

NEURONAL SELECTIVITY OF RABIES VIRUS INFECTION STUDIED BY PRE-EMBEDDING ULTRASTRUCTURAL IMMUNOCYTOCHEMISTRY Orlando Torres-Fernández (1), Gloria E. Yepes (1), Ladys Sarmiento (1), Javier E. Gómez (1), Hernán J. Pimienta (2). (1) Laboratorio de Microscopía, Instituto Nacional de Salud, Bogotá, Colombia. (2) Centro de Estudios Cerebrales, Facultad de Salud, Universidad del Valle, Cali, Colombia. E-mail: otorresf@ins.gov.co

Rabies virus (RV) infection in the pyramidal neurons of the cerebral cortex has been easy to demonstrate by means of the histological observation (1). Nevertheless, if RV infects cortical interneurons (non pyramidal neurons) is unknown. These cells cannot be distinguished for routine histological methods neither by means of the conventional electron microscopy. Therefore, it is necessary to use specific neuronal markers. Three calcium binding proteins (CaBPs): calbindin (CB), calretinin (CR) and parvalbumin (PV) are found in cortical interneurons but not in pyramidal cells. In general each type of interneuron contains only one of the CaBPs (2). In this work a pre-embedding ultrastructural immunocytochemistry method was used to determine if RV infects the interneurons of the mouse cerebral cortex and the rate of infection in each cellular population distinguished by CaBPs immunoreactivity. Female, 4-week-old ICR mice were inoculated intracerebrally with RV isolated from a rabid dog. Moribund animals were anesthetized and they were sacrificed by means of transcardiac perfusion with buffered 4% paraformaldehyde/0.1% glutaraldehyde solution. The brains were removed and cut at 100 μ m on a Vibratome. Rostrocaudal coronal sections at level of the frontal cortex were obtained. Free-floating sections were processed by immunohistochemical method using specific antibodies for each one of the CaBPs (3). The immunoreactions were revealed by incubating the sections for 2 minutes in diaminobenzidine (DAB) then the immunostaining was intensified with 1% osmium tetroxide (OsO_4) during 30 minutes. After washing, sections were dehydrated in a graded ethanol series and flat-embedded in Durcupan resin between glass slides and coverslips coated with silicone. After polymerization the coverslips were removed with a razor blade and the sections were examined by light microscopy. Parts of the sections containing immunostained neurons were cut out with a razor blade and attached to epoxy resin blanks with cyanoacrylic glue (4). The semi-thin sections were stained with 1% toluidine blue. Thin sections were stained with uranyl acetate and lead citrate and examined in a Phillips CM10 electron microscope. The immunostained neurons were easily distinguished in the semi-thin sections by their dark brown color (Figure 1). These same neurons were then observed and photographed in the electron microscope at low magnification. They exhibited electro-dense nucleus and cytoplasm and were easily distinguished of the surrounding neuropil and of the non immunostained neurons (Figure 2). Some neurons showed less electro-dense content probably because they contained low concentrations of CaBPs (Figure 3). When increasing the magnification it was possible to observe the viral particles inside the immunostained cells (Figures 4-6). Rabies viral particles within all types of the CaBPs immunoreactive interneurons were observed in the following rates: in 81% of PV+ neurons (17/21) as well as in 74% of CR+ cells (14/19) and in 46% of CB+ neurons (6/13). In total the RV was observed in 70% of the interneurons examined (37/53). With this work it has been demonstrated, for the first time, that the RV infects the non pyramidal neurons of the cerebral cortex. Therefore, the presence of RV receptor molecules in the interneurons is suggested. These results constitute a significant contribution to the knowledge of the rabies pathogenesis. Also, this work has showed the utility of the CaBPs as neuronal markers that can help in the ultrastructural co-localization of infectious agents, especially virus, inside specific neurons.

References

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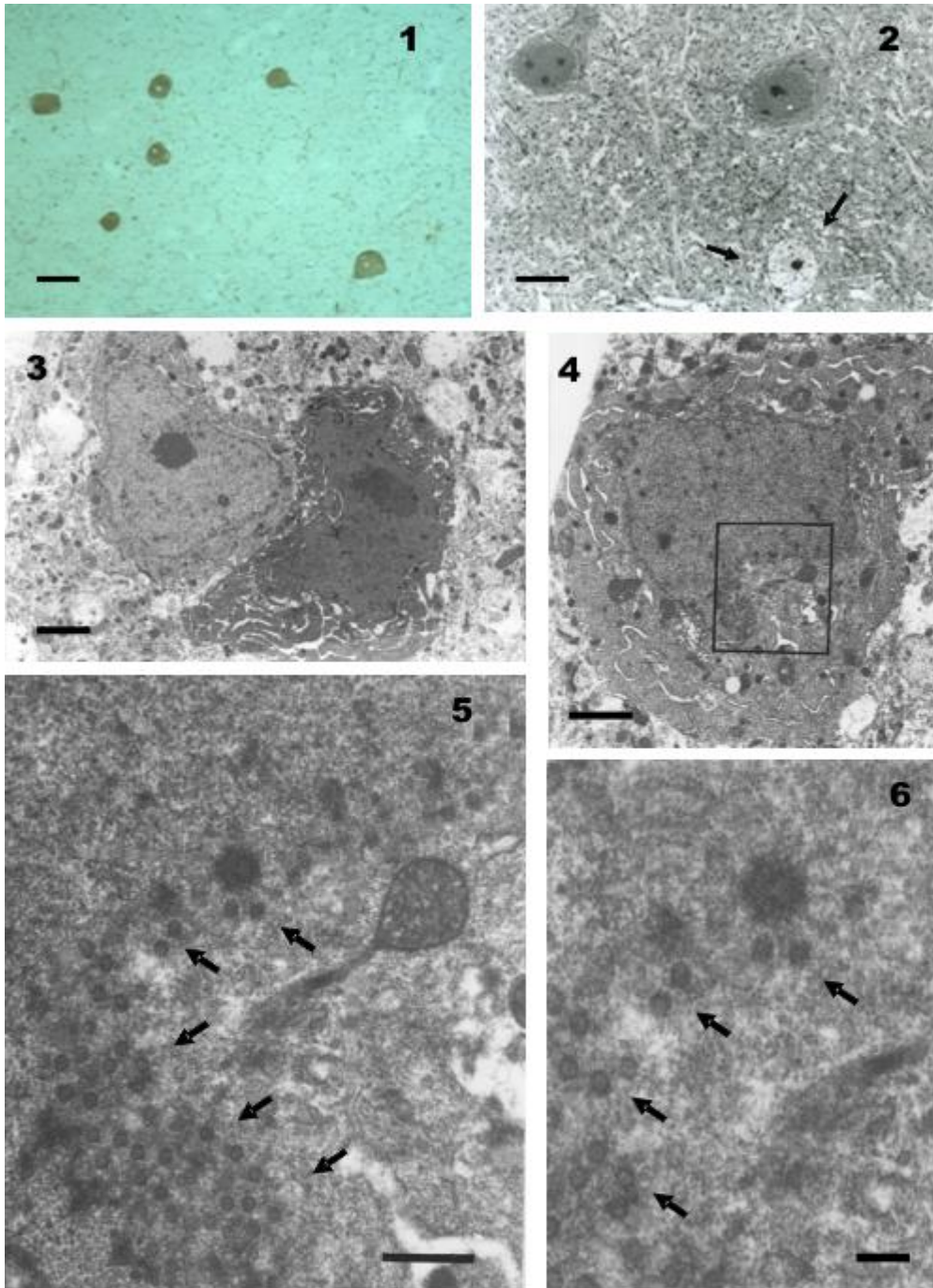


Figure 1. PV+ neurons are showed in a semi-thin section. **Figure 2.** Two electro-dense PV+ neurons and a non immunostained cell (arrows). **Figure 3.** Two CR+ cells show different staining intensity. **Figures 4-6.** Sequence of images from a CR+ neuron infected with RV. The area framed in the Fig 4 is magnified in the Figs 5 and 6. RV particles are pointed out by arrows. Scale bars: Fig 1 = 15 μm ; Fig 2 = 7 μm ; Fig 3 = 1.7 μm ; Fig 4 = 1.5 μm ; Fig 5 = 0.47 μm ; Fig 6 = 0.25 μm .