSVZ, vSVZ and SGZ in Formosan leaf-nosed bats (E). $Ki67^+$ cells were detected in these brain regions. $Ki67^+$ proliferative cells were found mostly concentrated in the ventral part of the SVZ. There was less $Ki67^+$ proliferative cells in the SGZ than those in the ventral part of the SVZ. Scale bar: 40 μm. Data are presented as mean ± SEM. *, compared to vSVZ. $^{**}p < 0.01$. $^{***}p < 0.001$.

Tables

Brain regions	Staining	Cell densities (mean ± SEM x10^5 cells/mm^3)		
		BrdU 1w	BrdU 2w	BrdU 3w
SEZ	$BrdU^+$	0.36 ± 0.19	0.48 ± 0.59	1.22 ± 0.35
GCL	$BrdU^+$	0.05 ± 0.02	0.01 ± 0.02	0.13 ± 0.05
	$BrdU^+$ $NeuN^+$	0	0	0.07 ±0.06

Table 1. Quantification of postnatal bulbar neurogenesis in Chestnut bats.

Brain regions	Staining	Cell densities (mean ± SEM cells/mm^3)		
		BrdU 1w	BrdU 2w	BrdU 3w
SGZ	$BrdU^+$	0.05 ± 0.01	0	0
GCL	$BrdU^+$	0.02 ± 0.01	0	0
	$BrdU^+$ $NeuN^+$	0	0	0

Table 2. Quantification of postnatal hippocampal neurogenesis in Chestnut bats.

Brain regions	Staining	Cell densities (mean ± SEM x10^5 cells/mm^3)			Statistics (p values)		
		BrdU 1w	BrdU 2w	BrdU 3w	BrdU 1w vs BrdU 2w	BrdU2w vs BrdU3w	BrdU 1w vs BrdU3w
SEZ	$BrdU^+$	1.47 ± 0.53	0.68 ± 0.11	0.51 ± 0.16	0.295	0.946	0.199
GCL	$BrdU^+$	0.24 ± 0.03	0.25 ± 0.09	0.18 ± 0.06	0.958	0.638	0.794
	$BrdU^+$ $NeuN^+$	0.11 ± 0.04	0.02 ± 0.007	0.10 ± 0.05	0.068	0.144	0.830

Table 3. Quantification of postnatal bulbar neurogenesis in Japanese long-winged bats.

Brain regions	Staining	Cell densities (mean ± SEM cells/mm^3)			Statistics (p values)		
		BrdU 1w	BrdU 2w	BrdU 3w	BrdU 1w vs BrdU 2w	BrdU2w vs BrdU3w	BrdU 1w vs BrdU3w
SGZ	$BrdU^+$	0.09 ± 0.03	0.11 ± 0.04	0.08 ± 0.03	0.963	0.855	0.953
GCL	$BrdU^+$	0.01 ± 0.002	0.04 ± 0.01	0.03 ± 0.01	0.317	0.870	0.522
	$BrdU^+$ $NeuN^+$	0.01 ± 0.002	0.03 ± 0.01	0.02 ± 0.01	0.451	0.925	0.621

Table 4. Quantification of postnatal hippocampal neurogenesis in Japanese long-winged bats.

Brain regions	Staining	Cell densities (mean ± SEM x10^5 cells/mm^3)			Statistics (p values)		
		BrdU 1w	BrdU 2w	BrdU 3w	BrdU 1w vs BrdU 2w	BrdU2w vs BrdU3w	BrdU 1w vs BrdU3w
SEZ	$BrdU^+$	1.30 ± 0.84	2.03 ± 1.49	1.60 ± 1.12	0.901	0.964	0.982
GCL	$BrdU^+$	0.13 ± 0.07	0.34 ± 0.19	0.28 ± 0.06	0.495	0.951	0.660
	$BrdU^+$ $NeuN^+$	0.07 ± 0.02	0.23 ± 0.14	0.13 ± 0.02	0.443	0.726	0.863

Table 5. Quantification of postnatal bulbar neurogenesis in Formosan leaf-nosed bats.

Brain regions	Staining	Cell densities (mean ± SEM cells/mm^3)			Statistics (p values)		
		BrdU 1w	BrdU 2w	BrdU 3w	BrdU 1w vs BrdU 2w	BrdU2w vs BrdU3w	BrdU 1w vs BrdU3w
SGZ	$BrdU^+$	0.07 ± 0.05	0.03 ± 0.02	0.09 ± 0.01	0.666	0.480	0.938
GCL	$BrdU^+$	0.01 ± 0.002	0.01 ± 0.01	0.03 ± 0.03	0.999	0.583	0.611
	$BrdU^+$ $NeuN^+$	0.01 ± 0.002	0.01 ± 0.01	0.02 ± 0.02	0.997	0.643	0.682

Table 6. Quantification of postnatal hippocampal neurogenesis in Formosan leaf-nosed bats.

Bat species	Cell densities (mean ± SEM x10^6 cells/mm^3)			Statistics (p values)	
	dSVZ	vSVZ	SGZ	dSVZ vs vSVZ	SGZ vs vSVZ
Chestnut bats	1.20 ± 0.23	1.14 ± 0.11	0.14 ± 0.01	0.773	0.001
Japanese long-winged bats	0.94 ± 0.20	1.16 ± 0.15	0.43 ± 0.05	0.827	0.062
Formosan leaf-nosed bats	1.24 ± 0.12	1.71 ± 0.18	0.25 ± 0.03	0.082	0.000

Table 7. Quantification of NSCs in Chestnut bats, Japanese long-winged bats and Formosan leaf-nosed bats.

Bat species	Cell densities (mean ± SEM x10^5 cells/mm^3)				Statistics (p values)		
	RMS	dSVZ	vSVZ	SGZ	RMS vs vSVZ	dSVZ vs vSVZ	SGZ vs vSVZ
Chestnut bats	1.64 ± 0.21	1.84 ± 0.22	0.75 ± 0.12	0	0.060	0.028	-
Japanese long-winged bats	2.22 ± 1.17	0.42 ± 0.15	1.44 ± 0.54	0.16 ± 0.07	0.427	0.138	0.022
Formosan leaf-nosed bats	7.01 ± 0.86	2.68 ± 0.46	8.70 ± 0.74	0.58 ± 0.15	0.282	0.001	0.000

Table 8. Quantification of neuroblasts in Chestnut bats, Japanese long-winged bats and Formosan leaf-nosed bats.

Bat species	Cell densities (mean ± SEM x10^5 cells/mm^3)				Statistics (p values)			
	RMS	dSVZ	vSVZ	SGZ	RMS vs vSVZ	dSVZ vs vSVZ	SGZ vs vSVZ	
Chestnut bats	0.41 ± 0.05	1.43 ± 0.38	1.17 ± 0.34	0	0.322	0.844	-	
Japanese long-winged bats	2.12 ± 0.44	1.12 ± 0.18	3.32 ± 0.96	0.03 ± 0.01	0.912	0.581	0.006	
Formosan leaf-nosed bats	0.75 ± 0.19	1.27 ± 0.27	3.07 ± 0.35	0.11 ± 0.02	0.001	0.003	0.000	

Table 9. Quantification of proliferating cells in Chestnut bats, Japanese long-winged bats and Formosan leaf-nosed bats.

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