**Figure Legend:** Figure 1 Incidental thalamic mineralization in a female F344/N rat from a subchronic study. The arrows identify focal mineral deposits. Figure 2 Incidental thalamic mineralization in a male B6C3F1 mouse from a chronic study. Note the irregular shape of the deposits at this high-magnification image. Figure 3 A focus of mineral deposition in a site of former cerebellar neuronal necrosis (arrow) in a control male F344/N rat from a subchronic study. Figure 4 Higher magnification of a focus of mineralization (calcospherites) at a site of former cerebellar internal granule cell necrosis (arrow) in a male F344/N rat from a subchronic study. Note the concentric lamellar nature of the concretions. Figure 5 Brain necrosis with ossification in a male F344/N rat from a chronic study. Note the osteoid (arrow) and osseous metaplasia (arrowhead) in a zone of former brain necrosis.

**Comment:** Mineralization in the CNS comprises either incidental multifocal microscopic deposits of mineral or secondary dystrophic calcification occurring after necrosis. Figures 1 and 2 show incidental multifocal microscopic deposits of mineral in the thalamus of rat and mouse, respectively. The thalamic distribution is usually bilateral but not necessarily symmetrical. It is a more common finding in B6C3F1 mice than in rats. The particles of mineral are often found, on close inspection, to have a multilamellar structure and are referred to neuropathologically as calcospherites. They appear to be located in the neuropil, or commonly associated with the intimal basement membrane of capillaries and small blood vessels. However, it is unusual to find any evidence of thalamic microinfarcts as a result of their presence, even when vessels are affected and the lumen is narrowed. The lesion is probably the result of focally abnormal tissue homeostasis with respect to calcium metabolism, and in these incidental cases, it is unlikely to represent dystrophic mineralization secondary to necrosis. More recently, thalamic lesions in aging mice have been associated with mice lacking vitamin D receptor. The occurrence of thalamic mineralization as calcospherites in all NTP rodent study groups confirms the incidental idiopathic nature of this phenomenon. When such lesions are seen in treated groups only, or when noted at other sites in the nervous system, careful evaluation for evidence of dystrophic mineralization, systemic hypercalcemia, vitamin D excess, or other forms of abnormal calcium homeostasis and metabolism should be considered.
On the other hand, mineral deposits, secondary to necrosis, are attributed to the commonly observed but complex process of dystrophic mineralization. Dystrophic mineralization is often seen as lamellar calcospherites found secondary to small clustered regions of neuronal necrosis at various neural sites, such as depicted in Figures 3 and 4. Figure 3 shows the aggregation of mineralized bodies (arrow) in sites of former granule cell necrosis in the cerebellum. Note the loss of neural tissue represented by spaces formerly occupied by internal granule cells, and the presence of multiple small, dark, round bodies. The presence of such mineralized foci may lead to disruption of the tissue during microtomy. The mineralized foci shown in Figure 3 should not be confused with the artifactual presence of boney spicules often carried into brain from overlying skull fragments during brain extraction/trimming or with heterotopic bone tissue. The round, often concentric, lamellar appearance of calcospherites, shown at higher magnification in Figure 4, is typical of late stages of mineralization of necrotic brain tissue. Calcospherites are deposited in a region of former cerebellar internal granule cell necrosis. Note the concentric laminated structures (arrow) of the larger bodies that are apparently absent in smaller ones. The deposition of mineral in the form of calcospherites in these cases requires several weeks to form after the necrotizing insult.

Figure 5 shows a region of prior necrosis adjacent to piriform cortex with an uncommon reactive focus of osteoid (arrow) and ossification (arrowhead). A large zone of brain necrosis is replaced by a progression of mineralized bodies of several types. To the right are some smaller basophilic calcospherites, and to the left are larger zones of necrotic brain tissue transformed into both osteoid, composed of pink hyaline material, and a more advanced stage of osseous metaplasia, where dense basophilic mineral and osteocytes are apparent.

**Recommendation:** Mineralization in the thalamus is not interpreted as a treatment effect unless it is absent in controls. If treatment or dose related, the lesion is diagnosed and graded based on the extent of the lesion. Dystrophic mineralization should be diagnosed simply as mineralization regardless of the variation in the spectrum of residual lesions such as calcospherites, or osteoid or osseous metaplasia. Such variations should be included in the narrative. The diagnosis should include the subsite unless the incidence is multifocal across
brain regions. Severity grading is based on the extent of the lesion. In the presence of concurrent lesions, the most severe lesion is typically diagnosed. Other concurrent lesions may be diagnosed separately, if warranted by the severity.

References:


Authors:

Peter Little, DVM, MS, PhD, DACVP
Neuropathology Consultant
Experimental Pathology Laboratories, Inc.
Research Triangle Park, NC

Deepa B. Rao, BVSc, MS, PhD, DABT, DACVP
NTP Pathologist (Contractor)
Integrated Laboratory Systems, Inc.
Research Triangle Park, NC