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INVESTIGATION INTO THE NEUROGENESIS IN DENTATE GYRUS AND OLFACTORY BULB OF THE ADULT BACTRIAN CAMEL

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ABSTRACT

The neurogenesis was explored in the adult bactrian camel using immunohistochemistry approach. The numerous cells were found in the subgranular zone of the dentate gyrus and the olfactory bulb by antibody against Doublecortin (DCX), which demonstrates that the neurogenesis also occurs in the adult bactrian camel.

Key words: Bactrian camel, dentate gyrus, neural stem cell, neurogenesis, olfactory bulb

In the central nervous system of adult mammals, neuroblasts are mainly generated in two specific regions, the dentate gyrus and the subventricular zone of the lateral ventricles (Liu *et al,* 2008). The newborn neurons generated in subventricular zone migrate *via* rostro-migratory stream to olfactory bulb, where they differentiate into interneurons. The newborn cells in the adult dentate gyrus are generated in subgranular zone and differentiate into neurons and glial cells in the granular layer. Adult neurogenesis has been mainly investigated in several mammalian species, such as rat (Altman and Das, 1965a, b, 1966; Kaplan and Hinds, 1977; Gould *et al,* 1992), rabbit (Guéneau *et al,* 1982), cat (Wyss and Sripanidkulchai, 1985), mouse (Kempermann *et al,* 1997a), non- human primates (Gould *et al,* 1999a b; Kornack and Rakic, 1999) and also in the human brain (Eriksson *et al,* 1998; Maækowiak *et al,* 2004).

Doublecortin is a microtubule-associated protein which is expressed predominantly in migrating neurons within the developing mammalian brain (Francis *et al,* 1999; Gleeson *et al,* 1999; Capes-Davis *et al,* 2005) and is frequently used as a marker of newly generated neurons (Hwang *et al,* 2007; Zhao *et al,* 2007). Neuronal nuclear antigen (NeuN) antibody recognises a neuron-specific nuclear protein in vertebrates (Mullen, 1992; Wolf *et al,* 1996; Sarnat *et al,* 1998). It is regarded as a universal marker for the postmitotic neuronal population and is used to distinguish neurons from glia because of the specificity of its expression (Kumar and Buckmaster, 2007).

However, the data of neurogenic studies in bactrian camels are very poor. In the present study, newborn neurons were identified in the dentate gyrus and olfactory bulb of the adult bactrian camel by means of immunohistochemistry staining with DCX and NeuN-antibody. The present study demonstrates the adult neurogenesis in the bactrian camel.

Materials and Methods

Tissue preparation

Two healthy and adult Alashan bactrian camel's heads were collected from the local slaughterhouse in the Inner Mongolia, China. The right hippocampus with dentate gyrus and the olfactory bulb were separated from the brain and fixed in 4% formaldehyde (pH 7.4).

Immunohistochemistry

Coronal sections of 50-µm fixed dentate gyrus and the ventromedial part of the olfactory bulb were cut with a vibratome. The sections were incubated in the first antibody, goat anti-DCX ((1:1,000, Santa Cruz, Heidelberg, Germany) containing 3%BSA, 0.3%Triton and 0.1% NaN_3 for 24h at 4ºC. After being washed in 0.1M PB, the sections were incubated in the secondary antibody (donkey anti-goat Alexa 488; dilution 1:300, Molecular Probes) containing 3%BSA, 0.1% NaN_3, 0.1M PB. The sections were then incubated in mouse anti-NeuN (1:1000, Chemicon, Hofheim, Germany)

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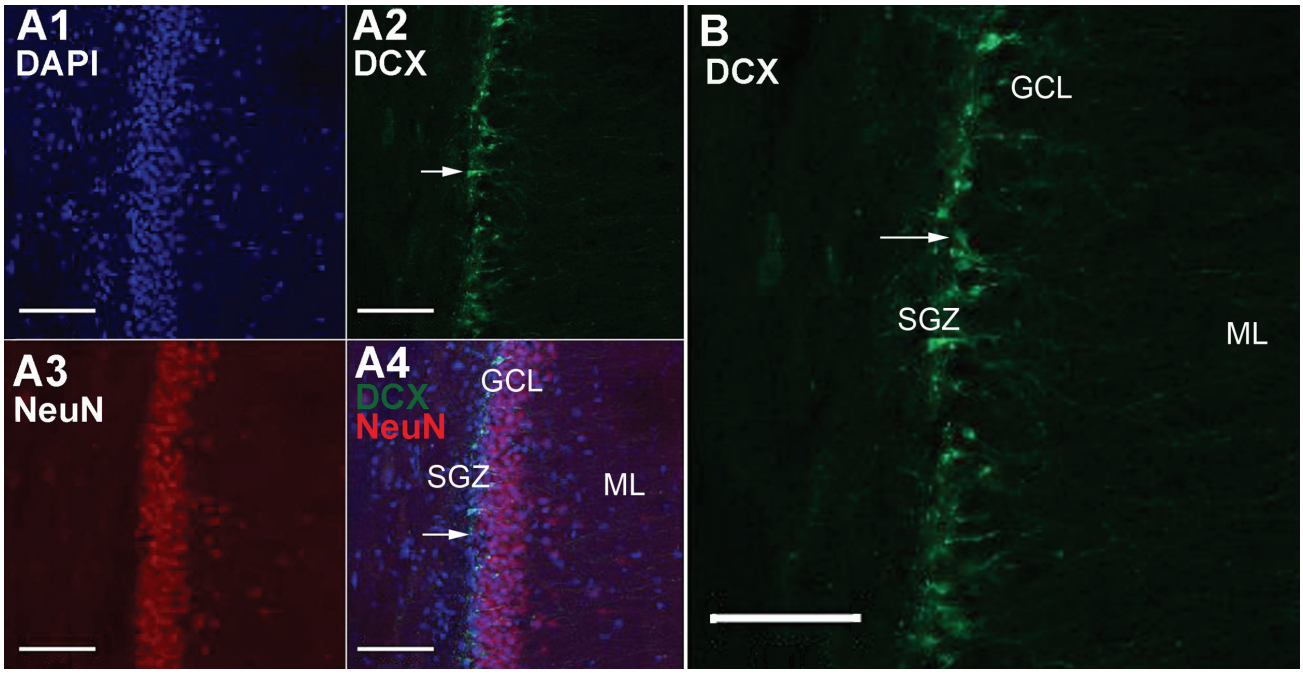

Fig 1. Generation of neurons in the adult bactrian camel's dentate gyrus (DG). Double immunofluorescence staining for DCX (green in A2, B), NeuN (red in A3,) and merged images (A4) revealed the presence of newly generated neurons in subgranular zone (white arrows) and matured cells (red) in granule cell layer . sgz, subgranular zone; gcl, granule cell layer, ml molecular layer. Scale bars = 100µm in A1, A2, A3, A4 and B.

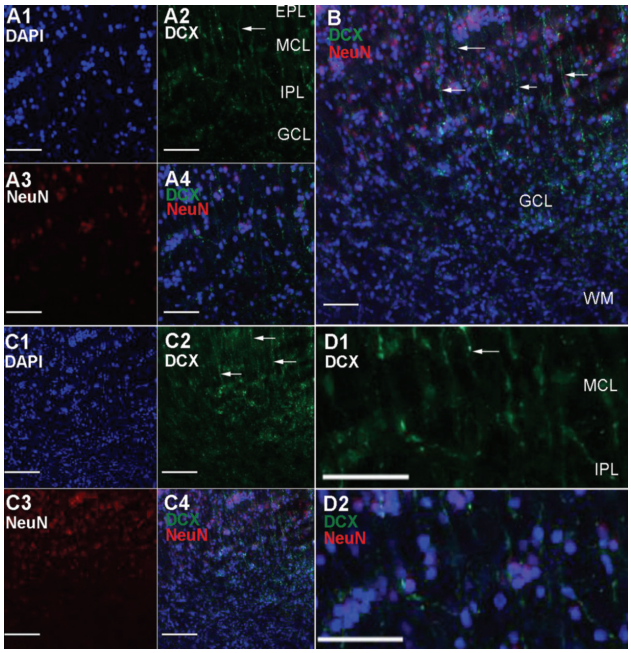

Fig 2. Generation of neurons in adult bactrian camel's olfactory bulb (OB). DCX-positive cells (green) indicate the newly generated neurons (A2, C2 and D1) with the moniliform neurites (white arrows in A2, B, C2 and D1). The merged images (A4, B, C4 and D2) revealed the topography of newborn and mature neurons. EPL, external plexiform layer; MCL, mitral cell layer; IPL, internal plexiform layer; GCL, granule cell layer; WM, white matter. Scale bars = 100µm in C1, C2, C3 and C4; 50µm in A1, A2, A3, A4, B, D1 and D2.

containing 3%BSA, 0.3%Triton and 0.1% NaN_3 for 24h at 4°C. After being washed in 0.1M PB, the sections were incubated in the secondary antibody (goat anti-mouse, Alexa 568; dilution 1: 300, Molecular Probes, Göttingen, Germany) containing 3%BSA, 0.1% NaN_3, 0.1M PB and DAPI overnight at 4°C. After rinsing in 0.1M PB for 2h, they were mounted in Moviol.

Imaging and Quantification

The sections double-labeled with NeuN and DCX were observed and analysed using a confocal scanning laser microscope (Zeiss LSM 510) to study morphologically and quantify the fate of newly generated cells.

Results

Three concentric layers were discernible in the coronal section of the dentate gyrus. These were the inner polymorphic granule and outermost molecular layers. DCX-positive cells were found exclusively near the border of the subgranular zone (Fig 1, A4 and B). Their dendrites directed from the subgranular zone to the granule and molecular layers. The NeuN+cells were found distributing throughout the granule cell layer, but none of them were found in the subgranular zone. It revealed that newborn neurons are generated near the border of the subgranular zone. They undergo maturation during their migration to the granule cell layer.

In contrast to the dentate gyrus, the olfactory bulb presents a cellular architectonic with 6 layers. Outwards from the core, in which a ventricle exists, the layers can be distinguished as the granule cell, internal plexiform, mitral cell, external plexiform, glomerular and nerve fibre layers. The part located ventromedially to the ventricle was sectioned for the immunohistochemistry assay. DCX positive cells mainly gathered in the granule layer and the DCX positive dendrites extended through all the layers (Fig 2, A2, C2 and D1). These dendrites were moniliform and own complex spines (Fig 2, A2, B, C2 and D). The double labeling with DCX and NeuN showed that the granule layer accumulated only newly generated and immature neuronal cells.

Discussion

Newborn neurons were continuously found in the subgranular zone of the hippocampus throughout adulthood (Altman and Das, 1965b; Cameron and Mckay, 2001; Doetsch and Hen, 2005; Ming and Song, 2005; Song *et al,* 2005; Aimone *et al,* 2006; Overstreet-Wadiche and Westbrook, 2006; Kee *et al,* 2007). They express a series of transient markers such as doublecortin, which are found in immature neurons during development (Doetsch and Hen, 2005). These newborn neurons could have developed from the neuron-specific progenitors present therein. Furthermore, they could also have originated from neural stem cells that exist in the ventricular supendyma around the hippocampus (Maækowiak *et al,* 2004). Neural progenitor cells generated from these stem cells migrate into the dentate gyrus (Seaberg and van der Kooy, 2003; Maækowiak *et al,* 2004). The present research showed the existence of immature neurons in the dentate gyrus of the bactrian camel. This indicates that adult bactrian camel's dentate gyrus is a rich source of newborn neurons. The newborn neurons can integrate into the existing circuitry of the hippocampus as evidenced by the development of functional synaptic inputs provided by the medial perforative path and growth of axons to target cells in CA3 (Hastings and Gould, 1999; Van Praag *et al,* 2002; Jessberger and Kempermann, 2003; Doetsch and Hen, 2005; Esposito *et al,* 2005; Ge *et al,* 2006; Tashiro *et al,* 2006; Zhao *et al,* 2006; Kee *et al,* 2007). Kee *et al* (2007) documented that new neurons make a unique contribution to memory processing in the dentate gyrus.

Similar to the dentate gyrus, new neurons were also found within the olfactory bulb throughout adult life (Altman, 1969; Doetsch and Hen, 2005). They originated in the subventricular zone and emigrate into both the granule cell and glomerular layers, where they differentiated into interneurons (Altman, 1969; Lois and Álvarez-Buylla, 1994; Carleton *et al,* 2003; Doetsch and Hen, 2005; Valero *et al,* 2007). These newly generated interneurons establish synaptic connections predominantly with mitral cells, the main projection neurons of the olfactory bulb (Petreanu and Álvarez-Buylla, 2002; Carleton *et al,* 2003; Valero *et al,* 2007). These neurons play a critical role in the modulation of the olfactory signal by modifying MC activity (Mori *et al,* 1998; Mori *et al,* 1999; Carleton *et al,* 2002; Lledo *et al,* 2004). The observation in the present study that numerous DCX-positive cells exist in the olfactory bulb revealed that this neural tissue is rich in newborn neurons. The incorporation of these new neurons into the olfactory bulb circuitry has been found to be essential for the maintenance of the structure and function of the olfactory bulb (Gheusi *et al,* 2000; Rochefort *et al,* 2002; Zheng *et al,* 2004; Valero *et al,* 2007).

The neurogenesis in adult dentate gyrus and olfactory bulb, demonstrate that the bactrian camel brain retains a potential for self-renewal throughout life. Based on the observation, that the environmental stimulation can influence the adult neurogenesis in rodent dentate gyrus (Kempermann *et al,* 1997a, b; Eriksson *et al,* 1998), it has been demonstrated that the adult neurogenesis might be a result of arid and semi-arid condition and could provide a prerequisite for surviving of camels in these hard conditions.

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