The Neuropathology of Autoimmune Encephalitis and PANS and Development of Specialized Brain Banks for these Disorders

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Disclosures:

Unpaid board membership for:

Neuroimmune Foundation

Pink Concussions

Medical Expert for:

Dept of Justice/HHS Vaccine Injury Comp Program

DC Office of the Chief Medical examiner

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Dedicate this lecture to the patients, families, and care-givers who battle neuroimmune disorders

Topics

- Brain disorders and neuroinflammation
- Brain banking and establishment of the PANDAS/ PANS & OTHER NEUROIMMUNE RELATED DISORDERS (POND) Brain Bank at Georgetown University
- Case discussion
- Questions

What is inflammation in the brain or neuro-inflammation?

What is inflammation in the brain or neuro-inflammation?

- Circulating immune cells originating from the blood:
  - Lymphocytes
  - Macrophages
  - Neutrophils, Eosinophils, Mast cells, NK cells, etc

What is inflammation in the brain or neuro-inflammation?

- Circulating molecules from the blood:
  - Antibodies
  - Chemokines and cytokines
  - Other proteins
  - Exosomes

What is inflammation in the brain or neuro-inflammation?

- Cells and circulating molecules originating in the CNS:
  - Microglia
  - Astrocytes and oligodendroglia
  - Neurons
  - Vessels and perivascular cells
  - Secreted molecules

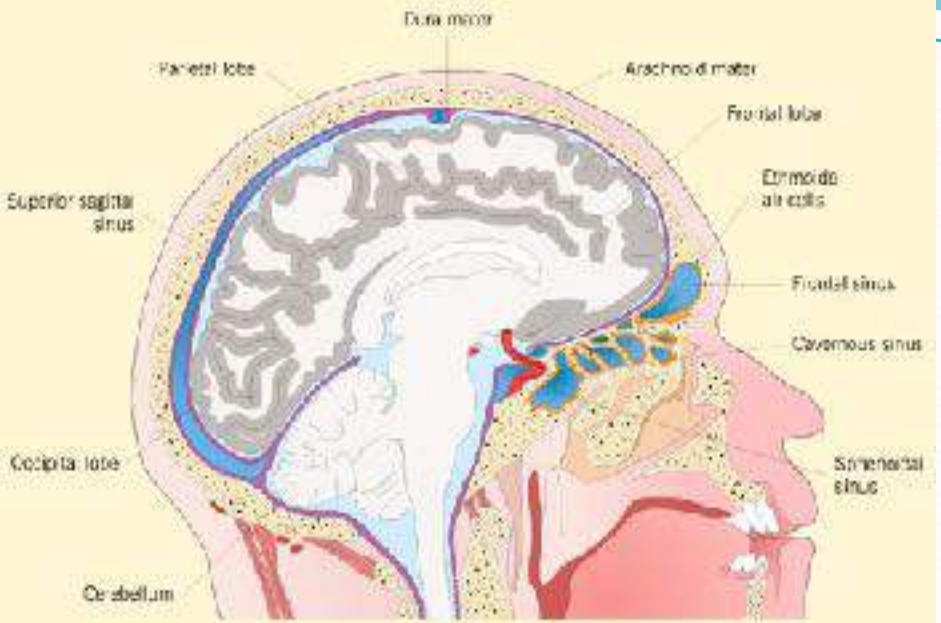
Glossary:

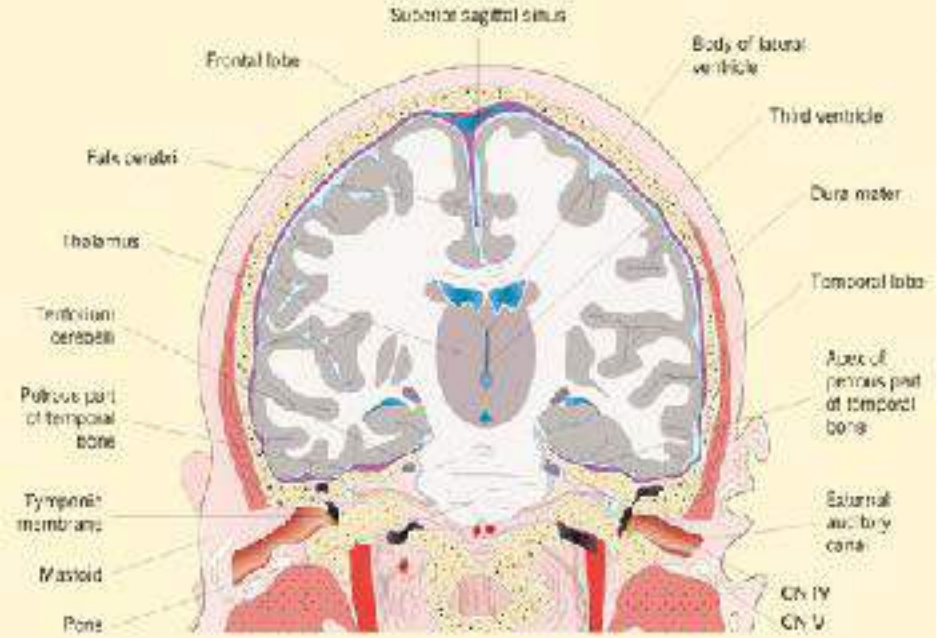
- ***Meningitis:*** **Inflammation of the <u>membranes</u> of the brain or spinal cord**
- ***Encephalitis:*** **Inflammation of the <u>brain</u>**
- ***Myelitis:*** **Inflammation of the <u>spinal cord</u>**
- ***Other:***
  - **Encephalopathy**
  - **Encephalomyelitis**
  - **Meningoencephalitis**
  - **Innate and Adaptive responses**

Brain has impaired host resistance:

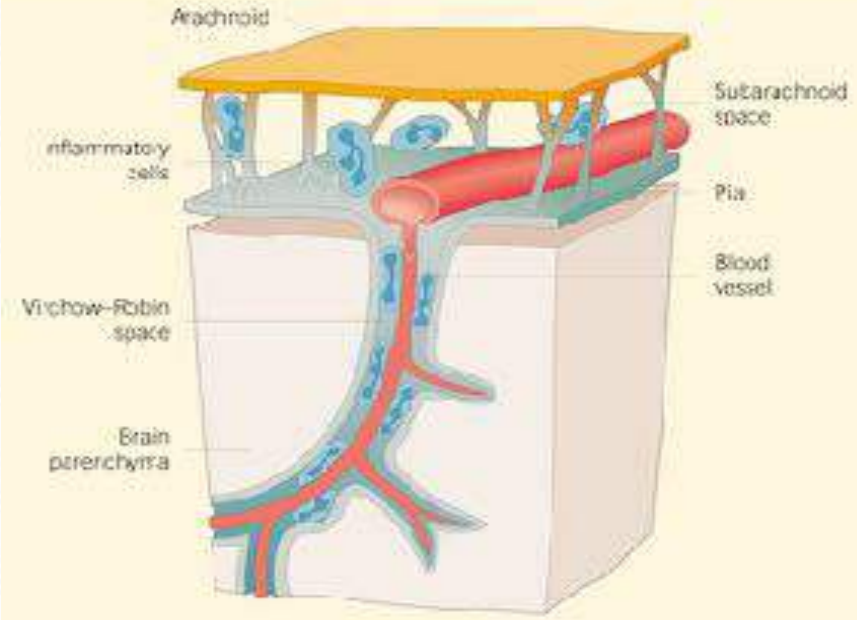
- Narrower array of defenses:
  - Lack of specific antibody and complement
  - Inefficient phagocytosis
  - >Immunocompromised hosts
- Drug penetration limited
- Closed space

RELEVANT CNS ANATOMY





SUBARACHNOID AND PERIVASCULAR INFLAMMATION



What conditions elicit neuroinflammation? VITAMINS

What conditions elicit neuroinflammation? VITAMINS

- **V**ascular
- **I**nfectious
- **T**oxic/Trauma
- **A**utoimmune
- **M**etabolic disorders
- **I**diopathic/Iatrogenic
- **N**eoplastic
- **S**ychiatric (a little liberty w/ spelling) ?

CNS infectious syndromes

- Diverse range of pathogens
- Acute to chronic; “trivial to fatal”
- Four cardinal manifestations;
  - Fever, headache, altered mental status, focal neurologic signs
- Syndrome-recognition approach to diagnosis

Adapted from Scheld WM ed. Infections of the CNS.

Viruses: Enteroviruses, Mumps, arboviruses, herpesviruses, LCM virus, HIV, HTLV,adenovirus, paraflu, influenza, measles, rabies, JC virus, hemorrhagic fevers, Zika

Rickettsia: Rocky Mountain Spotted Fever, Erhlichia, + others

Bacteria: Neisseria meningitidis, Haemophilus influenzae, Streptococcus pneumoniae, Listeria, Klebsiella, Salmonella, Brucella, Mycoplasma, Bartonella, Nocardia, Actinomyces, Mycobacterium tuberculosis

Bacterial + other toxins: Botulism, Tetanus, Pertussis, Marine

Spirochetes: Syphilis, Lyme disease, Leptospirosis

Fungi: Cryptococcus, Histoplasmosis, Coccidiodomycosis, Blastomycosis, Candida, Aspergillus + other molds

Protozoa and helminths: Toxoplasma, Naegleria fowleri, Angiostrongylus, Strongyloides, Acanthomoeba, Cysticercosis, Trypanosomiasis, Schistosomiasis, Malaria

Encephalitis in the community

- Incidence: ~20,000 cases/yr in U.S.
- More common in children
- Nearly 100 different infectious causes
- Of fatal cases:
  - Acute viral encephalitis (67%) vs. post-infectious encephalomyelitis (10-33%)

Johnson RT. Clin Infect Dis 1996;23:219-26.

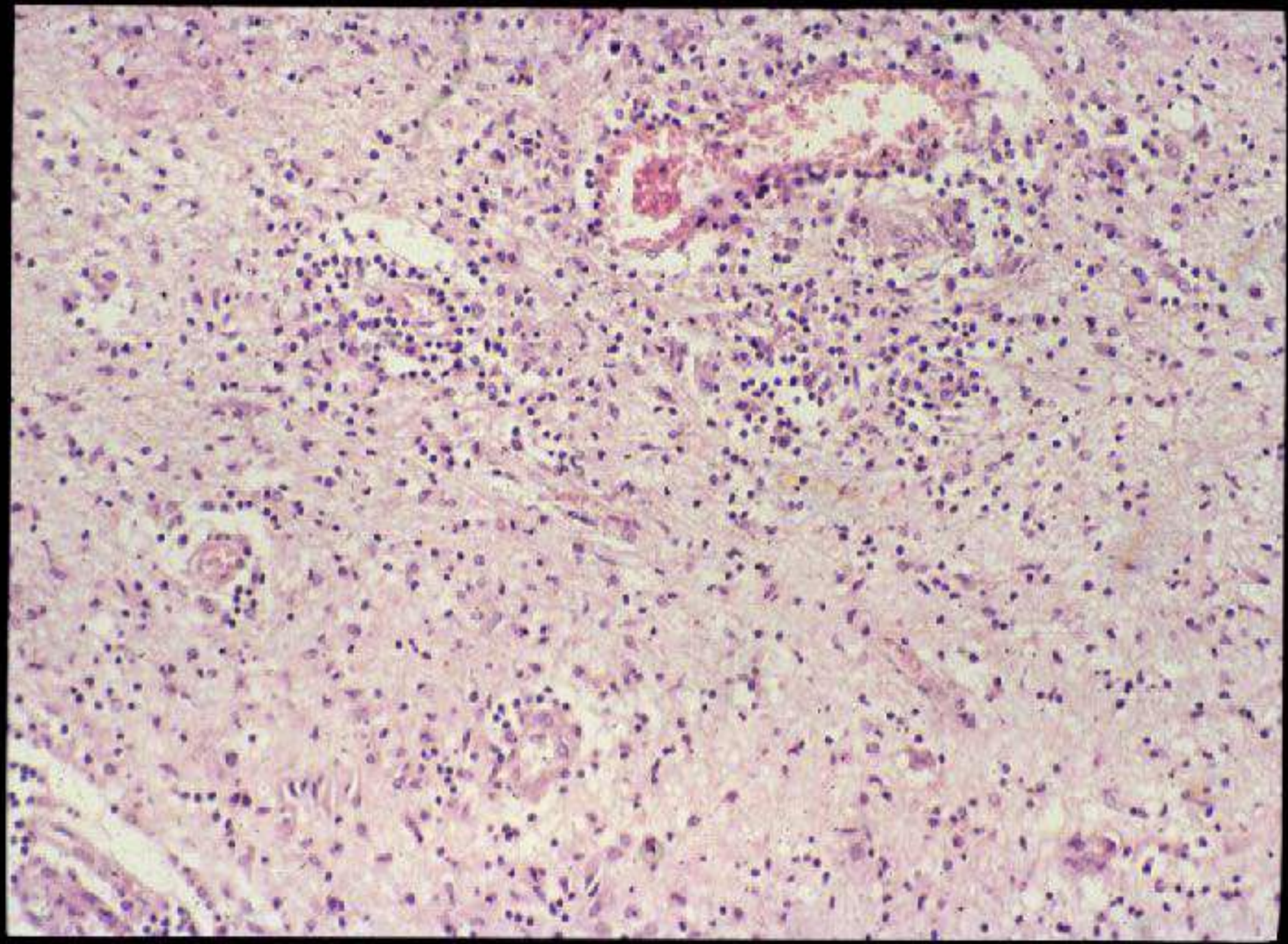
Encephalitis viruses

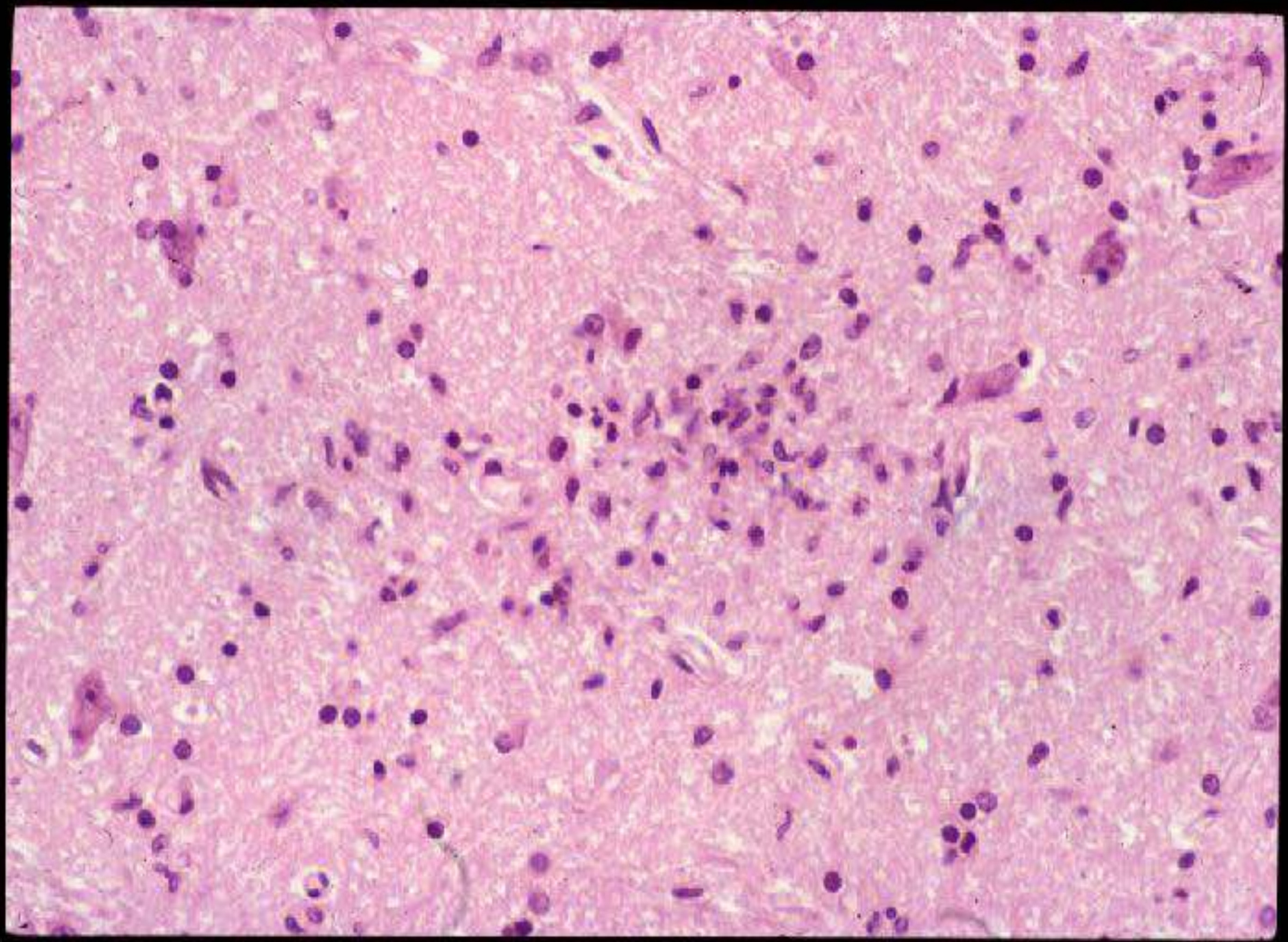
- Coxsackie and echovirues: *Rare fatalities in neonates*
- Human herpes virus 6: *Mild encephalitis is children*
- HIV: *Rare with primary infection*
- Adenoviruses: *Occasional serious encephalitis in children*
- EBV: *Occasional encephalitis with infectious mononucleosis*
- CMV: *Occasional encephalitis with infectious mononucleosis*
- Mumps: *Common mild encephalitis, rare deaths*
- Lymphocytic choriomeningitis virus: *Common mild encephalitis, rare deaths*
- Arboviruses: *1-50% cases are fatal*
- Herpes simplex viruses: *> 70% untreated cases are fatal*
- Rabies: *>99% cases are fatal*

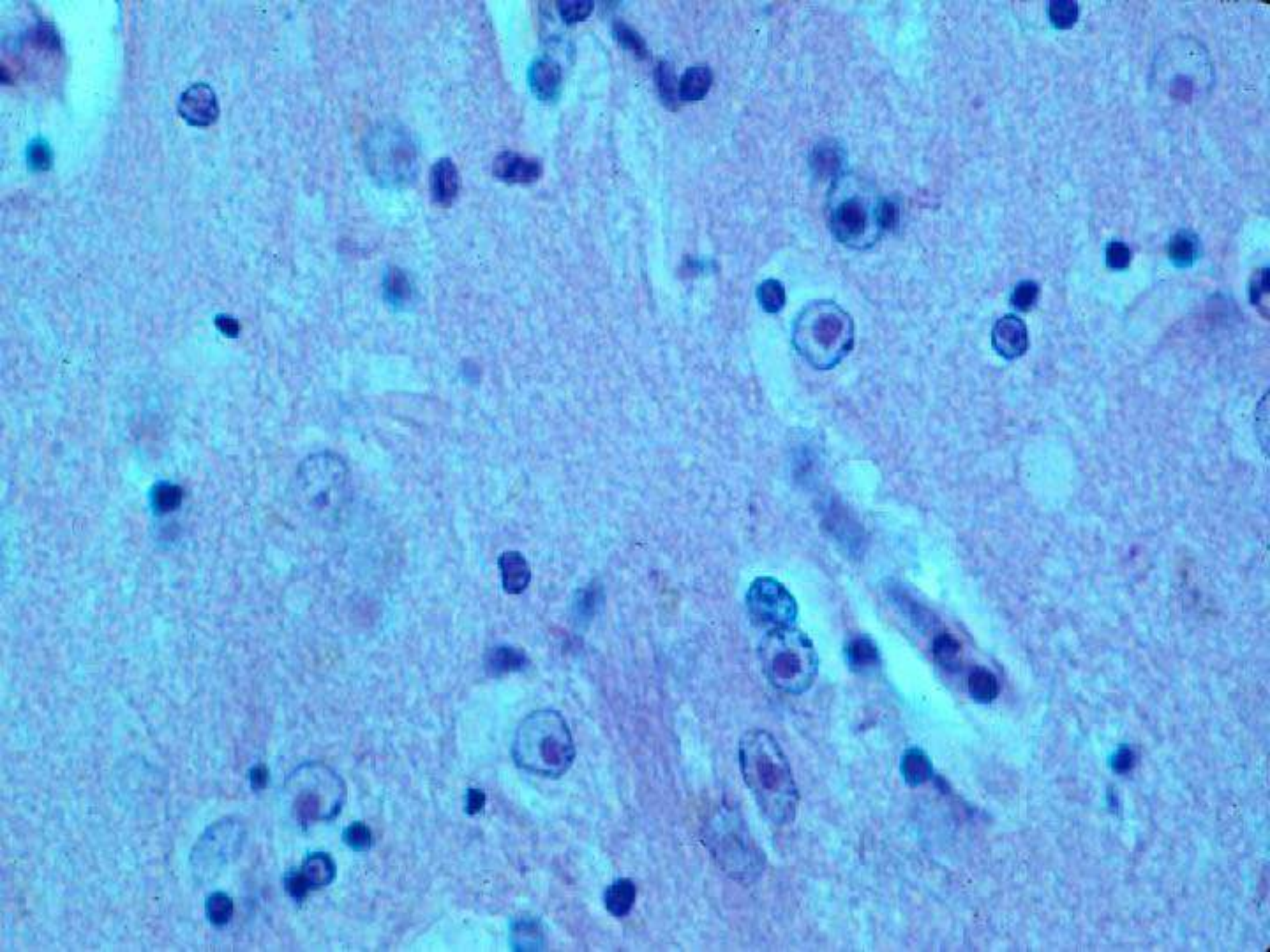
Johnson RT. Clin Infect Dis 1996;23:219-26.

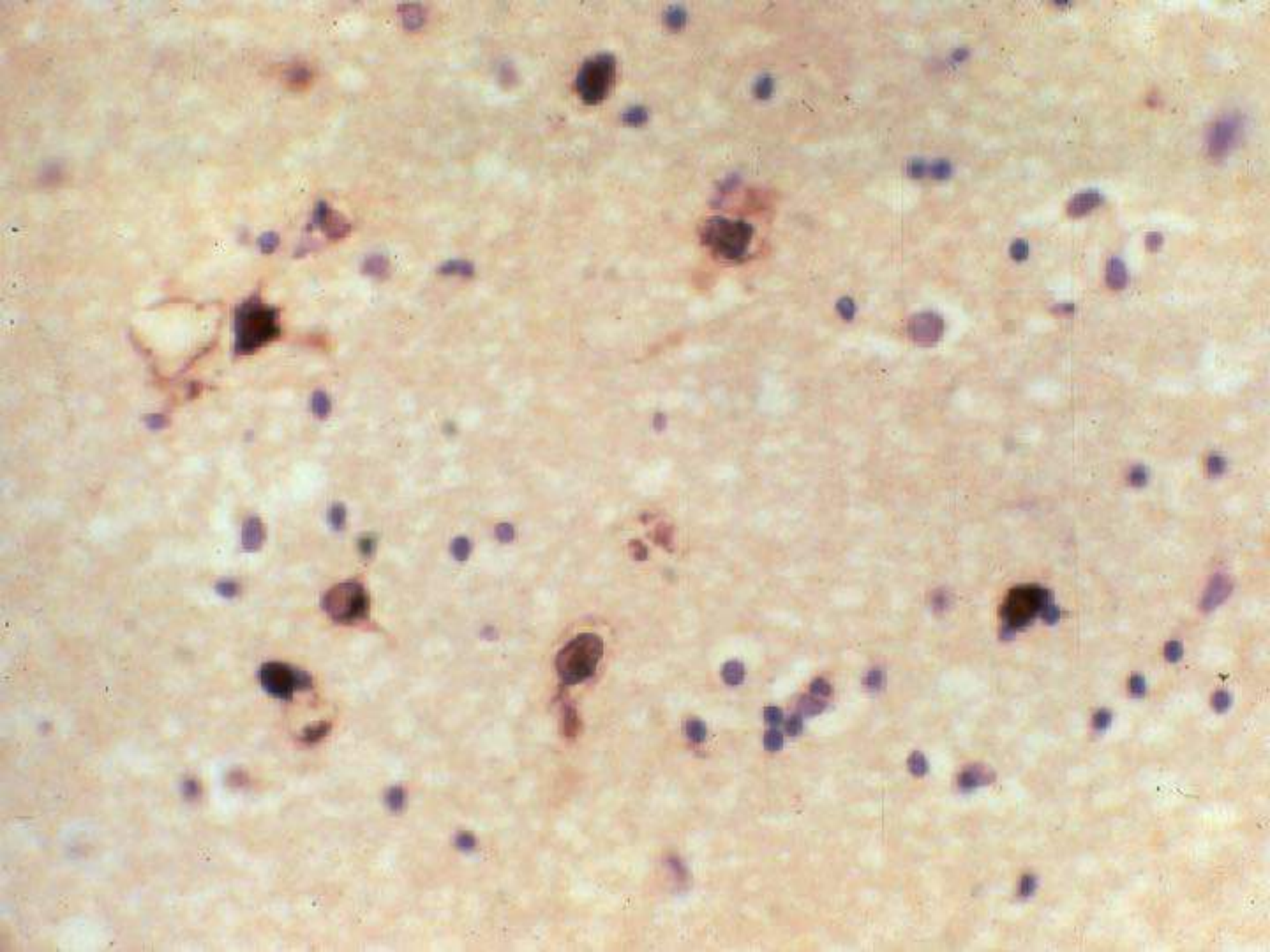
Post-infectious encephalomyelitis

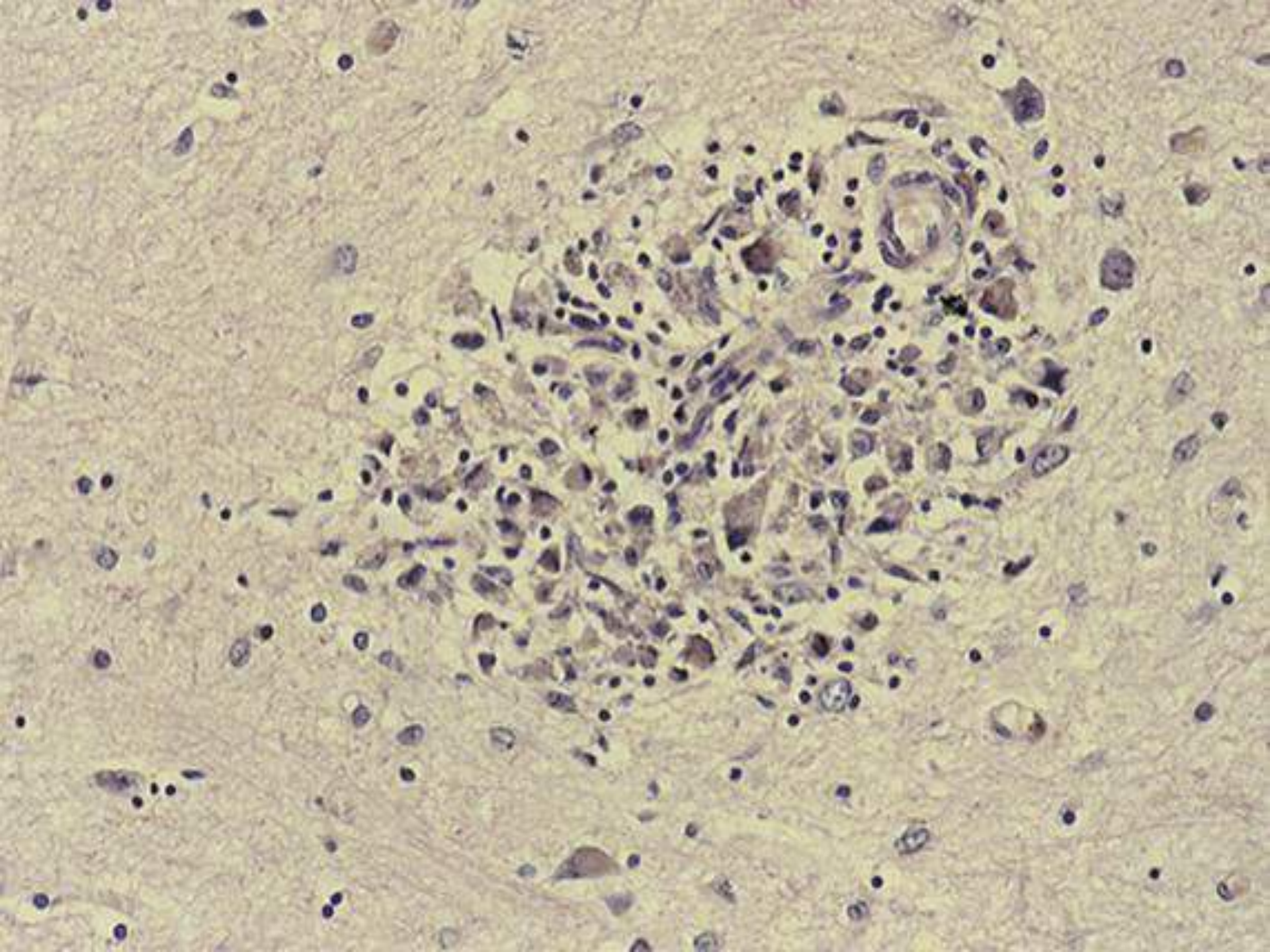
- **Autoimmune demyelinating disease**
- **Mimics acute viral encephalitis clinically:**
  - **Additional history of rash, nonspecific respiratory, or GI illness days to weeks preceding**
- **CSF often normal or nonspecific**
- **MRI:**
  - **Enhancing multifocal white matter disease c/w demyelination**

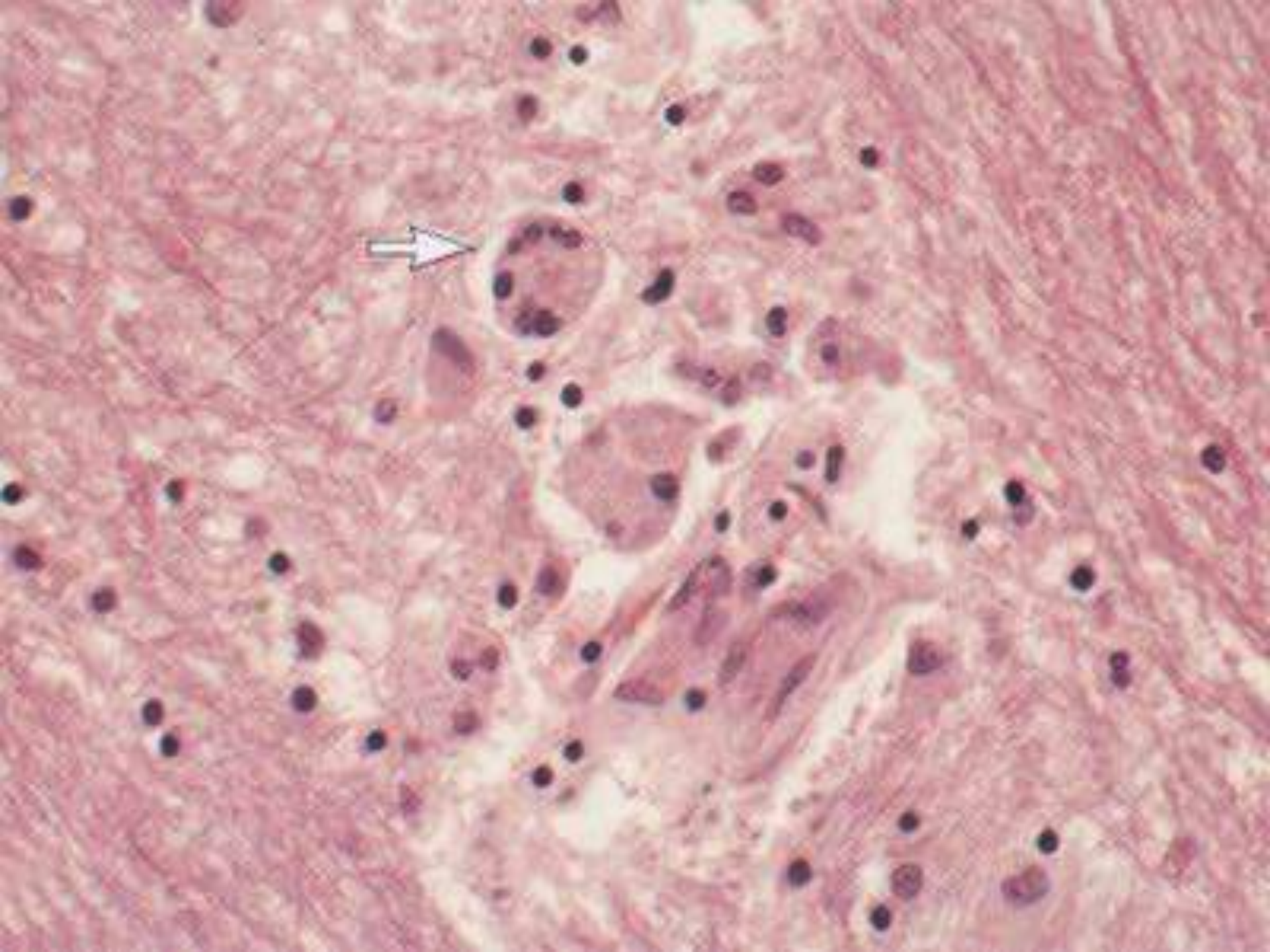




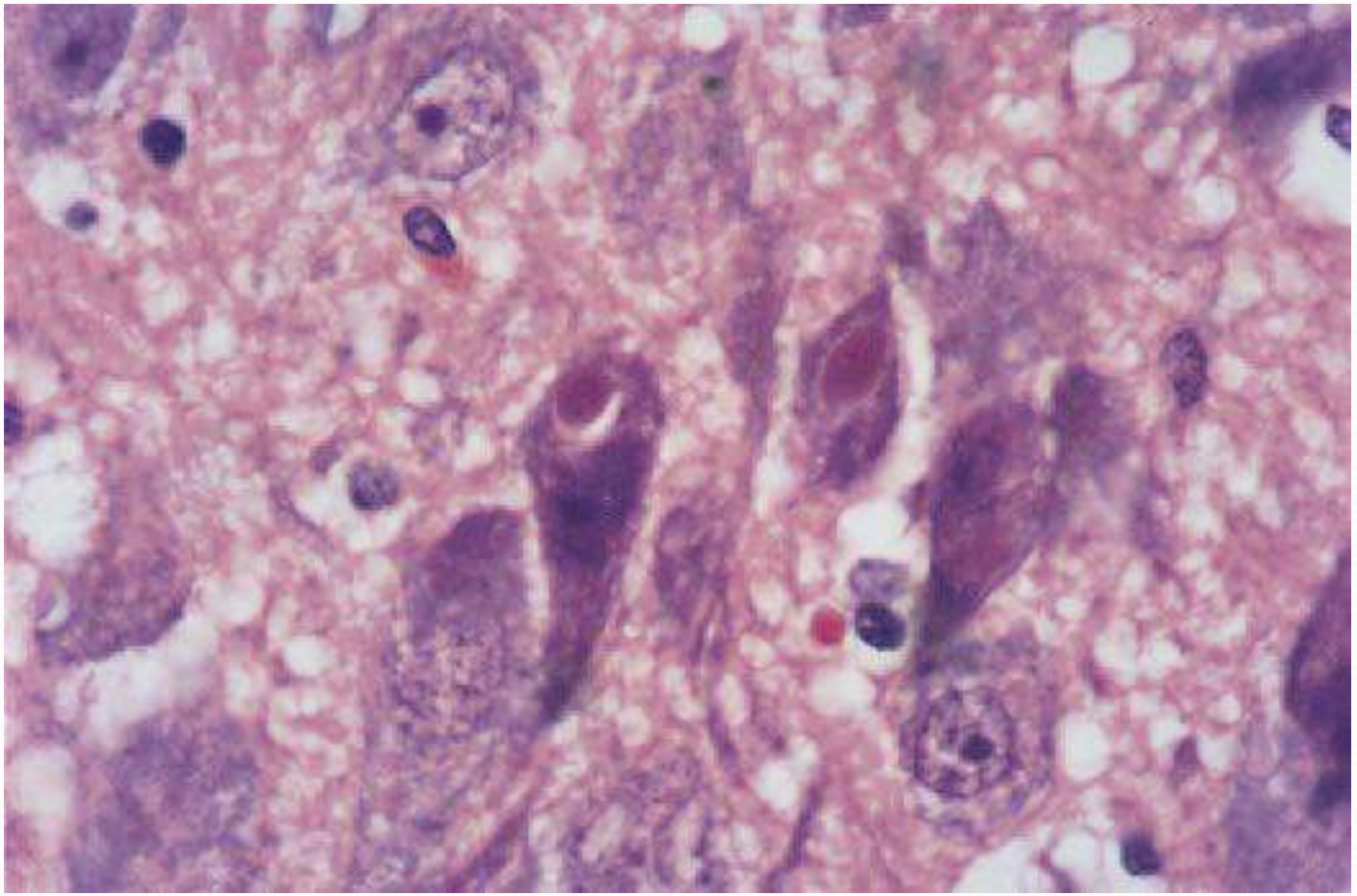


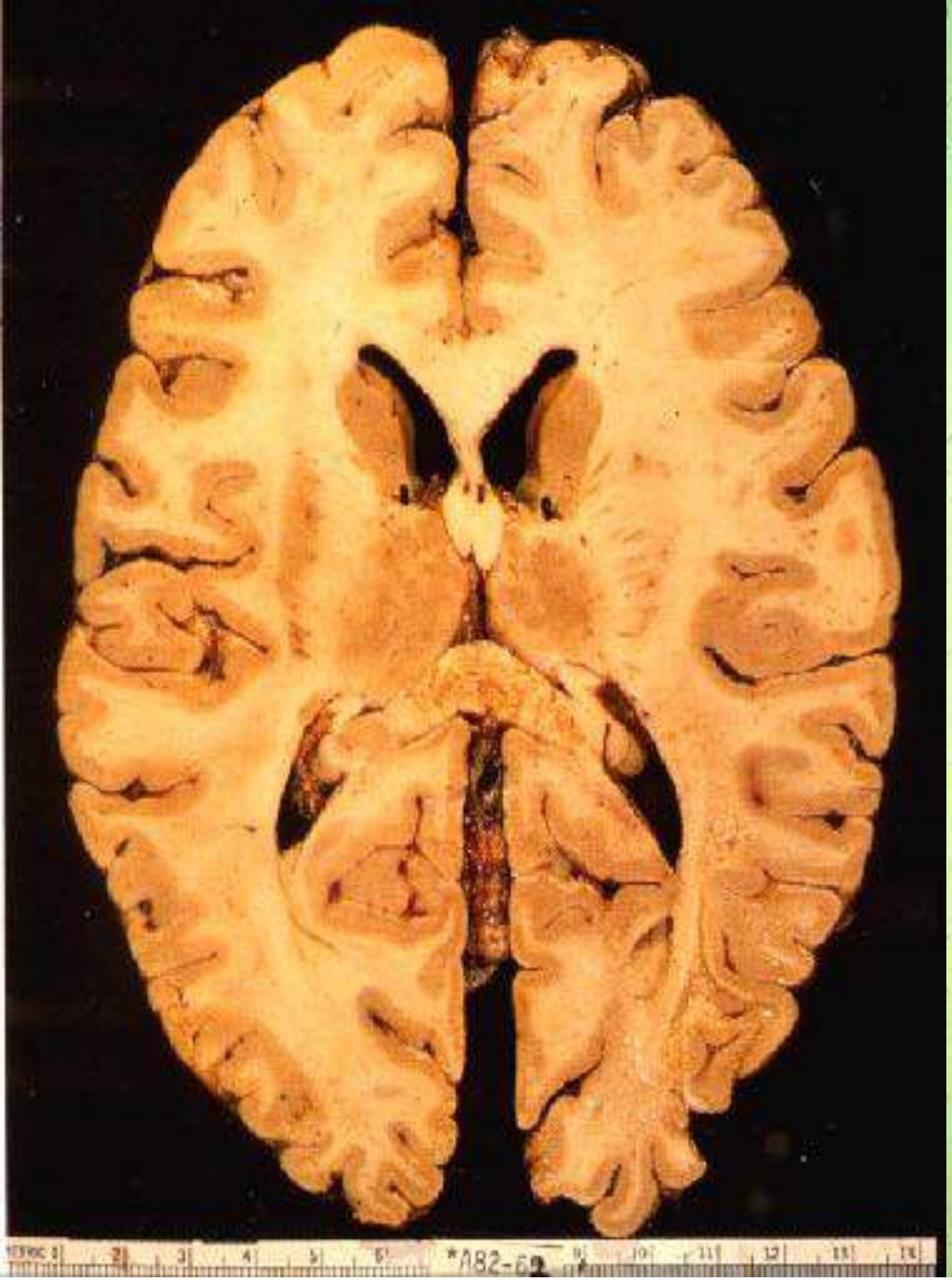


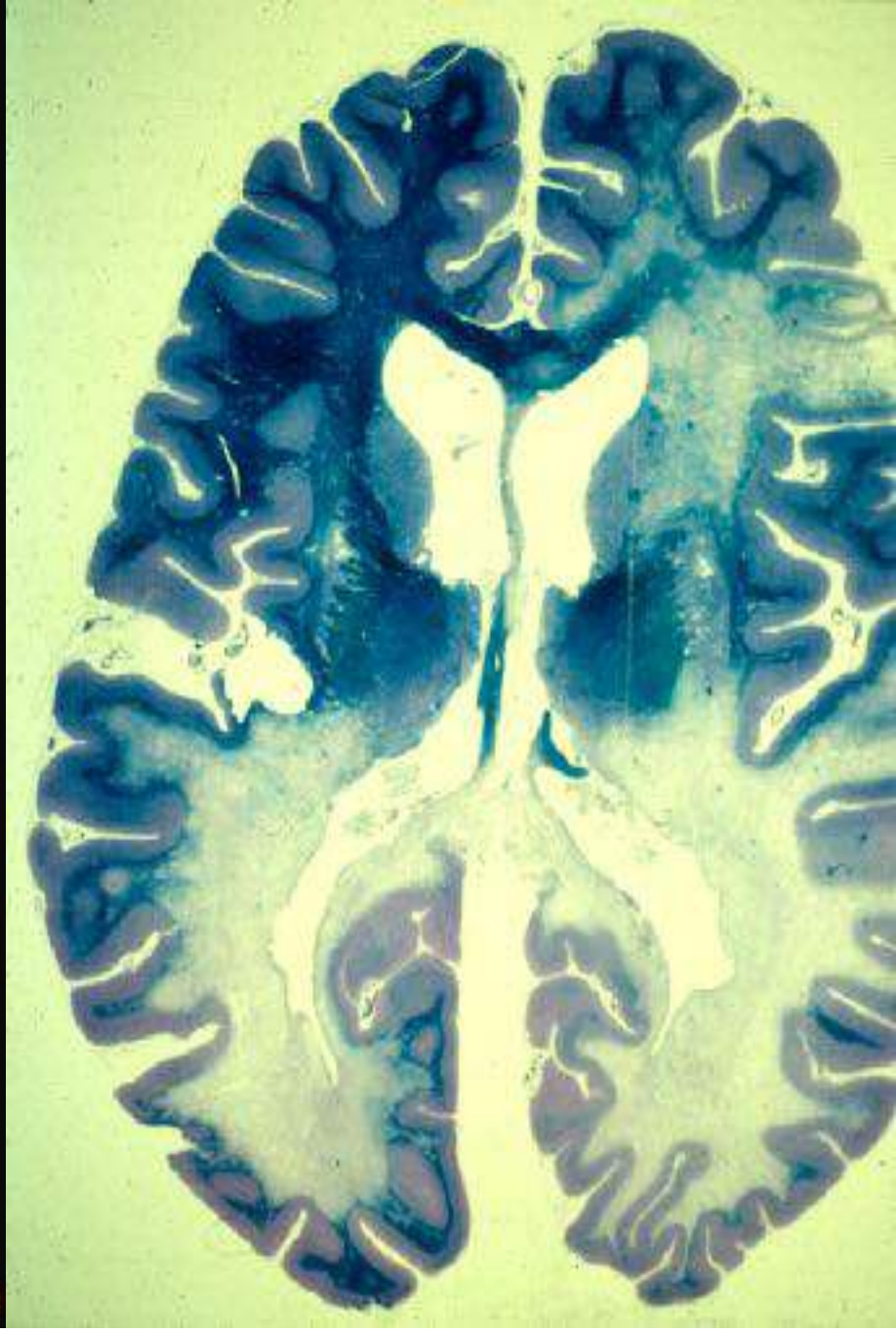


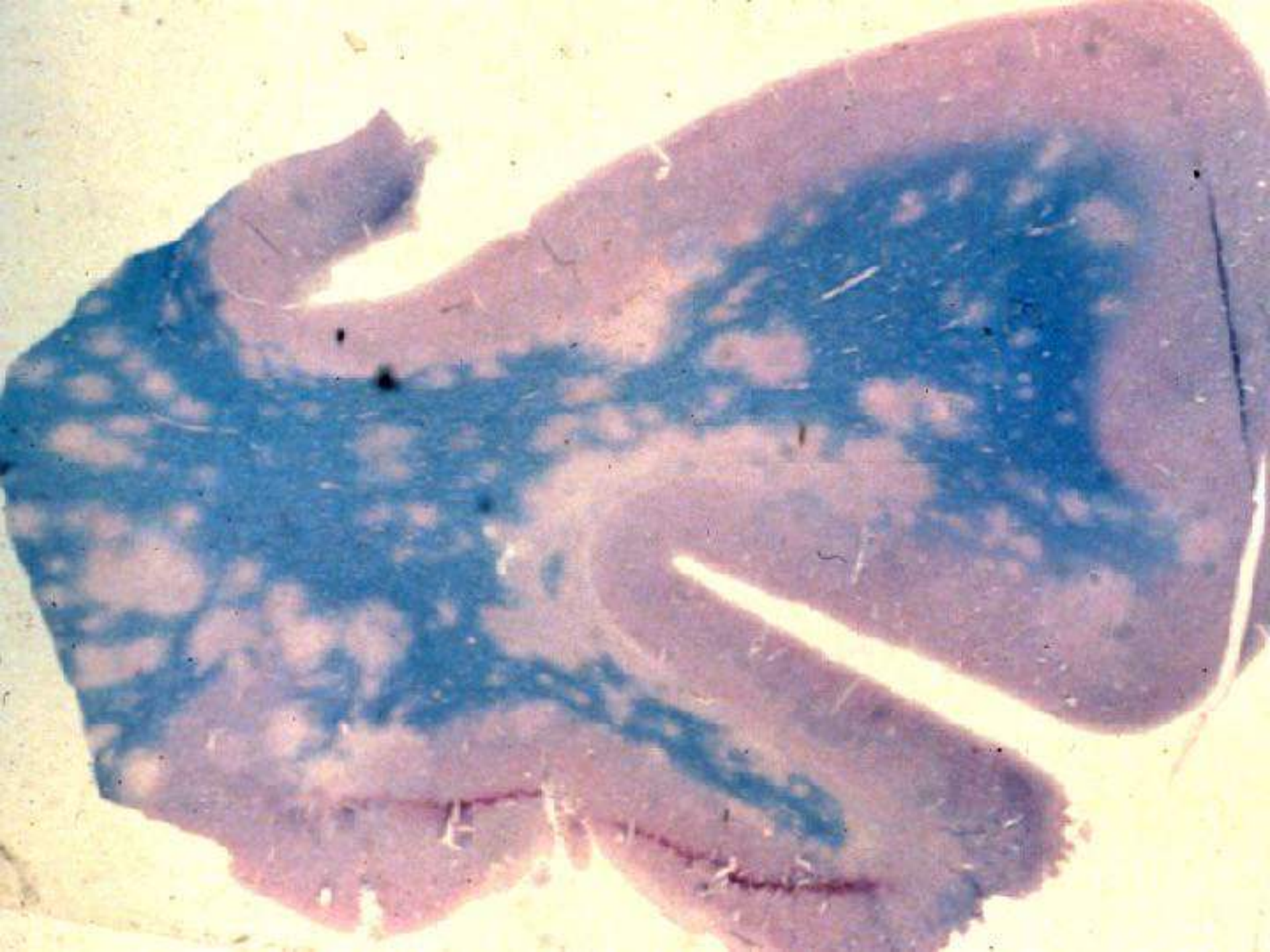


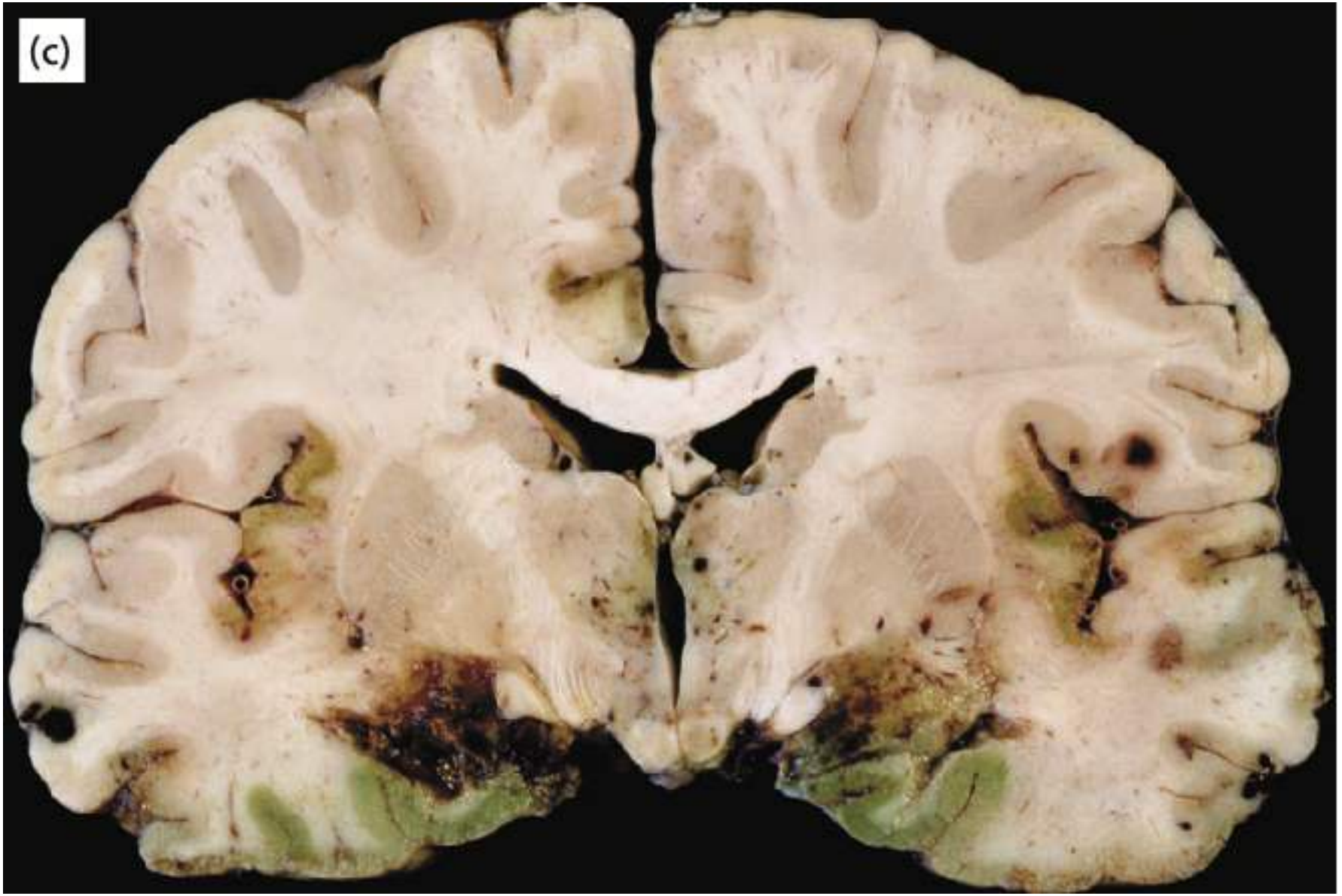
Negri bodies (eosinophilic viral inclusion bodies), cytoplasm of hippocampus







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Non-infectious or Auto-immune

- **Tumor associated**
- **Post-infection associated**

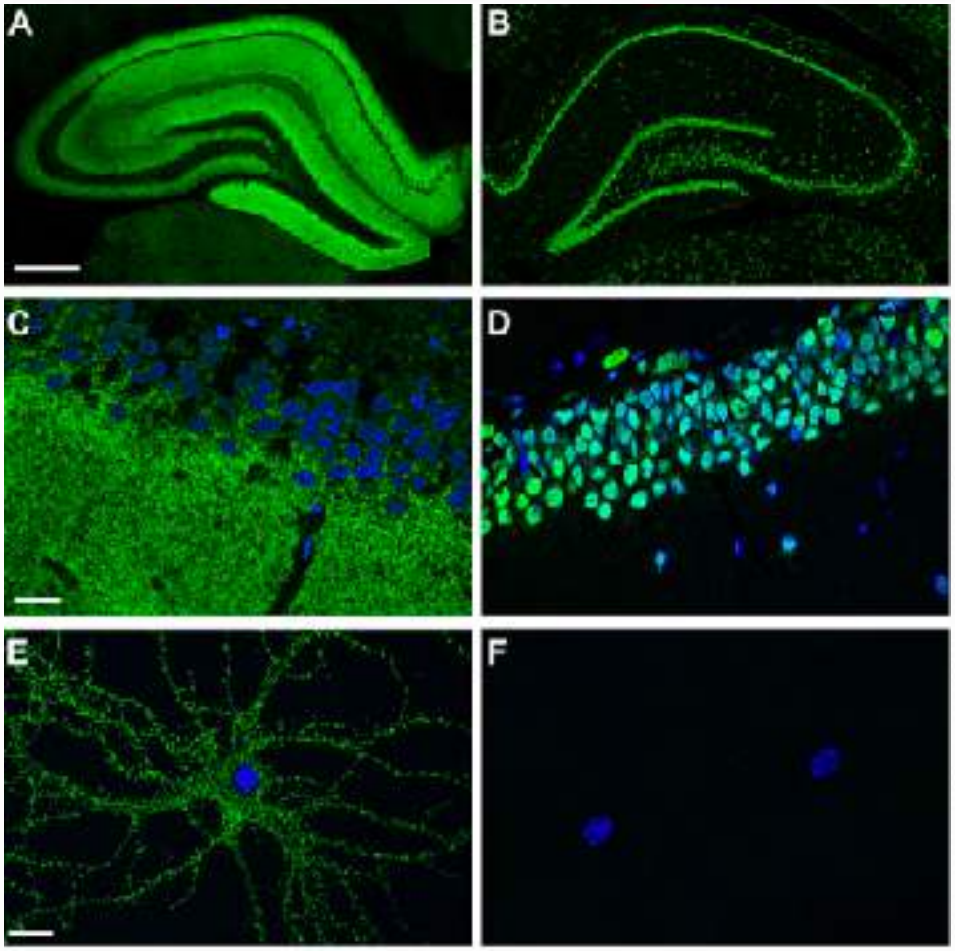
Table 1 Antibodies to intracellular antigens

Antibody	Oncological association	Frequency of tumour	Response to immunotherapy	Neurological manifestations
ANNA-1 (anti-Hu)	Small-cell carcinoma.	>90%	Poor	Limbic, cortical encephalitis. Autonomic neuropathies, sensory neuronopathy and other peripheral neuropathies.
ANNA2 (anti-Ri)	Small-cell carcinoma, breast adenocarcinoma and bladder cancer.	>60%	Poor	Brainstem encephalitis (opsoclonus–myoclonus, laryngospasm, trismus and cranial neuropathy) and cerebellar degeneration.
ANNA3	Small-cell carcinoma.	>60%	Poor	Limbic and brainstem encephalitis, sensory and sensorimoto neuropathies and myelopathy.
PCA2	Small-cell carcinoma.	>90%	Poor	Brainstem or limbic encephalitis and cerebellar degeneration.
Ma1, Ma2	Testicular (Ma2); breast, colon and testicular (Ma1).	>90%	Moderate	Ma2 Limbic encephalitis, diencephalitis, brainstem encephalitis; Ma1 and Ma2 brainstem encephalitis and cerebellar degeneration.
CRMP-5	Small-cell carcinoma and thymoma.	>75%	Poor	Encephalitis. Optic neuritis and retinitis, myelopathy, neuropathy and Lambert–Eaton myasthenic syndrome.
Amphiphysin	Small-cell carcinoma and breast adenocarcinoma.	>90%	Poor	Limbic encephalitis. Myelopathy, stiff-man syndrome and cerebellar degeneration.
GAD65	Thymoma; neuroendocrine tumours, breast or colon adenocarcinoma.	<10%	Moderate	Stiff-person syndrome, stiff-person phenomena, brainstem encephalitis and cerebellar degeneration.
GFAP	None described to date.		Good	Meningoencephalomyelitis, headache, papillitis and cerebellar ataxia.

Table 2 Antibodies targetting neuronal cell surface and synaptic proteins

Antibody	Oncological association	Frequency of tumour	Response to immunotherapy	Neurological manifestations
VGKC complex LGI1	Thymoma, small-cell lung cancer.	<10%	Good	Limbic encephalitis, hyponatremia and faciobrachial dystonic seizures.
CASPR2	Thymoma	40%	Good	Isaacs syndrome, Morvan's syndrome and limbic encephalitis.
NMDAR	Ovarian teratomas, testicular germinoma and neuroblastoma.	Varies with age, sex, and ethnicity	Good	Psychiatric disturbances, dyskinesias, catatonia, central hypoventilation and autonomic instability, and opsoclonus–myoclonus.
AMPAR	Thymic tumours, lung carcinoma and breast adenocarcinoma.	70%	Good	Limbic encephalitis and nystagmus.
GABA-A receptor	Thymoma, small-cell lung cancer and rectal cancer.	40%	Good	Status epilepticus, epilepsia partialis continua, psychosis, behavioural disturbances, orolingual dyskinesias and chorea.
GABA-B receptor	Small-cell lung carcinoma and other neuroendocrine neoplasia.	70%	Good	Limbic encephalitis and orolingual dyskinesias.
mGluR5 receptor	Hodgkin's lymphoma.	>90%	Good	Cerebellar ataxia and limbic encephalitis (Ophelia syndrome).
GlyR	Thymoma, breast cancer and Hodgkin's lymphoma.	<10% of published cases	Moderate	Progressive encephalomyelitis with rigidity and myoclonus, oculomotor disturbances, dysautonomia, hyperekplexia and respiratory failure.
DPPX	None described to date.		Moderate	Encephalitis, sleep disturbances, myoclonus, hyperekplexia, dysautonomia and gastrointestinal dysmotility.
IgLON5	None described to date.		Poor	Non-REM parasomnias, REM sleep behaviour disorder, apnoea, stridor and cognitive decline.

Experimental models



ANTI-NMDA

ANTI-HuD

Physiol Rev 97: 839–887, 2017
Published March 15, 2017; doi:10.1152/physrev.00010.2016

AUTOANTIBODIES TO SYNAPTIC RECEPTORS AND NEURONAL CELL SURFACE PROTEINS IN AUTOIMMUNE DISEASES OF THE CENTRAL NERVOUS SYSTEM

Josep Dalmau, Christian Geis, and Francesco Graus

Post-infectious encephalomyelitis

- Vaccinia virus [1:60-100,000]: *Smallpox vaccine virus*
- Measles virus [1:1000]: *Almost eliminated by introduction of vaccine*
- Varicella-zoster virus [1:4000]: *Acute cerebellar ataxia*
- Rubella virus[1:20,000]: *Reduced (99%) in U.S. by vaccine*
- EBV [Rare]: *Early weeks of infectious mononucleosis*
- Mumps virus [Rare]: *Reduced (99%) in U.S. by vaccine*
- *Influenza virus [Rare]*
- *Zika*
- Nonspecific respiratory illness
- Bacterial – streptococcus and others

Case

- A 45 year old man returns from visiting his family in Miami 2 weeks ago, presents with 1 week history of tingling, numbness of the lower extremities. Over the last 3 days, it has progressively worsened, and he started having bilateral lower extremity weakness and gait issues.
- His PMHx was unremarkable except for a mild rash which he attributed to “a heat rash” and low grade fever of 99.5 about 5 days before onset of symptoms.

Zika Virus (Zika)

- Single stranded RNA virus.
- Genus *Flavivirus*, family *flaviviridae*.
- Closely related to dengue, yellow fever, Japanese encephalitis, and West Nile viruses.
- Primarily transmitted through the bite of an infected *Aedes* species mosquito (*Ae. aegypti* and *Ae. albopictus*).

- 2 geographically distant lineage of the Zika virus – African and Asian – are known
- Current circulating one in the Americas are of Asian lineage
- African lineage is not felt to be neurotropic

Aedes aegypti

Aedes albopictus
(Asian Tiger Mosquito)

Clinical Manifestations of Zika infection

- Only 20% become symptomatic
- Illness is mild
- Incubation period: 2 – 14 days
- Symptoms resolve within 2 – 7 days
- Nothing to suggest that the symptoms of infection is more severe in pregnant women

- *After symptom onset, the duration of Zika viremia is usually up to 1 week*
- *In non-pregnant persons, Zika virus RNA can be detected in the serum 11 - 13 days after symptom onset*
- *In pregnant women can persist 10 weeks after symptom onset*

Neurologic problems

- Adverse fetal outcomes – the full spectrum is yet to be determined.
  - Microcephaly
  - Fetal abnormalities detected by ultrasound in 29% of women with Zika infection during pregnancy in Brazil
  - Early Fetal loss and Fetal death
  - Ocular and hearing abnormalities
- Guillain-Barré syndrome
- Acute myelitis and meningoencephalitis

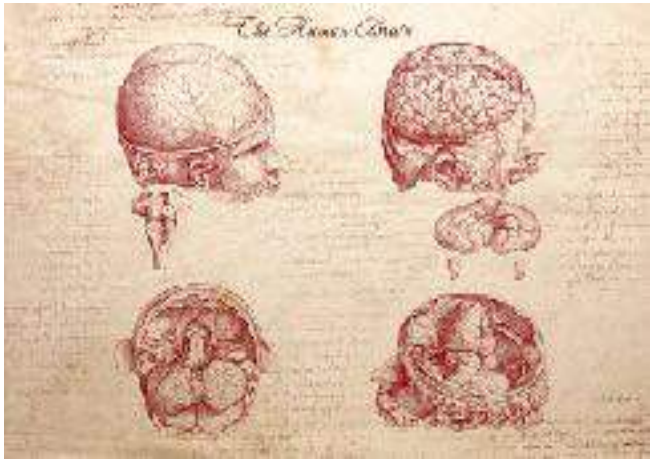
Other post-infectious or vaccine associated

- Acute disseminated encephalomyelitis (ADEM)
- Acute hemorrhagic encephalomyelitis
- Hemophagocytic lymphohistiocytosis (HLH) syndrome
- Influenza-related encephalopathy/encephalitis and acute necrotizing encephalopathy

- Pediatric autoimmune neuropsychiatric disorders associated with streptococcal infection (PANDAS/ PANS)

History of Brain Collecting and Banking

- Alcmaeon of Croton (ca. 520-450 B.C.)
- Hippocrates of Cos (460-370 B.C.)
- Herophilus of Chalcedon (330-260 B.C.) and Erasistratus of Ceos (304-250 B.C.)
- Galen, 2nd-century AD Rome
- Leonardo da Vinci (1452-1519).
- EXTENSIVE STUDY 18-20TH CENT



MY INSPIRATION TO BECOME A BRAIN BANKER…

Young Frankenstein (1974)
YES, IT'S ON Amazon Prime!

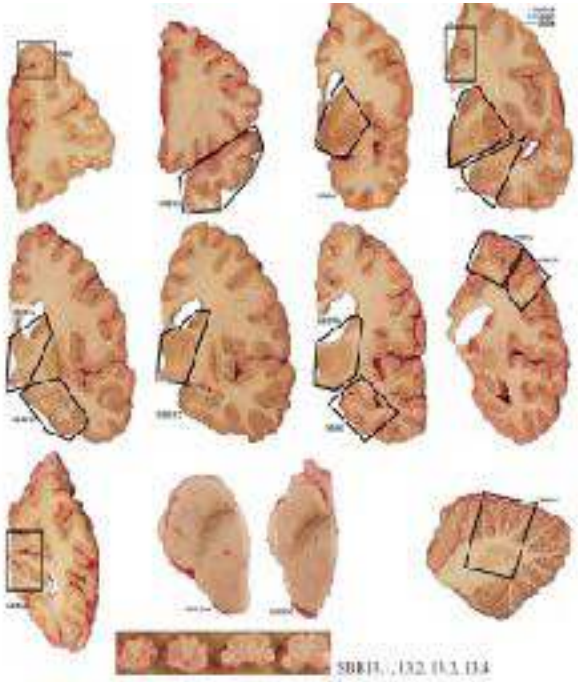
Acta Neuropathol (2008) 115:509–532
DOI 10.1007/s00401-007-0311-9

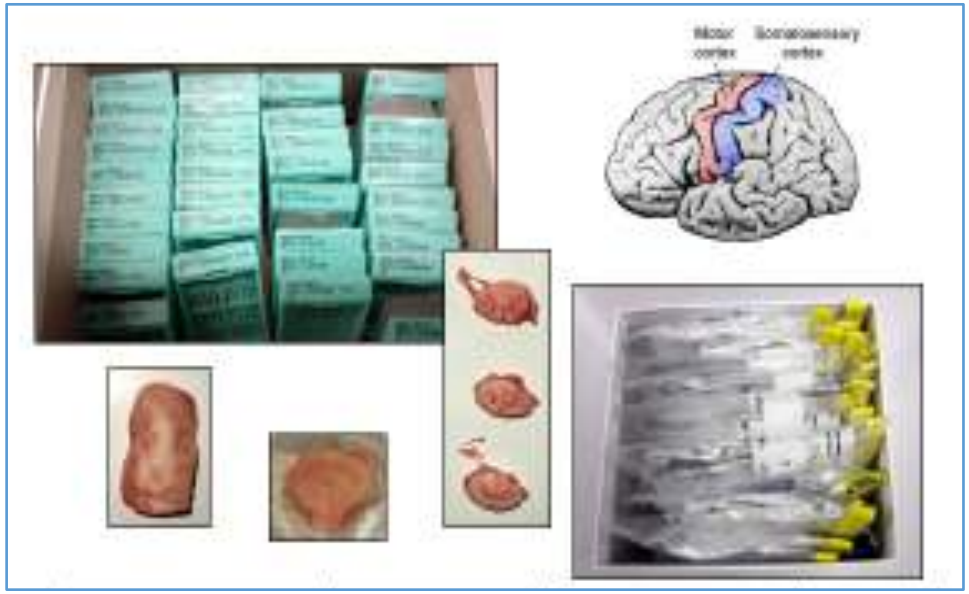
METHODS REPORT

Twenty-first century brain banking. Processing brains for research: the Columbia University methods

Jean Paul G. Vonsattel · Maria Pilar del Amaya · Christian E. Keller

Tissue Sampling





Storage of tissues in fixative and blocks and slides

Frozen tissues

- Genomics
- Proteomics
- Transcriptomics
- Metabolomics
- Any ”omics” you can think of

Staining

Block	H&E	H&E + Luxol FB (or Nissl + LFB)	TDP43 and/or pTDP43	Tau (AT8 or Davies PHF-1)	Ubiquitin Optional (Dako or other)	Optional	
						Silver	P62
Midfrontal gyrus	YES		YES	YES		YES	
Superior & Middle temporal	YES					YES	
Occipital Cortex	YES					YES	
Motor Cortex	YES		YES		YES		
Basal ganglia/basal forebrain	YES						
Hippocampus + Entorhinal cortex (medial temporal lobe)	YES		YES	YES	YES	YES	YES
Midbrain	YES						
Pons	YES						
Medulla		YES					
Spinal Cord, cervical + thoracic	YES	YES	YES		YES		
Spinal Cord, lumbosacral	YES	YES	YES		YES		
Cerebellum	YES		YES		YES		YES
Inferior parietal lobule	YES					YES	
Amygdala	YES			YES		YES	

PANDAS/PANS & OTHER NEUROIMMUNE RELATED DISORDERS (POND) Brain Bank at Georgetown University

Established in 2022 with the support of Georgetown University and philanthropic support from the

Alex Manfull Research Fund for PANDAS/PANS and other Neuroimmune Disorders

POND Mission Statement

- The POND Brain Bank was founded to aid researchers, medical students, clinicians, our patients, and their families to advance understanding of PANS/ PANDAS and other related neuroimmune disorders. Our goal is to assist research efforts to ensure that no person will lose their life to PANS/PANDAS, or lose the years it can take to successfully treat these neuroimmune disorders.

POND Biospecimen Use Committee

Committee members:

- Jennifer Frankovich
- Beth Latimer
- Beth Stevens
- Kyle Williams
- Brent Harris
- Barbara Weinstein

Moderator:

- Marina Selenica

POND Development Committee

- Susan Manfull
- Barbara Weinstein
- Brent Harris
- Marina Selenica

Currently we have tissue/biofluids from 3 brains in the POND Brain Bank

Case – Clinical history

- Previously healthy 19yo female had mononucleosis and strep infection
- New allergies, new plaque psoriasis
- Developed new neuropsychiatric conditions that year which worsened in the next several years
  - OCD
  - Depression
  - Insomnia
  - Eating disorders
  - Alcohol abuse

Case – Clinical history

- After graduating from an Ivy league school she worked in several prestigious, high stress jobs on East coast
- Diagnosed with PANDAS at age 26
- Treated for this illness but several months later seemed to have an acute episode of fever, aches and went to Urgent care. Seemed to be doing better, communicating with family, but then tragically took her own life

CNS Postmortem Disposition and Findings

- The case was handled by the local Medical Examiner's Office and brain sent to the NIMH per collaborative program and consent from the family.
- In 2020, the family requested that the biobanked brain, slides, and blocks be sent to the Georgetown Brain Bank for review and research banking and teaching.

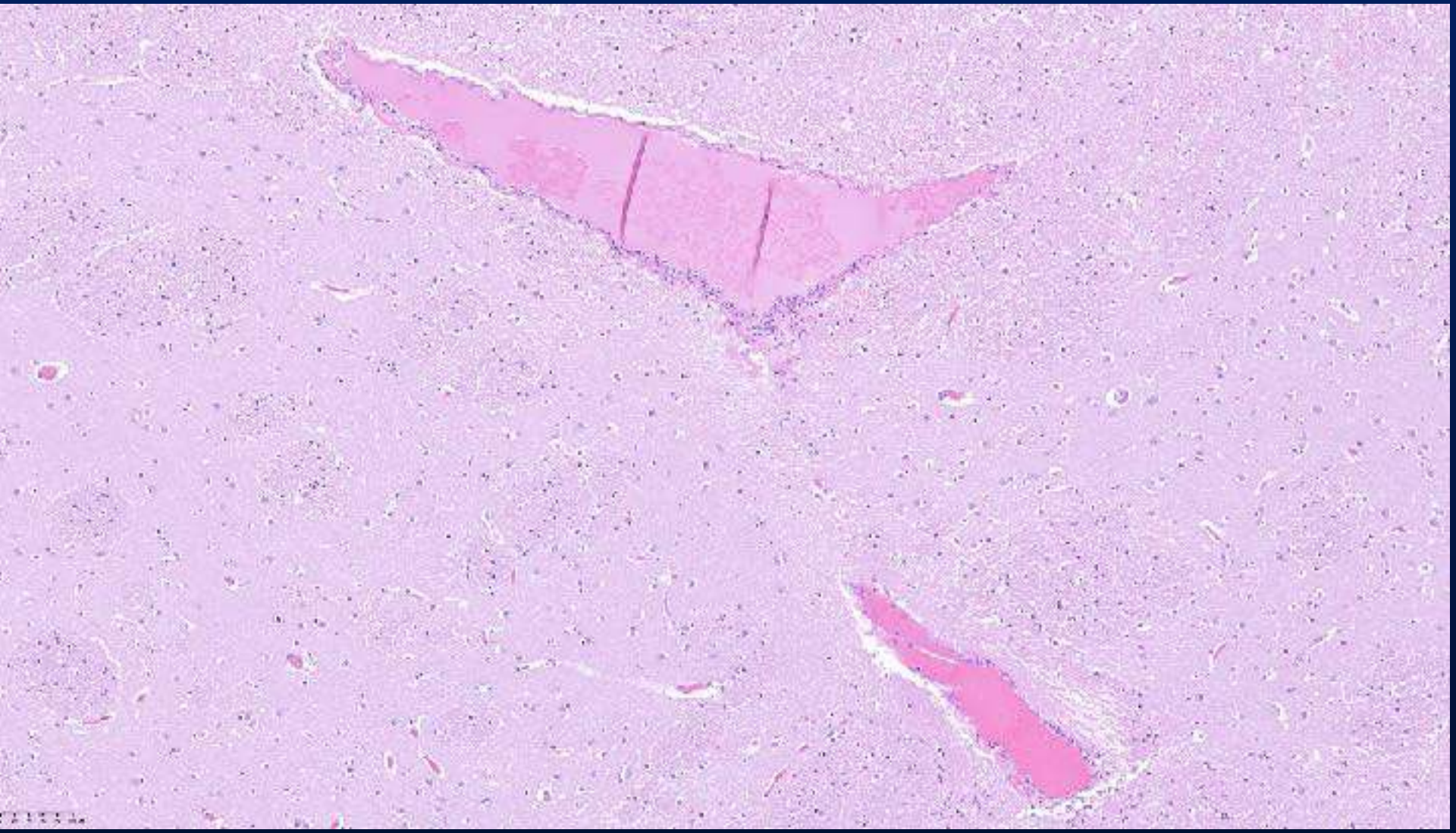
Neuropathological gross evaluation

- Brain weight of 1400g (normal)
- Meninges – normal
- Vasculature – mild, patchy atherosclerosis
- Coronal sections revealed no gross abnormalities

Neuropathological microscopic evaluation

- H&E findings were largely normal with exception of finding in the basal ganglia and thalamus :
  - Occasional vessels with perivascular lymphocytic cuffs
  - Scattered metabolic glia
  - Mild reactive astrocytosis
- No evidence of demyelination
- No abnormal tau on IHC
- No viropathic changes
- No microglial nodules

Basal ganglia – perivascular cuffs

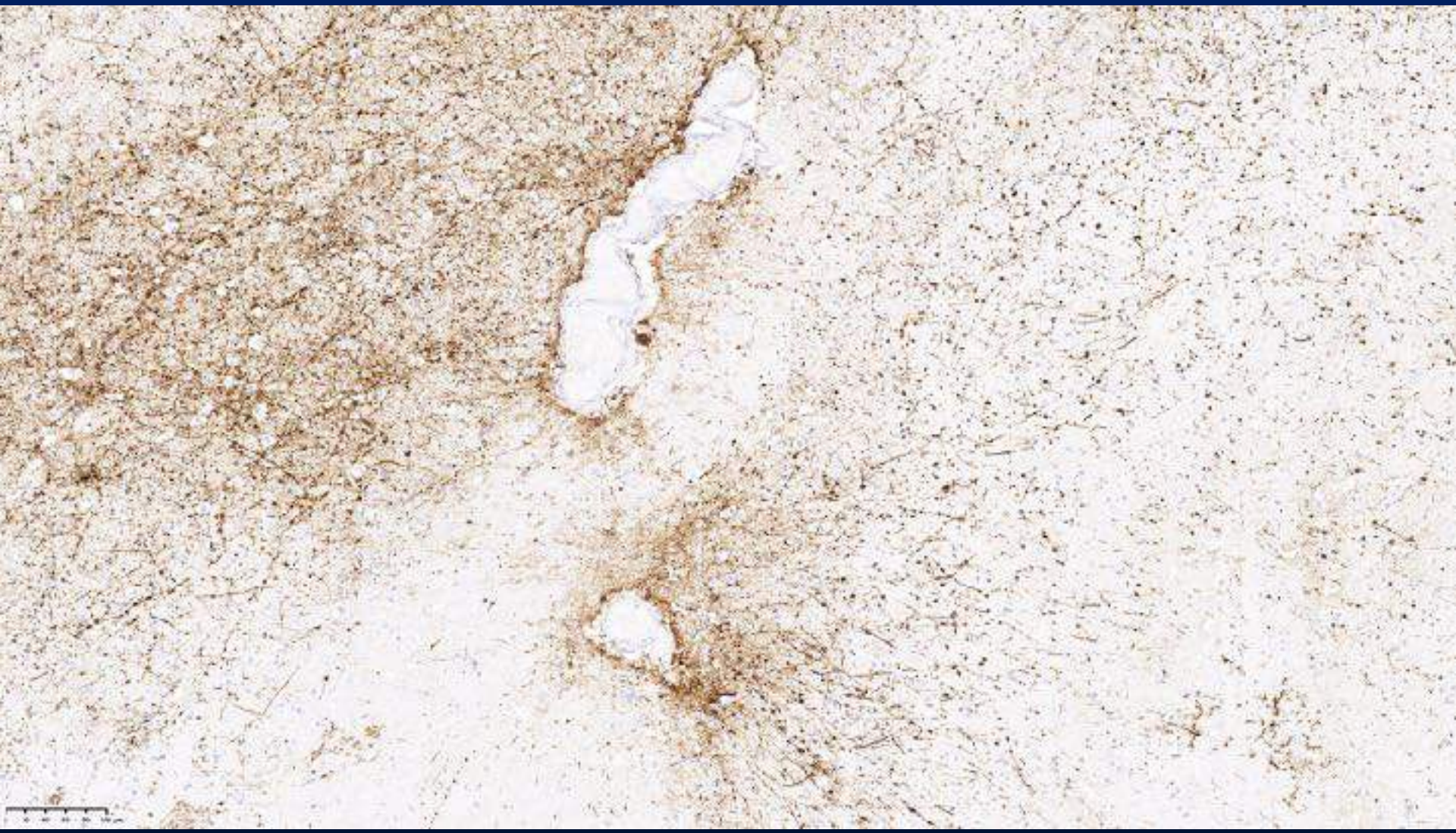


Basal Ganglia CD3 and CD4 IHC

Basal Ganglia CD68 and CD20 IHC

STAT3 - Early effector Th17 cell development relies on key cytokines that signal through STAT3

Basal Ganglia – GFAP IHC



Questions?

Contact info to request Tissues or Slides or to discuss collaborations

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More info about POND and the Georgetown Brain Bank

https://thealexmanfullfund.org

https://neurology.georgetown.edu/patientcare/georgetownbrainbank/