Infection of Hemagglutinating Encephalomyelitis Virus (HEV) at the Visual Pathways of Rats

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1. INTRODUCTION

Hemagglutinating encephalomyelitis virus (HEV) is neurotropic coronavirus causing vomiting and wasting disease or encephalitis in piglets (Andries and Pensaert. 1981). In the infected animals, the virus reaches the CNS through the nerve pathways from peripheral nerve. Our previous studies have demonstrated that HEV propagated through nervous route and its infection was restricted to neurons after inoculation into sciatic nerve or footpad of rats (Hirano et al., 1995, 1998). Our findings suggest that HEV is useful as a trans-synaptic tracer for analyzing neuronal connections in the CNS. The present study was performed to examine and evaluate the usefulness of HEV as a trans-synaptic tracer in the visual pathway.

2. MATERIALS AND METHODS

Plaque-purified HEV 67N strain was propagated and assayed for infectivity in SK-K cells as described previously (Hirano et al., 1990). Specific pathogen free of 6- to 8-week-old Wistar male rats were used. Using microsyringe, 5 μl (5 x 10^4 PFU) was inoculated into vitreous body of

The Nidoviruses (Coronaviruses and Arteriviruses).
Edited by Ehud Lavi *et al.*, Kluwer Academic/Plenum Publishers, 2001.

the right eye of rats under deep anesthesia with halothane. Animals were perfused with a fixative containing 4% paraformaldehyde in 0.1M phosphate buffer under deep anesthesia with pentobarbital. The brain sections were obtained on a freezing microtome, reacted with anti-HEV 67N mouse antibody (1:1000) at 4C overnight, and then stained with FITC-conjugated goat serum (anti-mouse IgG) at room temperature for 2 hours. The stained sections were examined under a confocal laser scanning microscope.

3. RESULTS

On day 3 after inoculation, the virus antigen was found in the retina (RT), dorsal lateral geniculate nucleus (DLG), superior colliculus (SC), and primary visual cortex (VC). In the inoculated eye, immuno-reaction was detected in vitreous body. Retinal ganglion cells as well as neurons in the inner nuclear layer were antigen positive (Fig. 1a). No antigen was found in other layers including pigmental epithelial cells. A small number of neurons in DLG were shown to be positive (Fig.1b). A few neurons in SC were immuno-positive (Fig. 2a). The morphology of antigen positive neurons in SC and DLG exhibited typical shape demonstrated with Golgi-impregnation as described in a textbook (Sefton and Dreher, 1994). Only few neurons in VC were positive (Fig. 2b). On day 7, antigen-positive neurons in SC and VC increased in number (Figs. 3a and 3b). In SC, the antigen-positive neurons and their dendrites located in the optic layer and superficial grey (Fig. 3a). In VC, positive neurons distributed in the cortical column with about 300μm in width in a patchy like fashion (Fig. 3b). The virus antigen was detected predominantly in neurons but not in glial cells including ependymal cells. During this experiment, HEV infected neurons appeared to be without cytopathological changes.

4. DISCUSSION

After direct inoculation of HEV into eyeball, the antigen positive neurons were found in the RT, DLG, SC and VC on day 3. Latter, the number of HEV-positive neurons in DLG, SC, and VC increased with time dependent pattern. These nuclei are known to be retino-geniculo-cortical visual pathway. Such findings described above demonstrate that HEV propagation and progression is based on anterograde/trans-synaptic transport.

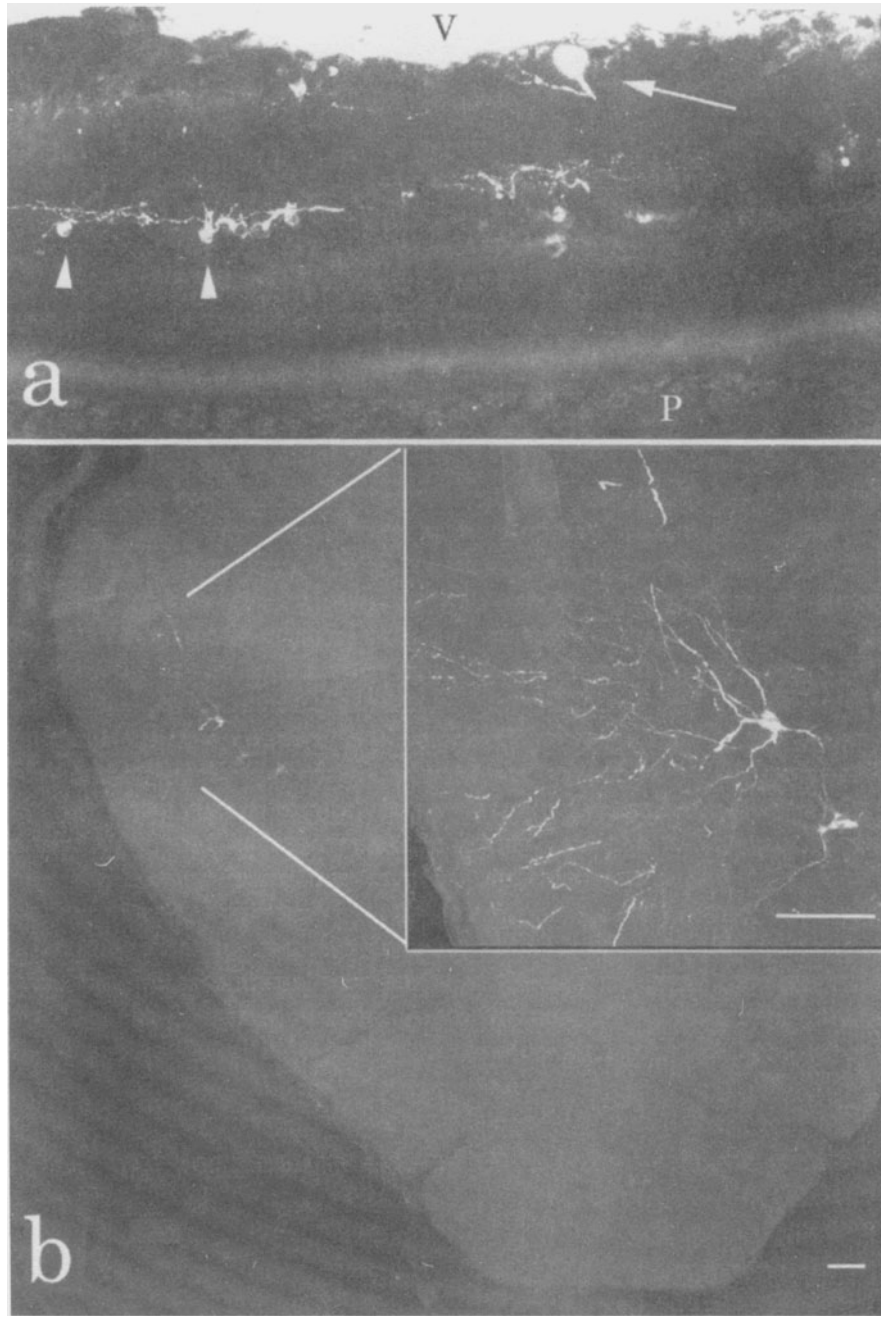

Figure 1: Day 3, a) Immuno-positive ganglion cells (arrow) and neurons in the inner nuclear layer (arrow heads) of retina. P: pigmented epithelial cells; V: vitreous body; b) A typical Immuno-positive neuron in dorsal lateral geniculate nucleus, Bar: 100 μm

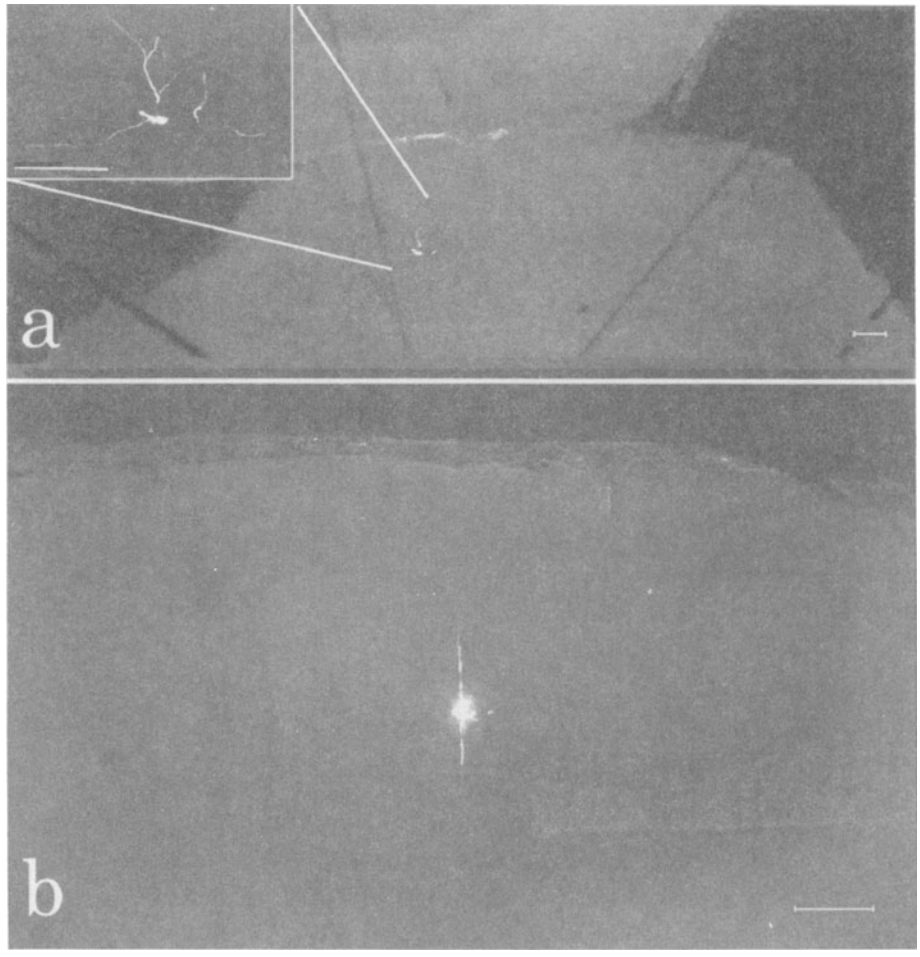

Figure 2: Day 3, a) An antigen positive neuron in the superior colliculus. b) Primary visual cortex, Bar: 100 µm

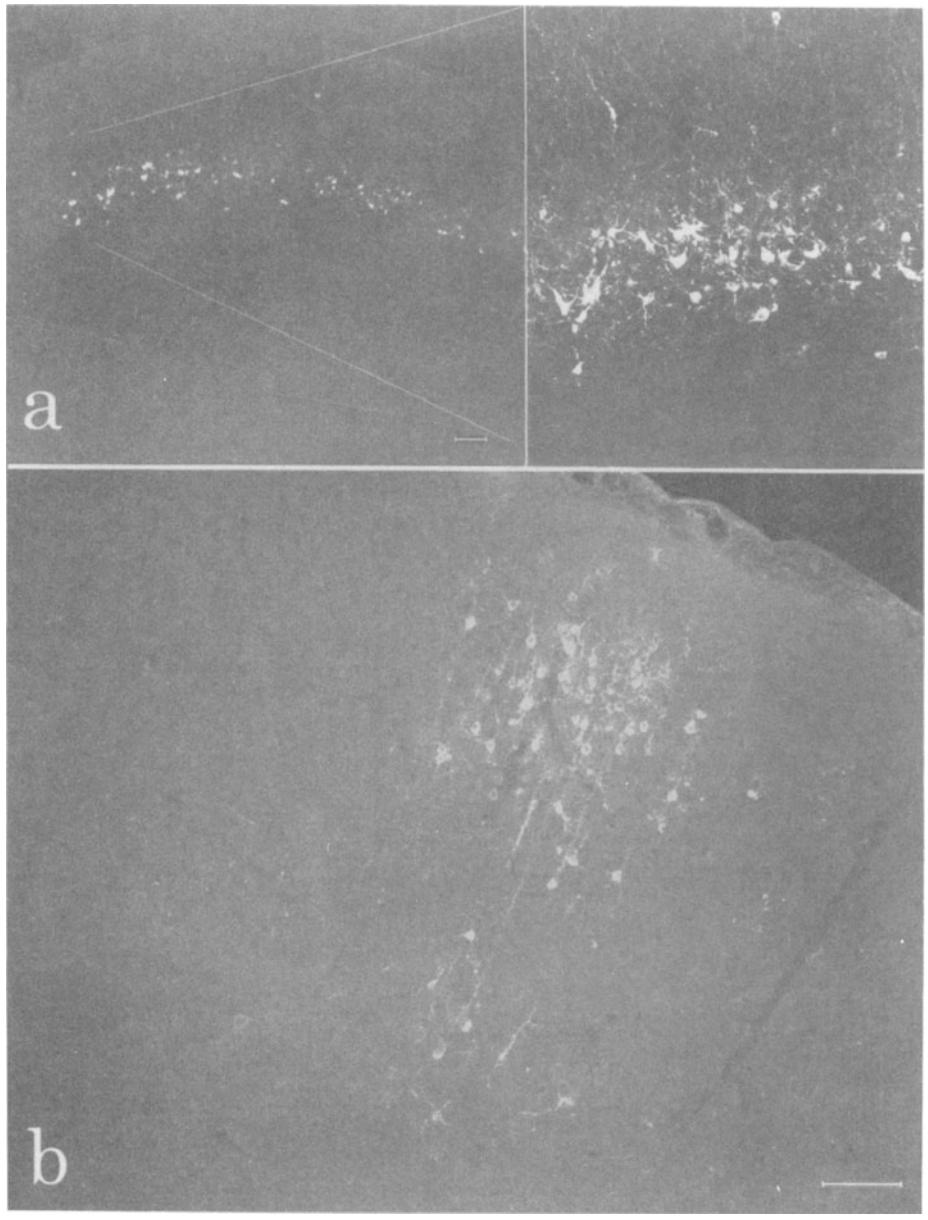

Figure 3: Day 7, a) Superior colliculus; b) Primary visual cortex. Immuno-positive neurons increased in number (see Fig. 2). Bar: 100 μm

Our observations in the present study is coincident with the results of the report on the visual system of mice infected by intraocular route with herpes simplex virus (Sun et al., 1996). HEV antigen was detected predominantly in neurons, but not in glial cells, of visual pathway nuclei in the present study. This result indicates that HEV is confirmed to be extremely neurotropic different from other viruses including mouse coronavirus (MHV), which infects not only neurons but also surrounding glial cells (Lavi et al., 1988). In other word, HEV is a causative agent which has strict neurotropism. In addition, no distinct degenerating changes were found in HEV infected neurons, suggesting that the fiber connections along visual pathway kept intact as far as the period examined. Such properties of HEV have the advantage of analyzing neuroanatomical connections in the visual pathways as a trans-synaptic tracer.

ACKNOWLEDGMENTS

This study was supported by Grands-In-Aids (No.11660309) from the Ministry of Education and Cultures of Japan and by Japan Society for the Promotion of Science.

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