

Short Communication

## Age-related Histological Findings in the Pineal Gland of Crl:CD(SD) Rats

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**Abstract:** To provide background data as the pathologic basis, the pineal glands of 190 male and 193 female Crl:CD(SD) rats at ages of 0–7, 51–58, 70–85 and 111 weeks were examined histologically in this study. Mineralization and fibrosis were common findings in the aged rats, whereas they were rarely found in the young ones; mineralization was present in 7, 44, 67 and 79% of males and in 0, 32, 67 and 79% in females, and fibrosis was present in 0, 29, 48 and 44% of males and 0, 18, 40 and 35% of females at ages of 0–7, 51–58, 70–85 and 111 weeks, respectively. Striated muscle fiber appeared regularly in the fibrosis region from 51–58 weeks of age when fibrosis increased, while the origin of this fiber remained unclear. Vacuolation of pineal cells also increased with age in both sexes, though the total incidence was low. There was a low incidence of lymphocytic infiltration in both sexes, but this was not related to age. (DOI: 10.1293/tox.25.287; J Toxicol Pathol 2012; 25: 287–291)

**Key words:** pineal gland, spontaneous lesion, age-related finding, striated muscle fiber, rat

The pineal gland of mammals is quite a small tissue formed mainly by pinealocytes, which are cells with a neurosecretory function, and glial-like interstitial cells<sup>1–3</sup>. This organ is located at the top of the brain at the intersection of the median line and cerebral transverse fissure and is covered by the pia mater and connected to the third ventricle by the stalk. The pinealocyte secretes melatonin, and this plays a role in the maintenance of homeostasis involving the gonads, hypophysis and melanin<sup>1–3</sup>. The actions of melatonin on tumor metabolism and growth have also been shown in recent *in vivo* and *in vitro* studies<sup>4–6</sup>. Therefore, histopathological evaluation of the pineal gland is meaningful to understand the non-neoplastic and neoplastic lesions in some organs. To date, toxicological pathologists have rarely observed the pineal gland tissue of experimental animals, especially in ordinary toxicological or carcinogenicity studies. Some previous reports revealed spontaneous histological changes associated with aging of the pineal glands in rats<sup>7–9</sup>. However, there are no reports comparing the incidences of histological changes in the pineal glands of rats of either sex over a wide age range. We are reporting spontaneous lesions observed in pineal glands from 190 male and 193 female rats with the aim of providing background data as the

pathologic basis for recognizing the adaptive physiological or morbid morphology of the pineal gland.

A total of 383 Crl:CD(SD) rats purchased from Charles River Laboratories Japan Inc. (Kanagawa, Japan) were used in the present study to collect house data. They were kept at a temperature of  $22 \pm 3^{\circ}\text{C}$  with a humidity of  $55 \pm 20\%$  and 12-hr light and 12-hr dark cycle and were fed a normal diets and unrestricted drinking water. At 0–7 (days 0, 4, 10, 17, 21, 29, 42 and 49), 51–58, 70–85 and 111 weeks, the rats were anesthetized and euthanized to collect the pineal gland and other organs. The numbers of animals in each age group are shown in Tables 1 and 2. The glands were fixed in 10% neutral phosphate-buffered formalin, embedded in paraffin, sectioned and stained with hematoxylin-eosin (HE), Masson's trichrome (MT) and phosphotungstic acid hematoxylin (PTAH). Immunohistochemical stainings for glial fibrillary acidic protein (GFAP: rabbit anti-cow glial fibrillary acidic protein, DAKO, Japan, 1:100), smooth muscle actin (SMA: monoclonal mouse anti-human smooth muscle actin, DAKO, Japan, 1:200), myogenin (monoclonal mouse anti-rat myogenin, DAKO, Japan, 1:50) and desmin (monoclonal mouse anti-human desmin, DAKO, Japan, 1:100) were performed. The animals were cared for according to the principles outlined in the guide for the care and use of laboratory animals prepared by the Japanese Association for Laboratory Animal Science and our institution.

The incidences of histological findings are summarized in Tables 1 and 2. Focal mineralization was mostly found in the superficial part of the meningeal connective tissue or submeningeal part of the glands and was occasionally inside the glands (Fig. 1). Inside the glands, it appeared

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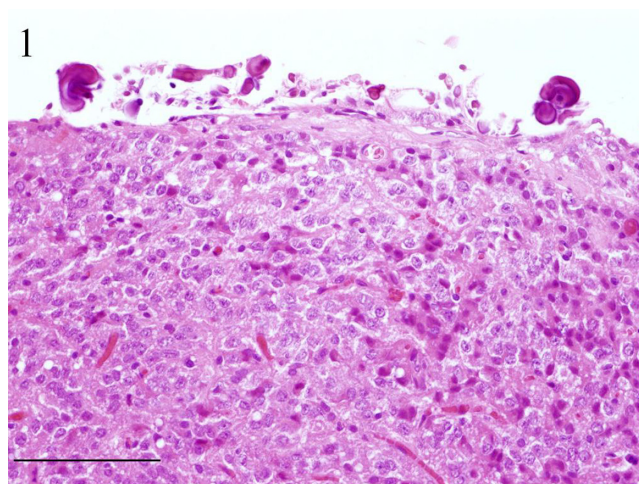
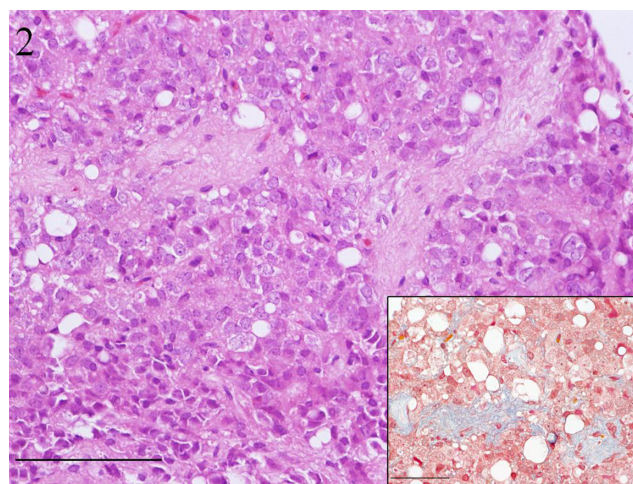
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**Table 1.** Incidences of the Histological Findings in the Pineal Gland of Male Crl:CD(SD) Rats

Age (weeks)	0–7		51–58		70–85		111	Total	
Number of animals examined	28		59		46		57	190	
Findings	n	Rate	n	Rate	n	Rate	n	n	Rate
Mineralization	2	7%	26	44%	31	67%	45	104	55%
Fibrosis	0	0%	17	29%	22	48%	25	64	34%
Striated muscle fiber	0	0%	4	7%	4	9%	5	13	7%
Vacuolation	0	0%	0	0%	2	4%	6	8	4%
Lymphocytic infiltration	0	0%	6	10%	1	2%	1	8	4%

**Table 2.** Incidence of the Histological Findings in the Pineal Gland of Female Crl:CD(SD) Rats

Age (weeks)	0–7		51–58		70–85		111	Total	
Number of animals examined	26		62		48		57	193	
Findings	n	Rate	n	Rate	n	Rate	n	n	Rate
Mineralization	0	0%	20	32%	32	67%	45	97	50%
Fibrosis	0	0%	11	18%	19	40%	20	50	26%
Striated muscle fiber	0	0%	0	0%	0	0%	0	0	0%
Vacuolation	0	0%	1	2%	11	23%	16	28	15%
Lymphocytic infiltration	0	0%	3	5%	3	6%	1	7	4%

**Fig. 1.** Laminated bodies of mineralization are found on the meningeal connective tissue of the pineal gland. 111-week-old male. HE. Bar=100 μm.**Fig. 2.** Fibrosis is found on the periphery of the gland. 103-week-old female. HE. Bar= 100 μm. Inset: Dense collagen fibers are present. MT. Bar=50 μm.

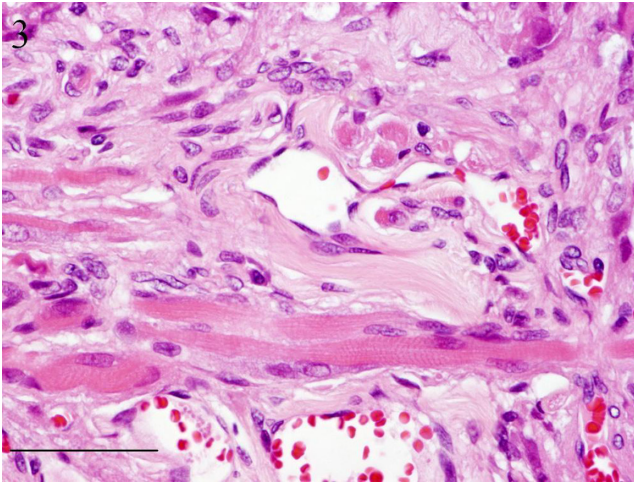
at the interstitial connective tissue with small blood vessels. The size and shape of the mineralization varied, although those of aged rats tended to be large, laminated and large in number. The incidence of mineralization increased with age; the incidence rates were 44%, 67% and 79% in males and 32%, 67% and 79% in females at 51–58, 70–85 and 111 weeks of age, respectively. Concerning the immature to younger rats at 0–7 weeks of age, mineralization was present in only two males at 4 and 6 weeks of age and was not present in females.

Focal fibrosis was found on the periphery of the gland and near the pineal stalk, and it sometimes extended to inside the gland. Frequently, small blood vessels were seen in the fibrosis region of aged rats and were infrequently hyalinized (Fig. 2). In other words, the small blood vessels were surrounded with a slight fibrous connective tissue in the younger rats or with a lot of fibrous tissue in aged rats,

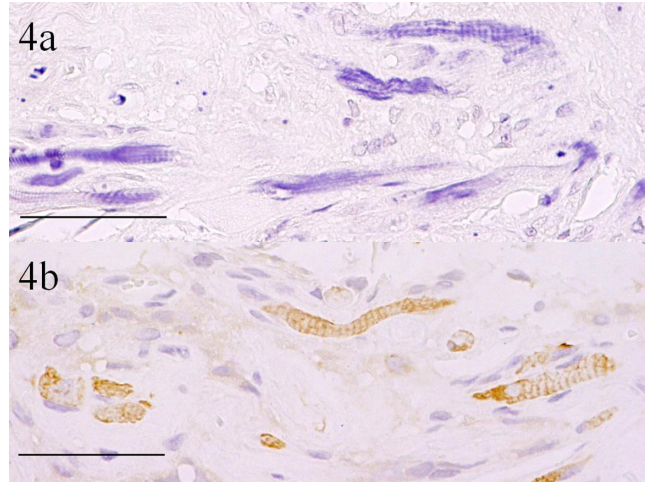
and the latter was regarded as fibrosis. The incidence of fibrosis increased with age and peaked at 70–85 weeks of age in both sexes; the incidence rates were 0%, 29%, 48% and 44% in males and 0%, 18%, 40% and 35% in females at 0–7, 51–58, 70–85 and 111 weeks of age, respectively.

Striated muscle fibers were rarely found in the pineal gland. The fibers were present in the connective tissue, and this tissue was frequently involved in fibrosis (Fig. 3). The muscle fibers did not seem to come into contact with the pinealocytes. Cross striation was observed in the cytoplasm with the HE stain and was distinct with the PTAH stain (Fig. 4a). Immunohistochemically, these fibers were positive for desmin (Fig. 4b) and negative for myogenin, SMA and GFAP. Striated muscle fibers were found in a total of 13 male rats but not in the females. Though the incidence of striated muscle fibers was low, they were present only in aged males with pineal fibrosis.

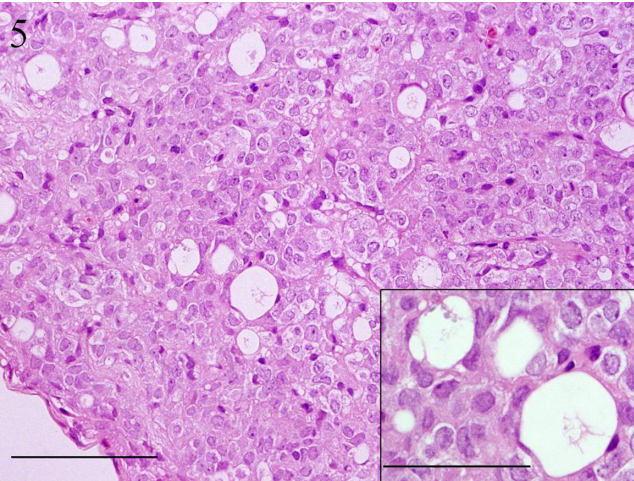




**Fig. 3.** The striated muscle fibers are present in the area of the fibrosis in the pineal gland. Cross striation is barely found in the cytoplasm. 85-week-old male. HE. Bar=50  $\mu$ m.



**Fig. 4.** The cross striation is distinct with PTAH stain (4a). Immunohistochemically, these fibers are positive for desmin (4b). 85-week-old male. Bar=50  $\mu$ m.

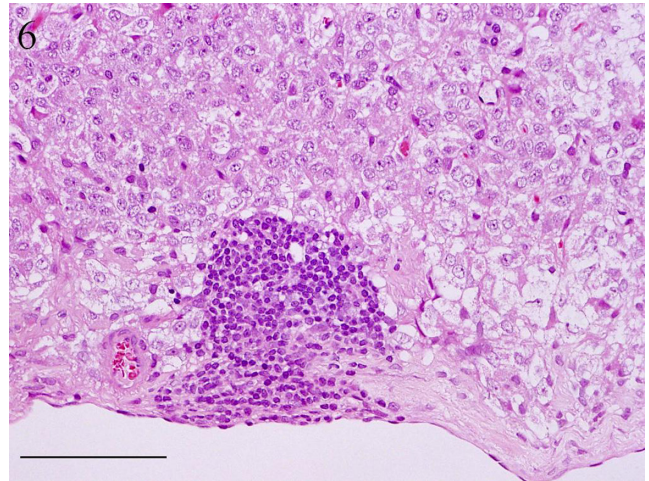


**Fig. 5.** Scattered vacuolation of the pineal cells is found. 105-week-old female. HE. Bar= 100  $\mu$ m. Inset: These vacuoles occur in the cytoplasm and are range in size up to 4–5 times the size of a normal pineal cell. The large vacuoles press the nuclei to the rims of the cells. The vacuoles occasionally contain a flocculent substance. HE. Bar=50  $\mu$ m.

Vacuolation of pineal cells was found scattered throughout the gland (Fig. 5). Single vacuoles were present ones in the cytoplasm and ranged in size up to 4 to 5 times the size of a normal pineal cell. The large vacuoles pressed the nuclei to the rim of cells and occasionally contained flocculent substances. The incidence of vacuolation was low and tended to increase with age in both sexes.

Lymphocytic infiltration was observed from the pia mater to the parenchyma of the pineal glands (Fig. 6). The incidence of lymphocytic infiltration was low in both sexes and not related to age.

We noted no neoplastic lesions in the examined pineal glands in the present study.



**Fig. 6.** Lymphocytic infiltration is found from the pia mater to the parenchyma of the pineal glands. 51-week-old male. HE. Bar=100  $\mu$ m.

Mineralization (calcium deposits) found in the extracellular space of the pineal gland in humans is called corpus arenaceum (corpora arenacea; “brain sand”), appears early at a young age and increases in number up to around thirty years of age or more<sup>10</sup>. The present study showed an age-related increase in mineralization in the rat pineal glands, although mineralization was previously reported mainly in aged rats and young to aged gerbil<sup>7, 8, 11</sup>. The incidence of this feature was shown clearly to increase from 70–111 weeks of age in both sexes. Superficial deposition was confirmed to be a histological characteristic of pineal mineralization in our study as described in the previous reports of laboratory animals<sup>7, 8, 11</sup>, whereas this positional characteristic has not been observed for human brain sand. Moreover, as another histological characteristic of mineralization, large areas of

mineralization in aging rats tended to show laminations according to previous reports<sup>8</sup>.

Fibrosis in the pineal gland of rats was reported to be an age-associated alteration<sup>7, 9</sup>. Wegiel *et al.* revealed that the fibrosis in the pineal glands occurred only sporadically, at an incidence of 1.1 percent<sup>7</sup>. On the other hand, the incidence of fibrosis was about 45 percent in the aged males and 37 percent in the aged females in our study. Similar to the histological characteristics stated in the previous reports<sup>7, 9</sup>, fibrosis in our study was observed in the periphery of the gland and near the pineal stalk. Blood vessels were present within the lesions in our study. So the increase in perivascular connective tissue was considered to have resulted in fibrosis in those areas, and this process was surmised not to have been involved in inflammation.

The appearances of striated muscle fibers in the pineal glands have previously been reported in some mammals, including the human, bovine, swine, bat and rat<sup>9, 12–21</sup>. In our study, striated muscle fiber was a rare finding with an age-related increase like previous reports, though the incidences varied among the reports<sup>9, 12, 15</sup>. Interestingly, these fibers were only found in male rats in our study; however, there are a few reports referring to the incidence in females. Quay reported the discovery of striated muscle fibers in the pineal glands of two males and one ovariectomized female out of a series of about 1200 rats<sup>15</sup>. Dill reported the distribution of striated muscle in the pineal glands of one female and one bilateral adrenalectomized female<sup>13</sup>. Other reports dealt with only male rats except for ones without a description discerning the sexes. The histological characteristics of the striated muscle fibers in our study were similar to those in the previous reports, as the fibers appeared in the connective tissue near the stalk of the pineal glands, especially with fibrosis<sup>9, 12, 15</sup>. The immunohistochemical results in our showed positive and negative staining for desmin and myogenin, respectively, suggesting that the fibers were mature. After examination of the fibers and consideration of the arguments of several other authors<sup>12, 13</sup>, the origin of the fibers remains unknown.

Neoplastic lesions in the pineal gland such as pinealomas and pineocytomas have been reported in some strains of rats in previous reports<sup>22–25</sup>; however, they are very rare tumors and are not classified in detail in rats. We noted no neoplastic lesions in the pineal glands of the examined 383 rats.

In the present study, we compared the incidences of the histological findings in the pineal glands of Crl:CD(SD) rats, which are used frequently in toxicity studies, among various ages of both sexes. Mineralization, fibrosis and vacuolation of the pineal cells were age-related findings. In addition, striated muscle fiber was a rare finding appearing only in male rats, but with an age-related increase.

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