available at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/brainres

BRAIN RESEARCH

Research Report

A brain slice culture model for studies of endogenous and exogenous precursor cell migration in the rostral migratory stream

Mette Tanvig[a], *Morten Blaabjerg*[a], *Rikke K. Andersen*[a], *Ana Villa*[b,1], *Ann Mari Rosager*[a], *Frantz R. Poulsen*[a], *Alberto Martinez-Serrano*[b], *Jens Zimmer*[a], *Morten Meyer*[a,*]

[a]*Department of Anatomy and Neurobiology, Institute of Medical Biology, University of Southern Denmark, Odense, Winsløwparken 21, DK-5000 Odense C, Denmark*

[b]*Center of Molecular Biology Severo Ochoa, Laboratory CX-450, Autonomous University of Madrid, 28049 Madrid, Spain*

ARTICLE INFO

Article history:
Accepted 22 July 2009
Available online 30 July 2009

Keywords:
Neural stem cell
Mesenchymal stem cell
Forebrain
Subventricular zone
Olfactory bulb

ABSTRACT

The rostral migratory stream (RMS) is the main pathway by which newly born subventricular zone (SVZ) cells reach the olfactory bulb (OB) in rodents. This migration has been well studied *in vivo*, but an organotypic *in vitro* model would facilitate more experimental investigations. Here we introduce a slice culture preparation of the rat forebrain including en suite the rostral part of the lateral ventricle, the RMS and the OB. The preparation was validated with regard to endogenous cell proliferation and migration by tracking bromodeoxyuridine (BrdU)-labelled cells in newly established and 3 and 6 week old cultures. For testing the migratory abilities of exogenous precursor cells, rat SVZ neurospheres and human neural (HNS1 cells) and mesenchymal (hMSC-TERT) stem cell lines were micrografted to the rostral SVZ of 1 and 7 day old cultures. Two weeks later graft derivatives were identified by immunohistochemical staining for human nuclei (HNS1/hMSC-TERT cells) and BrdU (HNS1 cells/neurospheres). Numerous HNS1 cells and BrdU-positive neurosphere cells were found in the RMS. Having reached the OB, subpopulations of the cells expressed the astroglial markers glial fibrillary acidic protein/hAM and the neuronal markers NeuN/tyrosine hydroxylase. Interestingly, the hMSC-TERT cells remained at the implantation site, demonstrating a diversity in migratory capability of different precursor cells. In conclusion, the RMS in rat forebrain slice cultures retains its ability to support migration of endogenous and exogenous neural precursors, making the cultures highly feasible for studies of conditions and factors regulating cell migration.

* *Corresponding author*. Fax: +45 65906321.
E-mail address: mmeyer@health.sdu.dk (M. Meyer).

Abbreviations: bFGF, basic fibroblast growth factor; β-tubIII, β-tubulin III; BrdU, bromodeoxyuridine; DAB, 3,3′-diaminobenzidine; EGF, epidermal growth factor; FA1/dlk, fetal antigen 1/delta like; FBS, fetal bovine serum; GABA, gamma-aminobutyric acid; GBSS, Gey's balanced salt solution; GFAP, glial fibrillary acidic protein; HRP, horse radish peroxidase; hAM, human astrocytic marker; hN, human nuclei; -ir, immunoreactive; NeuN, neuronal nuclei; NS, neurosphere; OB, olfactory bulb; PSA-NCAM, polysialylated neural cell adhesion molecule; RMS, rostral migratory stream; 2× SSC, 2×standard saline citrate; SVZ, subventricular zone; SVZa, anterior SVZ; TBS, tris buffered saline; TH, tyrosine hydroxylase

[1] Present affiliation: Centro de Investigación Sanidad Animal (CISA-INIA), Valdeolmos, 28130-Madrid, Spain.

0006-8993/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.brainres.2009.07.075

1. Introduction

Cell migration and neurogenesis were previously believed to end soon after birth. New neurons are, however, now known to form continuously in localized parts of the adult vertebrate brain. Regarding cell migration, neuroblasts born in the walls of the lateral ventricles in adult songbirds migrate long distances before final differentiation in several regions of the telencephalon (Alvarez-Buylla et al., 1994; Goldman and Nottebohm, 1983). In rodents, migration is most prominent for neural progenitor cells migrating from the anterior subventricular zone (SVZa) to the olfactory bulb (OB) forming the so-called rostral migratory stream (RMS). Once within the OB, the cells differentiate into granule cells and periglomerular cells important for odor discrimination (Bolteus et al., 2005; Corotto et al., 1993; Lois and Alvarez-Buylla, 1993; Lois et al., 1996; Soares and Sotelo 2004).

The postnatal tangential migration of cells in the RMS is different from the well-studied radial migration, which primarily occurs prenatally during brain development. In contrast cells in the RMS migrate in close association with each other in so-called chain migration (O'Rourke et al., 1995; Thomas et al., 1996; Wichterle et al., 1997). It is only after arrival in the OB that the cells disperse into the different layers in a radial manner. When studied *in vivo* in adult rodents chains of migrating cells, expressing the polysialylated neural cell adhesion molecule (PSA-NCAM), extend from the SVZ into the RMS (Bonfanti and Theodosis, 1994) enwrapped by tube-forming astroglial cells (Peretto et al., 1997).

The RMS has been well characterized *in vivo* (Altman, 1969; Fasolo et al., 2002; Garcia-Verdugo et al., 1998; Law et al., 1999; Menezes et al., 2002; Thomas et al., 1996), but despite some studies on guidance cues (Belvindrah et al., 2007; Conover et al., 2000; Hack et al., 2002; Jacques et al., 1998; Kirschenbaum et al., 1999; Mason et al., 2001; Murase and Horwitz, 2002; Paratcha et al., 2006; Wu et al., 1999) knowledge is still insufficient regarding factors and conditions regulating the migration.

Here we present a rat forebrain slice culture preparation where the SVZa and the RMS extending to the OB are organotypically and functionally retained. The purpose of establishing this preparation is to provide an easily accessible and long-term functional forebrain culture for experimental studies of the RMS and testing of migratory abilities of neural stem cells and cell lines. Slice culture studies of cell migration in various brain regions have been performed previously by Bolteus et al. (2005), Dayer et al. (2008), De Marchis et al. (2001), Mancini et al. (2009), Murase and Horwitz (2002), Nam et al. (2007) as well as Soares and Sotelo (2004), but they used acute brain slices or short culture periods of a few days only, in contrast to the present study, where cultures were kept functionally active for up to 6 weeks. In order to validate the potential use of our slice culture preparation, we also tested the migratory ability of three different types of precursor cells after micrografting into the SVZ of the cultures. The test samples consisted of rat SVZ-derived neurospheres/neural tissue spheres (Andersen et al., 2007, 2008), immortalized human neural stem cells (hNS1) (Villa et al., 2000) and immortalized human mesenchymal stem cells (hMSC-TERT) (Simonsen et al., 2002).

2. Results

2.1. *Proliferating cells in the slice cultures*

In 7, 21 and 42 day old cultures, exposed to the cell proliferation marker bromodeoxyuridine (BrdU) for 24 h at the end of the culture period, numerous BrdU-immunoreactive cells were found in the subventricular zone (SVZ), the rostral migratory stream (RMS) and in the olfactory bulb (OB) (Figs. 1A and B). Comparative analysis of the BrdU distribution, performed at 1 and 21 days, after BrdU treatment of 7 day old cultures, revealed that a large number of BrdU-positive cells had accumulated in the OB indicating that the migratory capacities were preserved in the cultures.

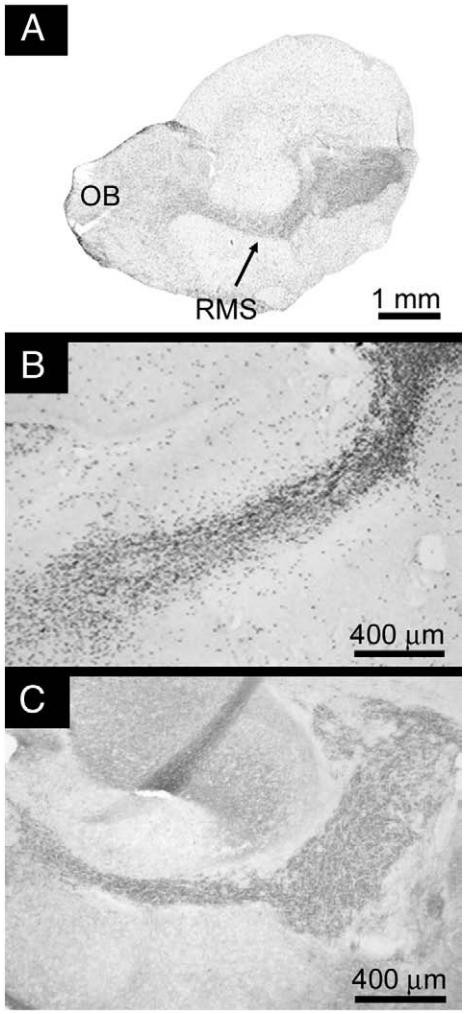

Fig. 1 – Incorporation of bromodeoxyuridine (BrdU) and expression of PSA-NCAM in rat forebrain slice cultures. In forebrain slice cultures grown for 7 days (A) and 21 days (B) BrdU-labelled cells were detected in the rostral migratory stream (RMS) and olfactory bulb (OB) by immunostaining. The BrdU was added to the culture medium 24 h before histological processing. (C) In 21 day old cultures migrating cells in the RMS expressed the neural cell adhesion molecule PSA-NCAM.