Characteristics of the Septohippocampal Cholinergic GABA-ergic Neurons Using Retrograde Marker and Immunochemical Tests

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UDC 612.825.266.017.1.083.3

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol.117, № 2, pp. 143-145, February, 1994. Original article submitted September 27, 1993.

It is shown that a large number of immunopositive neurons possess hippocampal projections; there are many immunonegative cells on sections as well. The correlation between choline acetyltransferase- and parvalbumin-positive neurons and retrograde and double-labeled neurons is demonstrated to be much the same, thus favoring the method used.

Key Words: *mouse hippocampus; cholinergic and GABA-ergic neurons*

Routine immunocytochemical methods do not make it possible to identify two neurotransmitter systems simultaneously and their projections from one brain structure to another. The recent introduction into the research practice of the WAHG-complex (Wheatgerm Agglutinin apo-Horseradish peroxidase - Gold), transported retrogradely only [2], has made it feasible to determine and characterize simultaneously both choline- and GABA-ergic neurons of nuclei in the medial septum/diagonal fascicle (MS/DF) complex which possess hippocampal projections.

MATERIALS AND METHODS

Experiments were carried out on 10 albino mice 10-14 weeks old. After anesthesia (ketamine in a dose of 260 mg/kg) and craniotomy, the animal was placed in a stereotaxic apparatus and WAHG label was injected unilaterally into the CA1 and CA2 hippocampal regions chosen according to the atlas of the mouse brain [8]. Each animal received 3-5 injections of 0.3-0.5 μl WAHG complex over 3-5 min to avoid the reuptake of the complex into a capillary.

Five days postoperation the animals were anesthetized with nembutal (300 mg/kg) and perfused transcardially (for 15 min). The brain was then removed and postfixed in solution without glutaraldehyde for 2 h. Brain sections (28-32 sections of 40 μ) of the MS/DF region were obtained using a vibratome (Vibratome, series 1000, USA).

The immunocytochemical reactions were performed using monoclonal antibodies (MA) against choline acetyltransferase (CAT, dye: diaminobenzidine, DAB) and against parvalbumin (PA, dye: 4-chlorine-1-naphthol) as labels of cholinergic- and GABA-ergic [6] brain neurons, respectively.

In accordance with the standard protocol [3], reactions were performed first for CAT visualization and then for PA. Staining with silver was performed finally for visualizing the WAHG complex injected into the hippocampus and transported retrogradely to the septum. The procedure of immunochemical determination was described in detail previously [5].

The count of labeled cells of MS/DF nuclei was performed under a light microscope (×160). The total number of retrogradely labeled neurons and the number of double-labeled neurons (retro-

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0007-4888/94/0002-0142$12.50

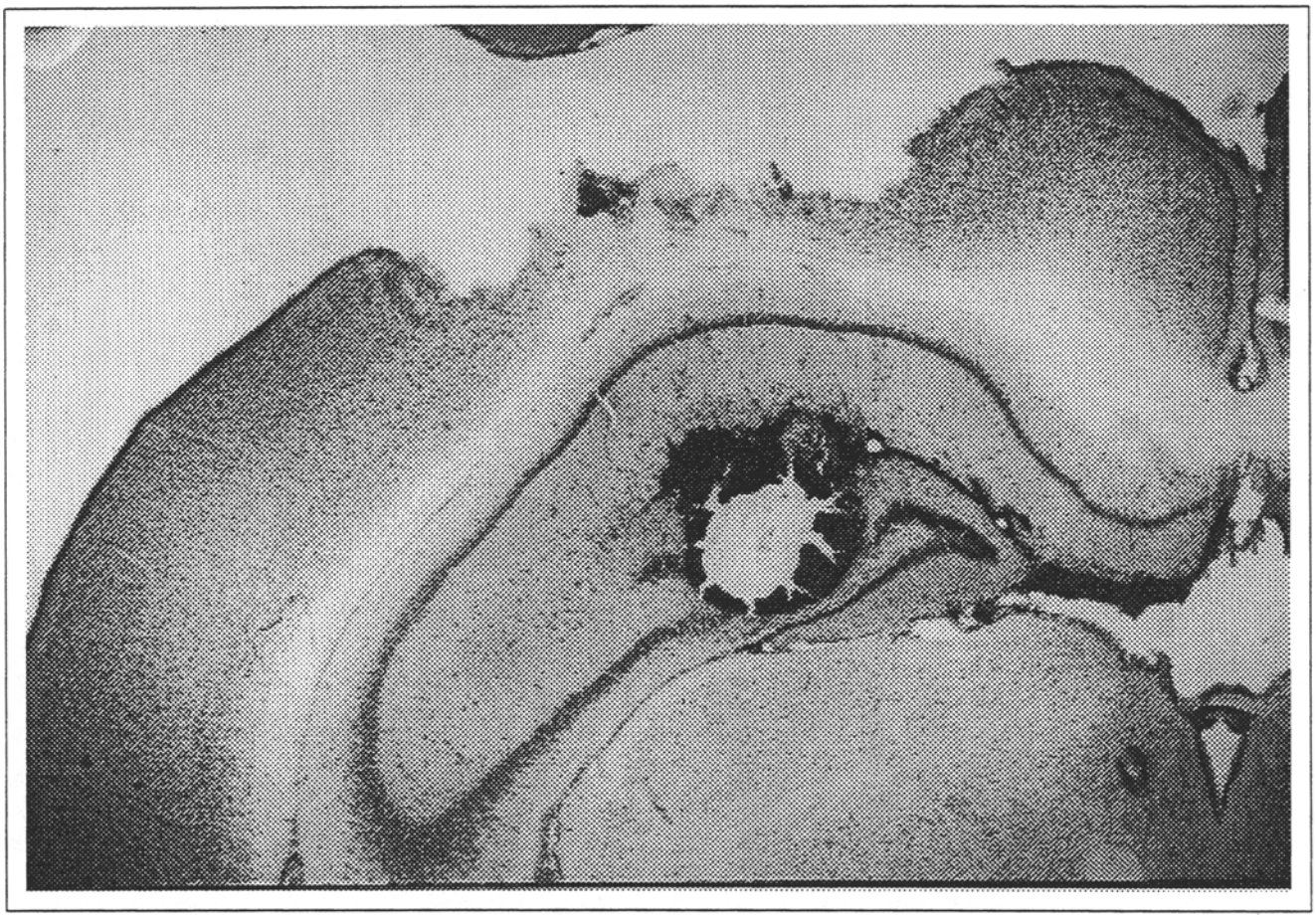

Fig. 1. Section of mouse hippocampus (40 μ) in the region of injection of WAHG complex.

gradely labeled and immunopositive) and their percent ratio were calculated for quantitative assessment on 7 rostracaudal equidistant successive preparations chosen from all septum sections of one animal.

RESULTS

Cholinergic and GABA-ergic neurons with projections to the hippocampus were found on the same sections of the MS/DF nucleus complex after injection of WAHG complex into the hippocampus (the region of injection is seen on Figure 1. Retrogradely labeled neurons (Fig. 2) contain striking black granules that are clearly noticeable both in CAT-positive neurons stained brown with DAB and in blue PA-positive neurons stained with 4-chlorine-1-naphthol. We did not find neurons containing both transmitters, which agrees with data obtained previously by other methods [1,4].

The results of the quantitative assessment showed that nearly 30% of all retrogradely labeled neurons are CAT-positive (30.3±4% unilateral and 27.9±5% contralateral), whereas 5-8% (4.6±2% unilateral and 8.2±2% contralateral) are PA-positive. Among the total number of immunopositive neurons 40±2% unilateral and 7.4±2% contralateral CAT-containing septal cells project to the hippocampus, while PA-containing neurons have 18.4±4 and 6.5±2% hippocampal projections, respectively.

Thus, we were able to reveal and quantitatively assess CAT- and PA-positive neurons on the same preparation due to the use of the WAHG complex, which is transported retrogradely and just requires a silver reaction for its visualization (rather than additional staining), together with the method of dual immunohistochemistry for CAT and PA. Analogous experiments carried out on rats also found that around 35-45% of MS neurons are cholinergic and project to the hippocampus [1,7]. At the same time, the number of PA-positive neurons proved to be markedly greater. The cause of these differences may be the different anatomy of the mouse and rat brain.

The findings attest that rather a large number of immunopositive neurons have projections to the hippocampus. However, such connections may also exist in the hippocampus itself, and it is not possible to conclude from data whether these projections are direct or contralateral as related to unilateral projections.

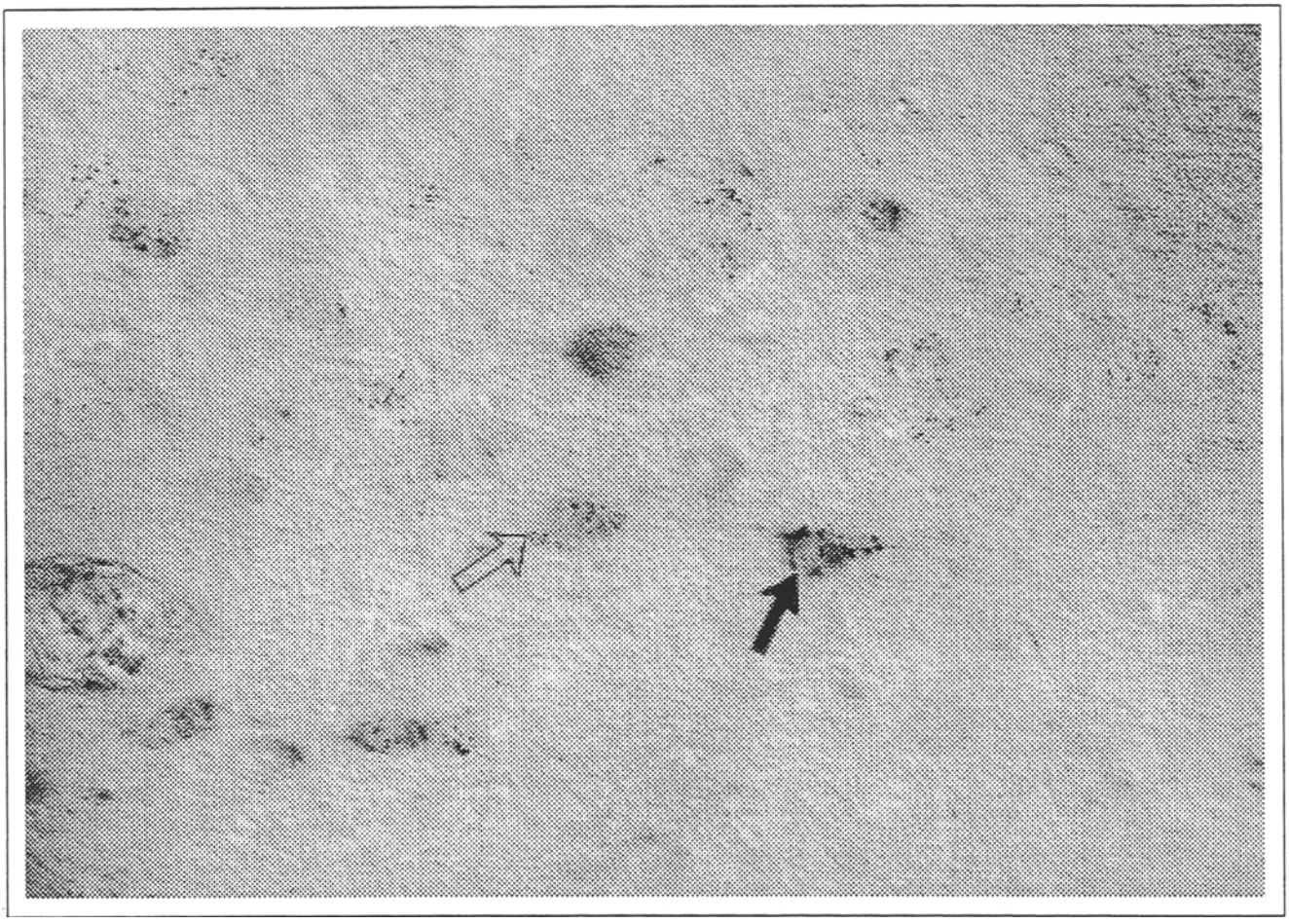

Fig. 2. Mouse medial septum. Retrogradely labeled (the black arrow points to black granules) and PA–positive (white arrow) neurons.

There are also many immunonegative cells on the sections. It is known [1] that some 22% of MS/DF neurons contain galanin, in some cases colocalized with CAT. Its presence may hamper the staining. Moreover, broad individual variations in nonlinear animals may make for a high error of determination and obscure the picture. Nevertheless, the correlation between CAT- and PA-positive and retrogradely labeled and double-labeled (retrogradely and immune) neurons is much the same. This proves the informativness of the method and its usefulness for characterizing the brain of various genetic lines of mice.

The authors are indebted Dr. G. Wiener and D. T. Laroux for their advice and technical assistance in WAHG-complex injecting; the Hoffman-Laroche Foundation for financial support; R. Linke, G. Schmidt, and G. Bance for admirable methodological assistance and M. Winter for fine pictures. The Schwegler study was financed by the German Society of Naturalists (SFB 325-B12).

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