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A new method of embryonic culture for assessing global changes in brain organization

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Abstract

While dissociated, reaggregated cells and organotypic slice cultures are useful models for understanding brain development, they only partially mimic the processes and organization that exist *in vivo*. Towards bridging the gap between *in vitro* and *in vivo* paradigms, a method for culturing intact brain tissue was developed using whole cerebral cortical hemispheres in which the anatomical and cellular organization of nervous system tissue is preserved. Single, free-floating telencephalic hemispheres were dissected from embryonic mice and placed into defined culture medium on an orbital shaker. Orbital shaking was necessary for optimal growth, and cortices grown under these conditions closely approximated *in vivo* parameters of cell division, differentiation, migration and cell death for up to 24 h. In addition to wild-type cultures, the method was compatible with genetically altered tissues. One particular advantage of this method is its ability to reveal global anatomical alterations in the embryonic brain following exposure to soluble growth factors. This method should thus be helpful for assessing a wide range of soluble molecules for their systemic effects on the embryonic brain.

Keywords: Development; Embryonic; Cerebral cortex; *Ex vivo*; Cell culture

1. Introduction

The size and shape of the cerebral cortex are determined during neurogenesis, when cells undergo proliferation, differentiation, migration and cell death in the developing brain (Caviness et al., 1995; Haydar et al., 1999a,b; Pompeiano et al., 2000). These various neurogenic processes depend on local cellular interactions that are altered by the disruption of an intact central nervous system (Bittman et al., 1997; Linden et al., 1999; Murciano et al., 2002).

Dissociated, reaggregated embryonic brain cells and organotypic slice cultures are simple models for understanding cerebral cortical development (Berglund et al., 2004; Ghosh et al., 1994; Haydar et al., 1999a,b). While these systems allow for precise control of culture conditions, they do not retain the spatial organization of the brain, and consequently, they only partially mimic the underlying neurogenic processes that occur *in vivo*. Here, the potential use of cultured whole cerebral cortex hemispheres was explored for studying mechanisms dependent on the anatomical organization of the nervous system. This model takes advantage of the controlled environment of *in vitro* systems while preserving the integrity of cerebral cortical tissue.

In 1961, Moscona described the use of gyratory rotation for culturing suspensions of embryonic cells (Moscona, 1961). While this orbital shaking was necessary to promote the aggregation and formation of three-dimensional spheres from the cell suspensions, it also improved the aeration and diffusion of nutrients to the cells. To promote the aeration and diffusion of nutrients while culturing whole brain hemispheres, orbital shaking was explored here.

The present model also permits the study of soluble factors for their possible effects on cortical architecture. Extrinsic signaling molecules such as neurotrophins, fibroblast growth factor 2 (FGF2), epidermal growth factor, pituitary adenylyl cyclase activating peptide, and insulin-like growth factor 1 are known

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to alter cell cycle, migration, differentiation and cell death in neuronal cells (Barde, 1994; Bartlett et al., 1994; Bondy and Cheng, 2004; Dicicco-Bloom et al., 1998; Farinas et al., 2002; Ferguson and Slack, 2003; Ford-Perriss et al., 2001; Fukushima et al., 2002; Ghosh and Greenberg, 1995; Kingsbury et al., 2003; Meirer et al., 2001; Ostenfeld and Svendsen, 2004; Temple and Qian, 1995; Vaccarino et al., 1999; Waschek, 2002; Wong and Guillaud, 2004); however, the roles these molecules play in shaping cerebral cortical morphology are poorly understood.

Using orbital shaking in defined media, morphology and neurogenic processes in whole cerebral hemispheres were assessed. The novelties of this free-floating preparation are that it approximates *in vivo* organization while preserving an intact cortical hemisphere and permits exposure of intact brain tissue to a wide range of soluble factors that can be delivered under controlled conditions.

2. Material and methods

2.1. Animals

Animal protocols were approved by the Animal Subjects Committee at The Scripps Research Institute and conform to National Institutes of Health guidelines and public law. Timed-pregnant BALB/c or C57Bl/6 females were anesthetized by halothane inhalation and sacrificed by cervical dislocation.

2.2. Dissection of cortical hemispheres

Embryos were removed at embryonic day 14 (E14) and placed into a 100 mm × 15 mm tissue culture (TC) dish containing serum-free medium consisting of Opti-MEM I (Gibco-BLR, Cat. #31985-070) with 20 mM glucose, 55 mM 2-mercaptoethanol and 1% penicillin/streptomycin. Brains of embryos were then dissected in individual 60 mm × 15 mm dishes containing serum-free medium. Specifically, an anterior horizontal cut above the eyes and a posterior horizontal cut through the brainstem were performed using fine forceps (#5, Fine Science Tools). Starting at the lateral edge, skull tissue was gently removed using forceps to free the entire brain from the head of the embryo (Fig. 1A). The two cerebral cortical hemispheres were separated along the midline (white dashed line, Fig. 1B) and cut away from the remaining brain using a No. 11 scalpel blade (black dashed line, Fig. 1B). Excess midbrain tissue along the midline of each hemisphere was removed to expose the lateral ventricles for ample diffusion of medium and nutrients (black arrow, Fig. 1C). The meninges were kept intact. This procedure takes approximately 30 min per pregnant female (approximately 6–9 embryos) to be completed.

2.3. Ex vivo cultures

Using a P1000 Pipetman and a cut pipette tip, each cortical hemisphere was transferred with 1 ml of medium from the 60 mm × 15 mm dish to an individual well of a 12-well TC plate containing 1 ml of fresh medium (total volume within each individual well was 2 ml). TC plates were then placed on a shaker

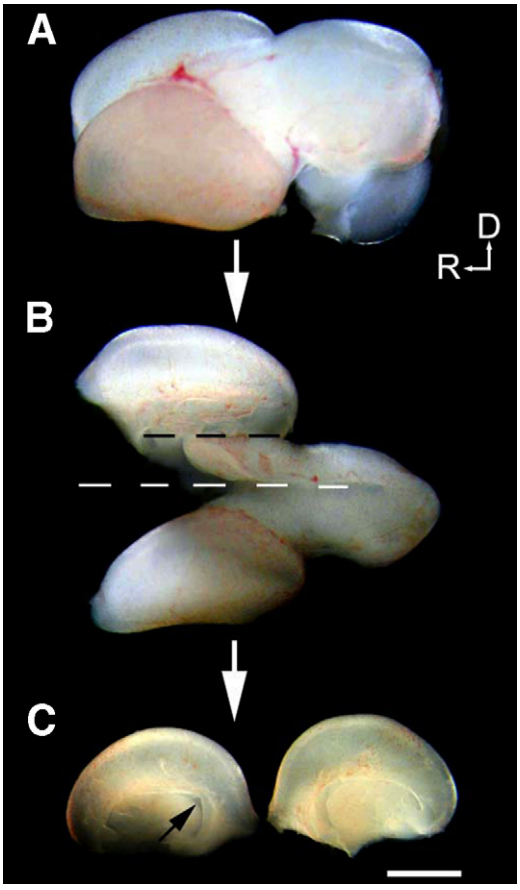

Fig. 1. Dissection of cortical hemispheres. (A) E14 brains were removed from the skull. (B) Cortical hemispheres were divided along the midline (white dashed line). (C) Hemispheres were dissected away from the rest of the brain (black dashed line) and the lateral ventricles were exposed (black arrow) to allow adequate diffusion of nutrients. The meninges were left intact. D: dorsal; R: rostral. Scale bar, 1 mm.

table inside a tissue culture incubator. Cortices were cultured for 24–48 h at 37 °C in 5% CO_2 with mild agitation (approximately 70 rpm), similar to previous descriptions (Rehen et al., 1996).

For growth factor experiments, hemispheres were cultured in medium containing 1 μM lysophosphatidic acid (LPA; Oleoyl-LPA; Avanti Polar Lipids, Alabaster, AL) in 0.1% fatty-acid free bovine serum albumin (FAFBSA; Sigma) to serve as a carrier for LPA, or 40 ng/ml of FGF2 (R&D Systems, Minneapolis, MN) while the opposite hemisphere was cultured in control medium containing 0.1% FAFBSA or serum-free medium alone (Fig. 2).

2.4. Preparation of growth factors

For LPA, 220 μl sterile water was added to 1 mg LPA powder to make a 10 mM solution. This 10 mM solution was then diluted with 10% FAFBSA to make a 100 μM solution. Either 20 μl of 100 μM LPA in 10% FAFBSA or 20 μl of 10% FAFBSA was added to each well containing a cortical hemisphere in 2 ml final volume of defined medium. The final concentration of LPA per well is 1 μM in 0.1% FAFBSA. For FGF, FGF powder is