Chem. Senses 30 (suppl 1): i113–i114, 2005 doi:10.1093/chemse/bjh140

Adult Neurogenesis and Gliogenesis in the Rat Olfactory Nervous System

Nanae Fukushima, Takashi Kato, Zhiyou Li, Kumiko Yokouchi and Tetsuji Moriizumi

Department of Anatomy, Shinshu University School of Medicine, Matsumoto 390-8621, Japan

Correspondence to be sent to: Tetsuji Moriizumi, e-mail: moriizum@sch.md.shinshu-u.ac.jp

Key words: cell fate, granule cells, olfactory bulb, periglomerular cells, rostral migratory stream

Introduction

In the olfactory nervous system, it is well established that neural stem cells continually proliferate in the periventricular region, migrate along the rostral migratory stream (RMS) and differentiate into neurons of the glomerular (OB-GL) and granule cell (OB-GCL) layers of the olfactory bulb (OB). In this paper, we will answer the following questions regarding adult neurogenesis of the olfactory nervous system. (i) What is the fate of newly generated bulbar neurons? (ii) Does neuronal regeneration occur after neuronal cell death of the forebrain regions in and around the RMS? (iii) Does neurogenesis occur within the OB independently of migrating neural stem cells? If intrabulbar neurogenesis occurs, are there some differences in neurogenesis and gliogenesis by the neural stem cells of different origins?

The fate of newly formed bulbar neurons in adult rats

The study was undertaken to discover whether newly formed bulbar neurons are added as a new population or replaced in the adult OB (Kato *et al.*, 2001). Since calretinin(+) cells are the main neuronal populations of the OB-GL and OB-GCL, we used calretinin as a neuronal marker. Adult rats (Wistar, female, 9 weeks old) were given systemic multiple injections of [^{3}H]thymidine in order to label proliferating stem cells, and were allowed to survive for 24 h, 2 weeks and 8 weeks. [^{3}H]thymidine(+)/calretinin(+) cells were counted in the OB-GL and OB-GCL, and area measurements were done in these bulbar layers. Then the number per unit area (mm^2) was calculated in each layer and regarded as the cell density.

The cell densities of [^{3}H]thymidine(+)/calretinin(+) cells were 0.3 ± 0.0 in the OB-GL and 0.1 ± 0.0 in the OB-GCL at 1 day survival. At 2 weeks survival, those cells were greatly increased in number, and the cell densities were 5.2 ± 0.4 (OB-GL) and 10.1 ± 0.5 (OB-GCL). At 8 weeks survival, the cell densities remarkably decreased both in the OB-GL (1.2 ± 0.1) and OB-GCL (3.5 ± 0.3). Regarding the cell densities at 2 weeks survival as 100%, those cells showed progressive declines to 23.1% in the OB-GL and 34.7% in the OB-GCL.

We have revealed that three-quarters (76.9%) of bulbar calretinin(+) periglomerular cells and two-thirds (65.3%) of bulbar calretinin(+) granule cells undergo neuronal cell death during the short survival time, indicating that neuronal replacement is a common phenomenon in the OB with active neurogenesis. It is interesting that the olfactory nervous system, where neurogenesis occurs throughout life, is made up of neuronal networks by continually recruited cells (olfactory receptor, bulbar periglomerular and granule cells) (Kato *et al.*, 2000).

The cell dynamics of calretinin(+) neurons following ibotenate-induced lesions in adult rat forebrains

The RMS contains different stages of cells (nestin, polysialylated-NCAM and calretinin), suggesting that neurogenesis is ongoing within the RMS. The study was aimed to examine whether calretinin(+) cells in the RMS might change dynamically following lesions by an excitotoxin (Li *et al.*, 2002). To make lesions on the RMS and its adjacent forebrains, adult rats (Wistar, male, 11–13 weeks old) were injected with ibotenate into the rostral and caudal forebrains. They were allowed to survive for 2, 5, 10 and 14 days after the injections. Non-lesioned forebrains were used as control. For sham-lesioned control, the rats received vehicle (isotonic saline) injections. The cell density, the number of calretinin(+) cells per unit area (mm^2), was calculated in the RMS from the control, saline-injected and ibotenate-injected animals.

The RMS is surrounded by several forebrain structures, such as the striatum, accumbens nucleus (Acb) and anterior olfactory nucleus (AO). Neuronal cell loss by ibotenate occurred in the Acb, AO and ventral part of the striatum. The cell densities of the RMS calretinin(+) cells were 163.4 ± 2.9 cells/mm^2 in the control group and 173.3 ± 7.6 cells/mm^2 in the saline-injected group. The cell density greatly decreased to 42.0 ± 5.8 cells/mm^2 at 2 days survival. In the forebrains adjacent to the RMS, calretinin(+) cells in the Acb and AO were severely damaged. At 5 days survival, the cell density (52.1 ± 7.5 cells/mm^2) did not change in the RMS. At 10 and 14 days survival, calretinin(+) cells in the RMS markedly increased in number, and the cell densities increased to 215.9 ± 5.2 cells/mm^2 (10 days) and 261.5 ± 16.9 cells/mm^2 (14 days). Although calretinin(+) cells were found not only in the RMS but outside the RMS, these calretinin(+) cells were mostly localized in the border areas between the RMS and its adjacent forebrain nuclei, indicating that neuronal regeneration does not occur far in the forebrain areas. At this stage, extensive neuronal cell loss and glial cell proliferation were evident in these nuclei.

In the RMS, it appears that calretinin(+) cells are the only neural components that are sensitive to ibotenate. Regarding the cell density of the RMS calretinin(+) cells of the control group as 100%, the cell densities actually decreased to 25.7% (2 days) and 31.9% (5 days). Thereafter, calretinin(+) cells repopulated in the RMS at 10–14 days survival and increased beyond the control value (10 days: 132.1%; 14 days: 160.0%). Interestingly, 61.8% of the calretinin(+) cells in the RMS still express polysialic acid, an essential molecule for the migration of the neuronal precursor cells via the RMS (Kato *et al.*, 1999). However, the repopulated calretinin(+) cells were preferentially restricted in and around the RMS without those cells deep into the surrounding forebrain nuclei. The abnormally increased glial components might have prevented the radial migration of calretinin(+) cells into the forebrain nuclei.

The differential neurogenesis and gliogenesis by local and migrating neural stem cells in OBs of adult rats

The RMS is a unique forebrain structure that provides a long-distance migratory route for the neural stem cells of the periventricular region towards the OB. The study was undertaken to demonstrate neurogenesis by the local neural stem cells of the intrabulbar origin, and to examine the degrees of neurogenesis and gliogenesis by

the neural stem cells of the different origins (periventricular vs intrabulbar) (Fukushima *et al.*, 2002). After making surgical lesions on the RMS, the adult rats (Wistar, male, 12–14 weeks old) received systemic multiple injections of 5-bromodeoxyuridine (BrdU) and were allowed to survive for 2 weeks. Neuronal and glial differentiation of the BrdU(+) cells in the OB-GCL and OB-GL were examined immunohistochemically using antibodies to neuronal (NeuN, neuronal nuclei) and glial (GFAP, glial fibrillary acidic protein) markers in the RMS-lesioned and unlesioned, control OBs.

Immunohistochemistry for BrdU revealed remarkable differences in the cell densities of the BrdU(+) cells in the OB. On the control side, there were a large number of BrdU(+) cells in the OB-GCL (225.0/mm^2) with a few BrdU(+) cells in the OB-GL (10.5/mm^2). Contrarily, the BrdU(+) cells were remarkably small in number in the OB-GCL (8.1/mm^2) and OB-GL (6.7/mm^2) on the RMS-lesioned side.

Double immunohistochemistry for NeuN and BrdU showed great differences in the ratios of expression of NeuN among the BrdU(+) cells between the control and RMS-lesioned OBs. On the control side, 36.7% of the BrdU(+) cells were NeuN(+) in the OB-GCL and 8.8% of them were NeuN(+) in the OB-GL. On the RMS-lesioned side, 3.7% of the BrdU(+) cells were NeuN(+) in the OB-GCL and 0.6% of them were NeuN(+) in the OB-GL. Double immunohistochemistry for GFAP and BrdU did not show major differences in the ratios of expression of GFAP among the BrdU(+) cells between the control and RMS-lesioned OBs. On the control side, 22.5% of the BrdU(+) cells were GFAP(+) in the OB-GCL and 32.2% of them were GFAP(+) in the OB-GL. On the RMS-lesioned side, 37.1% of the BrdU(+) cells were GFAP(+) in the OB-GCL and 44.9% of them were GFAP(+) in the OB-GL.

Complete inhibition of migration of the periventricular neural stem cells was evident by the finding that the BrdU(+) cells of the RMS-lesioned OB remarkably reduced to ~1/28 of those cells of the control OB in terms of the cell densities of the BrdU(+) cells in the OB-GCL. We showed the presence of the BrdU(+) NeuN(+) cells in the RMS-lesioned OB, suggesting neurogenesis by the pre-existing, intrabulbar neural stem cells. However, we cannot exclude the possibility that neural stem cells in the core of the OB, which were of the periventricular origin and existed at the site distal to the RMS lesion, might have participated in intrabulbar neurogenesis in the strict sense.

There were notable differences in the degrees of neurogenesis between the RMS-lesioned and control OBs. Considering the scantiness of the BrdU(+) cells in the RMS-lesioned OB, it is reasonable to think that newly generated neurons in the control OB were mostly derived from the migrating neural stem cells of the periventricular origin. The ratios of neurogenesis by the migrating neural stem cells were ~10 times higher than those by the local neural stem cells both in the OB-GCL and OB-GL. This suggests that neuronal differentiation of the migrating neural stem cells can be accelerated during migration.

There has been no report on glial differentiation of migrating neural stem cells in the adult olfactory nervous system *in vivo*. Gritti *et al.* (2002) revealed not only neuronal, but also astrocytic and oligodendrocytic differentiation of the neural stem cells of the mouse olfactory system *in vitro*. Our *in vivo* studies indicate that astrocytic differentiation is continually taking place in the OB at the adult stage. Furthermore, the rates of gliogenesis did not differ significantly between the RMS-lesioned and control OBs (22.5–44.9%), indicating that the local and migrating neural stem cells are both actively engaged in gliogenesis in the OB.

References

Fukushima, N., Yokouchi, K., Kawagishi, K. and **Moriizumi, T.** (2002) *Differential neurogenesis and gliogenesis by local and migrating neural stem cells in the olfactory bulb.* Neurosci. Res., 44, 467–473.

Gritti, A., Bonfanti, L., Doetsch, F., Caille, I., Alvarez-Buylla, A., Lim, D.A., Galli, R., Garcia-Verdugo, J.M., Herrera, D.G. and **Vescovi, A.L.** (2002) *Multipotent neural stem cells reside into the rostral extension and olfactory bulb of adult rodents.* J. Neurosci., 22, 437–445.

Kato, T., Yokouchi, K., Li, Z., Fukushima, N., Kawagishi, K. and **Moriizumi, T.** (1999) *Calretinin-immunoreactive neurons in rostral migratory stream: neuronal differentiation.* Neuroreport, 10, 2769–2772.

Kato, T., Yokouchi, K., Kawagishi, K., Fukushima, N., Miwa, T. and **Moriizumi, T.** (2000) *Fate of newly formed periglomerular cells in the olfactory bulb.* Acta Otolaryngol., 120, 876–879.

Kato, T., Yokouchi, K., Fukushima, N., Kawagishi, K., Li, Z. and **Moriizumi, T.** (2001) *Continual replacement of newly-generated olfactory neurons in adult rats.* Neurosci. Lett., 307, 17–20.

Li, Z., Kato, T., Kawagishi, K., Fukushima, N., Yokouchi, K. and **Moriizumi, T.** (2002) *Cell dynamics of calretinin-immunoreactive neurons in the rostral migratory stream after ibotenate-induced lesions in the forebrain.* Neurosci. Res., 42, 123–132.