

The studies on neurogenesis induced by brain injury in adult ring dove

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ABSTRACT

It was the first time demonstrated by us that the number of newborn neurons was increased after making lesion in forebrain of adult ring dove (*Streptopelia risoria*) by means of autoradiography and immunohistochemistry. Neurogenesis in the adult avian is restricted to the telencephalon. In doves with bilateral electrolytic lesion of nucleus ectostriatum (E), the mean number of proliferating cells in the lateral ventricular zone (LVZ) and newborn neurons in the forebrain increased by 1.95 times and 2.38 times respectively as compared with that in intact doves. The most remarkable increase of neurogenesis induced by nucleus ectostriatum lesions was found at the anterior-posterior level 3 (L₃), where the lesion site was located. These results showed that the electrolytic brain lesion altered the distribution pattern of proliferating cells in the LVZ and resulted in increase of the number of newborn neurons in the non-VZ areas of forebrain. The changes in number and distribution pattern of proliferating cells in LVZ and newborn neurons in forebrain may be dependent on site of lesion. Studies on the relationship between proliferating cells in LVZ and newly generated neurons in non-VZ areas may help to understand the mechanism of brain plasticity and development.

Key words: *Autoradiography, neurogenesis, brain lesion, ring dove.*

INTRODUCTION

It is a common statement that in higher vertebrates the CNS completes neuronal

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development during the pre- and prenatal periods. Preliminary work showed that neurogenesis continued in the brain of songbird during adulthood. Many newborn neurons are incorporated into the vocal-control nucleus of telencephalon, replacing old neurons and making new synaptic connections with distant targets within the forebrain[1, 2]. It is suggested that the newborn neurons in the vocal-control nucleus may play a role in the plastic modification of neuronal circuits related to song learning. The newborn neurons originate only from LVZ and migrate along the fibers of radial glial cells to their terminal fields[3, 4]. Our recent studies demonstrated that neurogenesis persists throughout adult life in the ring dove[5, 6]. The ring dove is a non-songbird, and there is no well-defined area controlling song learning and memory in the telencephalon. It implies that neurogenesis during postnatal life may be an universal phenomenon in avian. Therefore, it is possible that newborn neurons may serve functions other than those associated with song learning. It is well known that brain injury can induce reactive gliogenesis and regeneration of neuronal fibers, and new synapses were formed to help functional recovery. However, there is no report so far that the number and distribution pattern of newborn neurons can be changed after forebrain lesion in the adult bird. In a recent study in ring dove, the author suggested that reactive neurogenesis could be induced after a nucleus in hypothalamus of adult ring dove was injured. The processes of cell proliferation, migration and their final location in specific brain areas were changed after brain lesion[7]. It would be very interesting to compare the effects of brain-injury in different brain regions on proliferation, migration and differentiation of precursor neurons and to clarify if the distribution pattern of neurogenesis is region-dependent. The purpose of this work is to study the relationship between distribution of proliferating cells in LVZ and location of newborn neurons in the telencephalon in intact and brain injury birds by using combined autoradiography and immunocytochemistry. This may provide clues to understand the presence of newborn neurons in the adult avian and plasticity of CNS in the adult vertebrate brain.

MATERIALS AND METHODS

Animal, surgery and treatments

Thirty ring doves of different ages (from 10 ~ 18 months old) were used in these experiments. All doves were bred and maintained in the indoor dove colony of the Institute of Animal Behavior, Rutgers University, USA, under a photoperiod of 12 h light: 12 h darkness (light on Am:0700 and off Pm:0700 EST). The birds were deeply anesthetized with chloropent (0.025 ml/kg, body weight) and placed in a stereotaxic instrument. The coordinates were determined by using the pigeon atlas[8] with adjustment. For all the lesion and sham birds, a teflon-insulated electrode with a diameter of 0.14 mm was pushed into the center of nucleus ectostriatum. A lesion was produced by passing 1.2 mA of anodal current, using a current meter (Grass CCU 1-A), through the electrode for 40s. The procedure for the sham lesion animals was the same except that no electrolytic current was given. The intramuscular injection of [³H]thymidine (24.79×10^{10} Bq/M, 14.8×10 Bq/g, body weight; England Nuclear), a marker for DNA synthesis and used as an indicator for cell proliferation was started one day after surgery for all the lesion doves. This study consists of two

major paradigms, the short-term and long-term experiment. In the short-term experiment (n=18), doves were sacrificed 1h after a single [³H]thymidine injection. The birds in this group were used to observe the distribution of proliferating cells in LVZ after brain lesion. In the long-term experiment (n=12), each bird received a daily [³H]thymidine injection for seven days. The birds were sacrificed 3 months after the last injection. The three-month survival time allows newborn neurons migrating to final location, differentiating and developing new connections. The birds in this group were used to examine the distribution pattern of newborn neurons after brain lesion.

Combined autoradiography and immunocytochemistry

The birds were killed under deep chloropent anesthesia. Brains were fixed by intracardial perfusion with 4 % paraformaldehyde in 0.1 M phosphate buffer and embedded in polyethylene glycol(PEG)[9]. Coronal sections (6 μm thick) were cut on a rotary microtome and one section from every 300 μm was selected for the following double-labeling steps. A cell labeled with [³H]thymidine was considered as a newly proliferated cell, but only the cell that labeled with [³H]thymidine and stained by one of neuronal specific antibodies can be identified as a newborn neuron. Three antibodies, anti-microtubule-associated protein-2 (MAP-2), anti-neuron specific nuclear protein (NeuN, from Dr. Mullen) and anti-Tau were used in these experiments for immunohistochemistry. The working solutions of MAP-2, Tau and NeuN were 1:250, 1:250 and 1:100, respectively. The working solutions for biotinylated anti-mouse IgG and ABC complex were 1:200 and 1:100. Antigen staining was performed using a standard ABC method. The sections were dehydrated, delipidized, coated with KODAK NTB2 nuclear track emulsion and exposed at 4 °C for 30 d. The autoradiographs were developed and counterstained with the fluorescent DNA dye, Hoechst 33258, or cresyl violet. To test the specificity of the primary antisera used in this study, replace control experiments were performed where primary antisera were replaced by normal mouse serum. No staining was seen in the later cases.

Data analysis

All sections were mapped at a magnification of 600X using a computer-microscope system[10]. The criterion for a labeled cell was that it had 7 or more exposed silver grains over the nucleus, this was 10 or more times above background. The number of labeled cells was counted, and the location of labeled cells was mapped and analyzed. The data were expressed as means ± S.D. and T-test was used to evaluate the differences between experiments.

RESULTS

Short-term experiments

The lateral ventricular zone is the only region where many proliferating cells were found. There was no labeled cell in the third, or fourth ventricular zone. In this short-term experiment, the newly proliferated cells could not complete DNA synthesis and migration within 1h after [³H]thymidine injection. All newly proliferated cells were not reactive to three specific antibodies (MAP-2, Tau and NeuN) and were morphologically immature. We found that electrolytic lesion of nucleus ectostriatum promoted cell proliferation, altered both the distribution pattern and the number of proliferating cells in the LVZ. Compared with the intact birds, the mean number of proliferating cells/mm in the LVZ increased 1.95 times 1h after [³H]thymidine injection. Fig 1 showed the distribution patterns of proliferating cells in the LVZ of the intact birds and the birds with electrolytic brain lesion. The anterior-posterior levels were numbered from 1(L¹) to 13(L¹³) in the series of hemisections with a lesion

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site in L₃. The concentration of proliferating cells varied along the anterior-posterior axis. Many more proliferating cells were distributed at L₃-L₉, reaching a peak of 13.23 cells/mm at L₃ where the lesion site was located. Fig 2b showed the labeled cells with [³H]thymidine grains in LVZ at L₃. Tab 1 showed the distribution density of proliferating cells at different brain levels of LVZ. In addition, many proliferated cells in LVZ were also observed at L₁-L₁₃ where the number of proliferating cells was very low in intact doves.

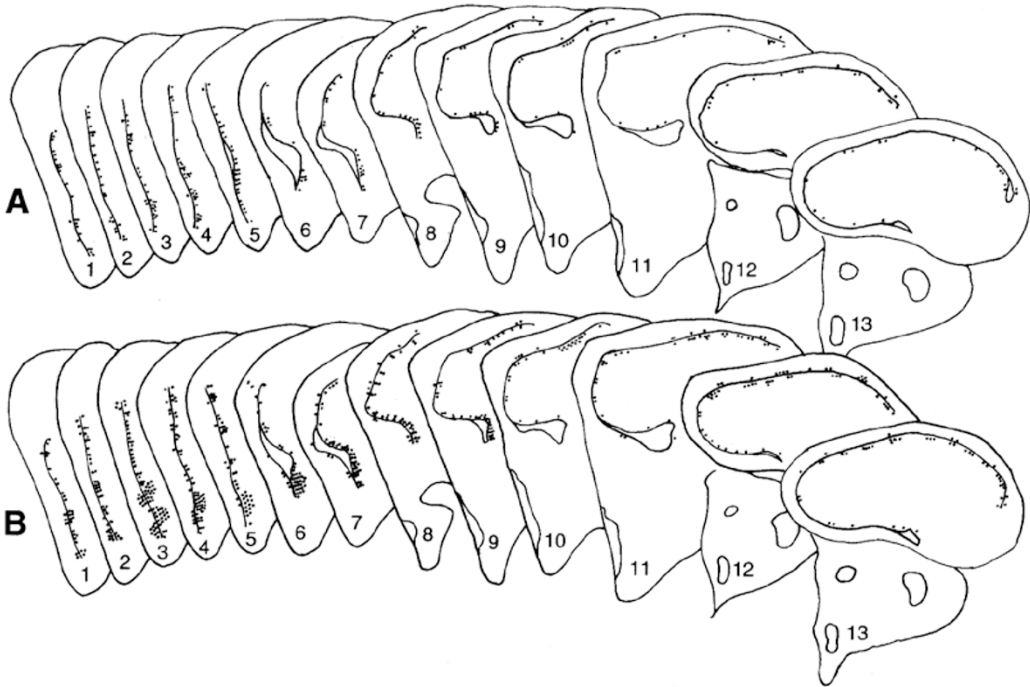


Fig 1. Distribution patterns of proliferating cells in LVZ (L₁ -L₁₃) of the intact birds and the birds with brain lesion. Each dot represents a labeled cell in LVZ 1h after receiving a single injection of [³H]thymidine. A: control group; B: lesion group.

Long-term experiments

Electrolytic brain lesion of nucleus ectostriatum induced significant increase in the number of newborn neurons in non-VZ areas, and newborn neurons were unevenly distributed throughout the forebrain (Tab 2). No newborn neuron was found out of telencephalon.

The newborn neuron in PEG sections stained with cresyl violet had a clear large nucleus without heterochromatin in it, so that the size was larger and the nucleus was paler than that of glial cell (Fig 2 c). The cells immunoreactive to neuron specific antibodies and labeled by [³H]thymidine were also considered as newborn

Tab 1. Differences in the densities of [³H] labeled cells in LVZ at different brain levels(L₁-L₁₃) between the intact birds and the birds with brain lesion(cell number/mm)

Brain level	Treatment		
	Control (n=6)	Lesion (n=8)	Sham (n=4)
L1	5.48 ± 0.42	8.12 ± 0.96**	5.92 ± 0.83
L2	5.29 ± 0.75	7.79 ± 0.70**	5.94 ± 0.18
L3	6.49 ± 0.741	3.23 ± 1.54**	6.57 ± 0.31
L4	5.95 ± 0.83	9.21 ± 1.10**	6.36 ± 0.78
L5	5.76 ± 0.29	9.64 ± 0.93**	5.81 ± 0.35
L6	2.91 ± 0.22	8.95 ± 0.84**	3.02 ± 0.15
L7	3.49 ± 0.42	8.18 ± 0.91**	3.33 ± 0.47
L8	2.97 ± 0.55	6.43 ± 0.78**	3.42 ± 0.49
L9	2.21 ± 0.57	8.37 ± 0.95**	2.79 ± 0.53
L10	1.73 ± 0.86	3.58 ± 0.44**	2.66 ± 0.57
L11	1.61 ± 0.26	3.00 ± 0.20*	1.85 ± 0.17
L12	1.59 ± 0.34	2.88 ± 0.53*	1.66 ± 0.28
L13	1.26 ± 0.09	1.90 ± 0.39	1.21 ± 0.09
total average	3.595	7.021	3.888

* P < 0.05; ** P < 0.01.

Tab 2. Differences in densities of newborn neurons in non-VZ areas at different brain levels (L₁-L₁₃) between the intact birds and the birds with brain lesion(cell number/mm²)

Brain Level	Group	Type of section			
		Cresyl	NeuN	MAP-2	Tau
L ₃	Control	1.15 ± 0.27	1.05 ± 0.10	0.94 ± 0.11	0.96 ± 0.17
	Lesion	5.20 ± 0.39**	6.18 ± 0.94**	5.84 ± 1.00*	5.16 ± 0.73**
L ₅	Control	1.16 ± 0.06	1.19 ± 0.27**	1.19 ± 0.26	1.23 ± 0.23
	Lesion	4.07 ± 0.30**	4.75 ± 0.41**	3.92 ± 0.53**	4.21 ± 0.64**
L ₇	Control	1.53 ± 0.15	1.45 ± 0.14	1.52 ± 0.19	1.59 ± 0.11
	Lesion	3.49 ± 0.53**	3.77 ± 0.20**	3.29 ± 0.58**	3.14 ± 0.32**
L ₉	Control	1.52 ± 0.48**	1.66 ± 0.30	1.41 ± 0.33	1.48 ± 0.26
	Lesion	4.40 ± 0.53**	4.75 ± 0.51**	4.41 ± 0.83**	4.91 ± 0.63**
L ₁₁	Control	2.45 ± 0.69	2.01 ± 0.04	1.40 ± 0.31	1.25 ± 0.12
	Lesion	3.93 ± 0.42**	4.80 ± 0.36**	4.54 ± 0.54**	4.46 ± 0.50**
L ₁₃	Control	3.33 ± 0.83	2.36 ± 0.26	2.27 ± 0.43	2.83 ± 0.49
	Lesion	5.48 ± 0.85**	6.12 ± 0.57**	6.07 ± 0.54**	5.82 ± 0.49**
In average	Control	1.856	1.620	1.455	1.557
	lesion	4.420	5.060	4.678	4.617

* P < 0.05; ** P < 0.01

Cresyl: the cells stained with cresyl violet; NeuN, MAP-2, Tau: cells labeled with [³H]thymidine were also immunoreactive to one of the neuron specific antibodies.

neurons (Fig 2d, e, f). Compared with intact birds, the distribution pattern of newborn neurons in non-VZ area was changed after lesioning of the nucleus ectostriatum. The number of newborn neurons increased significantly throughout the entire telencephalon. The most remarkable increase of newborn neurons in non-VZ

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area were found at brain level L_3 , where the number of newborn neurons was 4.52 times as high as that of intact birds. In addition, many newborn neurons were also distributed in the medial to caudal neostriatum at brain level L_{13} where a few newborn neurons were found in intact birds.

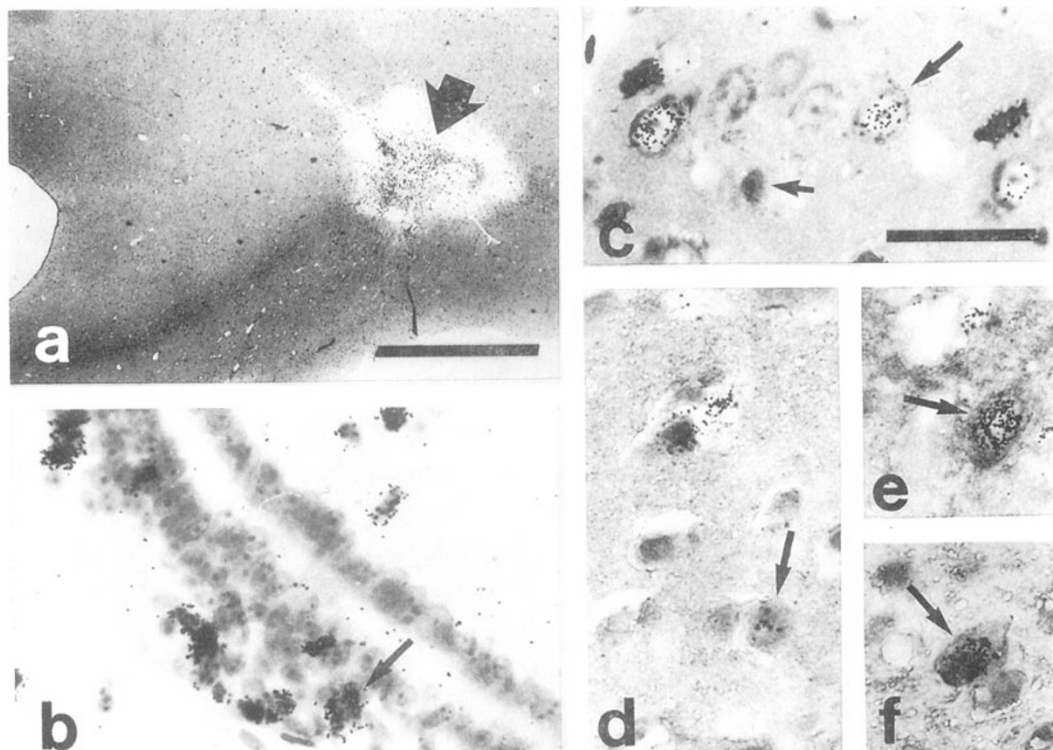


Fig 2. Photomicrographs of coronal sections depicting the [^3H]-labeled cells in the LVZ and newborn neurons in the non-VZ areas. a: Arrow showing the lesion site in nucleus ectosriatum. b: Cells labeled in LVZ. c: Cresyl-staining section showing the neurons (long arrow) and glia cells (short arrow). d-f: sections showing the double-labeled neurons (arrows) by [^3H] thymidine and immunohistochemical staining of Tau (d), MAP-2 (e) and NeuN (f). Bar=30 μm .

DISCUSSION

Our previous work demonstrated that electrolytic lesion of the nucleus ventromedial hypothalamus (VMN), a nucleus in hypothalamus of ring dove, promoted neurogenesis[7]. The present results extend this finding to the region of telencephalon in the ring doves, indicating that forebrain lesion not only induces the increase of newborn neurons but also alters the distribution pattern of both proliferating cells in LVZ and newborn neurons in non-VZ areas. The results in this experiment also

showed that sham lesion did not have this effect.

Although the lesion of both VMN and nucleus ectostriatum resulted in a large number of labeled proliferating cells and newborn neurons in the telencephalon, the distribution pattern resulted by lesion of different brain regions were significantly different. In short-term experiments, for example, the highest density of proliferating cells in LVZ after the lesion of nucleus ectostriatum was found in the anterior pole of telencephalon at level L_3 where lesion site was located. In the cases of VMN lesion, however, although the highest density of proliferating cells was also found in the same region of brain at level L_3 , the most remarkable increase of proliferating cells was found in neostriatum caudale (NC) at level L_{12} - L_{13} , where the proliferating cells were respectively 2.93 and 3.56 times more compared with that of intact birds. In intact birds, the neostriatum caudale is one of the regions where very few proliferating cells in LVZ are located.

In long-term experiments, the effects of lesions of the nucleus ectostriatum appear to be stronger than that in the birds received VMN lesion. Compared with the lesion of VMN, several areas in the telencephalon after lesions in the nucleus ectostriatum had particularly high density of labeled neurons, including regions such as lobus parolfactorius (LPO) at level L_3 - L_4 , hyperstriatum ventrale, and neostriatum caudale at level L_{11} - L_{13} , but most remarkable increase of newborn neurons was in the region of LPO at level L_3 where there were very few newborn neurons in intact birds. In the cases of the birds having VMN lesion, the density of labeled neurons increased gradually from anterior to posterior telencephalon, reaching a peak in the neostriatum caudale at level L_{13} , where the density was 2.30 times higher than that at level L_3 . In all our experiments, no matter where the lesion site was located, the vast majority of newborn neurons was found in the region adjacent to the lesion site. The present data suggests that the effects of brain lesions which altered the distribution pattern of neurogenesis may be region-dependent.

Previous evidence showed that the commitment of most newborn neurons to their final location occurs in the ventricular zone of higher vertebrate during the embryonic stage[11]. In songbird, newborn neurons originate from LVZ and then migrate and invade most regions of the telencephalon during postnatal life[1, 12]. The patterns of migration and location of newborn neurons in non-songbird are similar to that in songbird[5]. The results of our previous experiments also suggest that in ring dove newborn neurons may originate from LVZ and migrate to different brain regions during postnatal life. In the present experiments, we found that the distribution patterns of labeled proliferating cells both in LVZ and in non-VZ areas were significantly changed after brain lesion. In normal adult songbird, the highest concentration of proliferating cells was found in ventral portion of the brain at level L_3 , a region with high rates of cell division and was termed as "proliferative hot spots". Precursors of newborn neurons may originate from this region in adult songbird as some authors suggested[12]. A very interesting finding of this study is that after brain lesion the highest density of both proliferating cells in LVZ and

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newborn neurons in non-VZ area occurred at the same level of L₃, i.e. the "hot spots" in the song bird. However, little is known about the relationship between the site of origin and the final destination of newborn neurons, and how the newborn neurons changed their migrating pathways and terminated in specific brain regions after brain lesions. The present data from our experiments of lesioning both VMN and nucleus ectostriatum showed that there were parallel changes between cell proliferation in the LVZ and neurogenesis in the telencephalon. These parallel changes may support indirectly that newborn neurons originate from LVZ and migrate to distant locations in the forebrain[13]. The newborn neurons occurred during adult life could be of particular interest for further research not only in histogenesis but also in neural plasticity.

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REFERENCES

- [1] Goldman SA, Nottebohm F. Neuronal production, migration, and differentiation in a vocal control nucleus of the adult female canary brain. *Proc Natl Acad Sci* 1983;**80**:2390-4.
- [2] Paton JA, Nottebohm F. Neurons generated in the adult brain are recruited into functional circuits. *Science* 1984; **225**:1046-8.
- [3] Alvarez-Bulla A, Nottebohm F. Migration of young neurons in adult avian brain. *Nature* 1988; **335**:353-4.
- [4] Nordeen KW, Nordeen EJ. Projection neurons within a vocal motor pathway are born during song learning in zebra finches. *Nature* 1988;**334**:149-51.
- [5] Zuo MX. The newborn neurons in the brain of adult ring dove. *Science Bulletin China* 1995; **40**:1133-6.
- [6] Ling CY, Zuo MX, Alvarez-Buylla A, Cheng MF. Neurogenesis in Juvenile and adult ring doves. *J Comp Neurol* 1997; **379**:300-12.
- [7] Zuo MX. The effects on neurogenesis induced by brain injured of nucleus ventromedialis hypothalami in the brain of adult ring dove. *Acta Physiologica Sinica*, 1996; **48**:209-14.
- [8] Karten HJ, Hodos W. A Stereotaxic atlas of the brain of the pigeon (*Columba livia*). The John Hopkins Press: Baltimore Maryland 1967: 31-256.
- [9] Zuo MX. Application and technique of embedding animal tissue with polyethylene glycol. *J BJ Nor Univ (Nat Sci)* 1996; **32**:271-3.
- [10] Alvarez-Bulla A, Vicario DS. Simple microcomputer system for mapping tissue sections with the light microscope. *J Neurosci Meth* 1988; **25**:165-73.
- [11] Rakic P. Specification of cerebral cortical areas. *Science* 1988; **240**:170-6.
- [12] Alvarez-Buylla A, Theelen M, Nottebohm F. Proliferation "hot spots" in adult avian ventricular zone reveal radial glia cell divisions. *Neuron* 1990; **5**:101-9.
- [13] Kirn JR, Nottebohm F. Direct evidence for loss and replacement of projection neurons in adult canary brain. *J Neurosci* 1993; **13**:1654-63.

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