

Melanosis of the Dentate Nucleus in Two Cynomolgus Macaques



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Abstract

Introduction

Melanin deposits occasionally occur in central nervous system tissues. In cynomolgus macaques, spontaneous perivascular melanin pigment occurs in the meninges and in the cerebral cortex. Additionally, neuromelanin pigment accumulates with age in the substantia nigra and locus coeruleus in human and non-human primate brains. We report pigmentation consistent with melanin specifically within the cerebellar dentate nucleus of two juvenile cynomolgus macaques. Melanosis in the dentate nucleus has been reported in people but to our knowledge has not been previously reported in non-human primates.

Methods

Two 18-month-old male Chinese origin cynomolgus macaques were part of a study in which animals received a single dose of an AAV gene therapy via direct brain administration, remote from the cerebellum. On study, animals had regular detailed clinical observations and had neurologic assessments. Euthanasia occurred at scheduled study termination, 58 days post dose.

Results

There were no notable in life findings. At necropsy, both subjects had gross observations of symmetrically bilateral black discoloration in the cerebellar white matter. Microscopically, this correlated to dark brown granular pigment in the neuropil of the dentate nucleus of the cerebellum. The pigment was not within neuronal soma.

Conclusion

Pigmentation of the dentate nucleus in these two macaques closely resembles melanosis of the dentate nucleus reported in people. The underlying pathogenesis of this condition is unknown. No relationship to the test article was determined in this study. This may represent a rare spontaneous condition in cynomolgus macaques.

Introduction

- Pigment in the central nervous system (CNS)
 - In cynomolgus macaques, melanin pigment occasionally occurs in the meninges, as a product of leptomenigeal melanocytes, and in the cerebral cortex, where it is often perivascular and associated with astrocytes.¹⁻³
 - Neuromelanin is a type of melanin pigment produced in catecholaminergic neurons, where it accumulates in the neuronal cytoplasm with age, most notably in the substantia nigra and locus ceruleus.^{4,5}
 - Non-melanin pigments, such as hemosiderin and lipofuscin, can also occur in the CNS.
- Melanosis of the dentate nucleus (DN) is a rarely reported condition in people.
 - Between 1941-2017, there were 19 reported cases (13 females, 6 males) ranging in age from 26-104 years (mean 70.2, median 72).⁶
 - There are no common clinical conditions or histories among affected individuals.
- Pigment in the DN has not been previously reported in non-human primates.

Methodology

- Both affected animals were 18-month-old male Chinese origin cynomolgus macaques
- A single dose of an AAV gene therapy was administered direct to the brain in a region remote from the cerebellum
- On study, the animals had regular detailed clinical observations. Neurologic assessments were also performed.
- Euthanasia occurred at scheduled study termination, 58 days post dose.
- At necropsy, animals were perfused with saline. The unfixed brains were coronally sectioned followed by immersion fixation in 4% paraformaldehyde.
- Fixed brain samples were routinely processed for paraffin embedding and sectioning. Tissue sections were stained with hematoxylin-and-eosin (H&E).

Conclusions

- This finding was not associated with any neurologic abnormalities.
- No relationship to the test article was determined in this study.
- This may represent a rare spontaneous condition in cynomolgus macaques.
- **The location and microscopic appearance of the pigment in these two macaques closely resembles cases of melanosis of the dentate nucleus reported in people.**
- **The underlying genesis and clinical significance remains unknown.**
- Melanin in the DN has been demonstrated within astrocytic processes.⁶⁻⁸ Melanin deposits in the cerebral cortex in macaques have also been associated with astrocytes², suggesting a potential shared mechanism of formation.
- A process of involution and/or a relationship to lipofuscin has been postulated for melanosis of the DN, based on the generally advanced age of affected individuals. However, the young age of these affected cynomolgus monkeys suggests other factors, perhaps even congenital factors, play a role in this condition.
- Identification of additional cases of melanosis of the DN may shed further light on the pathogenesis of this rare condition in both cynomolgus macaques and humans.

References

1. Chamanza R, Marxfeld HA, Blanco AI, Naylor SW, Bradley AE. (2010) Incidences and range of spontaneous findings in control Cynomolgus monkeys (*Macaca fascicularis*) used in toxicity studies. *Toxicol Pathol.* 38:642-657.
2. Ito T, Chatani F, Sasaki S, Ando T, Miyajima H. (1992) Spontaneous lesions in cynomolgus monkeys used in toxicity studies. *Jikken Dobutsu.* 41(4):455-69.
3. Sato J, Doi T, Kanno T, Wako Y, Tsuchitani M, Narama I. (2012) Histopathology of incidental findings in Cynomolgus monkeys (*Macaca fascicularis*) used in toxicity studies. *J Toxicol Pathol.* 25(1):63-101.
4. Moses HL, Ganote CE, Beaver DL, Schuffman SS. (1966) Light and electron microscopic studies of pigment in human and rhesus monkey substantia nigra and locus coeruleus. *Anat Rec.* 55(2):167-83.
5. Herrero MT, Hirsch EC, Kastner A, Luquin MR, Javoy-Agid F, Gonzalo LM, Obeso JA, Agid Y. (1993) Neuromelanin accumulation with age in catecholaminergic neurons from *Macaca fascicularis* brainstem. *Dev Neurosci.* 5(1):37-48.
6. Hopley RT, Haller E, Rojiani MV, Rojiani AM. (2017) Morphologic and elemental analysis of primary melanosis of the dentate nucleus: review and correlation with neuromelanin. *J Neuropathol Exp Neurol.* 76(11):949-956.
7. Ule G, Berlet H. (1979) [Melanosis cerebelli. Ultrastructure and infrared absorption of the pigment (author's transl)]. *Acta Neuropathol.* 15:46(3):215-20.
8. Fan KJ, Kovi J, Duhaney SD. (1978) Melanosis of the dentate nucleus: fine structure and histochemistry. *Acta Neuropathol.* 15:41(3):249-51.

Results

- Both animals were euthanized at scheduled study termination. There were no notable in life findings for either animal.

Macroscopic Observations

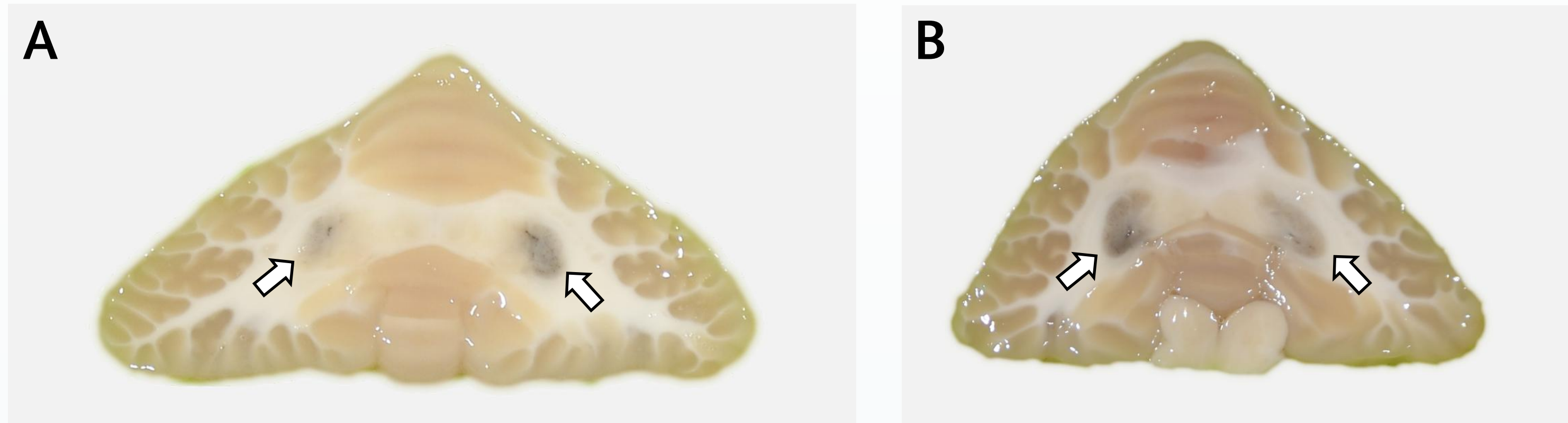


Figure 1. Unfixed brain slabs, male cynomolgus macaques. There is symmetrical, bilateral black-brown discoloration in the cerebellum in the area of the dentate nucleus (arrows). **(A)** Monkey 1, **(B)** Monkey 2.

Microscopic Findings

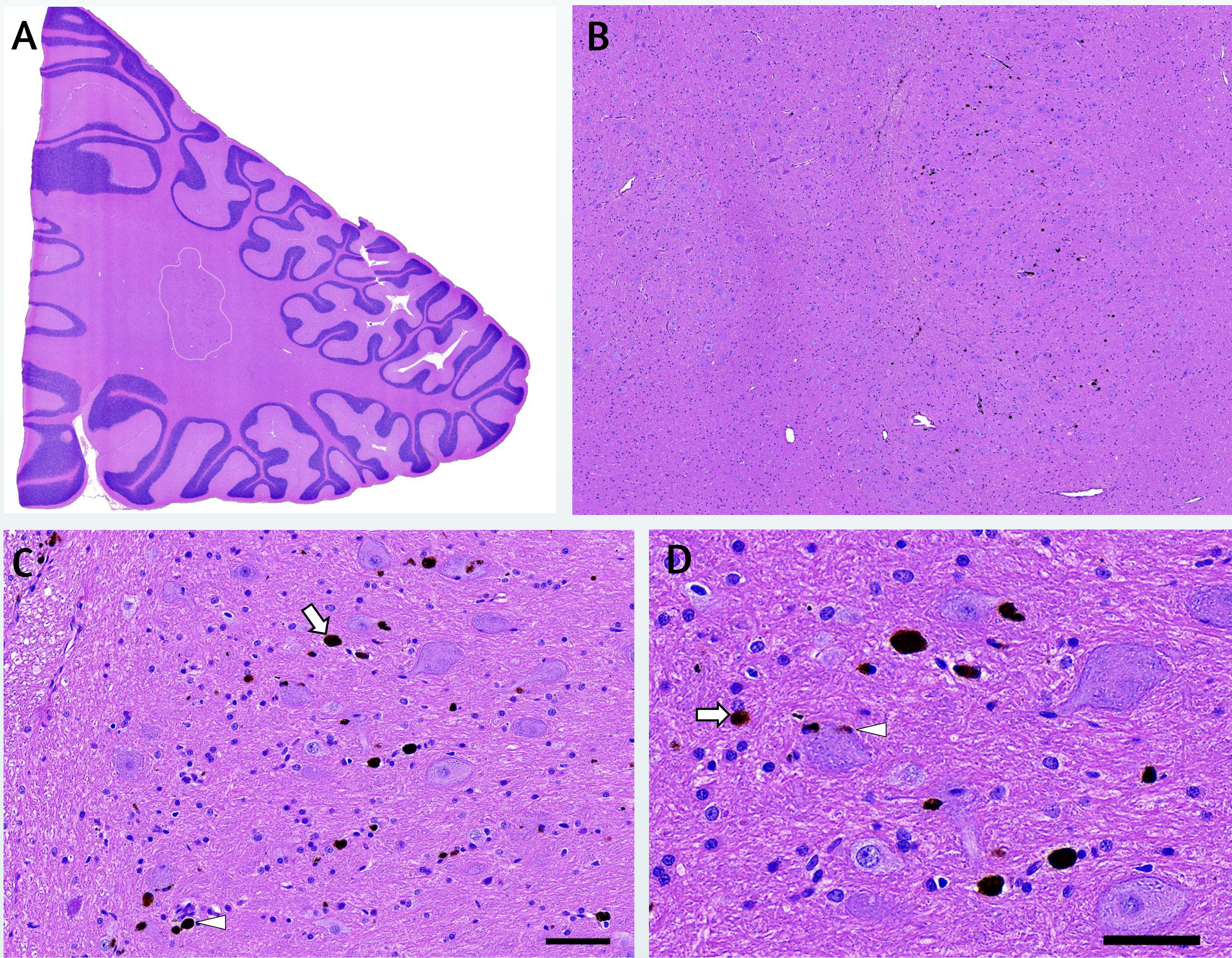


Figure 2. Brain, male cynomolgus macaque, H&E. **(A)** Subgross of the cerebellum from Monkey 1. The dentate nucleus is outlined in white. **(B)** Low magnification of the dentate nucleus from Monkey 1 showing scattered dark brown pigment deposits. **(C)** Higher magnification of the dentate nucleus from Monkey 1. Roughly spherical dark brown pigment deposits are present in the neuropil (arrow) and occasionally perivascular (arrowhead). Bar = 100 µm. **(D)** Pigment is present in close proximity to glial nuclei (arrow) and less often near the periphery of neuronal soma (arrowhead). There are no morphologic alterations of neuronal soma in the vicinity of the pigment. Bar = 50 µm.

- The pigment did not autofluoresce.
- Pigment deposits were not identified elsewhere in the examined brain or spinal cord of these animals.