affairs (Fig. 3). The discreteness of organization of the ventricular zone of the brain shows similarity with the organization of the germinative zone of the olfactory epithelium and epidermis of the skin, which consists of proliferative loci, each of which forms a vertical column of cells [8, 11].

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MORPHOLOGICAL CHARACTERISTICS OF HIPPOCAMPAL NEURONS DEVELOPING IN CELL CULTURE

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Dissociated and reaggregated cultures of cells from different parts of the CNS of mammalian embryos are widely used nowadays to study the principles governing morphogenesis of neurons, glioneuronal interactions, oriented growth of axons, and the formation of interneuronal connections. Objects widely used for research of this kind are cell cultures of the embryonic hippocampus [3, 6, 7, 9, 10].

The aim of this investigation was to study the morphogenesis of neurons in cell cultures from the hippocampus of mouse embryos.

EXPERIMENTAL METHOD

A homogeneous cell suspension obtained by enzymic and mechanical dissociation of the hippocampal tissues of 18-19-day C57BL mouse embryos was applied to collagen-coated coverslips (about 3000-4000 cells/cm^2 of glass) and cultured in Maximow's chambers [1] at 35°C. The nutrient medium was changed once after the 3rd-4th day of culture. The cultures were investigated intravitally at various times in vitro by photomicrography and by time-lapse motion picture filming in a light field and in phase contrast, stained by Nissl's method, and impregnated with silver by the method of Holmes and Wolff.

EXPERIMENTAL RESULTS

Most cells immediately after application of the suspension to collagen were spherical in shape, and only a few were observed to give off short single processes. In the course of a

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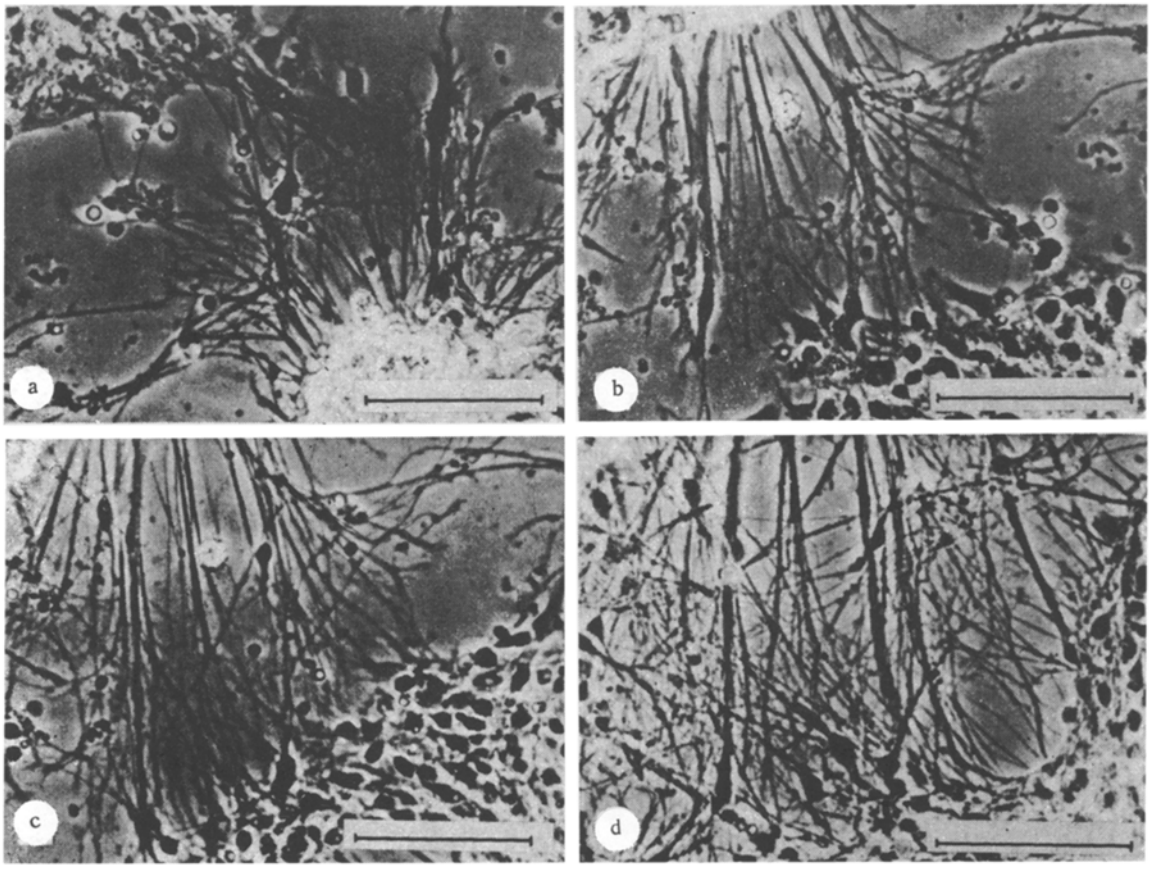

Fig. 1. Formation and fasciculation of processes accompanied by "drift" of aggregates in hippocampal cell culture. Fragments of time-lapse photomicrograph of the same region of a culture 48 h (a), 52 h (b), 60 h (c), and 84 h (d) from beginning of culture. Phase contrast. 200 ×. Scale 30 μ.

few hours of culture partial aggregation of the cells took place, with fixation to the supporting substrate. Toward the end of the 2nd day in vitro intensive formation of processes growing from the aggregates and single cells, and with cones of growth on their distal ends, was observed in vitro (Fig. 1a, b). During the next 2 or 3 days the processes grew longer and, in the form of bundles and separate fibers, bound the aggregates together (Fig. 1c, d). As the fibers grew and their fasciculation increased, the "drift" of some of the aggregates, described previously [11], was observed.

The formation of processes (dendrites) whose transverse diameter gradually decreased with an increase in the distance from the cell body and as division into smaller branches took place, was observed in neurons located mainly outside the aggregates. Cells in which one of the dendrites had a greater initial diameter and was longer than the rest (Fig. 2a, d) were morphologically similar to pyramidal neurons of the hippocampus developing in situ [3]. Besides neurons which acquired a dendritic system with well-defined polarity, multipolar and bipolar nerve cells also were formed, with an irregular system of processes. In some cells located outside the aggregates growth of only one process was observed: its transverse diameter, unlike that of the other processes, decreased sharply immediately after it left the body of the neuron, and thereafter remained constant throughout its length (Fig. 2d); this process could therefore be identified as an axon.

On further culture, diffuse networks and bundles of nerve fibers continued to be formed in and between the aggregates. Meanwhile the aggregates became paler and thinner, and were converted into a monolayer (Fig. 3a, b), containing concentrations of bodies of neurons and plexuses of their processes. Meanwhile discrete groups were formed from several independent neurons (Fig. 3c). In the later stages in vitro granule cells could be found in certain cultures (Fig. 3d). It must be emphasized that a Nissl's substance characteristic of neurons

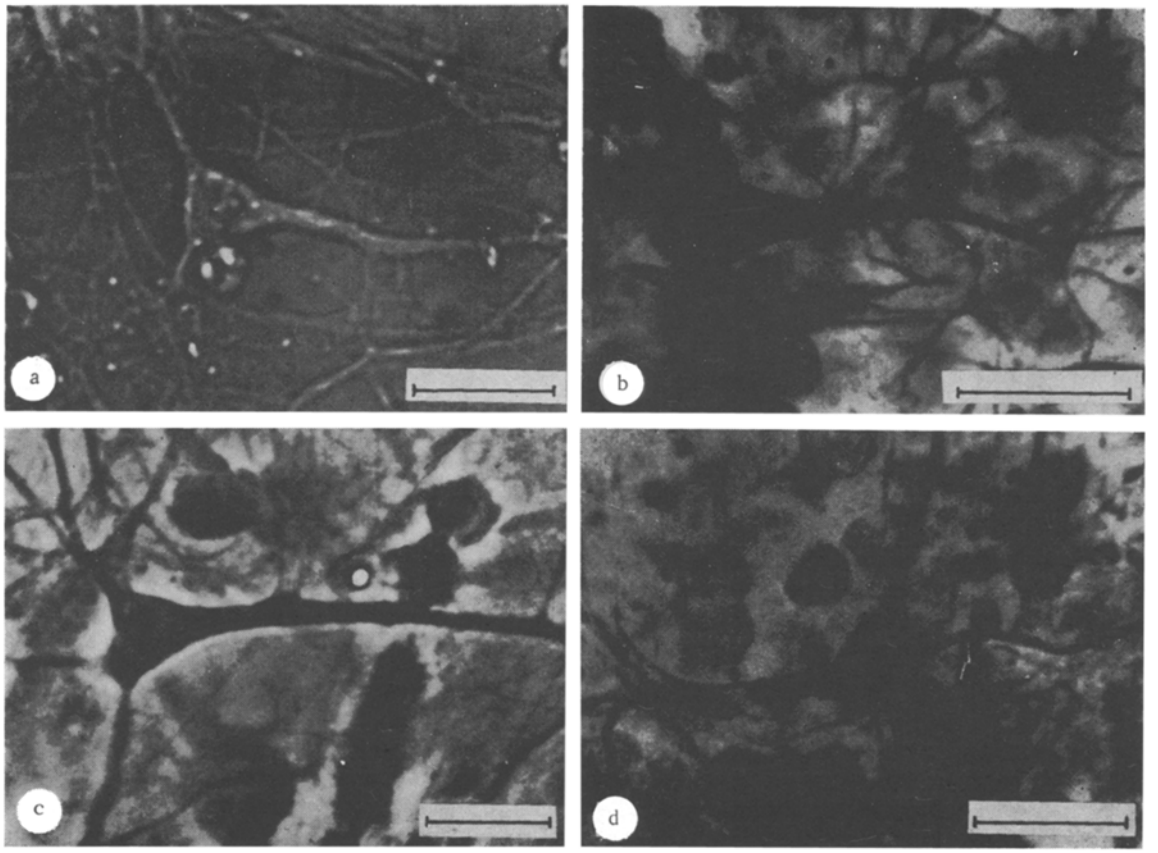

Fig. 2. Morphogenesis of pyramidal neurons in hippocampal cell cultures. a, b) Four days; c, d) 12 days in vitro. a, c) Living unstained cultures (a — light field, c — phase contrast); b, d) impregnation with silver by method of Holmes and Wolff. Arrow indicates axon. Magnification 400 ×. Scale 20 μ.

could be detected in the majority of the cultured cells, stained with cresyl violet, which were identified intravitally as nerve cells because of the size and shape of their body and the structure of the nucleus and character of the processes (Fig. 3a, b).

The results of time-lapse microfilming showed that the formation of the principal morphological features of isolated pyramidal neurons was complete by the 10th-12th day of culture, for during the next days in vitro no appreciable change took place in their shape or in the number and character of branching of the dendrites. It must also be pointed out that an invariable condition of long-term (more than 3 weeks) culture of both grouped and isolated cells was the presence of a flattened glial monolayer, formed between neurons and collagen during development of the cultures.

The results show that nerve cells of embryonic hippocampal tissue, developing in vitro in the absence of direct influences from afferent connections of the hippocampus, are capable of expressing a number of specific morphological features even in the early stages of culture (4th-6th day). The most distinct manifestation of this is the formation of neurons with the characteristic spatial organization of the dendrites, one of which, converted into an apical dendrite, gives the cell its pyramidal shape observed in the hippocampus in situ. It must be emphasized that we isolated the neurons at the stage of embryogenesis (18th-19th day) when the first afferent fibers are found in the hippocampus [8]. It can therefore be tentatively suggested that the growth of afferents toward the hippocampal pyramidal cells developing in situ, if it takes place before the beginning of culture, induces a process of dendrite formation and is one of the condions for subsequent development of dendrite systems specific for these neurons under conditions of deafferentation in vitro. This hypothesis is in agreement with data obtained on tissue and cell cultures of the cerebellum [2] and which are evidence of the triggering role of afferent fibers in the realization of programs of dendrogenesis,

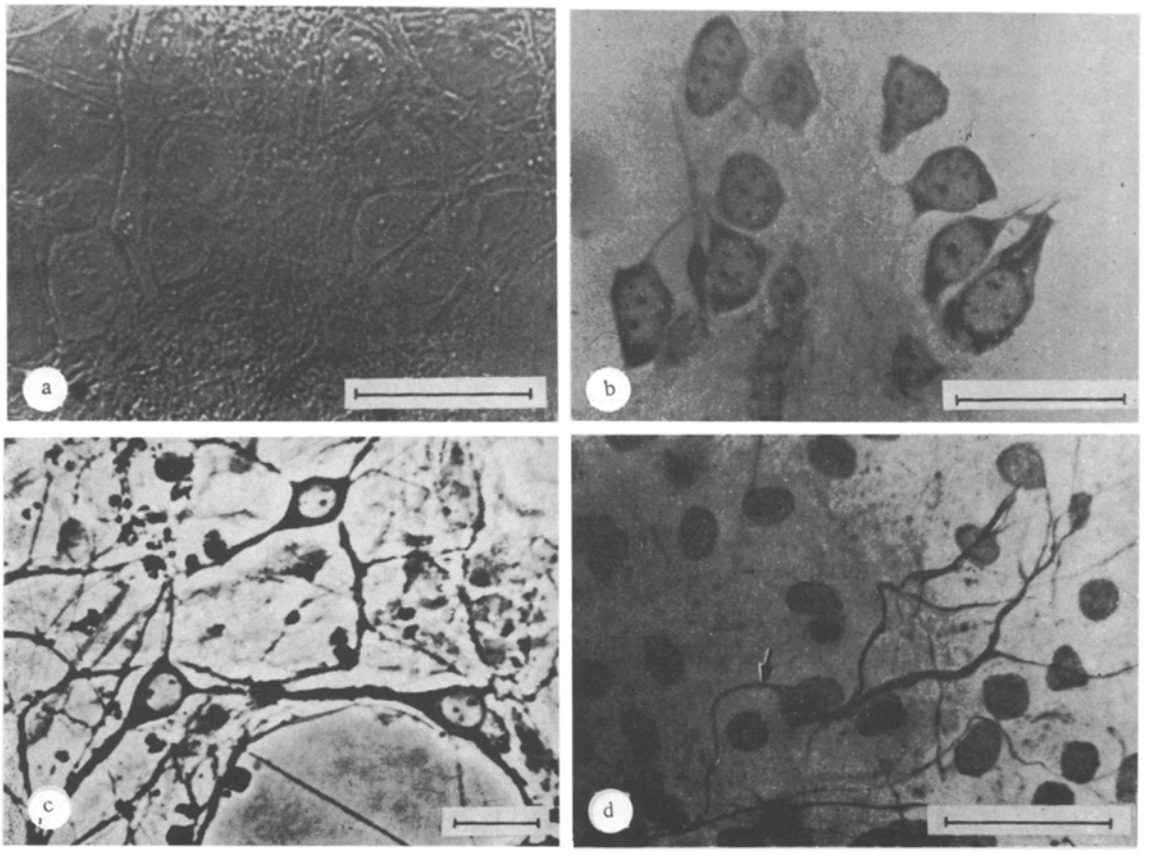

Fig. 3. Grouped and isolated neurons in hippocampal cell cultures. a, b) Groups of neurons in monolayer formed from an aggregate (a – living unstained culture, b – the same culture stained by Nissl's method; 19 days in vitro); c) isolated neurons (living unstained culture, phase contrast; 15 days in vitro); d) granule cell (impregnation with silver by the method of Holmes and Wolff, arrow indicates axon, 22 days in vitro). Magnification: a, b, d) 900 ×; c) 400 ×. Scale 30 μ.

which leads to the formation of neurons with a definite morphological phenotype. Meanwhile the development of other dendritic systems of the nerve cells, including some atypical for the hippocampus, accompanied by rapid and irregular growth and fasciculation of their axons, is evidence that plastic restructuring of neurons developing in culture can take place.

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