

Histopathology and Immunohistochemistry for Rabies Diagnosis

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Postmortem diagnosis

- What to sample?
- Which is the ideal test?

Human rabies postmortem diagnostic tests

| Test | Target | Clinical samples |
|---|--|-------------------|
| Direct fluorescent antibody test (DFAT) | Viral antigen | Brain |
| Histopathology and immunohistochemistry | Inclusion bodies (aggregates of viral particles) | Brain |
| Nucleic acid amplification (NAA) | Viral RNA | Brain |
| | | Nuchal skin/ CSF |
| Anti-rabies antibodies RFFIT/FAVN/ELISA | Virus neutralizing antibodies | Blood(serum)/ CSF |

Brain tissue - ideal sample

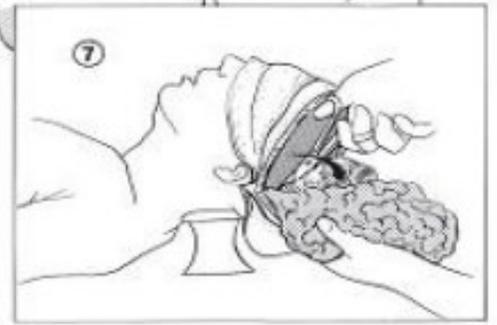
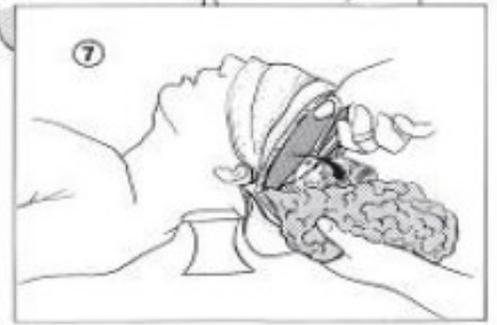
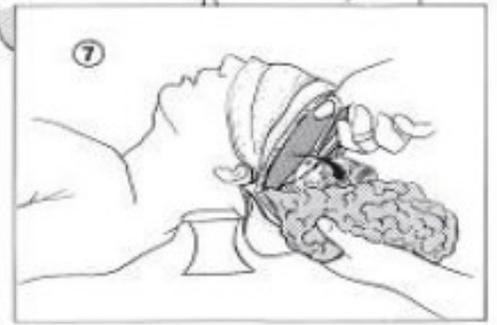
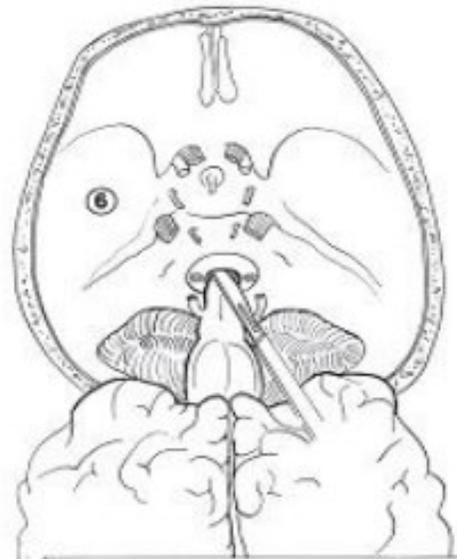
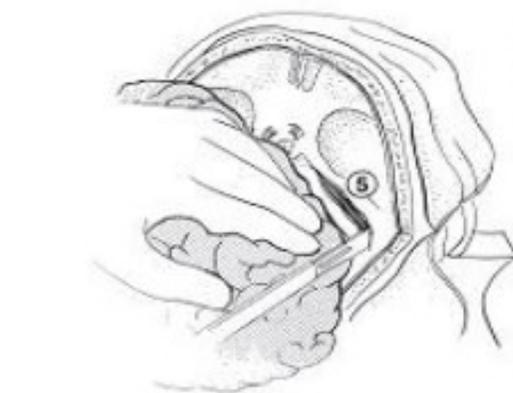
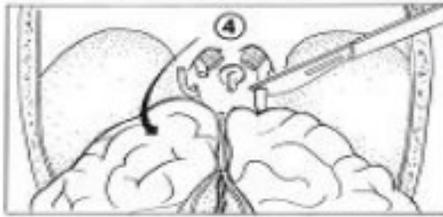
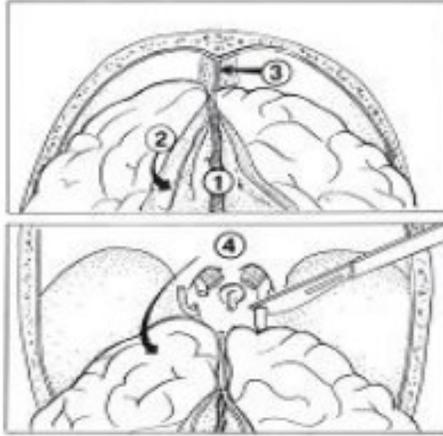
| Sample | Test | Factors affecting result |
|--------------|--|--|
| Brain tissue | Viral antigen by FAT (fresh)/ IHC (formalin fixed) | <ul style="list-style-type: none">- Temperature maintenance- Safe transportation- Infrastructure |

What do we need to know?

- How to obtain brain tissue?
- How to send tissue?
- Which areas to sample?
- What tests to do to diagnose?
- What are factors affecting sensitivity and specificity?

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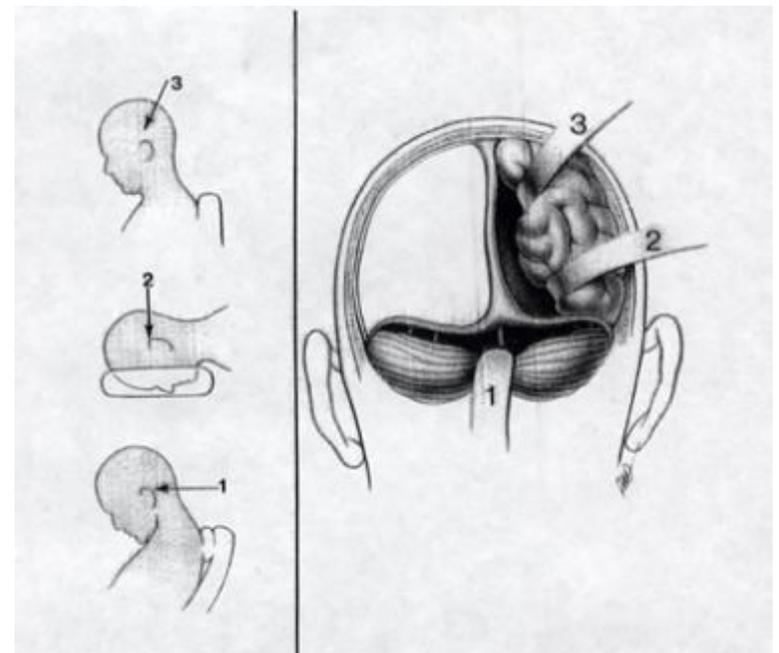
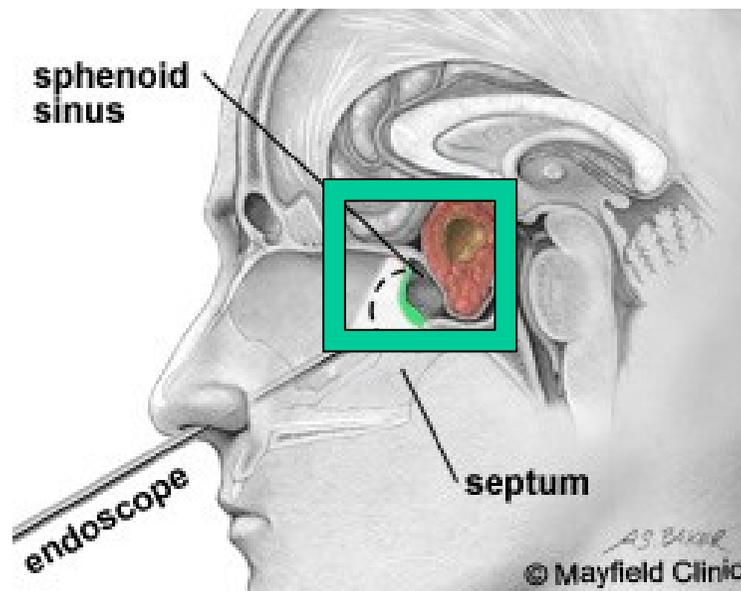
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- Cerebellum, brain stem, hippocampus.
- Superior frontal gyrus, orbitofrontal gyrus, cingulate gyrus, basal ganglia, amygdala, thalamus, inferior parietal lobule, striate cortex.

When you cannot...needle biopsy

- Transnasal/ orbital route using Tru-Cut biopsy needles - orbitofrontal
- Occipital route through foramen magnum with use of lumbar puncture - cerebellum and brain stem



What do we need to know?

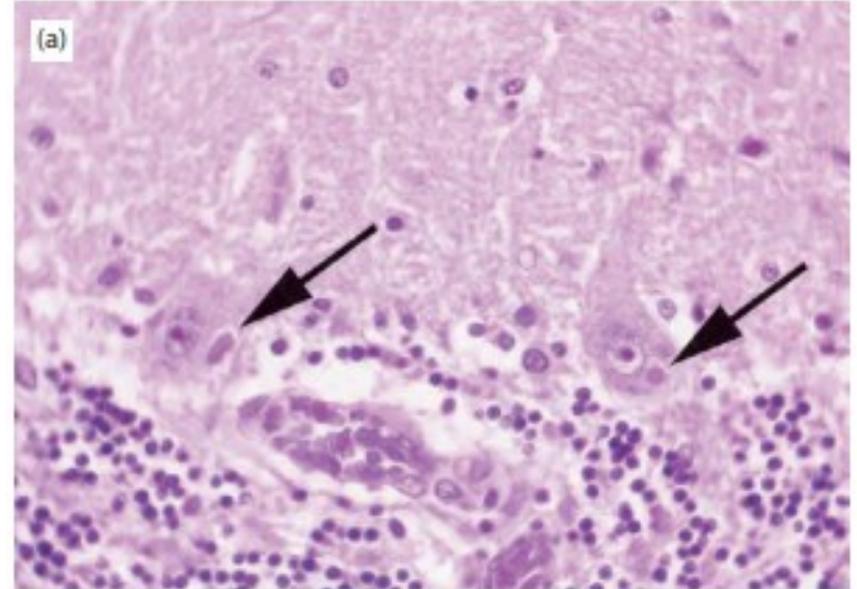
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Gross Pathology

- Macroscopic lesions in the CNS are mild or absent
- Brain and spinal cord may appear swollen

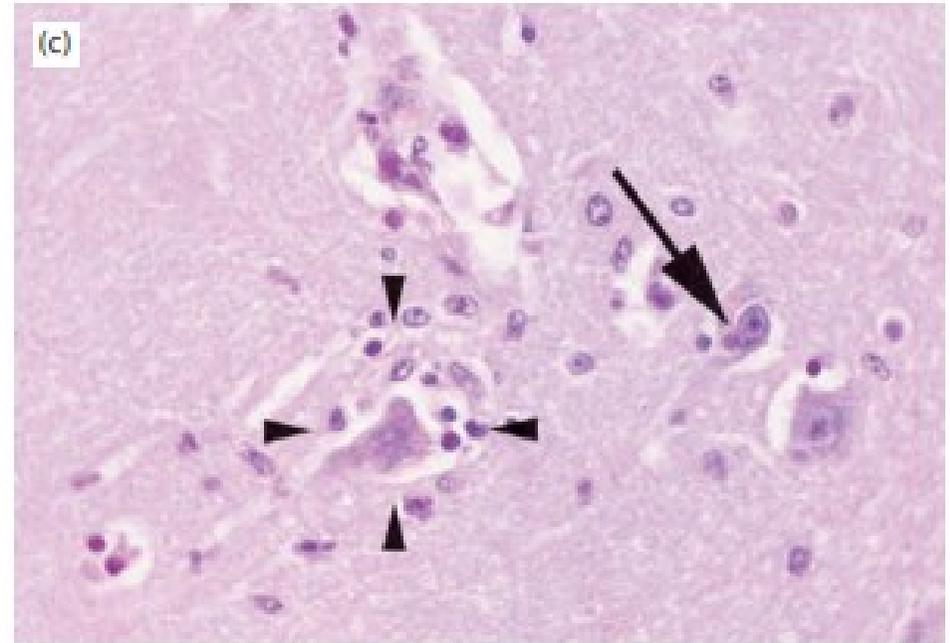
Histology

- Encephalomyelitis:
 - (i) mononuclear cell infiltrates of lymphocytes, plasma cells, and macrophages
 - (ii) perivascular lymphocyte cuffs
 - (iii) microglial nodules

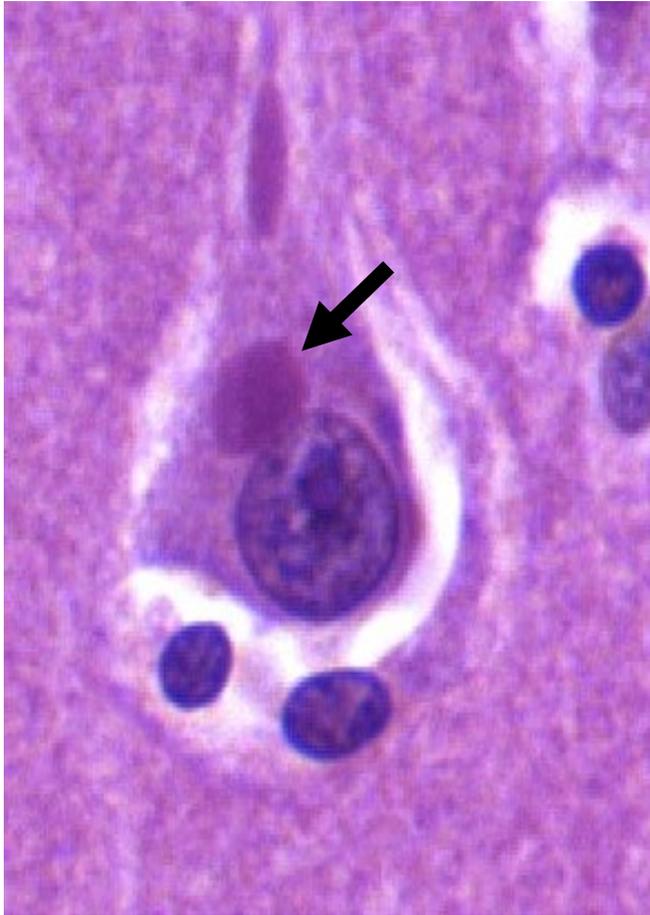


Histology

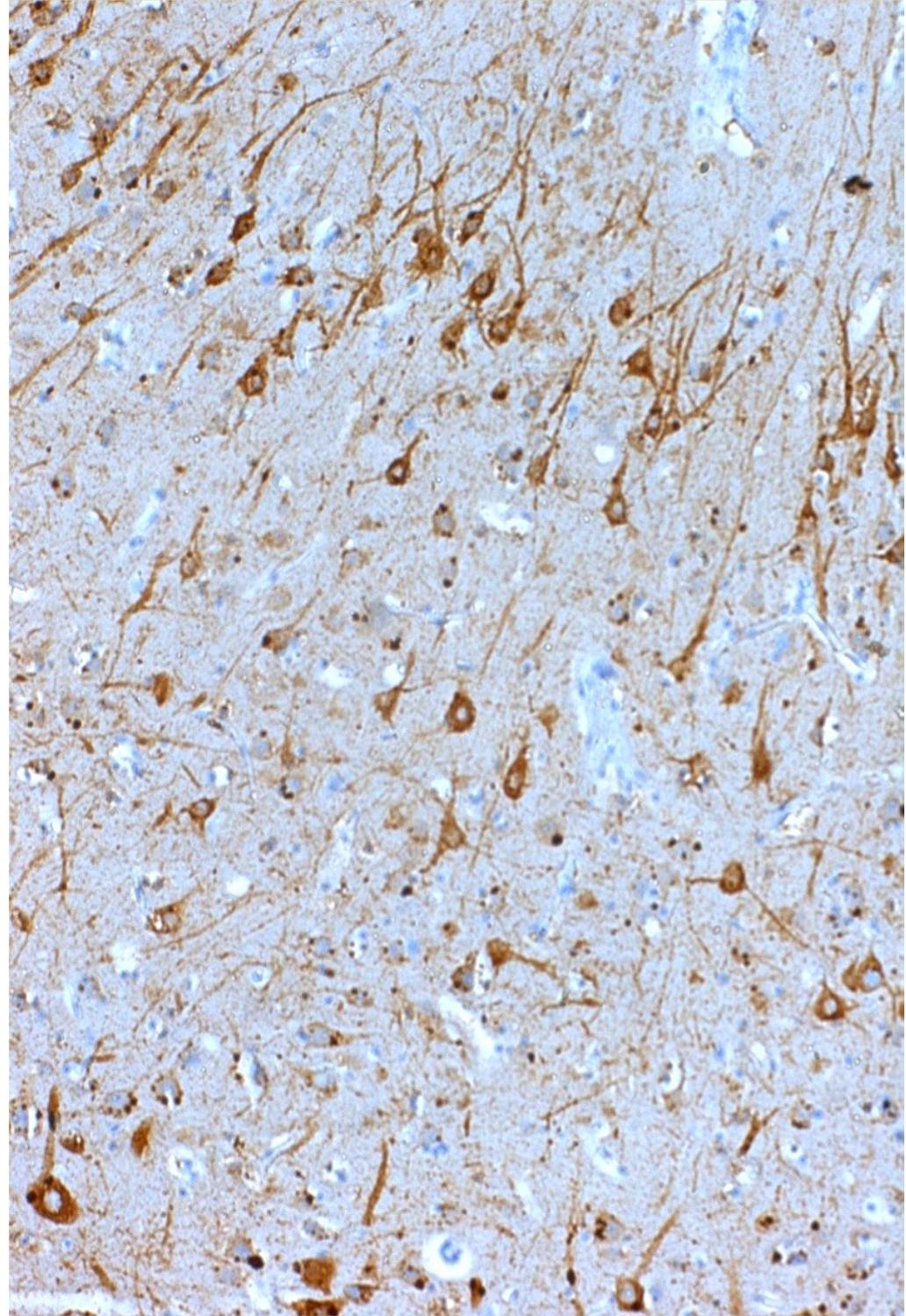
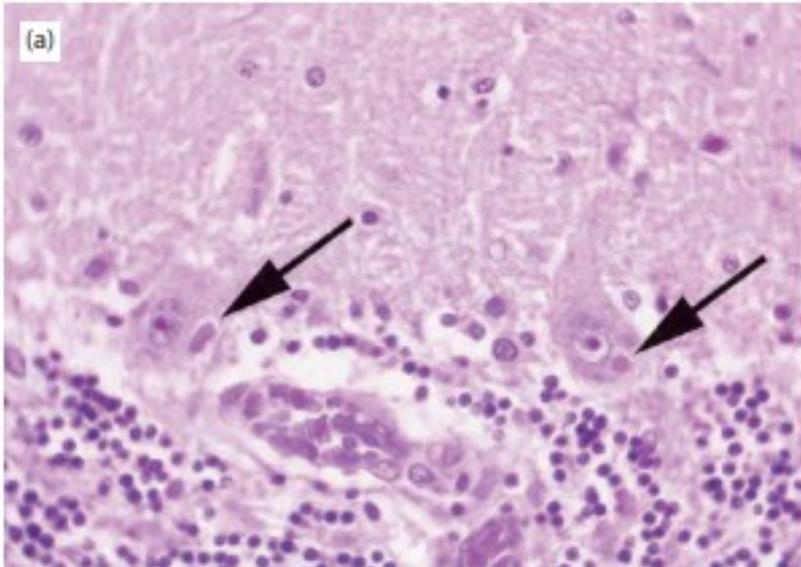
- Babes' nodules
- Babès (1887), described 'rabid nodules' in the motor nuclei of the medulla and the brain stem that represent microglial nodules



Negri bodies



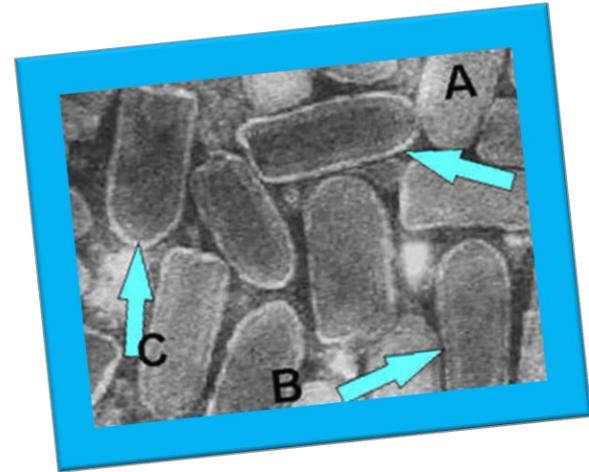
- Intracytoplasmic round or oval acidophilic inclusions—aggregates of nucleocapsids and are detected in neuronal cytoplasm in the brainstem, hippocampus, cerebellum, and several other brain regions.
- Sites: Seen in Purkinje neurons (cerebellum), hippocampus, brain stem
- Timing: IP > 3months
- Low sensitivity (30%)



**Immunohistochemistry:
widespread antigen**

Electron microscopy

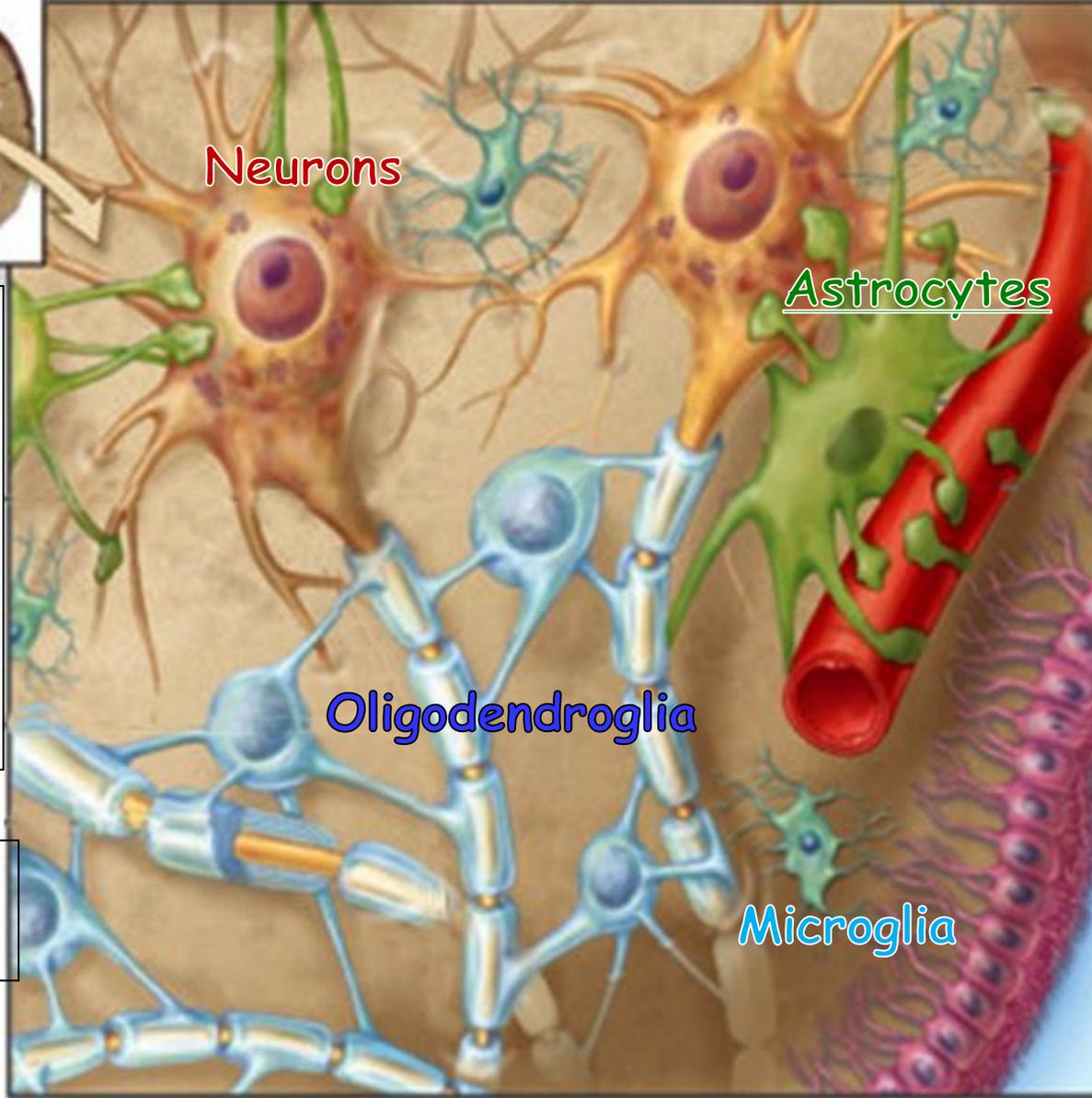
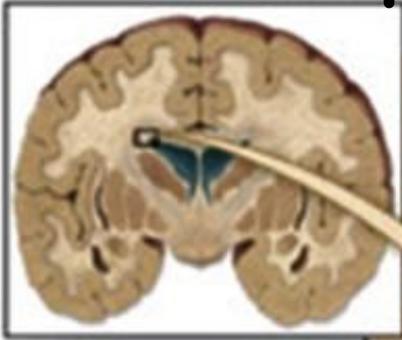
- Negri and lyssa bodies to contain a matrix of granular or filamentous nucleocapsid material
- Bullet-shaped virus particles



Viral infections

- ✓ **Distinctive clinical features**
 - Cell tropism (receptors)
 - Mode of entry
- ✓ **Characteristic neuroimaging** (reflects the pathology)
- ✓ **Diagnostic tests** (variable accuracy)
 - Serology – antibodies/ antigen
 - PCR
 - Viral isolation

Favourite hangout...tropism



Neurons

HSV & VZV
(DRG)

Rabies

(Ach - limbic),

Polio

(CD155: gut +
motor neurons)

Neurons

Astrocytes

Astrocytes

HSV, JCV,
CMV

Endothelial

JEV, CMV,
EBV

Oligodendroglia

Microglia

Microglia:

HIV

Oligodendroglia

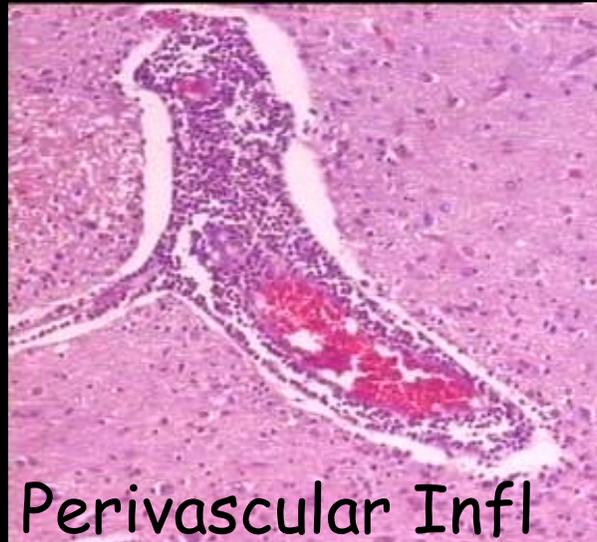
JCV, Measles

Host response/tissue injury

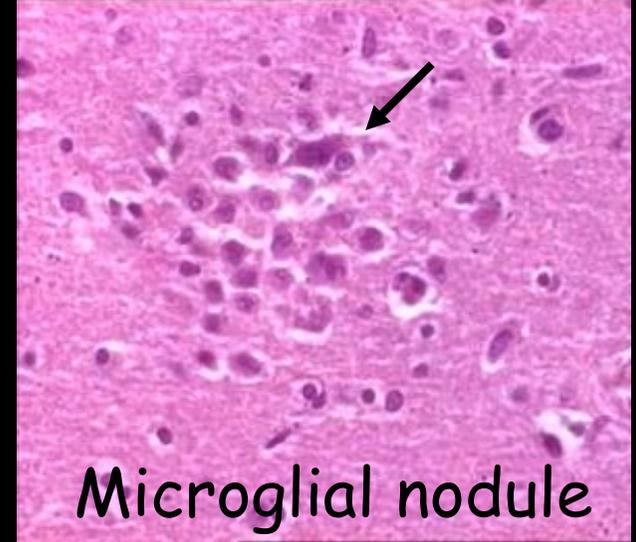
- Regardless of infecting virus, changes in brain are remarkable similar



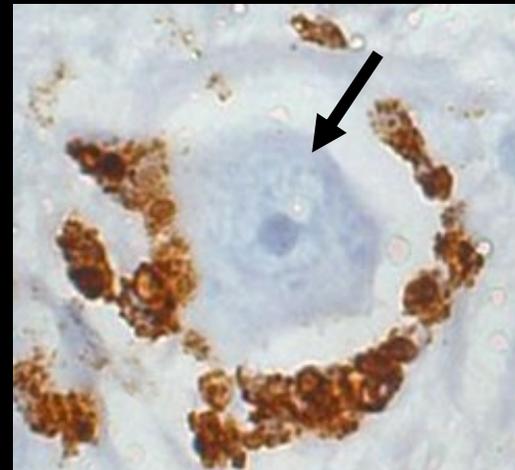
Cerebral Edema



Perivascular Infl



Microglial nodule



Neuronophagia

But...unlike other viral encephalitides

➤ Distinctive clinical features

- Hydrophobia: uncommonly seen
- History of dog bite: not often forthcoming

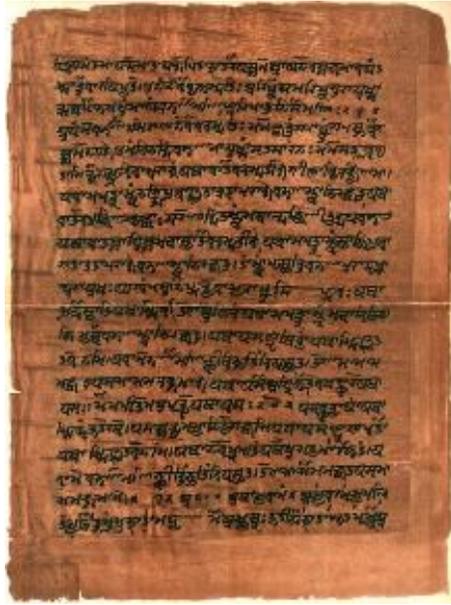
➤ Characteristic neuroimaging

- ✓ Absent/inconsistent

➤ Diagnostic tests

- Antibody tests: late appearance of antibodies, short survival and late seroconversion
- PCR: Negative test - does not rule out rabies
- Viral isolation: time consuming
- **Postmortem brain: gold standard of diagnosis!**

Historically...one of the oldest viruses known to man



Sanskrit "rabhas"
(to do violence)

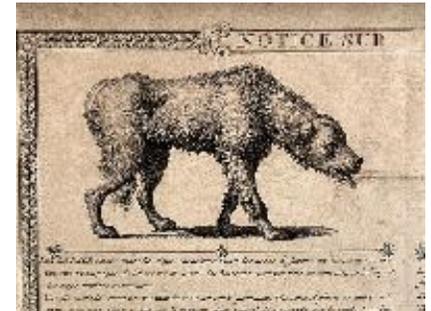
Vedic period (1500-500 BC) and is described in the ancient Indian scripture Atharvaveda



A woodcut from the Middle Ages



Ancient Greeks called it "lyssa" (violence).



Latin "rabere" (to rage).

Pathogenesis

Pathology beyond diagnosis!!!

Pathology
(Brain)

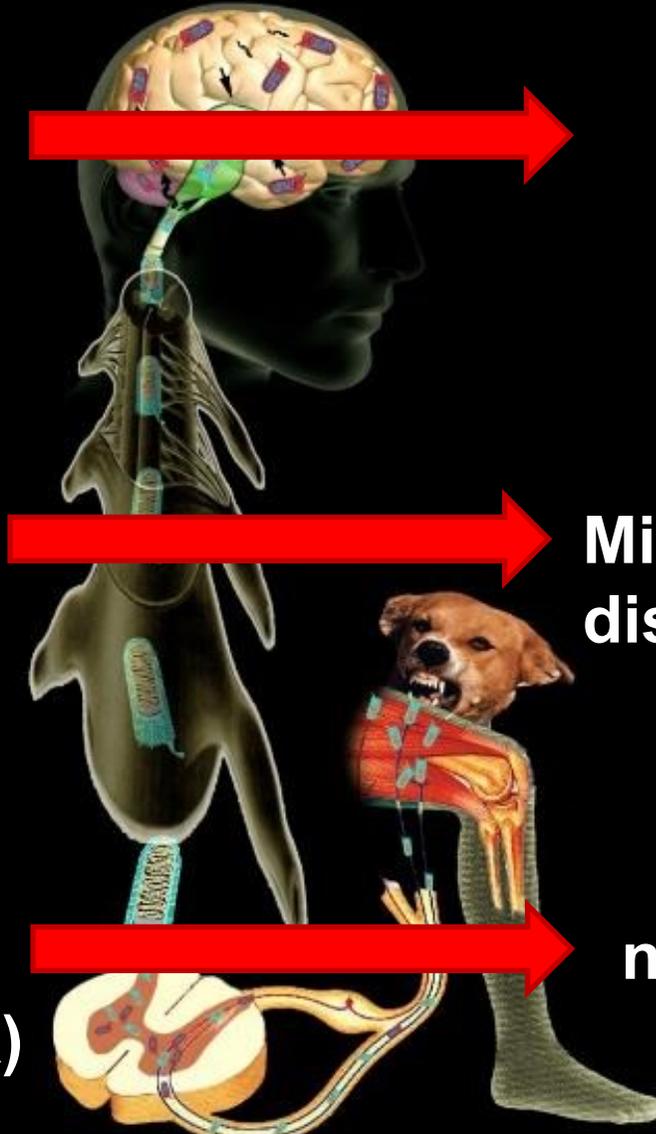
???

Transport
(intraaxonal)

**Microtubule
disrupting agent**

Viral Entry
(receptor- nAChR)

nAChR antagonist



Limited Brain Metabolism Changes Differentiate between the Progression and Clearance of Rabies Virus

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Abstract

Central nervous system (CNS) metabolic profiles were examined from rabies virus (RABV)-infected mice that were either mock-treated or received post-exposure treatment (PET) with a single dose of the live recombinant RABV vaccine TriGAS. CNS tissue harvested from mock-treated mice at middle and late stage infection revealed numerous changes in energy metabolites, neurotransmitters and stress hormones that correlated with replication levels of viral RNA. Although the large majority of these metabolic changes were completely absent in the brains of TriGAS-treated mice most likely due to the strong reduction in virus spread, TriGAS treatment resulted in the up-regulation of the expression of carnitine and several acylcarnitines, suggesting that these compounds are neuroprotective. The most striking change seen in mock-treated RABV-infected mice was a dramatic increase in brain and serum corticosterone levels, with the later becoming elevated before clinical signs or loss of body weight occurred. We speculate that the rise in corticosterone is part of a strategy of RABV to block the induction of immune responses that would otherwise interfere with its spread. In support of this concept, we show that **pharmacological intervention to inhibit corticosterone biosynthesis**, in the absence of vaccine treatment, **significantly reduces the pathogenicity of RABV**. Our results suggest that **widespread metabolic changes, including hypothalamic-pituitary-adrenal axis activation, contribute to the pathogenesis of RABV and that preventing these alterations early in infection with PET or pharmacological blockade helps protect brain homeostasis, thereby reducing disease mortality.**

PLOS one 2013

- The most striking change seen in rabies infected mice was a dramatic increase in brain and serum corticosterone levels
- **Speculate that rise in corticosterone is viral strategy to promote spread.**
 - block the induction of immune responses
 - Disabling IFN- signalling pathways
 - Reduce BBB permeability preventing entry of inflammatory cells into brain
 - Unchecked spread of virus

Ray of hope??

- Metyropone, inhibitor of corticosteroid synthesis
- Preventing clinical symptoms in rabies infected mice
- Reduced mortality???