Basic CNS Anatomy and Histology, and Basic Neuropathology of Selected Diseases and Conditions in Laboratory Animals

The Hong Kong University of Science and Technology

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What are the goals of The HKUST?

- Is the goal to take a device or compound from benchside to bedside?
- Is this driven by monetary profit, or the health and welfare of the population.
- A problem often is from bed to bench.
- Do the PIs and management understand GLP studies?
- Do they understand “Spirit of GLP” studies?
- Yardsticks are often number of publications, impact factor, patents and other KPIs that really are not meeting the goals of the institution.
- There will no longer be many if any “block buster” drugs because of precision medicine and immunotherapies

Major Responsibilities of Laboratory Animal Pathologists

- Assist lab animal veterinarians and their vivaria staff survey for and monitor natural disease agents in the colony.
- Assist lab animal vets in executing an effective sentinel program (if there isn't one, there likely are problems). Culture of staff important. Absence of sentinel program suggests PI non-compliance.
- Assist PIs to determine if pathological changes found in animal tissues are due to natural disease or experimental manipulation or both.
- Never charge PIs for diagnostic workups.
- If don't have a veterinary pathologist (as is the situation in many places), use available off-site diagnostic services
- Major issue is that PIs are not required to publish the health status of animals used in generating scientific publications
- The older I have gotten, the more skeptical I have become about histological changes published in the biomedical literature. I believe less and less of what I read regarding lab animal pathology

Topics to Contemplate When Doing CNS Pathology

- How to harvest brains and spinal cords
- How to fix brains and spinal cords
- How to trim brains and spinal cords
- How to process brains and spinal cords for histopathology
- How many sections to cut from each block

Topics to Contemplate When Doing CNS Pathology – cont.

- How to harvest brains and spinal cords
  - Do you, or should you, weigh the brain?
    - If so, weigh the animal first
    - Ideally, fast the animal first
  - What cuts do you make thru the calvarium?
    - Will you save the olfactory bulbs?
  - Do you do take the brain out when fresh?
  - What do you do with the pituitary gland?
  - How do you harvest the spinal cord?

Topics to Contemplate When Doing CNS Pathology – cont.

- How to fix brains and spinal cords
  - Do you do intravascular perfusion?
    - Do you first flush with heparinized saline?
    - Or do you first heparinize the animals?
  - If so, do you also immersion fix afterward?
  - Proper fixation and handling prevents “red” or “dark” neuronal cell body (soma) problem

Topics to Contemplate When Doing CNS Pathology – cont.

- How to trim brains and spinal cords
  - Do you make “perfect” coronal sections?
  - Do you make tangential sections of spinal cords?

Topics to Contemplate When Doing CNS Pathology – cont.

- How to process brains and spinal cords for histopathology
  - Do you “batch” controls with treatment or experimental groups in tissue processor?
  - Do you mix CNS tissue with other tissues?
  - Do you have a special tissue processing protocol for rodents, and especially mice?

Topics to Contemplate When Doing CNS Pathology – cont.

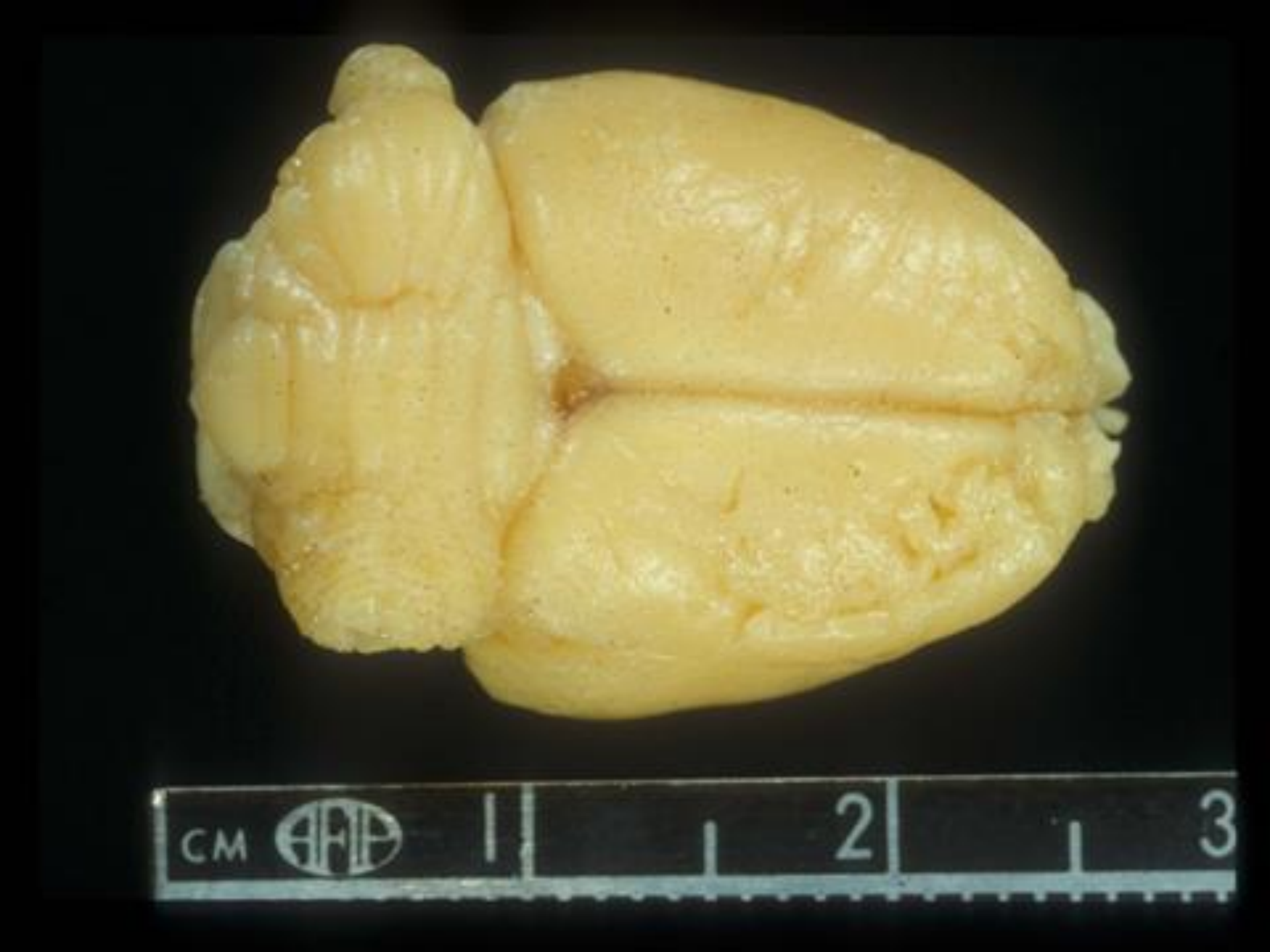
- How many sections to cut from each block
  - Depends on how many special stains are or might be needed
  - Best to over estimate the number
  - Plus consider publication needs

Topics to Contemplate When Doing CNS Pathology – cont.

- How many sections to cut from each block
  - Depends on how many special stains are

CNS Normal Anatomy – General Features

- Rodent brains are lissencephalic ("smooth brain") except in large rodents (e.g. beavers, capybaras). Also seen in other species (e.g. duck-billed platypuses, birds, bats, rabbits, prosimians, marmosets, squirrel monkeys).
- Other laboratory animals and other mammals (e.g. man) are gyrencephalic – have gyri and sulci.
- Lissencephaly is a pathological condition when present in a gyrencephalic species
  - Affects parts or all of the brain
  - Also called agyria or pachygyria
  - Due to defective neuronal cell migration (often due to viruses or hypoxia)
- Should not cut into or slice the brain until after immersion-fixation, even after intravascular perfusion.
- Pituitary glands in rodents are left in situ until after fixation
- Can use different intravascular fixatives. Best after perfusion first with heparinized saline (or heparinize the whole animal first)
- Do not touch the brain until after complete fixation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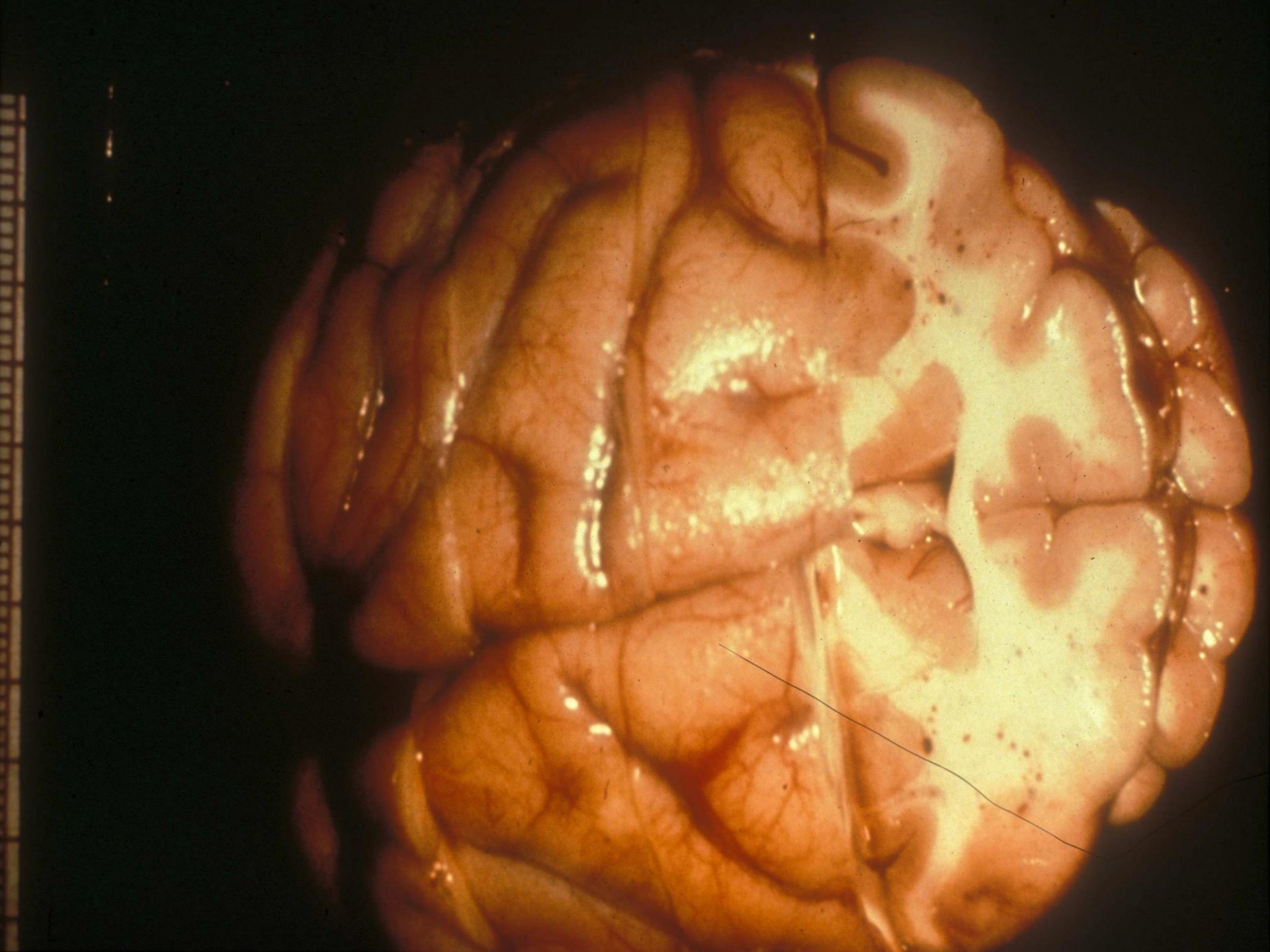
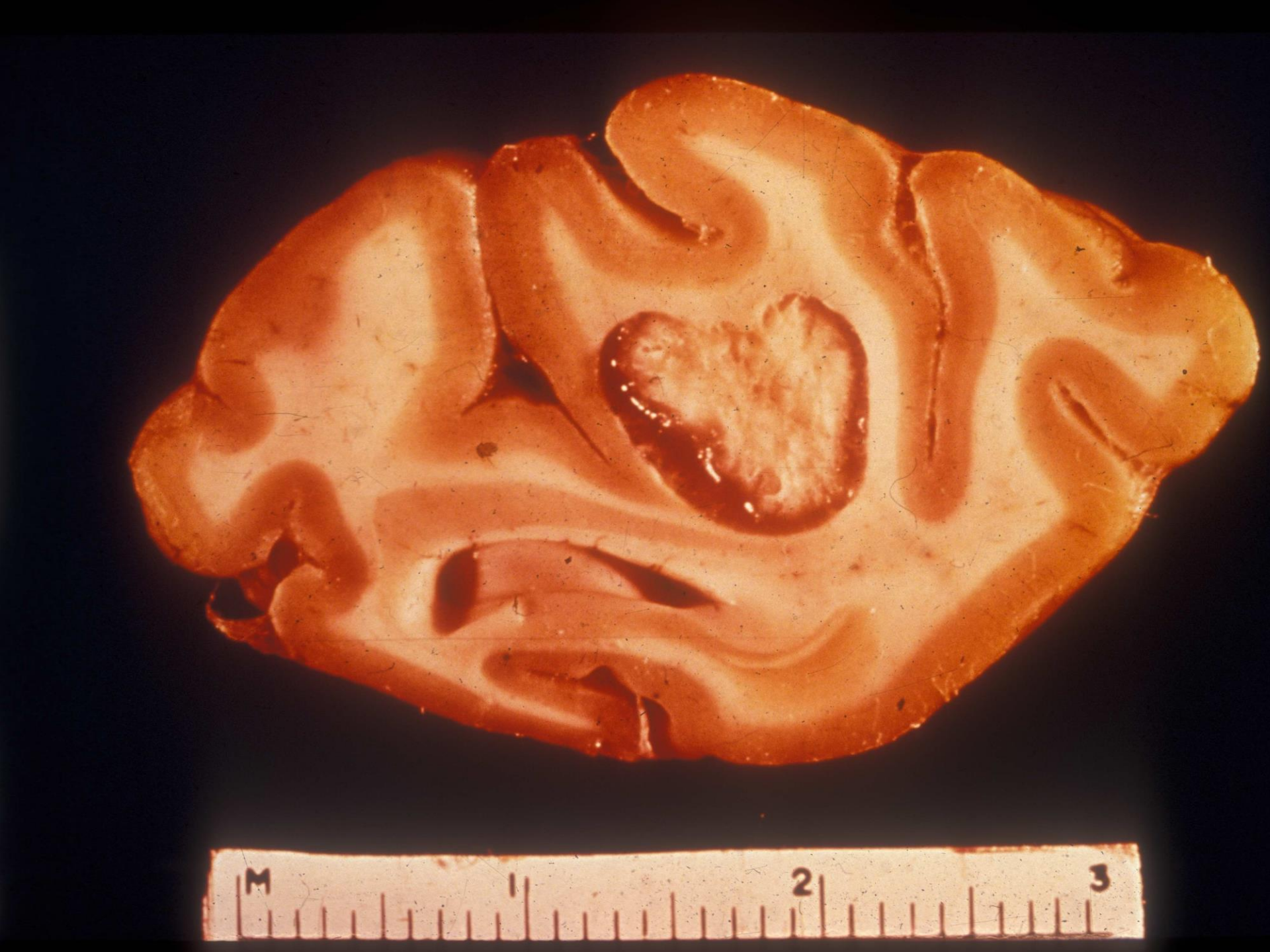
CNS Normal Anatomy – General Features - continued

- Brain has grey matter on outside (and also deep) with white matter in the interior
- Spinal cord has grey matter on inside (shaped like a butterfly) with white matter on the outside (columns containing tracts)
- Diseases affecting only the grey matter are prefixed with “polio” (e.g. poliomyelitis, polioencephalomalacia or poliomyelomalacia), whereas diseases affecting the white matter are prefixed by “leuco” or “leuko” (e.g. leukodystrophy or leukoencephalomalacia)

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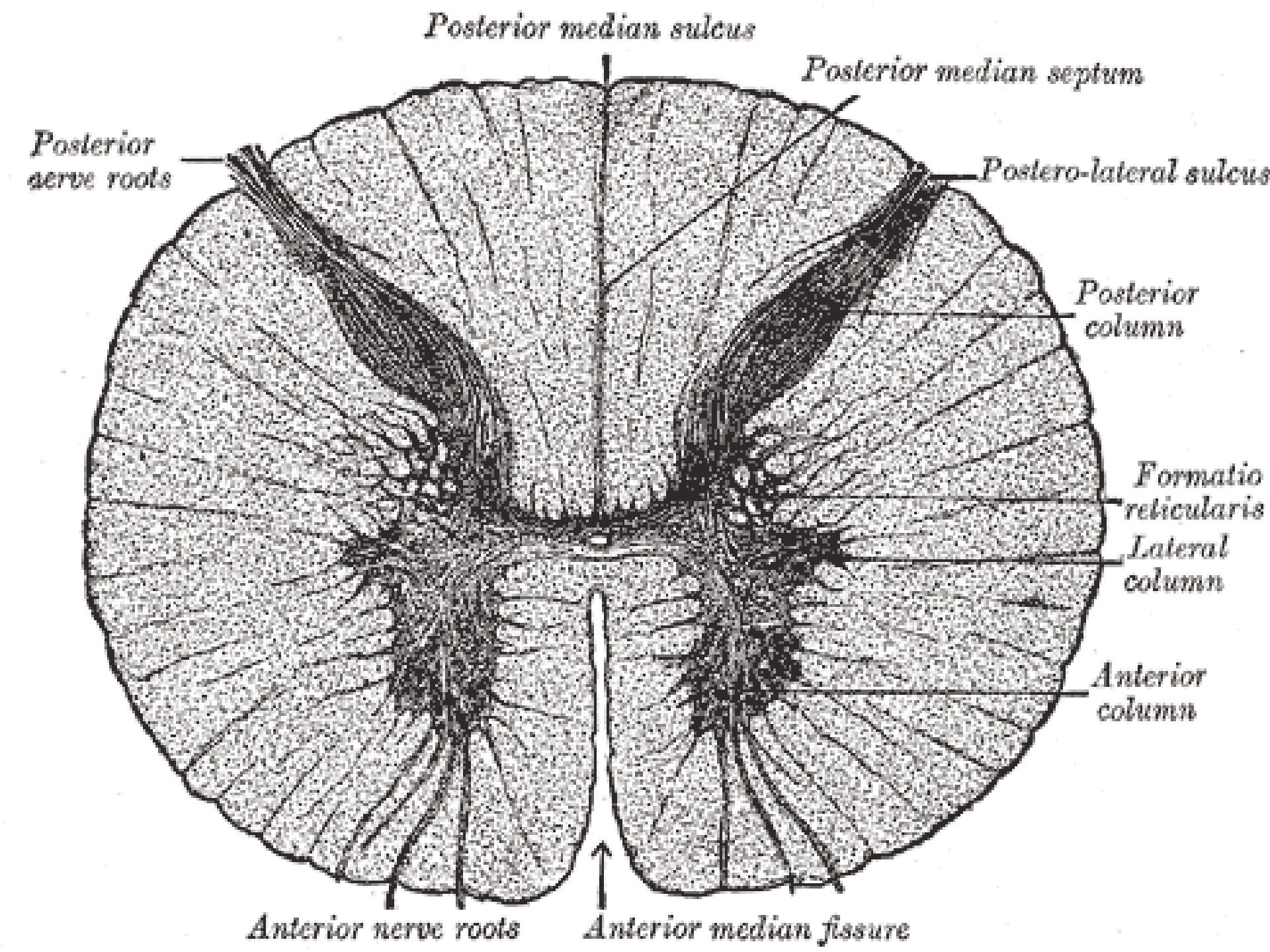
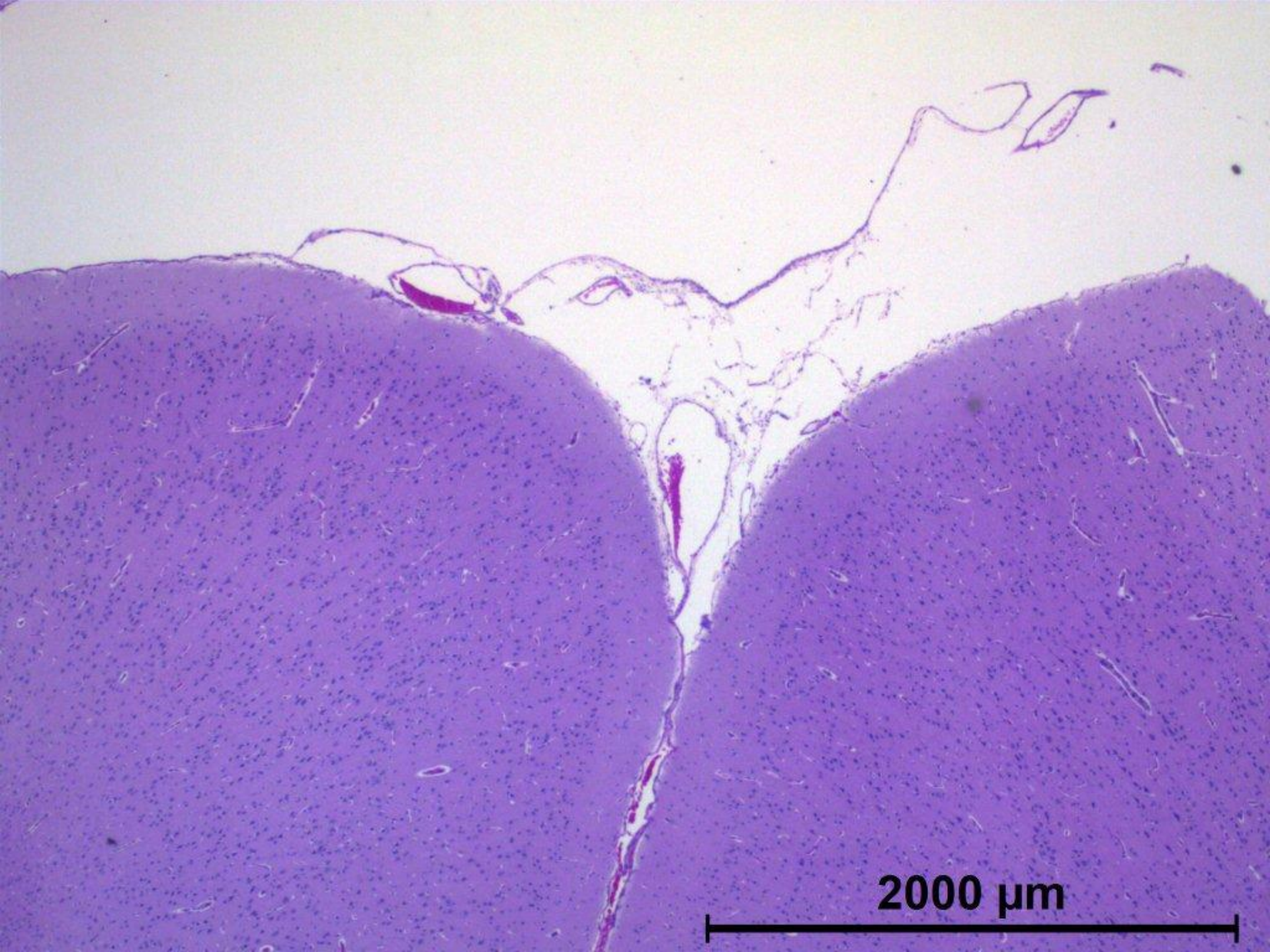

Posterior median sulcus
Posterior median septum
Posterior nerve roots
Postero-lateral sulcus
Posterior column
Formatio reticularis
Lateral column
Anterior column
Anterior nerve roots
Anterior median fissure

CNS Normal Histology – Meninges, Ventricles, Ependyma, Choroid Plexus

- Meninges
  - Dura Mater – tough outer covering
  - Arachnoidea (covering is the Arachnoid Mater)
  - Pia Mater – simple squamous cell, very thin
  - (Last two together are the leptomeninges and dive into the CNS and form the Virchow- Robin spaces)
- Ventricles (4 of them), Cisterns, Foramina, Aqueduct, and Canal in spinal cord
- Ependymal cells – line ventricles, spinal canal
- Choroid Plexuses – primary source of CSF (by both filtration and secretion)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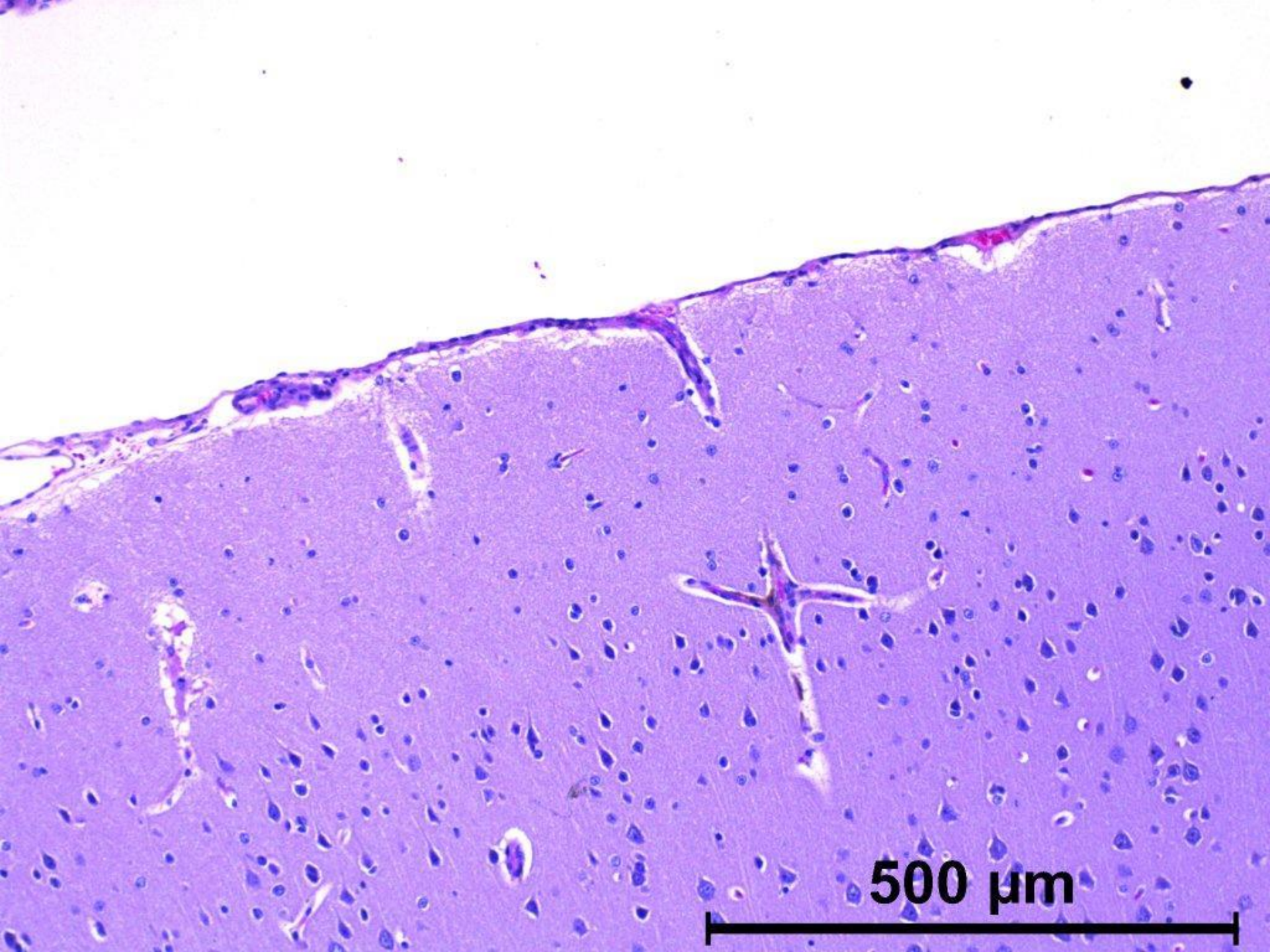
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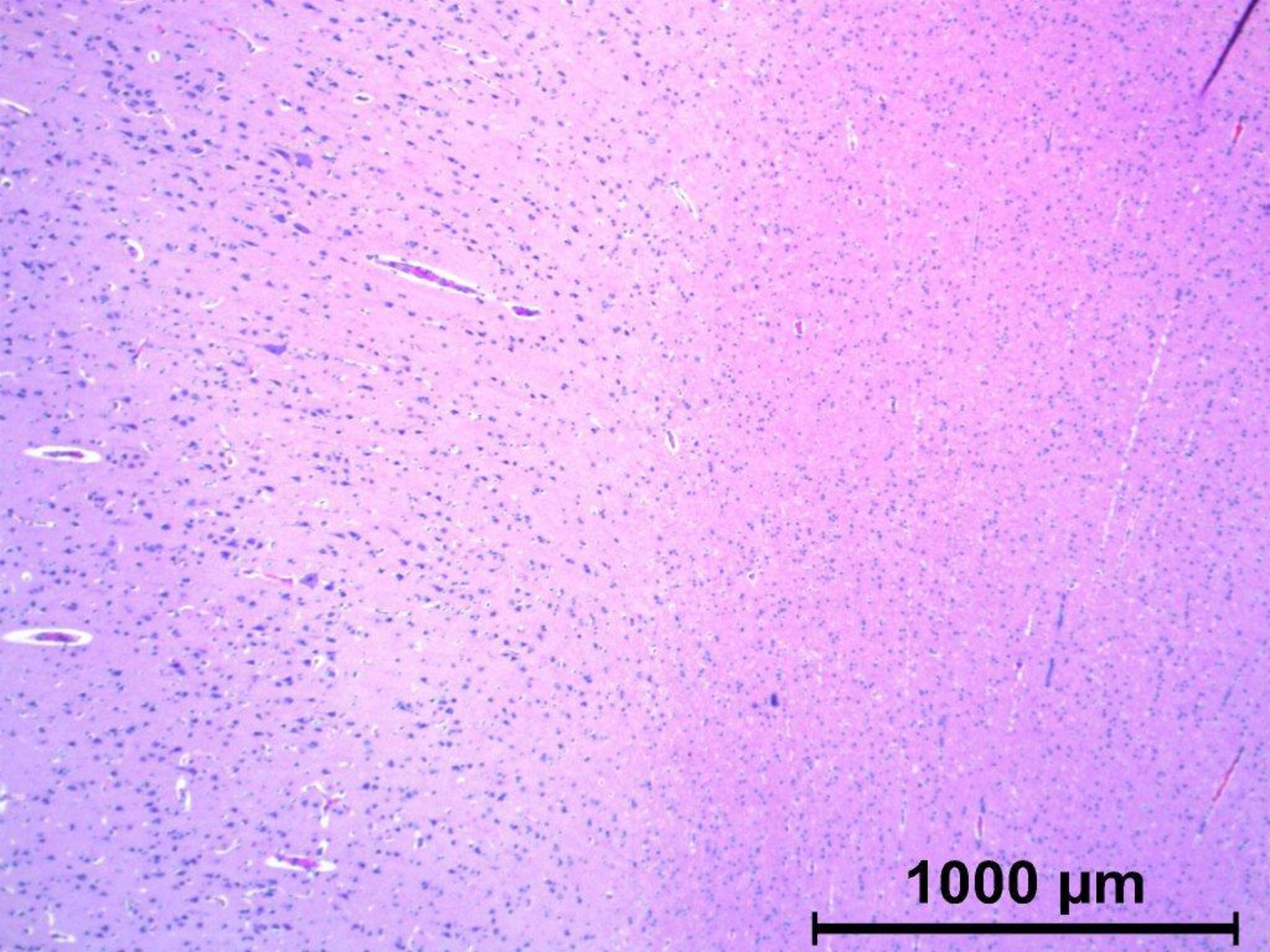
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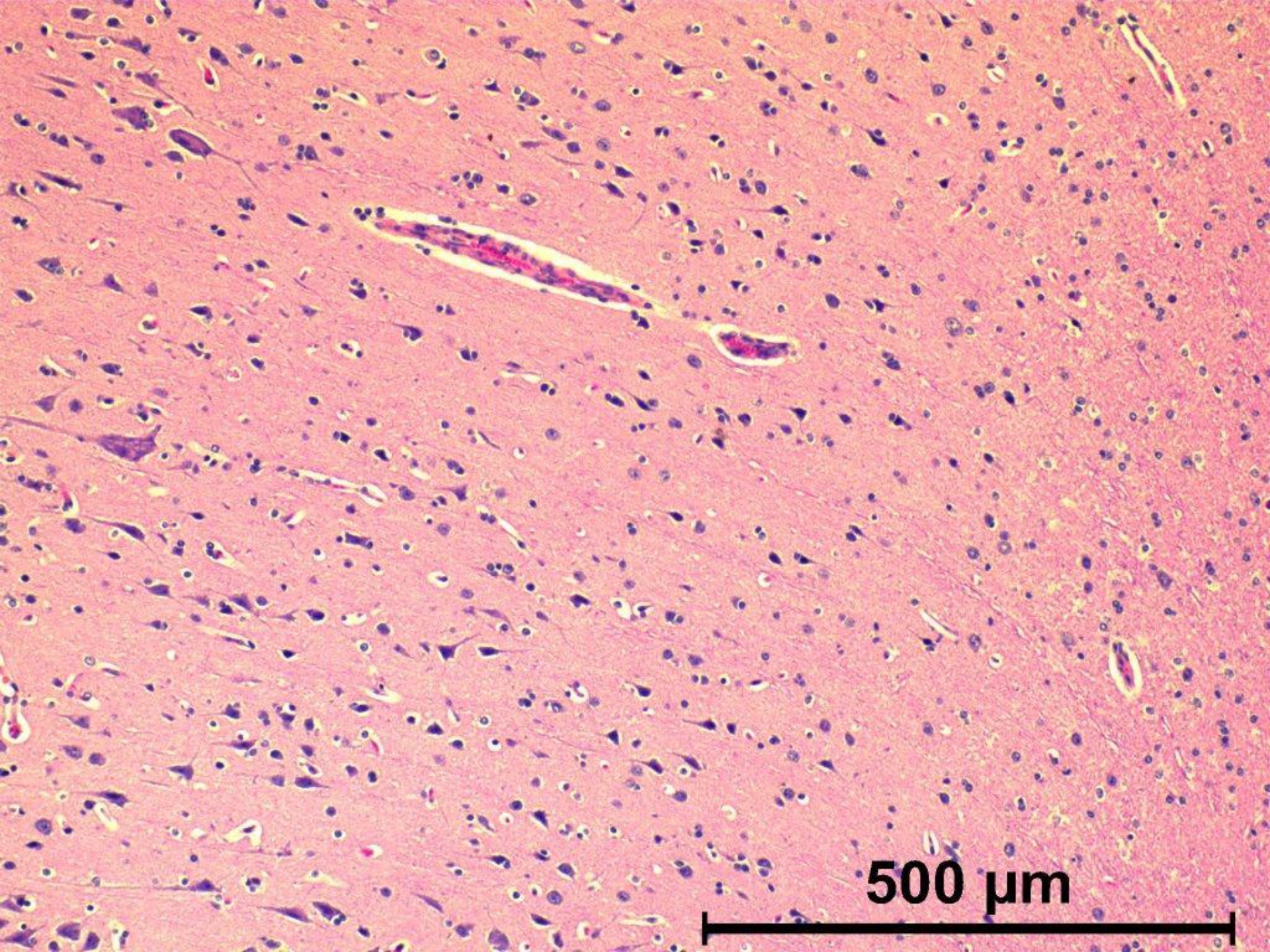
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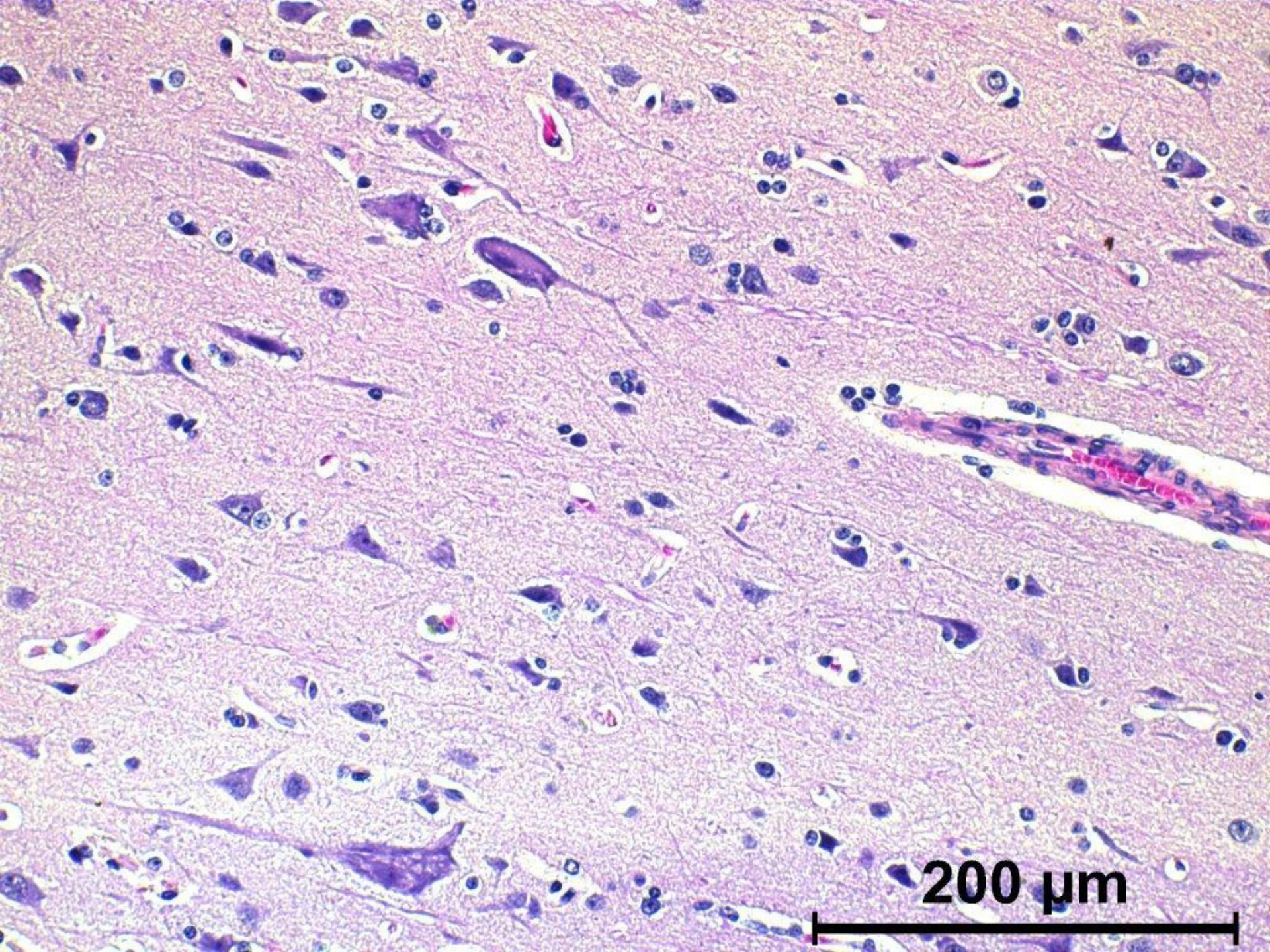
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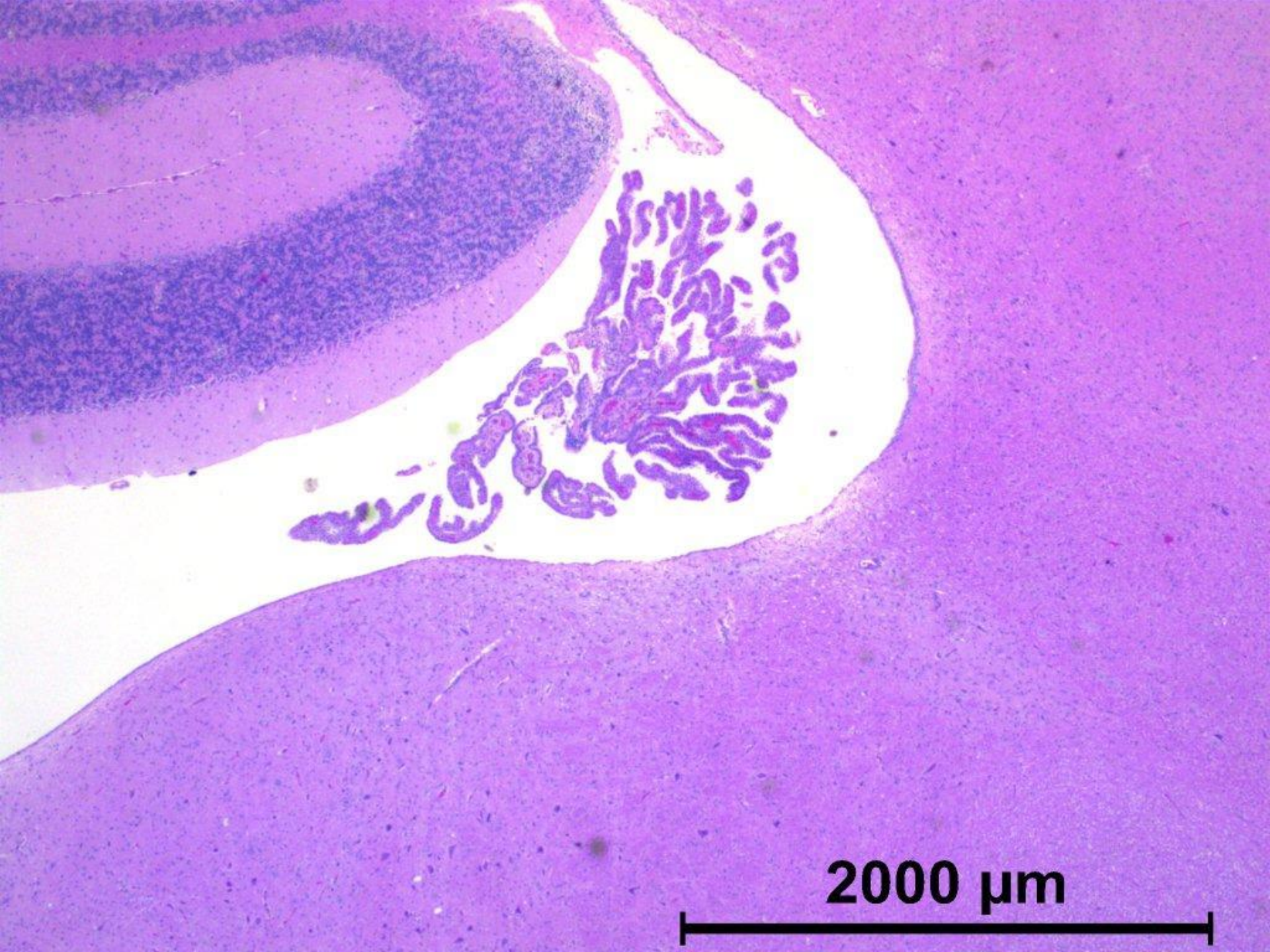
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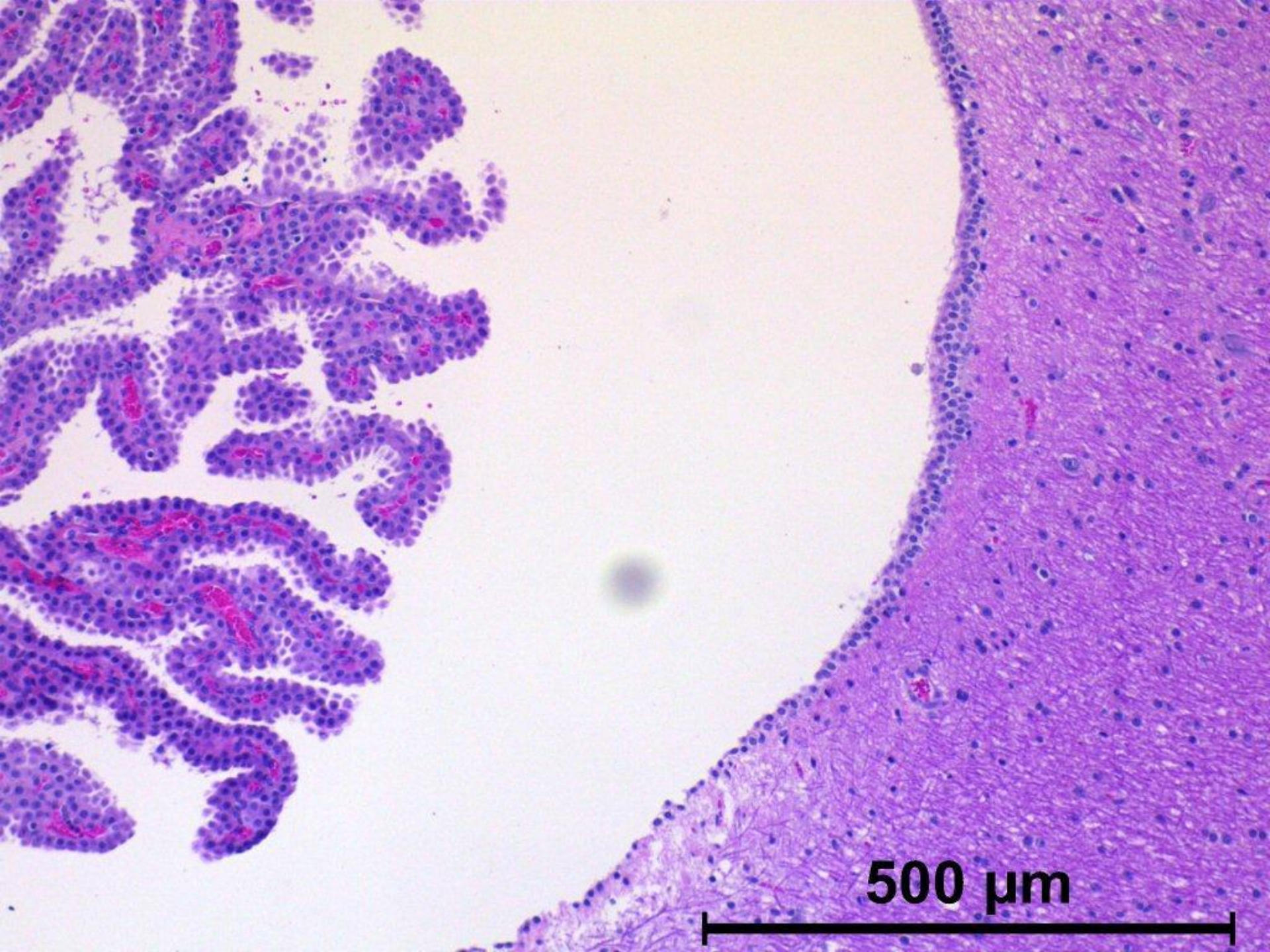
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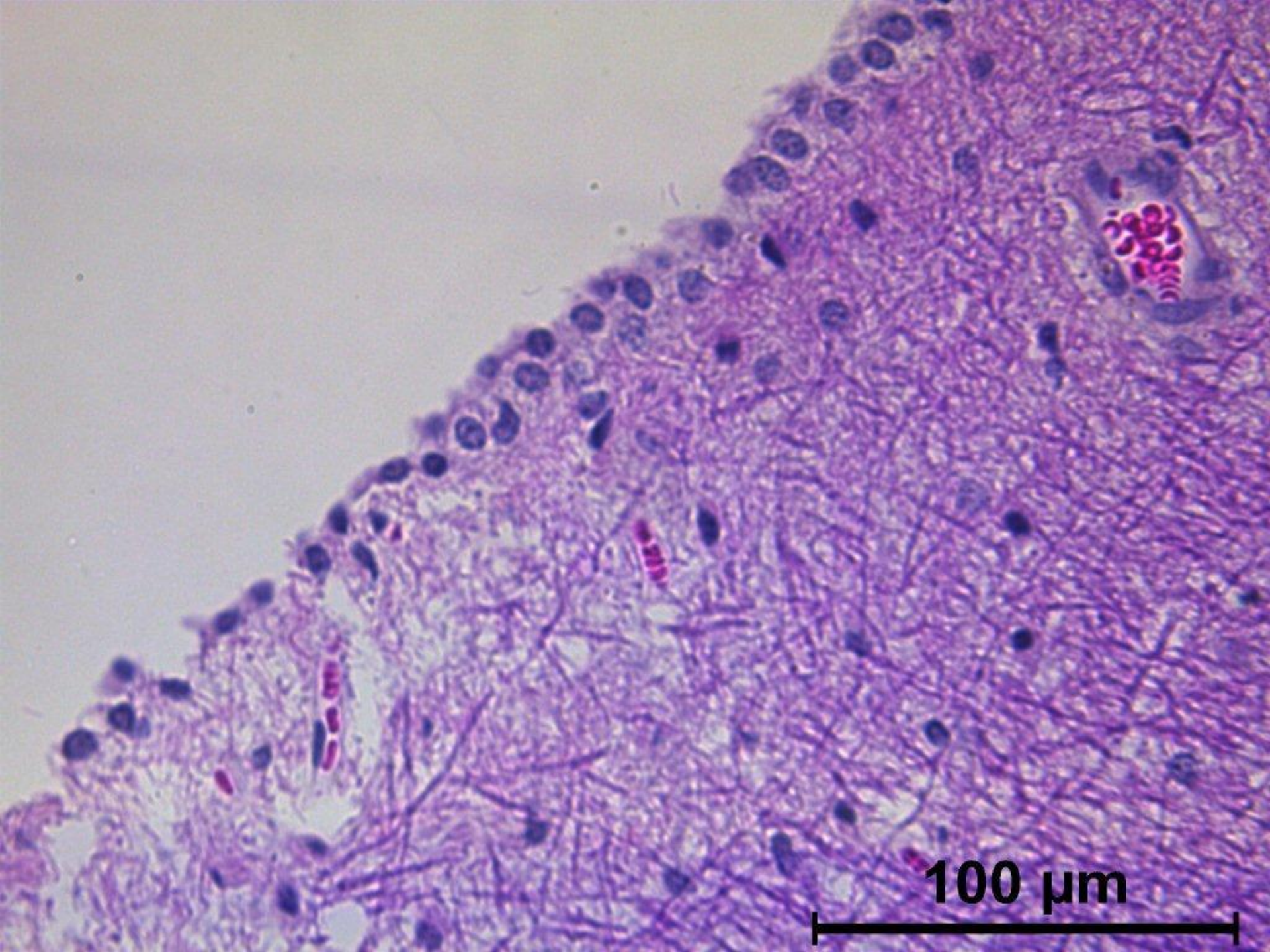
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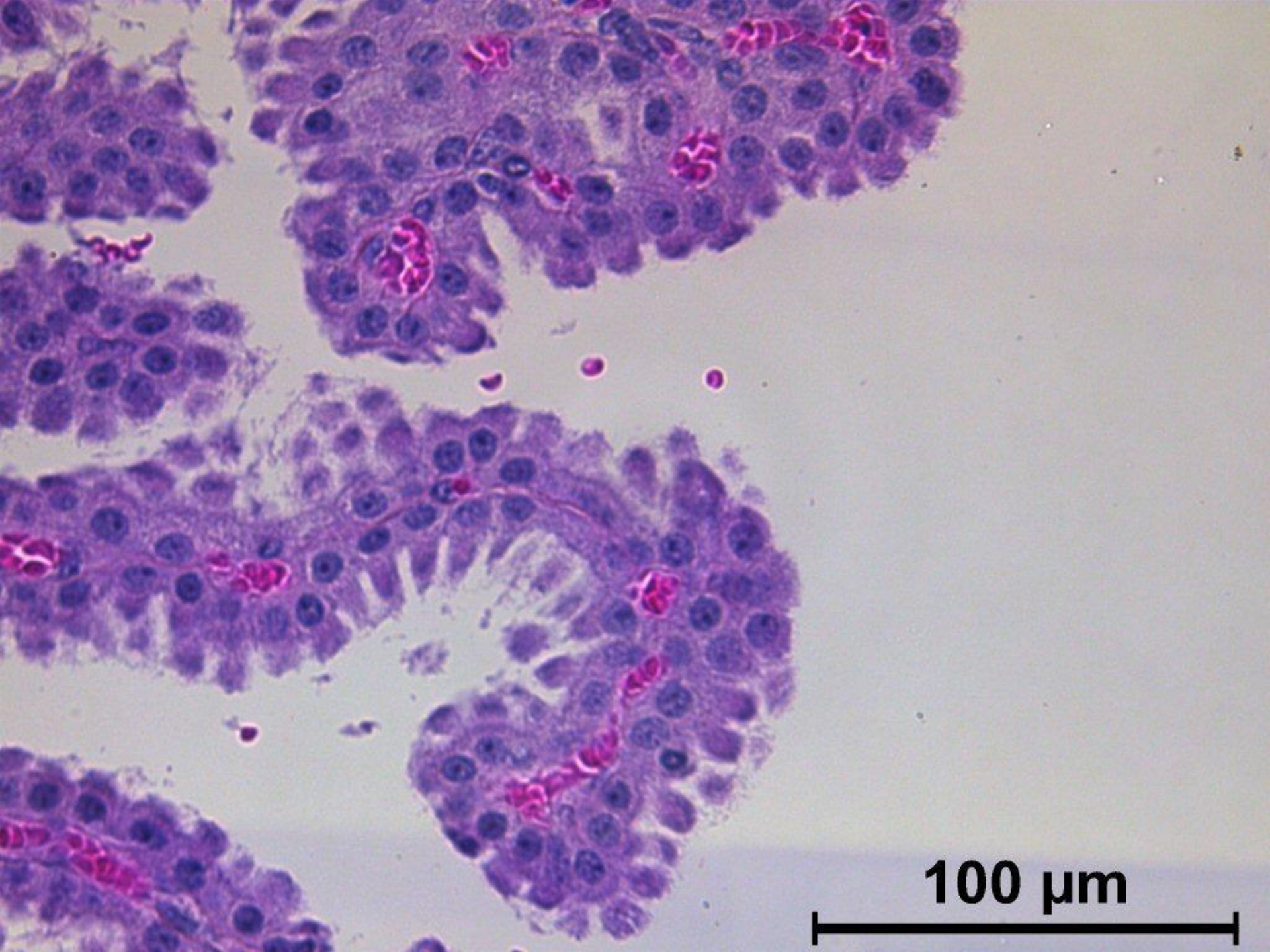
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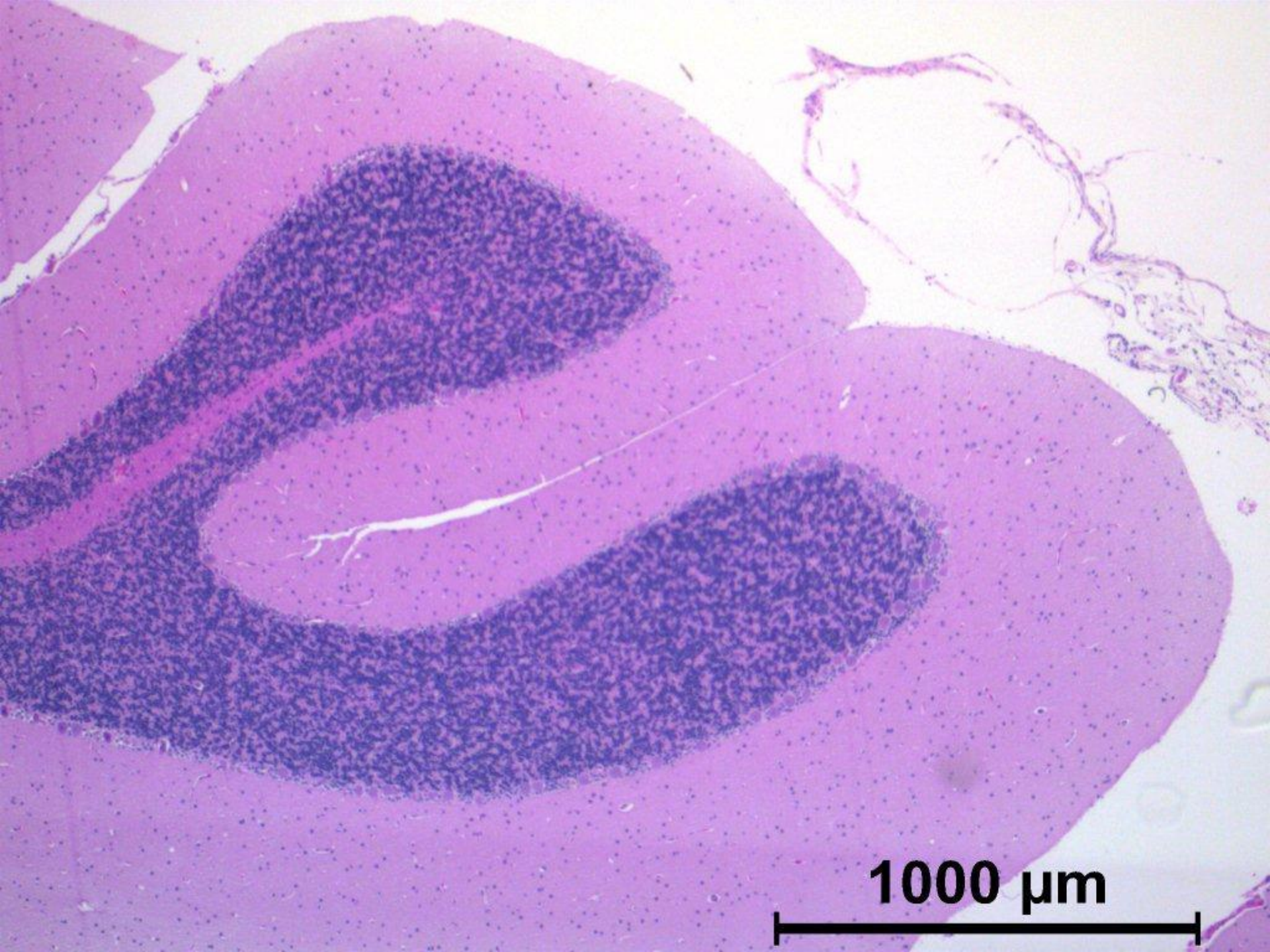
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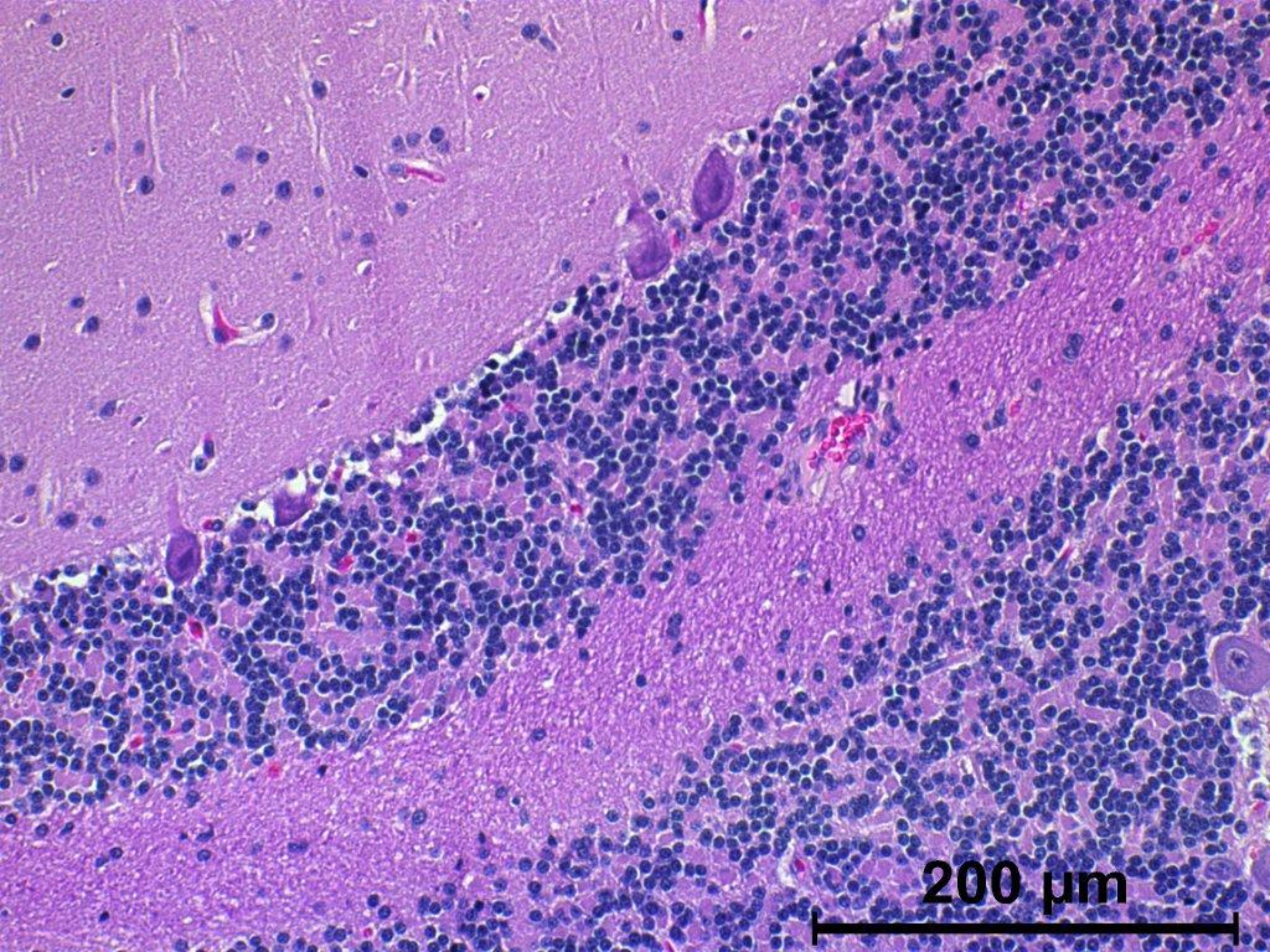
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CNS Normal Histology

- CNS Grey Matter (in cerebrum, cerebellum, brain stem, and spinal cord)
  - Neuronal cell bodies – grey matter only, are of varying shapes and sizes. Can see with light microscopy (lm). With small ones, can only see nuclei, not the cytoplasm or cell borders. Can confuse with astrocytes
  - Neurites (dendrites, axons, and proximal portions of long axons) also only in grey matter and of varying lengths and configurations. Often can see large axons with lm, but not small ones, or dendrites
  - Dendrites, and some axons, not myelinated or poorly myelinated
  - Axon terminals and nerve synapses also only in grey matter. Can not see with lm
  - Neuropil (or neuropile) – areas between neuronal cell bodies that contain unmyelinated axons, dendrites, and glial cell processes

- CNS White Matter (in cerebrum, cerebellum, brain stem, and spinal cord)
  - Contain distal portions of axons that are always myelinated
  - Contain no neuronal cell bodies dendrites, axon terminals, or nerve synapses

CNS Normal Histology – cont.

- Neuroglia (in both grey and white matter)
  - Macroglia
    - Astrocytes – are subtypes

      Fibrous - structural support for the CNS (more in WM)

      Protoplasmic – shorter processes (more in GM)

      Radial (regulate chemical environment for neurons; involved in neuronal migration during corticogenesis)

      Are what cause difference in blood flow as detected by fMRI
    - Oligodendroctyes – have subtypes also (dark, medium, dark)
      - Primarily make myelin
      - Are more in the white matter
      - Myelinate more than one axon (Schwann cells only myelinate one)
  - Microglia
    - Microglia are "resting" macrophages
    - Originate from bone marrow during embryogenesis
    - When activated function like other macrophages
    - When ingest a lot of lipid material are "gitter" cells
    - Are joined by monocytes from the blood
- Ependymal cells
  - Some folks consider them to also be glial cells
  - Are attached to underlying astrocyte foot processes
  - Have tight junctions, cilia, and microvilli
  - Form a barrier similar to the blood brain barrier (BBB)

CNS Normal Histology - cont.

- Above are the CNS Parenchymal Cells (functional cells)

- CNS Stromal Cells (support cells)
  - Are “utilities”, such as capillaries, venules, fibroblasts (few, near surface, no FCT scarring deep)
  - Pericytes – around vessels, contractile and help sustain BBB

CNS Normal Histology - cont.

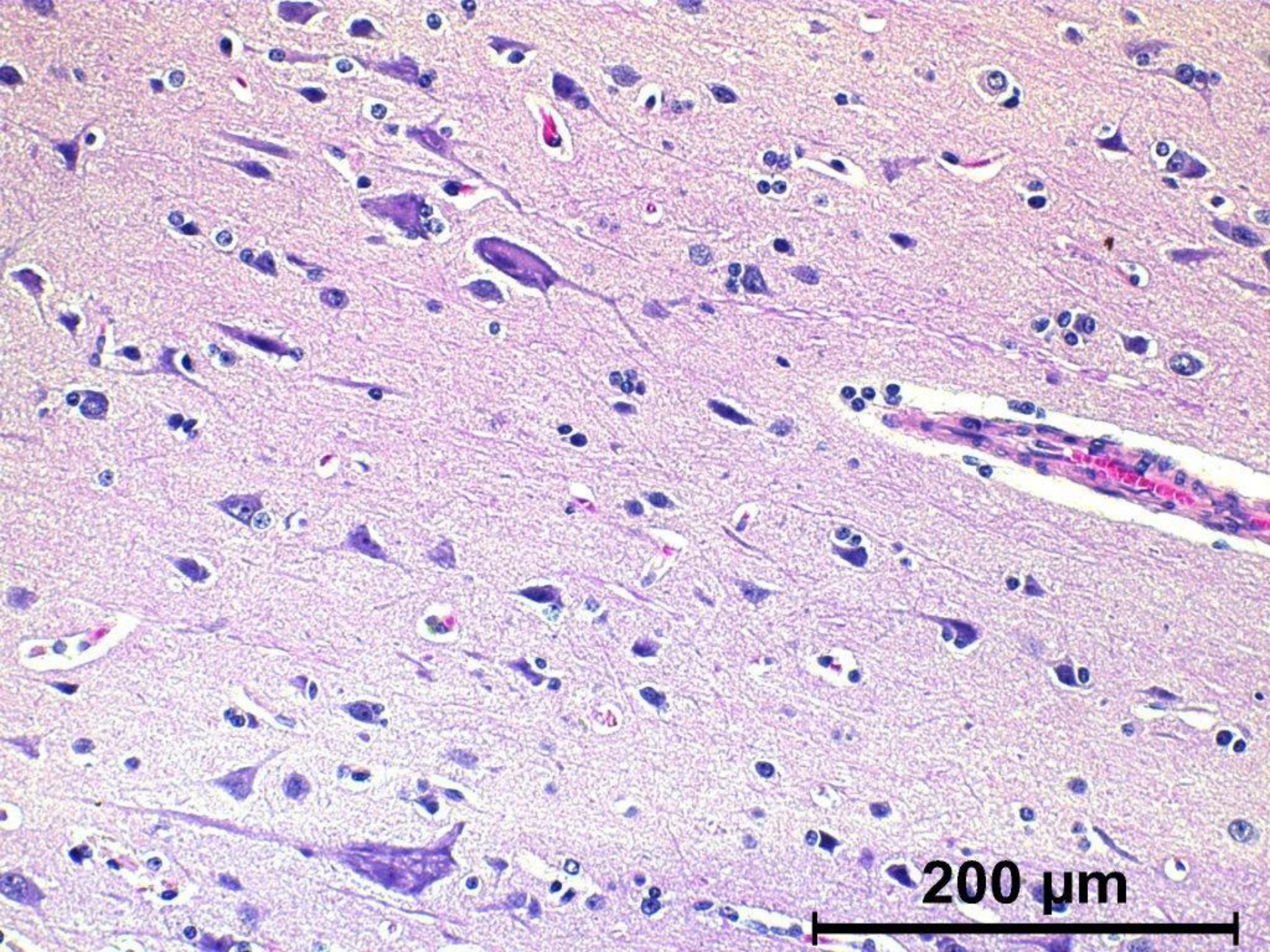
- Bood Brain Barrier (BBB)
  - Very important
  - Has 4 components
    - Endothelial cells – have tight junctions and reduced vesicular transport
    - Extracellular matrix – basement membrane
    - Astrocyte foot processes
    - Pericytes – are contractile
  - There also are other barriers

CNS Normal Histology - cont.

- A special cell not described in the literature is the skipocyte. It is present in normal tissue, but is very common when there are pathological changes.
- There are many kinds of normal for a pathologist, especially histologically
  - Textbook normal
  - Reality normal
  - Abnormal normal
  - Postmortem normal

CNS Normal Histology - cont.

- A pathologist wants good quality slides that best represent the tissues' histomorphological appearance.
- A pathologist wants to see if the all the cells that are supposed to be there are present, that they appear normal, and that there are no other cells or any kind of cell products or foreign material that shouldn't be there.
- A pathologist mostly identifies cells by nuclear size and shape.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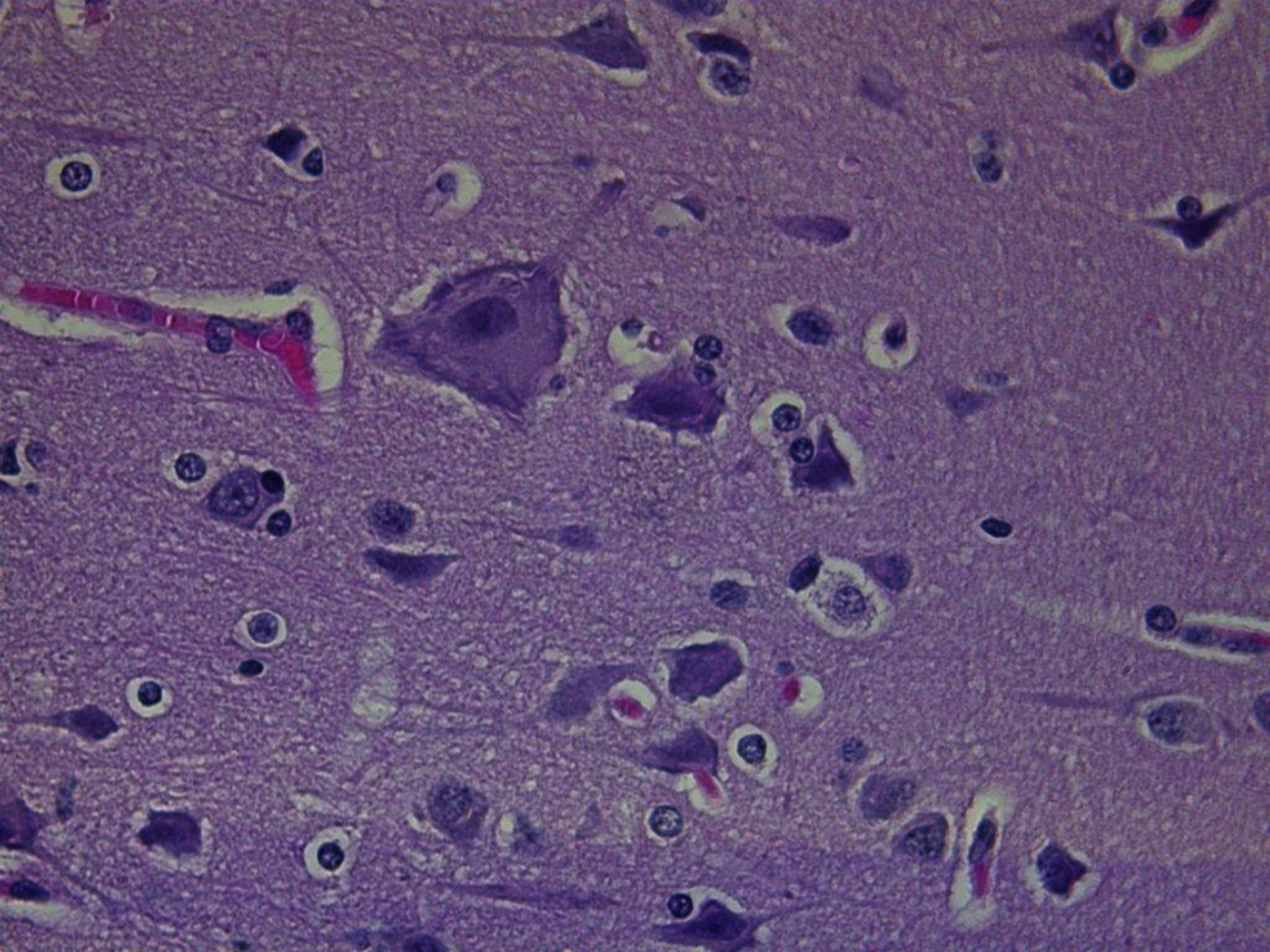

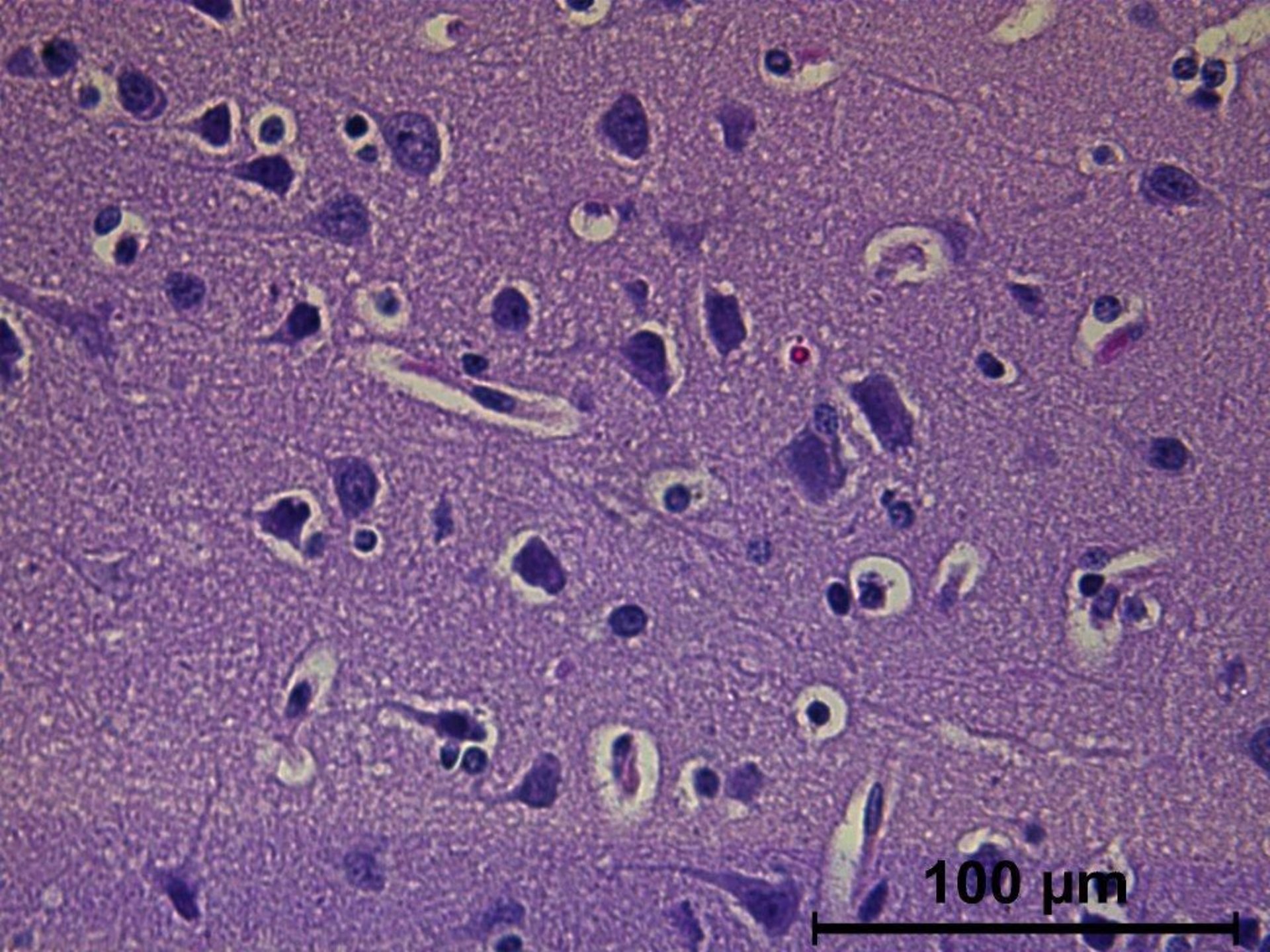
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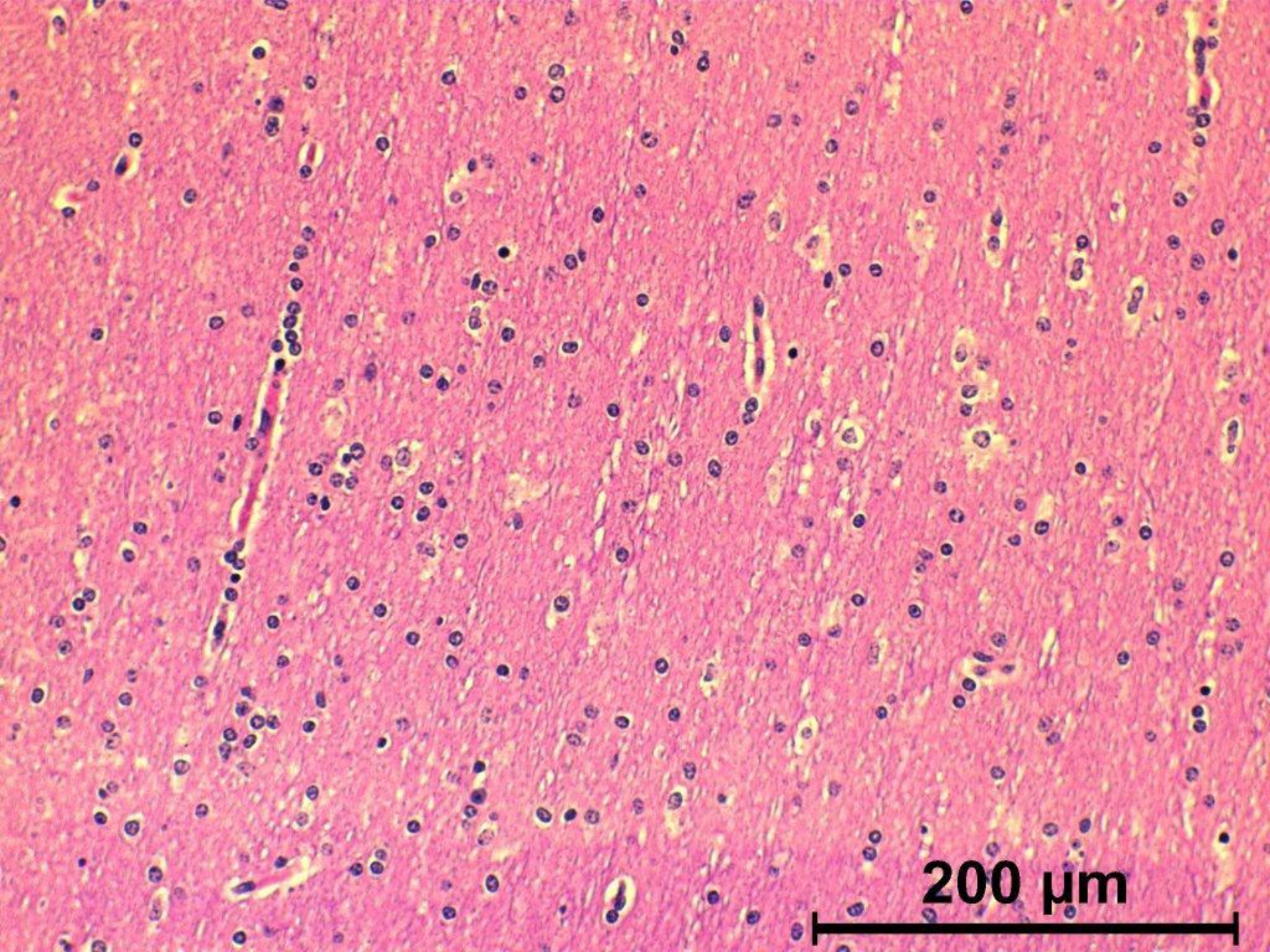
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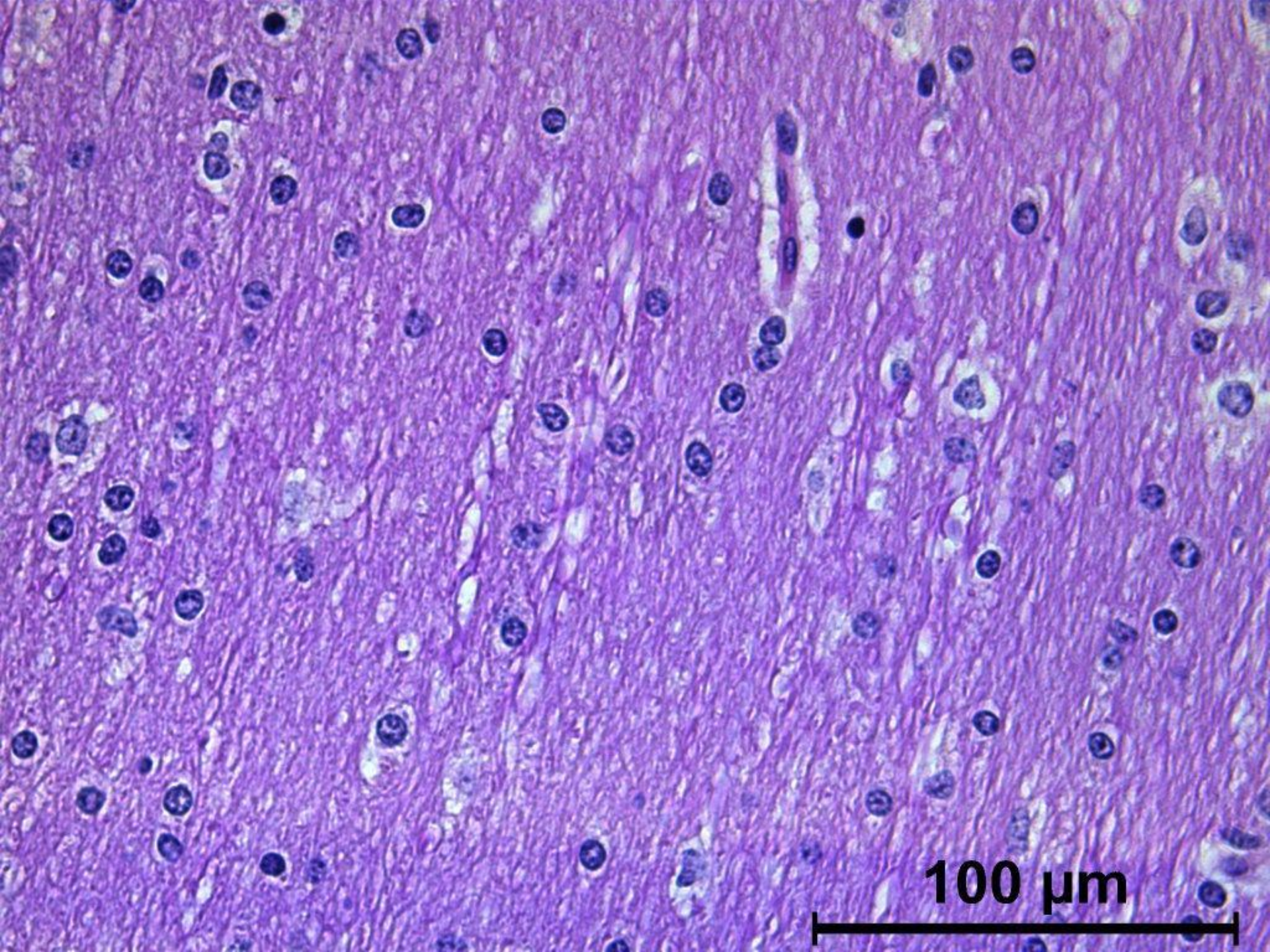
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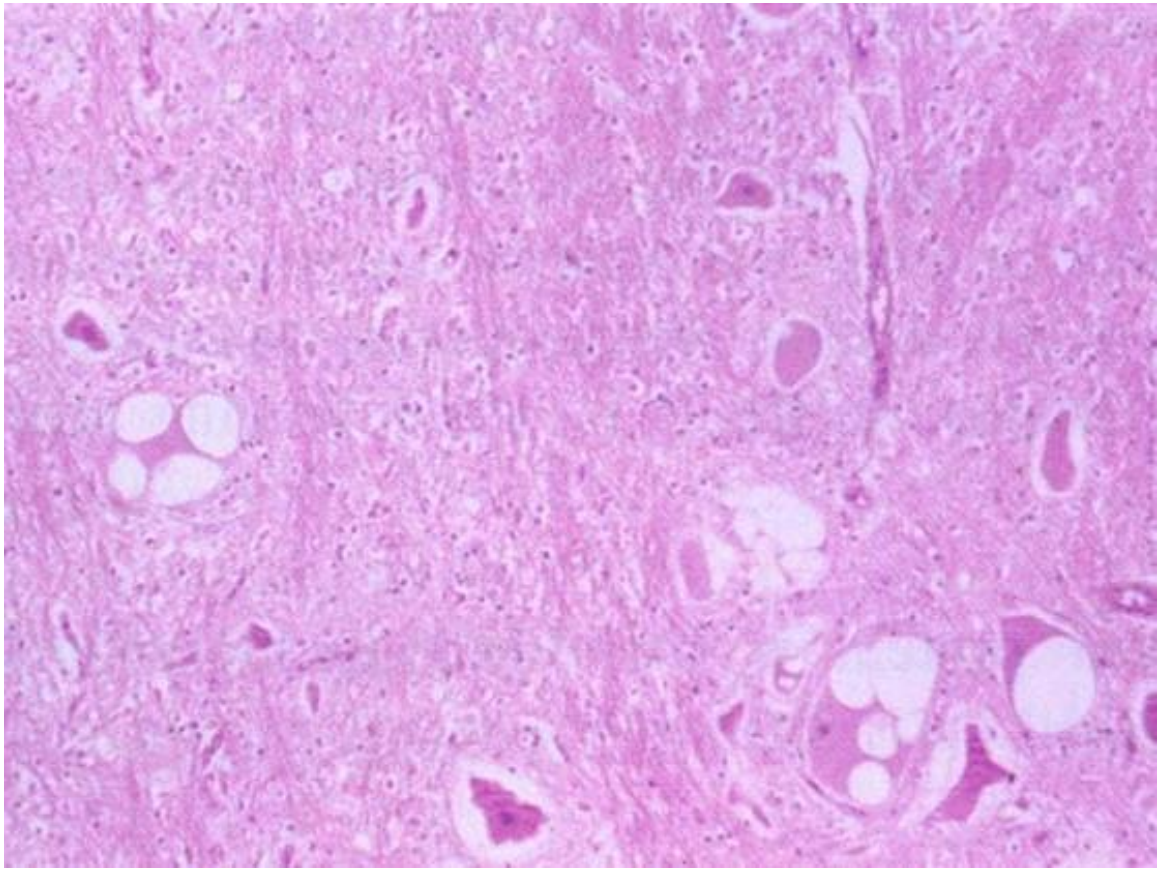
CNS Histologic Changes

- Vacuolation of grey and/or white matter
  - In soma of neurons is classic “spongiform encephalopathy”
  - “Status spongiosis” or spongiform change
  - May be demyelination (common and important)
    - Hopefully see macrophages in holes
    - Maybe see dystrophic (swollen) axons in holes
  - Can also confuse with degeneration of any cells
    - Cell degeneration is manifested as cytoplasmic and/or nuclear swelling and vacuolation
  - Must make sure can correctly differentiate from either

- Dark (Red) neurons
  - Inexperienced pathologists think these are necrotic cells
    - Necrotic cells – have karyolysis, pyknosis, or karyorrhexis of nuclei and usually eosinophilic cytoplasm

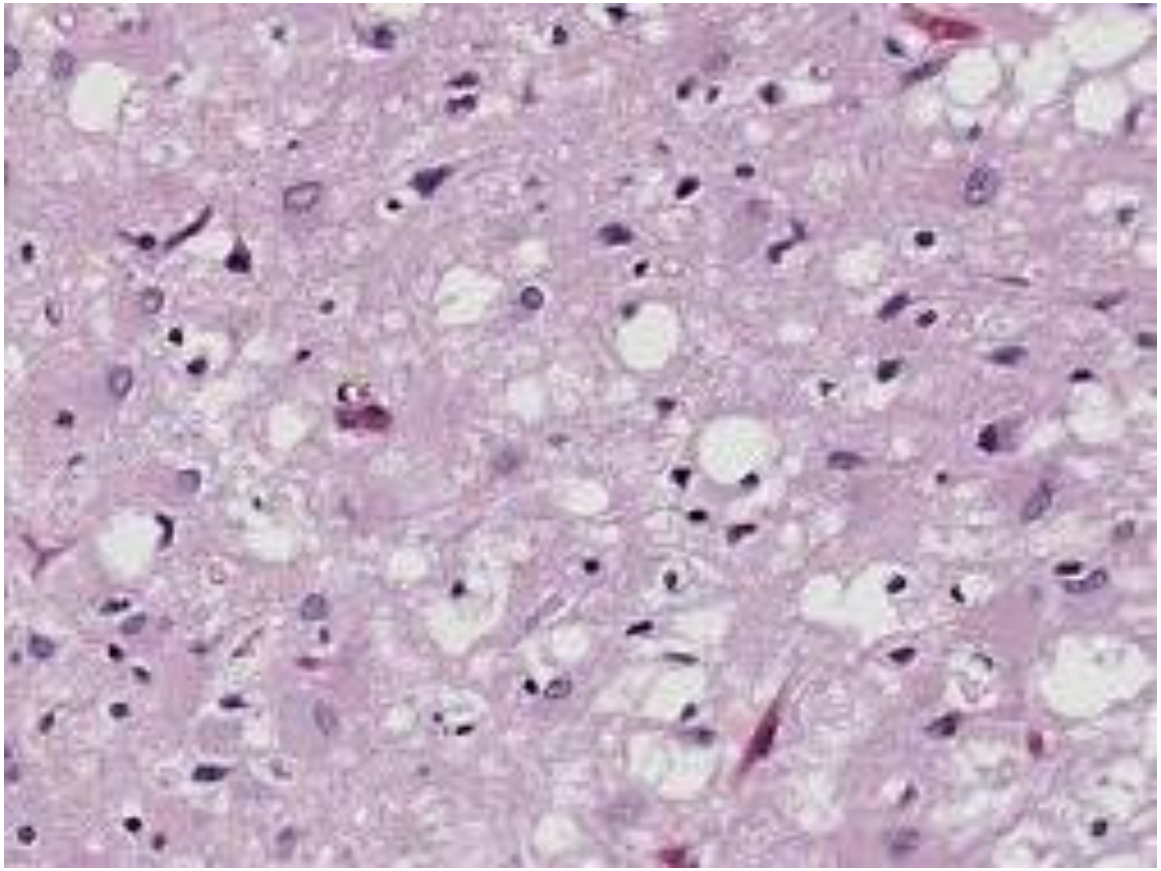
CNS Histology Artifacts

- Vacuolation of grey and white matter
  - "Status spongiosis" or spongiform change
  - Can confuse with demyelination
  - Can also confuse with degeneration of any cells
    - Cell degeneration is manifested as cytoplasmic and/or nuclear swelling and vacuolation
  - Must make sure can correctly differentiate from either
  - Can be differentiated from "spongiform encephalopathy"
- Dark (Red) neurons
  - Inexperienced pathologists think these are necrotic cells
    - Necrotic cells – have karyolysis, pyknosis, or karyorrhexis of nuclei and usually eosinophilic cytoplasm



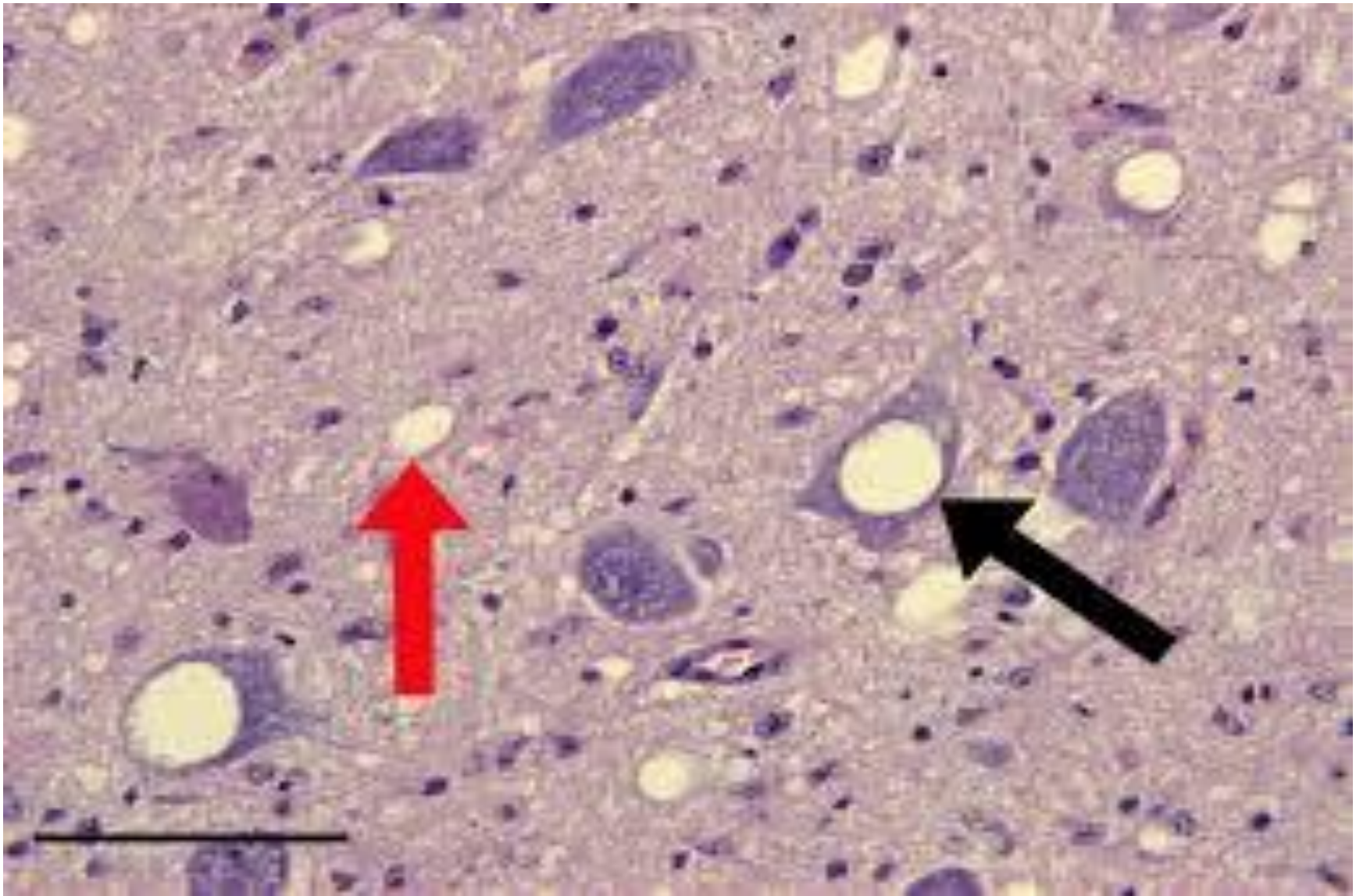
Bovine Spongiform Encephalopathy

Courtesy Veepro Holland



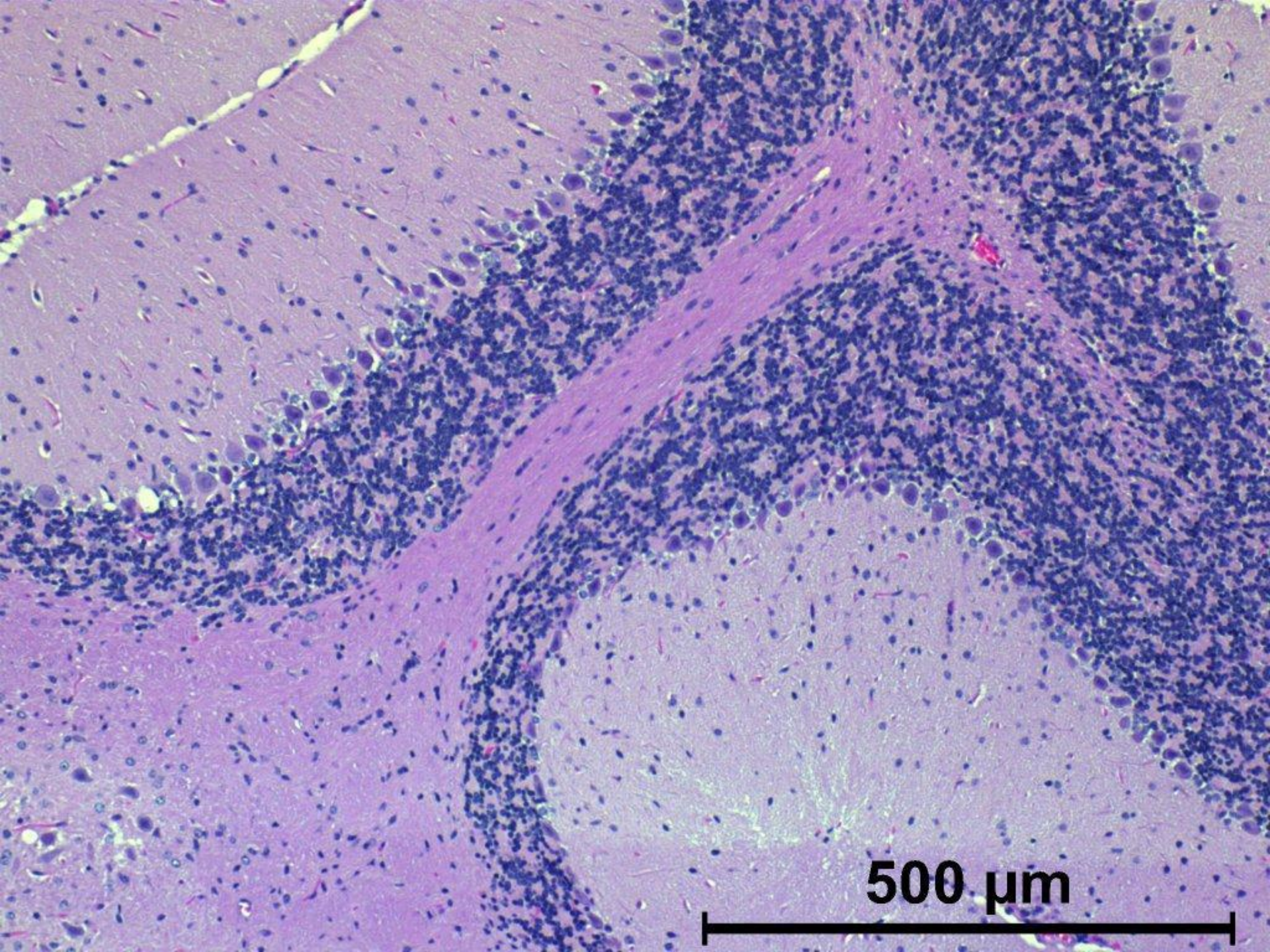
Creuztfeldt-Jakob Disease

Courtesy Encyclopedia Britannica



Demyelination

- Of major concern
- Must make sure it is real and not artifact
- Most common in white matter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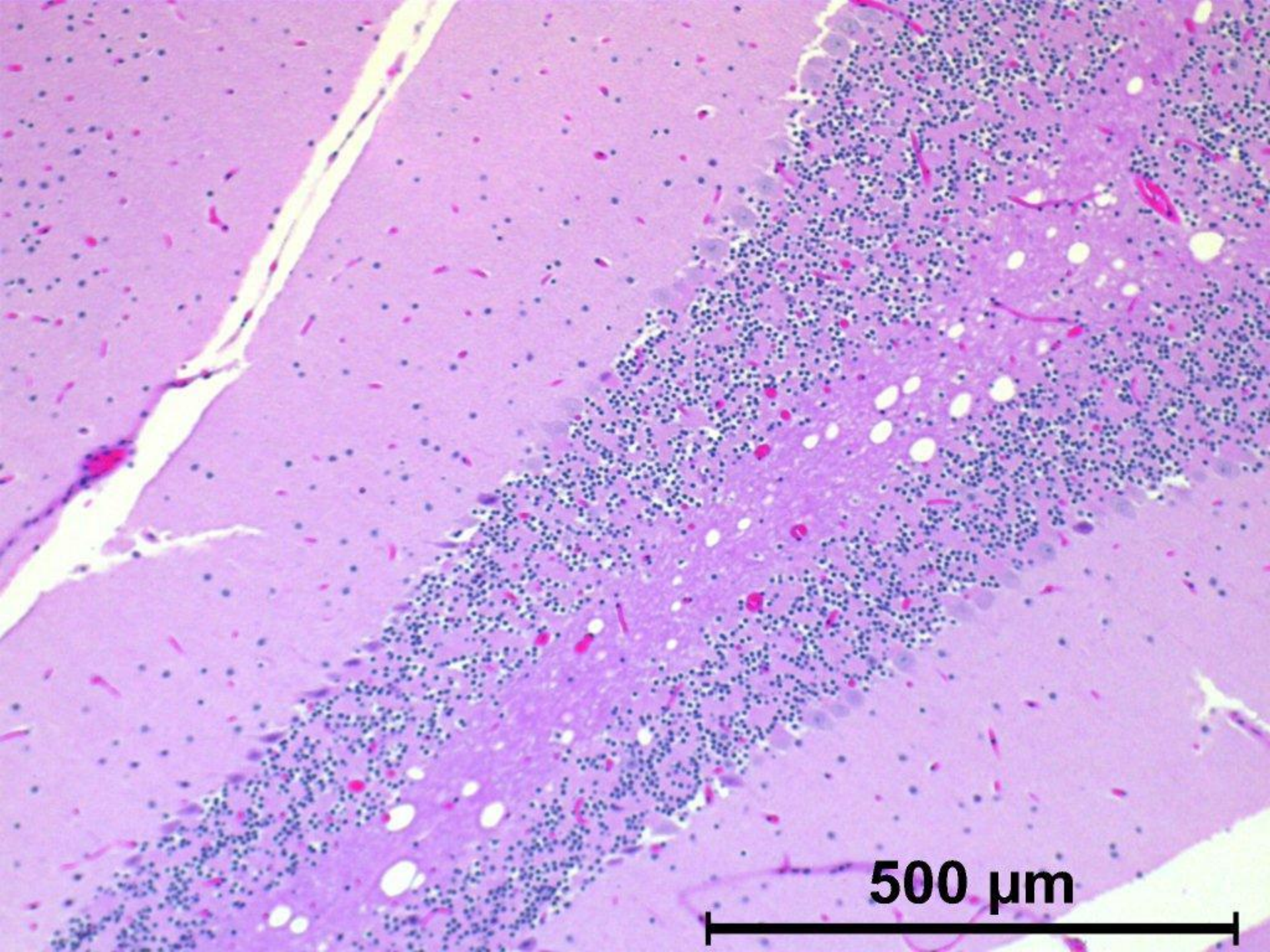
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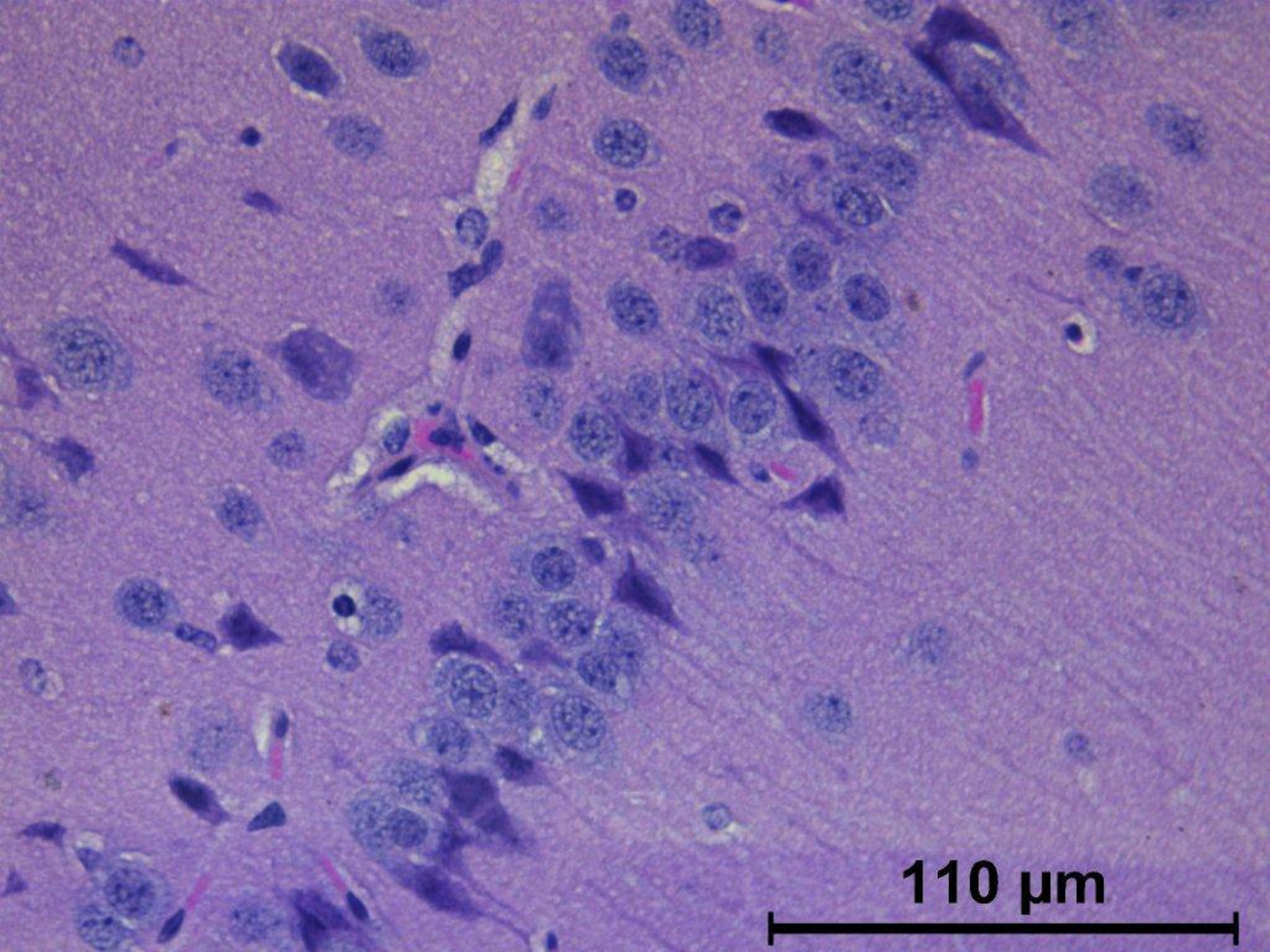
Dark (Red) Neuronal Cell Bodies

- Thought to be due to pressure or trauma before fixation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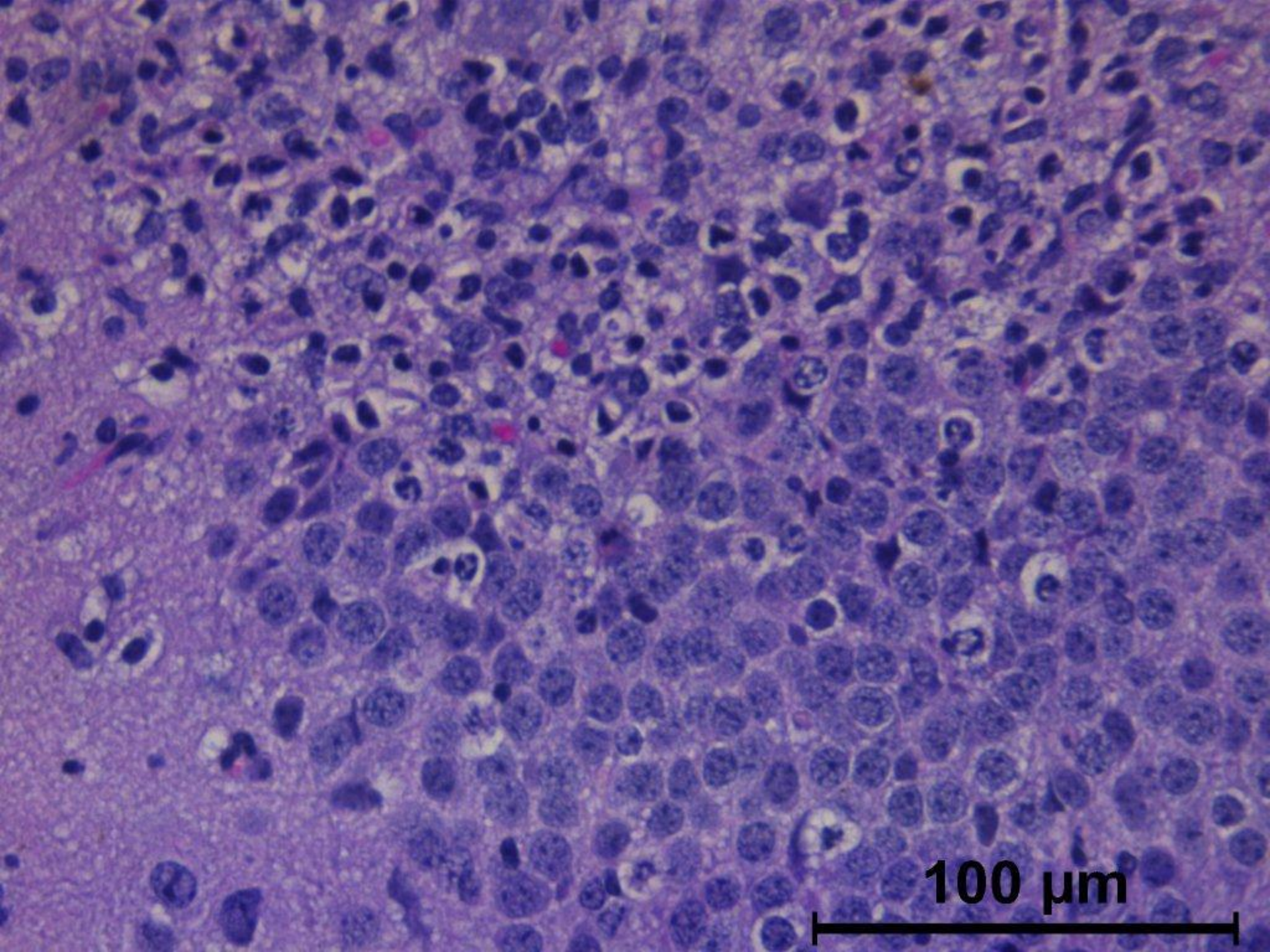
Necrosis of cells

- Called malacia when in brain
- Cell degeneration may proceed it
- Macrophages must phagocytose the debris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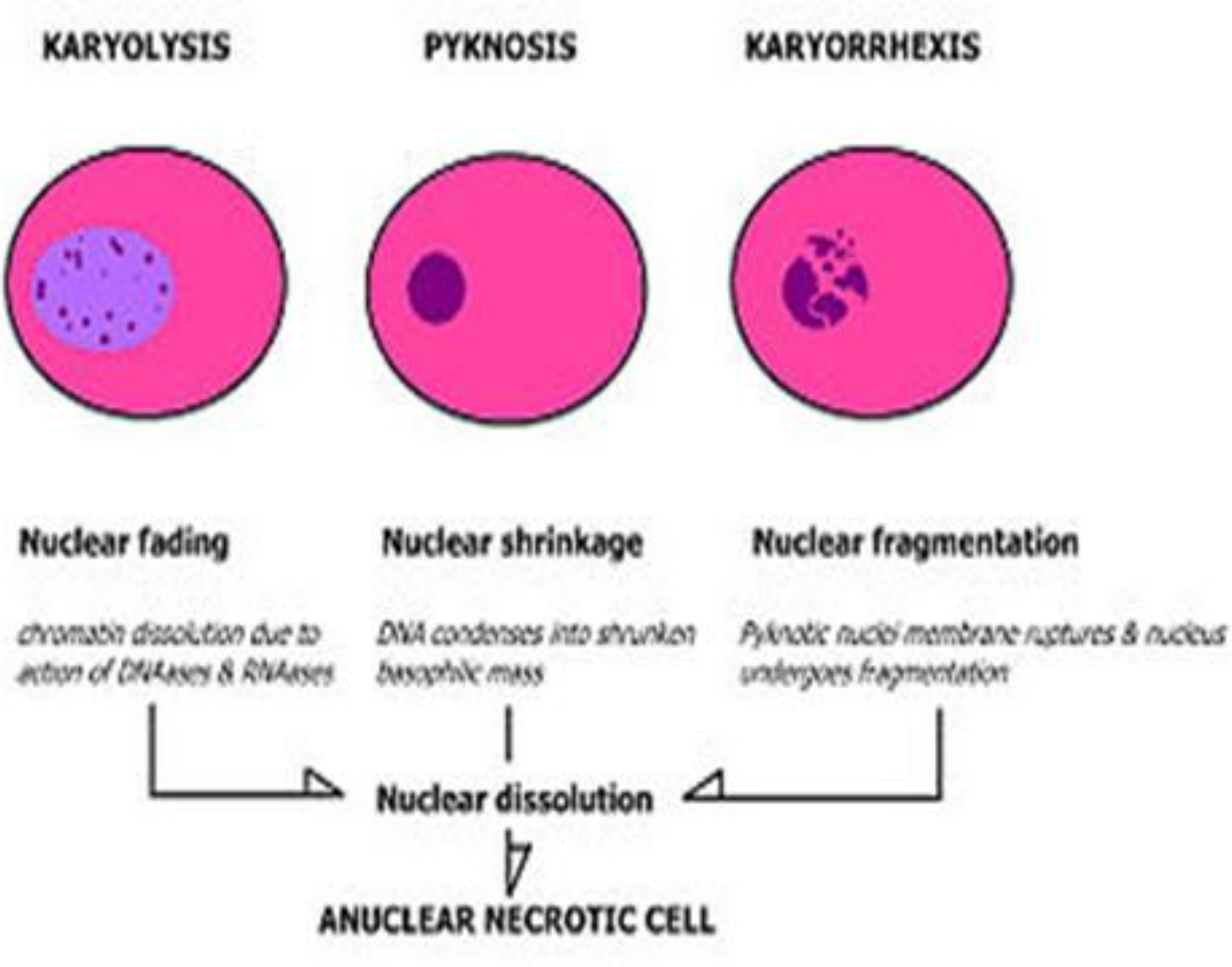

KARYOLYSIS
PYKNOSIS
KARYORRHEXIS
Nuclear fading
chromatin dissolution due to action of DNAases & RNAases
Nuclear shrinkage
DNA condenses into shrunken basophilic mass
Nuclear fragmentation
Pyknotic nuclei membrane ruptures & nucleus undergoes fragmentation
Nuclear dissolution
ANUCLEAR NECROTIC CELL

Cell necrosis

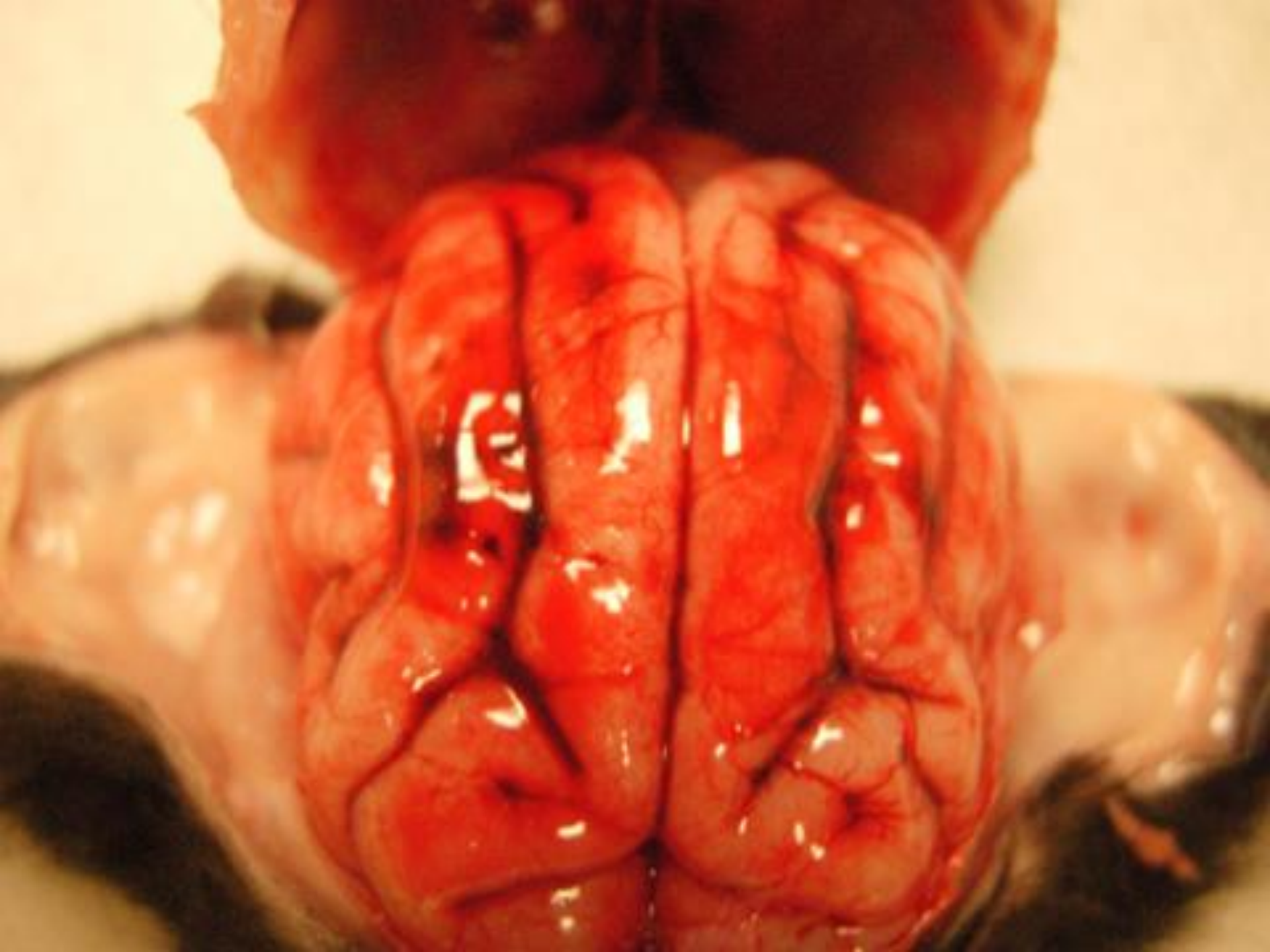
- There first may be degeneration, but it can happen so fast that there may be little, if any, degeneration (when this happens it is called coagulation necrosis)
- The cytoplasm has a reddish color (increased eosinophilia)
- The nucleus undergoes one of three changes
  - Pyknosis is when the nucleus condenses into one smaller homogeneous "dot" (also with apoptosis)
  - Karyorrhexis also is when the nucleus condenses, but it breaks up into pieces such as smaller homogeneous "dots"
  - Karyolysis is when the nucleus (and especially nuclear envelope) fades away

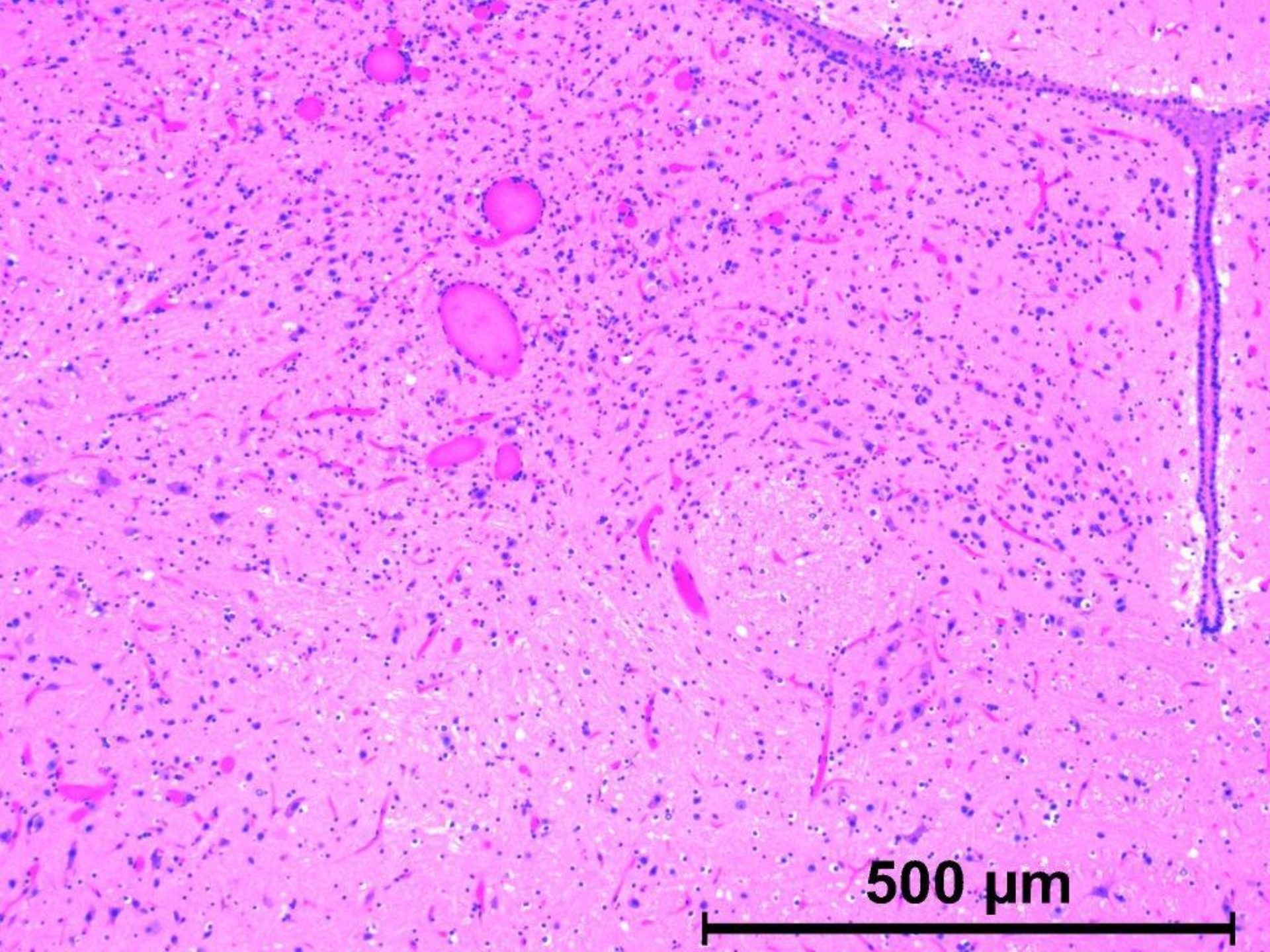
CNS Histopathological Changes

- Congestion
- Perivascular cuffing
- Glial nodule
- Glial nodule with necrosis
- Gliosis – diffuse
- Demyelination with axonal swelling using Luxol Fast Blue stain with Bodian silver impregnation technique
- Spongiosis
- Gitter cell (from German Gitterzelle)

Congestion

- Only at capillary level
- There is reserve capillary capacity in many organs and tissues of the body
- Determine if pathologic or not
- Non-pathologic is usually postmortem change
- Pathologic is hemodynamic or inflammatory

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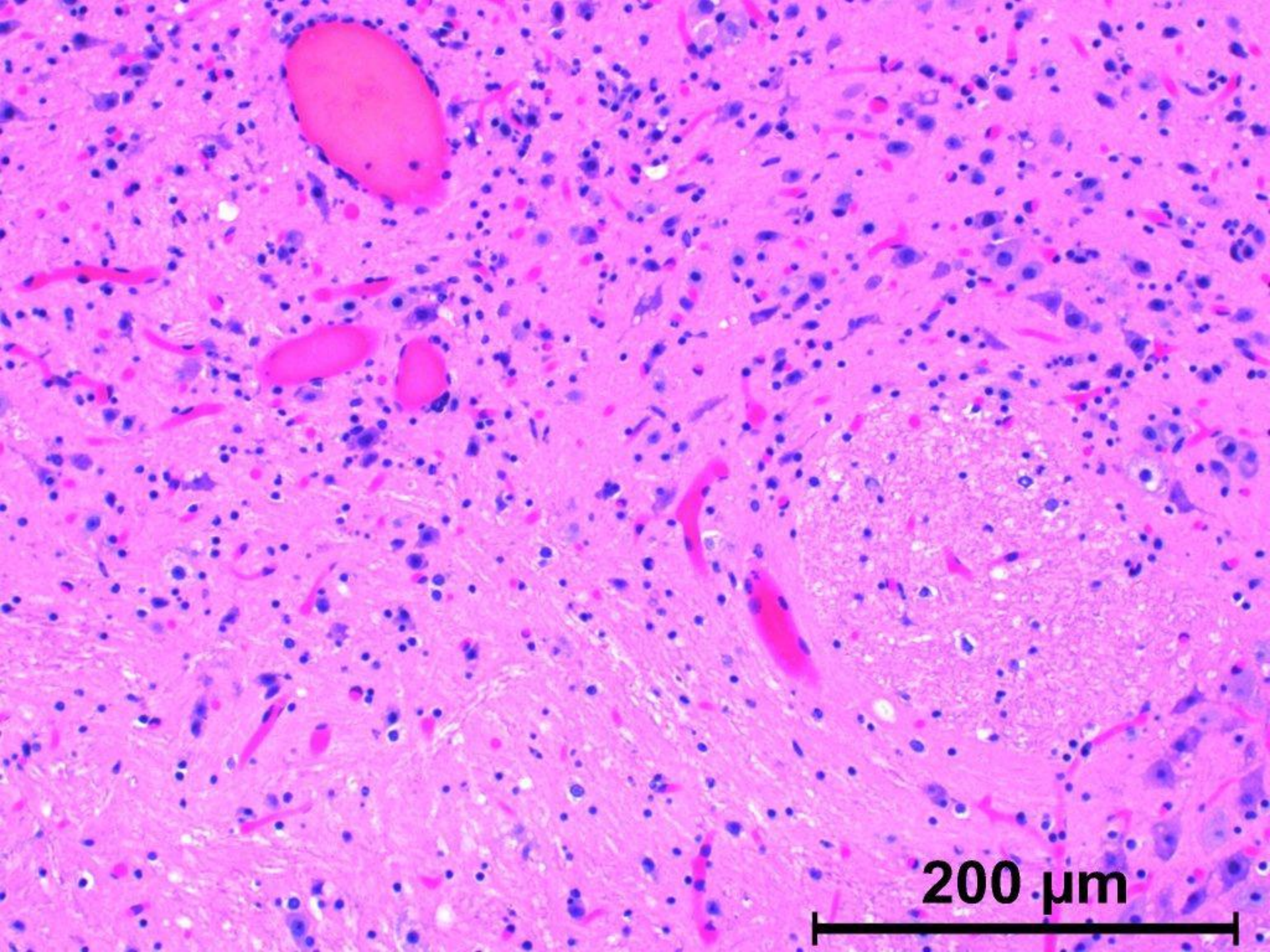
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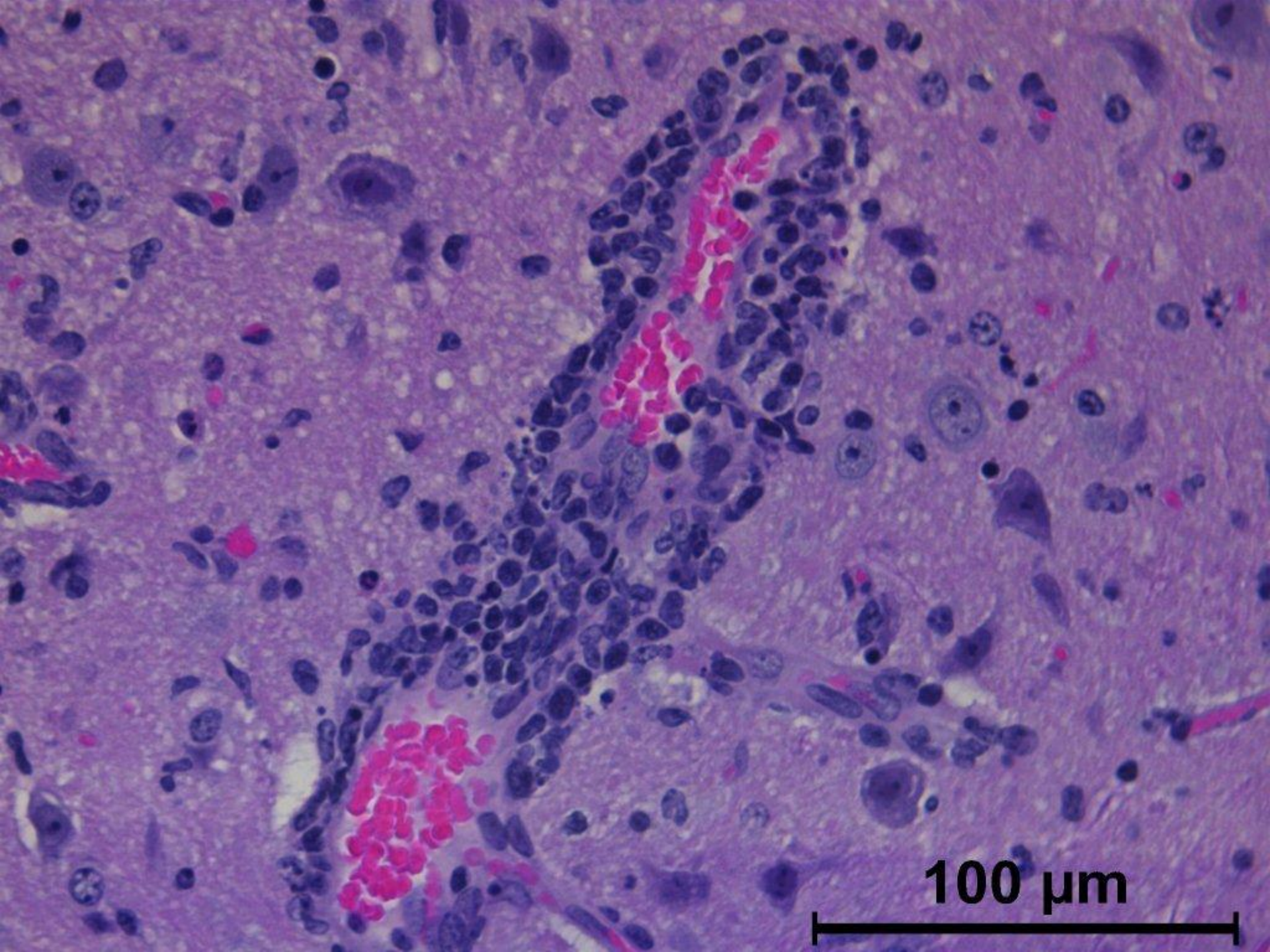
Perivascular cuffing

- Very common change
- Indicates an immunological reaction
- Should not call perivascular encephalitis, or perivascular inflammation.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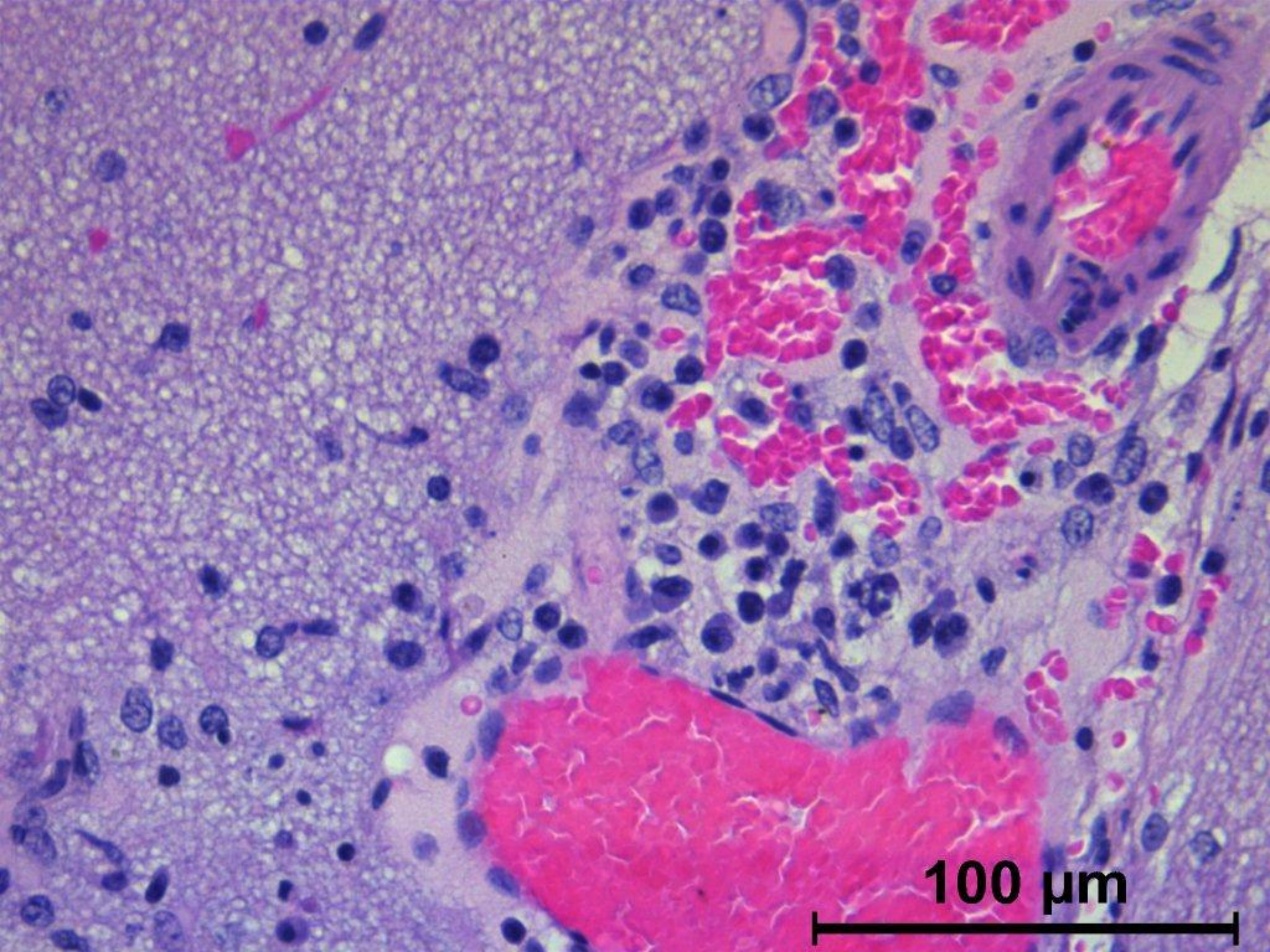
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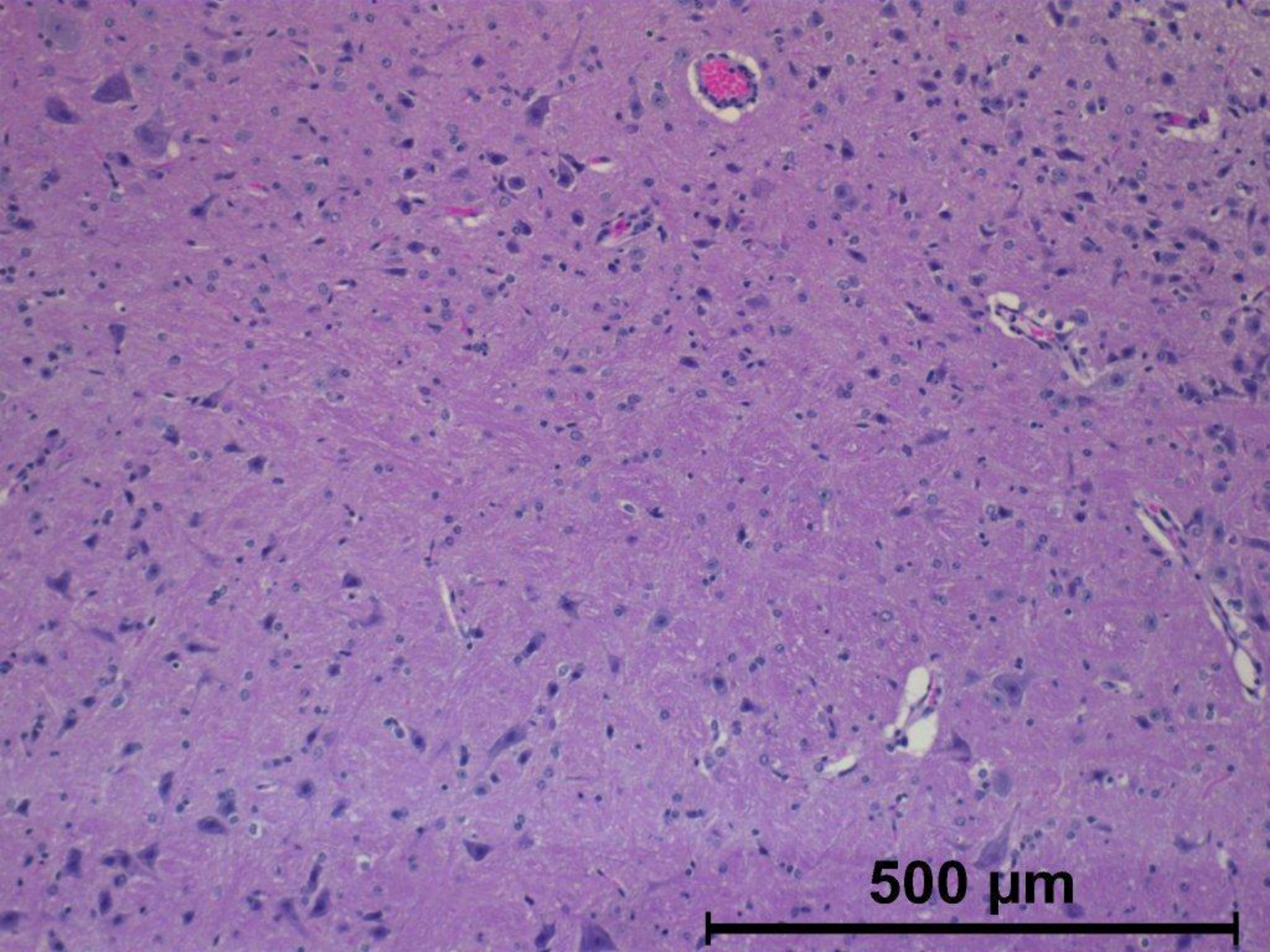
Gliosis

- Can be microgliosis or macrogliosis
- Astrocytes often called astrocytic scarring
- Is diffuse, while glial nodules are diffuse and poorly defined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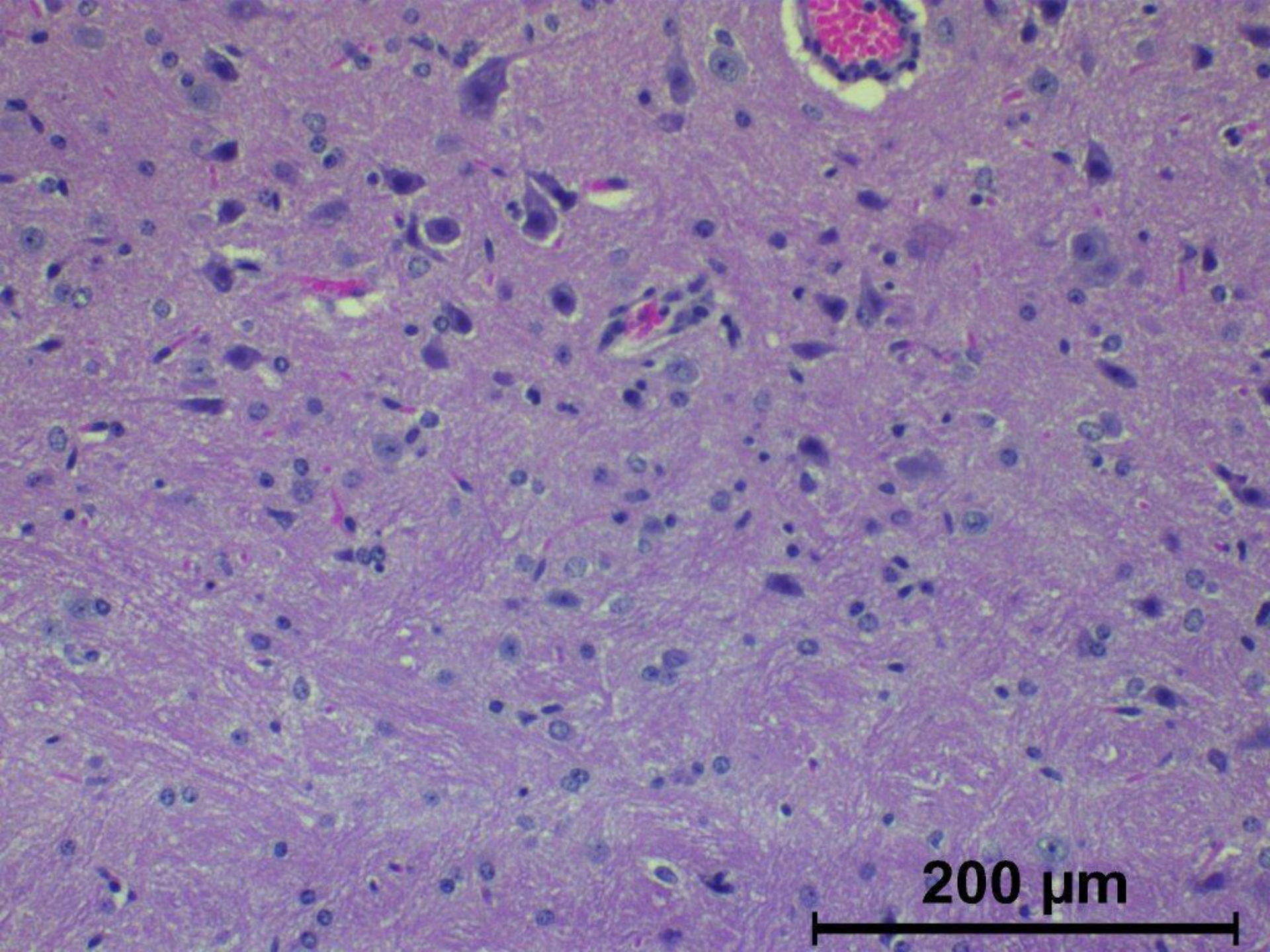
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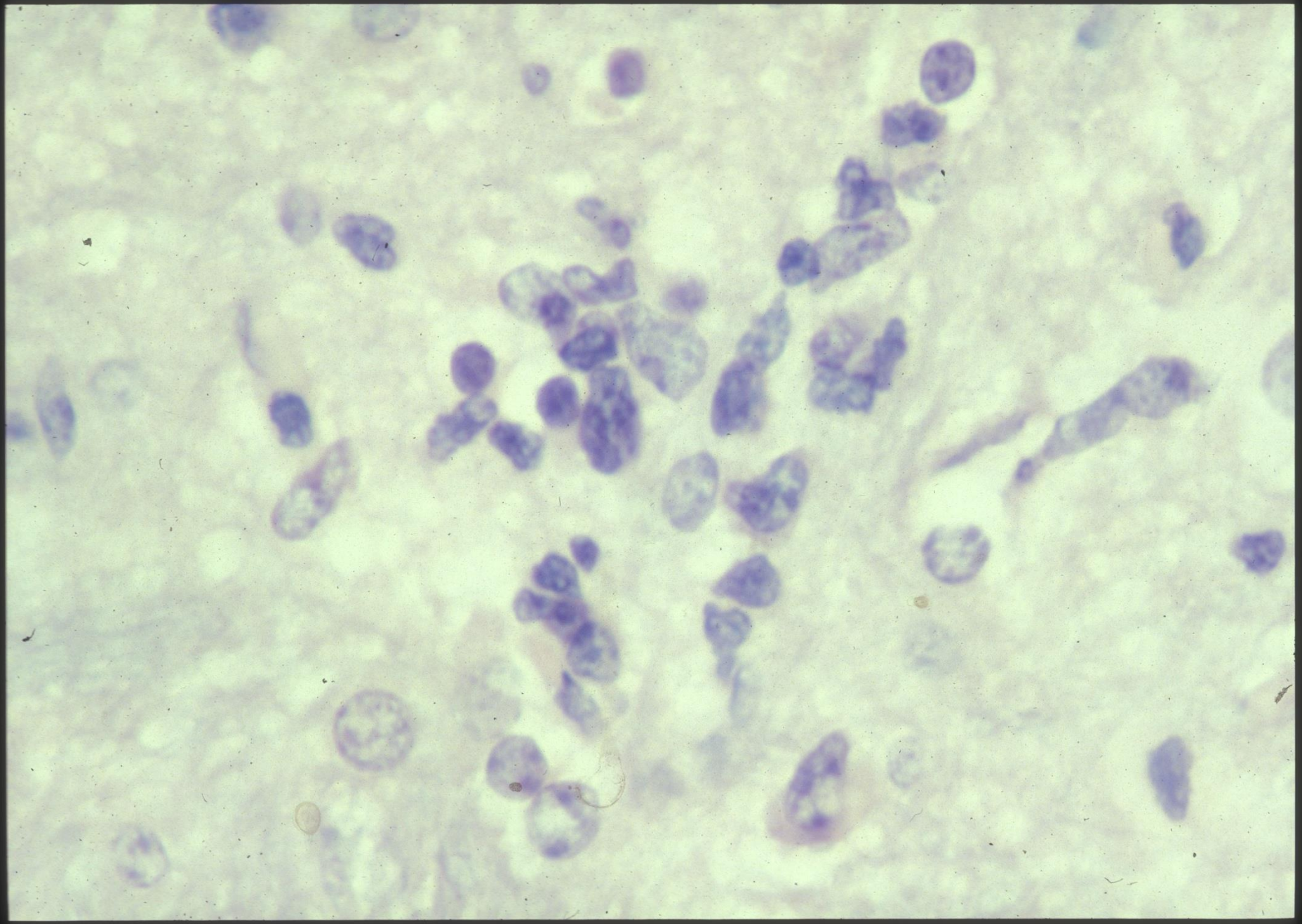

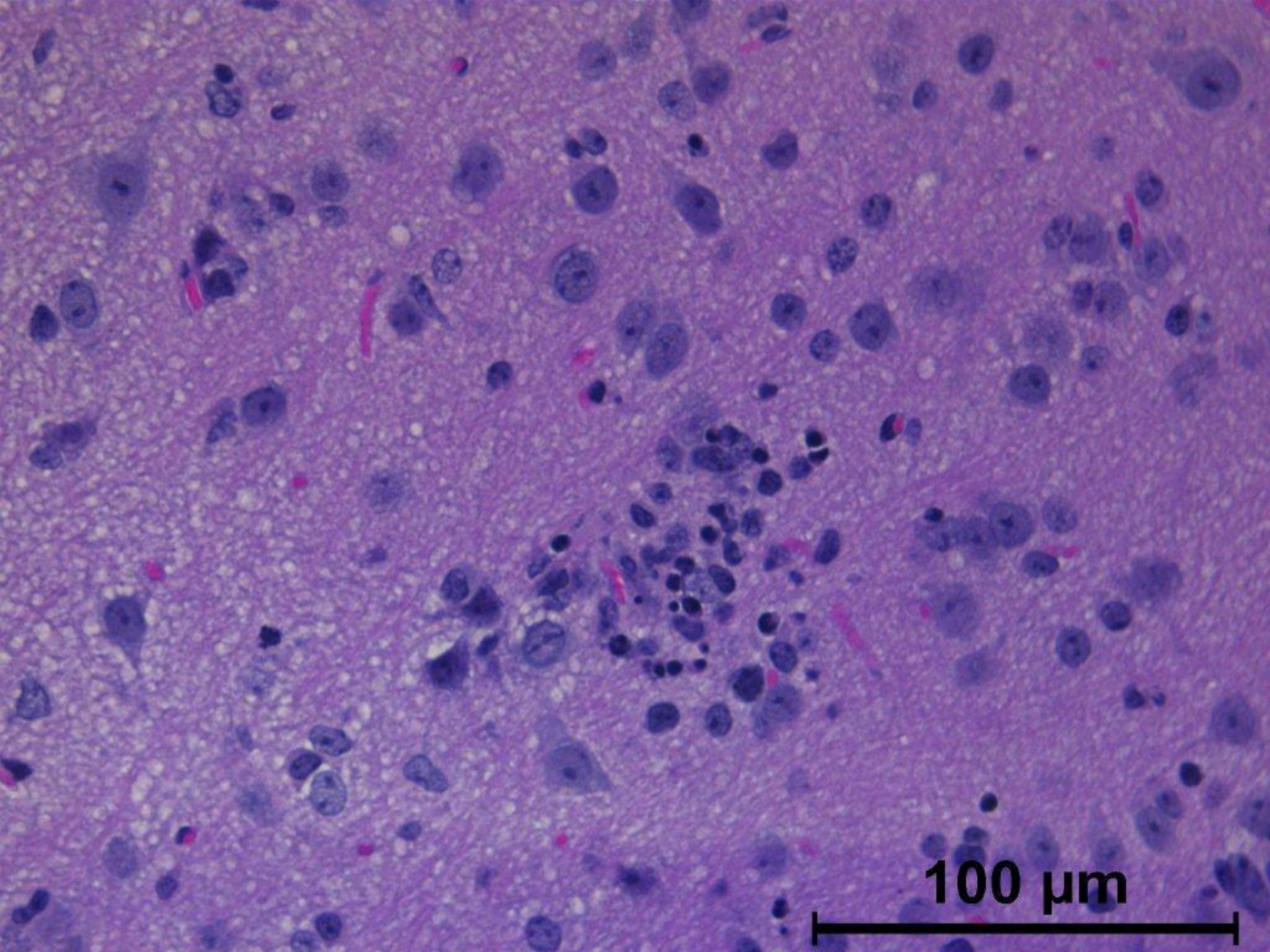
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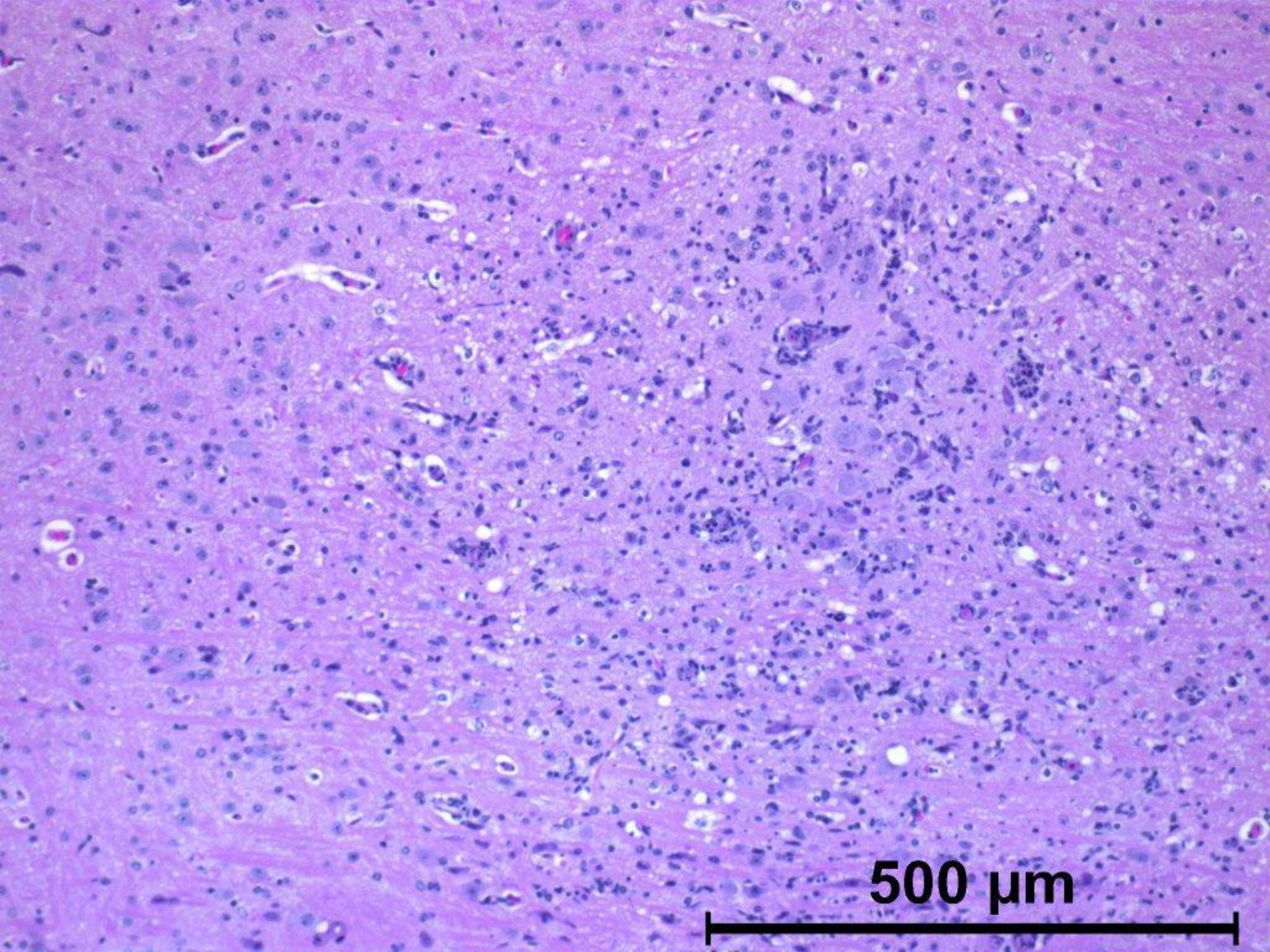
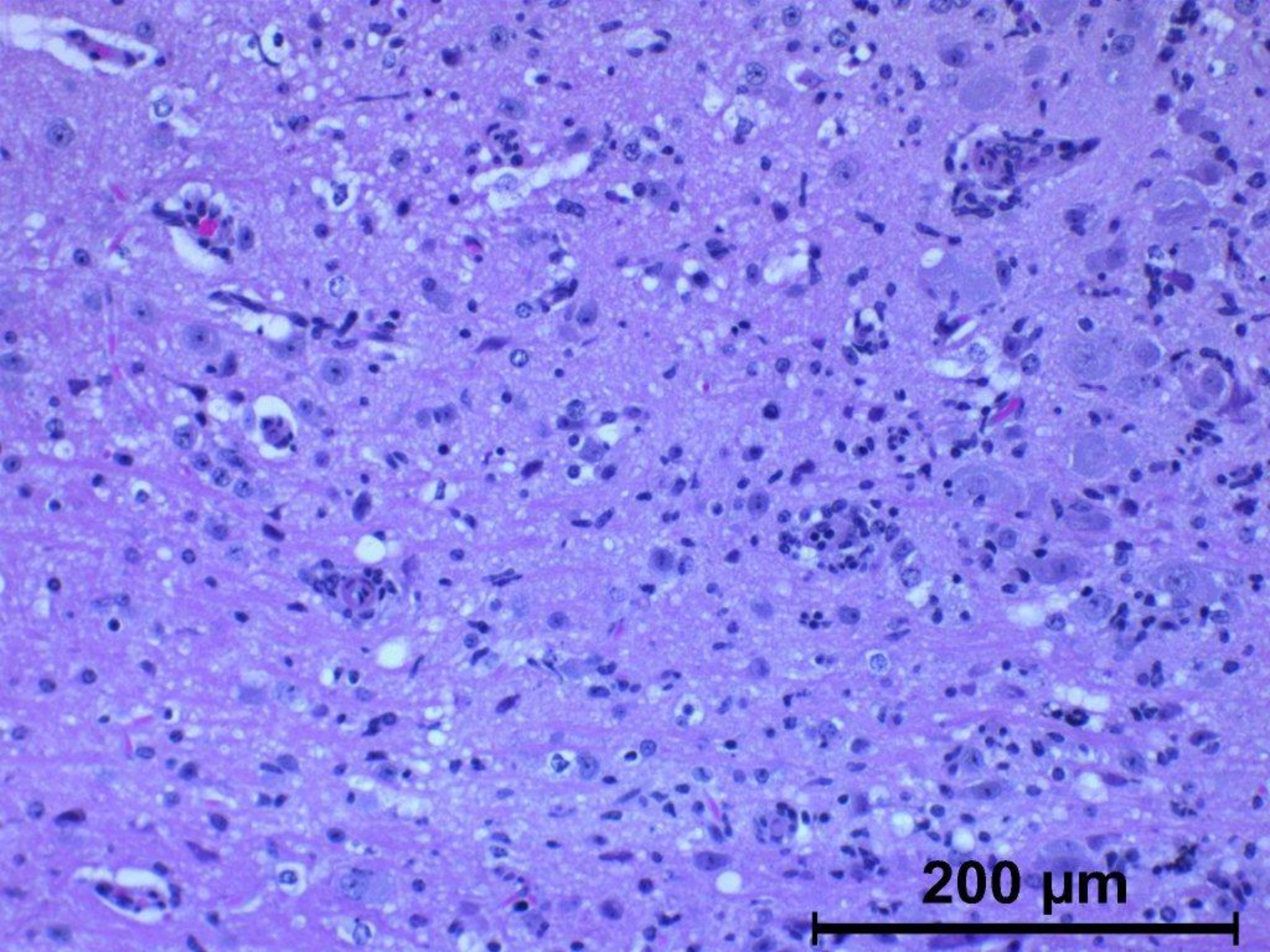
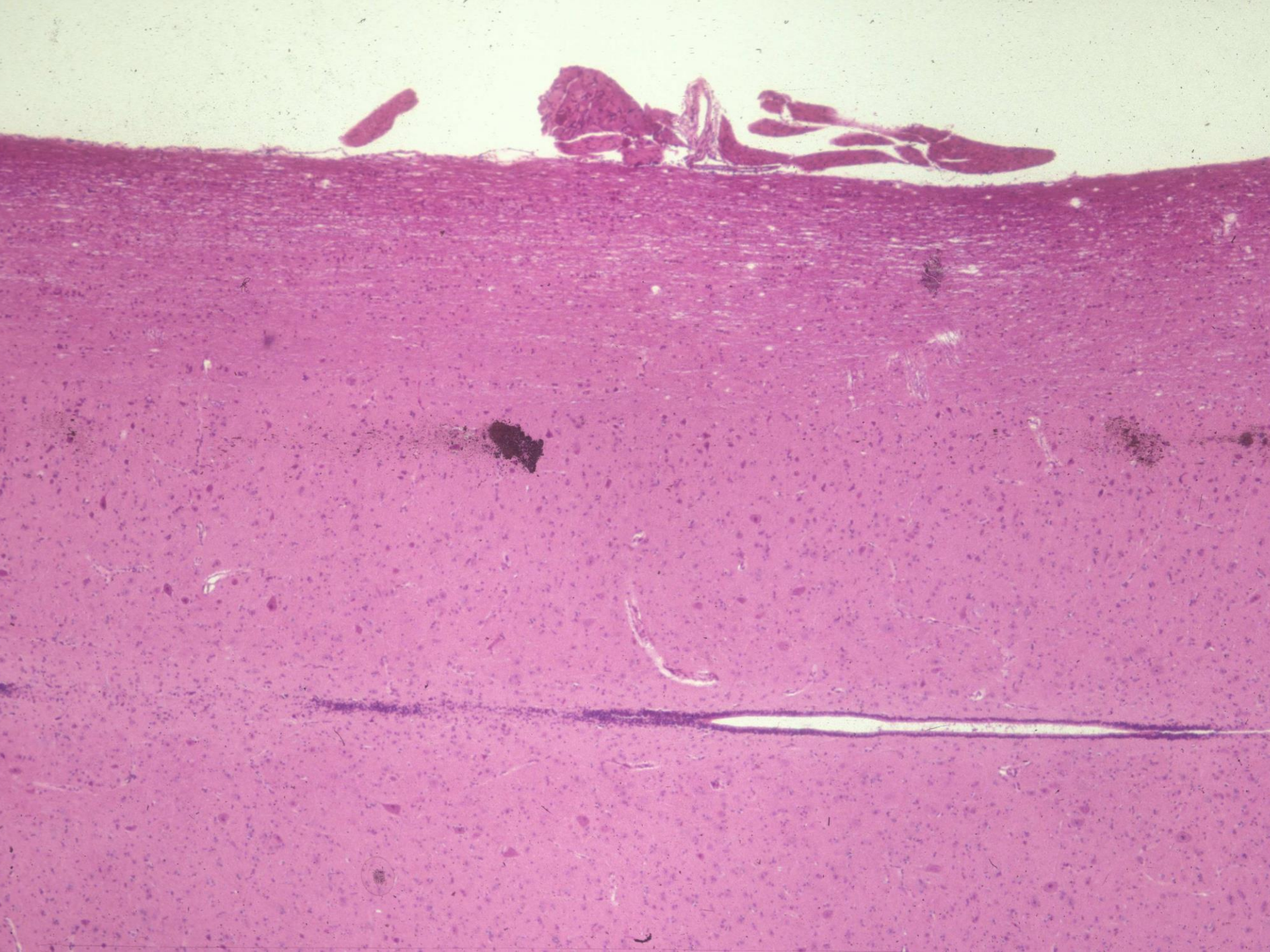
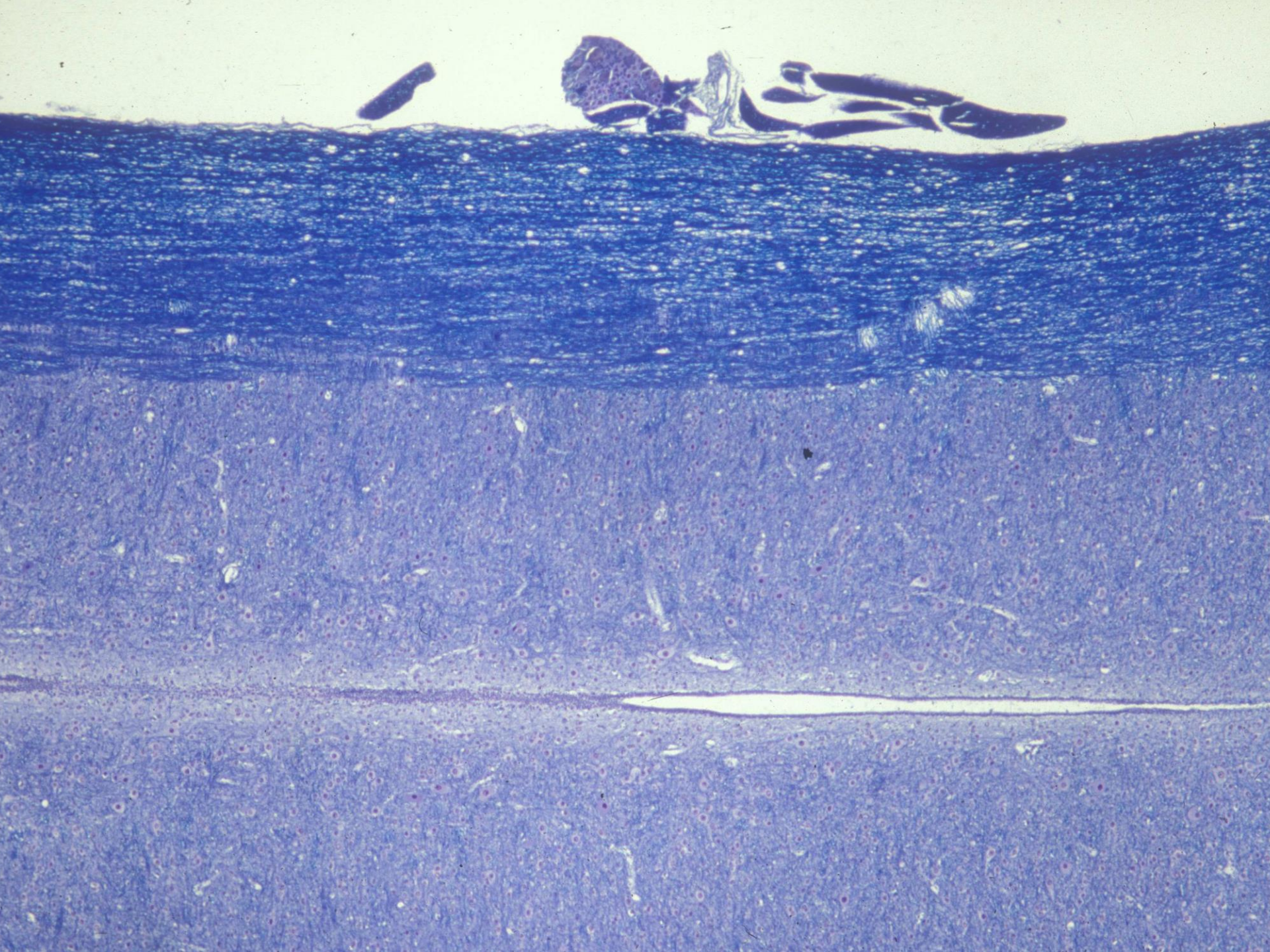

500 µm

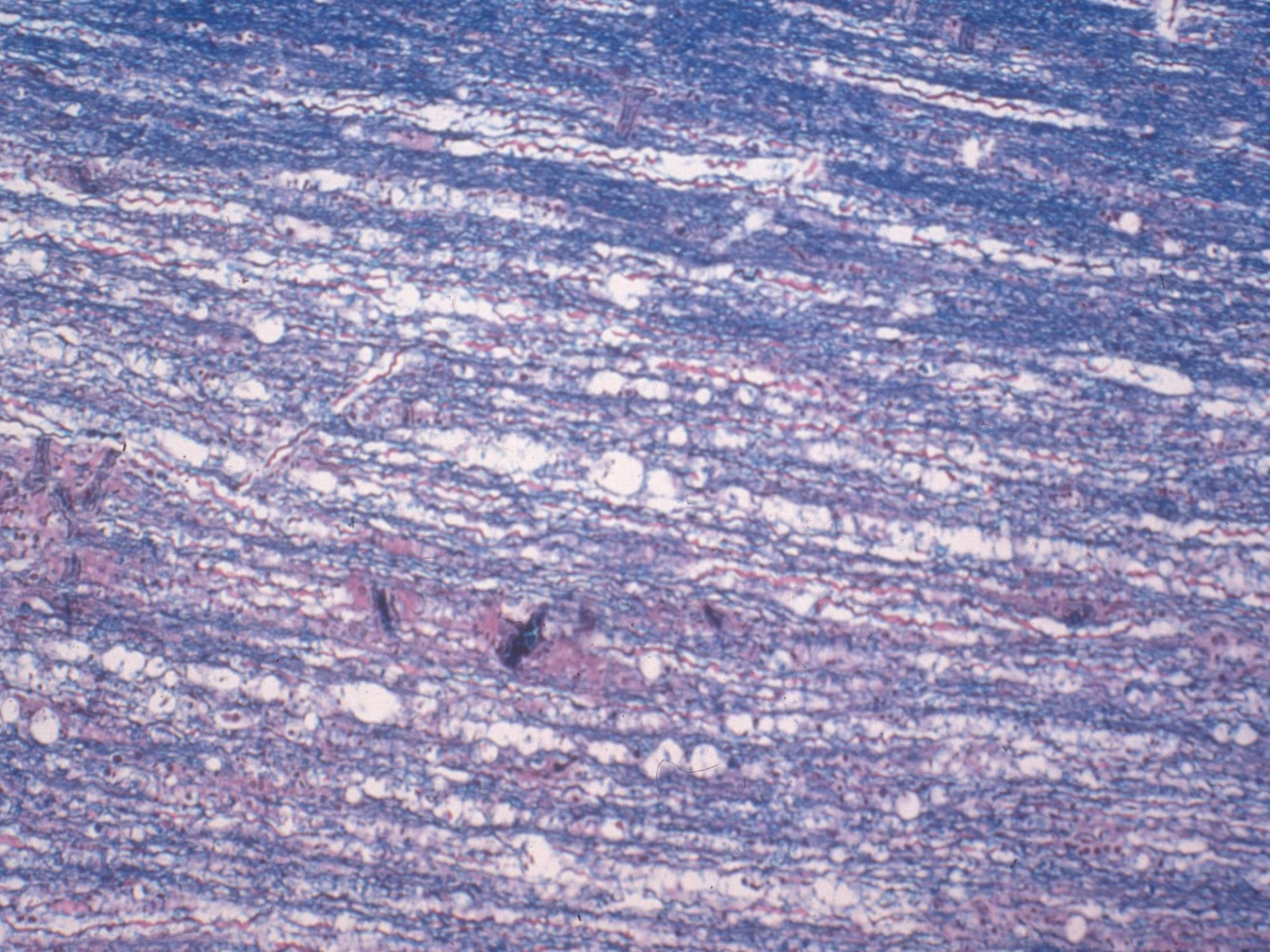

200 µm

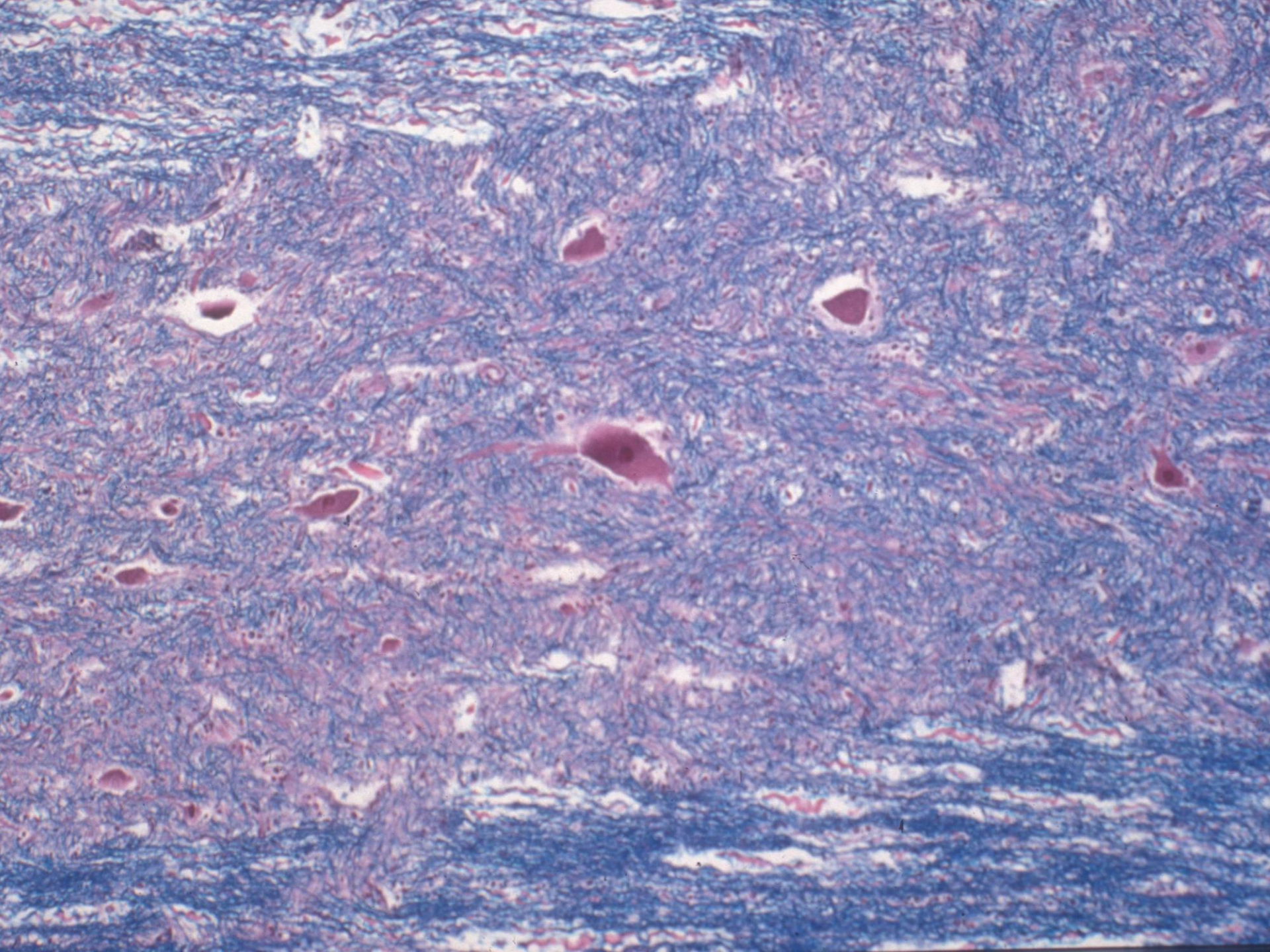
Demyelination in Spinal Cord

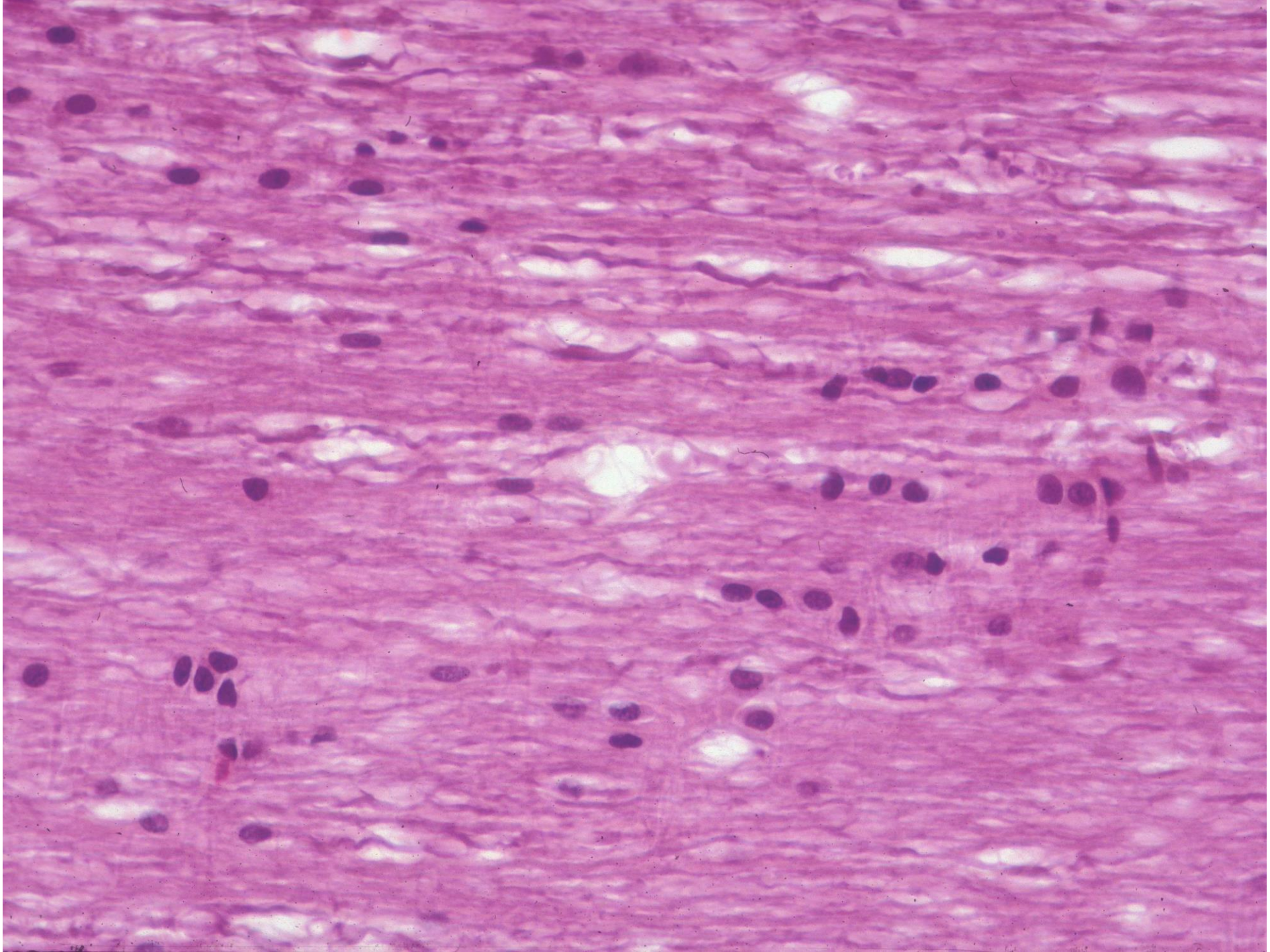
- Orientation of sections important
- Use special stains (e.g. Bodian and Luxol Fast Blue)
- Bodian (or other silver impregnation procedure) stains neurties
- Luxol Fast Blue stains myelin

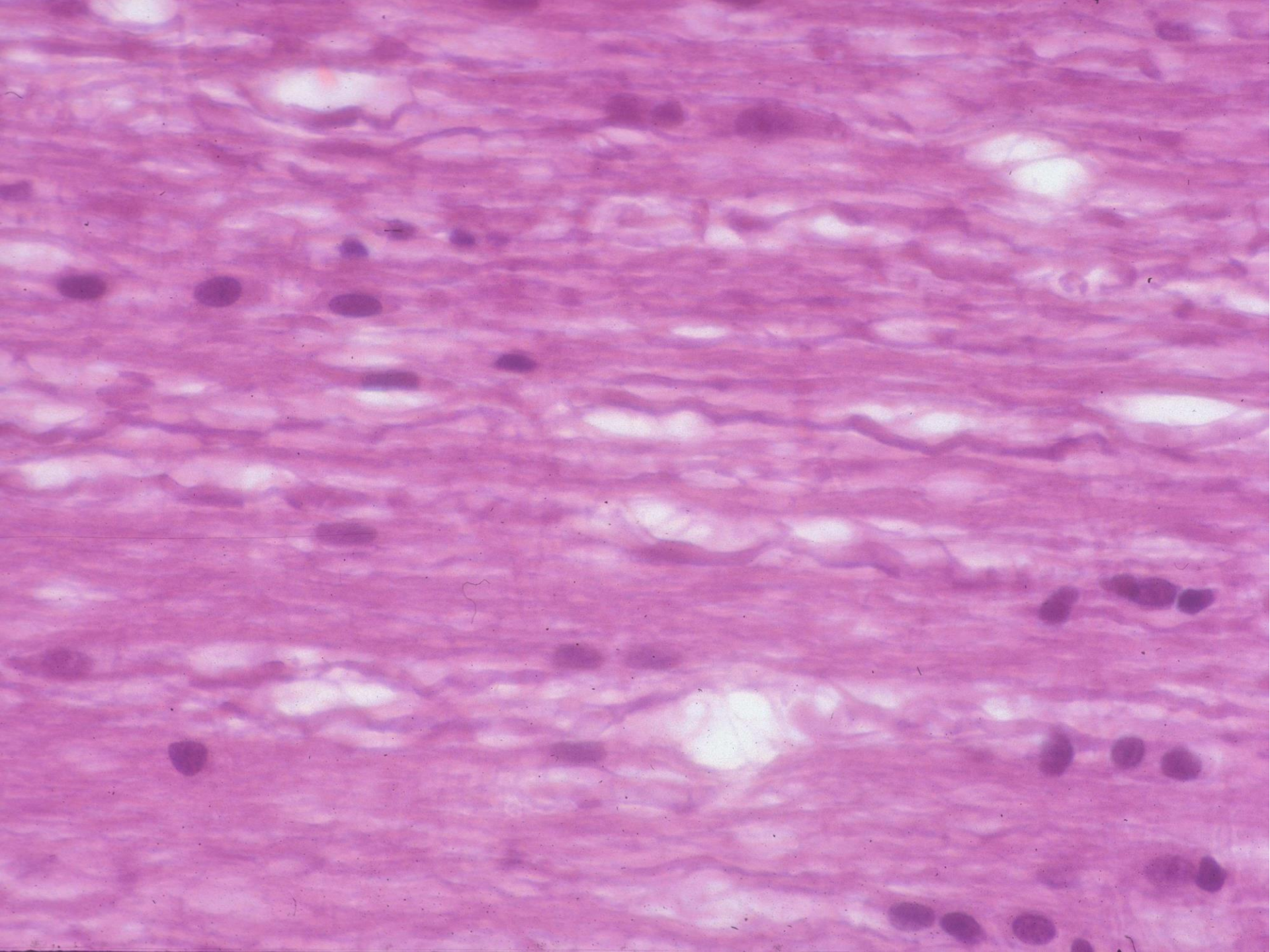


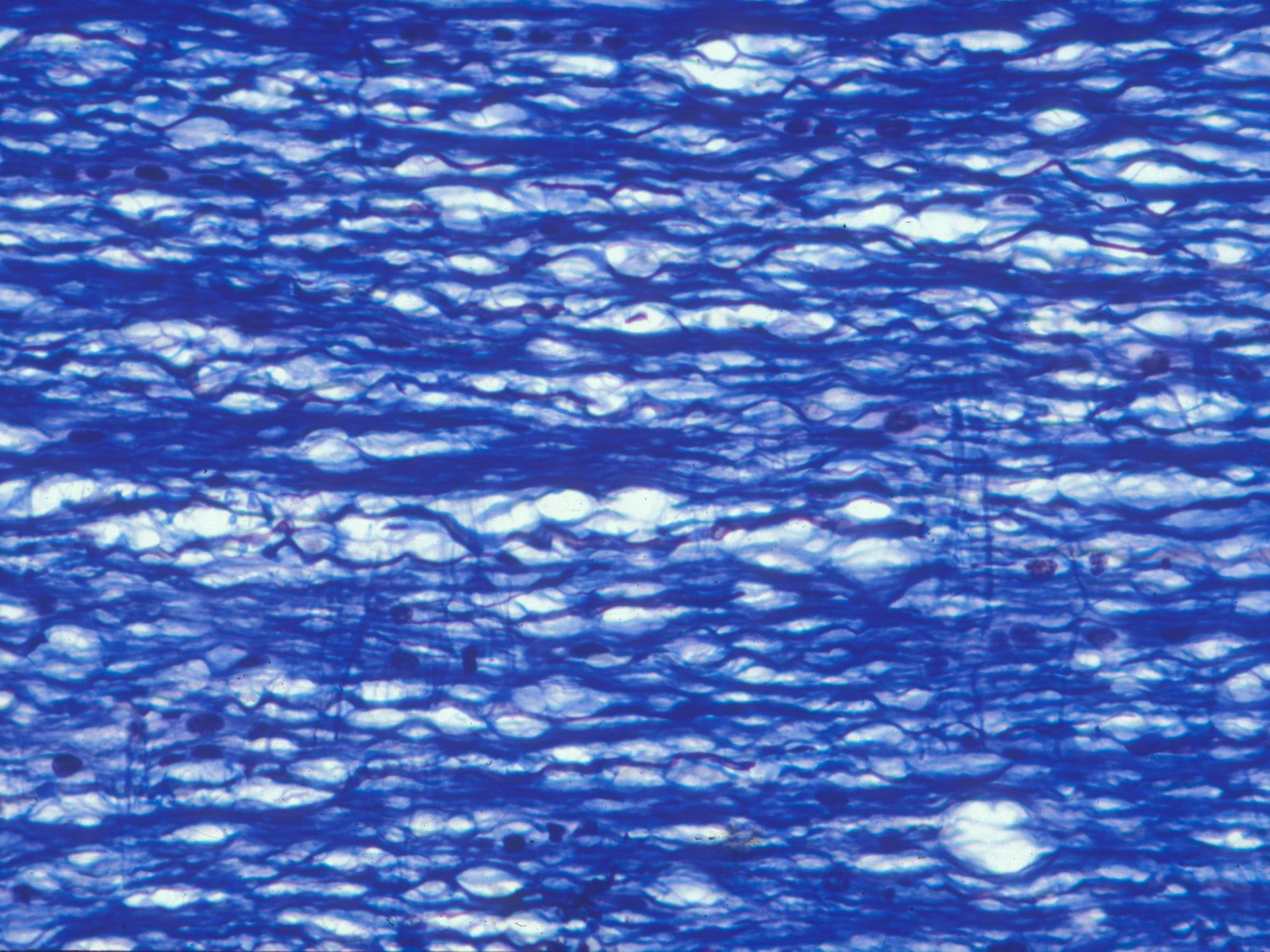


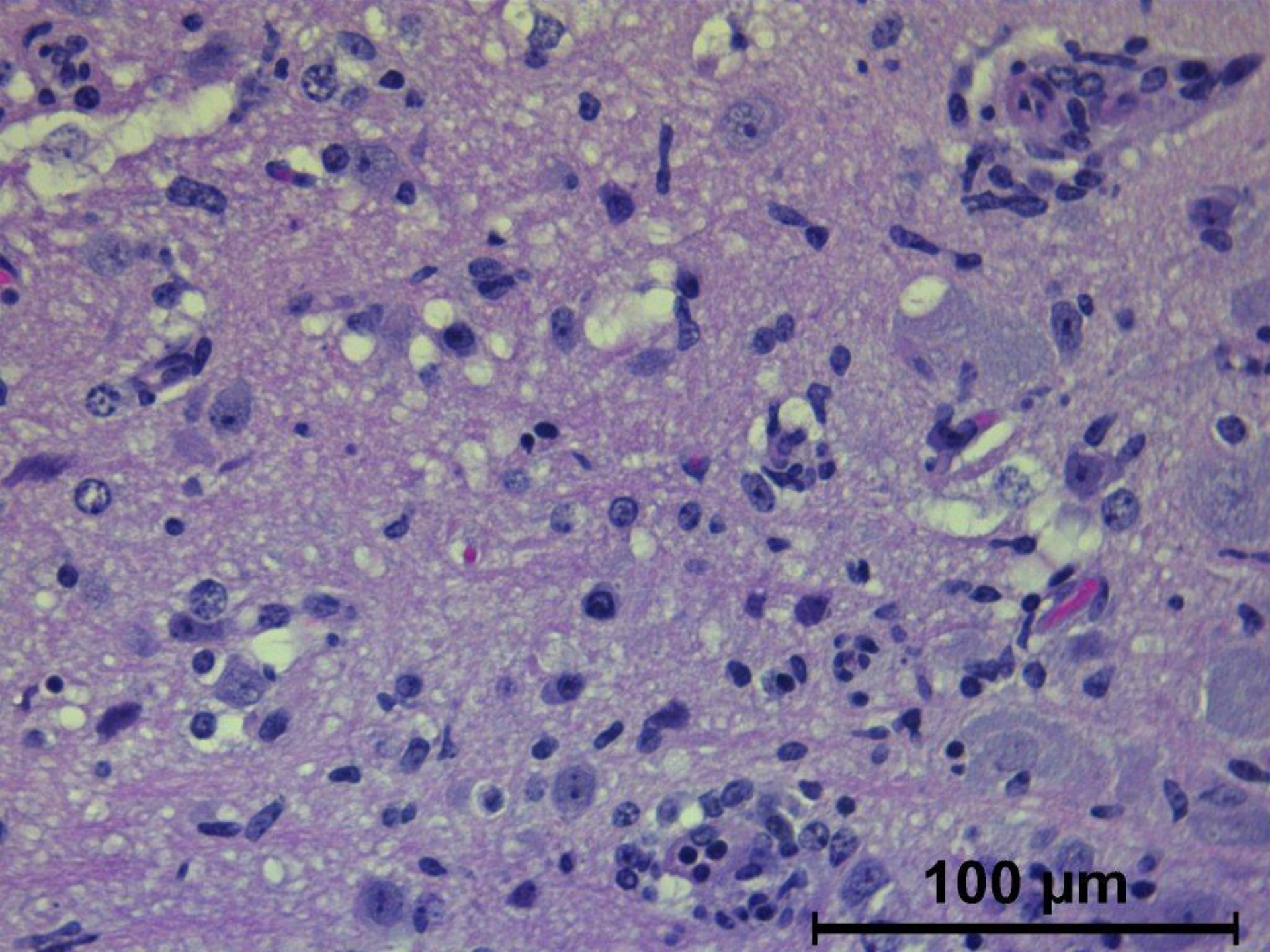








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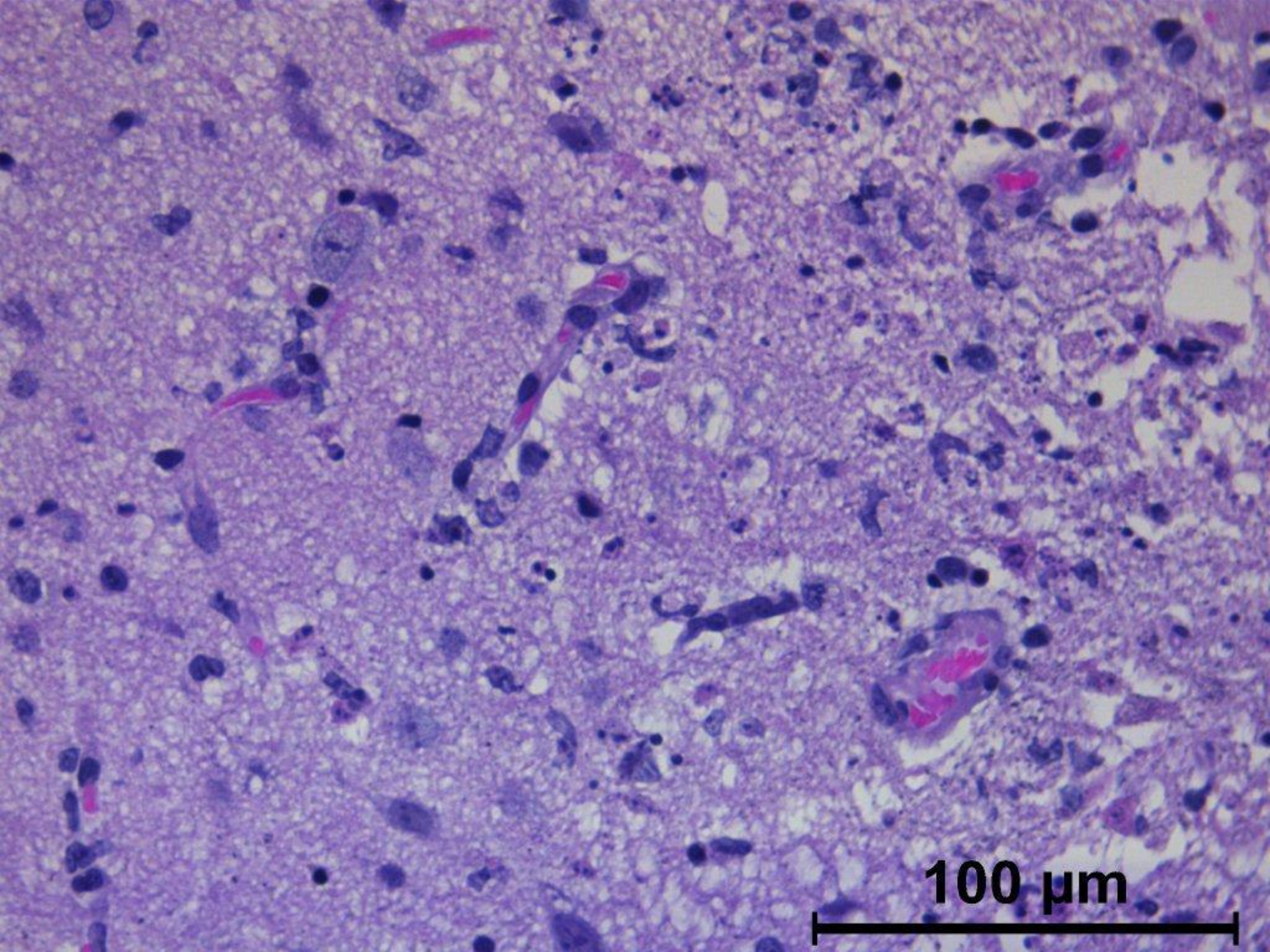
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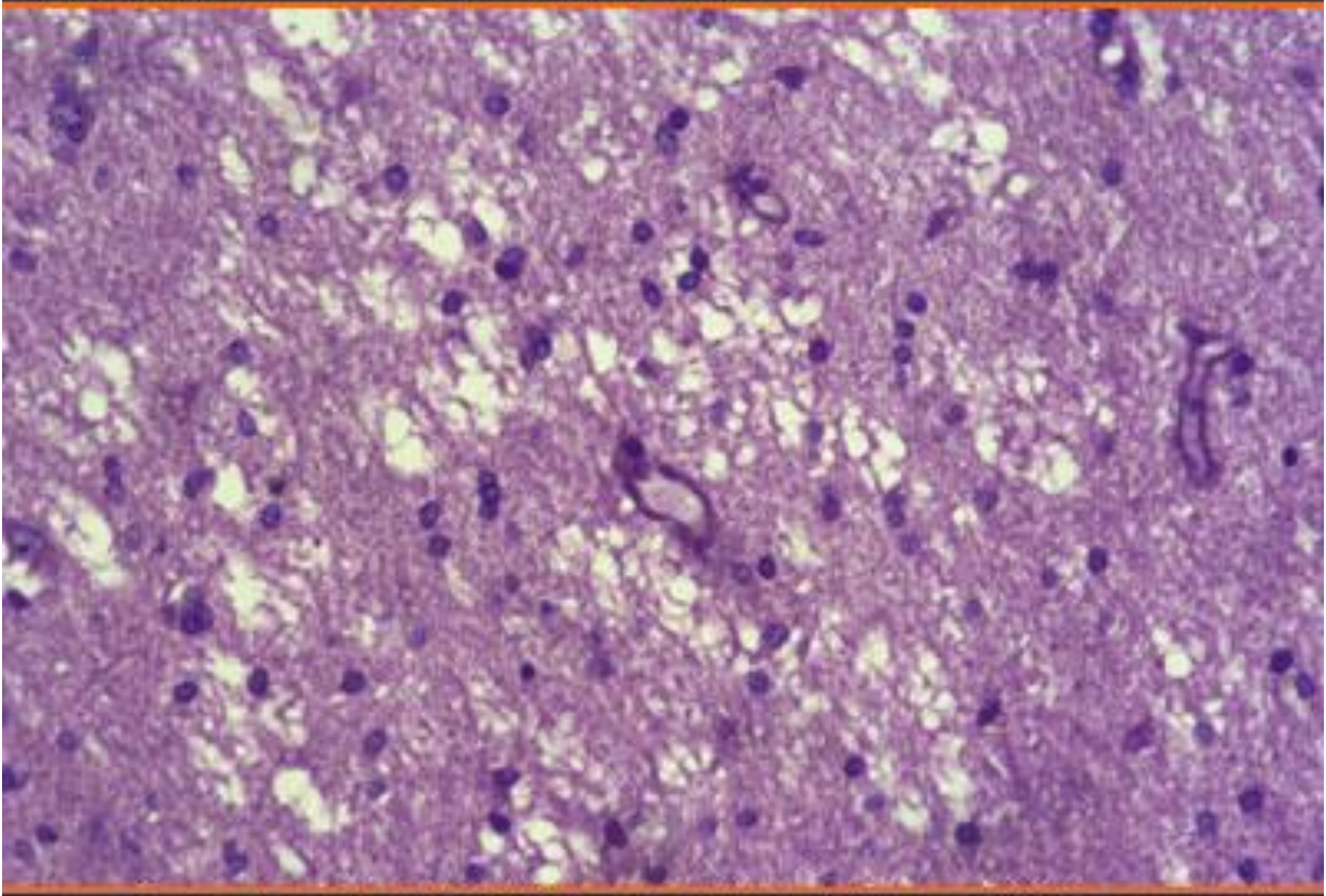

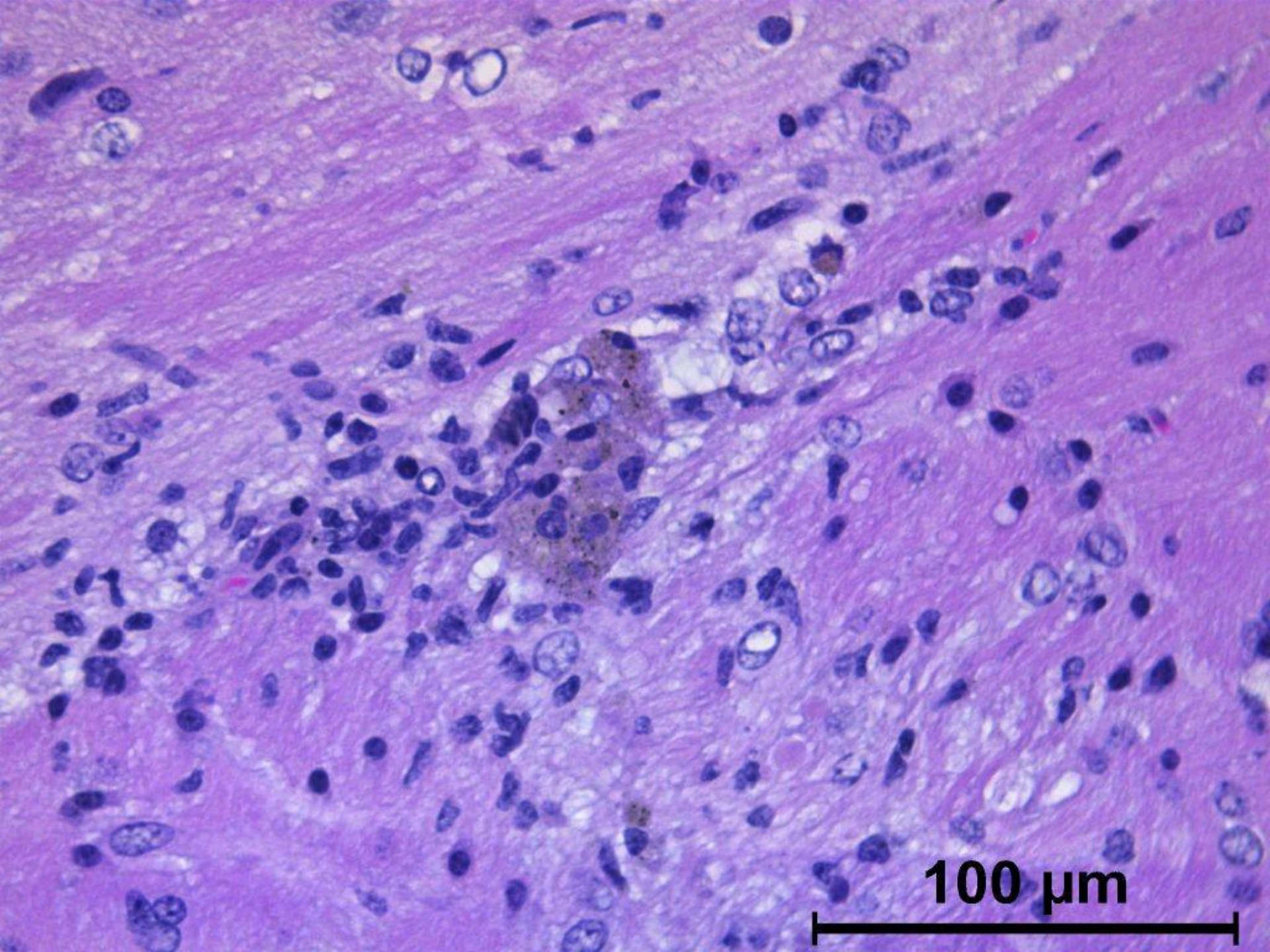
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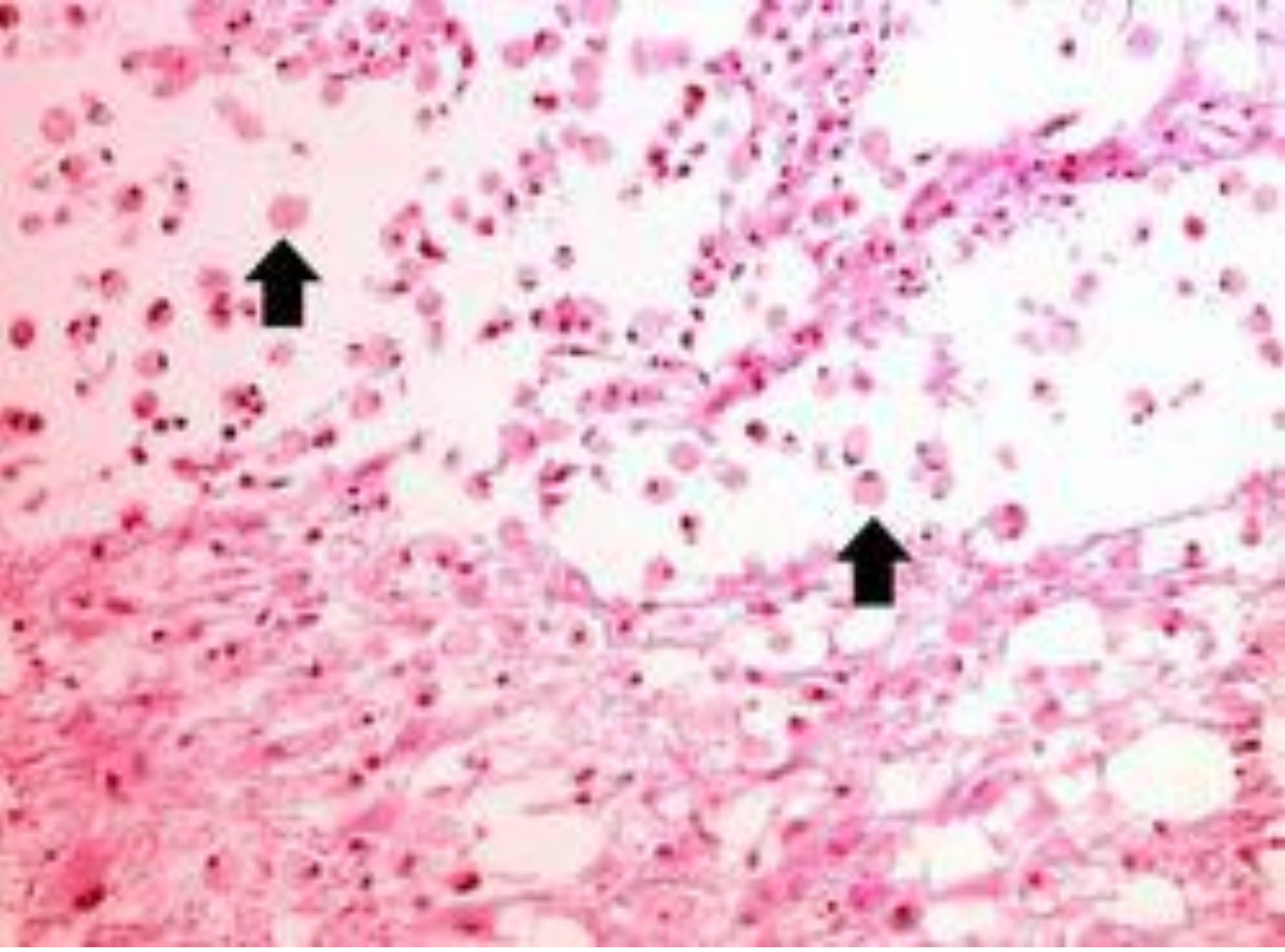
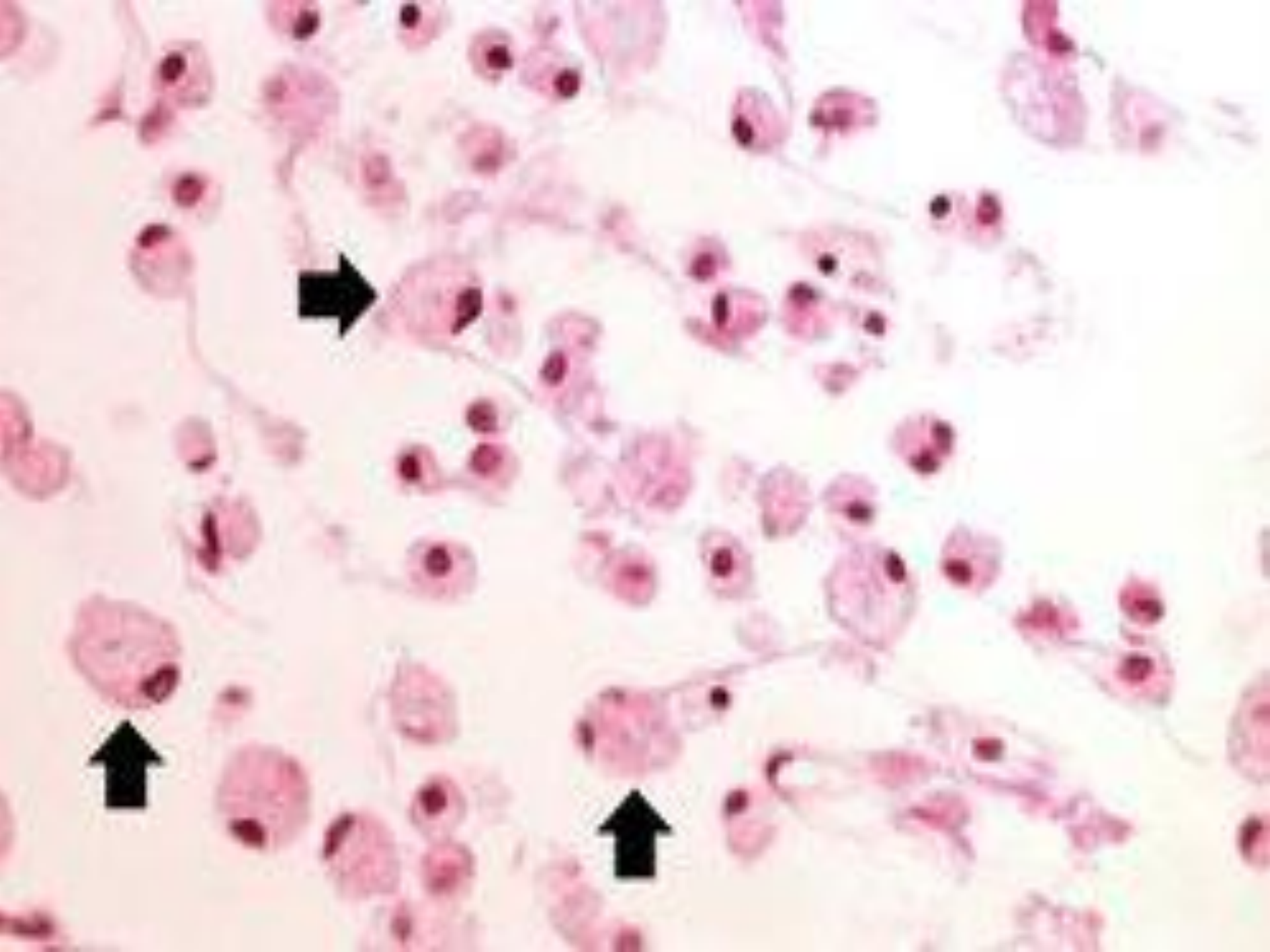
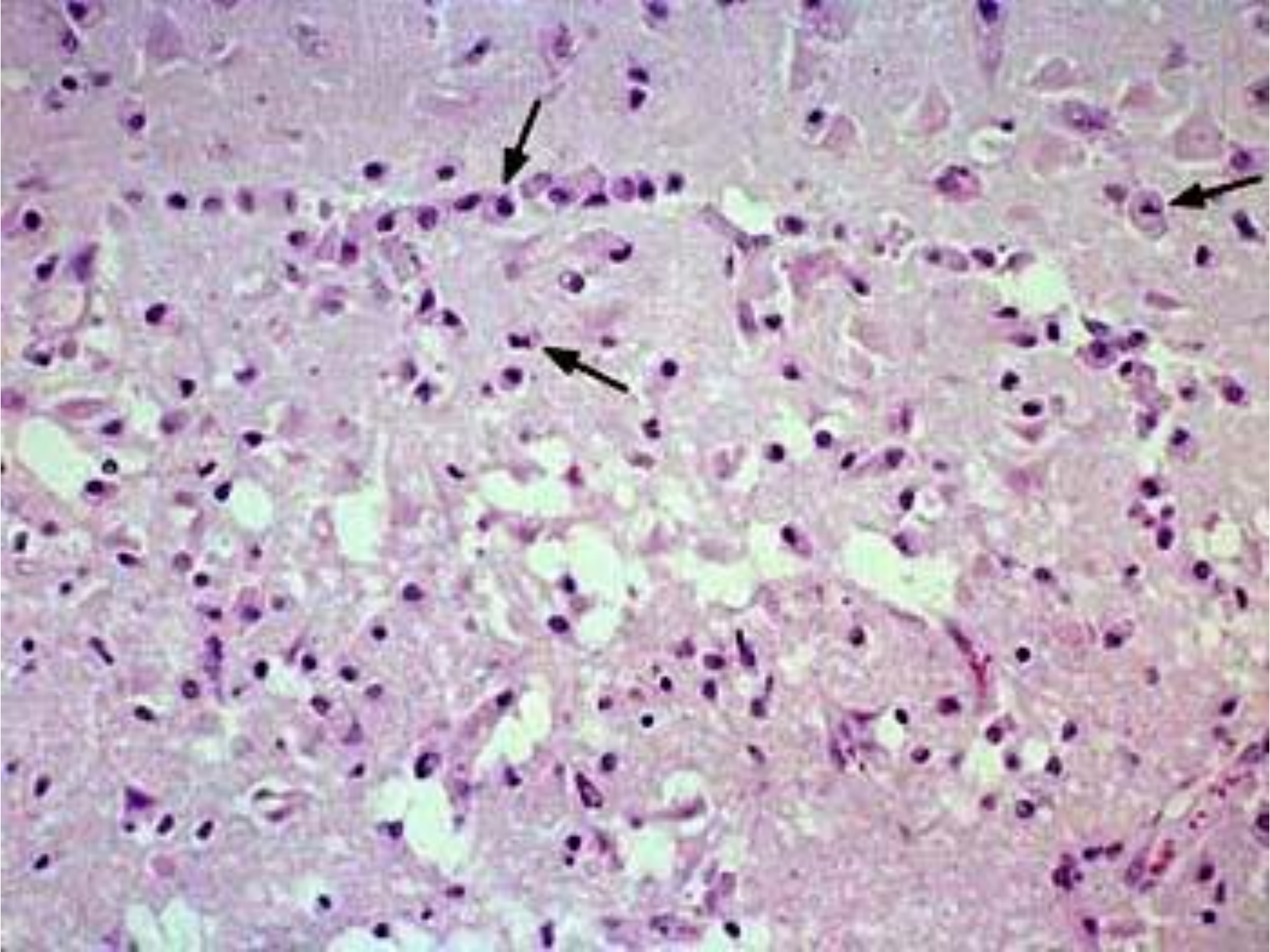
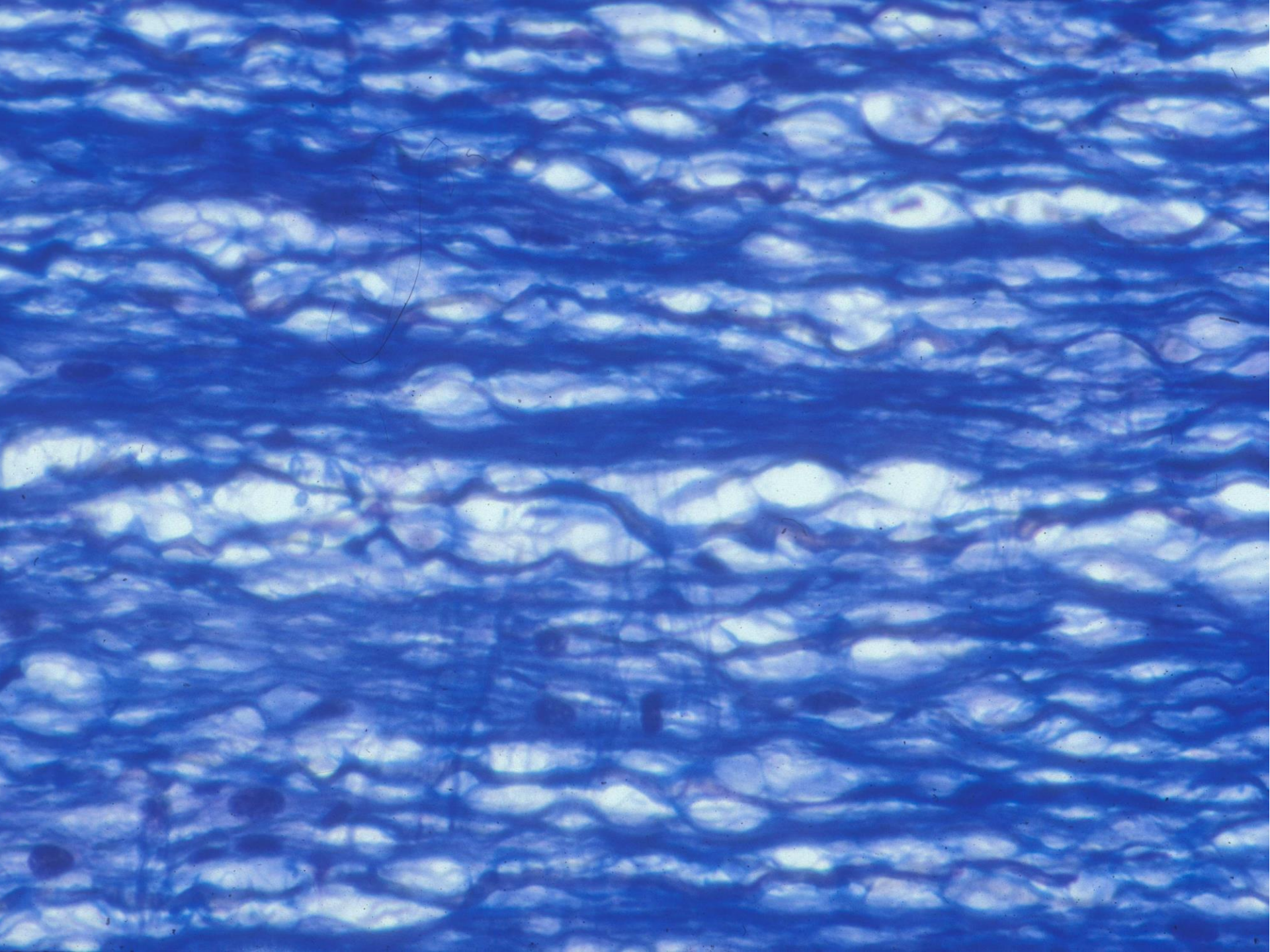


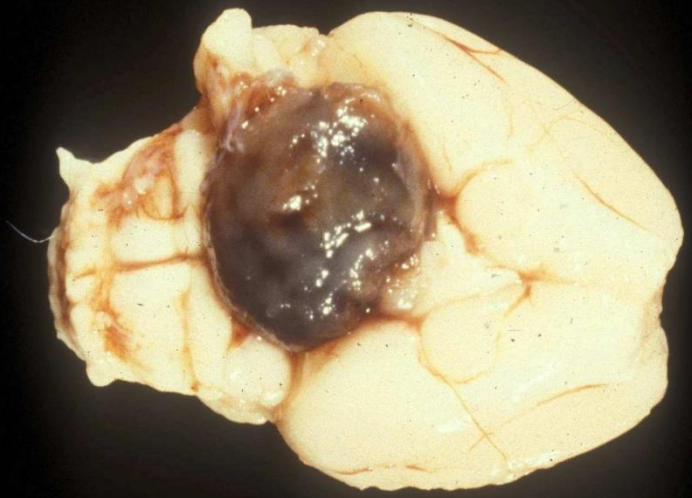


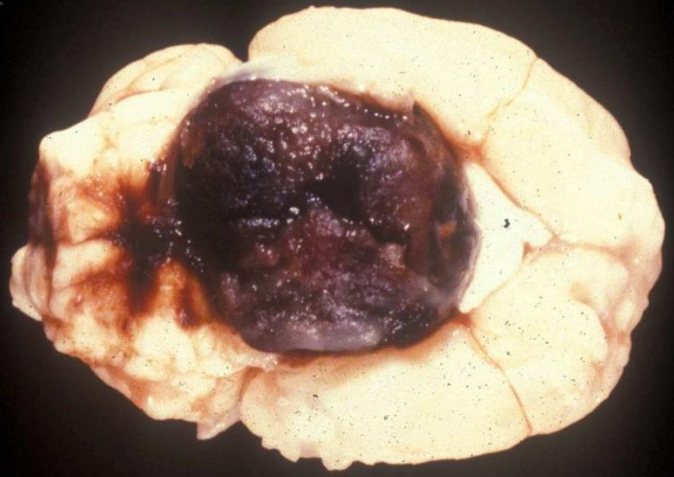


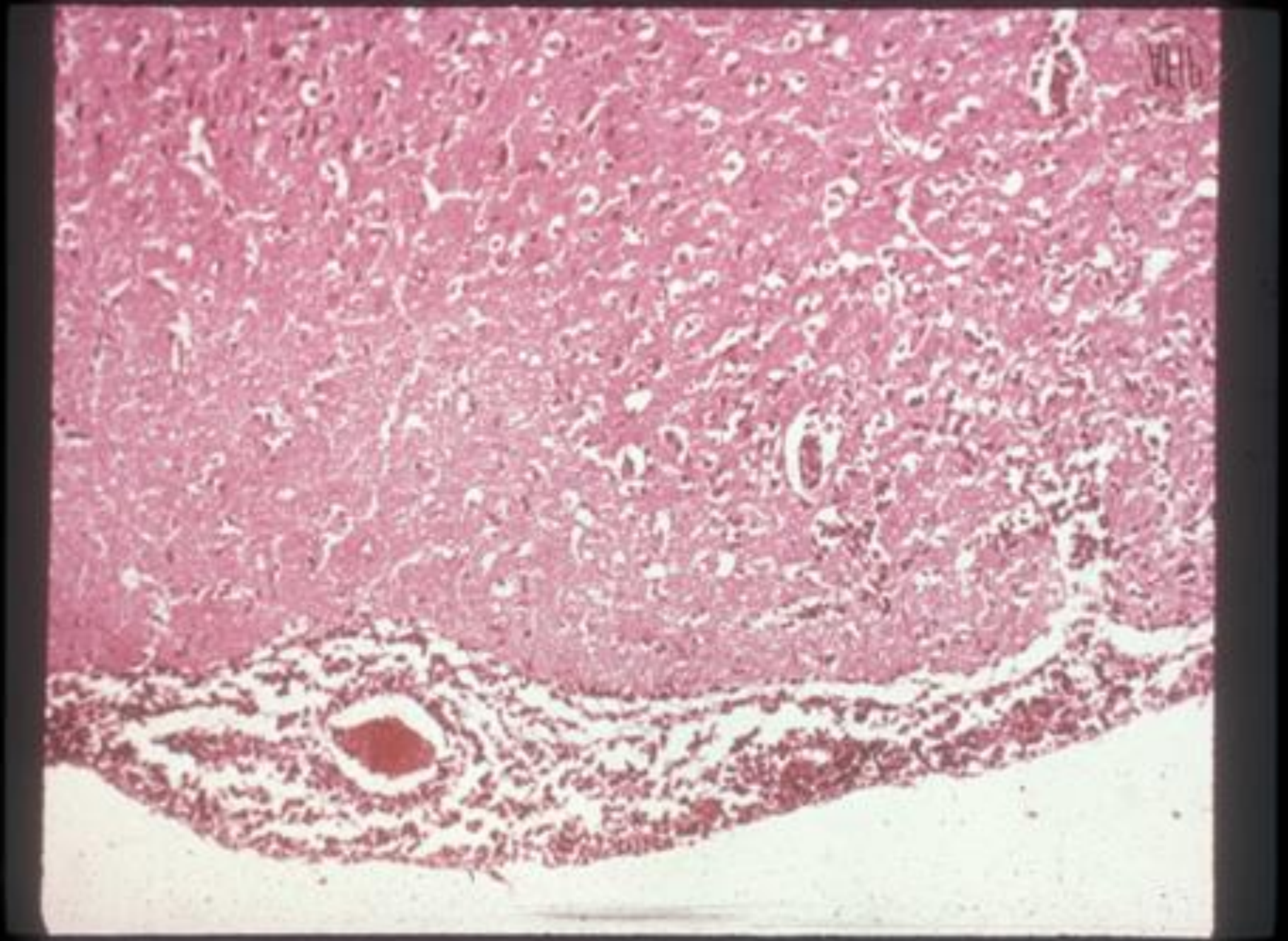


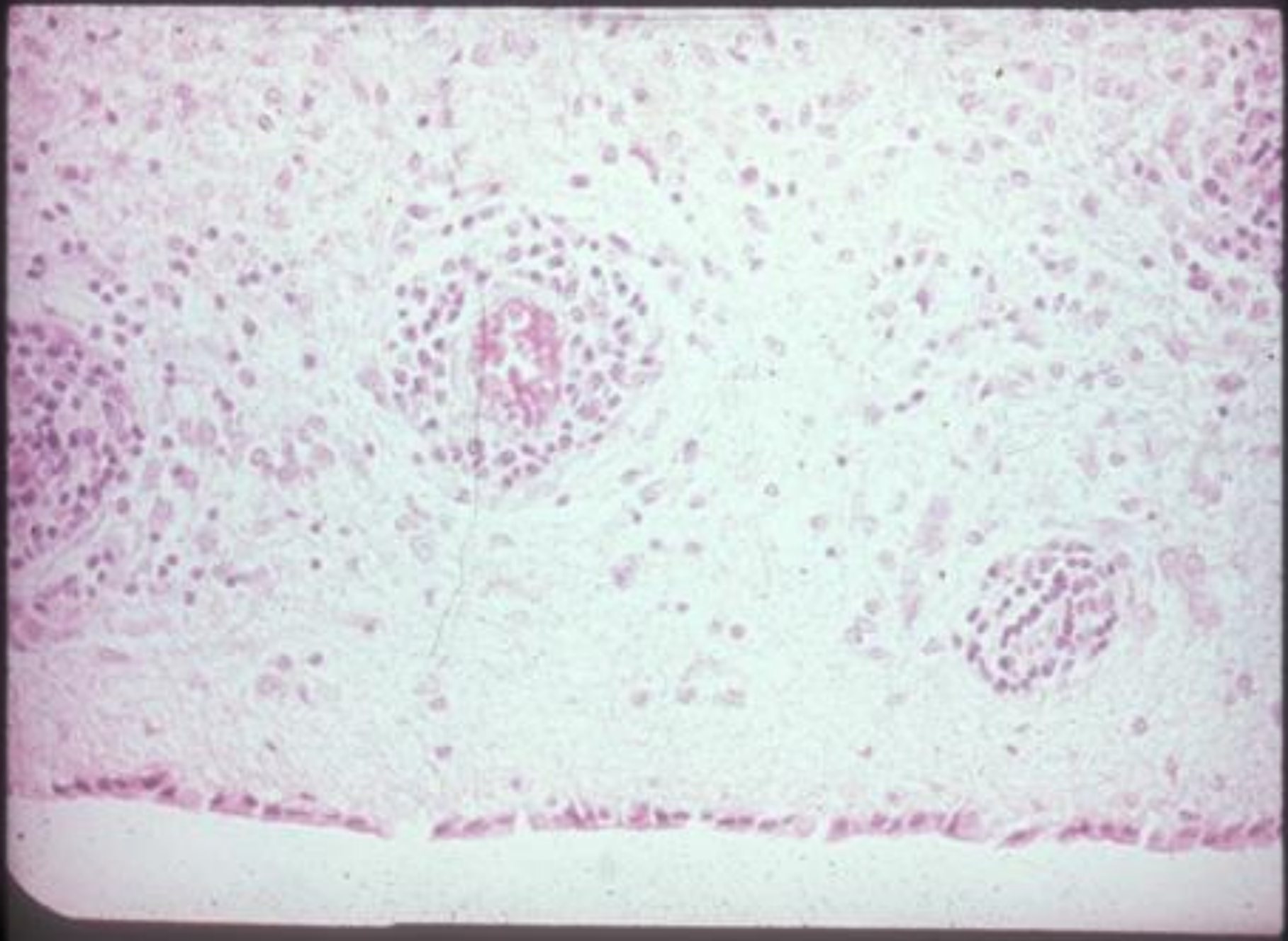
Pituitary Adenoma

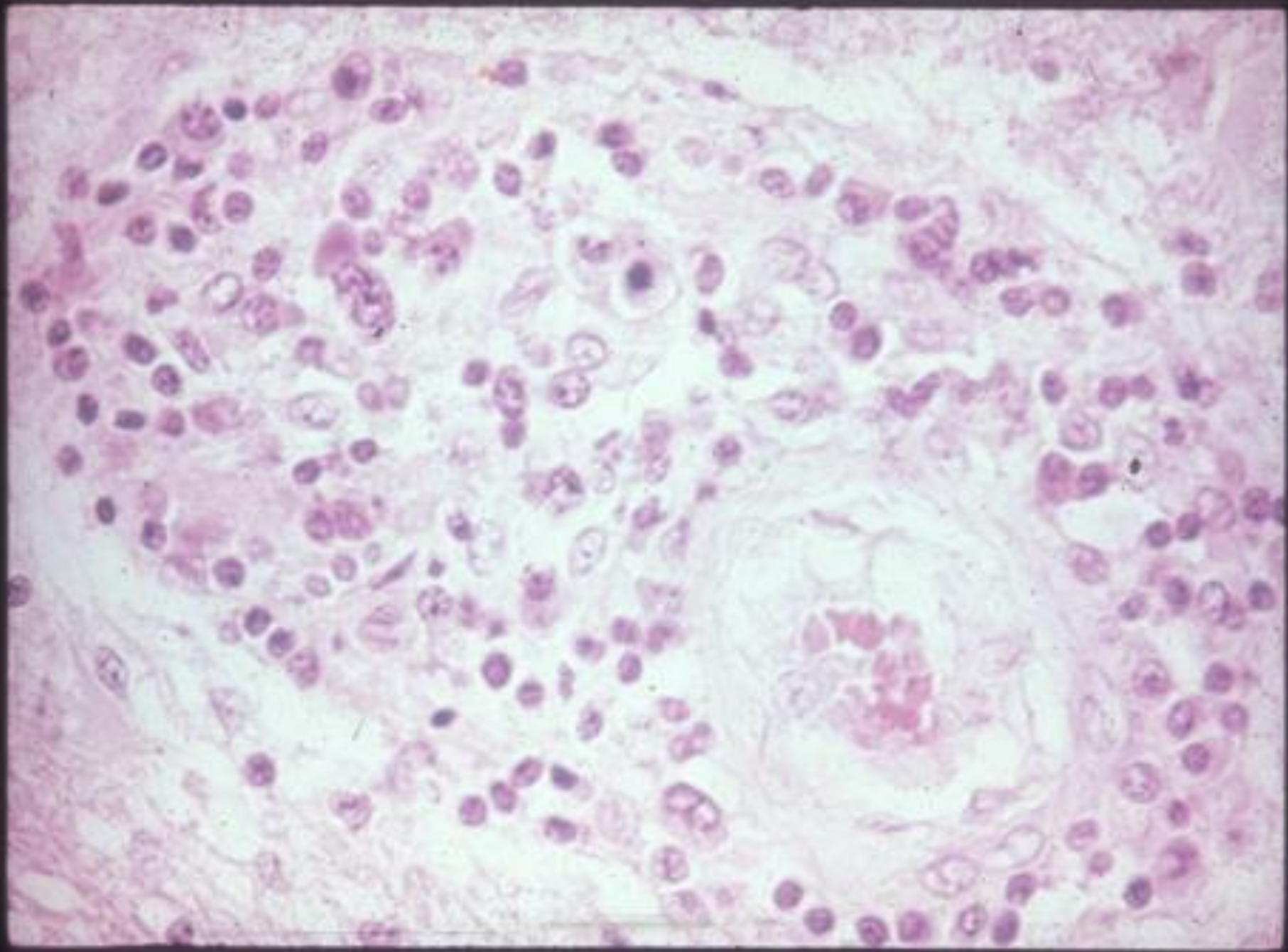
- Very common in rats
- Usually chromophobe type
- Incidence varies with strain
- Not common in mice





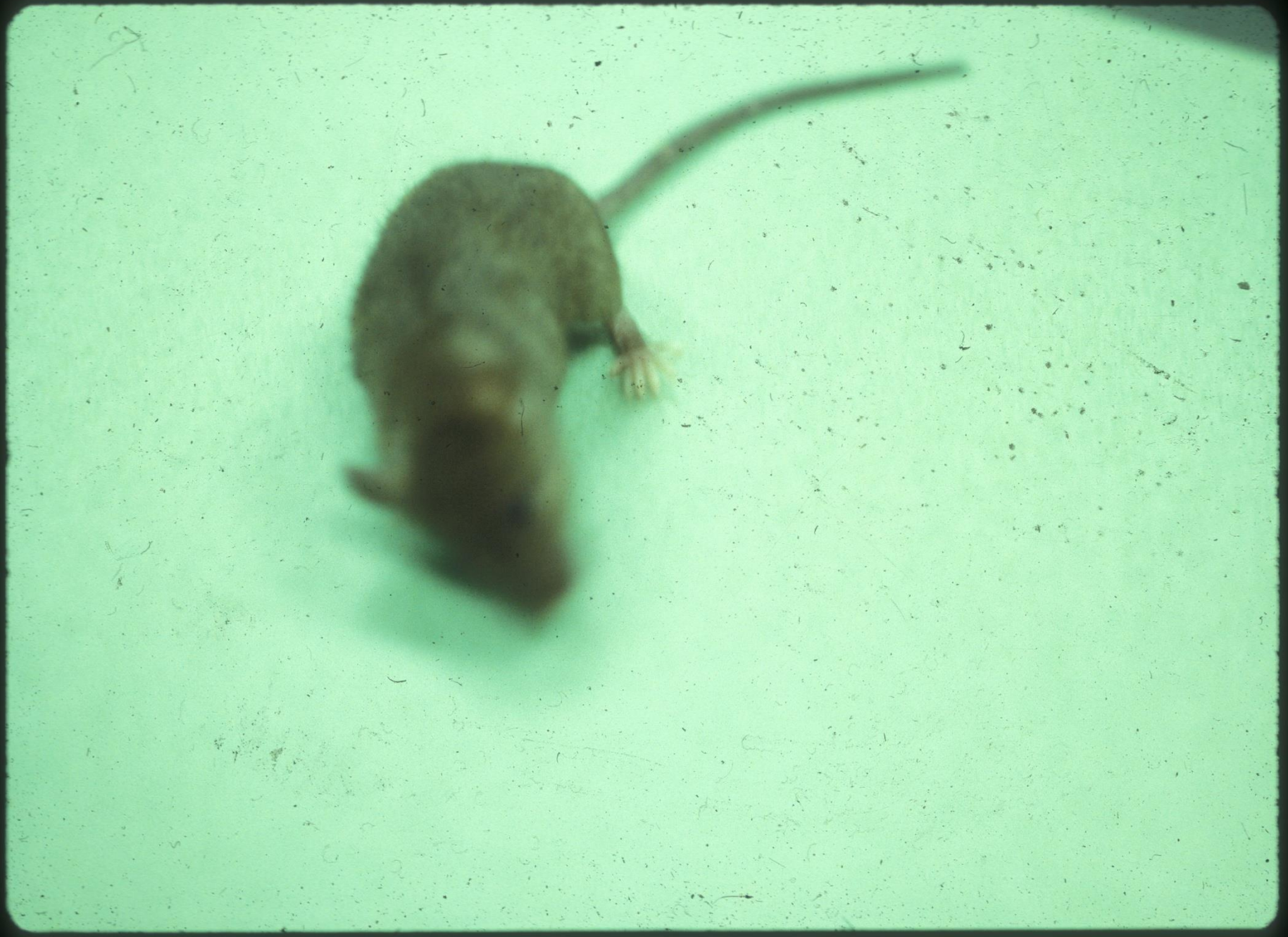


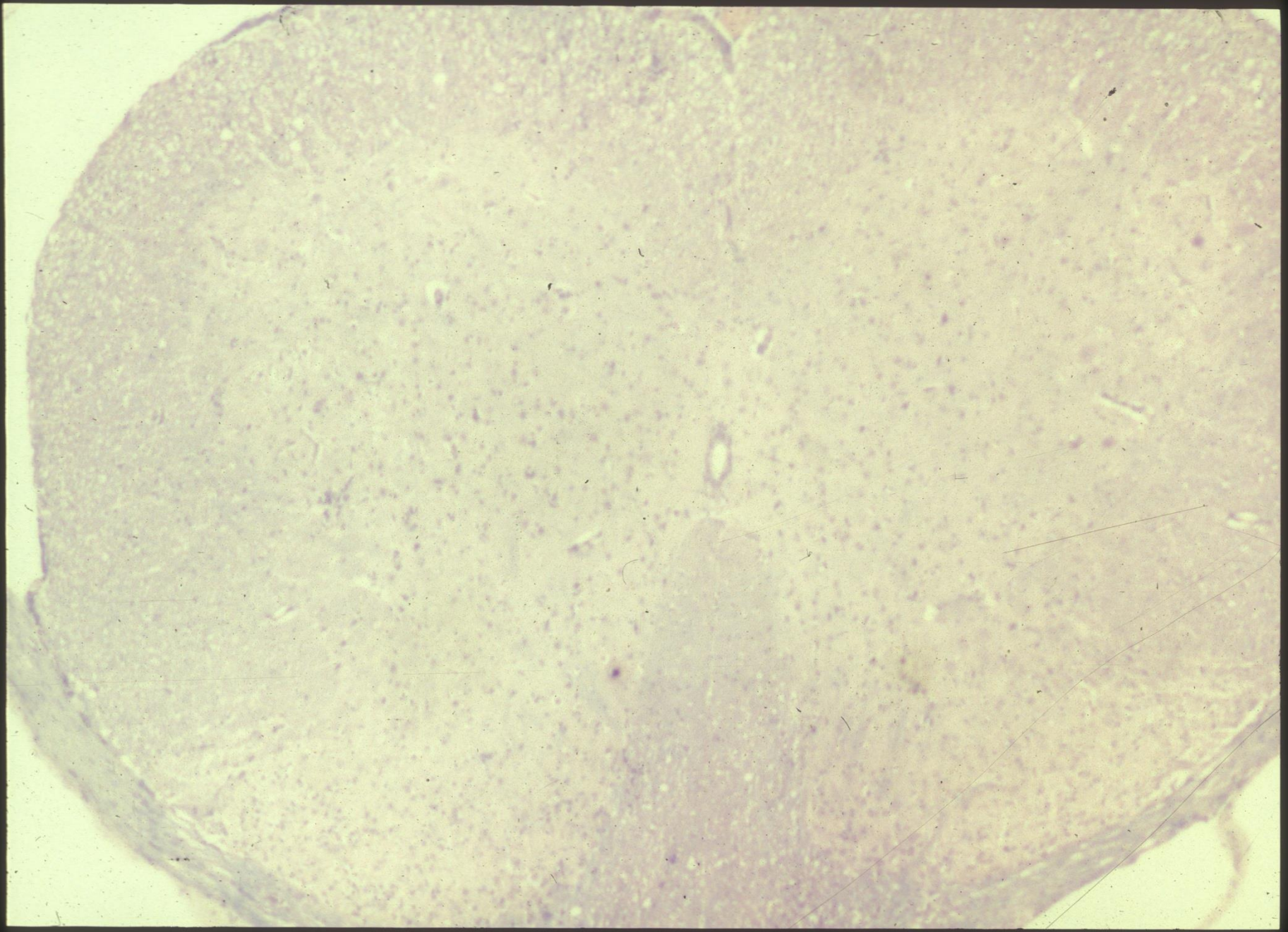


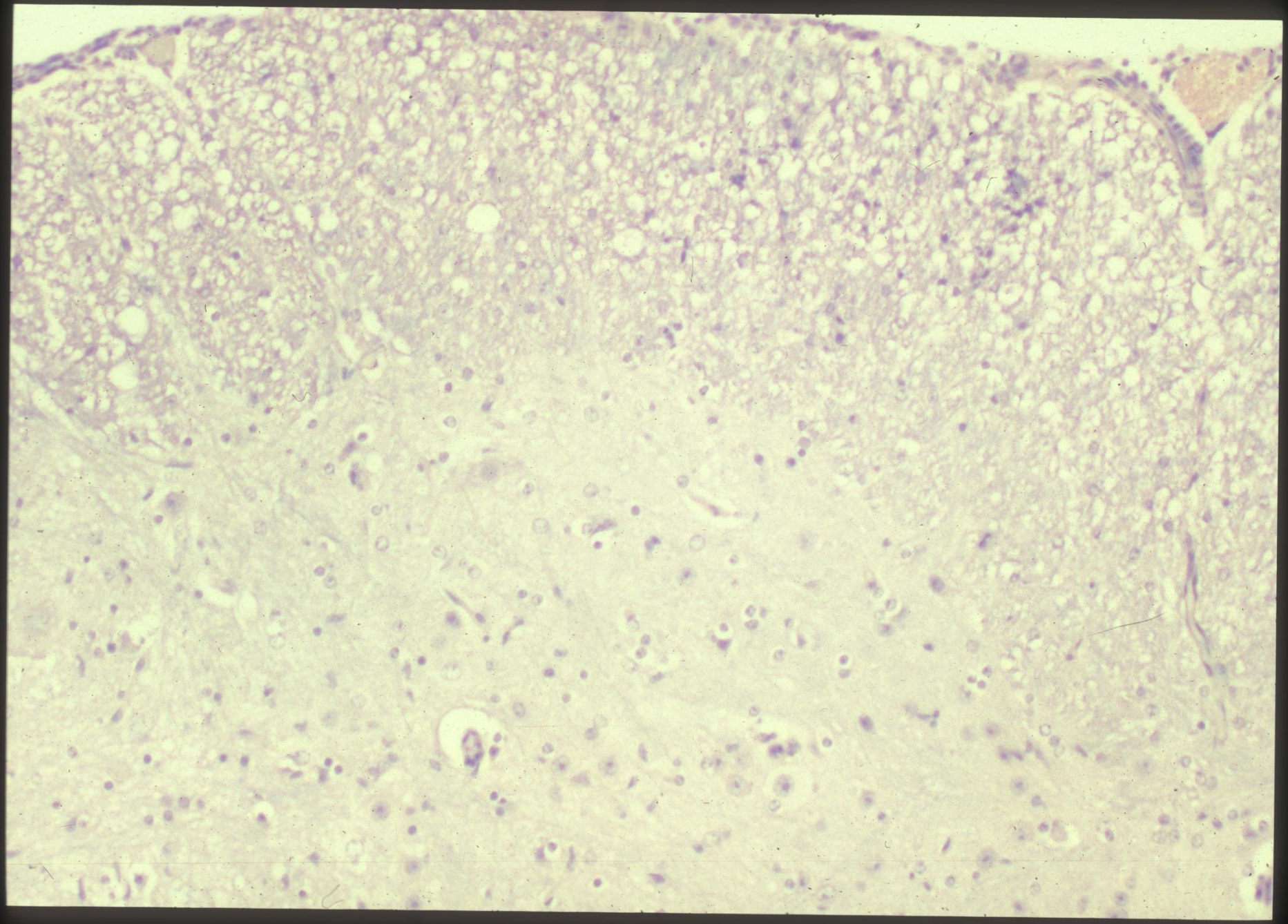


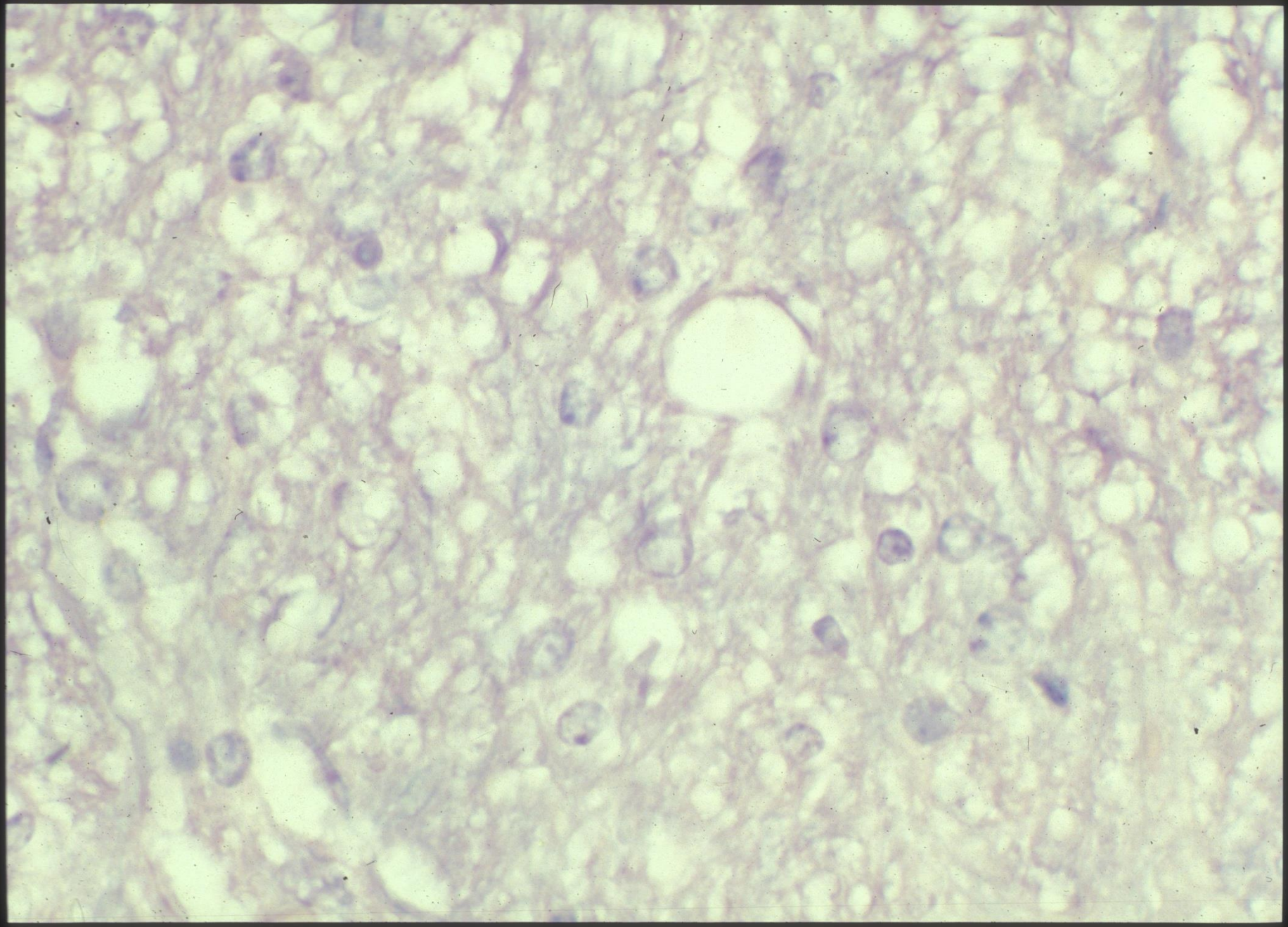
Mouse Encephalitis Virus

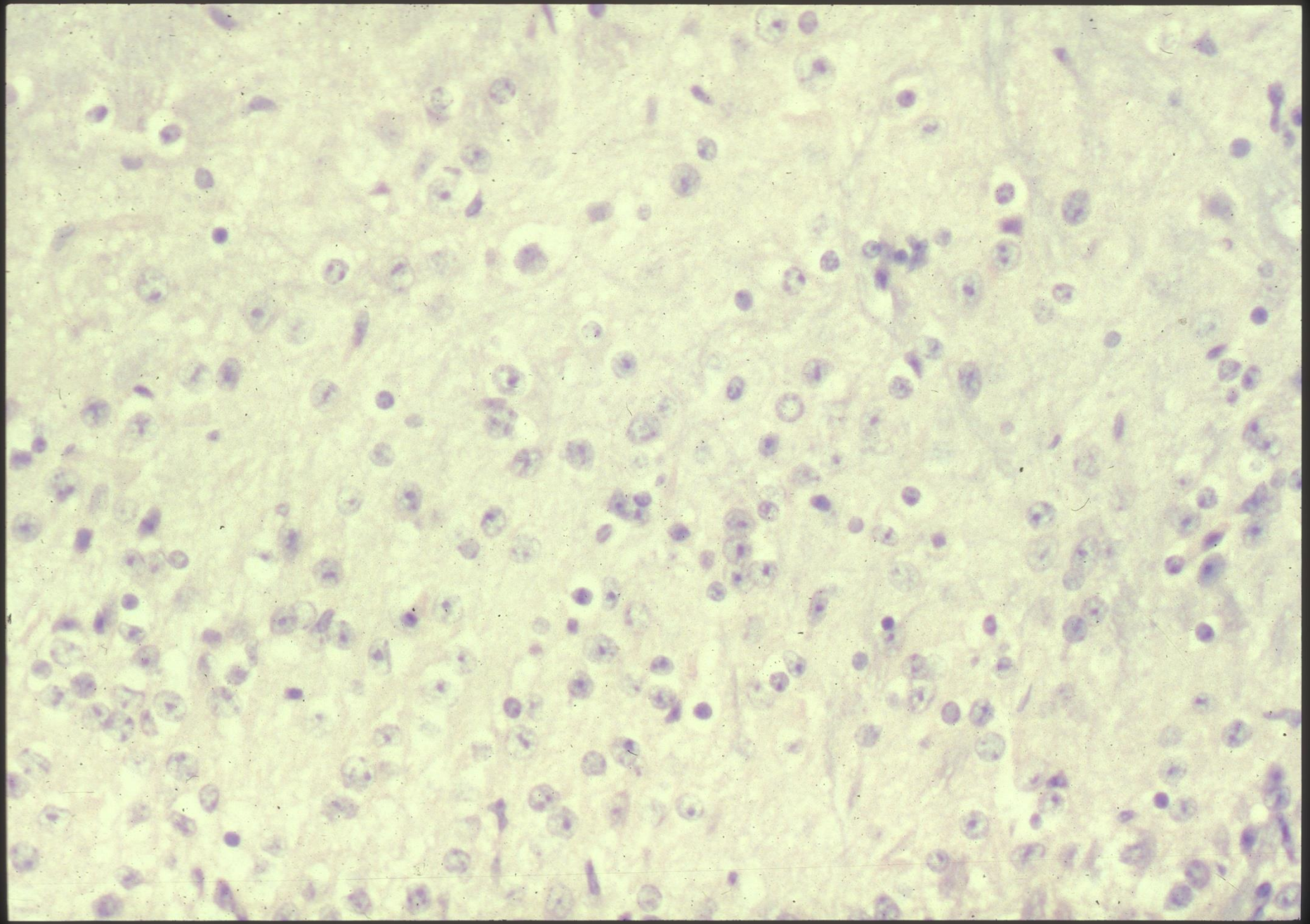
- A Picornavirus, genus Cardiovirus
- aka Theilovirus, mouse polio
- Many strains, e.g. (TO), GDVII
- Primarily an enterovirus, but no gut lesions
- Of interest because of CNS lesions
- Attacks neurons and glia
- Produces neuronolysis and demyelination and other changes

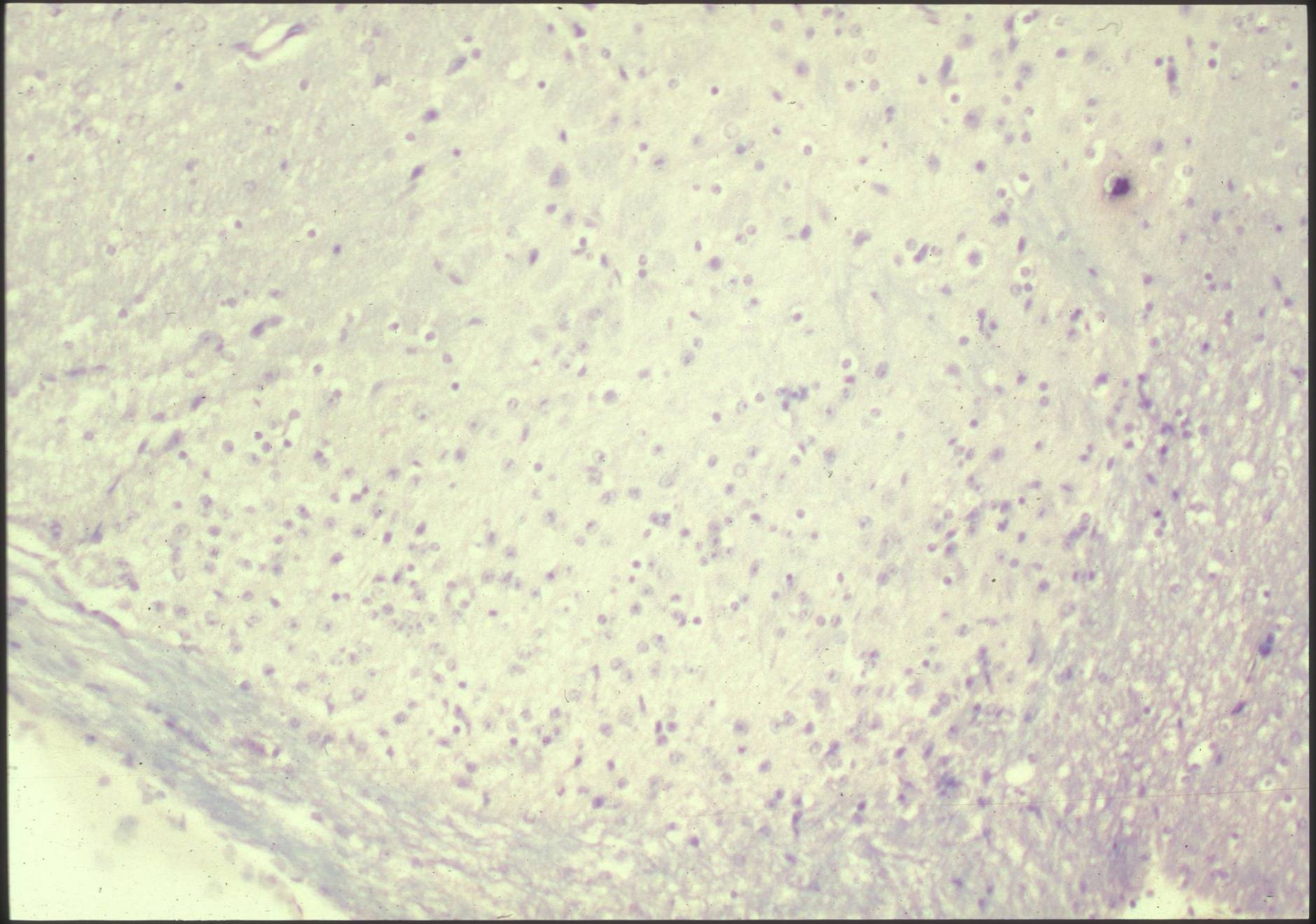


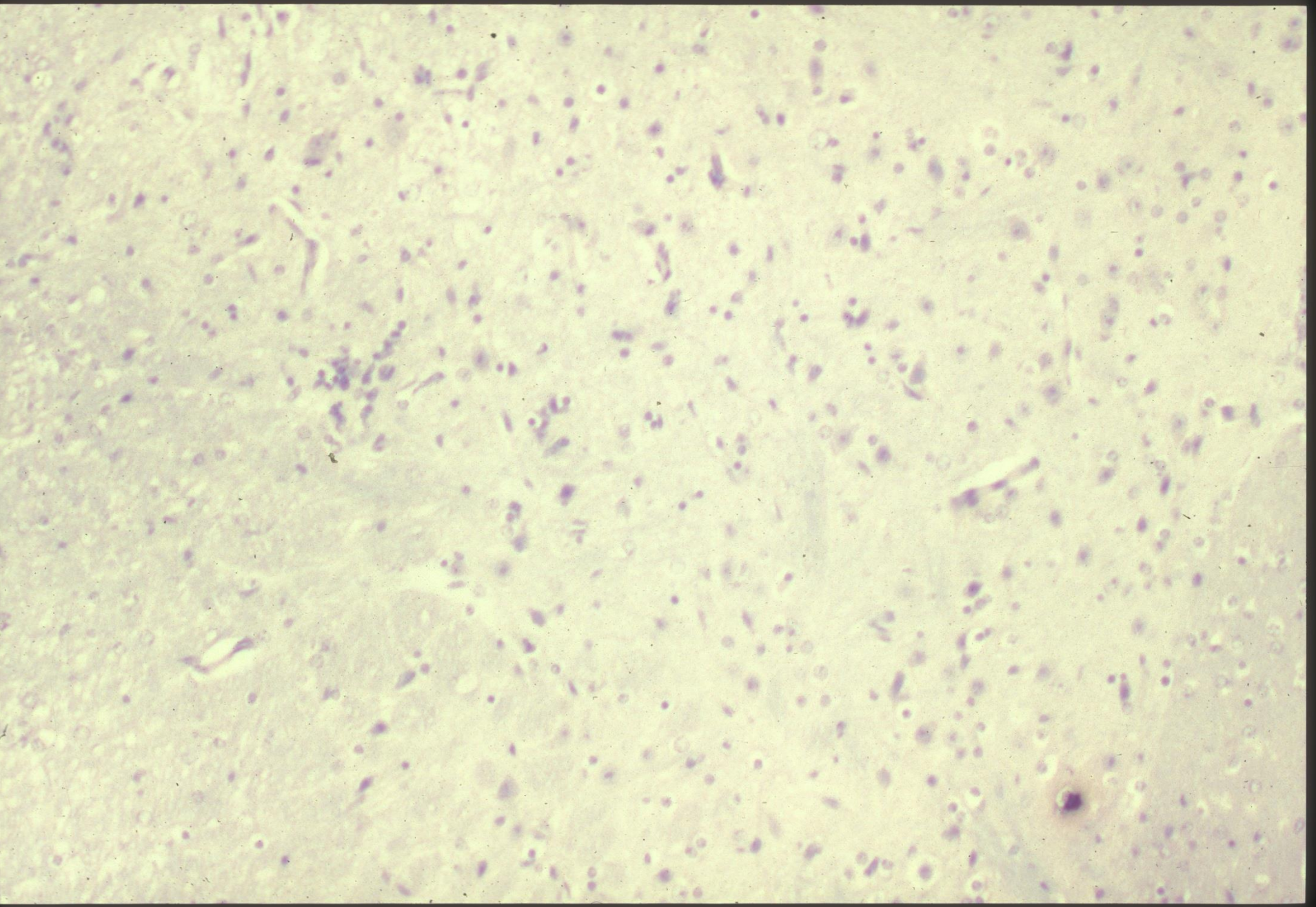






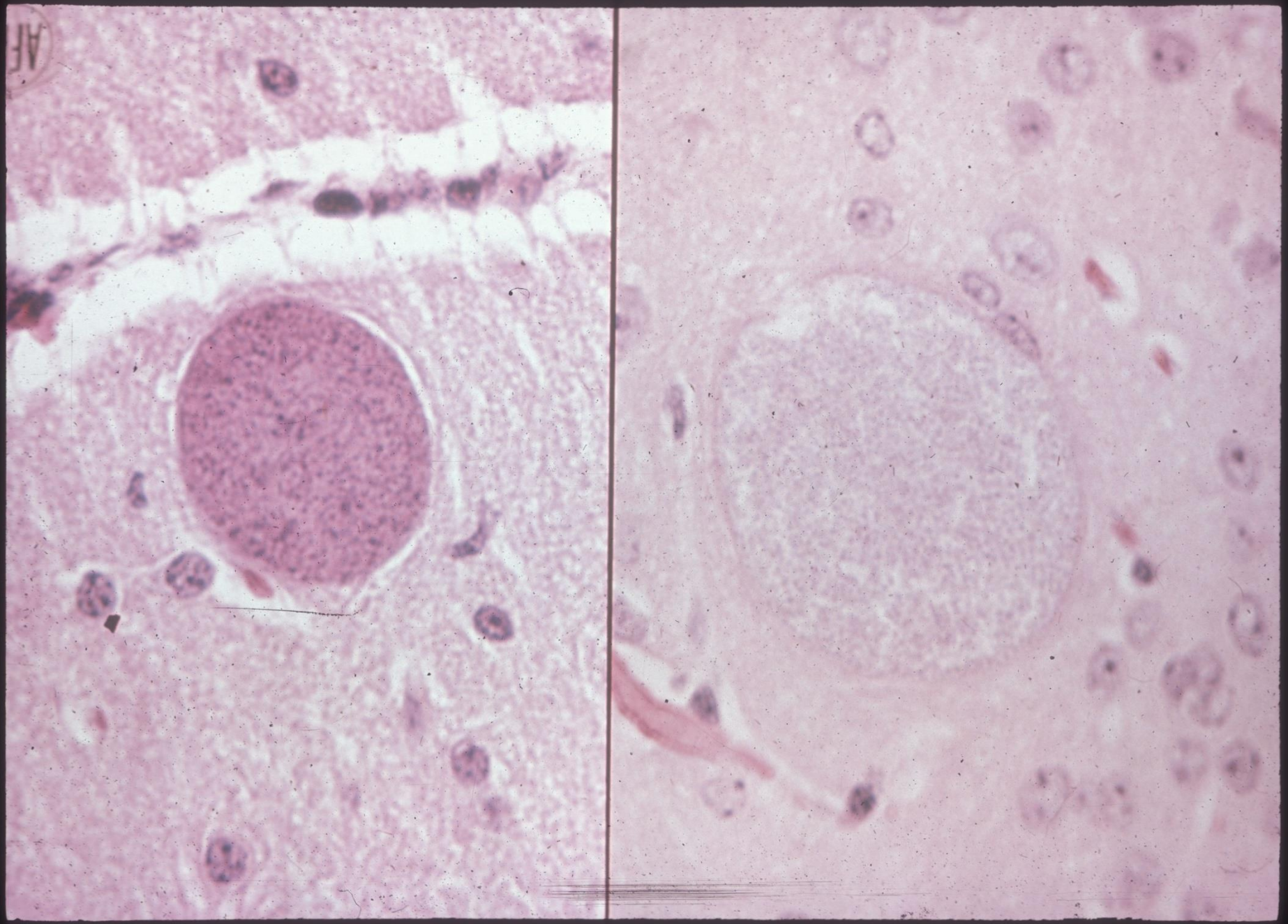


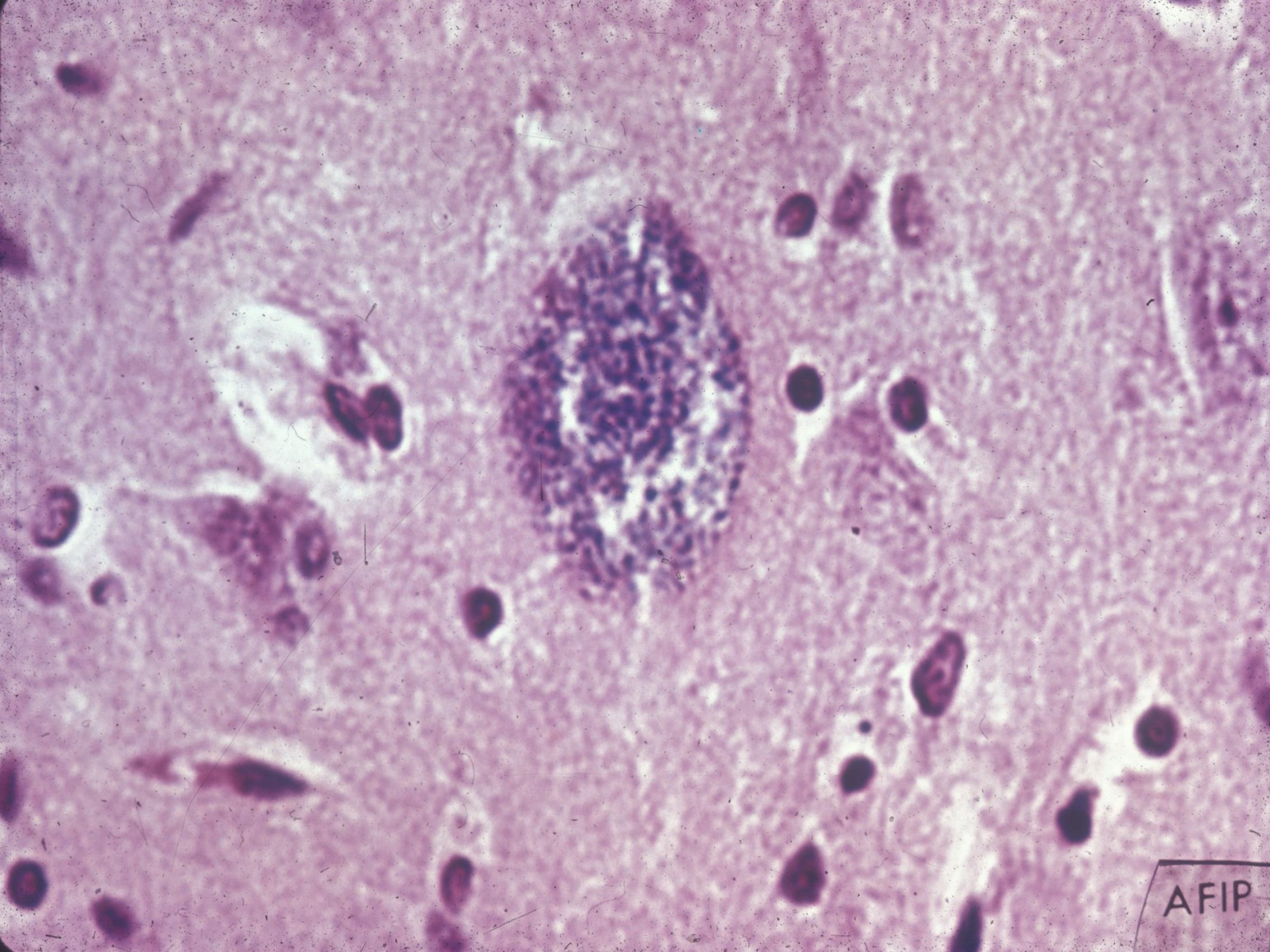




Toxoplasmosis versus Encephalitozoonosis

- Systemic microsporidial infections caused by *Toxoplasma gondii* and *Encephalitozoon cunuculi*
- Make sure rabbits (and other species) don't have *Encephalitozoon cuniculi*
- E. cuniculi causes systemic disease
- In the chronic phase produces brain cysts, which when rupture cause




AFIP

Mouse Hepatitis Virus

- Caused by a coronavirus
- Highly contagious
- Usually no clinical signs
- Usually no gross lesions
- Usually no microscopic lesions