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Presence of Melanin in the Cat Pineal Gland

Key Words

Pineal gland
Cat
Pigment
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Abstract

Light- and electron-microscopic features of pigmented cells in the cat pineal gland are described. These cells were observed throughout postnatal life from the second postnatal day to the oldest cats studied (up to 13 years old). No apparent relationship was observed among the amount of pigment and the animal age or sex. Pigmented cells showed a preferential localization at the ventral surface of the pineal gland near its distal end. The pineal pigment was histochemically identified as melanin. Pineal pigment granules showed ultrastructural features similar to melanocyte melanin granules.

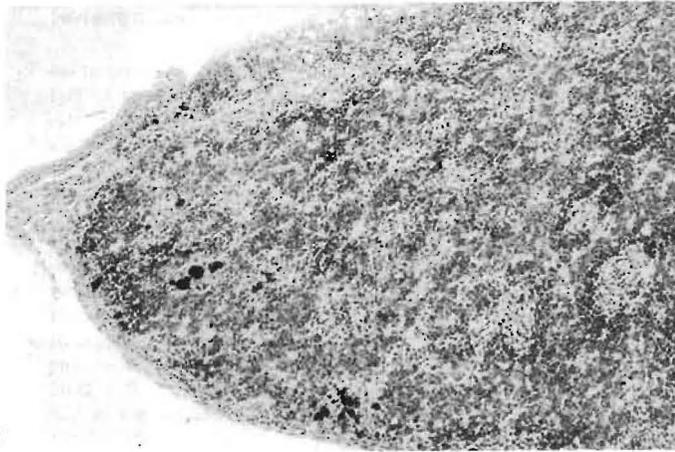
Introduction

Morphological studies have demonstrated the presence of cells endowed with pigment granules in the pineal gland of many mammalian species [1-10]. The chemical nature of this pigment is otherwise largely unknown. In bovines, the pineal pigment has been identified as melanin [1], while in equines [8] and dogs [10], it appears to be rather similar to neuromelanin.

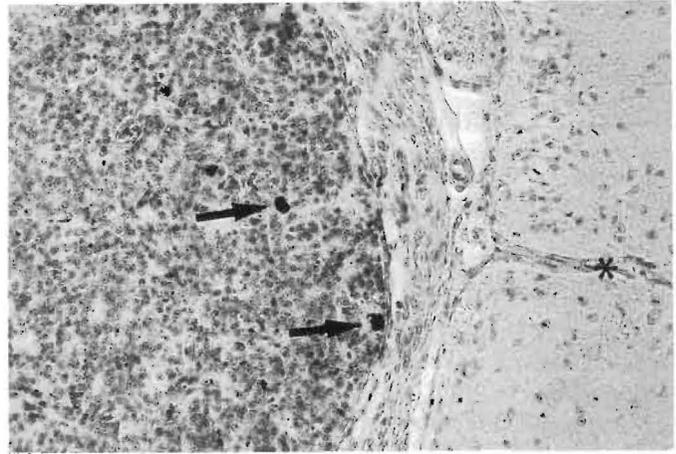
In a study on the postnatal maturation of the cat pineal gland, we detected the presence of cells with pigment granules. In the present study, we describe morphological and histochemical characteristics of the pigment in the cat pineal gland.

Materials and Methods

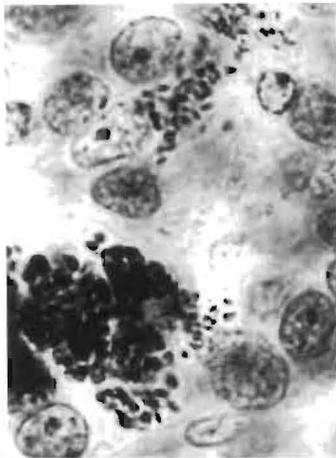
Thirty-nine clinically healthy cats living under a natural photoperiod (approximately 40° N latitude) were used for this study. The animals were killed under sodium pentobarbital anaesthesia at 11.00 h a.m. over a period between April and September. For the light microscope study, 2 cats (1 male and 1 female) were studied at each of the following ages: 2, 5, 10, 20 and 30 days; 2, 6, 9, 12 and 30 months. The pineal glands were fixed by immersion either in Bouin solution or in 10% formalin. After embedding in paraffin, 7- μ m-thick serial sections were obtained. Some sections were stained with a haematoxylin and eosin for routine light-microscopic observations. The identification of melanin was carried out by the methods recommended by Pearse [11]: bleaching with hydrogen peroxide and peracetic acid and the Masson-Fontana silver method. The 'in block' DOPA reaction [12] was performed on two additional pineal glands from 3- and 30-day-old cats.



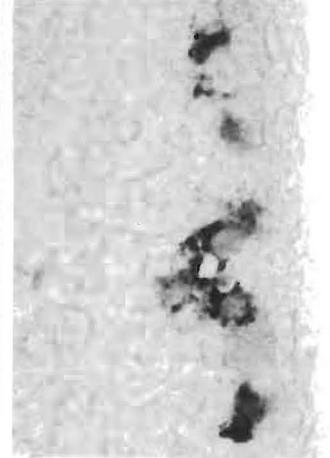
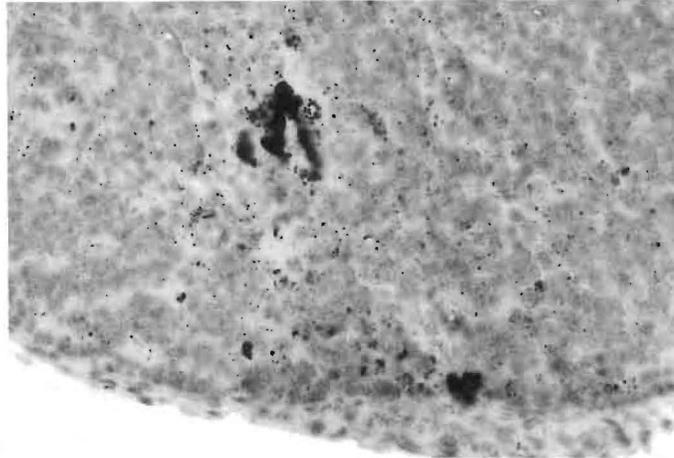
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Fig. 1. Sagittal section of the pineal gland. Clusters of pigmented cells located on the ventral surface near the distal end of the gland. 10-day-old male cat. Haematoxylin-eosin. $\times 100$.

Fig. 2. Frontal section of the pineal gland. Clusters of pigmented cells (arrows) close to the sagittal mean plane. Asterisk = Median sulcus between superior colliculi. 30-day-old female cat. Haematoxylin-eosin. $\times 185$.

Fig. 3. High magnification of pigment granules. 6-month-old male cat. $\times 1,450$.

Fig. 4. Argentaffin reaction on pigment granules. Masson-Fontana silver method. 2-month-old male cat. $\times 150$.

Fig. 5. DOPA reaction on pigmented cells. 'In block' DOPA method. 30-day-old female cat. $\times 750$.

For the electron-microscopic study, 2 cats (1 male and 1 female) were studied at each of the following ages: 2, 10, 20 and 30 days; 2 and 6 months; 1, 2 and 13 (only a female cat at this age) years. Pineal glands were immersion fixed in cold 3% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4. Tissue blocks were washed in 0.1 M phosphate buffer, postfixed in 1% osmium tetroxide in the same buffer and embedded in Vestopal W. Pigmented cells were localized on toluidine-blue-stained semithin sections. Ultrathin sections stained by uranyl acetate and lead citrate were studied in a Philips EM201 electron microscope.

Results

Both with the light and the electron microscope, pigment was observed in the pineal gland at all the ages studied, from the second postnatal day to 13 years old. The amount of pigment, though generally small, shows individual variations. No apparent relationship was observed among the amount of pigment and the animal's age or sex.

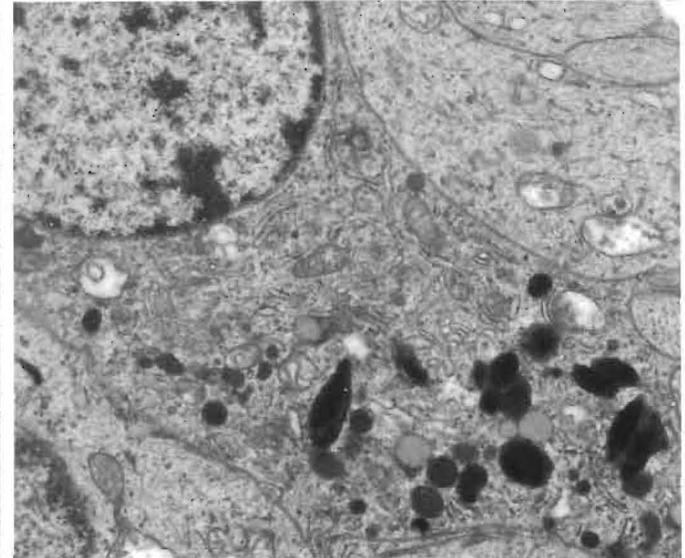
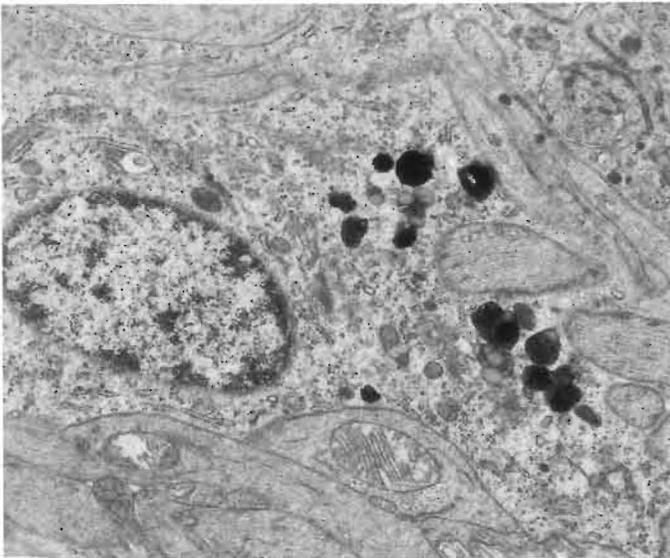
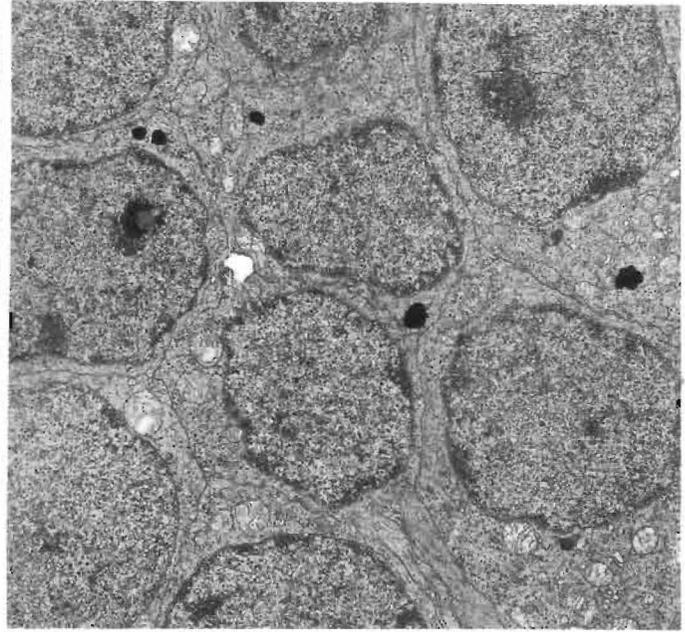
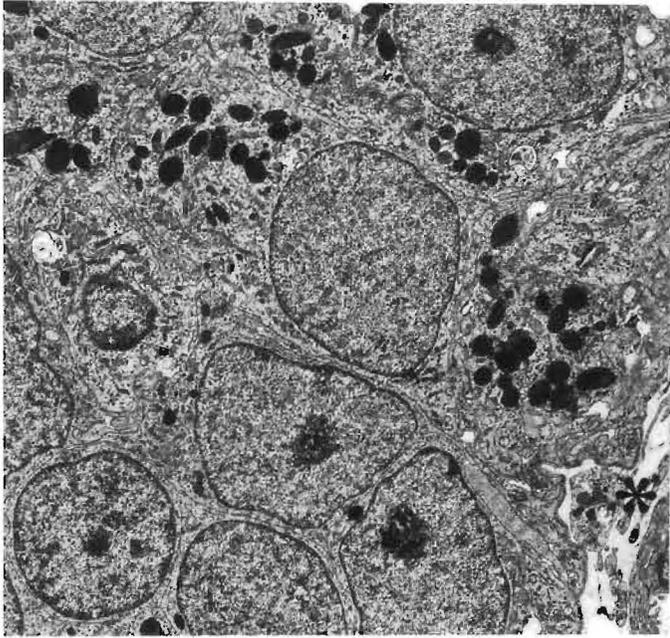


Fig. 6. Low magnification of a cluster of pigmented cells. Asterisk = Meningeal space. 2-day-old male cat. $\times 5,200$.

Fig. 8. Pigment granules within a pineal astrocyte. 1-year-old female cat. $\times 11,600$.

Fig. 7. Pigment granules within pinealocytes. 6-month-old female cat. $\times 5,800$.

Fig. 9. Pigmented cells rich in pigment granules, featuring ultrastructural differences compared to both pinealocytes and pineal astrocytes. 2-year-old male cat. $\times 22,000$.

Pigmented cells showed a characteristic localization in the cat pineal gland. These cells were found at the ventral surface of the gland, generally displaced towards its distal end (fig. 1). Pigmented cells were localized near the mean sagittal plane of the gland, forming small superficial clusters near the pineal gland capsule (fig. 1, 2). Occasionally, clusters of pigmented cells were also seen dispersed

throughout the pineal gland, near the connective tissue spaces (fig. 1). Pigmented cells were not seen outside the pineal gland, neither in the meningeal space nor in the neighbour posterior commissure.

In sections stained by haematoxylin and eosin, pigment granules were large, ovoid and brown in colour. Granules were detected in the perinuclear cytoplasm in variable

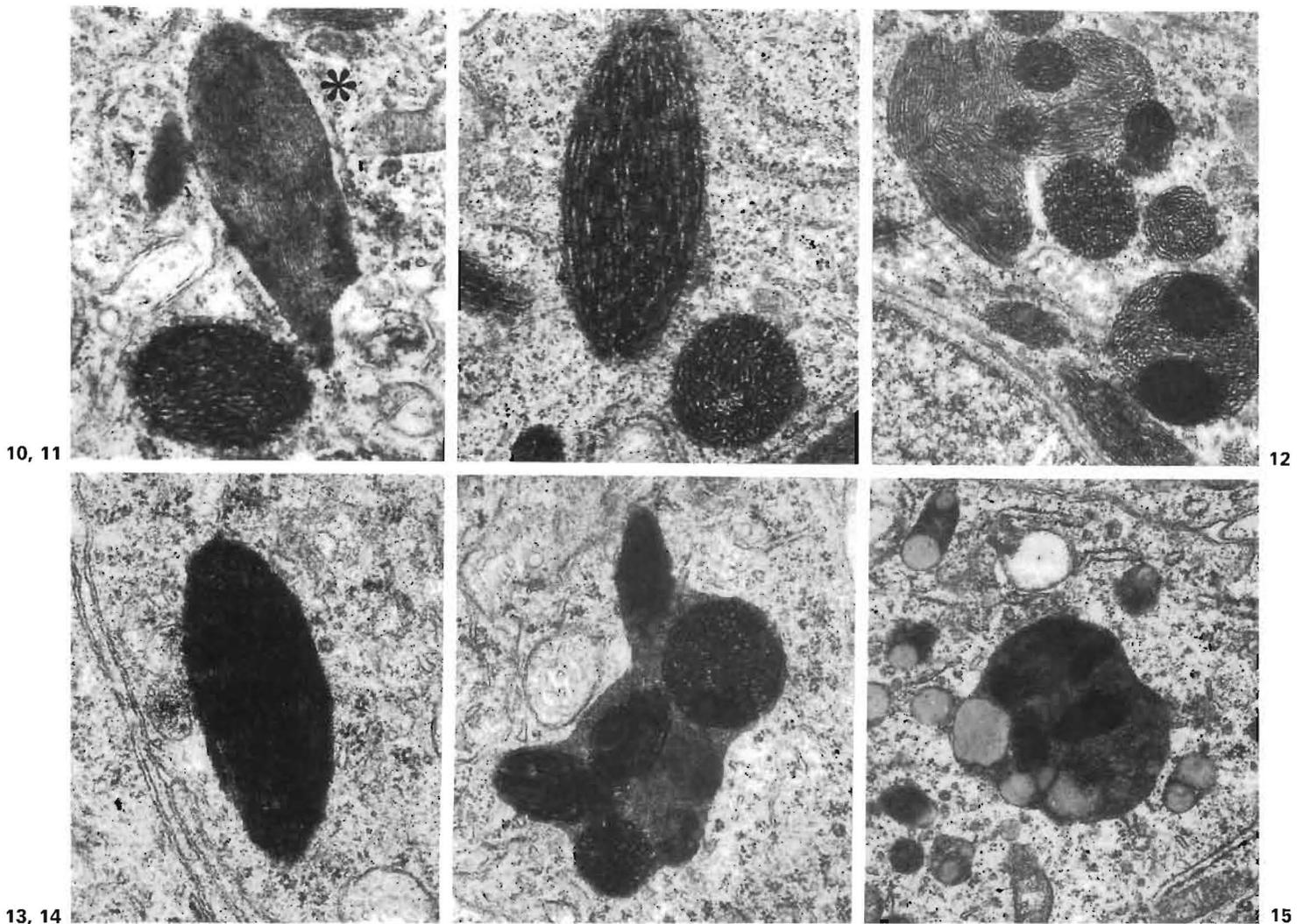


Fig. 10. Thin longitudinal fibrils within a pigment granule (asterisk). 30-day-old male cat. $\times 39,500$.

Fig. 11. Longitudinal and transversal sections of pigment granules containing thick fibrils. 6-month-old female cat. $\times 33,600$.

Fig. 12. Pigment granules showing electron-dense deposits on the fibrillar component. 20-day-old female cat. $\times 24,000$.

Fig. 13. Strongly dark content within a pigment granule. 1-year-old male cat. $\times 25,600$.

Fig. 14. Pigment granules within a lysosome-like dense body. 2-month-old female cat. $\times 30,700$.

Fig. 15. Pigment granules within a lipofuscin body. 13-year-old female cat. $\times 22,500$.

amounts among cells (fig. 3). The pigment granules on tissue sections bleached with hydrogen peroxide or peracetic acid and showed an argentaffin reaction as demonstrated by the Masson-Fontana silver method (fig. 4). A positive DOPA reaction was observed also in pigmented cells (fig. 5).

Ultrastructurally, pigmented cells formed groups generally abutting on the pineal surface, in contact with the basement membrane separating the pineal parenchyma from the adjacent meninx. In these groups, cells contained abun-

dant pigment granules (fig. 6). Isolated cells with few pigment granules were also occasionally found dispersed throughout the pineal parenchyma.

In the first postnatal week, the pigment is detected in immature-looking cells, similar to those forming the rest of the pineal gland parenchyma (fig. 6). From 30 days onwards, when well-defined cell types are evident, few pigment granules were found on cells identifiable as pinealocytes (fig. 7) and astrocytes (fig. 8). However, the largest clusters of pigment granules were observed in cells ultra-

structurally different from astrocytes and pinealocytes (fig.9). These cells presented a rounded or ovoid nucleus with small, abundant heterochromatin clumps. The cytoplasm is rich in organelles, particularly in elongated cisterns of the rough endoplasmic reticulum, surrounding small mitochondria with dense matrix. The Golgi apparatus is also well developed. The ultrastructure of these cells is more similar to that of astrocytes than pinealocytes, though they lack the typical astrocytic filaments.

Ultrastructurally, pigment granules showed characteristic features. Large and ovoid in shape, their electron-dense content is formed by longitudinally oriented fibrils. Several types of granules could be identified according to the thickness and darkness of the fibrillar granule content. These types were morphologically similar to premelanosomes, melanosomes and melanin granules described in cutaneous melanocytes (fig.10–13). The dark fibrillar content, characteristic of the pigment granules, was sometimes observed within lysosome-like dense bodies (fig.14). This finding, already present within the first postnatal days, is increasingly frequent with the aging of the animal. In the oldest cats studied, the dense fibrillar content appeared within typical lipofuscin granules (fig.15).

Discussion

Our results show that the presence of pigmented cells is a characteristic of the cat pineal gland throughout postnatal life. Therefore, the cat must be added to the already long list of mammals showing pigment on their pineal glands.

The amount of cells endowed with pigment granules is relatively small in the cat pineal gland. The localization of these cells in the pineal gland is fairly restricted, usually to the medial sagittal plane and displaced to the distal end of the pineal gland. This could explain the dearth of previous descriptions of cat pineal gland pigmented cells. Also, morphological studies on the cat pineal gland are scarce [2, 13–17].

The localization of pigment cells on the basal surface of the cat pineal gland is similar to that described for other domestic carnivores, e.g. the dog [2, 10]. However, in the dog, pigmented cells accumulate rather in the proximal region of the gland, near the posterior commissure [10, 18, 19]. By contrast, in the cat, they are displaced towards the distal end of the pineal gland. These differences between dogs and cats may probably be due to the different positioning of pigment cell precursors during the embryonic development in both species.

The differentiation of the pineal pigmented cells is apparently a prenatal process among carnivores, since these cells are seen in cats and dogs from the first postnatal days [18, 19]. Age-related or sex-related modifications of the pineal pigment are not described in any of these species.

In cats, the pineal bleaches with hydrogen peroxide or peracetic acid and shows an argentaffin reaction for the Masson-Fontana method. Pigmented cells are also positive for the DOPA reaction. All these characteristics identify the pigment as melanin. The ultrastructural appearance of the pigment granules is comparable to that of melanocyte melanin granules, including the premelanosome, melanosome and mature melanin granule stages [20]. Thus, cat pigmented cells seem to have a more complete melanogenic differentiation than is seen in dogs. Thus, dog pigmented cells seem to synthesize neuromelanin, perhaps due to their localization closer to the central nervous system [10].

Throughout postnatal life, melanin granules have been found within lysosome-like dense bodies. In the oldest animals, pigment granules are found within typical lipofuscin granules. This process of lysosomal digestion of the pigment granules has been demonstrated also in melanin-containing epidermal cells [20]. Since the amount of pineal pigment apparently does not vary with age, this might suggest a turnover of pineal pigment in the cat.

Our study demonstrates the presence of scarce pigment granules in both pinealocytes and pineal astrocytes. However, the largest clusters of pigment granules and the finding of a heterogeneous population of granules are observed in a cell type apparently different from pinealocytes and pineal astrocytes. The ultrastructure of these cells is very similar to that described for pigmented cells of the dog pineal gland [10]. It is appealing to suggest that this cell type is actually melanogenic and that the presence of pigment granules within pinealocytes and astrocytes is secondary to a transference process similar to that described for skin melanocytes and keratinocytes.

Additional studies on other mammalian species will be desirable to confirm that the pineal pigment is not an occasional and inconstant finding but a constant characteristic among mammals. We hope this will foster investigations on the functional role of the pineal pigment, up to now a non-explored field.

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