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Technical note

In vivo imaging of cortical interneurons migrating in the intermediate/subventricular zones

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ABSTRACT

We developed an imaging system that enables migrating cortical interneurons (CIs) through the lower intermediate zone/subventricular zone (IZ/SVZ) in mouse embryos. CIs were labeled by in utero electroporation performed at embryonic day (E) 11.5 and were observed, through the skull of living embryos, detached from the dam with the umbilical cord remain attached. To identify imaged cell locations, we used GAD67-GFP mice and GFP fluorescence was photo-bleached after the recording. We found that CIs in the IZ/SVZ migrated medially straight toward the midline on the tangential plane, while those in the marginal zone migrated in all directions.

1. Introduction

During development neurons exhibit dynamic changes in their shape and positions. It is therefore crucial to directly analyze their dynamic aspects to understand their development. Dynamic aspects of neurons have been traditionally studied using dissociated culture. However, recent technical progresses have allowed us to observe developing neurons in more physiological conditions such as living slices.

We and others studied the dynamics of CIs (Nadarajah et al., 2002; Ang et al., 2003; Tanaka et al., 2003, 2006, 2009; Martini et al., 2009) and precerebellar neurons in the hindbrain (Watanabe and Murakami, 2009; Shinohara et al., 2013). These studies led us to uncover several unexpected aspects of migrating neurons such as ventricle-directed migration (Nadarajah et al., 2002) and random walk behaviors (Tanaka et al., 2009).

However, results obtained from any kind of in vitro preparations might be associated with in vitro artifacts. Although some of them might be excluded by carefully observing fixed preparations, no information can be obtained regarding dynamic aspects. An ideal way to overcome this issue is to analyze dynamic phenomena in vivo. To this end we developed a preparation that enables visualization of migrating CIs in living embryos (Yanagida et al., 2012; Murakami, 2015). Using this preparation, we observed the migratory behaviors of CIs in the cortical surface, the marginal zone (MZ), for a long period of time in stable conditions and found that these neurons migrate in all direction in the tangential plane (Yanagida et al., 2012). However, such multidirectional migration is an atypical behavior. It is therefore important to analyze the behavior of migrating CIs in the IZ/SVZ, where these neurons might be executing directed migratory behaviors.

Here we have improved the previously developed techniques and succeeded in visualizing migrating CIs in the IZ/SVZ of mouse living embryos. We show that these interneurons migrate straight toward the midline and infrequently extending branched leading processes.

2. Materials and methods

2.1. Animals

Mice with ICR strain or *GAD67-GFP* knockin (delta-neo) mice (*GAD67-GFP* mice) (Tamamaki et al., 2003) maintained in an ICR background were used. Noon of the day of vaginal plug detection was termed embryonic day (E) 0.5. All experiments were performed

Abbreviation: CIs, cortical interneurons.

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in accordance with the Osaka University Guidelines for the Welfare and Use of Laboratory Animals.

2.2. In utero electroporation

To label GABAergic CIs, medial ganglionic eminence (MGE)-directed in utero electroporation (IUE) was performed, as described previously with some modifications (Tanaka et al., 2009; Yanagida et al., 2012). Five 45 V pulses with a duration of 50 ms were applied at 600 ms intervals to embryonic day E11.5 mouse embryos, using forceps-shaped electrodes (CUY650P2; NEPAGENE) and an electroporator (ECM830; BTX). In some cases electroporation was performed on *GAD67-GFP* mice. The electrodes were set at angles of 10–30° from the rostrocaudal axis of the animal body. Plasmids were dissolved in phosphate-buffered saline (PBS; 0.1 M, pH 7.4) and used at the following final concentrations: 3.0 μg/μL tandem dimer Tomato (tdTomato) (a gift from Dr. R.Y. Tsien) (Shaner et al., 2004) (pCAGGS-tdTomato) and 1.0 μg/μL tdTomato with a membrane anchoring signal of the growth-associated protein 43 (GAP-43) (subcloned by Mr. T. Kobayashi) (Liu et al., 1994; Moriyoshi et al., 1996) (pCAGGS-GAP-tdTomato). CAG promoter (pCAGGS) was a gift from Dr. J. Miyazaki (Niwa et al., 1991). After the end of the electroporation procedure, the dam was placed on a warming pad kept at 40 °C.

2.3. Time-lapse analysis

Time-lapse analysis was carried out as previously (Yanagida et al., 2012) with some modifications. The dam was anesthetized with urethane (SIGMA, 2.0 g/kg) and supplemental doses of urethane were administered as required. Embryos were removed from the dam at E14.5 such that the umbilical cord remained connected to the dam, and placed in a small container and embedded with 3% agarose in PBS. Prior to the embedding, the embryos was coated with gelatin (10% in PBS) to protect them from potential damage caused during the course of agarose gelling. The umbilical cord was carefully protected using superabsorbent polymer so that hardened agarose should not press it (Fig. 1). A small amount of superabsorbent polymer was also used adjust the position of the embryo's head. During these procedures embryos were warmed using a heater to maintain their body temperature.

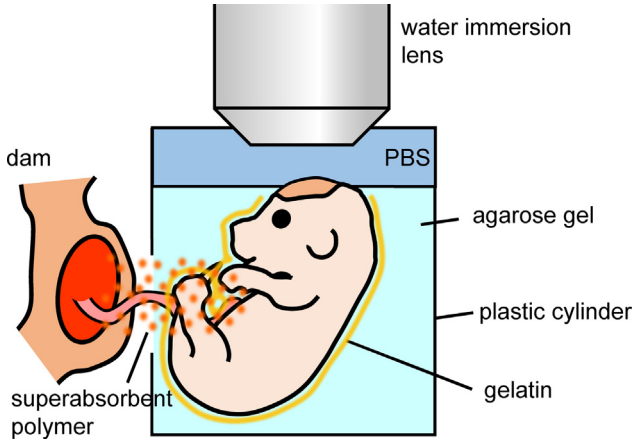

Fig. 1. Schematic illustration of mouse embryo in vivo imaging. The dam was anesthetized, and embryos were removed from the uterus while keeping the umbilical cord attached to the dam. For immobilization, an embryo was placed in a small plastic container filled with agarose solution. Migrating neurons in the cortex was observed through the skull after removing the skin. The umbilical cord was protected using superabsorbent polymer so that hardened agarose should not press it.

Scanning by a two-photon microscope (TCS-SP5 MP; Leica Microsystems) was performed using a 20× (N.A. 1.0) water immersion lens and 30–50 images were captured from the brain surface at an interval of 5 μm at every 15 min through the skull, allowing for imaging of CIs from the MZ thorough to the IZ/SVZ. Blood flow was monitored to examine viability of embryos during recording. In *GAD67-GFP* mice, a strong laser was illuminated to bleach GFP fluorescence thereby marking the position of the recording.

2.4. Image processing

10–20 z-series images that include labeled interneurons were chosen from the 30–50 images to create a projection image. Images that were distorted due the movement of embryos were excluded from the analysis. Movies were assembled using the MetaMorph (ver 7.7.0.0, Molecular device).

2.5. Fixed preparations

The forebrains of some of the embryos were immersion fixed using 4% paraformaldehyde before or after time-lapse imaging. They were then embedded in O.C.T compound (Sakura Finetechnical, Tokyo) and cut coronally at 20 or 30 μm using a cryostat (HM550; Microcom). Some of the sections were counterstained with 4′,6-diamidino-2-phenylindole (DAPI) (1:3000 in PBS).

3. Results and discussion

Most CIs originate from the MGE and the caudal ganglionic eminence (CGE) (Gelman and Marín, 2010; Rudy et al., 2011). Since most CGE-derived CIs are generated at E12.5 and later (Ma et al., 2012), IUE performed at E11.5 should almost exclusively label MGE-derived interneurons. We first observed labeled CIs in fixed embryos. Fig. 2A shows tdTomato-labeled CIs in a coronal section of an E14.5 embryo cortex. One can see that most labeled CIs migrate at a depth (~200 μm from the surface) of the cortex, likely the IZ/SVZ.

After confirming the depth of the migrating stream in E14.5 fixed embryos, we carried out time-lapse imaging using living embryos focusing on migrating IUE-labeled CIs at a depth of ~200 μm. In some experiments we used *GAD67-GFP* mice (Tamamaki et al., 2003) to identify imaged cell localization within the field of imaging (Fig. 2B–E). After the end of imaging, we applied illumination of strong laser light to breach the GFP fluorescence (Fig. 2C–E). This allowed us to localize the area of imaging. Once we identified the area of imaging, we could identify labeled CIs located within the frame of the imaging field after the imaging.

We found that many of these CIs were oriented medially in fixed embryos. Consistently, time-lapse imaging demonstrated that these CIs migrated straight toward the midline. Fig. 3 shows examples of migrating CIs in the IZ/SVZ of a living embryo. Curiously, unlike the CIs in the MZ (Yanagida et al., 2012), these cells exhibited directed migration toward the midline. Notably, the morphologies of these neurons were also different from those in the MZ (Yanagida et al., 2012). The CIs in the IZ/SVZ extended leading processes with a simple morphology infrequently exhibiting branching (Fig. 3A and B), although this might be due to a failure of imaging thin branches. The leading processes were oriented toward the midline (Fig. 4).

We have successful visualized migrating CIs at a depth of the brain using a two-photon microscope. Utilization of GAD67-GFP mice combined with bleaching by strong laser illumination allowed us to certify that images were captured from migrating CIs in the IZ/SVZ. We positioned the embryos using agarose, gelatin and superabsorbent polymer carefully maintaining animal's body temperature, enabling us to perform stable recording for a substantial period of time.