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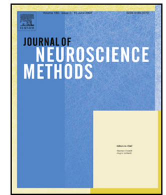

Short communication

New methods for investigation of neuronal migration in embryonic brain explants

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HIGHLIGHTS

- New immunocytochemistry (ICC) protocol for staining neurons migrating out of the MGE explant embedded in Matrigel was developed in this work.
- Our new protocol transfects MGE explants.
- Our microfluidic chambers allow visualization of the vectorial migration of individual neuronsfrom explants, and tracking of cellular organelles.
- Our methods provide new paradigms to study neuronal migration in real-time.

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ABSTRACT

Background: Proper migration of neurons is essential for the formation and normal functioning of the nervous system. Defects in neuronal migration underlie a number of neurologic diseases in humans. Although cell migration is crucial for neural development, molecular mechanisms guiding neuronal migration remain to be elucidated fully. Newborn neurons from the embryonic medial ganglionic eminence (MGE) migrate a long distance dorsally in the developing brain, giving rise to several types of interneurons in the neocortex.
New method: In this study, we developed an immunocytochemistry (ICC) protocol to stain neurons migrating out of the MGE explant embedded in Matrigel. We also established a protocol to efficiently transfect cells in MGE explants, achieving a transduction efficiency of more than 30%.
Comparison with existing method: In addition, we developed microfluidic chambers for explants that allow visualization of the vectorial migration of individual neurons from mouse embryonic MGE explants. Our microfluidic system allows monitoring of the distribution of cellular organelles (e.g. Golgi) within migrating neurons which have been stained with commercial molecular dyes or transfected with adeno-associated virus (AAV) expressing reporter proteins.
Conclusion: These methods provide new paradigms to study neuronal migration in real-time.
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1. Introduction

Neurological syndromes caused by disruption of neuronal migration can be devastating human disorders, which are mostly due to genetic causes (for an extensive review see reviews Reiner, 2013; Valiente and Marín, 2010). Studies have shown that disruption of cytoskeleton underlies a large spectrum of neuronal migration disorders (Métin et al., 2008). Migration defects of cortical interneurons can cause abnormal inhibitory responses in the developing cortex (Valiente and Marín, 2010). Ganglionic eminence (GE) neurons move tangentially and across the plane of the glial fiber system during brain development (Marín and Rubenstein, 2001). These GE-derived neurons contribute significantly to the

Abbreviations: AAV, adeno-associated virus; AEP, anterior entopeduncular area; GABA, gamma amino butyric acid; GE, ganglionic eminence; g.c., genome copy; GFP, green fluorescent protein; HP, hidden photon; ICC, immunocytochemistry; i.p., intraperitoneal; LGE, lateral GE; LV, lentivirus; MGE, medial ganglionic eminence; PFA, paraformaldehyde; PBS, Phosphate Buffered Saline; t.u., transducing units; VSV-G, vesicular stomatitis virus glycoprotein.

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GABAergic cortical cell population (Hernández-Miranda et al., 2010). The gamma amino butyric acid (GABA) interneurons must be produced and deployed to the cortex during fetal life in order for the neural circuits to function properly in the mature brain. Many GABA neurons migrate tangentially to reach their final destination. Tangential migration of GABA neurons is widespread in the embryonic rodent brain (Parnavelas, 2000), and three major routes of such migration have been recently characterized in detail: (1) from the medial GE (MGE) to the cortex; (2) from anterior entopeduncular area (AEP) to the neocortex and hippocampus; and (3) from the lateral GE (LGE) to the olfactory bulb· This latter migratory route persists into adulthood as the rostral migratory stream. In this study we have focused on MGE neurons because they have a unique migratory potential, and may have applications in cell replacement therapies in certain neurodegenerative diseases. MGE neurons are the only primary neuronal precursors known to be able to disperse when grafted into the adult brain (Wichterle et al., 1999).

Here, we describe novel methods to study MGE migration, including a microfluidic chamber to culture MGE explants. Our methods may open new avenues for informative neurobiological investigations. An advantage of the neurotechnology system we have developed is that, once validated, it could be used for screening of drugs, which can affect neuronal migration. In addition, immunocytochemical (ICC) staining methods can be used to detect changes in the distribution of proteins thought to contribute to migration defects, such as DISC1, Lis1, and Ndel1 (Richter and Loscher, 1999), and be used to express mutant proteins involved in neurological diseases instead of using transgenic animal models.

2. Materials and methods

2.1. Fabrication of the microfluidic microchambers

To probe the migration of neurons from a MGE explant toward a naturally chemotrophic cortical explant, we designed a microfluidic device with two compartments connected by an array a small channels. The two compartments, in the form of circular wells of 1.5 mm diameter and 3 mm depth were filled with gel matrix. Also filled with gel was the parallel array of microchannels of 25 μm width, 10 μm height, and average 150 μm length, connecting the two compartments. The cross section of the channels was chosen such that the migration and mechanical orientation of individual neurons could be recorded by time lapse microscopy. The device was fabricated by directly bonding polydimethylsiloxane (PDMS – Dow Corning Midland, MI) on glass coverslips, inside glass-bottom Petri-dishes (MatTek, Ashland, MA) after exposing the surfaces to be bonded to oxygen plasma for 30 s. The PDMS piece was cast on a microfabricated silicon wafer, to produce a replica of the photolithographic features on the wafer, baked for 12 h at 65 °C and punched using a 1.5 mm puncher (Harris Uni-Core, Ted Pella, Reading, CA) to define the two tissue compartments. The geometry of the array of microchannels and their connection to the two compartments was precisely defined using photolithography techniques, in a 10 μm thick layer of SU-8 photoresist (Microchem, Newton, MA). Immediately after fabrication, devices are kept on ice and filled with the liquid Matrigel (BD Biosciences, Franklin Lakes, NJ), which is then allowed to gel at room temperature, and covered with Neurobasal medium (BD Biosciences).

2.2. Animals

C57BL/6 breeding pairs were obtained from Charles River Laboratories (Wilmington, MA). C57BL/6 matings were set up and the females monitored for vaginal plugs. The day vaginal plug was observed was considered embryonic day 0 (E0), and the male was separated from the female. The pregnant dams were housed in a temperature- and humidity-controlled environment on a 12-h light/dark cycle with food and water available *ad libitum*.

2.3. Embryo collection and processing

For collection of embryos, the dams were anesthetized by intraperitoneal (i.p.) injections of a mixture of ketamine (50 mg/kg body weight) and xylazine (10 mg/kg body weight) and the embryos were removed by hysterotomy. The embryos were collected at E15 and decapitated immediately upon removal from the dam, as described previously (McCarthy et al., 2012).

2.4. Analysis of neuronal migration in MGE explants

Slice preparations were prepared from brains of E15 embryos as described with modifications (Crandall et al., 2007; McCarthy et al., 2011, 2012). Briefly, embryonic brains were removed and placed in 8% agarose (type VII; Sigma–Aldrich, St. Louis, MO). Coronal sections were cut at a thickness of 250 μm using a Vibratome. A sterile biopsy punch (Miltex Inc., York, PA) was used to collect disks of MGE from the vibratome sections (McCarthy et al., 2011). The majority of the neurons produced in the MGE are migratory neurons destined to become cortical GABA neurons (Butt et al., 2005; Rallu et al., 2002; Anderson et al., 2001). The disk explants from MGE additional punch-out from the cortical region were collected from the same embryonic slice. For staining experiments, MGE explants were cultured in 50 μl Matrigel covered with Neurobasal medium containing 2% B27 supplement, 0.25% glutamine, 0.0125% glutamate, 1× penicillin/streptomycin in glass bottom culture dishes (BD Biosciences). Following 48 h in culture, the explants were fixed with 4% paraformaldehyde (PFA) for 1 h, and stored in 1× Phosphate Buffered Saline (PBS) buffer at 4 °C until ICC was performed. For microfluidic devices experiments, both wells were filled with Matrigel and covered with Neurobasal medium, described above. MGE explant was placed in one of the wells and a cortical explant placed in the other well.

2.5. Immunocytochemistry (ICC) of MGE explants

PFA fixed explants were treated with warm (37 °C) 1× PBS for 90 sec (three 30 s incubations with warmed PBS), followed by incubation with 0.1% Triton X-100, 10% goat serum (Jackson Immuno Research) in 1× PBS for 1 h. After washing 3 times for 10 min with 1× PBS, the samples were incubated overnight at 4 °C with primary antibodies to βIII-tubulin and pericentrin (βIII-tubulin mouse monoclonal antibody, MAB55441, 1:1500; EMD Millipore, Billerica, MA; pericentrin rabbit polyclonal antibody, PRB-432C, 1:800; Covance, Dedham, MA) to reveal the processes and of the centrosome, respectively, in migrating cells. The next day, explants were washed three times with 1× PBS and incubated at room temperature for 1 h with secondary antibodies – anti-mouse Alexa 488 (1:1000) or anti-rabbit Alexa 555 (1:800) (Invitrogen/Life Technologies, Grand Island, NY). Nuclei were labeled with ToPro3 (1:1000; Invitrogen/Life Technologies, Grand Island, NY) by incubation for 15 min.

2.6. Lentiviral (LV) and adeno-associated virus (AAV) vectors and MGE transfection

Lentiviral vectors [LV-CMV-GFP (green fluorescent protein); Sena-Esteves et al., 2004] and AAV vectors (AAV-CBA-GFP; Broekman et al., 2006) packaged with serotype 8, 9, or rh10 capsids were kindly provided by Dr. Sena-Esteves (UMass Medical School, Worcester, MA). Viral titers of 5×10^7 transducing

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