Developmental Biology 332 (2009) 309–324

Contents lists available at ScienceDirect

Developmental Biology

journal homepage: www.elsevier.com/developmentalbiology

Four distinct phases of basket/stellate cell migration after entering their final destination (the molecular layer) in the developing cerebellum

D. Bryant Cameron [a], Kazue Kasai [b], Yulan Jiang [a], Taofang Hu [a], Yoshinaga Saeki [b], Hitoshi Komuro [a,*]

[a] Department of Neurosciences/NC30, Lerner Research Institute, The Cleveland Clinic Foundation, 9500 Euclid Avenue, Cleveland, OH 44195, USA
[b] Dardinger Laboratory for Neuro-oncology and Neurosciences, Department of Neurological Surgery and Comprehensive Cancer Center, The Ohio State University, Columbus, Ohio, 43210, USA

ARTICLE INFO

Article history:
Received for publication 21 January 2009
Revised 28 May 2009
Accepted 29 May 2009
Available online 3 June 2009

Keywords:
Stellate cell
Basket cell
Inhibitory interneurons
Neuronal cell migration
Cerebellum
Early postnatal mice
Time-lapse imaging
Brain slices

ABSTRACT

In the adult cerebellum, basket/stellate cells are scattered throughout the ML, but little is known about the process underlying the cell dispersion. To determine the allocation of stellate/basket cells within the ML, we examined their migration in the early postnatal mouse cerebellum. We found that after entering the ML, basket/stellate cells sequentially exhibit four distinct phases of migration. First, the cells migrated radially from the bottom to the top while exhibiting saltatory movement with a single leading process (Phase I). Second, the cells turned at the top and migrated tangentially in a rostro-caudal direction, with an occasional reversal of the direction of migration (Phase II). Third, the cells turned and migrated radially within the ML at a significantly reduced speed while repeatedly extending and withdrawing the leading processes (Phase III). Fourth, the cells turned at the middle and migrated tangentially at their slowest speed, while extending several dendrite-like processes after having completely withdrawn the leading process (Phase IV). Finally, the cells stopped and completed their migration. These results suggest that the dispersion of basket/stellate cells in the ML is controlled by the orchestrated activity of external guidance cues, cell–cell contact and intrinsic programs in a position- and time-dependent manner.

© 2009 Elsevier Inc. All rights reserved.

Introduction

In the developing brain, the majority of immature neurons migrate from their birthplace to their final destination (Rakic, 1990; Rakic et al., 1994; Nadarajah and Parnavelas, 2002; Komuro and Yacubova, 2003; Ghashghaei et al., 2006). This active movement of immature neurons is essential for the formation of neuronal cytoarchitecture and proper differentiation (Rakic, 2000; Guerrini and Filippi, 2005; Kerjan and Gleeson, 2007; Pang et al., 2008). Excitatory neurons and inhibitory neurons originate from different regions of the brain, and exhibit different modes of migration (Nadarajah and Parnavelas, 2002; Marin and Rubenstein, 2003; Tashiro et al., 2007; Tanaka et al., 2009). For example, in the developing cerebrum, excitatory neurons, which are born in the ventricular and subventricular zone, migrate radially to the cortical plate, while inhibitory neurons, which are born in the medial and lateral ganglionic eminence, migrate tangentially to the cortical plate (Marin and Rubenstein, 2001; Anderson et al., 2001; Ang et al., 2003; Kriegstein and Noctor, 2004). In the developing cerebellum, the migration of excitatory interneurons (such as granule cells) and inhibitory projection neurons (such as Purkinje cells) has been extensively examined (Rakic, 1971; Rakic and Komuro, 1995; Komuro and Rakic, 1998b; Ten Donkelaar et al., 2003; Yacubova and Komuro, 2003; Botia et al., 2007; Kumada et al., 2007; Jiang et al., 2008; Cameron et al., in press), but little is known about the migration of inhibitory interneurons (such as basket cells and stellate cells).

Both basket cells and stellate cells are GABAergic interneurons and located in the molecular layer (ML) of the cerebellum. They receive an excitatory synaptic input from granule cell axons (parallel fibers) and their axons make an inhibitory synapse with Purkinje cells. Both basket cells and stellate cells are scattered throughout the ML, but there is a bias in their allocation: the majority of basket cells are located in the bottom half of the ML, while the majority of stellate cells are located in the top half (Rakic, 1973). Until recently, the origin of basket cells and stellate cells has been controversial. The prevailing view has been that they share a common ancestry with cerebellar granule cells and originate from the external granular layer (EGL) (Miale and Sidman, 1961; Altman, 1972a,b). However, recent studies have clarified this long-standing conundrum of cerebellar histogenesis (Otero et al., 1993; Napieralski and Eisenman, 1993; Hallonet and Le Dourain, 1993; Zhang and Goldman, 1996a,b; Maricich and Herrup, 1999; Milosevic and Goldman, 2002, 2004; Yamanaka et al., 2004; Leto et al., 2006). The progenitors of both cells originate from the neuroepithelium of the fourth ventricle. They first migrate outward to reach the deep white matter (WM) of the embryonic cerebellum, and then continue to divide in the deep WM as well as in the folial WM during their migration.

* Corresponding author. Fax: +1 216 444 7927.
E-mail address: komuroh@ccf.org (H. Komuro).

0012-1606/$ – see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.ydbio.2009.05.575

It has been demonstrated that basket cells and stellate cells migrate from the deep WM through the folial WM, the internal granular layer (IGL) and the Purkinje cell layer (PCL) to the ML (their final destination) during early postnatal development (Zhang and Goldman, 1996a,b; Milosevic and Goldman, 2004), but little is known about how the cells complete their migration in the ML. In this study, with the use of real-time observation of cell movement and a herpes simplex virus (HSV) amplicon vector HGY, which expresses EGFP under the control of the HSV immediate-early (IE) 4/5 promoter, we examined the migration of basket cells and stellate cells in the ML of the early postnatal mouse cerebellum. We found that in the ML, basket cells and stellate cells sequentially go through four distinct phases of migration before completing their migration.

Materials and methods

All procedures were in strict accordance with the NIH Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of the Cleveland Clinic Foundation and the Ohio State University.

Plasmid construction

A HSV amplicon plasmid pHGY was constructed from pHGCX (Saeki et al., 2003; Kasai and Saeki, 2006) by removing the cytomegalovirus IE promoter and inserting a pair of *FRT* and *loxP* sites. pHGY is a pBR322-based amplicon plasmid and expresses enhanced green fluorescent protein (EGFP, BD Biosciences, San Jose, CA) under the control of the HSV IE4/5 promoter.

Cell culture and vector production

Vero 2–2 packaging cells (Smith et al., 1992) were kindly provided by Rozanne M. Sandri-Goldin (University of California, Irvine, CA) and cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), penicillin (100 U/ml), and streptomycin (100 μg/ml). G16-9 titration cells (Kasai and Saeki, 2006) were maintained in DMEM supplemented with 10% FBS, penicillin (100 U/ml), streptomycin (100 μg/ml), and hygromycin B (200 μg/ml). All cells were cultured at 37 °C in a CO_2 incubator (95% air and 5% CO_2). HSV amplicons were packaged using the improved helper virus-free packaging system as described previously (Saeki et al., 2001, 2003; Kasai and Saeki, 2006). Briefly, 2–2 cells (plated at approximately 90% confluence in 6-cm culture dishes) were transfected with 0.6 μg pHGY DNA, 0.2 μg pEBH-ICP27 DNA, and 2 μg fHSVΔpacΔ27-0+ DNA using LipofectAMINE Plus (Invitrogen, Carlsbad, CA) according to the manufacturer's protocol. Three days later, the transfected cells and medium were harvested, and the crude HGY vector stocks were concentrated by ultracentrifugation at 75,000×*g* for 3 h at 4 °C over a 25% (wt/vol) sucrose cushion. The pellet was then resuspended in Hanks' balanced salt solution and stored at −80 °C until use. The titer (green fluorescent protein transducing units [TU]/ml) of each vector stock was determined on G16-9 cells at 24 h after infection.

Monitoring cell migration in cerebellar slice preparations

Cerebella of postnatal day (P) 5–14 mice (CD-1, both sexes) were sectioned transversely or sagittally into 180 μm-thick slices on a vibrating blade microtome (VT1000S, Leica Instruments) (Komuro and Rakic, 1992, 1993, 1995, 1998a; Komuro et al., 2001). Cerebellar slices were placed on 24-mm diameter polyester membrane inserts (0.4 μm pore size, Corning Inc.) in 6 well plates (Corning Inc.). The bottom of each plate was filled with 2.5 ml of culture medium, which consisted of DMEM/F12 (Invitrogen) with N2 supplement, penicillin (90 U/ml) and streptomycin (90 μg/ml). Fifteen μl of the culture medium with 0.7 μl of 2×10^8 TU/ml of HGY amplicon vector was added to the center of the top surface of each slice. The slices were subsequently put in a CO_2 incubator (37 °C, 95% air, 5% CO_2). Twenty-four hours after sectioning, slices were transferred and placed on 35 mm-glass bottom microwell dishes (MatTek Co.) with 2.0 ml of the culture medium. The dishes were placed into the chamber of a micro-incubator (PDMI-2, Harvard Apparatus) attached to the stage of a confocal microscope (Leica). The chamber temperature was kept at 37.0 ± 0.5 °C, and the slices were provided with a constant gas flow (95% air, 5% CO_2). To prevent movement of the slices during observation, a nylon net glued to a small silver wire ring was placed over the preparations.

A laser scanning confocal microscope (TCS SP, Leica) was used to visualize EGFP-expressing cells in the slices (Komuro and Rakic, 1998a; Komuro et al., 2001; Komuro and Kumada, 2005; Kumada et al., 2006; Cameron et al., 2007). The use of this microscope permitted high-resolution imaging of EGFP-expressing cells up to 100 μm deep within the tissue slices. The tissue was illuminated with a 488-nm wavelength light from an argon laser through an epifluorescence inverted microscope equipped with a 40× oil-immersion objective, and fluorescence emission was detected at 530 ± 15 nm. Image data were collected at an additional electronic zoom factor of 1.0–2.0. To determine the location of EGFP-expressing cells within the ML, the EGL–ML border and the ML–PCL border, at the beginning and the end of each recording session, fluorescence images and transmitted images were simultaneously recorded with 40× magnification. To avoid the injured cells located near the sectioning surfaces, we examined the migration of EGFP-expressing cells located 15–50 μm below the surface of each slice. To monitor migration and morphological changes, images of EGFP-expressing cells in up to 40 different focal planes along the *z*-axis were collected with laser scans every 30 min for up to 70 h.

The long-term observation of cell movement allowed us to examine the behavior of EGFP-expressing cells (stellate/basket cells) from the entrance into the ML to the completion of the migration within the ML. Therefore, in this study, the average speed of migration and the average transit time in the four different phases were obtained from the same EGFP-expressing cells (stellate/basket cells).

Monitoring cell migration in microexplant cultures

Cerebella of P0–P3 mice (CD-1, both sexes) were placed in an ice-chilled Hanks' balanced salt solution, and freed from meninges and choroid plexus (Komuro and Rakic, 1996, 1999; Yacubova and Komuro, 2002a,b; Kumada et al., 2009). Cerebellar slices were made with a surgical blade. Rectangular pieces (50–100 μm) were dissected out from the slices under a dissecting microscope. Small pieces of cerebellum were placed on 35 mm-glass bottom microwell dishes (MatTek Co.), which had been coated with poly-L-lysine (100 μg/ml)/laminin (20 μg/ml), with 50 μl of the culture medium. Dishes were put in a CO_2 incubator (37 °C, 95% air, 5% CO_2). Two hours after plating, 1 ml of the culture medium with or without 2 μl of 2×10^8 TU/ml of HGY amplicon vector was added to each dish. The incubation medium consisted of DMEM/F12 (Invitrogen) with N2 supplement, penicillin (90 U/ml) and streptomycin (90 μg/ml). Ten hours after plating, the culture medium was replaced by 1 ml of the fresh culture medium without HGY amplicon vector. Twenty-four hours after plating, dishes were transferred into the chamber of a micro-incubator (PDMI-2, Harvard Apparatus) attached to the stage of a confocal microscope (TCS SP, Leica). The chamber temperature was kept at 37.0 ± 0.5 °C using a temperature controller (TC-202, Harvard Apparatus) and the cells were provided with constant gas flow (95% air, 5% CO_2). The cells were illuminated with a 488-nm wavelength light from an argon laser through an inverted microscope equipped with a 20× oil-immersion objective or a 40× oil-immersion objective.

Download English Version:

https://daneshyari.com/en/article/2174118

Download Persian Version:

https://daneshyari.com/article/2174118

Daneshyari.com