A Dissection and Tissue Culture
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40. Preparation of Primary Cultures of Mouse Cortical Neurons

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INTRODUCTION

This procedure is based on the techniques outlined by Yavin and Yavin [1980], Yu et al. [1984], and Hertz et al. [1985a, b]. The advantage of this preparation is that the cultures are highly enriched in well-differentiated GABAergic cortical neurons (Fig. 1); neurons constitute ≥ 90% of the cells in culture [see also Kuriyama et al., 1986]. The basic strategy used is to isolate the cerebrum at a time in development when large numbers of neurons have just entered their postmitotic stage of differentiation and only a few proliferative glial precursors are present. The majority of these glial precursors are eliminated in culture with the use of cytosine arabinoside.

PROTOCOL

1. Take out the cerebral hemispheres aseptically from 15-day-old mouse embryos and place them in tissue culture medium with 20% horse serum. Our medium is a slightly modified Dulbecco's minimum essential medium (MEM), described in detail in Hertz et al. [1982], aerated with 5% CO_2/95% air (v/v). The age of the mouse embryos is critical, because in older embryos and fetuses a large proportion of the neurons is differentiating and shows extensive process formation,

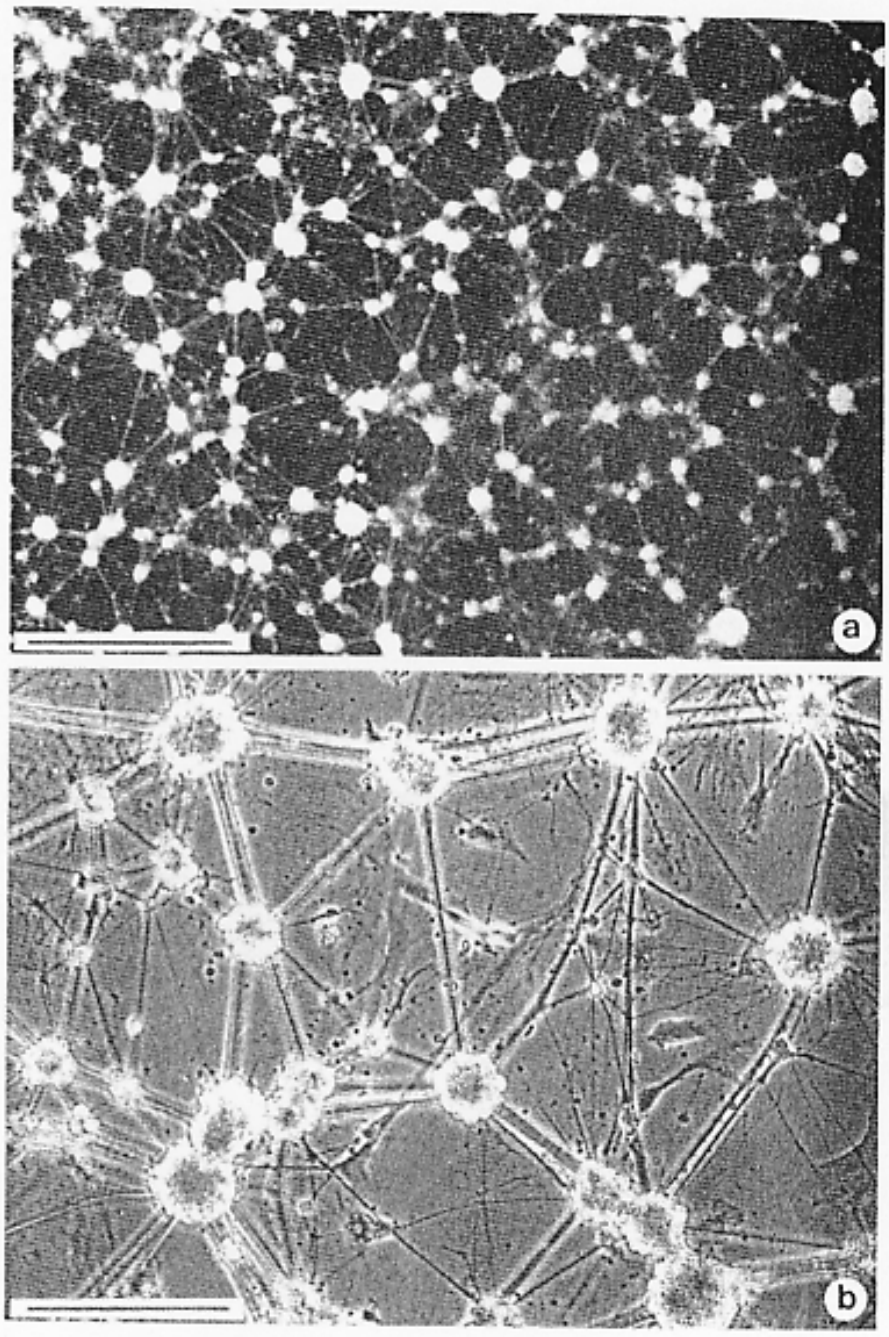

Fig. 1. Photomicrographs of living 12-day-old culture of mouse cerebral cortical neurons. **a:** Darkfield micrograph. Scale bar = 800 µm. **b:** Phase-contrast micrograph. Scale bar = 200 µm. (Reproduced from Hertz et al., 1985a, with permission of the publisher.)

which reduces the likelihood they will survive the dissociation procedure.

2. Remove the olfactory bulbs, hippocampal formations, basal ganglia, and meninges, thus isolating the neopallium, i.e., the cortical tissue above and lateral to the lateral ventricles. This step is not essential but probably leads to a more homogeneous culture of GABAergic neurons.
3. Replace medium with Puck's solution, i.e., a Ca^{2+}/Mg^{2+}-free balanced salt solution. Cut the neopallium into 1 mm cubes and add trypsin to a final concentration of 0.2% (Gibco, Grand Island, NY, 1:250).
4. Trypsinize for 2 min at room temperature and thereafter inactivate trypsin action by adding horse serum to a final concentration of 20%.
5. Triturate the tissue with a fire-polished pipette and centrifuge the cells for 2 min at 900*g*.
6. Resuspend the pellet in serum-free tissue culture medium in which the glucose concentration has been adjusted to 30 mM ($\simeq$ 3 ml per brain). Filter through a Nitex mesh of 80 μm pore size.
7. Plant cells in Falcon or NUNC tissue culture petri dishes (e.g., 3 ml, [cells from one brain] per 60 mm dish) that have been coated by exposure overnight to 12.5 μg/ml of L-polylysine in water.
8. Fifteen minutes later, remove medium with unattached cells (mostly nonneuronal cells) and add fresh medium with 30 mM glucose and 5% horse serum. Incubate at 37°C in a 95/5% (v/v) mixture of atmospheric air and CO_2 with a relative humidity of 90%.
9. After 3 days in vitro, expose the cultures to 40 μM cytosine arabinoside (added as 100 μl of a 1.2 mM solution per 3 ml) to eliminate the majority of proliferating nonneuronal cells.
10. Twenty-four hours later re-feed cultures with fresh growth medium with 30 mM glucose and 5% serum but without the cytotoxic agent. The cultures are maintained without further feeding for a total of 1.5–2.5 weeks. During this time the cells migrate to form clumps (Fig. 1), but they do not proliferate. After 2.5–3 weeks, the cultures degenerate.

These cultures are highly enriched in GABAergic neurons, as indicated by glutamic acid decarboxylase (GAD) activity and γ-aminobatyric acid (GABA) content, which in mature cultures (≥ 1.5 weeks) are two to three times higher than in the brain in vivo [Yu et al., 1984]. Approximately 90% of the cells in cultures prepared in a very similar manner stain for GAD [K. Kuriyama, personal communication].

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