Physiol. Res. 41: 459–462, 1992

RAPID COMMUNICATION

Are Embryonal Neurones Used for Transplantation "Sufficiently Immature"?

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Received September 23, 1992
Accepted October 19, 1992

Summary

Live neuronal suspensions, prepared from the hippocampal region of donors aged 20 embryonal days, were observed in the Nomarski Differential Interference Contrast. Many neurones displayed profiles, resembling dendrites or axons and both principal hippocampal neurones (pyramidal and granular cells) were identified. For transplantation studies, donors of a younger embryonal age are thus recommended.

Key words

Hippocampus – Embryonal neurones – Neuronal suspension – Dendritic development

Optimum neuronal survival in the suspension grafting method coincides with the period of proliferation, migration and early differentiation for each neurone type (Björklund *et al.* 1983). For transplantation of rat hippocampal neurones, embryonal donors aged 18-20 days are frequently used (Mudrick *et al.* 1989, Tonder *et al.* 1989). It is the time when proliferation terminates and differentiation starts (Angevine 1975). Routine microscopical methods of viability testing and cell counting can show only cell bodies; axons and dendrites are not visible. It is therefore difficult to assess the actual stage of neuronal maturation in the grafted material. We employed a modified method of the phase contrast for living and unstained cells to examine the degree of maturation in the material used in our experiments.

To determine the exact age of embryos, the proestrus phase of the oestrous cycle was estimated in females of the LEP inbred strain according to their vaginal impedance (Bartoš 1977). Animals were then paired for 16-18 hours. The day after mating was taken as embryonic day 0.

Neuronal cell suspension was prepared from embryos aged 20 days, delivered by Caesarian section (Björklund *et al.* 1983). The middle portion of the CA1 area with adjacent dentate gyrus of the right and left hippocampus were dissected under a preparation microscope. The samples from all fetuses of one mother were pooled in the basic medium (0.6 % D-glucose in 0.9 % saline). After cutting into small pieces, the tissue was incubated in trypsin (0.1 % trypsin in the basic medium) for 20 min at 37 °C, washed several times by replacing the basic medium, and then dissociated by repeated pipetting through a Pasteur pipette with a fine opening (10-15 times). The resulting suspension was kept at room temperature. The viability of suspended neurones was routinely tested (Björklund *et al.* 1983) and usually 85-95 % of the cells were "viable". This percentage remained stable for several hours. For microscopic observation, a drop of live suspension was placed on a slide, coverslipped and photographed (Microscope Olympus Vanox-S and Nomarski Differential Interference Contrast Attachment).

Most of the cells we observed had large round somas with various profiles emerging from them. Most of these fibres were of uneven thickness, with the thickest part originating from the cell body, sometimes they were beaded and frequently branched. In many respects they resembled dendrites (Fig. 1A,B). Some of the fibres were thinner, and branched less frequently. The majority of them were not visibly connected to any

cell. They might represent sectioned axons (Fig. 1B). In a well dispersed suspension, cells with a typical shape of soma and dendritic branching pattern (i.e. granular or pyramidal cells) were identified. (Fig. 1B).

The degree of maturation of embryonal neurones considerably differed in our suspension material (Fig. 1A). All of the cells manifested some signs of immaturity (Fig. 2B), however, many of them appeared to be much more developed than might be expected from the Golgi impregnation study (Fig. 2A) (Pokorný and Yamamoto 1981). In the transplantation studies, the ratio of surviving neurones also depends on their stage of maturity. The high proportion of well developed neurones in our material from twenty-day-old embryos can explain the comparatively low percentage of surviving neurones in our previous transplantation studies (Pokorný *et al.* 1991). It therefore seems important to use donors of lower embryonal age for transplantation studies. On the other hand, neuronal suspensions with such a high degree of developed and well preserved dendritic trees, might be promising material for various electrophysiological and morphological studies.

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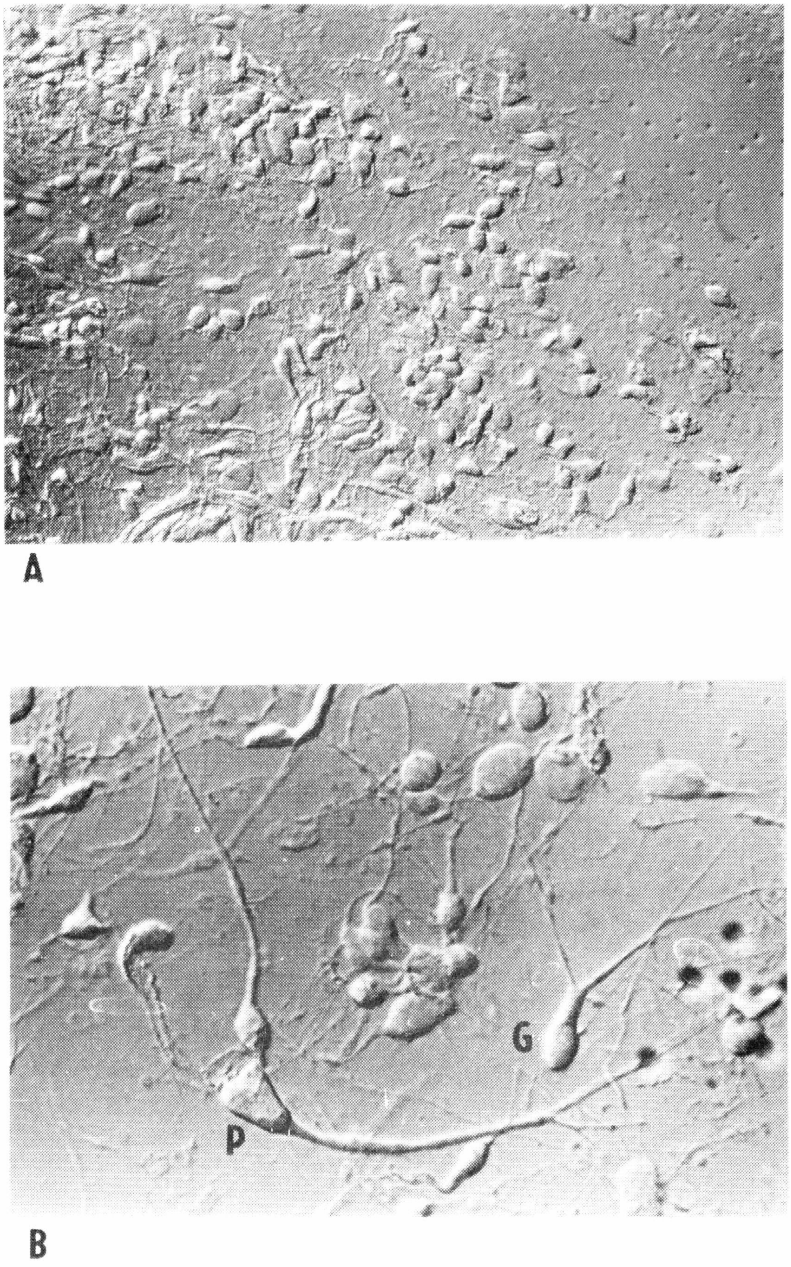

Fig. 1
Hippocampal cell suspension from embryos aged 20 days. A- Nomarski (x 250), B- pyramidal cell (P) and granular cell (G), Nomarski (x 500).

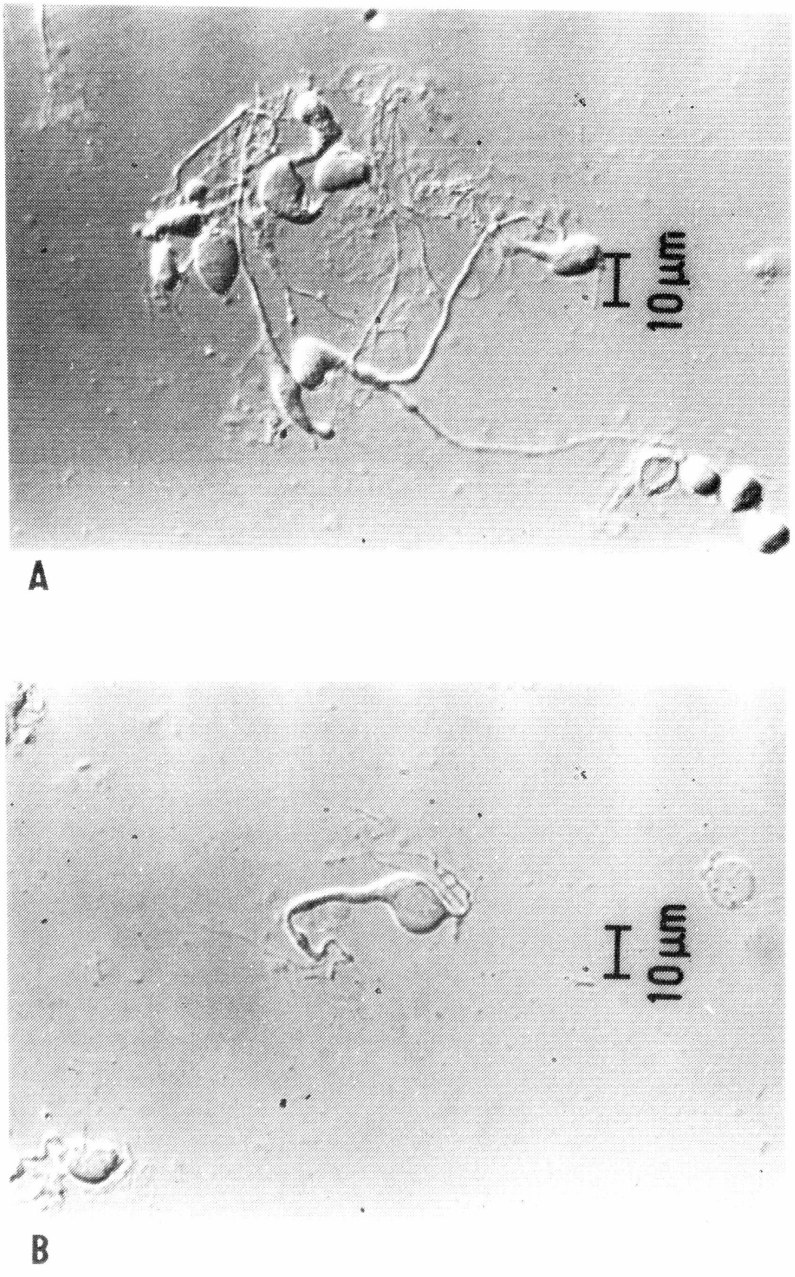

Fig. 2
Hippocampal cell suspension from embryos aged 20 days. A- "mature" granular cell, Nomarski (x 500), B- "immature" cell, Nomarski (x 500).