RESEARCH ARTICLE

Acute Encephalitis, a Poliomyelitis-like Syndrome and Neurological Sequelae in a Hamster Model for Flavivirus Infections

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Infection of hamsters with the murine flavivirus Modoc results in (meningo)encephalitis, which is, during the acute phase, frequently associated with flaccid paralysis, as also observed in patients with West Nile virus encephalitis. Twenty percent of the hamsters that recover from the acute encephalitis develop life-long neurological sequelae, reminiscent of those observed, for example, in survivors of Japanese encephalitis. Magnetic resonance imaging and histology revealed severe lesions predominantly located in the olfactory-limbic system, both in hamsters with acute encephalitis as in survivors. Prominent pathology was also detected in the spinal cord of hamsters with paralysis. Modoc virus infections in hamsters provide a unique model for the study of encephalitis, a poliomyelitis-like syndrome and neurological sequelae following flavivirus infection.

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Introduction

Flavivirus infections are a major cause of severe encephalitis in man. The West Nile virus (WNV) is mainly endemic in Africa, the Middle East and around the Mediterranean Sea. In 1999, an outbreak of WNV caused 62 cases of encephalitis in the New York City metropolitan area, with 7 deaths (12). In 2000 and 2001, increased WNV activity and westward spread of the virus was reported (4). In 2002, WVN severely hit the central United States with a nationwide total (as of December 31, 2002) of at least 4156 registered cases and 284 deaths (*http://www.cdc.gov/*). In recent years, WNV epidemics in humans have also been reported in Romania (1996) (15), in southern Russia (1999) (32), and in Israel (2000) (48). A poliomyelitis-like syndrome and flaccid paralysis have been recently reported to be associated with WNV infection (13, 28). The Japanese encephalitis virus (JEV), another flavivirus, causes one of the most common arthropod-borne encephalitides and occurs in a vast geographic area including India, China, and virtually the whole of Southeast Asia (17). Although it is estimated that only one in 25 to 1000 infections results in clinical illness, each year, at least 10 000 cases of symptomatic JEV (sporadic or epidemic) are reported (52). Morbidity can be as high as 30 to 50% (44), whereas neurological and psychiatric sequelae are seen in 25 to 40% of the survivors (50, 52). Two important subtypes of the tick-borne encephalitis virus (TBEV) exist, ie, the European and the Eastern subtype. The mortality rate associated with infection by the Eastern subtype (also referred to as Russian spring-summer encephalitis virus [RSSEV]) is about 20%; for infection by the European subtype (also referred to as Central European encephalitis virus [CEEV]), this value is 1 to 2% (16). Also the Saint Louis encephalitis virus (SLEV), endemic in the western United States (25), the Murray Valley encephalitis virus (MVEV), endemic in Australia (33), the Powassan virus (POWV), endemic in Canada (5), and several other flaviviruses are known to cause severe encephalitis in man.

We employed the Modoc virus (MODV) to establish a model for flavivirus infections in hamsters (30). The Modoc virus has been isolated from white-footed deer mice (*Peromyscus maniculatus*) captured in California (Modoc county) in 1958 (18). Later on, the virus was also isolated from deer mice trapped in the wild in Oregon, Montana, Colorado, and Alberta, Canada (57). Neutralization tests using blood samples isolated from mammals trapped in Alberta, as well as from humans, indicate the appearance of natural infection without disease (57). No arthropod vector has been demonstrated for MODV (18). Based on cross-serological reactivity, the virus has been classified as a Flavivirus (8, 10, 53). Recently, we have determined the complete genomic sequence of the Modoc virus. A detailed phylogenetic and taxonomic

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analysis of the entire coding region of the genome confirmed the classification of MODV in the cluster of flaviviruses with no known vector (29).

Here, we present a unique model for the study of acute flavivirus encephalitis, flavivirus-induced flaccid paralysis, a flavivirus-associated poliomyelitis-like syndrome and long-lasting neurological sequelae following flavivirus infection.

Materials and Methods

Cells and viruses. African green monkey kidney (Vero) cells were grown in minimum essential medium (MEM, Gibco, Paisley, Scotland) supplemented with 10% inactivated fetal calf serum (FCS, Integro, Zaandam, The Netherlands), 1% L-glutamine, and 0.3% bicarbonate. MODV was obtained from the American Type Culture Collection (ATCC VR-415) and cultured on Vero cells as previously described (30).

Animals. Eight- to 12-week old Gold hamsters (Mesocricetus auratus) were used throughout the experiments. All animals were bred at the Rega Institute. The hamsters were maintained under artificial diurnal lighting conditions with free access to food and water. The principles of good laboratory animal care were followed. All experiments were approved by the ethical committee on vertebrate animal experiments of the K. U. Leuven.

Animal experiments. Hamsters were inoculated with 10^4 PFU (plaque forming units) of Modoc virus either via the intraperitoneal route (i.p.) (10^4 PFU in 500 μl) or, following brief ether anesthesia, via the intranasal route (i.n.) (10^4 PFU in 50 μl). All animals were weighed daily and examined for clinical manifestations of encephalitis such as tremor, muscle weakness, paralysis, closed eye(s), and posture. Every day, the animals received a single dose of 16 mg/kg of oxytetracycline by subcutaneous (s.c.) injection to suppress bacterial infection of the gut (a condition seen in most MODV-infected hamsters), thus increasing the animal's comfort. Hamsters with severe encephalitis, that was judged to be very likely lethal, were subjected to magnetic resonance imaging shortly before fatal outcome of the disease was expected, after which the animals were euthanized and the brain and spinal cord dissected (see below). Animals with moderate encephalitis and that were assumed to survive the acute phase of the infection were kept for several months (up to 1.5 years) to allow evaluation of residual neurological brain damage by MRI and histopathology, long after the symptoms of the acute encephalitis had resolved.

Magnetic resonance imaging. The hamsters were anesthetized by intramuscular injection with a volume (2 μl/g) of a 1:2:2 mixture of atropine (0.5 mg/ml, 0.2 mg/kg, Sterop, Brussels, Belgium), ketamine (50 mg/ml, 40 mg/kg, Ketalar®, Parke-Davis, Zaventem, Belgium) and xylazine (20 mg/ml, 16 mg/kg, XYL-M® 2%, VMD, Arendonk, Belgium). T2-weighted MRI scans were generated with a 2.4 T MRI system (Bruker B – C 24/40, Ettlingen, Germany; magnetic center 588 mm) with the following parameters: 10 slices; slice thickness: 2 mm; interslice distance: 2 mm; field of view (FOV): 4 × 4 cm. A multi-spin-multi-echo sequence was used (TR = 3000 ms; TE = 8, 16, 24 up to 96 ms [a total of 12 echoes]; matrix 256 × 192). The raw data were processed with the ANALYZE™ software package (version 7.5, Mayo Biomedical Imaging Resource, Mayo Foundation, Rochester, Minn).

Histopathology. Following imaging of the brain, the animals were sacrificed by ether anesthesia and perfused transcardially with 20 ml of 4% buffered formaline. The brain and spinal cord were dissected and post-fixed in 4% buffered formaline, embedded in paraffin blocks, and processed following standard histological procedures. For each animal, six consecutive axial sections of 6 μm were cut at 40 different positions of the brain at a mutual distance of ±0.3 mm. This meticulous approach ensured that all major anatomical areas of the brain were represented in the tissue sections. For routine microscopic evaluation, one slide per position was stained according to the Luxol-Fast blue MBS stain (Klüver-Barrera). Remaining slides were available for supplementary staining, such as immunohistochemistry, when necessary.

Results

Modoc virus infection in hamsters. Following intranasal or intraperitoneal inoculation with the virus, 100% of the hamsters (59 animals; 4 independent experiments) developed acute MODV encephalitis, of which about 50% (30/59) succumbed during the acute phase of the infection. Hamsters with acute MODV encephalitis presented with closed eyes, muscle weakness and flaccid paralysis (Figure 4A) or a poliomyelitis-like syndrome (Figure 4B), often accompanied by tremor. Of the hamsters that survived the acute phase of the infection, about 80% (23/29) did not

Morphological area of the brain	Acute encephalitis following *intranasal* inoculation				Acute encephalitis following *intraperitoneal* inoculation			
	-	+	++	+++	-	+	++	+++
a Olfactory/frontobasal lobe	.	4*	2	[1]	1	2	1	.
b Temporal lobe	.	.	.	[7]	.	2	2	.
c (Hypo)thalamic area	1	2	3	[1]	2	1	1	.
d Cingulate gyrus	5	[2]	.	.	4	.	.	.
e Ventricles	4	1	1	[1]	1	2	1	.
f Hindbrain	7	.	.	.	3	[1]	.	.
	Total of 7 hamsters				Total of 4 hamsters			

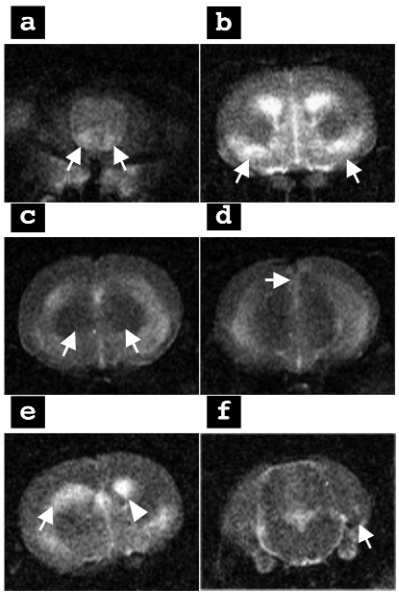

Figure 1. *Abnormalities in the brain of hamsters during the acute phase of Modoc virus encephalitis as monitored by MRI.* Intensity of the lesions (or widening in case of the ventricles): (-) = none; (+) = mild; (++) = moderate; (+++) = severe; * indicates the number of animals displaying none, mild, moderate, or severe MRI abnormalities. □ = T2-weighted MRI images that are presented in the panel of figures on the right. Arrows indicate the corresponding abnormality (abnormalities) on the MRI images. Animal identification codes: (a) R3N1; (b) R3N1; (c) R3N5; (d) R3N5; (e) R3N3 and (f) R4N5.

show obvious neurological deficits, whereas the remaining 20% (6/29) of the survivors presented with clearly visible neurological sequelae, ie, flaccid paralysis of one of the front legs or paralysis of one of the hind legs, and wasting of the muscles thereof, resembling a poliomyelitis-like syndrome (Figure 5A, B).

Cerebral MRI of hamsters with acute Modoc virus-induced encephalitis. MRI was employed to generate T2-weighted images of the brain of 7 hamsters that had been inoculated with Modoc virus via the intranasal route and that presented with signs of severe acute encephalitis. These images clearly demonstrate severe and nearly symmetrical tissue damage in both temporal lobes of all 7 animals (Figure 1B). Hyperintense signals of variable intensity were observed in the olfactory/frontobasal lobes (7/7 animals) (Figure 1A) and in the (hypo)thalamic area (6/7) (Figure 1C), suggestive of mild to severe brain lesions. In 2 animals, the MRI scans indicated the presence of mild tissue damage in the *cingulate gyri* (Figure 1D). Mild to severe widening of the lateral ventricles was distinguishable on MRI scans of 3 animals (Figure 1E). One of the latter hamsters showed blockage of the foramen of Monroe of a lateral ventricle accompanied by widening of the contralateral ventricle (Figure 1E).

The brain of 4 hamsters that had been inoculated with MODV via the intraperitoneal route and that had also presented with signs of severe encephalitis at the time of cranial MRI, showed a similar distribution pattern of lesions on T2-weighted images. However, the lesions appeared to be less pronounced and more focused as compared to those observed in animals that were inoculated via the intranasal route (Figure 1). MRI abnormalities were observed in the hindbrain of one animal (Figure 1F).

Histopathology of the brain and spinal cord of hamsters with acute Modoc virus-induced encephalitis. All hamsters, that were infected via the intranasal route, presented with a diffuse and moderate to severe leptomeningoencephalitis, which was characterized by a specific and recurrent topographic distribution pattern (Figure 2A). The lesions showed a remarkable olfactory-limbic predominance, with damage to the *bulbus*

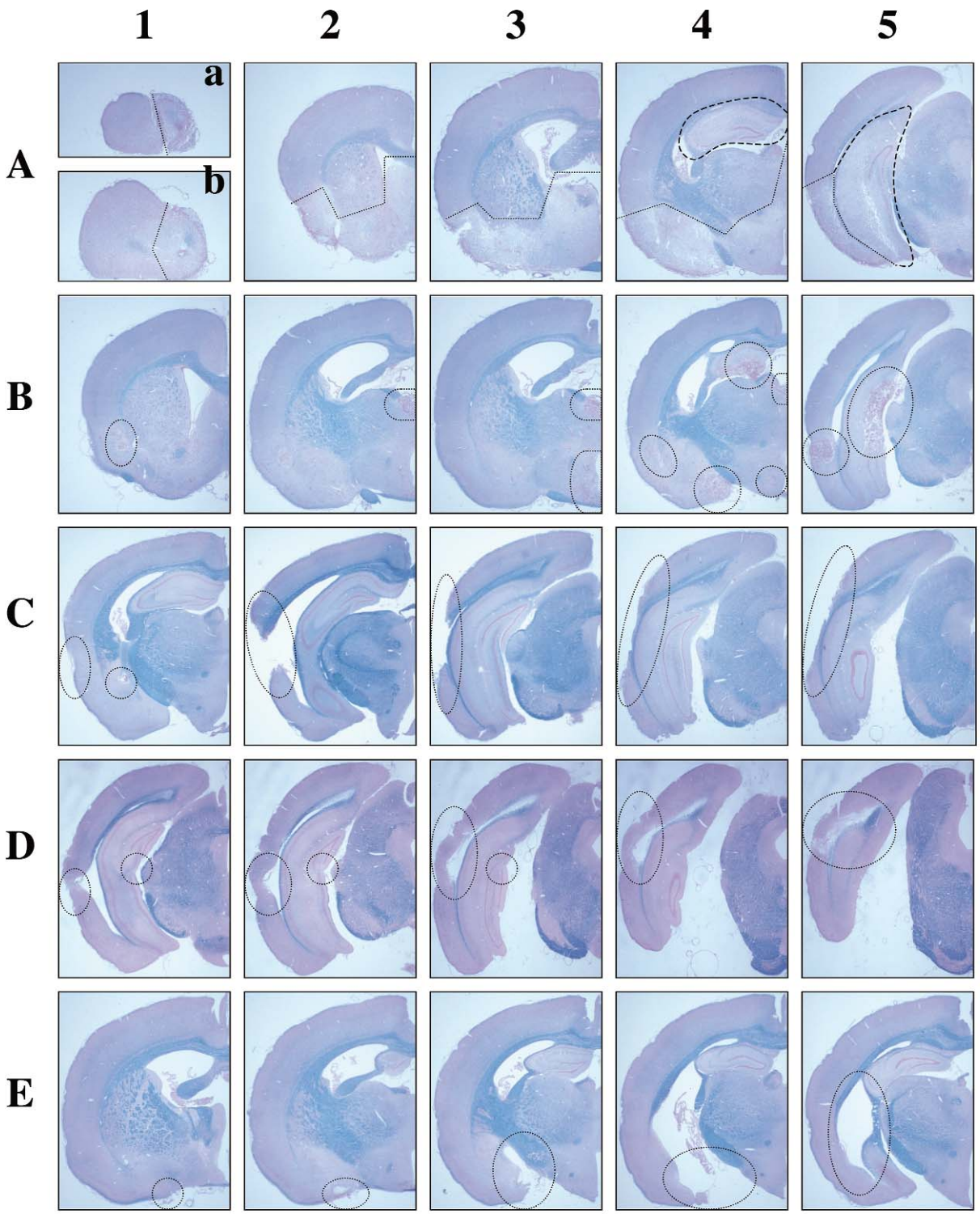
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Figure 2. (Opposing page) *Axial brain sections of hamsters with acute MODV encephalitis.*
A. Tissue sections of the brain of a moribund hamster (animal identification code R3N3) with acute MODV encephalitis that was inoculated via the intranasal route: (1a) Inflammation and necrosis of the olfactory nuclei with loss of the tissue architecture (right hand side of the image); (1b) Inflammation and necrosis below the rhinal fissure (right hand side of the image); (2) Inflammation and necrosis involving (from left to right below the dotted line) *cortex piriformis*, dorsal endopiriform nucleus, *tuberculum olfactorium*, medial forebrain bundle and the septal nuclei; (3) Inflammation and necrosis involving (from left to right below the dotted line) cortex piriformis, dorsal and ventral endopiriform nuclei, anterior amygdaloid area and nuclei of the preoptic, hypothalamic and thalamic area; (4) Inflammation and necrosis involving (from left to right below the dotted line) *cortex piriformis*, dorsal and ventral endopiriform nuclei, amygdaloid nuclei, nuclei of the hypothalamic and thalamic area, nuclei of the habenular area, damage to the CA1, CA2 and CA3 area of the hippocampus (encircled by the striped line); (5) Inflammation and necrosis involving the lateral entorhinal and perirhinal cortex, dorsal endopiriform nucleus, and nuclei of the amygdaloid area (from upper left to lower right below the dotted line), necrotic damage to CA1, CA2 and CA3 area of the hippocampus (encircled by the striped line).
B. Tissue sections of the brain of a moribund hamster (animal identification code R4N5) that was inoculated via the intraperitoneal route: Focus of inflammation and necrosis (1) in the dorsal endopiriform nucleus; (2) in the paratenial and paraventricular thalamic nucleus; (3) in the paratenial and paraventricular thalamic nucleus, and in the preoptic nuclei; (4) in the hippocampus, mediodorsal thalamic nucleus, dorsale endopiriform nucleus, medial amygdaloid area and anterior hypothalamic area; (5) in the perirhinal and lateral entorhinal cortex, and CA3 of the hippocampus.
C. Tissue sections of the brain of a hamster without neurological sequelae (animal identification code R1/G; dissection of the brain at 652 days postinfection): (1) Focus of cystic fibrosis in the agranular/granular insular cortex and secondary somatosensory cortex, and in the central amygdaloid nucleus; (2) Continuity between the lateral ventricle and the subarachnoidal space because of transmural necrosis of the temporal cortex with rupture of the ependymal cell layer and *pia mater*; (3-5) decortication and decerebration involving the agranular insular cortex, perirhinal cortex, primary auditory cortex, secondary and primary visual cortex.
D. Tissue sections of the brain of a hamster with apparent neurological sequelae (animal identification code R1/X; dissection of the brain at 575 days postinfection): (1-3) decortication in the perirhinal/primary auditory cortex with widening of the lateral ventricle, focus of neuronal loss in the CA3 area of the hippocampus; (4) decortication in the perirhinal/primary auditory/secondary visual cortex with widening of the lateral ventricle; (5) area of cystic fibrosis involving the ectorhinal/temporal association/secondary visual cortex.
E. Tissue sections of the brain of a hamster without apparent neurological sequelae (animal identification code R1/D; dissection of the brain at 652 days postinfection): (1-2) Area of cystic fibrosis in the anterior cortical amygdaloid nucleus (3-4) extending to involve the amygdaloid area; (4-5) widening of the lateral ventricle.

olfactorius, *cortex piriformis*, prelimbic cortices, *hippocampi*, *corpora amygdaloidea*, *corpora mamillaria*, hypothalamic nuclei, and preoptic nuclei. *Cerebellum*, *medulla oblongata*, *medulla spinalis*, and basal ganglia, with exception of the *nucleus accumbens*, were not affected. The meningitis component was rather limited to the leptomeninges covering the affected anatomical regions (Figure 3A, B). The worst affected regions, primarily the olfactory bulbs and *cortex piriformis*, were necrotic with neuronal cell loss and granular destruction of the white and gray matter architecture (Figure 3A, B). Adjacent to these necrotic areas, less affected regions were found to be spongiotic with increased cellularity because of infiltration by lymphocytes and reactive astrocytic proliferation (Figure 3A) with the formation of microglial nodules. Furthermore, satellitosis, neuronophagia, and singular cell necrosis were observed (Figure 3B). Infiltration of inflammatory cells in the ependymal cell layer and *plexus choroideus* was apparent, as was an increased cellularity of the cerebrospinal fluid (data not shown). Polymorphonuclear inflammatory cells were not observed. Intracellular viral inclusions or pathognomonic viral cytotoxic effects were not distinguishable. The acute phase of MODV encephalitis following intranasal inoculation is thus characterized by a moderate to severe non-specific viral leptomeningoencephalitis with a predominant tropism for the olfactory and limbic system, which is consistent with the route of inoculation and cerebral MRI findings.

In contrast to hamsters that were inoculated via the intranasal route, no specific or recurrent distribution pattern of CNS damage was noted for hamsters that were infected via the intraperitoneal route. Brain lesions were found to be more focal and not diffusely continuous as was observed in intranasally infected hamsters (Figures 2B; 3C, D). On one hand, the olfactory bulbs were completely spared, but on the other hand, the thalamic and hypothalamic nuclei, as well as the basal ganglia, were more severely affected in intraperitoneally infected hamsters. Occasionally, also the *nuclei habenulae* showed histopathological damage. The inflammatory response was not different from that described for hamsters infected via the intranasal route (Figure 3C, D), and is consistent with a mild to moderate non-specific viral encephalomyelitis with leptomeningitis. The lesions were predominantly observed in morphological regions belonging to the olfactory and limbic system, as was observed for hamsters infected via the intranasal route.

The spinal cord of hamsters that had been inoculated via the intraperitoneal route was characterized by

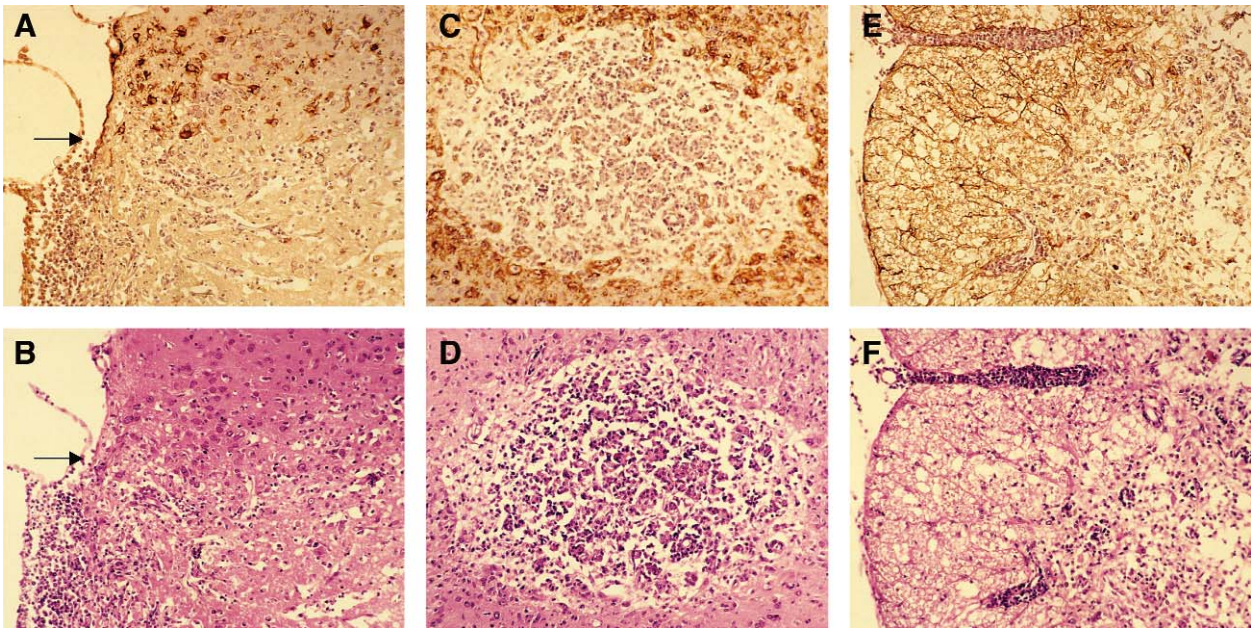

Figure 3. *Histopathology of MODV encephalitis.* (**A**) Immunohistochemical staining for glial fibrillary acidic protein (GFAP) and (**B**) staining with hematoxylin/eosin (H&E) of the brain of a hamster with acute encephalitis following intranasal inoculation with MODV (higher magnification of a tissue section adjacent to that depicted in Figure 2-A4). The rhinal fissure (arrow) represents an imaginary boundary between (i) affected parenchyma under the fissure, of which the leptomeninges show clear signs of meningitis and in which a clearly visible inflammatory infiltrate can be observed and (ii) above the rhinal fissure, less affected parenchyma with reactive astrocytic proliferation; (**C**) GFAP staining and (**D**) H&E staining of the brain of a hamster with acute encephalitis following intraperitoneal inoculation with MODV (higher magnification of a tissue section adjacent to that depicted in Figure 2-B1). Focal and well delineated lesion with severe tissue destruction and extensive inflammatory infiltrate in the dorsal endopiriform nucleus, surrounded by less affected gray matter with clear signs of reactive astrocytic proliferation at the border of the focal lesion; (**E**) GFAP staining and (**F**) H&E staining of the spinal cord of a hamster with acute encephalitis following intraperitoneal inoculation with MODV (higher magnification of a tissue section adjacent to that depicted Figure 4C). Perivascular cuffing, infiltration of the neuronal tissue by inflammatory cells, destruction of neurons and spongiosis are apparent.

extensive bilateral (Figure 4C) or unilateral (Figure 4D, E) damage to the grey matter. Perivascular cuffing, infiltration of the neuronal tissue by inflammatory cells, destruction of neurons and spongiosis were perceptible (Figure 3E, F). No signs of pathology were observed in the spinal cord of hamsters that had been infected via the intranasal route.

Cerebral MRI of long-term surviving hamsters with and without neurological sequelae. T2-weighted MRI images were obtained from the brains of 2 hamsters with neurological sequelae and of 7 hamsters without apparent neurological sequelae (on day 317 postinfection [2 hamsters], day 575 [3 hamsters], and day 652 [4 hamsters]). The residual neurological sequelae in 2 of the hamsters that survived acute MODV infection (examined on day 575 and 652 postinfection, respectively) were characterized by paralysis of one of the hind legs and wasting of the muscles thereof, resembling a poliomyelitis-like syndrome (Figure 5A, B). This was further visualized by the ink footprint trail of one of these hamsters (Figure 5C). Another hamster (not included in the MRI study) showed long-lasting flaccid paralysis of one of the front legs as visualized by the ink footprint trail (Figure 5D). The footprint trail of a healthy hamster is shown for comparison (Figure 5E). MRI abnormalities were moderate in the temporal lobes of all hamsters (Figure 6B), regardless of the presence of (observable) sequelae. Mild lesions were also observed in the orbitofrontal cortex of 2 animals (22%) (Figure 6A), in the (hypo)thalamic area of one animal (11%) (Figure 6C), and in the *cingulate gyrus* of another animal (11%) (Figure 6D). Mild widening of the lateral ventricles was visible in 4 hamsters (44%) (Figure 6E). No abnormalities were detected in the hindbrain of any of the animals (Figure 6F).

Histopathology of the brain and spinal cord of long-term survivors with and without neurological sequelae. Signs of mild residual meningoencephalitis and

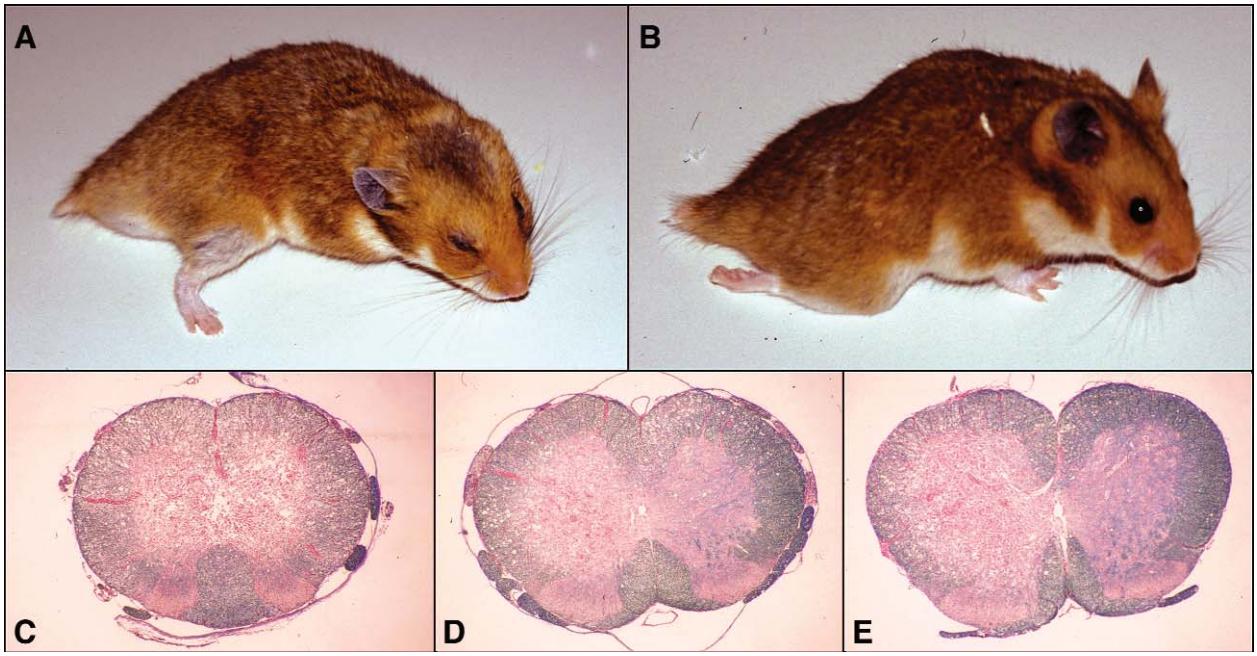

Figure 4. *Acute MODV encephalitis and MODV-associated spinal cord damage.* (**A**) hamster (animal identification code R4N4) with acute MODV encephalitis presenting with muscle weakness, flaccid paralysis of the 4 limbs and closed eyes; (**B**) hamster (animal identification code R4N5) with acute MODV encephalitis presenting with a poliomyelitis-like syndrome characterized by paralysis of the hind limbs; (**C**) axial section of the spinal cord of a hamster (animal identification code R4N4) with severe MODV encephalitis showing bilateral damage to the grey matter; (**D, E**) axial sections of the spinal cord of a hamster (animal identification code R4N5) with severe MODV encephalitis showing unilateral damage to the grey matter.

irreversible tissue destruction were observed in the brain of hamsters more than one year after they recovered from acute MODV encephalitis. In all hamsters, more or less pronounced dilatation of the ventricular system was noted, as was an inconspicuous meningeal lymphocytic infiltrate (data not shown). In one hamster, very severe irreversible tissue damage was observed with continuity between the lateral ventricle and the subarachnoidal space due to transmural necrosis of the temporal cortex (Figure 2C). In the majority of the hamsters, a more or less pronounced mass reduction of the temporal and frontobasal cortices was observed. Pseudocystic lesions, particularly located in the temporal and frontobasal cortices, indicated focal tissue loss due to infarction (Figure 2D, E). Cortical cell layers adjacent to the *cortex piriformis* and the cell layers of the hippocampus were less dense as a result of individual neuronal cell loss. The residual neurons presented with atypical, irregular and hyperchromatic nuclei (data not shown). Viral inclusions could not be observed with light microscopy. In the brain parenchyma, a sparse lymphocytic infiltrate was still present and a slight perivascular accumulation of inflammatory cells in the Virchow Robin spaces of small and medium-sized blood vessels (perivascular cuffing) was observed (data not shown).

Only minor histopathological changes, such as spongiosis around the central canal and small, pseudocystic lesions (Figure 5F), were noted in the spinal cord of several hamsters that survived the acute encephalitis for >1 year without neurological sequelae. Severe residual tissue damage, such as loss of grey matter, widening of the central canal and the presence of inflammatory cells in the spinal cord tissue, was, however, detected in hamsters that presented with apparent neurological sequelae such as a poliomyelitis-like syndrome (Figure 5G).

Discussion

Following intranasal or intraperitoneal inoculation with MODV, hamsters develop acute encephalitis, which is amongst other characterized by muscle weakness, flaccid paralysis or a poliomyelitis-like syndrome, and tremor. About 50% of the animals succumb during the acute phase of the infection, about 40% survive without neurological sequelae, and the remaining 10% recover with obvious long-lasting neurological sequelae.

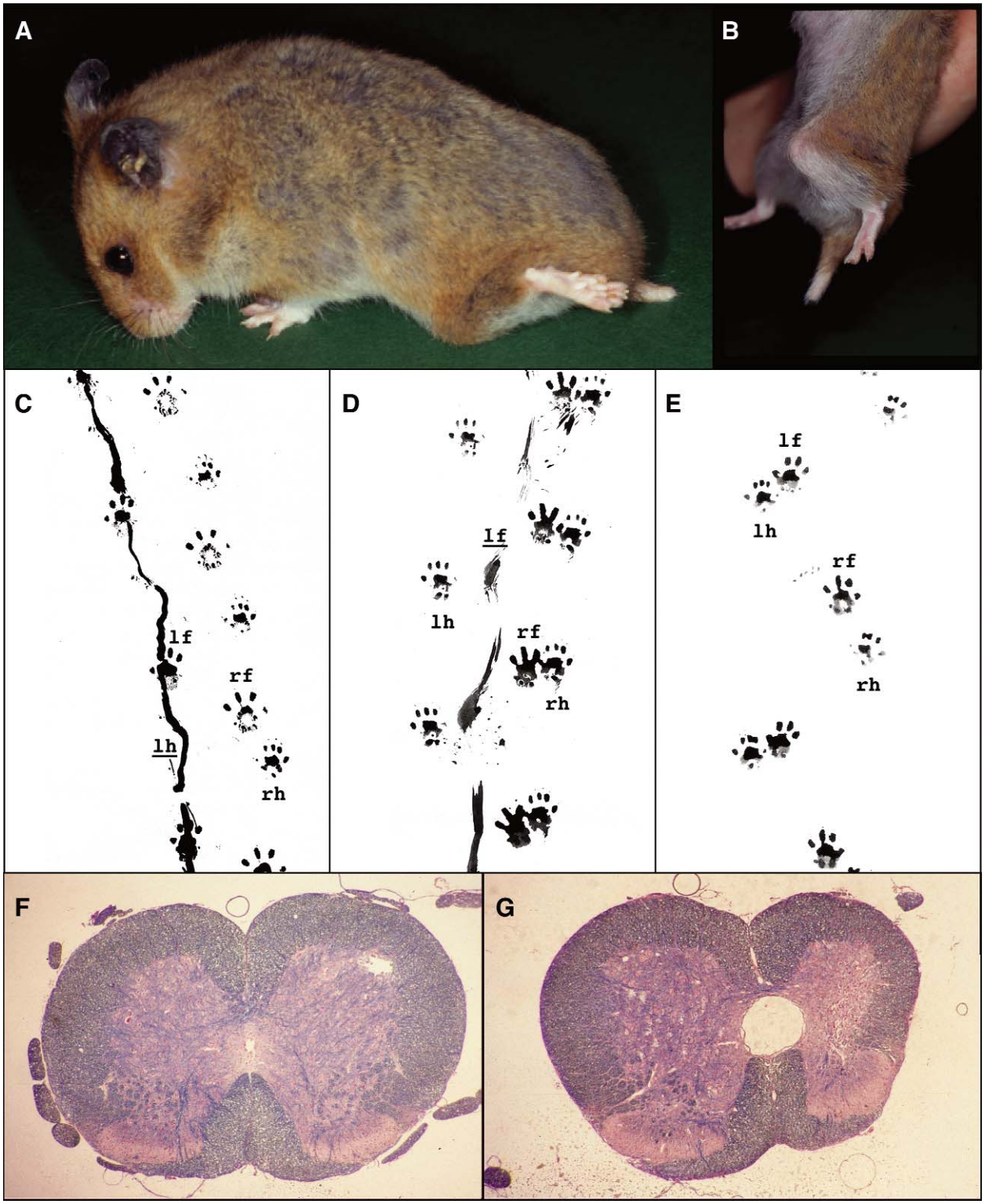
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Figure 5. (Opposing page) *Lifelong neurological sequelae and spinal cord damage following MODV encephalitis.* (**A, B**) Hamster (animal identification code R2/Z), >1 year postinfection, with long-lasting paralysis of the left hind leg and wasting of the muscles thereof; (**C**) ink footprints of a hamster with paralysis of the left hind leg >1 year postinfection; (**D**) ink footprints of a hamster with paralysis of the left front leg >1 year postinfection; (**E**) ink footprints of a healthy uninfected hamster; (**F**) pseudocystic lesion, suggesting tissue loss due to infarction, in the spinal cord of a hamster without neurological sequelae (animal identification code R1/G); and (**G**) severe, unilateral loss of grey matter, loss of cell bodies in the contralateral half, and widening of the central canal in the spinal cord of a hamster with residual neurological sequelae >1 year postinfection (animal identification code R2/Z).
lf = left front leg footprint, lh = left hind leg footprint, rf = right front leg footprint, rh = right hind leg footprint. Underlining marks the paralysed leg.

Morphological area of the brain	Long-term survivors *without* sequelae				Long-term survivors *with* sequelae			
	-	+	++	+++	-	+	++	+++
a Olfactory/frontobasal lobe	6*	1	.	.	1	[1]	.	.
b Temporal lobe	.	.	7	.	.	.	[2]	.
c (Hypo)thalamic area	6	[1]	.	.	2	.	.	.
d Cingulate gyrus	7	.	.	.	1	.	[1]	.
e Ventricles	4	3	.	.	1	[1]	.	.
f Hindbrain	7	.	.	.	[2]	.	.	.
	Total of 7 hamsters				Total of 2 hamsters			

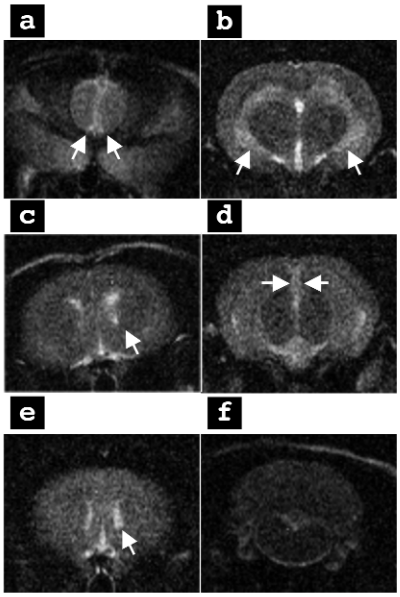

Figure 6. *Abnormalities in the brain of hamsters that survived the acute phase of MODV encephalitis as monitored by MRI.* Intensity of the lesions (or widening in case of the ventricles): (-) = none; (+) = mild; (++) = moderate; (+++) = severe; * indicates the number of animals displaying none, mild, moderate, or severe MRI abnormalities. [ ] = T2-weighted MRI images that are presented in the panel of figures on the right. T2-weighted MRI image are shown in panel of figures on the right. Arrows indicate the corresponding abnormality (abnormalities) on the MRI images. Animal identification codes: (a) R1/Q; (b) R1/Q; (c) R2/AC; (e) R1/Q; (f) R1/Q and (g) R2/AC.

MRI examination of the brain of hamsters during the acute phase of MODV encephalitis (either following intranasal or intraperitoneal inoculation), revealed moderate to severe lesions in both temporal lobes of all animals. Also the thalamic-hypothalamic area appeared to be generally affected and widening of the lateral ventricles was observed. Histological study of the brain of animals with acute encephalitis not only revealed severe tissue damage in the above mentioned areas, but also revealed that the brain pathology was more extensive than was expected from the MRI scans. Generally, a 1.5 T MRI scanner is used in the clinical setting. Because of the smaller brain size of hamsters, we used 2.4 T MRI equipment to obtain images with higher resolution. A good correlation was observed when the MRI data and the histopathological data were compared. However, the resolution of the images might still have been too low to adequately visualize less pronounced, but yet important brain lesions, that were only detectable microscopically. Diffuse (midbrain) lesions, that were observed with MRI in the brain of hamsters with MODV encephalitis, have also been reported in patients with Japanese encephalitis (39).

Multiple MRI studies on patients with Japanese encephalitis (11, 21, 24, 26, 38, 45), tick-borne encephalitis (3, 31), and Saint Louis encephalitis (54) sug-

gested that lesions located in the thalamic nuclei are highly indicative for flavivirus encephalitis. The MRI abnormalities and the corresponding histopathological lesions that we observed in the thalamic area of the brain of MODV-infected hamsters further corroborate the importance of these lesions as a hallmark of flavivirus encephalitis. However, thalamic lesions have not been observed in all patients (or hamsters) with flavivirus encephalitis and may therefore not be regarded as a prerequisite for the diagnosis thereof. For example, in some patients, lesions were found to be confined to the *substantia nigra*, phenotyped by Japanese encephalitis-associated Parkinsonism (23, 47). Similar findings have also been reported in patients with Saint Louis encephalitis (11). In another report, the MRI and EEG characteristics of Japanese encephalitis were observed in a patient with HSE (43). Similar to what we observed in MODV-infected hamsters, brain lesions in patients with flavivirus encephalitis have also been observed in the cortical grey matter, brain stem nuclei (19, 41, 46), white matter (45), and, less frequently, in the basal ganglia and cerebellum (11, 22, 36).

During the acute phase of MODV encephalitis, also spinal cord abnormalities were noted. In humans, flavivirus-associated spinal cord damage, as detected by MRI, has been observed in patients with acute tick-borne encephalitis (7, 11, 22), Japanese encephalitis (35) and West Nile virus (13, 28).

Long after the obvious symptoms of the acute MODV encephalitis resolved, all hamsters, even hamsters that appeared healthy, presented with residual lesions (eg, in the temporal lobes) that were detectable by MRI. Residual histopathological changes such as small cyst-like lesions of presumable encephaloclastic response and posthemorrhagic lesions with hemosiderin deposits were detectable in the brain of the hamsters. These abnormalities were similar to those reported to occur in the post-acute stage following severe Japanese encephalitis (38, 46). Decerebration and decortication, as observed in hamsters that survived the acute phase of MODV disease, are also frequently noted in patients that survive severe Japanese encephalitis (37, 41). In contrast to humans, on which a standard array of neurological tests can be performed to specifically identify these neurological deficits, animals need to be trained to perform certain tasks. The hamsters in this study were not trained to participate in any of such behavioral or cognitive experiments. Therefore, the actual percentage of animals with residual neurological deficits following acute MODV encephalitis may have been higher than the observed 10%, which only represents the percentage of animals with obvious neurological sequelae.

Although there are other hamster models available for the study of flavivirus encephalitis (51, 56), MODV infections in hamsters represent, to the best of our knowledge, the first model in which small laboratory animals develop long-lasting neurological sequelae following acute flavivirus encephalitis induced by intraperitoneal inoculation with the virus. Following intracerebral inoculation with West Nile virus, a poliomyelitis-like syndrome has been described in monkeys (34) and a similar syndrome has been described in horses following natural infection with West Nile virus (9). The situation in hamsters that survived the acute phase of MODV encephalitis is reminiscent of that of patients that survived severe Japanese encephalitis (1, 36, 38, 40, 46, 50), Saint Louis encephalitis (55), West Nile encephalitis (2) or tick-borne encephalitis (7, 14, 20, 27, 42). Movement disorders, or a poliomyelitis-like syndrome, have been reported regularly following severe acute Japanese encephalitis (6, 35, 36, 49), and muscle wasting was reported in up to 25% of these patients (38). Acute flaccid paralysis of the limbs, as well as a poliomyelitis-like syndrome have been observed in patients with severe West Nile virus infections (2, 13, 28). In addition, cognitive and emotional deficits have been observed in patients that survived acute flavivirus encephalitis.

In conclusion, we here presented a unique animal model, reminiscent of flavivirus encephalitis and a flavivirus-induced poliomyelitis-like syndrome in humans. Hamsters that survive the acute phase of the infection may develop long-lasting neurological sequelae. This model will be instrumental for the study of the pathogenesis and therapy of flavivirus infections involving the central nervous system.

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