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Original Article / Özgün Araştırma

Histopathological Analysis of The Eye And Optic Nerve Structure In The Blind Mole Rat

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Abstract

Objective: Blind mole rats (Nannospalax xanthodon Nehring, 1898) are subterranean mammals that are well-known for their high tolerance to hypoxia and resistance to cancer. Due to their unusual habitat, these animals have developed several adaptations during their evolution. Therefore, this study aimed to identify possible structural differences in Nannospalax visual system in comparison to other mammals that might have arisen as a result of adaptation to underground life.

Method: Six blind mole rats were used in the study. No procedure was performed on the rats. After the animals were anesthetized with ether, their eyes and optic nerves were removed. For this purpose, pseudo-eyes and optic nerves were harvested and fixed in 10% formaldehyde for a week. Tissues were embedded into paraffin and blocked via routine histological procedures. Five micrometer sections in thickness were taken and stained with Hematoxylin & Eosin (H&E) and Cresyl Violet.

Results: Histopathological analysis of the eye revealed the presence of cornea, retina, sclera, iris, zonula adherens, lacrimal gland and ducts, fatty tissue, muscle layer and the vascular structures. No pathology was observed of optic nerve.

Conclusions: We found that the visual system of N. xanthodon share some similarities with other blind mole rat species from Israel, while some histological properties were defined by our study for the first time in the literature.

Keywords: Nannospalax xanthodon, blind mole rat, eye, lacrimal system, optic nerve

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Kör Sıçanlarda Göz ve Optik Sinir Yapısının Histopatolojik Analizi

Öz

Amaç: Kör sıçanlar (Nannospalax xanthodont Nehring, 1898) hipoksiye yüksek toleransı ve kansere karşı dirençleri ile iyi bilinen yeraltı memelileridir. Alışılmadık yaşam alanları nedeniyle, bu hayvanlar evrimleri sırasında çeşitli adaptasyonlar geliştirdiler. Tüm bu nedenler göz önüne alınarak, Nannospalax görsel sistemindeki yeraltı yaşamına adaptasyon sonucu ortaya çıkabilecek diğer memelilere kıyasla olası yapısal farklılıkların belirlenmesi amaçlanmıştır.

Yöntemler: Çalışmada altı adet kör sıçan kullanılmıştır. Sıçanlara herhangi bir işlem yapılmadı. Hayvanlar eter ile anestezi uygulandıktan sonra gözleri ve optik sinirleri çıkarıldı. Çıkarılan dokular bir hafta boyunca %10 formaldehit içinde bekletildi. Dokular rutin histolojik prosedürlerden geçirilerek parafin bloklara gömüldü. Bloklardan 5 mikro metre kalınlığında kesit alındı ve Hematoksilin&Eosin (H&E) ve Cresyl Violet ile boyandı.

Bulgular: Gözün histopatolojik analizi kornea, retina, sklera, iris, zonula adherens, lakrimal bez ve kanallar, yağ dokusu, kas tabakası ve vasküler yapıların varlığını ortaya çıkardı. Optik sinir patolojisi gözlenmedi.

Sonuç: N. xanthodon görsel sisteminin İsrail'den gelen diğer kör sıçan türleriyle bazı benzerlikleri olmasına rağmen literatürde olmayan, bazı histolojik özellikler ilk kez çalışmamız tarafından tanımlandı.

Anahtar kelimeler: Nannospalax xanthodon, kör sıçan, göz, lakrimal sistemi, optik sinir.

INTRODUCTION

Blind mole rats (Nannospalax xanthodon Nehring, 1898) are wild rodents that originated in Anatolia some 40 million years ago[1,2]. These animals spend most of their lives in underground galleries and have developed significant adaptation to subterranean life. They are model organisms for hypoxia tolerance, cancer resistance and longevity. The stress factors related to living underground have driven the speciation of these animals at chromosomal and genomic levels, which also resulted in morphological changes[2]. Several studies investigated these genetic variations and biogeographical relations in areas where blind mole rats have expanded[3-5]. There are three main species of Nannospalax distributed in different regions of Turkey, namely N. leucodon, N. xanthodon and N. ehrenbergi with several cytotypes varying in chromosome numbers (2n=38-62)[2,6,7]. Similarly, within the past 30 years, Nannospalax species have been studied as model organisms for speciation and adaptive expansion[8].

One of the most significant results of living underground is the severe regression in the visual system due to deprivation of light stimulus, which practically renders the animal blind. In line with this, blind mole rats are congenitally microphthalmic animals with non-functional subcutaneous eyes that lack any image forming capability[9,10] (Figure 1).

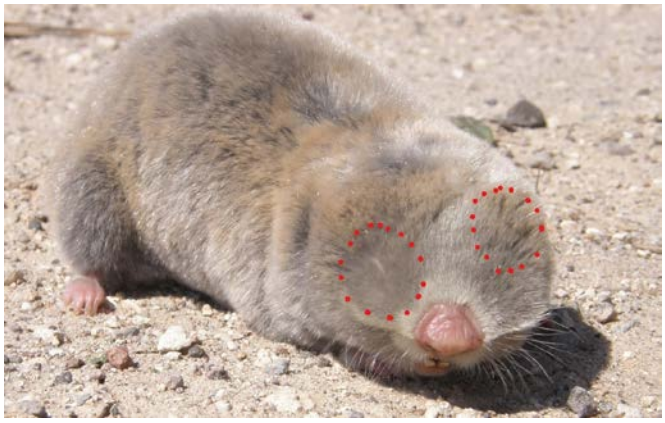

Figure 1. A photo of N. xanthodon species, with eye locations covered with skin

Visual adaptation has resulted in atrophied eyes and lack of short-wave opsin cones, while the role of retina in photoperiodic perception was reported to be preserved despite blindness[10,11].

There is only a limited number of studies investigating the morphological and cytological structures of the organs and tissues of blind

mole rats, which display significant adaptation to subterranean life. Therefore, this study provides the first data on the structure of visual system components in adult Nannospalax xanthodon.

METHODS

Ethics statements and animal All animal procedures were approved by the Institutional Ethics Committee at XXXX (Date: 10.09.2019, Protocol number: 2019/24) and were carried out in accordance with the principles of the Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, revised 1996). Wild type Nannospalax rats were obtained from Niğde/Turkey. Without any treatment, six animals were sacrificed via ether inhalation. Before sacrification, the animal was weighed. The structures that developed instead of functional eyes, as we name here "pseudo-eyes" (Figure 2) and the optic nerves were removed and weighed. Optic nerves and pseudo-eyes were placed in 10% formaldehyde and embedded in paraffin blocks following routine procedures. In order to measure the volume of the pseudo-eyes, Archimedes' principle[12-14] was used. For this, the pseudo-eyes were submerged into a graduated cylinder filled with water. The volume of the eye was equal to that of the displaced water (Figure 3).

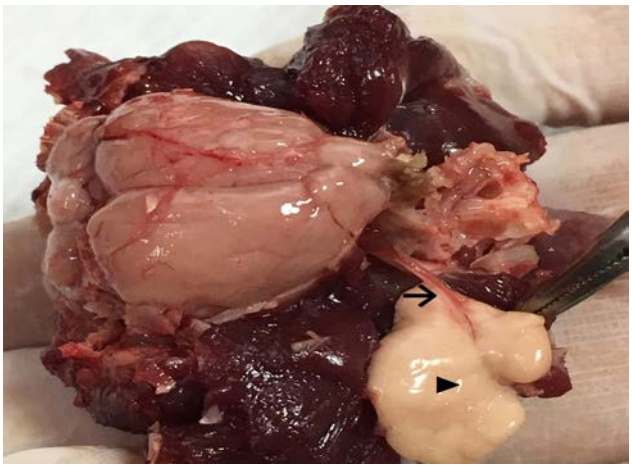

Figure 2. Dissection of the pseudo-eye (→) and the optic nerve (*) of N.xanthodon.

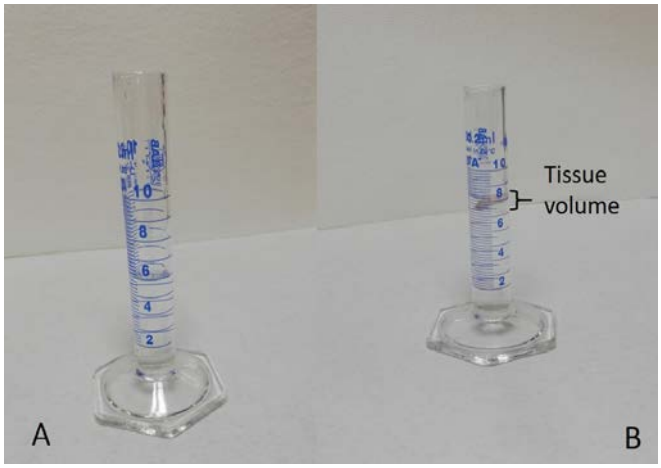

Figure3. Application of Archimedes' principle to measure volume. A) Graduated cylinder filled with water, B) Volume of the tissue calculated by the increase in total volume.

Histological procedures

Harvested tissues were fixed in 10% formaldehyde for a week. Paraffin embedded blocks were prepared through routine procedures. Five micrometer sections in thickness were cut using Leica Biosystems RM 2245 Microtom (Germany) and stained with Hematoxylin &Eosin (H&E) and Cresyl Violet.

Calculating length of the optic nerve

Optic nerve sections of were stained; length (long and short sides) was measured using a light microscope (Olympus, BX53, Japan) equipped with a digital camera (DP 80, Olympus, Japan) and Cellsens standard program (version1.17) at x4 magnification (Figure 3).

Statistic

Statistical Package for the Social Sciences (SPSS) (22.0 USA software) was used. Oneway ANOVA test was used for further data analysis. Values were calculated as Mean±Standard Error. For all comparisons $p < 0.05$ was considered statistically significant.

RESULTS

Measurements for the animal's body, pseudo-eye and optic nerve

The body weight, the weight and the volume of the right and left pseudo-eyes, as well as the measurements of the length of the optic nerve are given in Table 1. There was no statistically significant difference between right and left volume (p = 0.837) and right and left weight (p = 0.841) (Table 1).

Table I: Measurements for the animal's weight, pseudo-eye and optic nerve.

Measured parameters		
Blind mole rats (n=6)	**Mean ±Standard error**	
Animal Weight	215.33±20.97gr	
Pseudo-eye	**Right**	**Left**
Weight	0.23±0.55gr*	0.24±0.57gr*
Volume	0.29±0.13 ml*	0.27±0.12 ml*
Optic nerve		
Length short edge	742.56**±**15.63 µm	
Length **long edge**	2377.73**±**107.281µm	

** No statistically significant.*

Histopathological evaluation of the optic nerves and the pseudo-eyes

Our first observation was that the structures that we call "pseudo-eyes", which localized to where the eyes should develop, were completely buried under the skin and the visual system development was impaired. Surprisingly, we found that the optic nerve exists and serves as a connection between the pseudo-eyes and the brain. The optic nerve contained blood vessels that provided nourishment of the pseudo-eyes and histologically, no pathologies were identified. The optic nerve was anatomically at its appropriate place. Long and short edge lengths were measured to understand the exact dimensions of the figure. As a result, it had an elliptical shape, as opposed to its normal round structure. Myelinated nerve fibers were clearly visible as well (Figure 4).

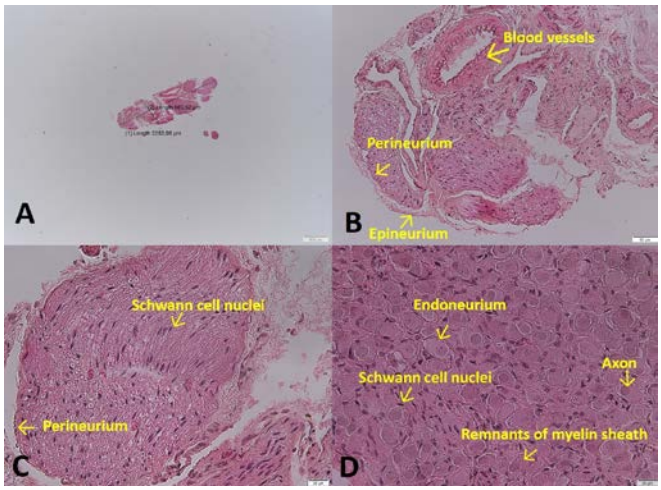

Figure 4. Panoramic images of the optic nerve, H&E. A) Measurements of the diameter. B, C and D General view of optic nerve (magnification respectively x2, x20, x40, x40).

The histopathological analysis of the pseudo-eyes revealed structures and the localizations of the cornea, retina, sclera, iris, zonula adherens, lacrimal gland and ducts (widely distributed within the pseudo-eye), fatty tissue, muscle layer and the vascular structures (Figures 5, 6, 7 and 8). In addition, we observed an empty space around the iris that we suspected it to be the vitreous cavity.

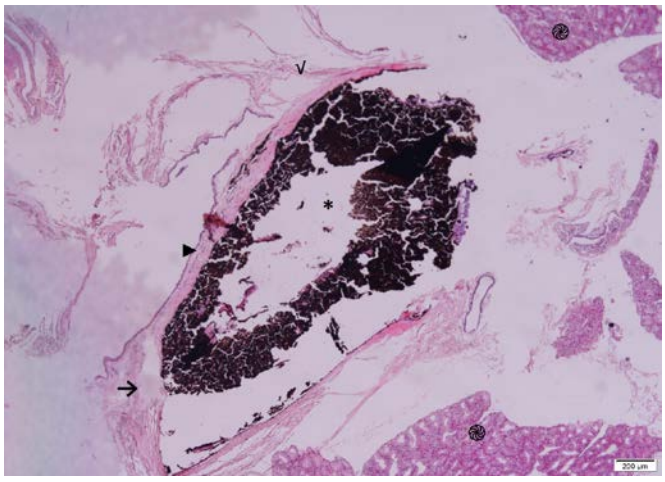

Figure5. Structure of the pseudo-eye (x4), H&E.Cornea (▶), sclera (→), iris (*), zonula adherens(√), lacrimal gland and ducts (֍) are indicated.

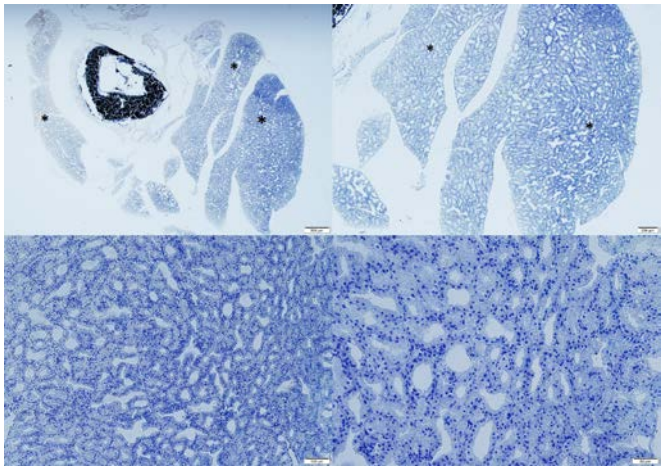

Figure6. Lacrimal gland and ducts (*) in the pseudo-eye (x2, x4, x10, x20), Cresyl violet.

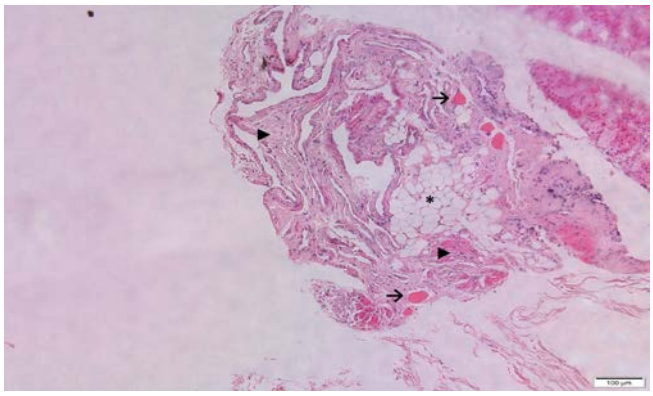

Figure 7. Fatty tissue (*), muscle layer (►) and the vascular structures (→) in the pseudo-eye (x10), H&E.

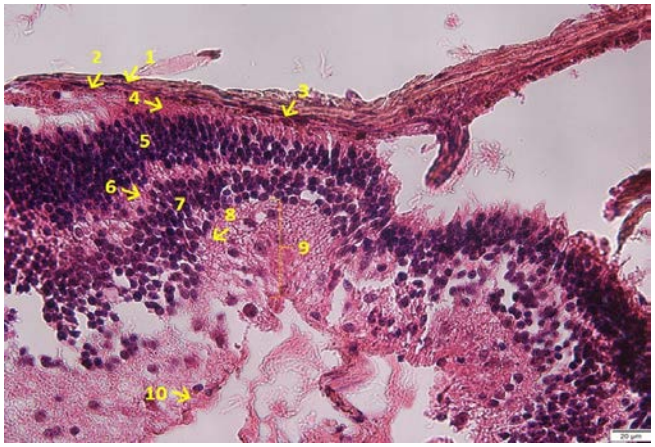

Figure 8. Histology of the retina (x40, H&E). The retina can be divided into 10 layers including, (1) the inner limiting membrane, (2) the nerve fiber layer, (3) the ganglion cell layer, (4) the inner plexiform layer, (5) the inner nuclear layer, (6) the outer plexiform layer, (7) the outer nuclear layer, (8) the outer limiting membrane, (9) the photoreceptor layer and (10) the retinal pigmented epithelium monolayer.

DISCUSSION

Blind mole rats (N. xanthodon) have subcutaneous eyes that do not respond to light in the form of image formation. However, these functionally regressed eyes have been suggested in the literature to still provide photoperiod perception, which is impaired by the removal of the eyes[11,15]. Although they are completely blind, their ability to distinguish between dark and light could be associated with sensing and localizing breeches in their underground tunnels[16-18]. Furthermore, the visual system in the blind mole rat has been implicated in the maintenance of the circadian rhythm[9]. In line with these previous findings, we identified several important structures of a functional eye such as cornea, retina, sclera, iris, zonula adherens, lacrimal gland and ducts, fatty tissue, muscle layer and the vascular structures of the eye in N. xanthodonin this study, despite the fact that their eyes are not functionally active. Our findings were also consistent with the ocular structure of Spalax ehrenbergi[19]. It was particularly interesting for us to detect retina in the blind mole rats, which could be explained by their photosensory perception ability[16]. Moreover, we found the N. xanthodon eye volume to be 0.13-0.4 mm3, which is smaller than the eye volumes described for rats (0.37-0.64 mm3)[20], indicating regression of the pseudo-eyes.

Another interesting finding of our histopathological analysis on the Nannospalax pseudo-eyes revealed that they were largely occupied by lacrimal glands and ducts. This is very surprising as the presence of these structures within the eye has never been observed for any organism elsewhere. Therefore, we believe that this is a special condition for the blind mole rat that we detected for the first time in the literature. Lacrimal apparatus, which is responsible for tear secretion and its drainage from the eye, consists of secretory and excretory systems. Secretory

system has two compartments; main lacrimal glands and accessory exocrine glands of Krause and Wolfring. Lacrimal gland is located in the orbital fossa in the upper outer part of the orbita[21]. Lacrimal gland is an exocrine gland, which is controlled by the autonomic nervous system. The secretory function of the lacrimal gland has effects on the eye such as cornea, conjunctiva, retinal over-stimulation by light and psychogenic stimulation, as well as on stimulating the nasal mucosa[22]. As the blind mole rats are subterranean rodents that live in underground galleries, they dig using their teeth, it is likely that they inhale soil up into their noses, which could explain the necessity for excess lacrimal gland development and increased tear secretion to clear it out.

Studies on the optic nerve have only focused on the blind mole rats S. ehrenbergi and S. leucodon so far[23]. Therefore, this study, for the first time, reports the presence of optic nerves in N. xanthodon, though they were atrophied and in elliptical shape. This is contradictory to the findings of Herbinet al.[23] showing that the optic nerve had a round structure. This observed condition may be congenital or due to adaptation. It is also possible that the elliptical shape has resulted as an artifact of experimental procedures. The optic nerve was observed to have a normal histological structure in terms of layers, general structure, axons and myelin sheath.

In conclusion, our histopathological analysis on the blind mole rat's eye and optic nerve revealed significant findings such as the lacrimal gland occupancy of the pseudo-eyes, which have not been reported elsewhere. These findings are important in having a better understanding of their evolution and adaptation to subterranean life, as well as their suggested cancer resistance.

Part of this work has been presented at International Turkic World Congress on Science and Engineering (UTUFEM) 2019.

Ethics Committee Approval: All animal procedures were approved by the Institutional Ethics Committee at XXXX (Date: 10.09.2019, Protocol number: 2019/24) and were carried out in accordance with the principles of the Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, revised 1996).

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Conflict of interest: The authors declare that there are no conflicts of interest.

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