The neuropathological examination: Do I need it?

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INTRODUCTION
The study of the pathology of the central nervous system (CNS) frequently constitutes an important step in helping the clinician to understand the morphological changes and the pathogenesis of canine and feline neurological diseases.

The neuropathological examination is part of the post-mortem procedure and the other organs of the body should always be examined in order to achieve a complete necropsy evaluation. In fact, even if the clinical signs are neurological, important information can be obtained by examining the major organs as well as the nervous system in case the animal was suffering from a generalised condition, such as septicaemia, viraemia, metabolic disorders or metastatic dissemination of tumours. In some cases, neurological signs can be the consequence of functional and anatomical changes of the nervous system due to severe extraneural disorders, such as liver and kidney failure, or metabolic alterations, such as hypoglycaemia and hypocalcaemia.

Samples for histopathological examination should be taken from any organ showing macroscopic abnormalities. Pathological examinations of peripheral nerves, autonomic nervous tissue and muscle tissue are performed, depending upon the case history, and require an accurate choice of samples and specific tissue treatment and fixation.

The neuropathological examination is considered the diagnostic ‘gold standard’ and is essential in cases where an in vivo diagnosis was not reached, and with a view to protecting other live animals and humans that may have been in contact with the animal in case of a fatal contagious disease (i.e. rabies, distemper, FIP). Thus, the neuropathological analysis is a key factor in driving further clinical studies, research and treatments. However, without the help of the primary responsible veterinary practitioner, the collection of samples and the further integration of the clinical and neurological signs with the anatomical and pathological findings are not possible.

PRINCIPLES OF NEUROPATHOLOGICAL EXAMINATION
The neuropathological diagnosis is based on the macroscopic and histopathological examination of representative areas of the CNS. This study includes three consecutive steps which are closely interrelated:
1. the morphological analysis of the lesions
2. their topographical distribution throughout the CNS
3. the critical integration of these findings with the clinical data and the necropsy findings.

With the exception of tumours, the morphological features of the diseases of the CNS are characterised by their association with the different cell types that constitute the nervous tissue. These lesions may involve the neurons, the astrocytes, the oligodendrocytes and the microglial cells. Vascular and meningeal cell changes, as well as alteration of neuroepithelial cells of choroid plexus and ependymal layer may also be associated in some diseases. All these tissue elements are closely interrelated and their reactions may contribute to determine massive tissue lesions, such as necrosis, spongiosis, demyelination and inflammatory lesions.

Topographical analysis requires the systematic examination of representative samples of brain and spinal cord. This can allow identification of different distributions of lesions and this is extremely useful when determining the possible aetio-pathogenesis of the disease. For instance, many inflammatory diseases show a preferential distribution of lesions in specific areas of the CNS, such as grey matter (polioencephalitis) or white matter (leukoencephalitis); most toxic/metabolic diseases show a bilateral and symmetrical distribution of lesions; some neurodegenerative disorders cause changes that involve selected, functionally-related neuro-anatomical structures (e.g. motor neuron disease).

Individual cell types of the CNS react to injury in non-specific ways, although it is the sum of the type (morphology) and distribution (topography) of these reactions which frequently gives specific nervous system diseases a recognisable, diagnostic appearance.
**REMOVAL OF THE BRAIN AND SPINAL CORD**

The post-mortem examination of the CNS should be performed following simple but precise procedures because of the fragility of the tissue and its anatomical intraosseal location. The animal’s body should be placed in ventral recumbency and the head is severed from the neck by cutting through the atlanto-occipital joint. The organs of the neck should have been previously removed during the routine post-mortem examination of the respiratory and alimentary tracts.

**Brain**

A midline incision is then made through the skin over the dorsum of the head from the nasal plate to the dorsal rim of the foramen magnum. The skin over the skull is then separated from the underlying tissue as far as the zygomatic arch and the temporal muscles removed. The skull is exposed and a transverse cut is made caudally to the orbital crests through the frontal sinus. This cut must be continued until the cranial cavity is entered. The brain could be slightly damaged at this point, but this damage is usually superficial and does not preclude an adequate neuropathological evaluation. Next, two cuts are made on each side of the skull from the lateral rims of the foramen magnum, dorsal to the occipital condyles. These cuts are extended rostrally, dorsal to the zygomatic ridge, to reach the first cut.

Removal of the cranial vault is accomplished by inserting a chisel into each lateral cut at the level of the nuchal crest and the transverse cut through the frontal sinus. With gentle leverage the cranial vault can be removed (Fig. 1). At this stage, the overlying dura and the tentorium cerebelli are severed and the brain is extracted by turning the skull upside down and cutting the cranial nerves with a scissors or a scalpel. This last procedure can be started either caudally or rostrally. The cranial nerves are cut in sequence, the brain being gently supported in the palm of the hand.

**Spinal cord**

Having exposed the vertebral column by removal of skin and muscle, the spinal cord is removed after cutting the dorsal arches of the vertebrae, from a rostral to caudal direction. Each arch is cut with bone forceps, avoiding damaging the cord. When the cord is exposed, it can be removed by cutting the nerve roots, distal to the dorsal root ganglia, while the cord is gently raised by forceps grasping the dura. Avoid bending the cord by excessive raising, since this will cause histological artefacts of the nervous tissue.

**EXAMINATION OF THE BRAIN, SPINAL CORD AND RELATED STRUCTURES**

Once the brain and the spinal cord have been removed, an overall examination can be performed. Haemorrhage and laceration are usually self-evident (Fig. 2), as well as changes in the bony structures, such as fractures, luxations and malformations. Presence of extra-axial, space-occupying lesions, such as tumours or abscesses, may be detected within
the cranial cavity (Fig. 3) or the epidural space (Fig. 4). Some tumours may invade the brain by spreading from the nasopharynx (Fig. 5) or the middle ear (Fig. 6) and penetrating the bones of the skull.

Brain swelling can be indicated by coning of the cerebellum, in which the caudal part of the vermis is flattened and elongated, being forced under the dorsal rim of the foramen magnum (Fig. 7). Flattening of cerebral gyri and underlying, fluctuating softening of the cerebral hemispheres, may indicate hydrocephalus. There may be single or multiple foci of induration, malacia or discoloration, perhaps caused by tumours (Fig. 8) or abscesses.

Any space-occupying lesion within the vertebral canal frequently causes indentation or distortion of the normal structure of the cord because of a compressive effect (Fig. 9). Swelling of the cord may be caused by an intramedullary tumour such as a glioma or a nerve-sheath tumour that invades the spinal cord. In this latter case, severe swelling, tortuosity and firmness of neoplastic nerve roots is a concurrent finding (Fig. 10).

The empty vertebral canal can be further examined for evidence of prolapsed discs or tumours, running a finger along the floor of the canal. Any abnormal tissue or neo-formation should be sampled for histopathological examination.
FIXATION OF THE CNS

Once the overall inspection of the CNS is completed, it should be fixed by immersion in a 4% isotonic formalin solution, in a volume equal to 10-20 times the volume of the brain. The solution can be made by adding nine parts of an isotonic solution of NaCl to one part of commercial formaldehyde (usually at 37-40%). Fixation should preferably be carried out as soon as possible after the death of the animal to avoid autolytic artefacts in the nervous tissue. If this is not possible, the CNS material (or the entire head of a dog or cat) can be stored in a refrigerator for a maximum of 24 hours at 4°C. The nervous tissue should never be stored in a freezer because it causes ice crystals to form and these will subsequently appear as empty spaces in the histological sections.

The spinal cord should be fixed avoiding excessive bending of the tissue. For this reason, cord segments of appropriate length should be cut and individually labelled as to level, or put in different labelled containers. In the absence of macroscopic changes, representative segments of cervical, cervicothoracic, and lumbosacral spinal cord should be sampled.

Fixation of the entire brain should take a minimum of five days for a cat and ten days for a large-breed dog. The fixing solution should be replaced with a fresh solution after 24 hours of fixation. After this period, the whole brain, spinal cord and samples of other tissues can be submitted to the neuropathologist for histological preparation and examination. The samples should always be accompanied by a detailed history, including complete signalment, clinical history and ancillary investigations.

SAMPLING OF THE CNS

After fixing is completed, the brain can be cut transversely into slices 3-4 mm thick. The period of fixation ensures that the brain will not become distorted by uneven shrinkage, which may occur if it is sectioned as a fresh specimen, with the slices put into fixative subsequently.

While the brain is being sliced, it is examined, looking for:
- any unilateral or bilateral, symmetrical or asymmetrical variation of shape and volume of the hemispheres (Fig. 11)
- evidence of dilated ventricular system (Fig. 12)
- atrophy of cerebral cortex or cerebellum (Fig. 13)
- presence of focal or multifocal space-occupying lesions (Fig. 14).

Other pathological features may include:
- haemorrhage
- discoloration of grey or white matter
- malacia (Fig. 15).

If changes can be detected macroscopically, the brain area involved should be sampled for histopathological examination. In the absence of gross changes, standard levels of brain and spinal cord are selected in our laboratory:
- the cerebral hemisphere at the level of caudate nucleus and internal capsule
- the contralateral cerebral hemisphere at the level of thalamus and hippocampus
- mesencephalon

![Fig. 11: Post-ischemic atrophy of the left cerebral hemisphere of a cat.](image1)

![Fig. 12: Congenital internal hydrocephalus in a kitten: asymmetric enlargement of the ventricles.](image2)

![Fig. 13: Congenital hypoplasia of the cerebellum in the brain on the right, compared with cerebellum of a normal cat on the left.](image3)

![Fig. 14: Dog - multiple metastases of haemangiosarcoma.](image4)
pons and cerebellum
caudal part of the medulla oblongata.

The transverse sections of the spinal cord should comprise the nerve roots and dorsal ganglia. Adjacent samples of the spinal cord should be sectioned longitudinally to detect white matter changes, such as slight Wallerian degeneration. Specimens of tissue that have not been sampled should be retained, in case it proves to be necessary to examine further regions of the CNS.

CONCLUSIONS
The anatomical pathological examination of the CNS requires specific techniques and procedures that may involve extra effort and time for the practicing veterinarian performing a necropsy. Nevertheless neuropathological examination is extremely useful in the understanding of individual cases, as well as for advancing overall knowledge of animal neurology.

The role of the referring veterinarian is the first and the fundamental step in reaching the final diagnosis and in helping a better understanding and consequent treatment of neurological diseases.

FURTHER READING