perpolarization with no apparent center-surround antagonistic polarization. An electron microscopic study of serial sections revealed that about 30 percent of the retinal cells, whose somata were located in the outer nuclear layer, were displaced bipolar cells (N. Kouyama and T. Ohtsuka, *Brain Res.*, in press).
16. Similar results have also been reported in other vertebrate retinas [D. I. Attwell, F. S. Werblin, M. Wilson, S. M. Wu, *J. Physiol. (London)* **341**, 74P (1983); D. A. Burkhardt, G. Hassin, J. S. Levine, E. F. MacNichol, Jr., *ibid.* **309**, 215 (1980)].
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Infection of the Basal Ganglia by a Murine Coronavirus

Abstract. *The coronavirus, mouse hepatitis virus strain A59 (MHV-A59), causes mild encephalitis and chronic demyelination. Immunohistochemical techniques showed that MHV-A59–infected C57BL/6 mice contained dense deposits of viral antigen in the subthalamic nucleus and substantia nigra, with fewer signs of infection in other regions of the brain. The animals showed extra- and intracellular vacuolation, neuronal loss, and gliosis in the subthalamic-nigral region. Such localization is unprecedented among known viral encephalitides of humans and other species. This infection by a member of a viral class capable of causing both encephalitis and persistent infection in several species may be related to postencephalitic parkinsonism.*

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Coronaviruses cause encephalitis in several animal species, although in humans they are recognized primarily as respiratory pathogens (*1*, *2*). In mice the A59 strain of mouse hepatitis virus (MHV-A59) causes a chronic demyelinating disease with minimal encephalitis (*3*). MHV-A59 replicates readily in glial cells in vitro but has little propensity to infect neurons (*4*). We wished to further examine the neural tropism of this virus in mice. Using immunohistochemical methods, we observed a strong tropism for the basal ganglia in the region of the subthalamic nucleus and substantia nigra.

Mice of strain C57BL/6 were infected with MHV-A59 (*5*) by intracerebral inoculation at 4 to 6 weeks of age (Table 1). Animals rated as unaffected appeared normal by routine observation. Mice rated as moderately affected showed piloerection and a hunched posture, while those rated as severely affected had marked reduction or difficulty in locomotion, with many appearing moribund. Deaths due to encephalitis or hepatitis usually occurred within 2 weeks of infection. At intervals the animals were killed and perfused with 10 percent buffered formalin or paraformaldehyde-lysine-periodate (PLP) fixative for immunohistochemical and light microscopic examination or with 4 percent buffered glutaraldehyde for ultrastructural examination. To locate viral antigens, we performed immunohistochemical analysis of fixed frozen sections (10 μm) and sections cut from paraffin-embedded material (6 to 8 μm), with comparable results. Antiserum raised in rabbits against detergent-disrupted MHV-A59 was used as the primary antiserum for the peroxidase-antiperoxidase staining technique (*6*), and diaminobenzidine was used as the chromagen.

No significant staining was seen in sections from uninfected control brains with immune antiserum to MHV-A59 or from infected animals incubated with preimmune serum. However, antigen-positive cells were clearly seen in sections from infected animals incubated with the antiserum. The number of immunoreactive cells was closely related to the clinical severity of the encephalitis and the interval between inoculation and death. Viral antigens were present in the brain in greatest amounts within the first 2 weeks after inoculation. In all animals in which sufficient numbers of antigen-positive cells were present for their distribution to be assessed (12 of 19 animals examined), a discrete cluster of infected cells was consistently found in the diencephalon in the region of the subthalamic nucleus and the adjacent substantia nigra. A low-power view of such an area (Fig. 1A) illustrates the discrete localization of viral antigen bilaterally in an animal showing few antigen-positive cells in surrounding brain regions. The immunoreactivity in this patch consists of cellular profiles of variable size, cellular debris, and diffuse extracellular antigenic material. Much of the antigen was associated with vacuolation of the region (Fig. 1B). These antigen-positive regions were usually bilaterally symmetrical with discrete borders, and were evident in animals killed as early as 4 days after inoculation. The region involved contains many large neurons, and most of the identifiable antigen-containing cells appeared to be neurons. Many cells containing antigen were fragmented and unidentifiable as to cell type. Antigen also appeared to be present in the extracellular space, particularly in sections from severely affected animals. Cell loss, vacuolation, and gliosis (in animals with long intervals between inoculation and death) were seen in the subthalamic-nigral region in all moderately to severely affected animals (Fig. 1C). The cellular changes in this region were typical of those associated with coronavirus infection (*7*, *8*). Intracellular vacuolation was common, and many neurons appeared swollen, with pyknotic nuclei and loss of cytoplasmic detail. Larger vacuoles were packed within the region of cell loss, and vacuoles containing cellular fragments were commonly seen, suggesting that the larger vacuoles may have resulted from cell lysis. In moderately affected animals killed at intervals longer than 4 weeks after inoculation, the involved regions showed little viral antigen but were characterized by neuronal loss, persistent vacuolation, and gliosis.

The location of the intense patch of immunoreactivity varied little among animals. The subthalamic nucleus was most consistently involved, with the le-

Table 1. Clinical status of infected mice. Each letter [U (unaffected), M (moderately affected), and S (severely affected)] represents an individual animal examined histologically. Of 28 animals inoculated intracerebrally with 3000 plaque-forming units (PFU), 4 died, 8 were killed, and 16 survived. Of 30 animals given 4500 PFU, 8 died, 9 were killed, and 13 survived. Of 15 animals given 6000 PFU, 8 died and 7 were killed.

Weeks between inoculation and sacrifice	Dose of virus (PFU)		
	3000	4500	6000
1		MS	SSSS
2	UUM	MM	SS
3			M
4	M		
>4	MMMU		

sion extending into the rostral portion of the pars compacta of the nigra and rarely into the small-cell pars reticulata. Ultrastructural examination of the antigen-positive region revealed numerous cells with abundant viral particles, most of which were within endoplasmic reticulum or Golgi membranes. Many of the infected cells showed organelle-depleted cytoplasm with various amounts of vacuolation. Infected cells were of several cell types, with neurons clearly affected (Fig. 2).

We also found infected cells in several other brain regions, but without the intensity of immunoreactivity, large number of neurons, and restricted distribution of the subthalamic-nigral region. These areas included the thalamus, tegmentum, pons, subiculum of the hippocampus, and cortex surrounding the forceps of the corpus callosum. Antigen in these regions frequently appeared as a diffuse patch, with fewer associated cellular profiles. Many of the antigen-containing cells in these regions also appeared to be neurons, while viral antigen was associated with glia in the corpus callosum and other fiber tracts. The animals showed mild demyelination of brain fiber tracts, but demyelinated regions were unrelated topographically to the basal ganglia lesions. In general, the degree of pathologic change in particular brain regions corresponded to the density of antigen-containing cells.

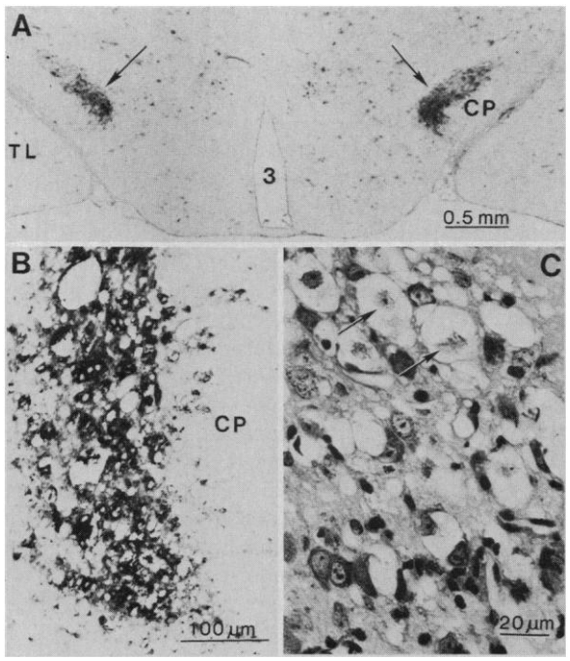

Fig. 1. (A) Photomicrograph of a coronal section through the brain of an infected mouse. This animal received 6000 PFU 1 week before it was killed. The section was incubated with immune serum against MHV-A59 (dilution 1:200), reacted with the immunoperoxidase technique, and counterstained with cresyl violet. Bilaterally symmetrical regions containing dense accumulations of viral antigen are apparent at the junction of the substantia nigra and the subthalamic nucleus (arrows). There are scattered antigen-containing cells in surrounding regions. (CP, cerebral peduncle; TL, temporal lobe of cerebral cortex; 3, third ventricle.) (B) Higher magnification view of the region of dense antigen accumulation from the same animal. Most of the antigen is within the cytoplasm of cells of the gray matter, and some of the antigen is associated with vacuoles. (C) Pars compacta of the substantia nigra from a mouse infected with 3000 PFU and killed 2 weeks later. The section was stained with cresyl violet and Luxol fast blue. While some of the neurons in the lower part of the field appear normal, many are vacuolated. Large vacuoles with cellular fragments are apparent (arrows). Mild lymphocytic infiltration is present.

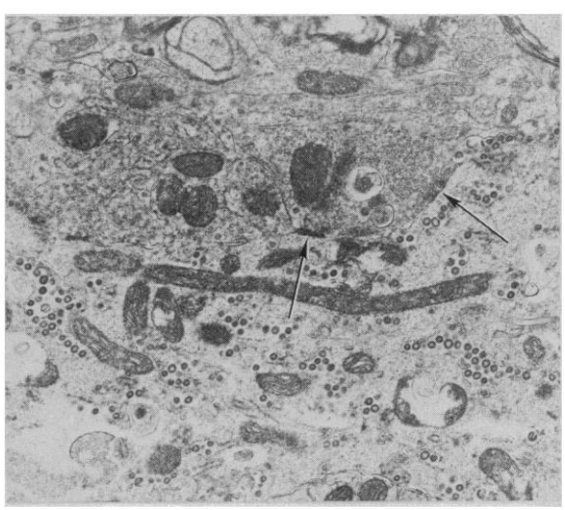

Fig. 2. Electron micrograph of affected tissue from an animal infected with 4500 PFU and killed 1 week later. Abundant viral particles are present in membranous cisternae of a subthalamic nucleus neuron. Although this cell has begun to vacuolate, sypnatic contacts with axon terminals remain (arrows). The magnification is ×38,500.

Tissue or cell tropism of a virus is the result of complex interactions between host and viral factors. There are several virus types that have strong predilections to infect particular components of the nervous system. Examples include herpes zoster and simplex viruses for sensory ganglia and poliovirus for anterior horn neurons (*9*). Involvement of the basal ganglia has been observed in humans as a rare component of several viral encephalitides, but has been a consistent aspect of only two (*10*). The most well known is encephalitis lethargica, or von Economo's disease, in which most affected individuals showed evidence of basal ganglia involvement, with many survivors developing postencephalitic parkinsonism (*11*). Although it is presumed that this epidemic of encephalitis during the period 1916 to 1925 was due to a viral infection, the agent has not been identified (*12*). Japanese B encephalitis virus also occasionally affects the substantia nigra, and viral antigen has been demonstrated in some nigral cells from patients who died from this disease and in virally inoculated mice (*13, 14*).

The factors responsible for the regional localization of MHV-A59 are unknown. MHV-A59 has been considered a weakly neuronotropic virus that has a much greater tendency to infect glial cells than neurons (*2–4*). Other coronaviruses, particularly the JHM strain of MHV, infect neurons readily, but to our knowledge the encephalitis associated with MHV-JHM has not been described as affecting the basal ganglia in particular (*8, 15*). Our infected animals showed immunoreactive cells in the white matter, and our observations are consistent with those of Lavi *et al.* (*3*), who observed demyelination, most prominently in the spinal cord. The subthalamic-nigral infection is the most striking exception to the nonneuronal tropism shown by MHV-A59 in other parts of the nervous system.

The potential of coronaviruses to cause nervous system disease in humans is beginning to be explored (*16*). These viruses are known to cause chronic central nervous system (CNS) disease in animals, and the genome of MHV-A59 persists in the nervous system of mice for at least several months (*17*). The ability of MHV-A59 to cause a persistent CNS infection as well as its propensity to

infect the substantia nigra makes it a potential animal model for postencephalitic parkinsonism. In light of the experience with encephalitis lethargica, in which parkinsonian symptoms progress for months to years after the initial infection, the relation of a previous virus infection to the development of idiopathic Parkinson's disease has long been considered (*18*). However, most Parkinson's disease patients have no history of encephalitis, and there is no evidence for infection by any of several studied viruses (*18, 19*). Although the tropism of MHV-A59 for the basal ganglia is reminiscent of encephalitis lethargica, there are differences in pathology between these two encephalitides. Neither demyelination nor cellular vacuolation are seen in encephalitis lethargica, while neurofibrillary tangles, commonly seen in postencephalitic parkinsonism, are not seen in MHV-A59 encephalitis. The antigen-dense, necrotizing lesions in MHV-A59 infection encompass a variable amount of the subthalamic nucleus and only the more rostral portion of the nigra. Von Economo's disease destroyed most of the nigra, although, like this experimental encephalitis, it affected other regions of the brain as well (*20*). A possible role for coronaviruses in the pathogenesis of postencephalitic parkinsonism or Parkinson's disease remains a subject for future investigation, as does the mechanism through which this coronavirus consistently and selectively infects this clinically important region of the brain.

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Depolarization and Muscarinic Excitation Induced in a Sympathetic Ganglion by Vasoactive Intestinal Polypeptide

Abstract. *The effects of vasoactive intestinal polypeptide (VIP) in the superior cervical ganglion of the cat were studied in vitro and in vivo with sucrose gap and multiunit recording, respectively. At a dose of 0.03 to 0.12 nanomole, VIP produced a dose-dependent, prolonged (3 to 15 minutes) depolarization of the ganglion and enhanced the ganglionic depolarization elicited by the muscarinic agonist acetyl-β-methylcholine. At a dose of 1.8 to 10 nanomoles, the peptide enhanced and prolonged the postganglionic discharge elicited by acetyl-β-methylcholine, enhanced muscarinic transmission in ganglia treated with an anticholinesterase agent, and enhanced the late muscarinic discharge elicited by acetylcholine. VIP did not affect the early nicotinic discharge elicited by acetylcholine or by electrical stimulation of the preganglionic nerve. It is concluded that VIP has a selective facilitatory action on muscarinic excitatory mechanisms in the superior cervical ganglion of the cat.*

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Recent studies (*1–3*) have focused attention on the synaptic interactions between vasoactive intestinal polypeptide (VIP) and acetylcholine (ACh). In the submandibular gland, VIP and ACh coexist in the parasympathetic postganglionic nerves and are released during nerve stimulation (*1*). VIP mediates neurally evoked vasodilation in the gland and also facilitates ACh-induced glandular secretion (*1, 2*). Radioligand receptor binding studies suggest that VIP enhances the secretory effect of ACh by increasing the affinity of ACh for muscarinic receptors on the gland cells (*3*). Thus VIP seems to function as a neuromodulator and a transmitter at certain cholinergic neuroeffector junctions.

A similar facilitatory effect of VIP on neuronal muscarinic mechanisms in vesical parasympathetic ganglia of the cat was shown by recent studies in our laboratory (*4*). Exogenous VIP enhanced muscarinic transmission and the ganglionic excitatory responses to muscarinic agonists but did not alter nicotinic transmission or the responses to nicotinic agonists. These observations suggested that VIP must have a very selective postsynaptic effect to alter the interaction of ACh with muscarinic receptors or to alter the transduction mechanisms leading to muscarinic depolarization and ganglion cell firing. Other investigators have reported that VIP also increases adenosine 3′,5′-monophosphate (cyclic AMP) concentrations (*5*) and tyrosine hydroxylase activity in autonomic ganglion cells (*6*). These observations, coupled with the immunohistochemical demonstration of VIP axons and varicosities in autonomic ganglia (*7*), indicate that VIP may be a transmitter or a modulator of cholinergic transmission at ganglionic synapses.

The effects of VIP were examined in eight ganglion preparations in vitro and ten preparations in vivo. For the in vitro experiments, superior cervical ganglia were removed from barbiturate-anesthe-