
Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

# Host and viral factors that influence viral neurotropism
# I. Viral cell attachment proteins and target cell receptors

Kenneth L. Tyler

*Since the time of Charcot, over a century ago, it has been recognized that the clinical signs and symptoms produced by viral illnesses involving the CNS are due in large part to the specific regions of the nervous system injured. As the list of viruses capable of infecting the CNS expanded rapidly during the earlier part of the 20th century, so too did the recognition that individual viruses exhibited specific affinities for particular regions of the nervous system, and often for particular cell types within these regions. In this part of our review we will focus specifically on the role played by the interaction between specific viral proteins ('cell attachment proteins') and receptors on target cells in determining the tropism of certain viruses for the CNS. In a subsequent article, the role of host genes, the site of entry and route of spread of virus, and the contribution of tissue-specific viral genes (enhancers) to viral neurotropism will be reviewed.*

*Kenneth L. Tyler is at the Department of Microbiology and Molecular Genetics, Harvard Medical School, and Department of Neurology, Massachusetts General Hospital, Boston, MA, USA.*

## Poliovirus

It has been nearly thirty years since J. J. Holland and L. C. McLaren showed that there was a strong correlation between the susceptibility of specific tissues to pathologic injury by polivirus *in vivo* and the capacity of tissue homogenates to bind virus *in vitro*. They also showed that if the receptor-binding step was bypassed by transfection of cells with poliovirus RNA, then many receptor-negative cells supported one cycle of poliovirus replication[1,2]. From these studies they concluded that the presence or absence of the appropriate cellular receptor was the major determining factor in cell and tissue susceptibility to poliovirus infection. However, they clearly recognized that the virus had to be capable of reaching the target cell population in the infected host, and that for cell injury to occur, the virus had to successfully complete steps in the viral replication cycle that followed receptor binding. Recent studies on the binding of radiolabelled poliovirus type 1 (Mahoney) to human regional CNS tissue homogenates appear to conflict somewhat with this earlier view by indicating that poliovirus binding activity within the CNS is much more widespread than the restricted distribution of pathologic lesions would lead one to predict[3]. This suggests that factors other than receptor distribution must play a significant role in determining poliovirus neurotropism.

The poliovirus receptor is an integral membrane present in about 3000 copies on HeLa cells[4]. All three serotypes of poliovirus compete for attachment to a common receptor, which is different from that utilized by other enteroviruses. The receptor is encoded by a gene present on human chromosome 19 (Ref. 5), and susceptibility to poliovirus infection can be transmitted to previously insusceptible cells (e.g. mouse L-cell fibroblasts) by transfection of these cells with specific DNA fragments (presumably encoding the poliovirus receptor) derived from susceptible HeLa cells[6].

The nature of the poliovirus cell attachment protein(s) has been partially clarified by the availability of high-resolution X-ray crystallographic studies of the three-dimensional structure of both poliovirus[7] and other picornaviruses, including human rhinovirus 14 (HRV14)[8] and mengovirus[9]. On the outer surface of HRV14, protrusions formed by part of the outer capsid protein VP1 are surrounded by a deep (25 Å) 'canyon', and in mengovirus by a deep 'pit'. The canyon and pit walls are composed primarily of stretches of VP1 and to a lesser extent by portions of the carboxy terminus of outer capsid protein VP3. It has been suggested that stretches of VP1 and VP3 along the floor of the canyon may provide the site of viral attachment to cellular receptors[8]. Although polioviruses do not use the same cellular receptor as HRV14, their crystallographic structure also shows 'peaks' on the outer surface of the virus composed of outer capsid protein VP1. These peaks are surrounded by a broad

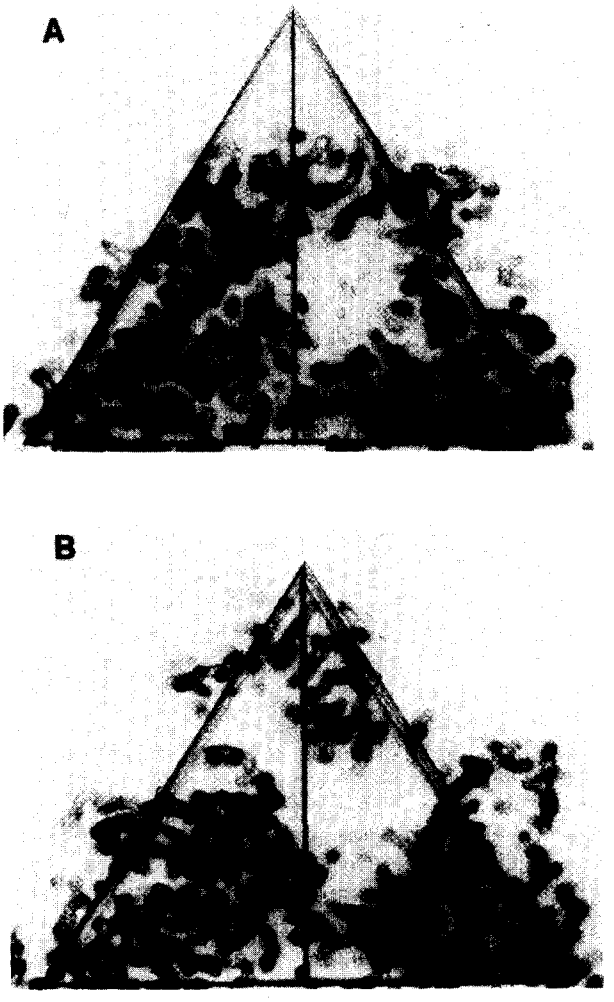


**Fig. 1.**
*Electron density map of* **(A)** *mengovirus and* **(B)** *human rhinovirus 14 (HRV14) showing the deep external pit in mengovirus and the canyon in HRV14 believed to be the receptor binding sites.* (Taken, with permission, from Ref. 9.)



0378 - 5912/87/$02.00

'valley' analogous to the HRV14 'canyon' and the mengovirus 'pit'[7]. Poliovirus receptor binding sequences may be located in this region, and would presumably differ from the homologous sequences in HRV14, thereby accounting for the differences in receptor specificity and cell tropism.

**The rabies virus**

Rabies virus provides another example of how cellular receptors and viral cell attachment proteins may interact to determine neurotropism. Following the bite of a rabid animal, rabies virus is inoculated into the subcutaneous tissues and muscles of the unfortunate victim. Virus replication in striated muscle cells may serve to amplify the size of the initial inoculum. In experimental models of rabies infection, virus rapidly localizes in neuromuscular and neurotendinal spindles and at the neuromuscular junction, before spreading via nerves to reach the spinal cord[10]. Lentz and colleagues demonstrated that in chick embryo myotubes, isolated murine diaphragm–phrenic nerve preparations, and muscles from mice inoculated with rabies virus *in vivo*, that rabies-virus binding sites appeared to co-localize with areas of acetylcholinesterase staining and α-bungarotoxin binding[11,12]. Pre-treatment of cultured chick myotubes with either α-bungarotoxin or (+)-turbocurare inhibited rabies infection, and pre-treatment of fixed chick myoblasts with a monoclonal antibody to the α-subunit of the acetylcholine receptor (AChR) blocked binding of radiolabelled rabies virus. These findings led the authors to propose that a rabies-specific host cell receptor was located at the neuromuscular junction, and that it was either identical to or closely associated with the nicotinic AChR[11,12].

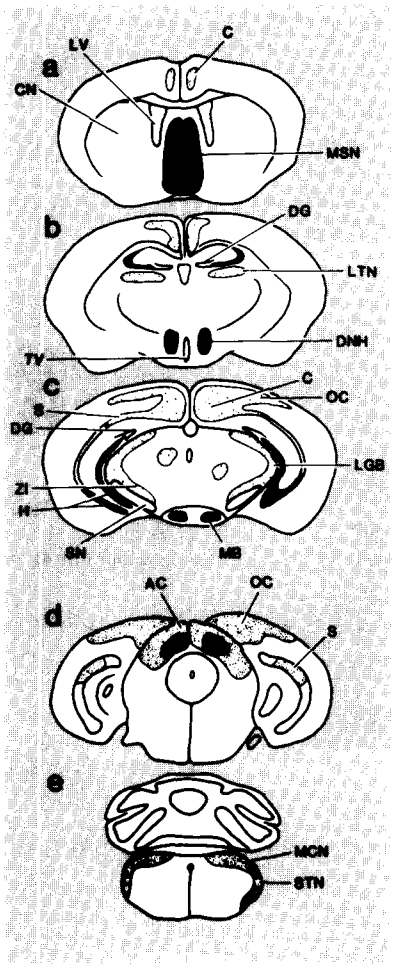


**Fig. 2.** *Location of lesions in coronal sections of mouse brain following intracerebral inoculation of reovirus type 3 (Dearing) (shaded and black areas), and of reovirus type 3 hemagglutinin variant (black area only). A single amino acid substitution in the hemagglutinin protein of the variant is responsible for its capacity to injure only a subset of the areas injured by its wild-type parent.* (Taken, with permission, from Ref. 23.)

Cells lacking AChR can be infected by rabies virus *in vitro*[13] and *in vivo*[14], suggesting that host cell structures other than the AChR can also serve as receptors for rabies virus. One such receptor may be a membrane phospho- or glyco-lipid[13]. Vesicular stomatitis virus (VSV), another rhabdovirus, competes with rabies virus for binding to some cell lines, and independent evidence exists that phosphatidyl serine may serve as a VSV receptor. Gangliosides may also play a role in rabies virus binding, as preliminary studies indicate that the insertion of exogenous gangliosides into the membrane of rabies-insusceptible asialyo-CER cells results in the restitution of susceptibility to rabies virus infection[15].

The rabies envelope glycoprotein (G) appears to serve as the viral cell attachment protein. Binding may depend on the conformation of the protein in the viral envelope, as isolated G does not compete with whole virus in *in-vitro* binding studies[13]. Lentz and his colleagues have drawn attention to the fact that portions of the amino acid sequence of G (amino acids 151–238) are homologous to regions of the amino acid sequence of certain curare-mimetic snake neurotoxins. They hypothesized that this region of the rabies glycoprotein could contain the recognition site for viral binding to the AChR[16].

Another approach to identifying biologically important regions of G has been to isolate and characterize rabies virus variants that are resistant to neutralization by anti-G monoclonal antibodies. Biological characterization of a large number of these variants combined with partial amino acid sequence data have indicated that an arginine residue located at position 333 is an important marker of the pathogenic viral phenotype[17,18]. Variants with a number of amino acid substitutions at this position (glutamine, glycine, isoleucine) are avirulent or have attenuated pathogenicity[17,18]. Conversely, variants isolated to date with amino acid substitutions at positions 330, 336, 338 are fully pathogenic[18]. The apathogenic rabies variants appear to compete for the same cellular receptor as the wild-type virus (*in vitro*)[13]. However, studies with one variant *in vivo* indicated that its rate of spread within the CNS, the number of neurons it infected, and the degree of cellular necrosis it induced were all considerably less than that of its wild-type parent, although the differences in pathology were felt to be quantitative rather than qualitative[19]. In another *in-vivo* study with two variants, it was found that unlike the wild-type virus, both variants were able to infect the lens of the rat eye after inoculation into the anterior chamber, indicating that qualitative differences in tropism may indeed exist between variants and wild-type rabies virus[14]. When compared to the wild-type virus, the variants also appeared to be incapable of spreading from the eye to the CNS via either parasympathetic oculomotor or pretectal retinopetal fibres, although they retained the capacity to spread via the sympathetic fibres in the ophthalmic nerve[14].

**Mammalian reoviruses**

The mammalian reoviruses have provided another example of a group of viruses in which the nature of the cell attachment protein and the viral receptor have been extensively investigated. Reoviruses of serotype 1 and 3 infect different populations of cells within the brain, pituitary gland, and retina. Using reassortant viruses containing different combinations of genes derived from type 1 and 3, it has been shown that the viral S1 dsRNA segment, which encodes the outer capsid protein, sigma 1, determines the capacity of these viruses to infect discrete populations of CNS cells[20,21]. In order to study the tropism of reovirus type 3 for neuronal cells, a

number of sigma 1 antigenic variants were isolated based on their ability to escape neutralization with anti-sigma 1 monoclonal antibodies[22]. These variants all have markedly attenuated neurovirulence and restricted CNS tropism when compared to the parental type 3 virus[22–24]. Comparison of the predicted amino acid sequence of the sigma 1 proteins of the variants with that of the wild-type parental virus indicates that each of the variants has a single amino acid substitution (amino acid 340 or 419)[24]. *In-vivo* investigation of the properties of a reassortant virus containing a S1 dsRNA segment derived from one of these variants clearly shows that the single amino acid substitution in the sigma 1 protein is responsible for the attenuated neurovirulence and restricted CNS tropism of the variants[25].

The nature of a receptor on cultured lymphoid and neuronal cells for reovirus type 3 has recently been identified[26]. The receptor is a monomeric glycoprotein containing sialic acid residues, which has a molecular mass of 67 kDa. The receptor appears to be structurally similar to the mammalian β-adrenergic receptor[27]. This conclusion is based on three lines of evidence: (1) anti-reovirus receptor antibody immunoprecipitates purified β-adrenergic receptor; (2) the molecular masses, isoelectric points, and trypsin digests of reovirus receptor and β-adrenergic receptor are identical; and (3) purified reovirus receptor binds β-antagonists, and this binding is blocked by β-agonists. Obviously a number of questions concerning the reovirus type 3 receptor remain to be answered. Can cells lacking β-adrenergic receptor be infected by reovirus type 3 (suggesting that more than one cellular receptor may exist)? Does the *in-vivo* tropism of reovirus type 3 depend on binding to the β-adrenergic receptor? Does reovirus type 3 bind to a pharmacologically active ligand binding site, and do β-agonists inhibit viral binding?

## Coronaviruses

Information has accumulated about specific aspects of the viral cell attachment proteins of a number of other neurotropic viruses. The coronaviruses are enveloped RNA viruses, which include several neurotropic species [e.g. mouse hepatitis virus (MHV) type 3 and 4, strains A59 and JHM]. Variants of several of these viruses have been generated based on their ability to escape neutralization by monoclonal antibodies (MAb) directed against the envelope peplomer glycoprotein (E2)[28,29]. Several of the E2 variants isolated to date have shown markedly attenuated neurovirulence. Instead of producing a severe acute encephalitis, a number of E2 variants produce demyelination due to infection and destruction of oligodendrocytes. Passive immunization of mice with certain anti-E2 MAbs can also alter the course of infection from encephalitis to demyelinating disease[30]. These findings suggest that the E2 peplomer glycoprotein is a major determinant of pathogenesis for neurotropic coronaviruses and that alterations in this protein may result in changes in viral neurotropism (e.g. sparing of neurons).

The receptor for neurotropic coronaviruses on CNS cells has not been identified, although a plasma membrane receptor for MHV-A59 on intestinal epithelial and liver cells has been partially characterized[31]. This receptor is a monomeric protein with a molecular mass of 100–110 kDa. The presence or absence of this receptor appears to account for some of the differences in susceptibility to MHV-A59 infection that occur in inbred strains of mice[31]. Since differences in the susceptibility of some inbred mouse strains have been mapped to chromosome 7 (Ref. 32), it will be interesting to see if this chromosome encodes the MHV-A59 receptor.

## Lymphocytic choriomeningitis virus

*In-vivo* studies of persistent infection of susceptible strains of inbred mice with lymphocytic choriomeningitis virus (LCMV) have provided important insights into factors that influence neurotropism in this viral system[33,34]. Some strains of inbred mice (e.g. C3H), infected at birth with LCMV (Armstrong), develop a syndrome of poor growth, retarded development and hypoglycemia, whereas others (e.g. BALB/WEHI, SWR/J) do not. Suscept-

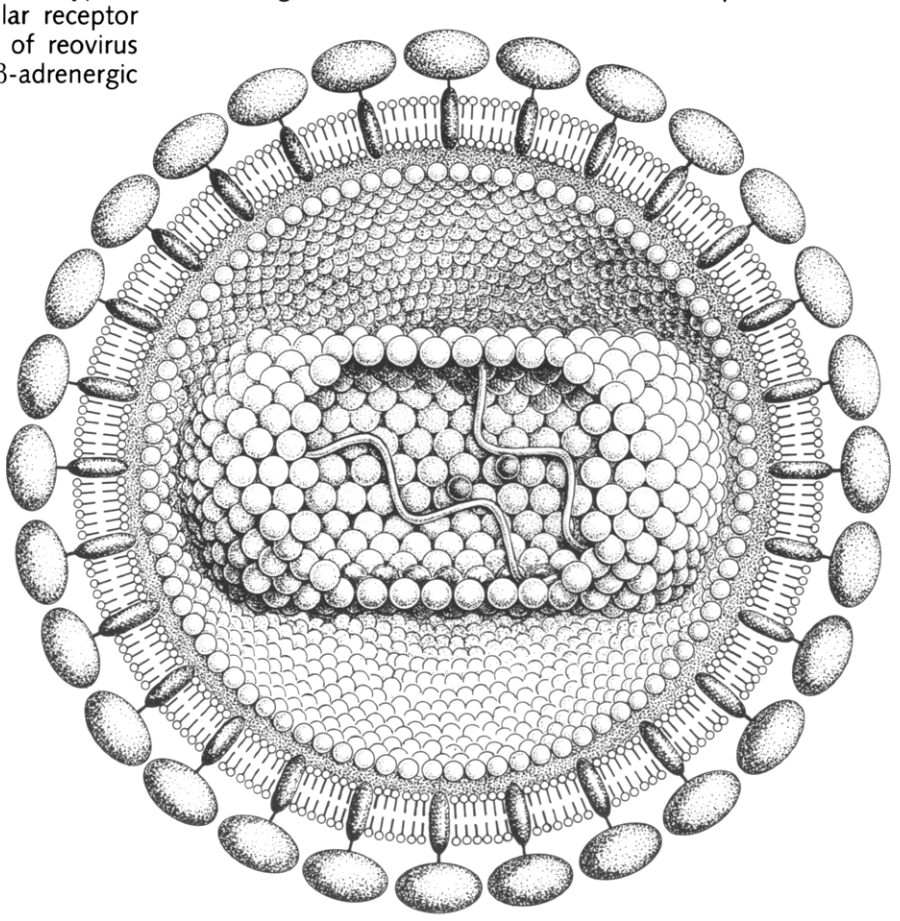

**Fig. 3.** *Schematic diagram of the structure of the human immunodeficiency virus (HIV), the agent responsible for AIDS, showing the gp120 envelope glycoprotein peplomers on the outer surface of the virion. The gp120 protein serves as the viral cell attachment protein.* (Redrawn from Ref. 51.)

ible mice have high concentrations of replicating LCMV in the anterior lobe of the pituitary gland, restricted primarily to the growth hormone-producing cells. Infection of these cells results in markedly diminished levels of growth hormone in the pituitary gland and hypoglycemia. Resistant strains of mice do not develop clinical illness, have only minimal evidence of LCMV infection of the pituitary gland, and show normal growth hormone and blood glucose levels. These results clearly exemplify the importance of host genetic factors in the development of LCMV-induced illness[33].

When different strains of LCMV are tested for their capacity to produce disease in susceptible C3H mice, striking variability is noted. Some LCMV strains (e.g. Armstrong) infect the majority of growth hormone-producing cells, whereas others (e.g. WE) replicate in far fewer of these cells and do not alter growth hormone synthesis[33]. In an effort to determine the genetic basis for this difference, reassortant viruses between LCMV Armstrong and LCMV WE were generated and studied for their ability to infect growth hormone-producing cells in the pituitary. The results of this study indicate that the LCMV small (S) RNA segment, which encodes the two viral surface glycoproteins, is responsible for determining the capacity of LCMV to infect these cells[34]. Thus, the LCMV system provides another example of the importance of proteins on the surface of virions in determining viral neurotropism.

**Mumps virus**

The use of mutants selected by their resistance to neutralizing monoclonal antibodies has also proved to be a fruitful approach to investigating the neurovirulence and neurotropism of mumps virus[35]. Monoclonal antibodies against the hemagglutinin-neuraminidase (HN) surface glycoprotein have been used to select variants of the Kilham strain of mumps virus, at least one of which (M13) has significantly attenuated neurovirulence after intracerebral inoculation into suckling hamsters. In this experimental system, inoculated animals develop a syndrome characterized by lethargy, wasting, and incoordination followed after 2–3 weeks by the development of hydrocephalus. There is neuropathologic evidence of widespread cellular necrosis in the cerebrum and brain stem, severe ependymitis with obstruction of the aqueduct of Sylvius, leptomeningitis, and perivascular cuffing. By contrast, the M13-infected mice show only minimal signs of early illness, but still go on to develop hydrocephalus. The M13 variant infects far fewer neurons in the hamster brain than the parental virus, and does not cause cellular necrosis. However, the severity of the ependymitis and subsequent obstructive hydrocephalus is identical to that of the wild-type virus. These results suggest that the mumps virus HN protein plays an important role in neurovirulence, and that this protein may also be involved in binding to or replication within neurons[35].

**TABLE I.** Homologies between HIV envelope proteins and other macromolecules

| Homologous molecule | Amino acids | gp120 amino acids | Ref. |
|---|---|---|---|
| Immunoglobulin γ-heavy chain (constant) | 71–115 | 49–92 | 43 |
| Vasoactive intestinal peptide (VIP) | 7–11 | 196–200 | 49 |
| Neuroleukin | 403–447 | 238–282 | 48 |
| Immunoglobulin α-heavy chain (constant) | 67–106 | 406–445 | 43 |
| Interleukin 2A | 34–39 | 850–855 | 50 |

**Human immunodeficiency virus (HIV-I)**

There has been an explosive growth in our understanding of the nature of the factors that influence the neurotropism of HIV (previously known as HTLVIII, LAV, ARV), the agent responsible for the acquired immunodeficiency syndrome (AIDS). Neuropathologists and neurologists quickly recognized that large numbers of patients with AIDS had evidence of central and peripheral nervous system dysfunction, including subacute encephalitis, vacuolar myelopathy, and polyneuropathy. Immunocytochemical procedures, *in-situ* hybridization, and culture of nervous system tissue obtained by biopsy or at autopsy, have provided unequivocal evidence for the neurotropism of HIV[36–40]. Using immunocytochemical staining techniques, HIV antigen has been localized primarily in the cortical grey matter, near microglial nodules, and both around and within the walls of cerebral blood vessels[38]. The involvement of the CNS is widespread, although the concentration of HIV antigen-positive cells within these regions is relatively low[38,39]. HIV-infected cells, as defined by *in-situ* hybridization, immunocytochemistry, and electron microscopy, are typically monocytes, macrophages, giant cells, and capillary endothelial cells[38–40]. Viral antigen has not been detected in neurons in most studies, and is only rarely found in glia[40].

A variety of studies have now established the fact that the HIV virus envelope glycoprotein (designated gp120 or gp110) is the cell attachment protein, and that the CD4 (T4) antigen is an essential component of the HIV-cell receptor[41–45]. The introduction of a functional cDNA encoding the CD4 antigen into a number of CD4 human lymphocyte and epithelial cell lines make these previously resistant cells susceptible to HIV infection, although the same does not appear to be true of non-human cell lines[43]. In order to identify the exact epitope of the CD4 antigen with which HIV binds, a large number of anti-T4 monoclonal antibodies have been tested for their ability to block HIV infection[45]. Competition binding experiments with these MAbs have indicated that the HIV-binding site on the CD4 antigen is relatively large, since antibodies to at least four distinct epitopes can interfere with virus infection. These experiments have also indicated that polymorphism of the CD4 antigen does not significantly alter the binding of HIV.

The identification of the CD4 antigen as the HIV receptor in lymphoid cells led to the search for this

antigen in the CNS. CD4 mRNA sequences can be detected in homogenates of human brain tissue by northern blot analysis[43]. This mRNA appears to be of two size classes (3 kb and 1.8 kb), the larger of which is of similar size to CD4 mRNA derived from T-lymphocytes. The rough distribution of the CD4 mRNA appears to parallel the distribution of HIV-infected cells, with larger amounts in the cortex and less in the cerebellum and brainstem. The exact population(s) of cells in the CNS that contain the CD4 mRNA has not yet been clearly determined.

Attempts have been made to identify the specific region(s) of the HIV envelope glycoprotein that are important in the binding of HIV to the T4 antigen. Comparison of HIV isolates from different patients, as well as sequential isolates from the same individual, have led to the identification of 'constant' and 'variable' regions within the HIV glycoprotein[46,47]. Systematic correlation of variations in the gp120 protein with differences in tissue tropism (e.g. neurotropism) of HIV isolates has not yet been described, so it remains unclear whether the observed variations in the gp120-cell attachment protein result in viruses with altered affinity for tissues such as the CNS.

A number of regions of the HIV envelope glycoprotein (gp120) have been found to show amino acid sequence homologies with various biologically interesting molecules (see below). Several of the regions are rather short (e.g. those homologous to interleukin 2A and VIP), some of the regions appear to fall within the 'highly variable'[46,47] regions of the gp120 sequence (e.g. the region homologous to VIP and part of the region homologous to the immunoglobulin $\alpha$-heavy chain constant region), and in one case (IL 2A) the homology is to the transmembrane region of the protein. Data supporting any significant biological role for these homologies, either on the tropism of HIV or its effects on the nervous and immune systems, is essentially non-existent, although this has not hampered speculation on these subjects.

One of the basic principles of neurotropism emerges from the several examples cited above. For many viruses, the interaction between specific cell attachment proteins on the surface of the virus particle and specific receptor proteins on the outer membrane of host cells is a fundamental determinant of viral neurotropism. In the subsequent article in this series we shall examine how a variety of other viral and host factors, including host genes, the site of viral entry, the route of viral spread, and the action of tissue-specific viral genes all exemplify other important determining principles of viral neurotropism.

***Acknowledgements***

*Bernard Fields, John Boyle, Steven Lynn, Skip Virgin, and Francisco Gonzalez-Scarano all provided helpful discussion of the issues addressed in this review. The author is supported by a NIAID Physician-Scientist Award (K11 AI00610) and an Alfred P. Sloan Research Fellowship. Research support includes NIH Program Project Grant (5 P50 NS16998).*

**Selected references**

1 Holland, J. J., McLaren, L. C. and Syverton, J. T. (1959) *J. Exp. Med.* 110, 65–80
2 Holland, J. J. (1961) *Virology* 15, 312–326
3 Brown, R. H., Jr, Johnson, D., Ogonowski, M. and Weiner, H. L. (1987) *Ann. Neurol.* 21, 64–70
4 Krah, D. L. and Crowell, R. L. (1985) *J. Virol.* 53, 867–870
5 Miller, D. A., Miller, O. J., Dev, V. G., Hashimi, S. and Tantravahi, R. (1974) *Cell* 1, 167–173
6 Mendelsohn, C., Johnson, B., Lionetti, K. A., Nobis, P., Wimmer, E. and Racanello, V. R. (1986) *Proc. Natl Acad. Sci. USA* 83, 7845–7849
7 Hogle, J. M., Chou, M. and Filman, D. J. (1985) *Science* 229, 1358-1365
8 Rossman, M. G., Arnold, E., Erickson, J. W., Fankenberger, E. A., Griffith, J. P., Hecht, H-J., Johnson, R. E., Kamer, G., Luo, M., Mosser, A., Rueckert, R., Sherry, B. and Vriend, G. (1985) *Nature* 317, 145–153
9 Luo, M., Vriend, G., Kamer, G., Minor, I., Arnold, E., Rossmann, M. G., Boege, U., Scrabe, D., Duke, G. and Palmenberg, A. (1987) *Science* 235, 182–191
10 Murphy, F. A. (1977) *Arch. Virol.* 54, 279–297
11 Lentz, T. L., Burrage, T. G., Smith, A. L., Crick, J. and Tignor, G. (1982) *Science* 215, 182–184
12 Burrage, T. G., Tignor, G. H. and Smith, A. (1985) *Virus Res.* 2, 273–289
13 Wunner, W. H., Reagan, K. J. and Koprowski, H. (1984) *J. Virol.* 50, 691–697
14 Kucera, P., Dolivo, M., Coulon, P. and Flamand, A. (1985) *J. Virol.* 55, 158–162
15 Tsiang, H. (1985) *Bull. Inst. Pasteur* 83, 41–56
16 Lentz, T. L., Wilson, P. T., Hawrot, E. and Speicher, D. W. (1984) *Science* 226, 847–848
17 Dietzschold, B., Wunner, W., Wiktor, T., Lopes, A. D., Lafon, M., Smith, C. L. and Koprowski, H. (1983) *Proc. Natl Acad. Sci. USA* 80, 70–74
18 Seif, I., Coulon, P., Rollin, P. and Flamand, A. (1985) *J. Virol.* 53, 926–934
19 Dietzscold, B., Wiktor, T., Trojanowski, J., MacFarlan, R., Wunner, W., Torres-Anjel, M. and Koprowski, H. (1985) *J. Virol.* 56, 12–18
20 Weiner, H. L., Powers, M. L. and Fields, B. N. (1980) *J. Infect. Dis.* 141, 609–616
21 Tyler, K. L., Bronson, R. T., Byers, K. and Fields, B. N. (1985) *Neurology* 35, 88–92
22 Spriggs, D. R. and Fields, B. N. (1982) *Nature* 297, 68–70
23 Spriggs, D. R., Bronson, R. T. and Fields, B. N. (1983) *Science* 220, 505–507
24 Bassel-Duby, R., Spriggs, D. R., Tyler, K. L. and Fields, B. N. (1986) *J. Virol.* 60, 64–67
25 Kaye, K., Spriggs, D. R., Bassel-Duby, R., Fields, B. N. and Tyler, K. L. (1986) *J. Virol.* 59, 90–97
26 Co, M. S., Gaulton, G., Fields, B. N. and Greene, M. (1985) *Proc. Natl Acad. Sci. USA* 82, 1494–1498
27 Co, M., Gaulton, G., Tominaga, A., Homcy, C. J. and Fields, B. N. (1985) *Proc. Natl Acad. Sci. USA* 82, 5315–5318
28 Fleming, J., Trousdale, M., El-Zaatari, F., Stohlman, S. and Weiner, L. (1986) *J. Virol.* 58, 869–875
29 Dalziel, R., Lampert, P., Talbot, P. and Buchmeier, M. (1986) *J. Virol.* 59, 463–471
30 Buchmeier, M., Lewicki, H., Talbot, P. and Knobler, R. (1984) *Virology* 132, 261–270
31 Boyle, J. F., Weismiller, D. G. and Holmes, K. V. (1987) *J. Virol.* 61, 185–189
32 Knobler, R. L., Taylor, B. A., Wooddell, M. K., Beamer, W. G. and Oldstone, M. B. A. (1984) *Exp. Clin. Immunogenet.* 1, 217–222
33 Oldstone, M. B. A., Ahmed, R., Buchmeier, M. J., Blount, P. and Tishon, A. (1985) *Virology* 142, 158–174
34 Riviere, Y., Ahmed, R., Southern, P. and Oldstone, M. B. A. (1985) *Virology* 142, 175–182
35 Love, A., Rydbeck, R., Kristensson, K., Gruell, C. and Norrby, E. (1985) *J. Virol.* 53, 67–74
36 Ho, D. D., Rota, T., Schooley, R., Kaplan, J., Allan, J., Groopman, J., Resnick, L., Felsenstein, D., Andrews, C. and Hirsch, M. (1985) *N. Engl. J. Med.* 313, 1493–1497
37 Shaw, G., Harper, M., Hahn, B., Epstein, L., Gajdusek, D., Price, R., Navia, B., Petito, C., O'Hara, C., Groopman, J., Cho, E-S., Oleske, J., Wong-Staal, F. and Gallo, R. C. (1985) *Science* 227, 177–182
38 Gabuzda, D., Ho, D., de la Monte, S., Hirsch, M., Rota, T. and Sobel, R. (1986) *Ann. Neurol.* 20, 289–295
39 Koenig, S., Gendelman, H., Orenstein, J., Dal Canto, M., Pezeshkpour, G., Yungbluth, M., Janotta, F., Aksamit, A., Martin, M. and Fauci, A. (1986) *Science* 233, 1089–1093

40 Wiley, C., Schrier, R., Nelson, J., Lampert, P. and Oldstone, M. B. A. (1986) *Proc. Natl Acad. Sci. USA* 83, 7089–7093
41 Dalgleish, A., Beverly, P., Clapham, R., Crawford, D., Greaves, M. and Weiss, R. (1984) *Nature* 312, 763–767
42 Klatzman, D., Champagne, E., Chamaret, S., Gruest, J., Guetard, D., Hercend, T., Gluckman, J-C. and Montagnier, L. (1984) *Nature* 312, 767–768
43 Maddon, P., Dalgleish, A., McDougal, J., Clapham, P., Weiss, R. and Axel, R. (1986) *Cell* 47, 333–348
44 McDougal, J., Kennedy, M., Sligh, J., Cort, S., Mawle, A. and Nicholson, J. (1986) *Science* 231, 382–385
45 Sattentau, Q., Dalgleish, A., Weiss, R. and Beverly, P. (1986) *Science* 234, 1120–1123
46 Hahn, B., Shaw, G., Taylor, M., Redfield, R., Markham, P., Salahuddin, S., Wong-Staal, F., Gallo, R., Parks, E. and Parks, W. (1986) *Science* 232, 1548–1553
47 Starcich, B., Hahn, B., Shaw, G., McNeely, P., Modrow, S., Wolf, H., Parks, E., Parks, W., Josephs, S., Gallo, R. and Wong-Staal, F. (1986) *Cell* 45, 637–648
48 Gurney, M. E., Heinrich, S., Lee, M. and Yin, H-S. (1986) *Science* 234, 566–574
49 Pert, C., Hill, J., Ruff, M., Berman, R., Robey, W., Arthur, L., Ruscetti, F. and Farrar, W. (1986) *Proc. Natl Acad. Sci. USA* 83, 9254–9258
50 Reiher, W., III, Blalock, J. and Brunck, T. (1986) *Proc. Natl Acad. Sci. USA* 83, 9188–9192
51 Gallo, R. C. (1987) *Sci. Am.* 256, 46–57

*We welcome comments from our readers. Short communications stand the best chance of publication, and the editor reserves the right to take extracts from the longer ones.*

## Neuronal calcium

SIR:

In a recent article[1], McBurney and Neering discussed the possible mechanisms involved in the maintenance of low levels of free calcium in neurons. Largely by analogy with other cell types, they defined four mechanisms: uptake of calcium into mitochondria, uptake into endoplasmic reticulum, calcium extrusion by the plasma membrane and calcium binding by cytosolic proteins. They did not, however, take account of two uniquely neuronal sites of calcium sequestration. In presynaptic nerve terminals, synaptic vesicles may be a major site of storage for calcium. The use of cytochemical techniques for the detection of calcium stores has demonstrated such a role for synaptic vesicles[2,3], and isolated brain synaptic vesicles have been shown to accumulate calcium through the action of a $Ca^{2+}$ ATPase[4].

At many synapses, the postsynaptic site of calcium sequestration may be a unique neuronal organelle, the spine apparatus[5]. In the dendritic spines of many central neurons, the spine apparatus (Fig. 1) is the only organelle present. Cytochemical studies have again pin-pointed the spine apparatus as a potential calcium store[2,3]. In addition, it has been speculated that the spine apparatus of dendritic spines could be the site of localization of receptors for inositol 1,4,5-trisphosphate[6], which in other systems releases calcium from intracellular stores. It is likely that neurons possess calcium regulatory mechanisms similar to those in other cell types, but also others with uniquely neuronal features.

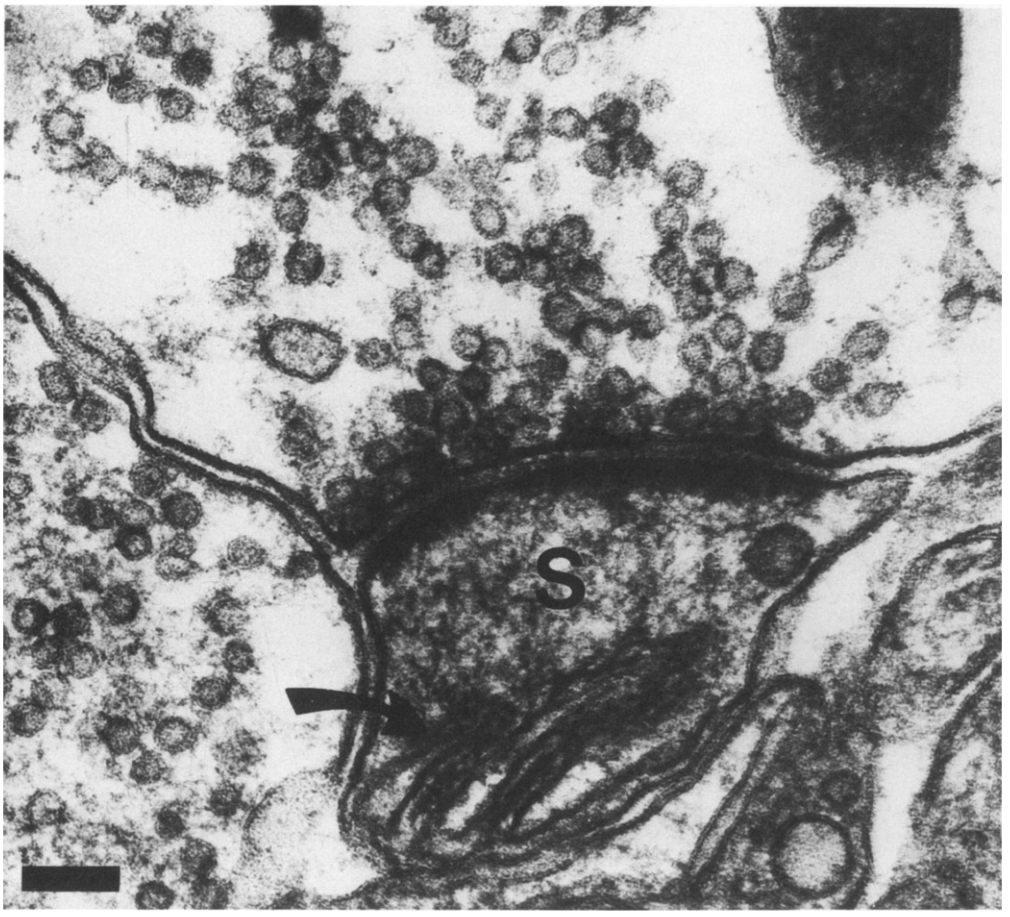


**Fig. 1.** *Electron micrograph of a synapse in the cerebral cortex of the rat. The characteristic spine apparatus (arrow) within the dendritic spine (S) consists of a series of membrane sacs and electron-dense plates. Scale bar is 0.1 μm.*

**Rogert D. Burgoyne**
*MRC Secretary Control Group, The Physiological Laboratory, University of Liverpool, PO Box 147, Liverpool L69 3BX, UK.*

### References

1 McBurney, R. N. and Neering, I. R. (1987) *Trends Neurosci.* 10, 164–169
2 Fifkova, E., Markham, J. A. and Delay, R. J. (1983) *Brain Res.* 266, 163–168
3 Burgoyne, R. D., Gray, E. G. and Barron, J. (1983) *J. Anat.* 136, 634–635
4 Rahamimoff, H. and Abramovitz, E. (1978) *FEBS Lett.* 92, 163–167
5 Gray, E. G. (1959) *J. Anat.* 93, 420–433
6 Worley, P. F., Baraban, J. M., Colvin, J. S. and Snyder, S. H. (1987) *Nature* 325, 159–161