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Herpes Simplex Virus Infection of Motor Neurons: Hypoglossal Model

HARRY OPENSHAW†* AND WILLIAM G. ELLIS

Department of Neurology and Pathology, University of California, Davis, Davis, California 95616

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Herpes simplex virus type 1 (HSV) was inoculated into the tongue muscle of A/J mice. Typical HSV vesicles developed on the tongue surface 4 days after HSV inoculation. Virus was isolated from hypoglossal nerve explants, and inflammatory cells appeared where the hypoglossal nerve exits from the ventral medulla. HSV viral capsids were present in astroglial cells near the point of nerve exit. A focal encephalitis ensued with immunoperoxidase staining of HSV antigens in neurons of the hypoglossal nucleus. These findings indicate that HSV can penetrate the neuromuscular junction, travel in a pure motor nerve, and produce a focal encephalitis in the corresponding central nervous system motor nucleus.

Herpes simplex virus (HSV) is known to spread in sensory axons and infect sensory neurons in ganglia of the peripheral nervous system (PNS) (17). Similar infections of autonomic nerves and ganglia have been documented in experimental animals and in humans (14, 22). In contrast to the sensory and autonomic nervous systems, HSV involvement in motor nerves and central nervous system (CNS) motor centers has been neglected since Goodpasture and Teague's early pathological studies of rabbits (4). The present report describes a hypoglossal model of HSV infection in mice. In this model, HSV spreads in a pure motor nerve and produces a focal infection in CNS motor neurons.

Female, 4- to 6-week-old A/J mice (Jackson Laboratories, Bar Harbor, Maine) were inoculated in the tongue muscle with 10^6 PFU of the F strain of HSV type 1 (American Type Culture Collection, Rockville, Md.). Vesicular lesions appeared on the tongue surface 4 days after HSV inoculation. HSV antigens were localized in tissue sections by the peroxidase antiperoxidase (PAP) technique (16). In brief, deparaffinized sections of tongue lesions were incubated for 16 h at 5°C with anti-HSV type 1 serum diluted 1:1,000 in Tris-buffered saline. The sections were then treated sequentially with sheep anti-rabbit immunoglobulin G, rabbit PAP (Cappel Laboratories, Cochranville, Pa.), and 3,3′-diaminobenzidine (Sigma Chemical Co., St. Louis, Mo.). Figure 1 shows a typical vesicle with immunoperoxidase staining of HSV antigens in the mucosa of the tongue. Adsorption of the primary antiserum with HSV-infected RK-13 cells prevented PAP staining. To determine the neural pattern of spread, homogenates and explant cultures (12, 15) were carried out on the brainstem, trigeminal ganglia, and hypoglossal nerve. The indicator cell line was RK-13 cells (seed culture kindly provided by Nathalie J. Schmidt, Berkeley, Calif.), and maintenance medium consisted of Eagle minimal essential medium containing 2% heat-inactivated fetal bovine serum, 0.03% glutamine, 100 U of penicillin per ml, and 100 mg of streptomycin per ml. Table 1 shows the percentage of virus recovery from these tissues in week 1 after HSV inoculation by the tongue route. For purposes of comparison, a second group of mice were inoculated with HSV by corneal scarification (12, 15). In both inoculation groups, virus was recovered from the trigeminal ganglia and brainstem. However, hypoglossal nerve explants yielded HSV only in mice inoculated by the tongue route.

This result indicates that HSV spreads in both sensory (trigeminal) and motor (hypoglossal) nerves after tongue inoculation of virus. To determine if hypoglossal spread results in a CNS infection, histopathological studies of the lower medulla were done. Mice were perfused by intracardiac injection of Karnovsky solution (phosphate-buffered 2% paraformaldehyde and 2.5% glutaraldehyde). The brainstem was dis-

† Present address: Department of Neurology, City of Hope National Medical Center, Duarte, CA 91010.

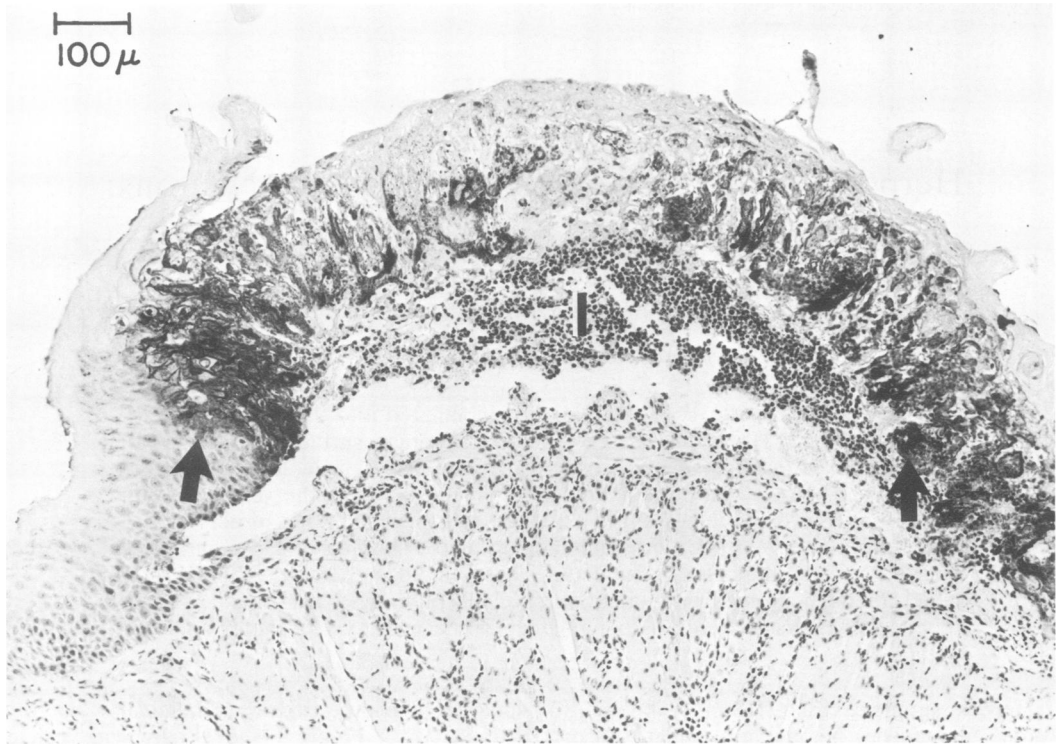


FIG. 1. Light micrograph of vesicular tongue lesion 4 days after HSV inoculation by the tongue route. Inflammatory cells (I) lie on either side of a fluid-filled chamber in the submucosa. The arrows mark immunoperoxidase staining of HSV antigens in the mucosa (PAP with hematoxylin counterstain).

sected free, trimmed, and fixed for an additional 2 h in Karnovsky solution. Specimens were then postfixed in 1% osmium tetroxide, dehydrated in graded ethanols, impregnated with propylene oxide, and imbedded in Quetol (Ted Pella, Inc., Tustin, Calif.). Sections (1 μm) were stained with toluidine blue and examined by light microscopy. Figure 2 shows the level where the hypoglossal nerve exits the brainstem in a mouse sacrificed 4 days after HSV inoculation by the tongue route. Inflammatory cells were present near the exit (arrow) but not more distally in the hypoglossal nerve.

Ultrathin sections were obtained in the two areas indicated on Fig. 2. These sections were stained with 4% uranyl acetate in 70% ethanol and 0.4% lead citrate in 0.1 N sodium hydroxide and then examined in a Phillips 400 electron microscope. In the intramedullary area marked with a circle in Fig. 2, viral capsids were observed in occasional astroglial nuclei (Fig. 3). Within the triangular area in Fig. 2, particles morphologically compatible with HSV capsids were noted inside motor axons (Fig. 4).

The motor nerve cell bodies of the hypoglossal nuclei are located dorsal and rostral to the exit level shown in Fig. 2. To determine if input virus from the tongue infects these motor neurons, transverse paraffin sections of medulla through the hypoglossal nuclei were stained for

TABLE 1. Virus recovery from nervous system tissue after HSV inoculation by the tongue or corneal route

Route of HSV inoculation	Virus culture					
	Brain stem homogenates (no. positive/ no. tested)	(%)	Trigeminal ganglia homogenates (no. positive/ no. tested)	(%)	Hypoglossal nerve explants (no. positive/ no. tested)	(%)
Tongue	20/24	(83)	27/29	(93)	12/16	(75)
Cornea	9/9	(100)	8/9	(89)	0/9	(0)

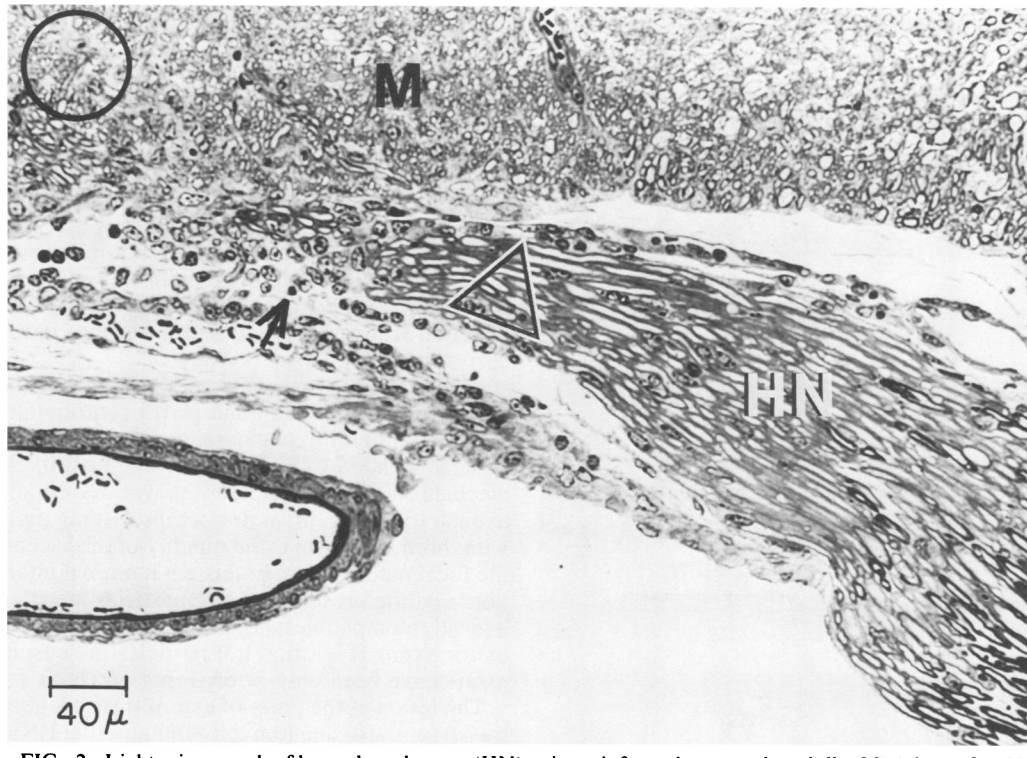


FIG. 2. Light micrograph of hypoglossal nerve (HN) at its exit from the ventral medulla (M) 4 days after HSV inoculation by the tongue route. The arrow marks inflammatory cells surrounding and infiltrating the nerve near its exit. The circle and triangle indicate regions studied in further detail by electron microscopy (see Fig. 3 and 4). (Section [1 μm thick] of epoxy-embedded osmicated tissue counterstained with toluidine blue.)

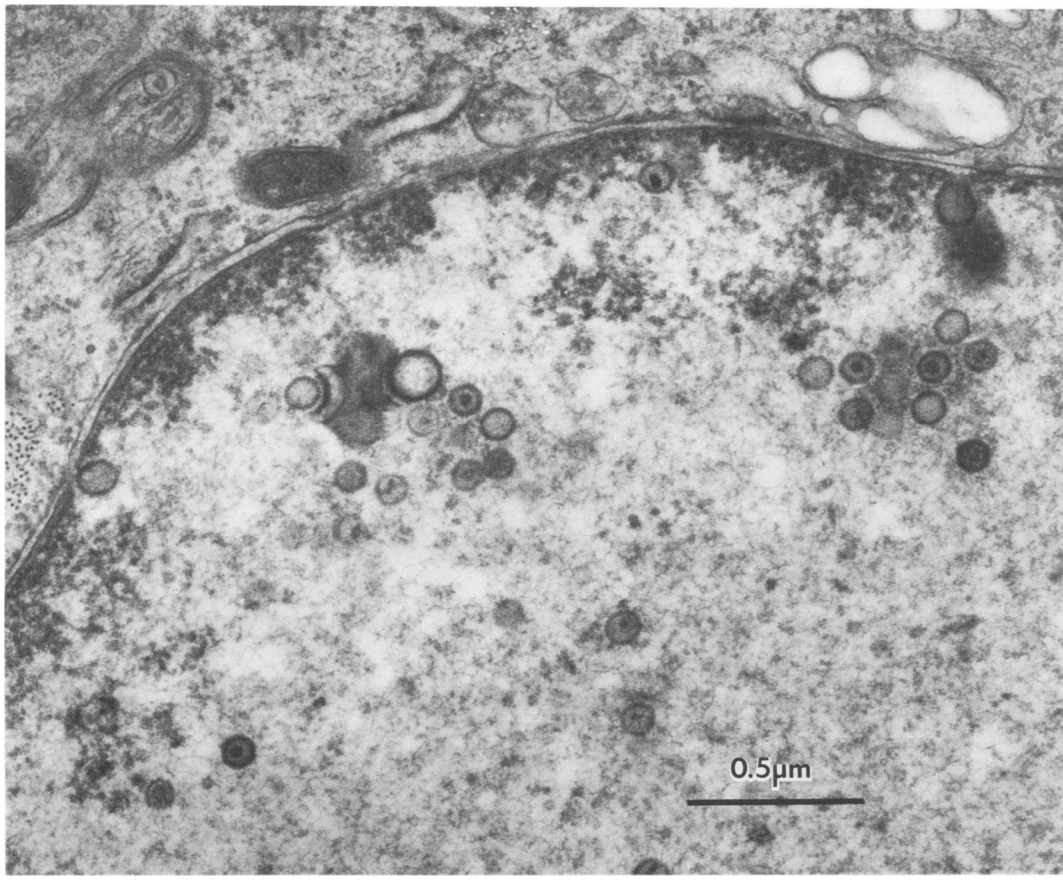


FIG. 3. Electron micrograph of hypoglossal root exit area in lower medulla 4 days after HSV inoculation by the tongue route. Intranuclear 100-nm viral capsids are present in astroglial cells. This micrograph was taken within the circular area shown in Fig. 2.

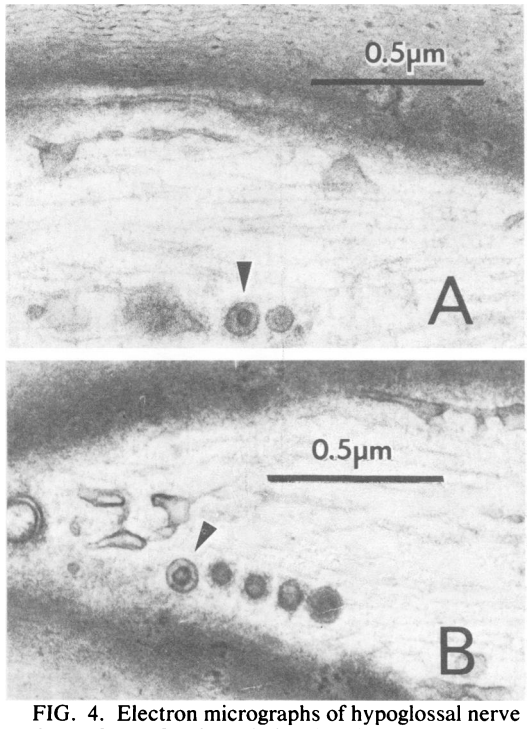
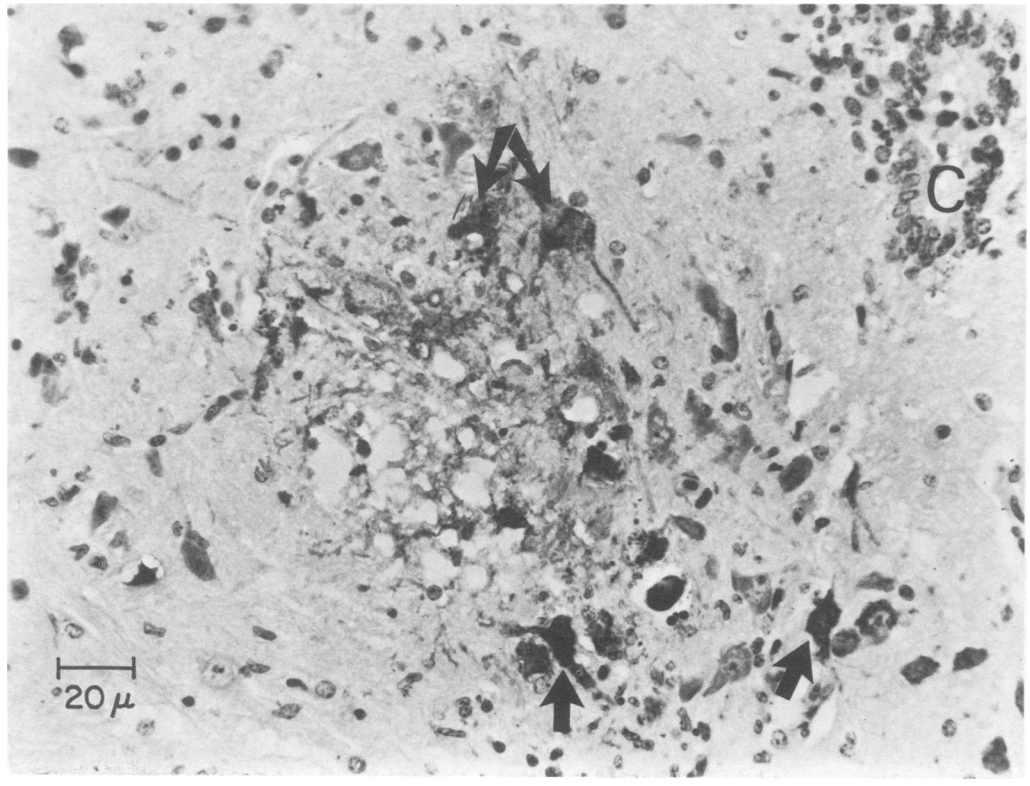


FIG. 4. Electron micrographs of hypoglossal nerve 4 days after HSV inoculation by the tongue route. Arrows mark intra-axonal 100-nm particles which are morphologically compatible with HSV viral capsids. These micrographs were taken within the triangular area marked on Fig. 2.

HSV antigens by the PAP technique. Figure 5 shows HSV antigens discretely localized to motor neurons in the hypoglossal nucleus 4 days after HSV inoculation by the tongue route. It is unlikely that HSV reached these motor neurons by the trigeminal route since a diffuse brainstem encephalitis rarely occurred. Rather, the CNS infection was restricted to motor neurons in the corresponding hypoglossal nucleus.

In summary, HSV appears to penetrate the neuromuscular junction and travel centripetally in the pure motor hypoglossal nerve. By analogy to sensory nerves (3, 7, 13, 23), the most likely mechanism of HSV spread is via retrograde axonal transport in motor axons. Compatible with this mechanism is the rapidity of the ascent, the focal nature of the brainstem neuronal infection, and the presence of rare particles morphologically compatible with HSV viral capsids in motor axons (Fig. 4). Viral particles in sensory axons have been only rarely reported (1, 5).

The lesion at the point of exit of the hypoglossal nerve is also qualitatively similar to the HSV-induced entry zone lesion reported in trigeminal roots (8, 9, 19–21). As noted in the sensory system (19), the Schwann cell basal lamina in the PNS may act as a barrier to axonal virions. However, at the PNS-CNS junction, astroglial foot processes abut directly on the axon. Apparently some intra-axonal virus particles enter these foot processes, replicate in junctional as-



troglial cells, and provoke a local inflammatory response. The analogous process probably occurs in the motor system since we can demonstrate HSV replication in astroglial nuclei near the hypoglossal nerve exit (Fig. 3) and inflammatory cells are clustered about the hypoglossal nerve at the point of exit from the ventral medulla (Fig. 2).

Varicella-zoster virus does occasionally produce segmental motor paresis (18), but there is no clinical evidence in humans that HSV affects CNS motor centers. Subclinical involvement in motor neurons remains a possibility. In fact, such involvement in the hypoglossal system would not be unexpected since tongue lesions as shown in Fig. 1 occur commonly in primary herpes gingivostomatitis (11).

Regardless of the relevance to human infection, the hypoglossal model has some distinct advantages in experimental work. HSV latency is known to occur in the CNS as well as in ganglia (2, 6, 10), but the cell type, degree of genome expression, and mechanism of reactivation in the CNS are unknown. Most studies of CNS infection have used the trigeminal model. This model can be cumbersome because (i) trigeminal neurons are scattered throughout the brainstem and upper cervical cord, making molecular and detailed histological studies difficult, and (ii) latency in the nearby trigeminal ganglia of the PNS makes it impossible to differentiate in situ reactivation in CNS trigeminal neurons from reactivation in ganglia and viral spread to the CNS. Trigeminal spread also occurs in the hypoglossal model (Table 1). However, by exploiting the motor nerve spread, the hypoglossal model provides the advantages of an infection in a discrete, well-localized CNS nucleus with no corresponding PNS ganglia. Thus, this model is being used in ongoing studies of reactivation of latent HSV in the CNS.

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FIG. 5. Light micrograph of lower medullar showing hypoglossal nucleus in transverse section 4 days after HSV inoculation by the tongue route. Neuronal fragmentation and neuropil vacuolization are present. The arrows mark immunoperoxidase staining of intraneuronal HSV antigens. Ependymal cells line the central canal (C) (PAP with hematoxylin counterstain).