

Nonhuman primate models of aerosol exposure to alphaviruses

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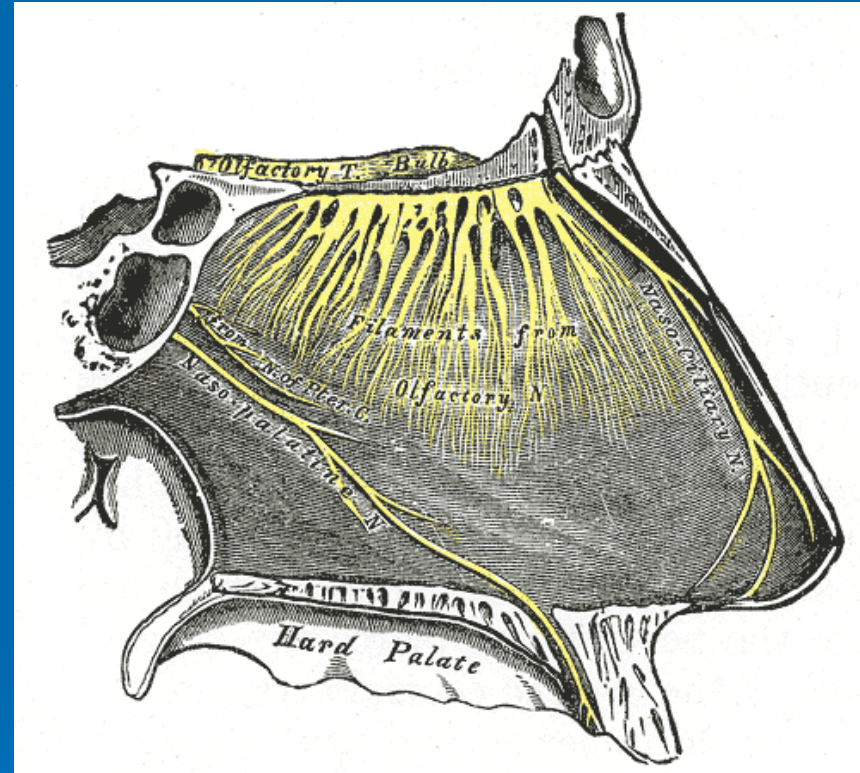
Alphaviruses

- Small (11.4 kb), positive-stranded RNA viruses
- Venezuelan (VEEV), western (WEEV), and eastern (EEEV) equine encephalitis viruses are naturally transmitted by mosquitoes, but are also highly infectious by aerosol.
- By mosquito, disease caused by VEEV and WEEV is incapacitating but rarely lethal.
- EEEV outbreaks are rare but have a high mortality (>30%) and a large percentage of survivors have long-term neurological complications.
- There are no licensed vaccines for humans. Current IND vaccines have problems with reactogenicity and poor immunological response.



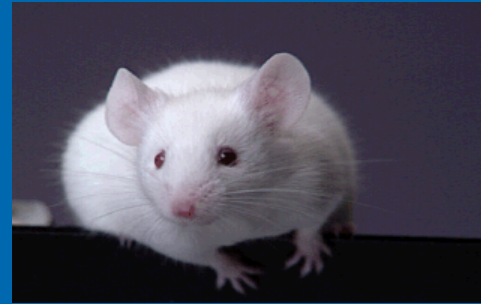
Aerosol exposure to alphaviruses

- Pathogenesis studies in animal models have shown that after natural infection, viremia leads to infection of the olfactory region and up through the olfactory nerves to the brain, causing encephalitis.
- Removing olfactory bulbs prevents or delays onset of encephalitis after subcutaneous inoculation of alphaviruses.
- Aerosol exposure may bypass the need for viremia because of direct infection of the olfactory region.



Rodent models

- Mice (VEEV, WEEV, EEEV)
By far the most used. Lethal infection; cause of death is viral encephalitis.
- Guinea pigs (EEEV)
Fatal viral encephalitis after aerosol exposure (Roy et al, submitted)
- Hamsters (VEEV, EEEV)
For VEEV infection, cause of death is endotoxic shock caused by sloughing of the intestinal wall. EEEV model was recently reported, cause of death was fatal encephalitis with vascular complications.



Nonhuman primate models

- Rhesus & cynomolgus macaques have been used for studies with VEEV. Rhesus were used in the 1930s for studies with WEEV and EEEV (no publications since 1939)
- In 1998, Pratt et al (Vaccine 16:1056-64) at USAMRIID used radiotelemetry devices to monitor fever after aerosol exposure of cynomolgus macaques to a virulent epizootic strain of VEEV.
- In 2004, Reed et al (JID 189:1013-7) at USAMRIID reported that enzootic strains could cause illness equivalent to epizootic strains in cynomolgus macaques exposed by the aerosol route.



Aerosol Exposure of Nonhuman Primates (NHP)



1 μm particle – deep lung

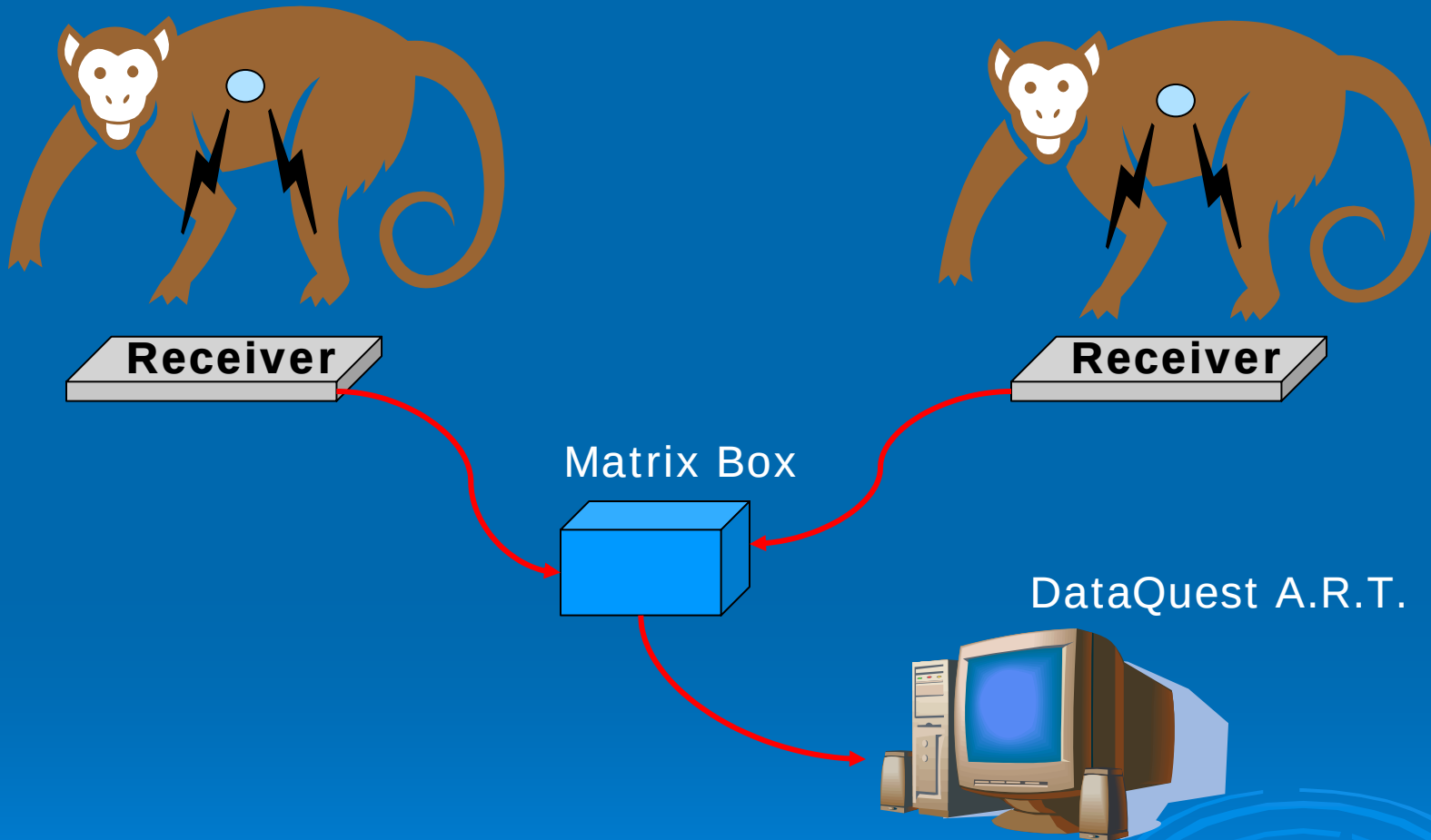
Whole-body plethysmograph
done prior to exposure

Dose confirmed by plaque assays
from starting concentrations and
aerosol sample collection



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Collection of telemetry data

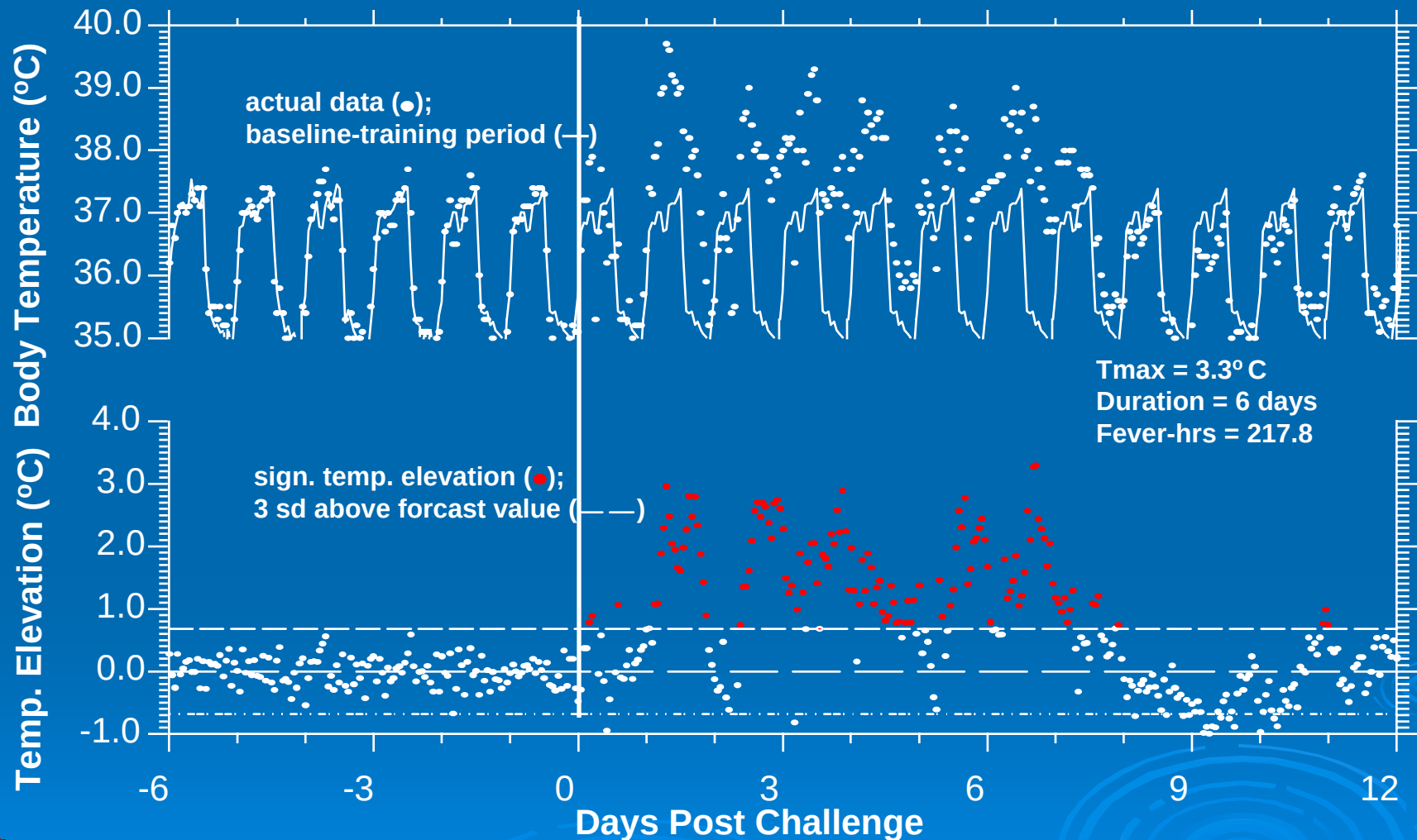


Instead of data collected once a day on an anesthetized monkey, physiological changes can be monitored continuously or intermittently at set intervals



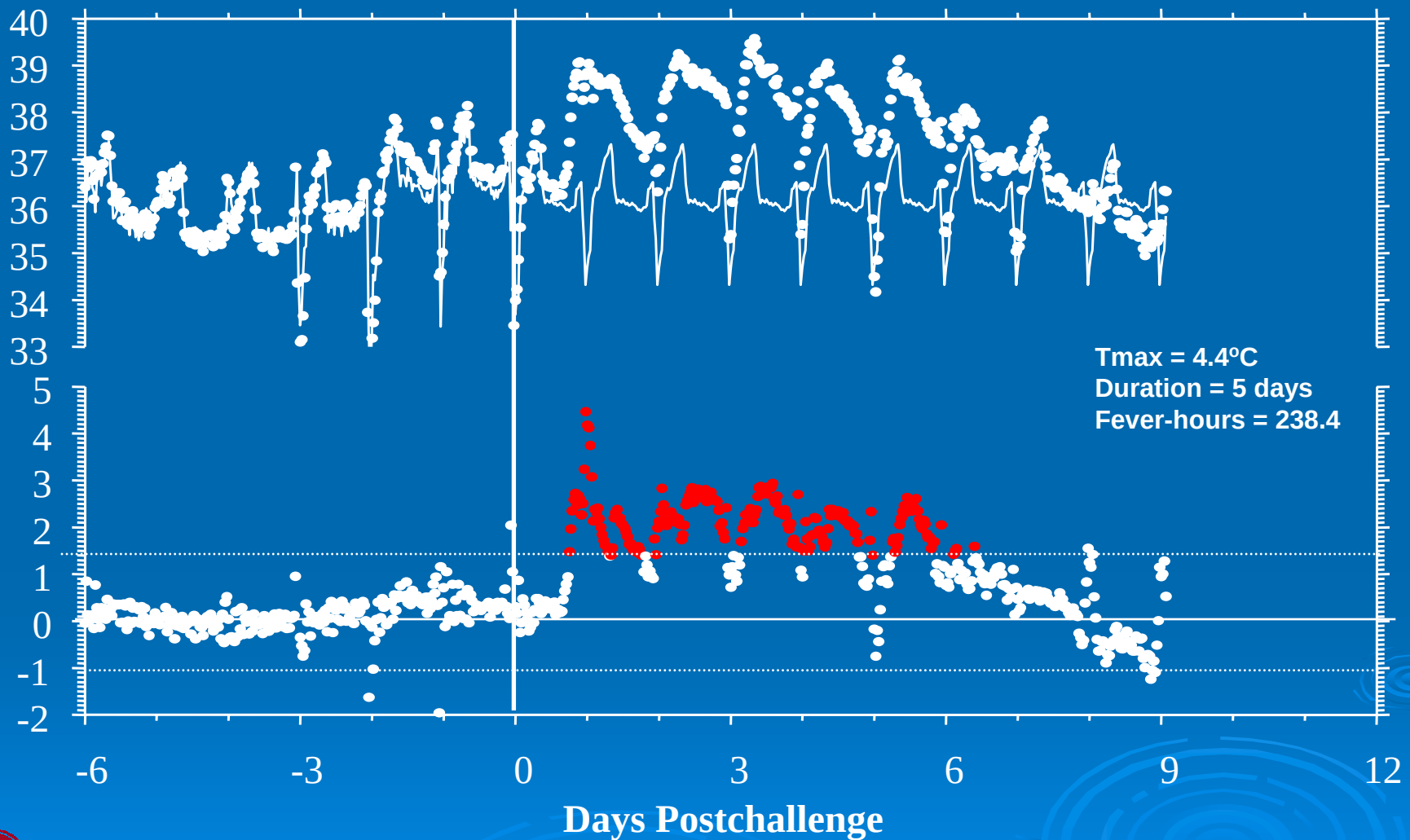
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Fever after exposure to epizootic VEEV-IA/B



**Naive macaque aerosol exposed to 10^8 pfu of
epizootic VEEV-IA/B**
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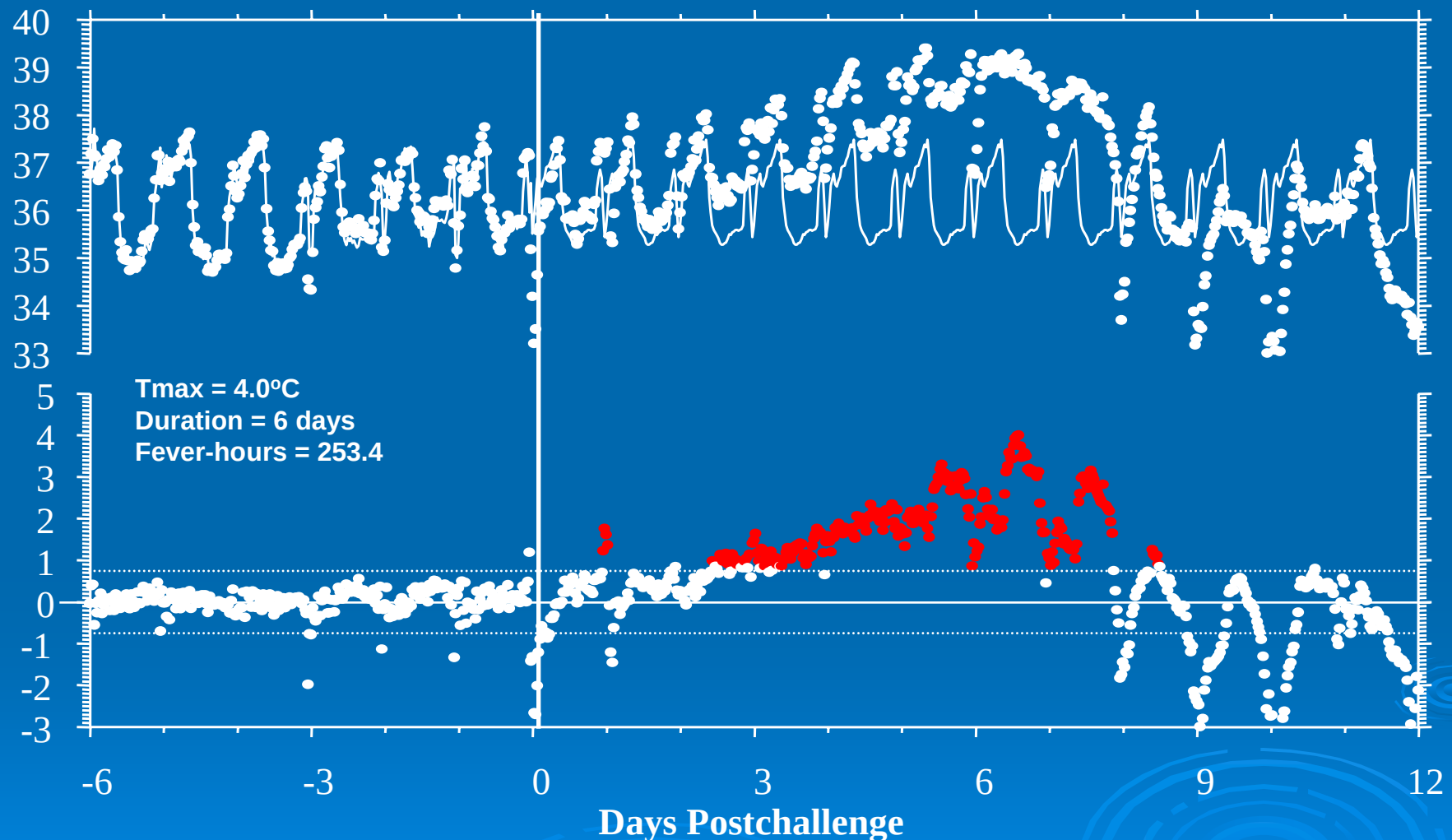
Similar fever responses to VEEV-IE



Naive macaque aerosol exposed to 10^8 pfu
enzootic VEEV-IE

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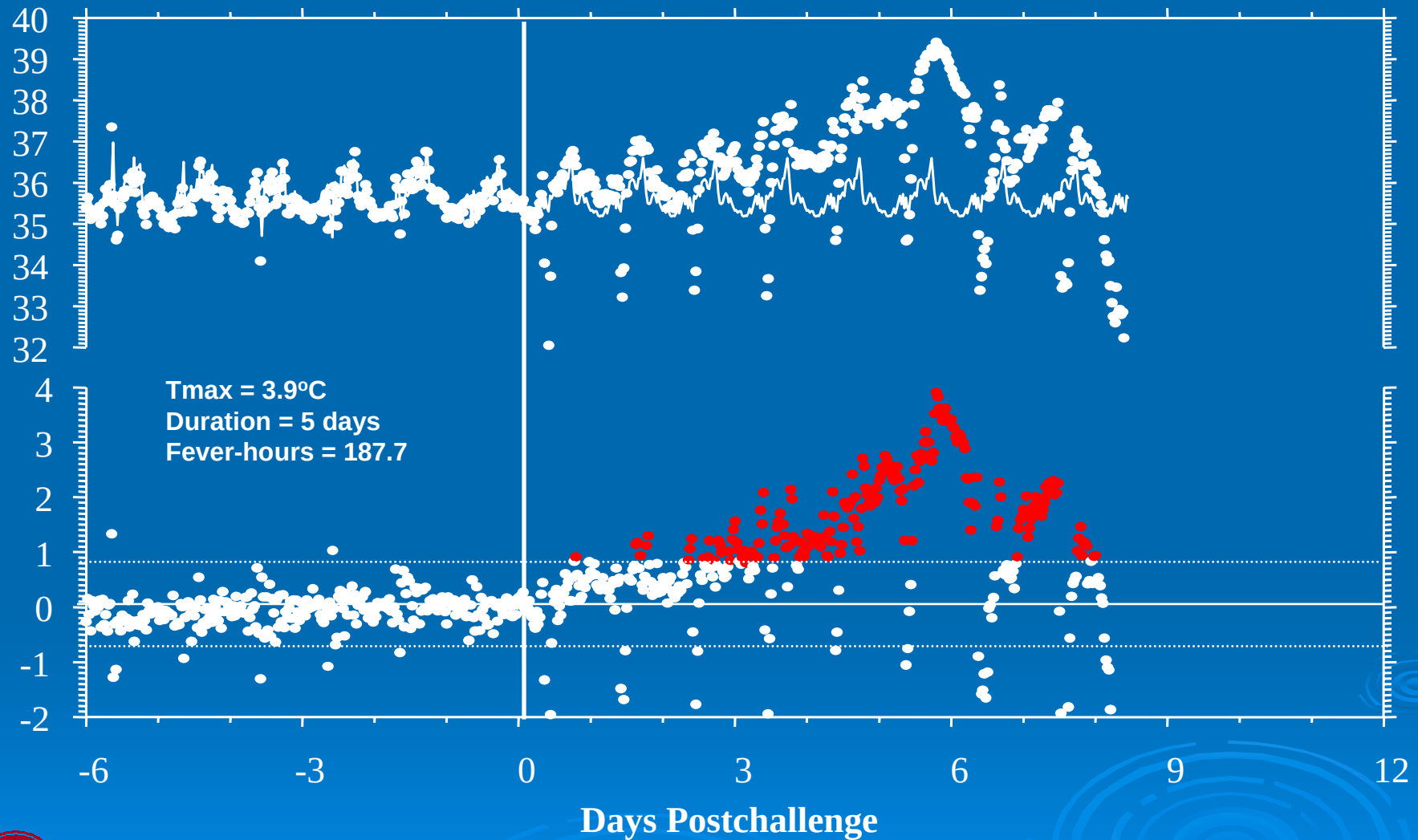
Delayed fever with VEEV-III A



Naive macaque aerosol exposed to 10^7 pfu

enzootic VEEV-III A
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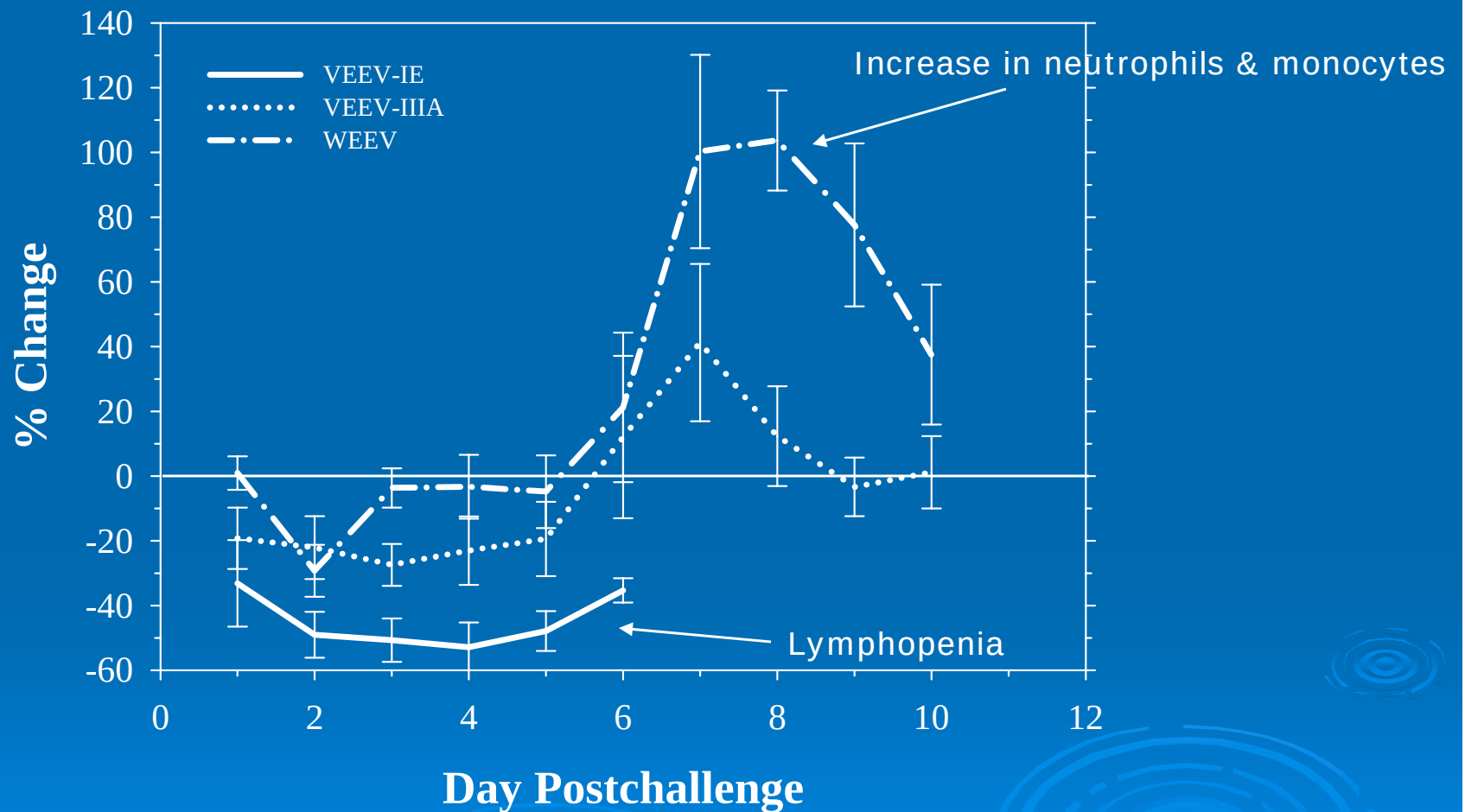
Fever response to WEEV



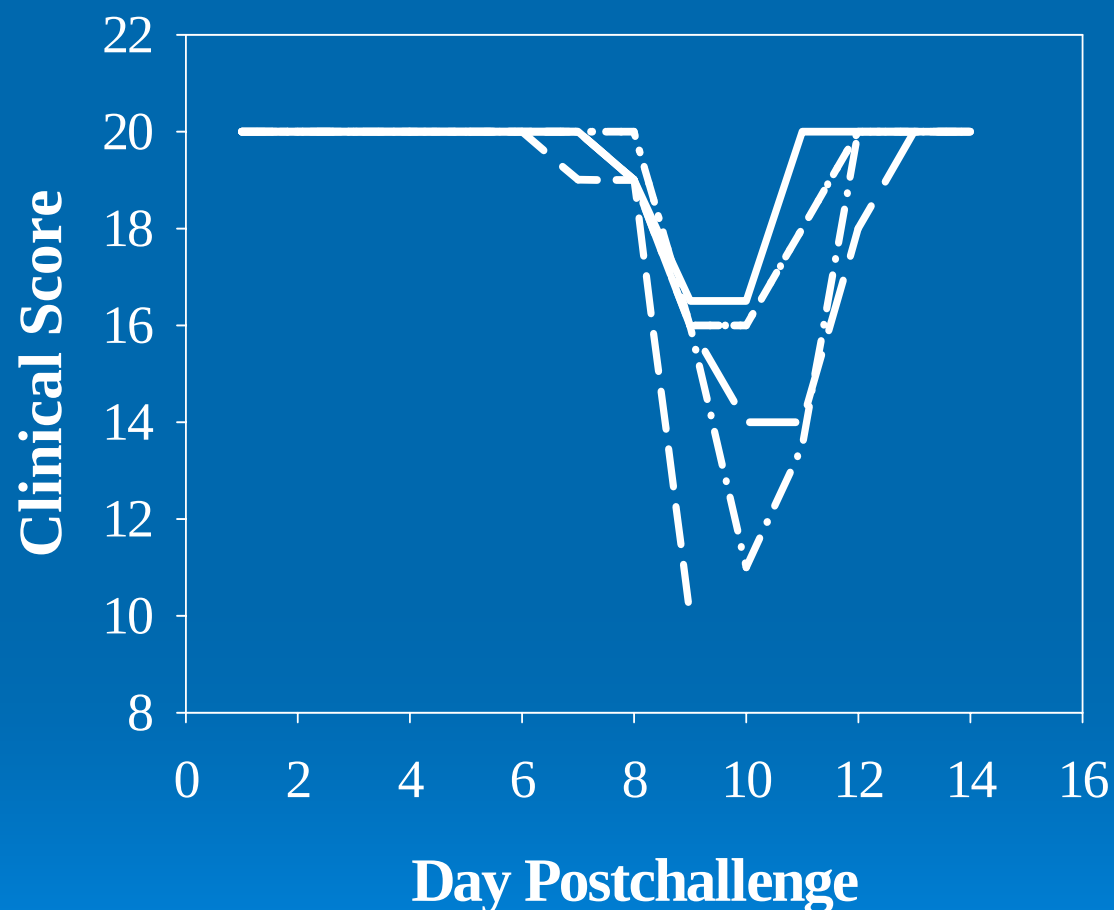
Naive macaque aerosol exposed to 10^7 pfu WEE

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Changes in peripheral blood leukocytes



Signs of encephalitis after aerosol exposure to WEEV



Clinical Score is the sum of:

- 1) Neurological signs
- 2) Activity
- 3) Behavior
- 4) Response to stimuli

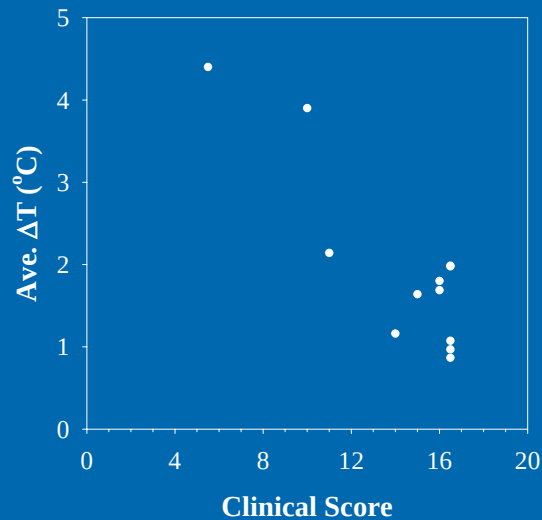
Each scored as 5 = normal



Animals that develop signs of encephalitis can survive infection

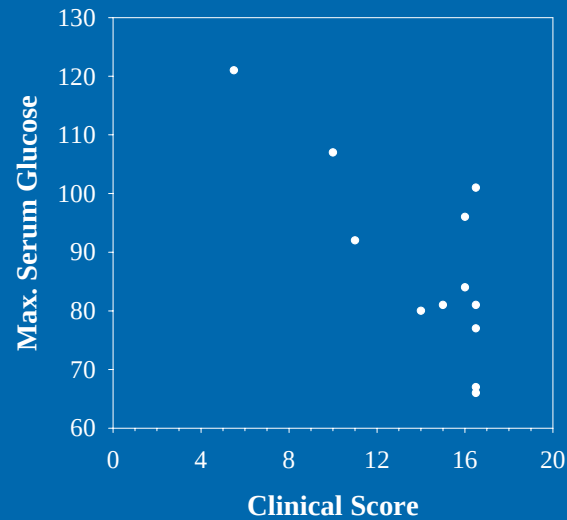
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Predicting outcome after exposure to WEEV



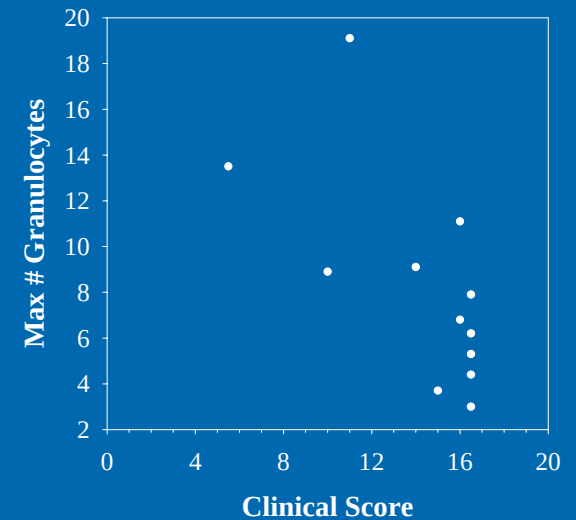
$$r = -0.87$$

Fever



$$r = -0.84$$

Glucose



$$r = -0.66$$

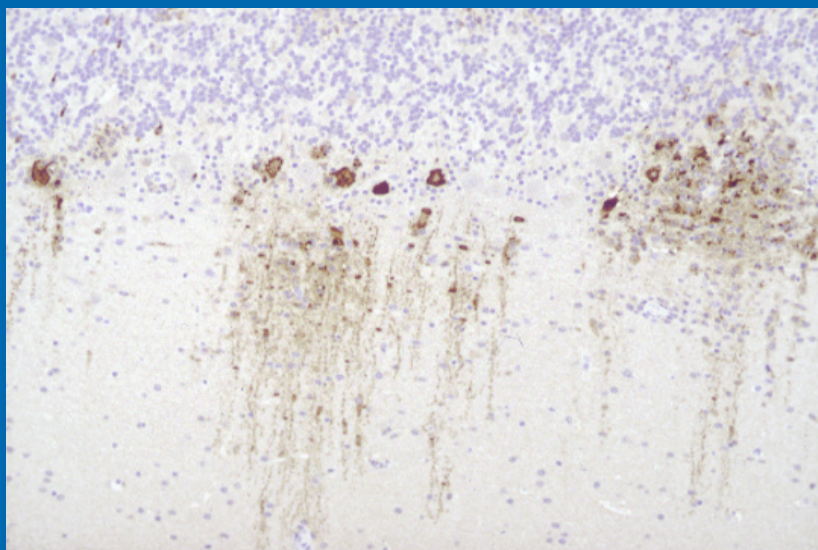
Granulocytes

n = 12



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Pathology in the CNS from aerosol exposure to WEEV

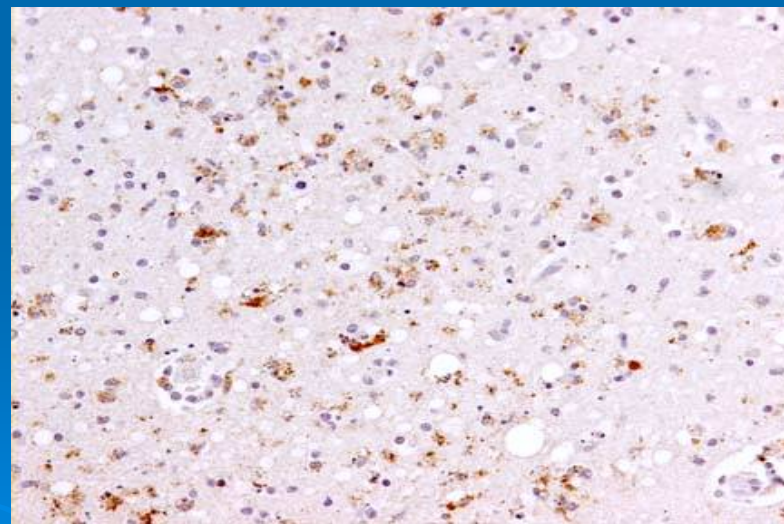


Viral antigen in Purkinje cells, cerebellum, rhesus macaque

Photo courtesy of Dr. Catherine Wilhelmsen

CNS, gray matter. Note the marked immunopositive tissue (brown staining) in this focal area of inflammation, increased cellularity of the neuropil, and apoptotic bodies. Immunoperoxidase stain, 10X & 4X.

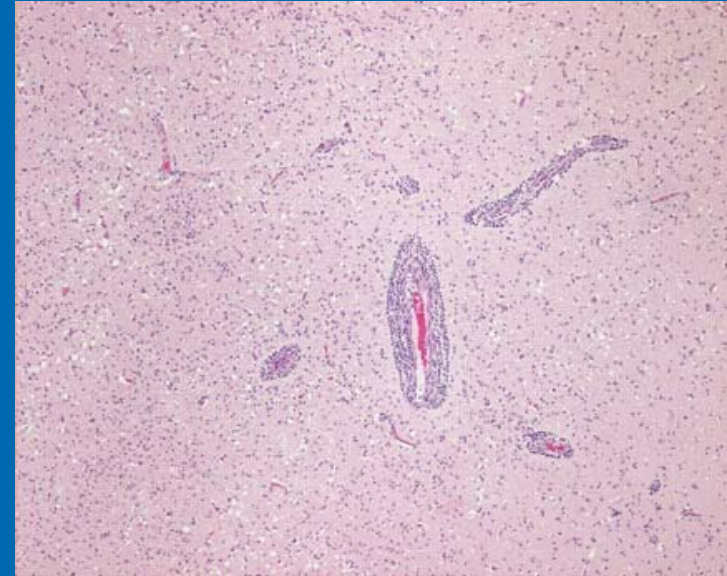
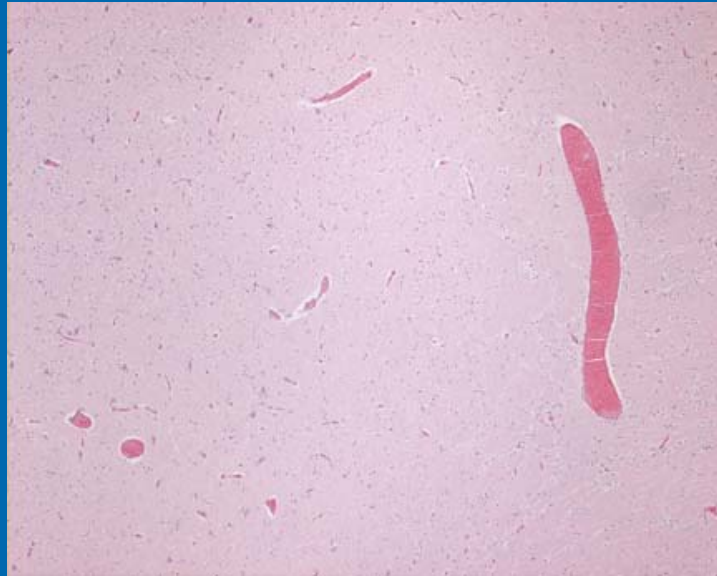
Photo courtesy of Dr. Tom Larsen



To date, virus has only been found in the brain & CNS of euthanized macaques

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WEEV Pathology, continued



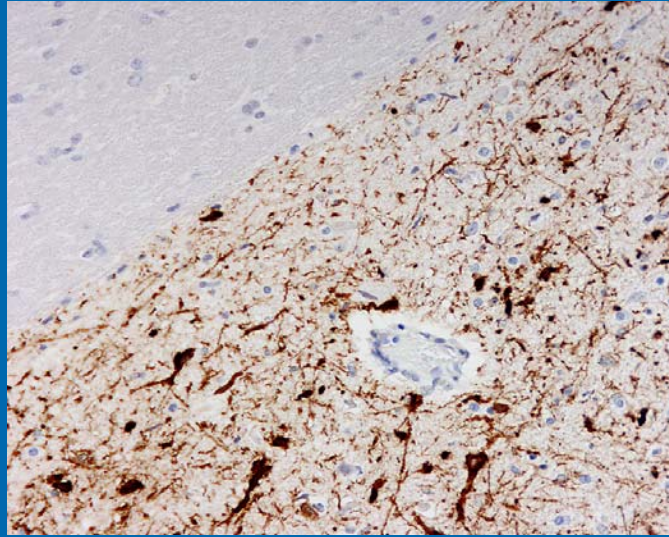
Photos courtesy of Dr. Tom Larsen

CNS, gray matter. Comparative slide demonstrating normal on the left and WEE-infected tissue on the right. Note the increased cellularity on the infected animal, the marked perivascular cellular infiltrate, and vacuolization of the neuropil. *Cynomolgus macaque*, H&E, 10X.



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Pathology from aerosol exposure to EEEV



Corpus striatum, cerebrum, 40x.
Note the prominent viral antigen staining in the gray matter of the brain.



Hippocampus, 10x.
Widespread antigen staining demonstrating viral antigen in neurons, their processes and astrocytes.

Photos courtesy of Dr. Jo Lynne Raymond



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Summary of NHP Response to Alphaviruses

	VEEV			WEEV	EEEV
	IA/B	IE	IIIA		
Fever	+++	+++	+++	+++	+++
Viremia	+++	++	+	-	IP
Lymphopenia	+++	++	+	-	IP
Leukocytosis	-	-	+/-	+++	IP
Serum glucose	ND	ND	ND	++	IP
Encephalitis*	+	+/-	+	++	+++

* -determined by sum of changes in neurological signs, activity, behavior and stimuli response

ND = not determined; IP = in progress

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Summary / Conclusions

- Cynomolgus macaques are a useful model of the human disease caused by aerosol exposure to alphaviruses.
- Aerosol exposure to enzootic VEEV caused disease in NHP.
- The duration & severity of the febrile response are comparable to epizootic strains of VEEV.
- Aerosol exposure to WEEV results in a more severe disease (in terms of fever, encephalitis and lethality) than VEEV in NHP.
- Outcome from exposure to WEEV correlates with increases in serum glucose levels, neutrophilia, and fever severity.
- EEEV is lethal by aerosol; pathology indicates a far more severe infection of the brain than is seen with WEEV.



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