MORPHOLOGY OF AMMON'S HORN OF THE RAT HIPPOCAMPUS IN TISSUE CULTURE

E. G. Antonova, L. V. Voskresenskaya, T. M. Pankova, and M. B. Shtark

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The principles of morphogenesis of Ammon's horn of the neonatal rat hippocampus in tissue culture (area CA_{1-2}) were studied by light-optical and electron-microscopic methods. Analysis of 5-, 12-, and 21-day cultures revealed maturation of the hippocampal pyramidal cells in vitro, the development of a protein-synthesizing system, and synaptogenesis. The synchronous development of the synaptic structures, of the protein-synthesizing system of the cell, and of electrogenesis suggests that these are interconnected factors.

KEY WORDS: nerve tissue culture; synaptogenesis; protein-synthesizing system.

It has been shown [2] and confirmed [1] that spontaneous unit activity is formed and maintained during tissue cultures of the rat hippocampus (area CA_{1-2}) during long periods in culture. These findings indicate that the central neurons preserve their basic functional properties in tissue culture. During an investigation of the formation of the brain-specific protein spectrum in vitro the present writers also found that it corresponds to the spectrum in vivo [3]. Evidence was thus apparently obtained of the "physiological" and "biochemical" differentiation of central neurons in vitro.

In the investigation described below a light-optical and electron-microscopic analysis was made of some morphological features of cell differentiation of Ammon's horn of the rat hippocampus (from newborn animals and in early postnatal development) in tissue culture.

EXPERIMENTAL METHOD

Explants of the hippocampus of noninbred rats and Wistar rats and their cultivation have already been described [2]. Cultures at different times were studied and photographed in phase contrast and after intravital staining with methylene blue by Konovalov's method. Fixed cultures were stained by the Nissl, Bodian, and Holmes–Wolf methods. Material for electron microscopy was fixed for 24 h with 2% glutaraldehyde in 0.1 M phosphate buffer, pH 7.3-7.4, and postfixed for 2 h with 1% OsO_4 with the addition of glucose (0.045 g/ml fixative). The tissue was stained with uranyl acetate and embedded in Araldite. Sections were stained with lead citrate by Reynolds' method and studied in the JEM-100B microscope. The tissue was studied after 5, 12, and 21 days in culture. Brain sections from animals at the same ages and also taken from newborn rats or young rats aged 2-3 days served as the control.

EXPERIMENTAL RESULTS

The peripheral zone of the explants after 1-3 days in culture usually consisted of growing and regenerating processes of neurons and glial cells. Processes of the gliocytes were distinguished by their higher refraction of light and their lower degree of arborization. Active migration of fibroblast-like and glial cells (fibrous astrocytes), distinguishable from neurons by the eccentric position of the nucleus and the less marked granular pattern of the cytoplasm, into the peripheral zone was observed. Mature nerve cells usually did not migrate into the zone of growth. Because of the opacity of the explants at these times, they were studied in sections

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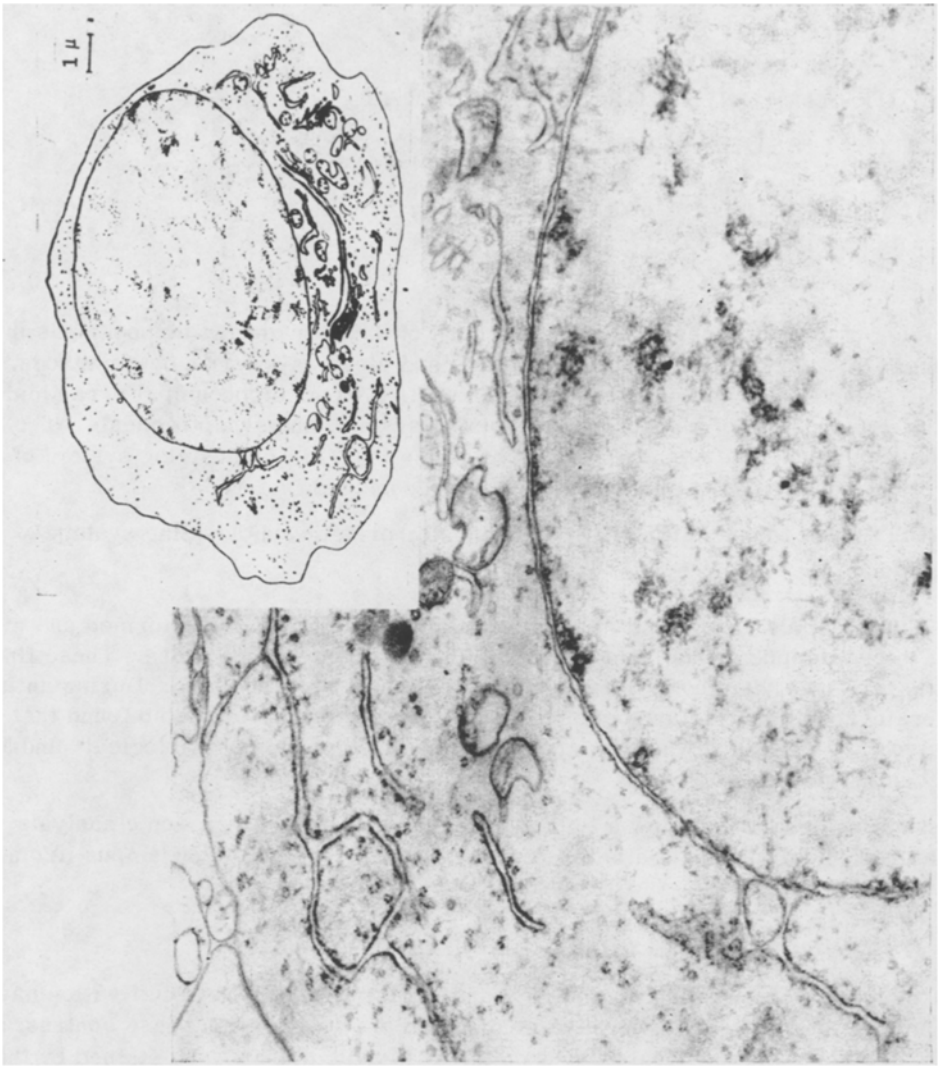

Fig. 1. Pyramidal neuron in Ammon's horn of hippocampus of 3-day-old rat. Scheme on left. Original magnification 4000×.

stained by Nissl's or Bodian's methods. In sections through explants at later stages of culture (1-2 weeks) three zones could be distinguished: an upper zone 40 μ thick, free from cells and containing neuropil, a middle zone 90 μ thick, including the principal cell concentrations, and finally, a deep region of necrosis, 40 μ thick. From the 12th-18th days of culture an "organotypical" pyramidal layer consisting of densely packed cells 15-22 μ in diameter, characterized by a slightly eccentric position of the large, pale nucleus, 1 or 2 nucleoli, and a definite orientation of the axon, could be identified at a depth of 60-80 μ.

At the 3rd week in tissue culture the explant was spread out, so that mature neurons with a perpendicular concentric arrangement of the Nissl's substance, characteristic of differentiated cells, could be observed.

On electron-microscopic investigation of the tissue of the rat hippocampus in the early postnatal period weak differentiation of the majority of neurons was observed (Fig. 1): They contained few organelles, the perinuclear spaces were wide, and the outer nuclear membrane had projections. The rough endoplasmic reticulum was often connected with the nuclear membrane. The mitochondrial cristae were ill-defined. Exclusively axodendritic synaptic boutons were observed (Fig. 1). After 3 weeks in culture most neurons were mature cells: Their bodies were clearly separated from one another by a narrow intercellular space, typical of their appearance in situ. The number of organelles in the cytoplasm was increased and neurofilaments appeared. The mitochondrial cristae were very well developed and the number of components in the Golgi complex was increased. The nuclei had the characteristic folded shape of neurons in tissue culture [5, 6] and the folds were filled with rosette-like polysomes. Many ribosomes were attached to the nuclear membrane (the nuclear

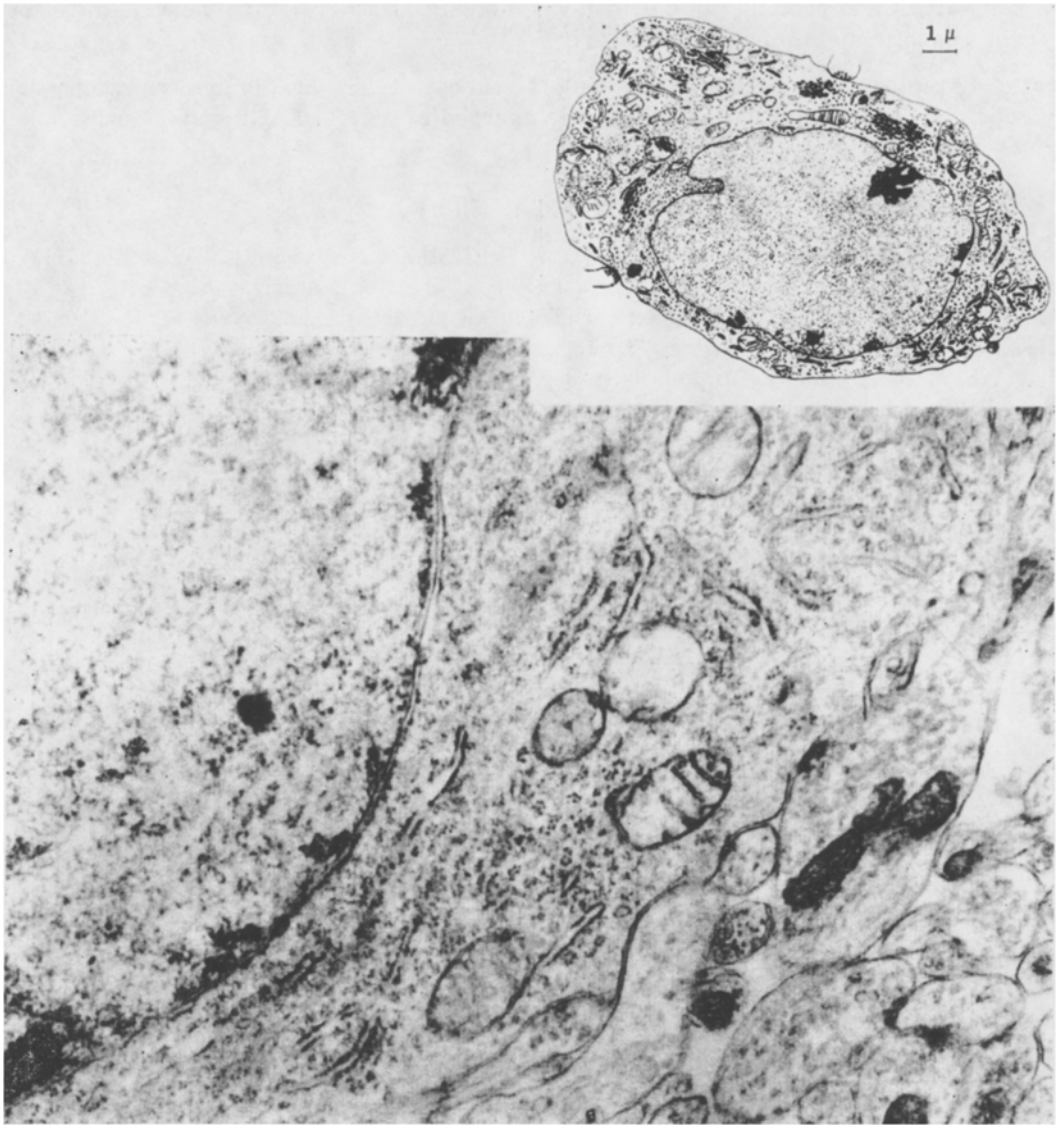

Fig. 2. Pyramidal neuron of Ammon's horn of hippocampus of 3-day-old rat, 18-day culture. Scheme top right. Initial magnification 4000×.

membrane in situ is most frequently free from ribosomes). Pores could be seen in the nuclear surface. The state of the organelles, the shape of the nuclei, and the presence of pores and ribosomes connected to the nuclear membrane are most likely evidence of increased protein synthesis in the neurons (Fig. 2).

In cultures at the same times (18th-21st days) numerous synaptic boutons were observed on the soma and large dendrites of the neurons (Fig. 2). Just as previously [9, 10], differentiation of the pyramidal neurons of Ammon's horn of the hippocampus was thus shown to occur in tissue culture, with the appearance of new synapses not present in the explanted material from newborn rats or rats in the early postnatal period. Granule cells of the dentate fascia were not specially identified in this study.

Synaptic axosomatic boutons must evidently be classed as inhibitory contacts owing their origin to axons of the hippocampal basket cells [4]. They were absent from the original material and were formed in tissue culture de novo. Their appearance in the 3rd week of culture coincided in time with the development of pyramidal cells in culture [8] and with activation of the protein-synthesizing system of the neurons. It is at this time that the brain-specific protein spectrum, which the writers studied immunochemically [3], is formed.

Three factors – synaptogenesis, intensification of protein synthesis, and the appearance of stable electrical activity – are synchronized in culture and develop independently of external afferent influences. There is reason to suppose that this is not accidental. Synaptogenesis and synaptic activity undoubtedly activate the

synthesis of polymers (RNA and proteins) in the postsynaptic cell, and this in turn facilitates the formation of the properties of the synaptic membranes of the neurons.

The formation of the basic properties of the central neurons of vertebrates in vitro independently of external factors means that a nerve tissue culture can be regarded as a closed cell system and a unique object for neurobiological research.

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