# FORMATION OF NERVE FIBRES BETWEEN ORGANOTYPIC EXPLANTS OF THE HIPPOCAMPUS AND MAMMILLARY BODIES OF NEWBORN MICE

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During combined organotypic culture of the neonatal mouse hippocampus and mammillary bodies, bundles of nerve fibers formed between the explants, some of them continuations of axons located in the alveus of Ammon's horn. It is suggested that hippocampal pyramidal neurons, deprived of their afferent inputs from other brain structures, in tissue culture remained capable of forming efferent projection connections characteristic of neurons of the intact hippocampus.
KEY WORDS: hippocampus; mammillary bodies; projection nerve fibers; tissue culture.

Previous investigations [1, 4, 5] showed that during combined culture of fragments of the spinal cord, brain stem, cerebellum, and forebrain, bundles of nerve fibers (axon bridges) form between the explants. On the basis of these observations, the processes of formation of projection connections between isolated explants of different brain structures, which are morphologically and functionally linked in vivo, have been reproduced in vitro and observations made on them.

One of the main projection systems of the hippocampus is formed by axons of pyramidal cells of Ammon's horn which terminate on neurons of the medial nucleus of the mammillary bodies [8]. The object of the present investigation was to study the potential ability of neurons of the isolated hippocampus, the postnatal differentiation of which was shown previously [3, 7] to continue in vitro, to form efferent projections to the mammillary bodies during combined culture of organotypic explants of the hippocampus and mammillary bodies of newborn mice.

## EXPERIMENTAL METHOD

The methods of organotypic culture of both the hippocampus and mammillary bodies were described previously [7, 9]. For combined explantation transverse sections of the hippocampus were arranged with the fimbria toward the dorsal edge of the explants of the mammillary bodies (Fig. 1). Paired explants were cultured under conditions similar to those used for the culture of single explants. The cultures were studied intravitally and impregnanted with silver by Wolf's modification of Holmes' method [10] and by Bodian's method [6].

## EXPERIMENTAL RESULTS

The morphology of the living neurons and the fibrous structure of the organotypic explants of the neonatal mouse hippocampus were described by the writer previously [2]. On the basis of the experimental results it was suggested that during culture bundles of nerve fibers characteristic of the intact hippocampus form in these explants, and that one of these bundles is a system of efferent projection connections of the entorhinal cortex (the perforant path), whereas the other, located in the alveus, is formed by axons of pyramidal cells of Ammon's horn.

In living cultures of the mammillary bodies single and grouped nerve cells located both in the central parts of the explants and in its peripheral zone could be observed after the 8th-10th day of culture. In explants of the mammillary bodies impregnanted with silver on the 10th-12th day of culture or later, nerve fibers were seen to be arranged diffusely or as bundles, and nerve cell bodies also were observed.

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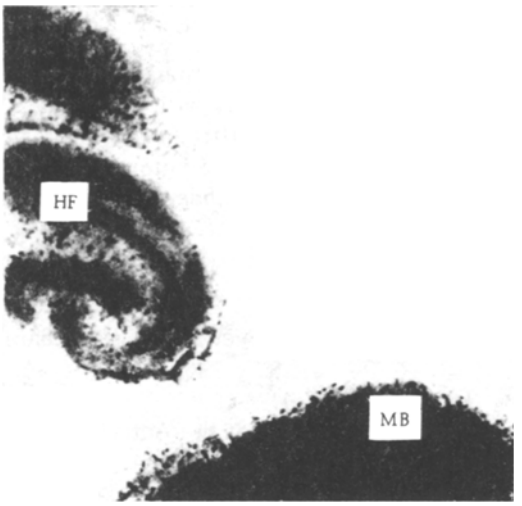


Fig. 1. Transverse section through hippocampal formation (HF) and mammillary bodies (MB) explanted for combined culture. Photomicrograph taken on day of explantation.

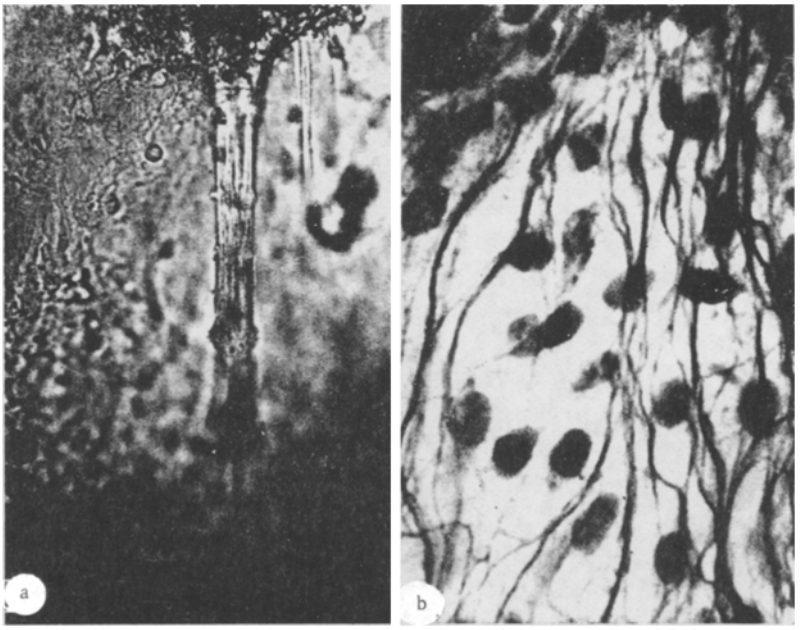


Fig. 2. Fibrous structures formed between explants of hippocampus and mammillary bodies: a) living unstained culture (7 days in vitro, 35×); b) nerve fibers impregnated with silver by Bodian's method (16 days in vitro, 900×).

During combined culture of the hippocampus and mammillary bodies fibrous structures began to form between the explants after the 4th-5th day in vitro (Fig. 2a). The most intensive migration of neuroglial cells took place from areas of the explants facing one another; according to Viktorov [1], this is evidence of the active participation of the neuroglia in the formation of "axon bridges" between the paired explants.

Impregnantion of the cultures on the 10th-12th day after explantation revealed bundles of nerve fibers leaving the hippocampus and penetrating to different depths into the mammillary bodies (Fig. 2b). Since tissue fragments used for combined explantation were completely isolated from each other it is evident that the formation of these bundles was due to growth of nerve fibers outside the explant and took place during culture. These bundles were observed to contain unbroken nerve fibers which were continuations of fibers located in the alveus of Ammon's horn and which thus were axons of hippocampal pyramidal neurons. It can tentatively be suggested that the nerve cells of the mammillary bodies induced the oriented growth of these axons, for if the hippocampus is cultured in the absence of the mammillary bodies, axons of the nerve cells were not

formed into bundles on leaving the explant, but spread from it in different directions.

The present results are evidence that neurons of the neonatal mouse hippocampus, under the conditions of organotypic culture, remain capable of forming projection nerve fibers not only with the explant itself (the perforant path), but also toward explants of the mammillary bodies. The possibility of the formation of projection connections characteristic of the hippocampus in vitro points to the relative autonomy and plasticity of the processes of oriented growth of axons of nerve cells despite the complete absence of afferent influences from surrounding brain structures.

## LITERATURE CITED


1. I. V. Viktorov, in: Functional and Structural Bases of Activity of Brain Systems and Mechanisms of Brain Plasticity [in Russian], No. 5, Moscow (1976), p. 78.
2. I. V. Viktorov and L. G. Khaspekov, Neirofiziologiya, No. 4, 384 (1976).
3. L. G. Khaspekov and I. V. Viktorov, in: Functional and Structural Bases of the Activity of the Brain System and Mechanisms of Brain Plasticity [in Russian], No. 4, Moscow (1975), p. 404.
4. C. Allerand, Exp. Neurol., 25, 482 (1969).
5. S. Crain and E. Peterson, Science, 188, 275 (1975).
6. S. Kim, Z. Zellforsch., 107, 454 (1970).
7. J. H. La Vail and M. Wolf, Am. J. Anat., 137, 47 (1973).
8. G. Raisman, W. Cowan, and T. Powell, Brain, 89, 317 (1966).
9. H. Sobcowicz, J. Neurocytol., 3, 431 (1974).
10. M. Wolf, J. Cell Biol., 22, 259 (1964).