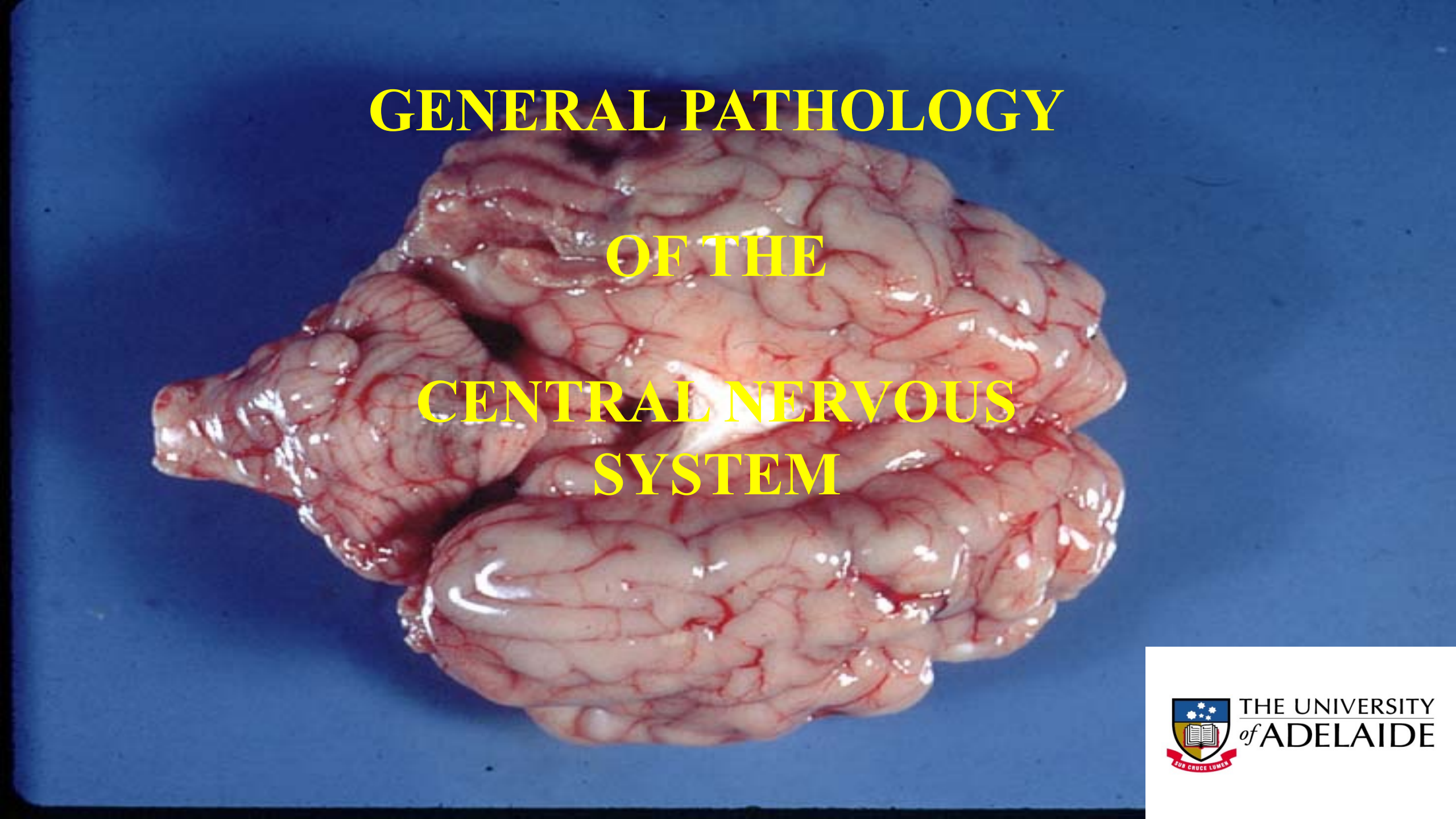
GENERAL PATHOLOGY

OF THE

CENTRAL NERVOUS SYSTEM



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GENERAL CONCEPTS

CNS RESPONSES

TO INJURY

Pathology is about pattern recognition

When a pathologist examines a tissue, particularly in histological sections, he/she will have in mind the normal architectural pattern

Then, any deviation from normality needs to be detected

CNS DISEASES

Is the CNS disease:

Inflammatory

Necrotising

Ischaemic-hypoxic

Demyelinating

Degenerative

Space-occupying lesion

Malformation

If, so what are the differential diagnoses?

CNS DISEASES

If a lesion is inflammatory, does the nature of the inflammatory reaction suggest a particular aetiology – infectious (and what organism) or degenerative

If a lesion is necrotic, is it confined to certain neuroanatomical sites and is it bilaterally symmetrical

CNS DISEASES

Inflammatory diseases tend to produce multifocal lesions, generalised neurological signs, and the clinical course progresses rapidly

Rapidly developing lesions tend to produce more severe neurological disturbance; when slower, may permit a degree of compensation by undamaged pathways

Congenital lesions are usually non-progressive

CNS DISEASES

If spongiform, in what neural element are the “holes” (neurons, glia, myelin)

If a space-occupying lesion, is it a tumour, haematoma or abscess and what are the sequelae

If a malformation, is it virally-induced or inherited

CNS DISEASES

Signalment (age, breed, and sex) is often important, especially with inherited degenerative disorders, many of which are breed-specific

If an intoxication is suspected, a thorough environmental examination is mandatory

Susceptibility of neural elements to injury

Cells of the CNS vary in their susceptibility to injury:

neurons > oligodendrocytes > astrocytes > microglia > blood vessels

Neurons have small energy reserves and a high metabolic rate and thus depend on an intact blood flow to supply oxygen and nutrients

There is scant regenerative capacity for neurons, but a small population of stem cells can potentially replenish neuronal populations

Blood-brain barrier protects against entry of harmful agents due to tight interendothelial junctions, paucity of micropinocytotic vesicles, and surrounding astrocytic end-feet

Nerve fibre regeneration in CNS/PNS

Nerve fibres in CNS have little regenerative capability, while those in PNS can regenerate under certain conditions

The outcome in the latter depends upon continuity of axoplasmic flow and correct alignment of proximal and distal portions of the endoneural tube in which the axon lies

Repair in the CNS

Healing in the CNS differs from other tissues and is achieved by proliferation of astrocytic processes

Fibroblasts are only found in leptomeninges and the outer few mm of the CNS where they are drawn into the cerebral cortex with blood vessels

Astrocytic capsules, unlike fibroblastic, tend to be poorly formed and easily broken down

Space-occupying lesions and ICP

The cranial cavity is nearly filled by brain, meninges and fluids and thus space-occupying lesions (tumours, abscesses, haemorrhages, and hydrocephalus) produce increased ICP and sometimes herniation of portions of brain into other cranial compartments

The CNS has an ability to resist infection but, once infection is established, it has a low degree of resistance

Poor outcomes are due, in part, to the complexity of the CNS and its vital role as a body organ

4 main portals of entry

Direct extension (e.g. from penetrating trauma, middle ear infection, nasal infection through cribriform plate of ethmoid bone separating nasal from cranial cavity, bacterial osteomyelitis)

Haematogenous – infectious agents, metastatic neoplasms

Leucocytic trafficking – macrophages/lymphocytes containing pathogens enter brain as part of normal cycling through CNS

Retrograde axonal transport – some viruses and bacteria

Defence mechanisms

Blood Brain Barrier

selective regulation of brain ECS and isolates brain from sudden biochemical changes in systemic circulation. Tight junctions prevent entry of proteins, hydrophilic molecules, and ions. Possesses transmembrane lipophilic pathways for small lipid molecules and numerous receptor-mediated transport systems for a range of molecules. Endothelium is negatively charged on its abluminal side (and basement membrane), impeding movement of anionic molecules such as chloride. Astrocytic foot processes cover >90% of abluminal capillary surface and astrocytic secretion of growth factors promotes BBB maintenance

Defence mechanisms

In non-BBB areas (area postrema, median eminence, neurohypophysis, pineal body, subfornical organ, commissural organ, and supraoptic crest), capillaries are fenestrated

Glia limitans – layer of astrocytic processes under pia mater

Blood-CSF barrier – formed by choroid plexus epithelium (but capillaries are fenestrated) and arachnoid membrane as both contain tight junctions

CSF-brain barrier (ependymal barrier) – but less efficient than BBB and materials in ventricles rather easily penetrate this barrier into the brain

Defence mechanisms

Innate and adaptive immune responses (see viral diseases for more detail)

Inflammation

BBB normally provides limited access to CNS for circulating cellular and humoral elements of the immune system, but monocytes and lymphocytes can penetrate an intact BBB and return to the circulation, thus providing immune surveillance of CNS

Inflammation disrupts the BBB and neutrophils enter (orchestrated by chemokines, selectins and integrins) and astrocytes/microglia become activated (via T lymphcyte-produced cytokines)

The diagnostic process

Clinical history

Signalment (age, sex, ethnicity/species&breed)

Clinical signs

Gross lesions

Microscopic changes

Pattern recognition

This is the key thought process in making a definitive diagnosis and involves

Recognition of deviations from the normal tissue architecture (pathological changes)

Gross examination

Record lesion size, shape, texture, colour, odour, and location

Distribution (random or symmetrical; focal, multifocal, coalescing, or diffuse)

Severity (mild, moderate or marked)

A morphological diagnosis should then be attempted

Pattern recognition: recognition of a lesion on the basis of previous experience

Hypothetico-deductive strategy: lesion is not immediately recognised (e.g. not previously seen or an atypical example of a well-known disease), but a hypothesis is formulated on the basis of background knowledge

Lesion categorised morphologically as

Degenerative/necrotic: cell swelling imparts pallor to tissue and necrosis may be distinguished by its focal or multifocal pattern and demarcation from adjacent viable tissue

Inflammation: readily appreciable when there is exudate, particularly on mucosal/serosal surfaces and, in absence of exudate, reddening or swelling

Vascular disturbance: e.g. haemorrhage, thrombosis, infarction

Growth disturbances

:

Tissue/organ is smaller (hypoplasia/atrophy)

Tissue/organ is larger (hyperplasia, hypertrophy, neoplasia)

Sometimes an aetiological diagnosis can also be made when the precise cause is evident at autopsy, especially if pathognomonic (i.e. specific for, or characteristic of, a particular disease)

Histology

10% formalin is the standard fixative – but prolonged fixation predisposes to cross-linking that masks antigens and interferes with IHC

The whole slide is first scanned under low power before focussing on lesions to avoid missing any other lesions

Haematoxylin and eosin (H&E) is the routine histological stain – negatively charged, acidic eosin has an affinity for cytoplasmic proteins and positively charged basic haematoxylin for nuclear structures

Fix large brains for >2 weeks, but not >4 weeks when formalin becomes acidic

With immersion fixation, unavoidable (especially mechanical) damage occurs during brain removal with development of artefacts – but intravascular pathology is undisturbed

Perfusion fixation via carotid arteries fixes neural tissue *in situ* in 2-3 h and minimises mechanical handling artefacts. Brain should remain *in situ* for >2 h – and up to 24 h with large brains to permit adequate penetration of the fixative

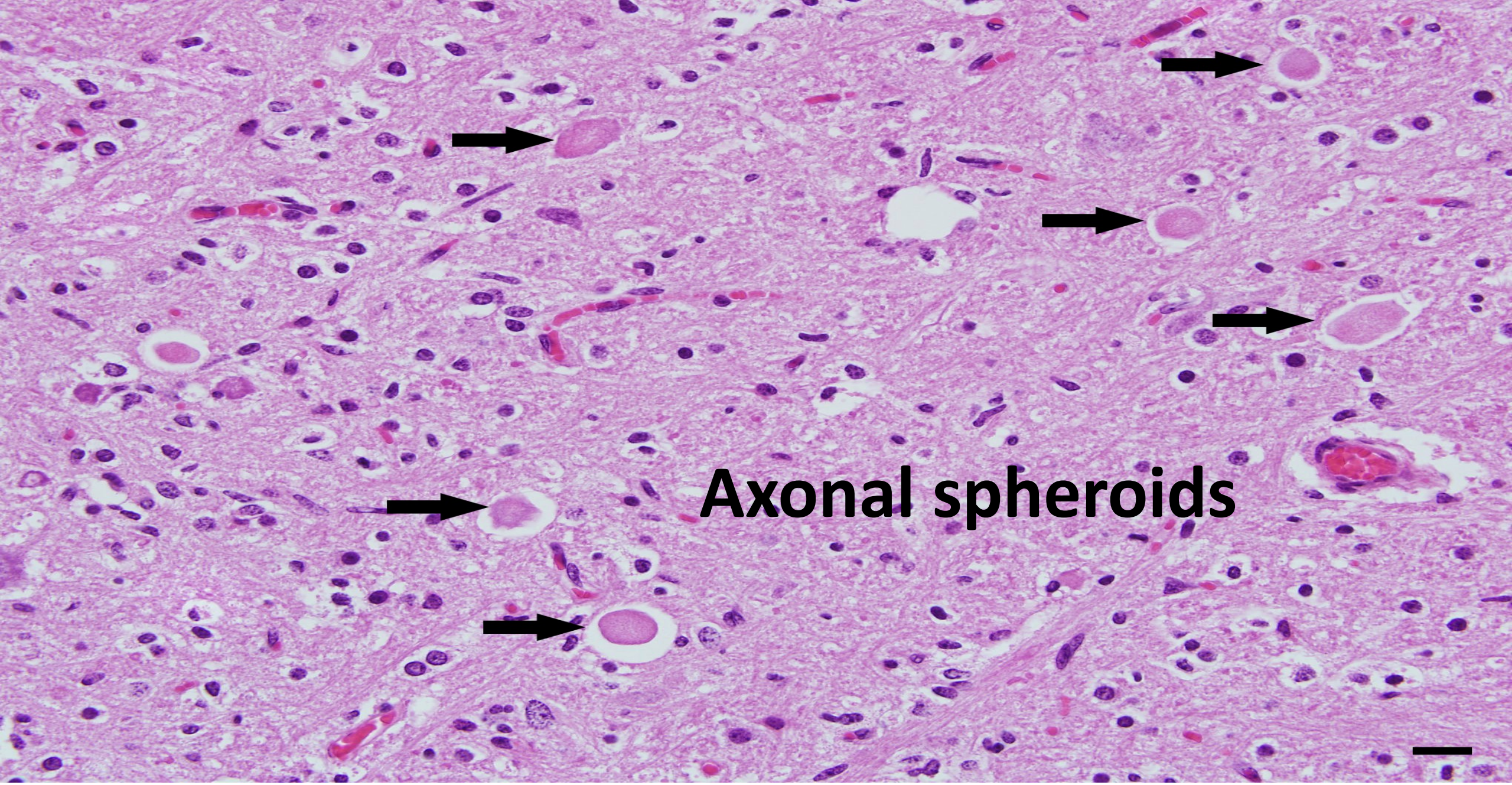
Post-mortem artefacts

Fresh brain is soft and easily damaged

Vacuolation of neuropil, neurons and glia is common

Perivascular and perineuronal spaces are enlarged due to swelling of astrocytic processes

Axonal spheroids are common in medullary cuneate and gracile nuclei in aged individuals


Axonal spheroids

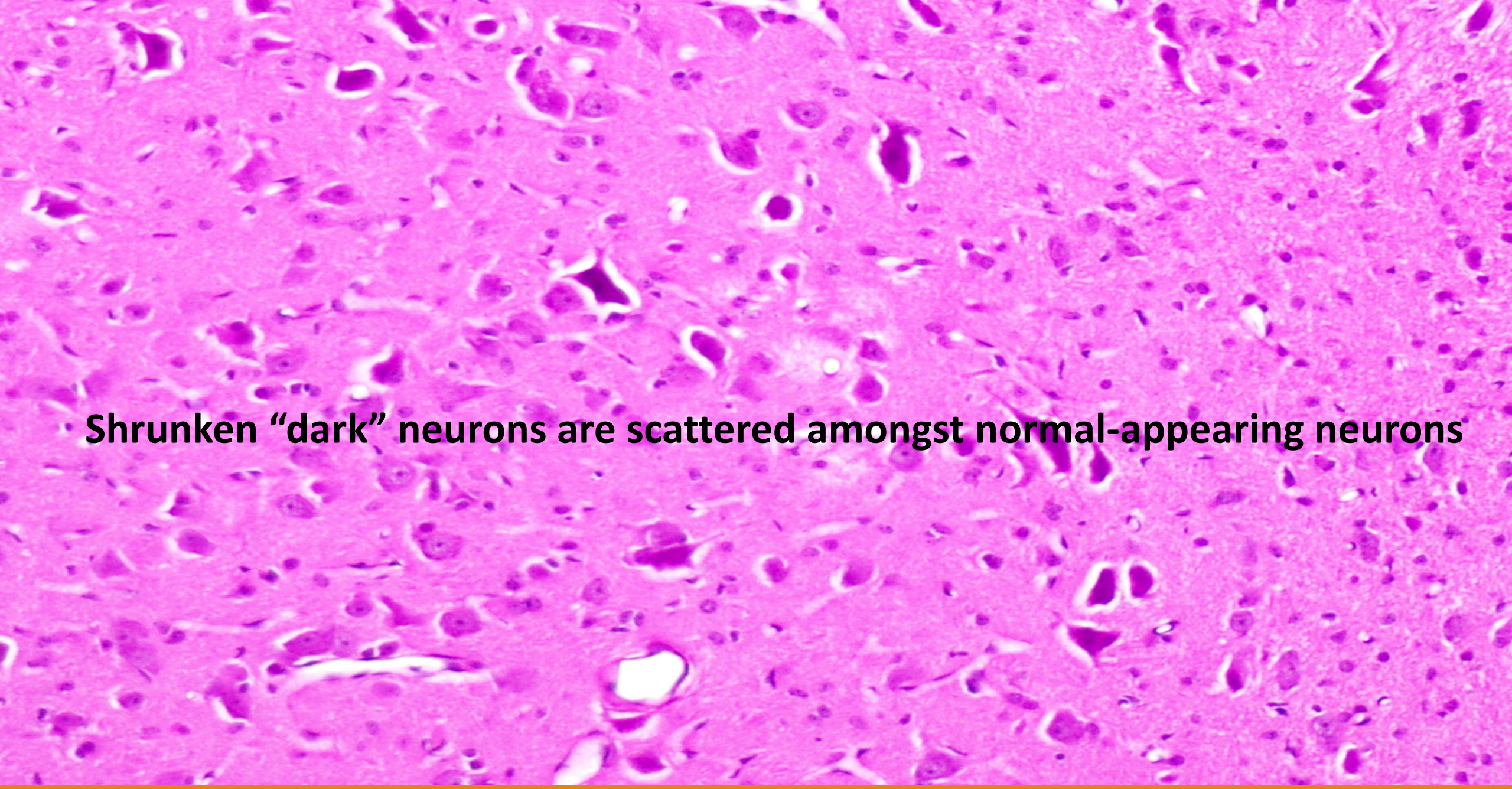
Shrunken, hyperchromatic “dark neurons”

Common, but decrease as P/M interval increases and minimised in humans by death-to-autopsy interval of >10 h before formalin fixation

Infrequently found in perfusion-fixed brains

Dark neurons have characteristic “corkscrew” dendrites

An early, potentially reversible, change with preservation of cellular substructure – serial studies show temporal reversal of cytoplasmic condensation


Shrunken “dark” neurons are scattered amongst normal-appearing neurons

Post-mortem decomposition of the brain

Sum of 3 processes:

Autolysis – cellular disintegration resulting from enzymatic autodigestion (normal brains tend to autolyse more rapidly than those with chronic disease)

Putrefaction – anaerobic degradation of tissue by invading intestinal bacteria

Decay – aerobic destruction of tissue by other microorganisms, sometimes abetted by arthropods and other necrophages

Histological diagnosis

Special stains can be used to identify, e.g. connective tissue, carbohydrate moieties, pigments, minerals, amyloid and microorganisms

Immunohistochemistry (IHC) can be performed on formalin-fixed, paraffin-embedded tissues, but usually requires some form of antigen retrieval to unmask tissue antigens and permit antibody binding

The autopsy

Remove the calvarium with hand/oscillating saw and collect intact brain (care with aerosols if infectious)

Incise dura over hemispheres and tentorium

Remove spinal cord – parasagittal cut (large animals) or dorsal laminectomy (small animals)

Immersion fix in 10-fold volume of formalin for 7-10 days (concentrated (40%) formalin for bovine brains or add to 10% until brain floats)

The autopsy

Handle fresh brain aseptically if bacterial culture indicated

Use minimal mechanical handling as produces histological artefacts such as “dark neurons”- much less in perfusion-fixed brains – potentially reversible as cell membranes/organelles intact

For electron microscopy, collect small blocks (2x2mm) of tissue and fix in 2.5% glutaraldehyde in buffer

Cytopathology of the nervous system

Neurons

Glia – astrocytes, oligodendrocytes, microglia

Microvasculature (blood-brain barrier)

Neurons

3 compartments – cell body (soma), which contains nucleus and cytoplasm (perikaryon), axons and dendrites

Neurons vary in size, shape and function and their cell bodies are organised into functional groups such as nuclei, grey columns, and cerebral lamina

Between neuronal cell bodies are intermingled neuronal and glial processes termed the neuropil

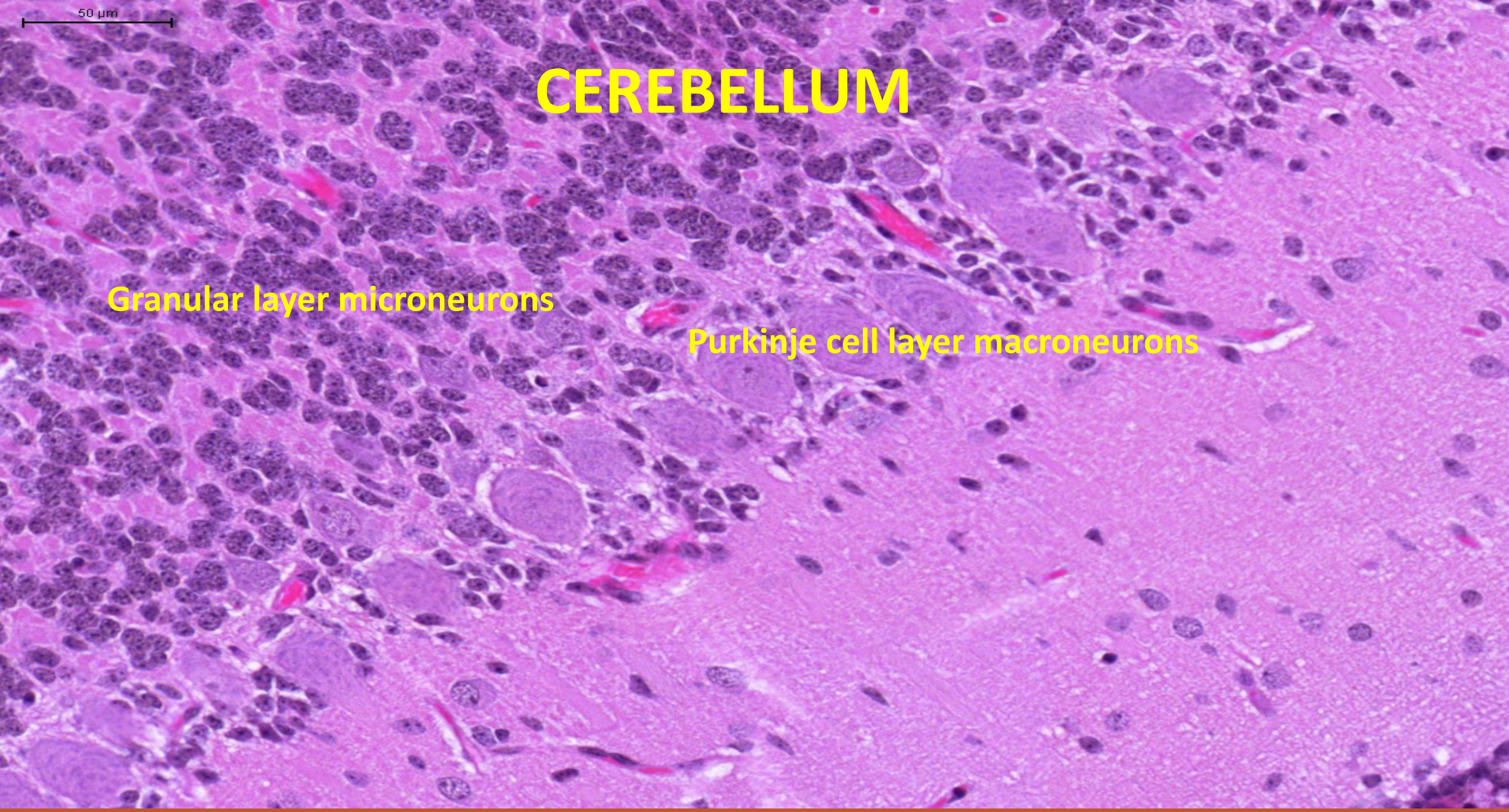
Neurons

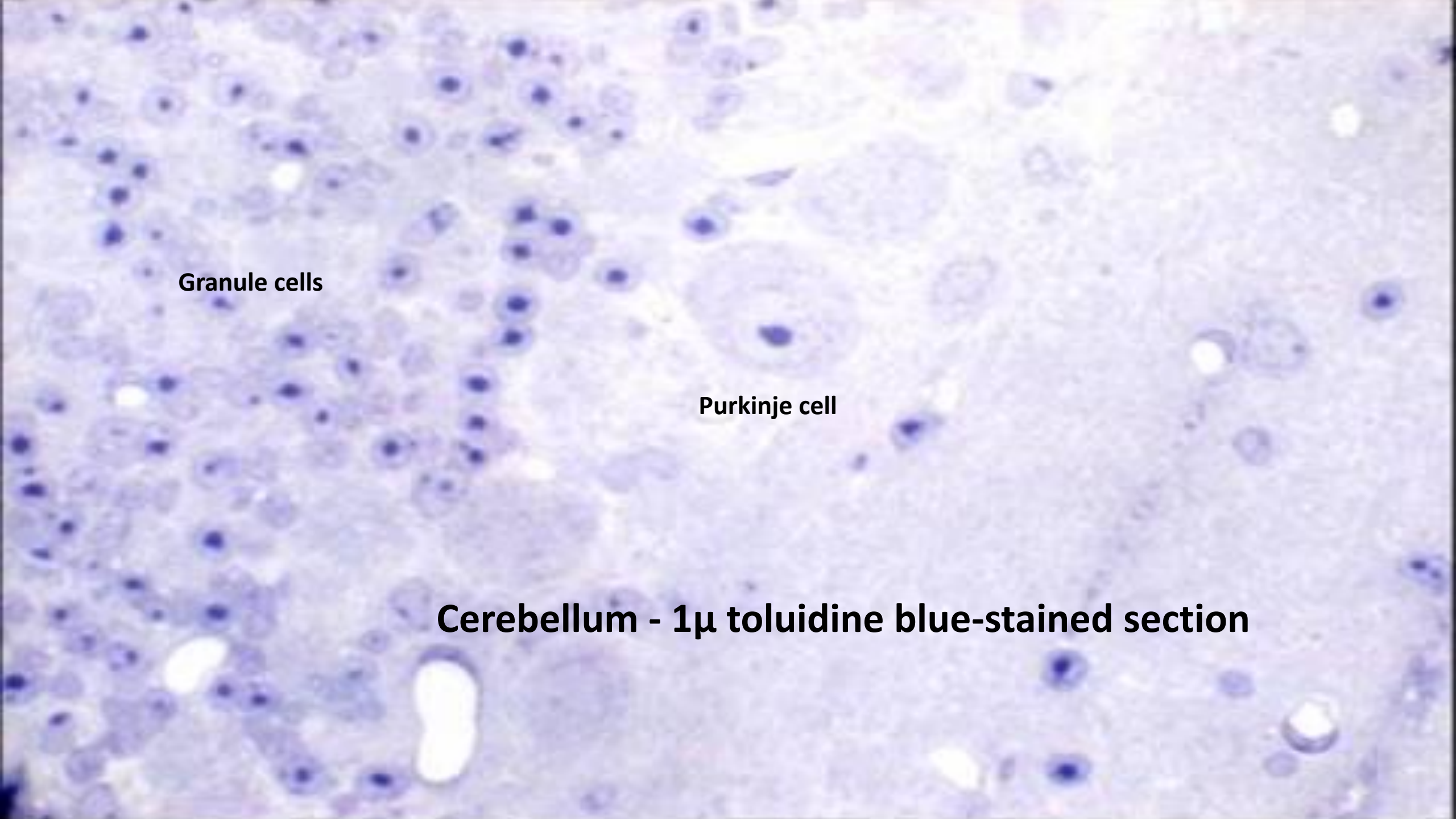
Neuronal cell bodies can be large (e.g. cerebellar Purkinje cells) or small (e.g. cerebellar granule cells)

Nuclei are generally centrally located with a prominent nucleolus in large neurons

Cytoplasm contains abundant rough endoplasmic reticulum (RER) (Nissl substance) that is responsible for protein synthesis

Grey matter is found in cerebral cortex; cerebellar cortex; central grey matter (basal nuclei or ganglia); and throughout the brainstem, often in nuclei


50 µm
CEREBELLUM
Granular layer microneurons
Purkinje cell layer macroneurons



Cerebellum - 1µ toluidine blue-stained section

Neurons

Generate and propagate electrical impulses – regulated by excitation and inhibition

Since neurons are the basic cell type in CNS, all neurological disease ultimately involves neuronal dysfunction

Post-mitotic cell with no regenerative ability, but stem cells found in certain areas with regenerative potential

Neuronal pathology

Central chromatolysis after axonal injury

Red cell change (“red-dead” neurons), especially after ischaemic-hypoxic insults

Vacuolar enlargement in lysosomal storage diseases

Cytoplasmic vacuolation in spongiform encephalopathies

Neuronal pathology (cont)

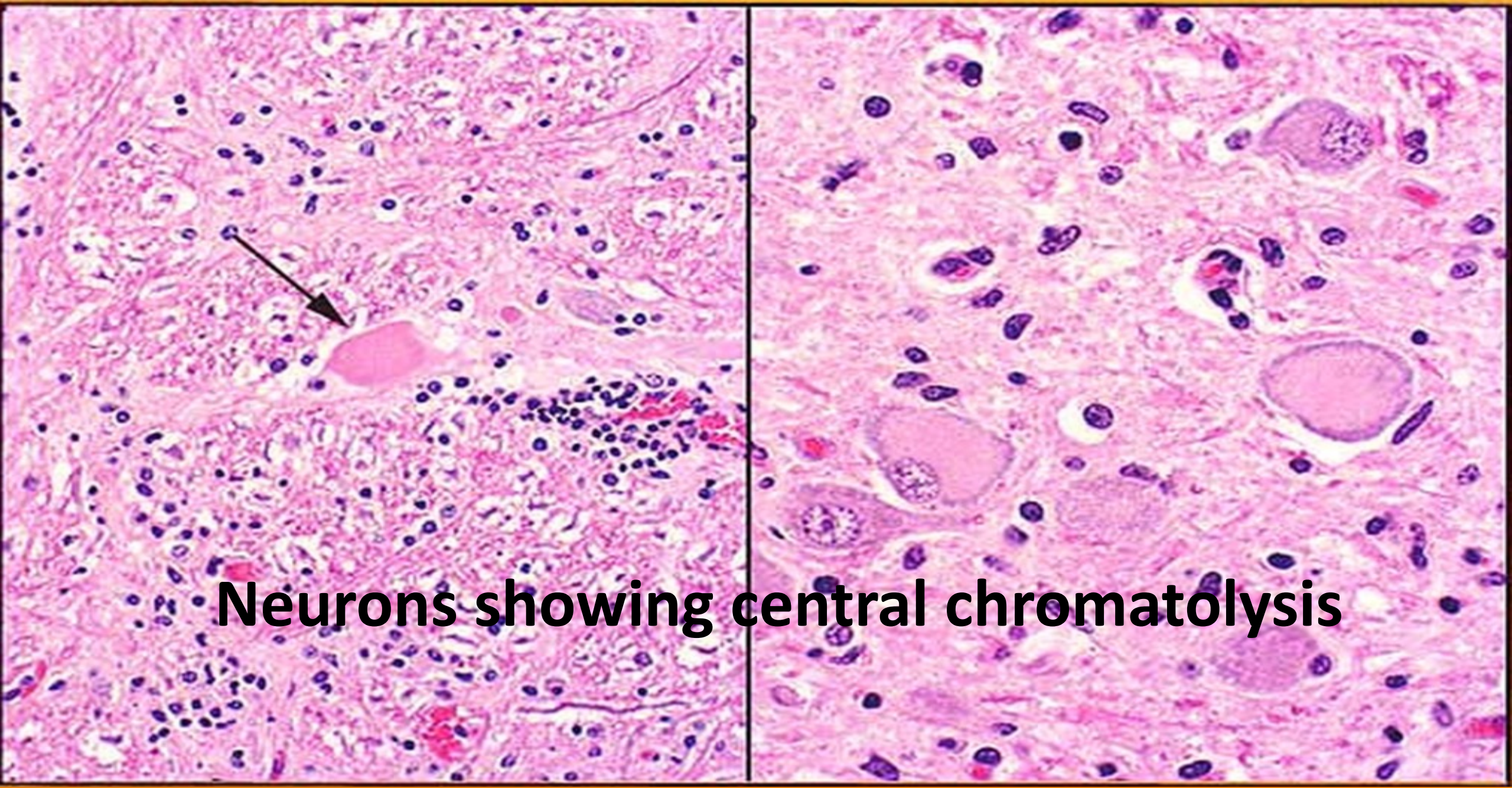
Accumulation of neurofilaments in neurodegenerative diseases

Inclusion body formation in certain viral diseases

Accumulation of lipofuscin in aged individuals

Central chromatolysis

Clear cytoplasmic space due to dissolution of Nissl substance (RER) – anabolic regenerative event during which neuronal metabolism is rearranged to support axonal regeneration (sometimes termed the axonal reaction)



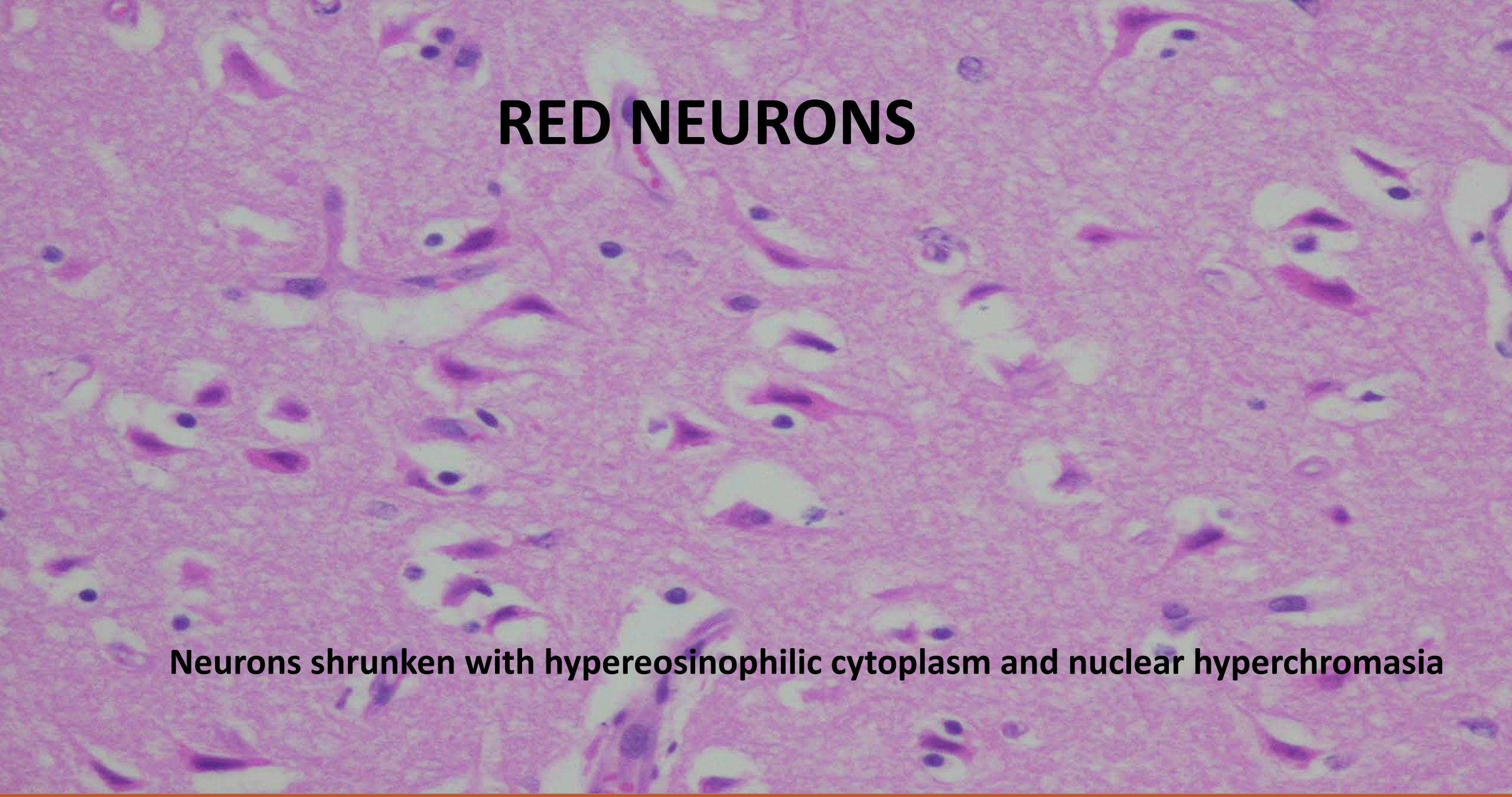
Neurons showing central chromatolysis

“Red-dead” neurons

Shrunken, hypereosinophilic cytoplasm with often nuclear pyknosis (frequently due to ischaemia-hypoxia)

Historically referred to as “ischaemic cell necrosis”, but also other causes

RED NEURONS



Neurons shrunken with hypereosinophilic cytoplasm and nuclear hyperchromasia

Excitotoxic neuronal damage

Neurons are very sensitive to excessive stimulation from excitatory amino acid neurotransmitters such as glutamate and aspartate when these are in excess

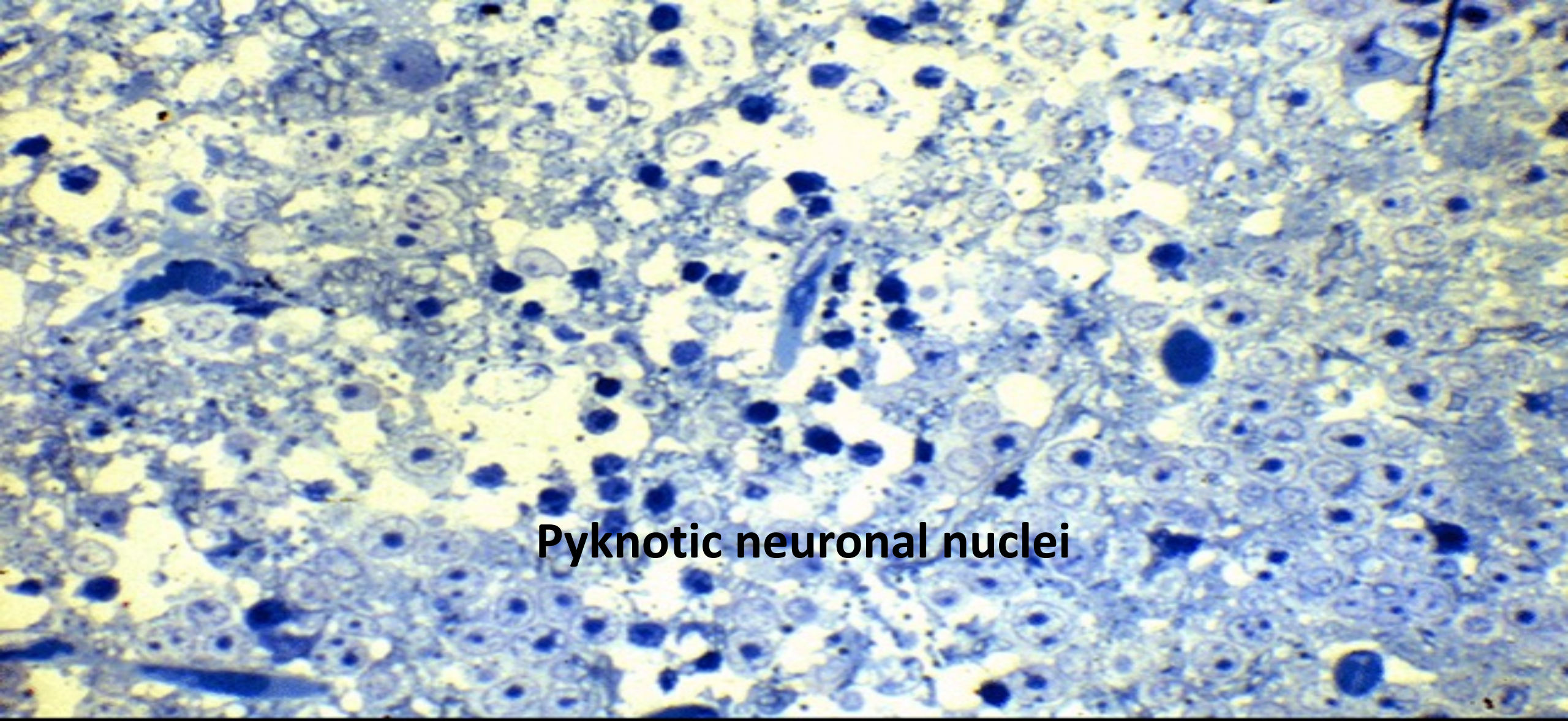
Since neurotransmitters can be toxic in excess, they are metabolised by astrocytes but, when this uptake capacity is exceeded, neuronal death can occur

Neuronal necrosis

Usually involves groups of cells (versus individual cells in apoptosis)

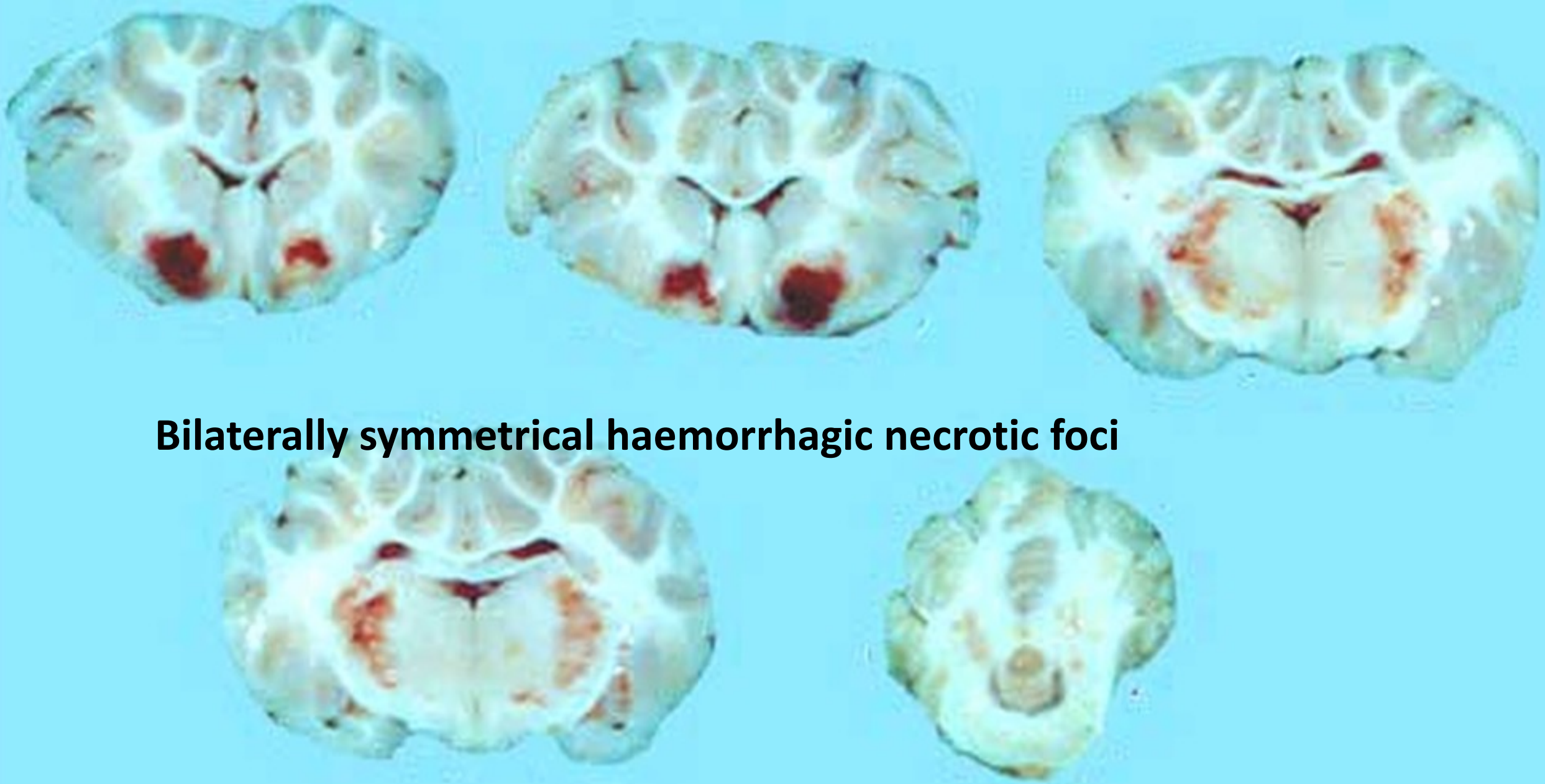
Sequential events are hydropic degeneration, mitochondrial swelling, nuclear pyknosis (nucleus small and shrunken and chromatin condensed) and karyorrhexis (fragmentation) and cell lysis (cell membrane damage and inability of the plasma membrane to control ion and fluid gradients)

Resulting cellular debris incites an inflammatory response (versus apoptosis)

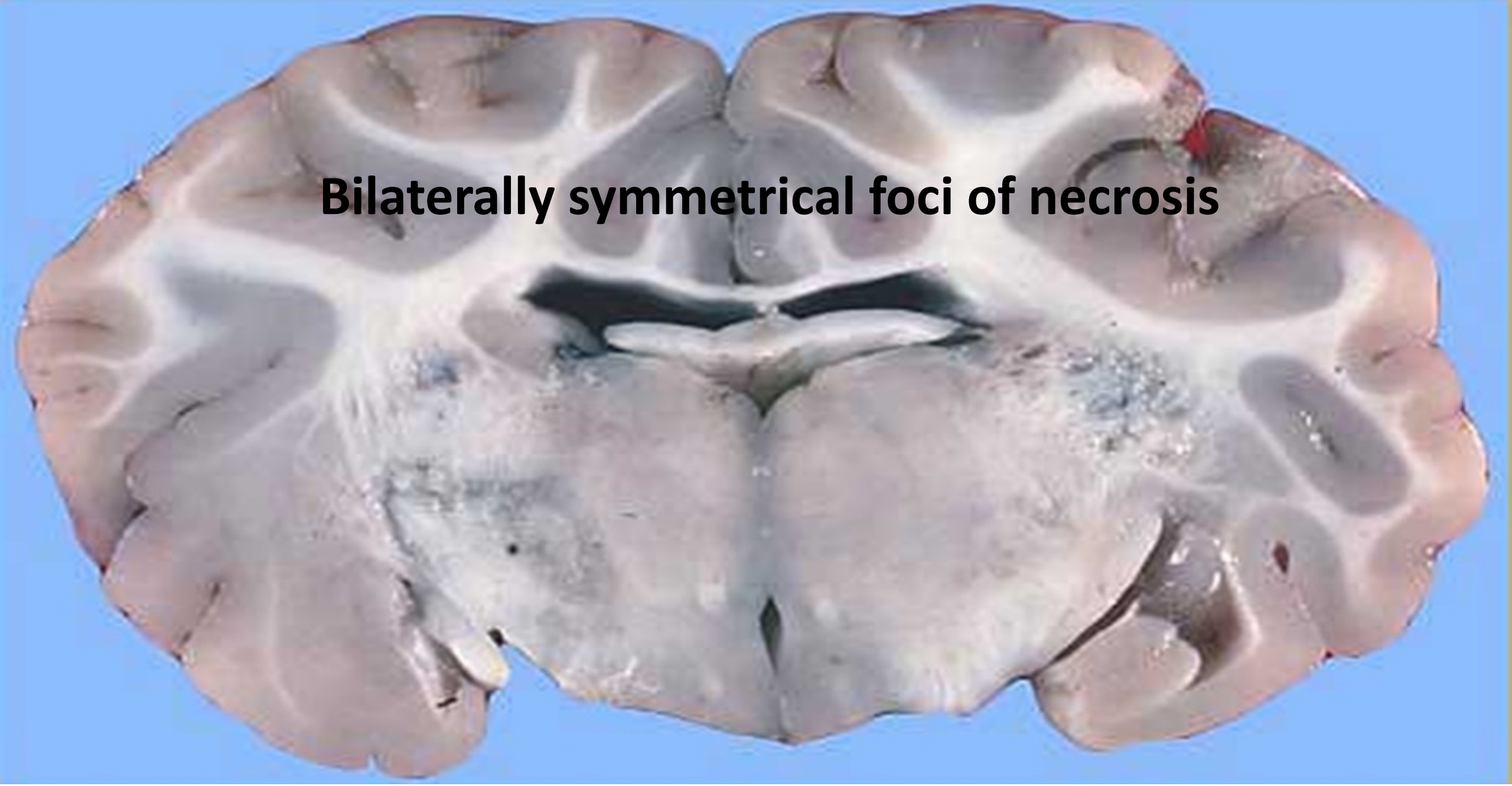


Focal necrosis of cerebellar granule cells [sheep]. [toluidine blue x200]

[Finnie, JW]



Bilaterally symmetrical haemorrhagic necrotic foci


Bilaterally symmetrical foci of necrosis

Apoptosis (programmed cell death)

Single cell-initiated, gene-directed cellular, self-destructive regulating mechanisms lead to "programmed" cell death

Used in CNS development to ensure correct migration and orientation of cell layers and removal of excess embryonic cells

To remove effete cells (i.e. cell turnover)

To maintain cell numbers in organ systems that have regenerative capability

Apoptosis

Signals are recognised and interpreted by cell membrane receptors (Fas, TNF) and a family of proteins (caspases) are activated, leading to destruction of cytoskeletal proteins and nuclear proteins such as DNA repair enzymes + activation of other degradative enzymes such as DNAases

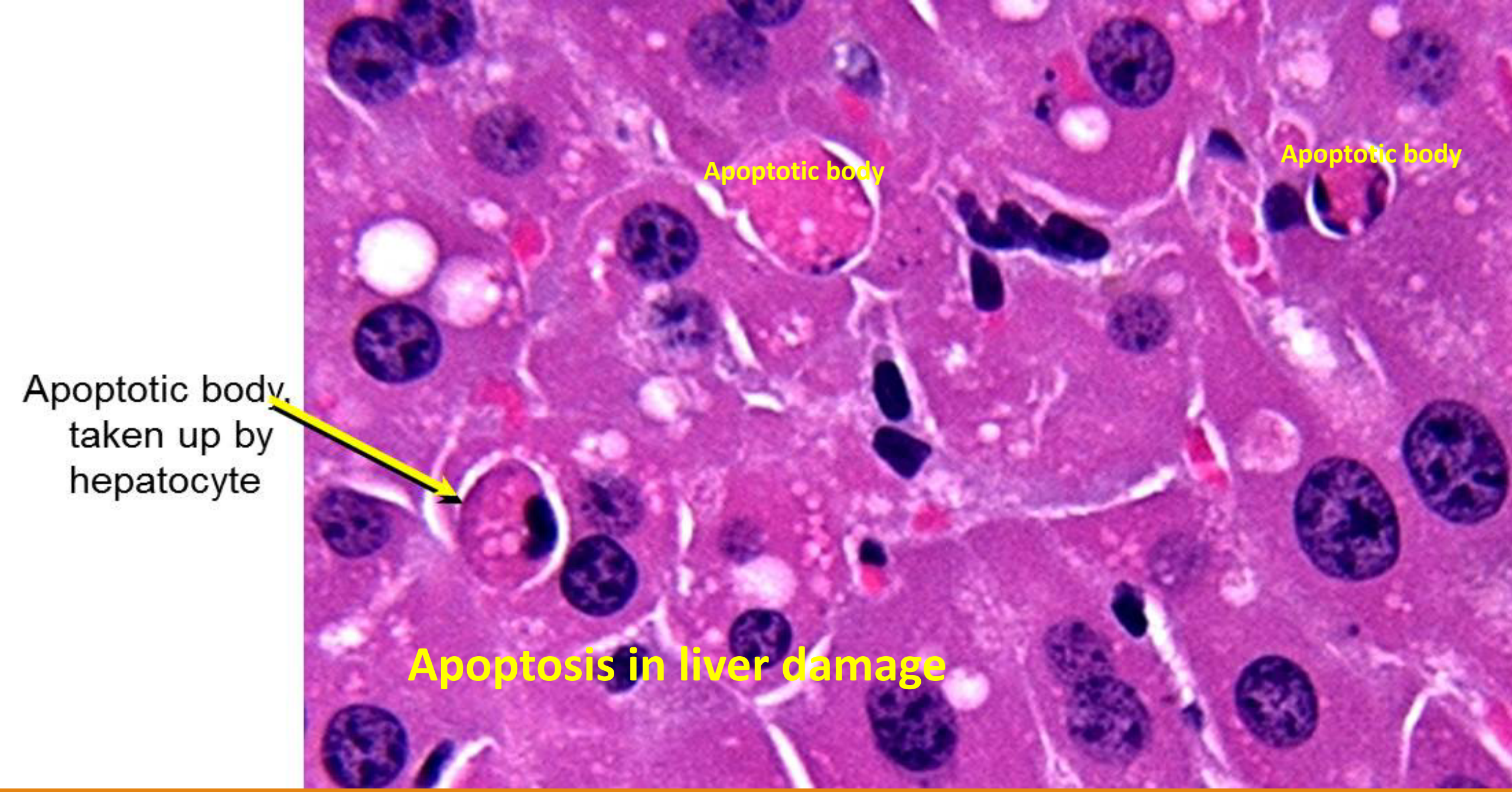
Morphological changes include shrinkage, cytoplasmic condensation and blebbing, and nuclear chromatin clumping and fragmentation (forming apoptotic bodies), which leads to removal by macrophages and sometimes contiguous viable parenchymal cells

Apoptosis does not incite inflammation

Apoptotic pathways

Intrinsic – cell kills itself when it senses stress. Mitochondria release cytochrome-c, which binds to apoptotic protease activating factor, then pro-caspase-9, which is cleaved to caspase-9, which then activates the effector caspase-3

Extrinsic - cell kills itself because of signals from other cells. TNF-α (cytokine produced by activated macrophages) is the principal effector. Binding of TNF-α to cell surface receptors initiates a pathway leading to caspase activation. Apoptosis can also be initiated by Fas (first apoptosis signal) leading to activation of caspases



Apoptosis in liver damage

Necroptosis

While necrosis is often viewed as passive cell death, it may be executed by a mechanism termed programmed necrosis, sometimes activated by the same molecules that activate apoptosis