

## Does the Manatee Have a Pineal Body?

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### Abstract

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The brain of an adult, female manatee (*Trichechus manatus*) was prepared for histological examination of the region of the diencephalon and mesencephalon, where the pineal body typically would be located. Careful examination of hematoxylin–eosin stained, interrupted serial microtomy sections revealed that this animal has a tubular epiphyseal recess apparently lacking organic pineal differentiation. There was no cytological evidence of pinealocytes in the vicinity of the atypical pineal rudiment.

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### Introduction

Our interest in manatees stems from the possibility that they lack a pineal gland. The few reports on the manatee that address the subject are superficial and confusing. We recently had the good fortune to examine a well-preserved brain of a West Indian manatee, which we subsequently sectioned for histological examination. This paper reports the results of our examination and summarizes the earlier literature regarding the pineal body of sirenians.

Manatees and dugongs (Class: Mammalia; Order: Sirenia) are the only completely aquatic herbivorous mammals. There are four living species of sirenians, all restricted to tropical or subtropical regions: the dugong, *Dugong dugon* (Dugongidae), of shallow, coastal marine waters of the Indo-Pacific from eastern Africa to northern Australia; the Amazonian manatee, *Trichechus inunguis* (Trichechidae), an Amazon River endemic; the West African manatee, *T. senegalensis*, of coastal lagoons and rivers of western Africa; the West Indian manatee, *T. manatus*. The West Indian manatee occurs in shallow bays, estuaries and large rivers in the southeastern United States, the Caribbean Islands and eastern Central and South America to at least 7°S in Brazil (Rathbun 1984). In the

United States the winter range of *T. manatus* is restricted to peninsular Florida, where they form aggregations at natural and industrial warm water discharge sources (Hartman 1979). Simpson (1945) placed the Sirenia in the Superorder Paenungulata, which also includes the proboscideans (elephants) and hyracoideans (hyraxes). Contemporary workers also assume a close evolutionary affinity among these groups (de Jong *et al.* 1981, McKenna 1975). Domning (1978) provided an overview of evolutionary patterns within the Sirenia. Nearly all fossil sirenians were tropical and marine in distribution (Domning *et al.* 1982).

### Material and Methods

The U.S. Fish and Wildlife Service salvaged a recently deceased, lactating female manatee (*Trichechus manatus*) on 17 August 1982 in Glynn County, Georgia, 31°6'N 81°23'W. The animal was 300 cm long and weighed 544 kg.

The head was removed and freed of most soft tissues. The skull was immersed in a large volume of 10% aqueous formalin solution for several days, after three transverse saw cuts had been carefully made to provide access for the solution to intracranial tissues. It was then wrapped in formalin-saturated cloth and trans-

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ported to the laboratory at Colorado State University. Cranial bone was cut away and the intact, well-fixed brain within its meninges was carefully removed.

The dura overlying each cerebral hemisphere was incised and reflected from the brain surface. The falx cerebri was severed at its rostral limit and the tentorium cerebelli at its lateral limits. These structures, together with dura overlying the cerebellum and caudal brain stem, were then carefully reflected from dorsal brain surfaces. Particular attention was given to the freeing of those tissues from structures of the epithalamic region. The pituitary gland was removed by transection of the hypophyseal stalk.

The brain was hemisected sagittally in the mid-line by hand with a microtome blade and the cut faces methodically examined. A rectangular tissue block, approximately 25 mm across and representing all structures encompassing the third ventricle, was isolated and excised from each sagittal face. These tissue blocks were processed for infiltration by paraffin and embedded. Interrupted serial sections, 8  $\mu\text{m}$  thick, were cut on a rotary microtome from each block at approximately 40  $\mu\text{m}$  intervals, starting from the median sagittal plane, and were stained with hematoxylin-eosin.

## Results

### Gross Examination

After removal of dura mater, falx cerebri and tentorium cerebelli, the dorsal aspect of the diencephalon was exposed by gentle, forward displacement of the occipital poles of the cerebral hemispheres. A substantial membranous meningovascular sheet, about 3 mm thick, invested the superior surfaces of diencephalic and mesencephalic structures and intruded below the caudal margin of the corpus callosum. No discrete, globoid pineal organ could be identified within this membrane. The right median sagittal face of the hemisected diencephalon and mesencephalon is illustrated (Fig. 1A). Gross inspection of this face permitted the ready identification of Sylvian aqueduct, third ventricle and thalamic massa intermedia (Fig. 1B). In the caudal, superior part of the third ventricle a narrow, slightly curved recess was identified which reflected a cone of epithalamic tissue caudally over the rostral part of the mesencephalic tectum. From the apex of this conical protrusion a collapsed, tubular appendix extended caudally in the midline into the meningovascular membrane overlying the mesencephalon. From its topographic associations, this epithalamic structure was considered to represent the epiphyseal recess of the third ventricle with the related rudiment of the epiphysis cerebri.

### Histological Examination

Microscopic examination of processed intracranial tissues indicated that fixation had been satisfactorily achieved. Cellular elements of the brain and the meninges retained good detail. The epiphyseal recess was readily identified as an ependyma-lined diverticulum in the median aspect of the roof of the third ventricle (Fig. 1C). The ependymal sheet lining the caudal surface of the recess was of columnar and cuboidal character and locally accompanied by numerous colloid-containing subependymal rosettes (Fig. 1D). This sheet became of low cuboidal character as it extended into the dorso-caudal part of the third ventricle, where it then became continuous with the modified ependymal elements of the subcommissural organ. The ependymal sheet lining the rostral surface of the epiphyseal recess was of simple, low, cuboidal character and without accompanying rosettes.

The distal, tubular extension of the epiphyseal recess could be followed histologically throughout its length only by studying serial sections through a depth of approximately 550  $\mu\text{m}$  from the sagittal face of the diencephalon. This was considered to be the result of the mid-line tubular structure having been deflected to one side at the time of brain hemisection. Throughout their length, the recess and its tubular extension were invested by a relatively narrow rind of neural and meningovascular tissues. Neural elements comprised cell populations that appeared identical to astrocytes and oligodendrogliaocytes in other brain regions, together with glial fibrillary processes. Neuronal perikarya were not identified and no myelinated or non-myelinated axons were observed. In no location, whether within the peri-ependymal neural tissues or their accompanying meningovascular sheath, were discrete aggregations of cells resembling pinealocytes observed. The search for pinealocyte populations was extended to all neighboring regions of the dorsal surfaces of the diencephalon and no such aggregations, whether of glandular or non-glandular character, could be found.

### Discussion

The literature relating to the presence or absence of a pineal body in manatees is confusing, shallow and non-critical. Murie (1872), in a gross study of West Indian manatee brain, figures a "pineal gland" in plate XXV but does not mention this body in the text. [Murie (1885, p. 39) later noted that in plate XXV of the 1872 work the same abbreviation was used to label both the

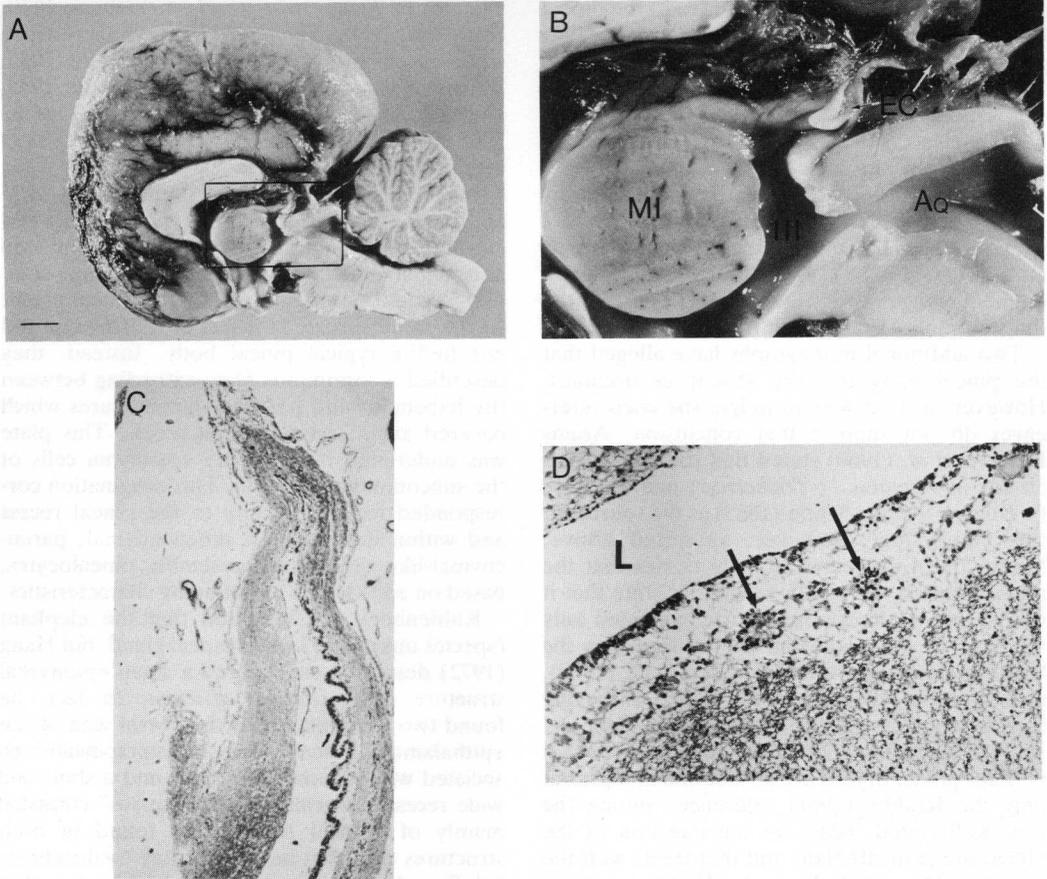


Fig. 1. (A) Median sagittal face of hemisected manatee brain. Box defines the area represented in B. The bar represents 1 cm. (B) Enlarged region of diencephalon and mesencephalon indicated in A. *MI* massa intermedia, *III* third ventricle, *Aq* aqueduct of Sylvius, *EC* epiphysis cerebri rudiment. (C) Epiphyseal recess of third ventricle. Note tall columnar ependymal sheet which lines the caudal surface of the lumen. Magnification 87.5 $\times$ . (D) Sub-ependymal rosettes (arrows) in the epiphyseal recess. *L* lumen. Magnification 300 $\times$ .

pituitary gland and the pineal gland.] Chapman (1875, p. 455) mentioned "the pineal gland with its peduncle" in his description of the same species, but his paper has no illustrations. Garrod (1879) stated in his notes on the West Indian manatee only that the pineal gland is small. Murie (1885) denied this, stating that the pineal gland cannot "be said to be small." These early works, based on examinations of three specimens, involved only gross dissection and no histological material. Quiring and Harlan (1953) described the gross anatomy of the brain of *T. manatus*, but failed to mention the pineal body. Beddard (1897) and Friant (1954) described gross brain morphology for *T. inunguis*, but unfort-

unately did not note either the presence or absence of a pineal body.

A series of transverse sections of a West Indian manatee brain is figured and described by Jelgersma (1934), but there is no mention of a pineal body. Jelgersma's sections were examined again by Verhaart (1972), who provided a detailed description of the tracts and nuclei of the manatee brain. Unfortunately, Verhaart's paper also does not mention the pineal body, nor is one visible in the photographs, although the sections shown include the habenular and posterior commissural regions. The author mentioned that the diencephalon was poorly stained and only the subthalamus and basal ganglia were distinct.

Krabbe (1961) could find no trace of the pineal body in the brain of a manatee (species not given) that he examined, but allowed that the body may have been torn away, since the brain was not complete. This is the only source we can find that specifically suggests the absence of a pineal body in the manatee.

Hill (1945, p. 173) described the gross anatomy of the brain of the dugong and stated that "there is no pineal body, but a large habenular apparatus occurs." Husar (1975), citing the monograph on the dugong brain by Dexler (1912), also stated that the pineal is absent in this species.

Two additional monographs have alleged that the pineal body may be absent in sirenians. However, as best we can judge, the cited references do not support that conclusion. Ariens Kappers *et al.* (1960) stated that the epiphysis is absent in *Manatus* (= *Trichechus*) and *Halicore* (= *Dugong*), citing Murie (1885) as the source for this conclusion. However, as noted above, Murie, in an indirect way, only denies that the pineal gland is not small; he did not state that it was absent. Furthermore, Murie examined only the brain of a manatee and did not report on the brain of a dugong. Ariens Kappers *et al.* (1960), in a footnote, cited Marburg (1920) as having traced remnants of the epiphysis in *Halicore*. Kelly in Wurtman *et al.* (1968, p. 1) allowed that perhaps pineal organs are absent in dugongs, but only the Krabbe (1961) reference, among the four Kelly cited, bears on the question of the pineal organ in sirenians and that treats with the manatee. As noted above, by Krabbe's admission, his specimen may have been incomplete.

As best we can determine then, the literature does not provide convincing evidence for or against the existence of pineal organs in the Sirenia. Indeed, the original reports are quite confusing and the confusion has been compounded by those who have cited them.

Our examination of a well-preserved brain of the West Indian manatee leads us to conclude that there is no typical pineal organ present, although there is a rudiment which may represent a very primitive epiphysis cerebri. Because we could not find cells that resembled pinealocytes in this structure or in the adjoining regions of the dorsal diencephalon, further doubt is cast on the possibility of this species having a functional pineal body.

As many investigators have observed, pineal organs are remarkably varied among the vertebrates in their cytology and morphology (Ralph 1975). It is especially striking that the organ is absent in a few of them. Some of the claims for absent pineal organs need to be re-examined, as we have done with *T. manatus*, but the evidence is substantial that the pineal gland is absent in the

alligator (*Alligator mississippiensis*) (Reese 1910, Roth *et al.* 1980). The pineal organ is alleged also to be absent in hagfish (*Myxine*) (Oksche 1965, Wurtman *et al.* 1968), *Torpedo* (Oksche 1965, Kuhlenbeck 1977), other edentates (Oksche 1965, Wurtman *et al.* 1968) and several species of cetaceans (Oksche 1965).

In an extensive study of the diencephalon of 14 adult and 2 embryo armadillos (*Dasypus novemcinctus*), using light microscopy and transmission and scanning electron microscopy, Phillips *et al.* (1985) could not identify a distinct pineal gland. In the same species Hofer *et al.* (1976) also did not find a typical pineal body. Instead, they described a continuous plate extending between the habenular and posterior commissures which covered an intercommissural recess. This plate was underlined by secretory ependyma cells of the subcommissural organ. This evagination corresponded topographically to the pineal recess and within it were seen subependymal, parenchymal-like cells which resemble pinealocytes, based on appearance and staining characteristics.

Kuhlenbeck (1977) stated that the elephant (species unknown) lacks a pineal gland, but Haug (1972) described and figured a small epiphyseal structure in *Loxodonta africana*. In fact, he found two evaginations of the dorsal wall of the epithalamus: a long recessus suprapinealis associated with the choroid plexus and a short and wide recessus pinealis. Both "recessus" consisted mainly of "pineal tissue". He found in both structures cells that he regarded as "wahrscheinlich Pinealocyten", based on the observation that they had fine-granulated, Gomori-positive cytoplasm. These cells were distinguished from nerve cells, which exhibit large Nissl granules.

The manatee's brain we have examined may have been inadequately preserved for a discriminating identification of such cells putatively resembling pinealocytes. However, one has to question what are the unique characteristics of pinealocytes, especially when they are not located within what can respectably be called a pineal body. There are significant structural differences among the pinealocytes of different kinds of mammals. Pinealocytes may consist of more than one subtype and show differences according to previous treatment and age (Quay 1974, pp. 28–32). The authors of this paper question whether, using simple light microscopy and a few staining methods, one can distinguish between pinealocytes and other neuroectodermal cells. Pinealocytes share most structural features common to neuroglia, sensory cells and neurons. Only by a combination of several techniques, such as fluorescence microscopy and electron microscopy, might one hope to prove that a cell is a pinealocyte (see Vollrath 1981, pp. 71–186, for details).

The absence of a pineal organ need not imply the absence of the *functions* commonly subsumed by pineal organs, for it is quite likely that some or all of the functions of the pineal organs are duplicated by other organs. This clearly appears to be so in the case of the production of melatonin, since the retina and other sites in many species are known to synthesize melatonin in daily patterns and in amounts similar to those of the pineal glands (Ralph 1980).

Just what the absence of a pineal gland may signify awaits further interpretation. It is noteworthy that those animals lacking a pineal organ, or having an atrophic one, are generally restricted in distribution to a more equatorial zone than those with large pineal organs (Ralph 1975). This may imply that pineal organs are essential for climatological adjustments or the seasonal adaptations required in environments at higher latitudes. Among the mammals, correlates of absent or atrophic pineal organs include a low precision of thermoregulation and a lack of a sharply defined seasonal reproductive cycle. The largely tropical sirenians are characterized by low metabolic rates and a limited capacity for endogenous heat production (Gallivan and Best 1980, Gallivan *et al.* 1983, Irvine 1983), as well as an absence of strong seasonality in the timing of reproductive events (Hartman 1979, Marsh *et al.* 1978).

## Summary

The West Indian manatee either does not have a pineal body or has only a rudimentary process which may represent an undeveloped epiphysis cerebri.

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