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- RESEARCH ARTICLE-

Comparative analysis of the different regions of skin tissue in *Nannospalax xanthodon*

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Abstract

Blind mole rats *(Nannospalax xanthodon* Palmer, 1903) 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 between different regions of the skin in *Nannospalax* as well as to characterize the histological organization of a specialized skin patch namely scrub sensory organ that have possibly arisen as a result of adaptation to underground life. Skin from the neck, nasal pad and the scrub sensory organ was harvested from wildtype blind mole rats and fixed in 10% formaldehyde. Tissues were embedded into paraffin and blocked via routine histological procedures. 5-micron sections were taken and stained with Hematoxylin & Eosin and Cresyl Violet. Histopathological analysis of the skin revealed that the nasal pad and the scrub sensory organ were significantly thicker compared to the neck skin. Skin epithelia was structurally normal, although it was thicker in the neck skin in comparison to others. We observed structures that are similar to pyramidal cells in the scrub sensory organ. In conclusion, we defined some histological properties of the scrub sensory organ in *Nannospalax xanthodon* for the first time in the literature. Specifically, the detection of structures that are similar to pyramidal cells is significant. These results indicate structural differences between different skin regions and suggest a role for the scrub sensory organ in somatosensation.

Keywords:
Blind mole rat, skin histopathology, somatosensation, pyramidal cell
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Introduction

Skin is the largest organ of the body, which acts as a protective barrier against mechanical impacts, infections, UV light, dehydration and extreme temperatures. It is also one of the sense organs; providing sensory perception in the form of touching and feeling. The environmental cues perceived by the skin are transformed into action potential, transmitted to the brain via somatosensory neurons and interpreted (Low et al., 2019). Pyramidal neurons are a subtype of neuronal cells that comprise more than half of all neurons in the cerebral cortex. They are particularly important in delivering environmental stimuli to the somatosensory cortex (Bekkers, 2011).

Blind mole rats *(Nannospalax* Palmer, 1903) are wild subterranean rodents of the Spalacidae family (Nevo et al., 1994; Nevo et al., 1995). Due to several external stress inducing factors related to living underground, such as darkness, limited food supplies, hypoxia and hypercapnia, these animals have evolved significantly and acquired critical features that provide adaptation to subterranean life (Nevo et al., 2013). These adaptations resulted in various morphological changes, including a cylindrical body shape, almost non-existent tail and outer ear, as well as underdeveloped, non-functional subcutaneous eyes (Sanyal et al., 1990). In line with the loss of eyesight, *Nannospalax* has developed sensory adaptations to living underground, such as auditory adaptation to maintain hearing sensitivity at low frequencies (<16kHz) within underground tunnels (Burda, 2006). Interestingly, blind mole rats have regularly arranged thick and long scrub-like hair follicle cells stretching from the nasal pad to each ear, which we name here as the *"scrub sensory organ"* (Figure 1), that acts as the first contact point of the animal with its environment while it digs and wanders around the underground tunnels. Somatosensory system, especially through mechanoreceptors, are highly developed in subterranean mammals e.g. star-nosed mole and naked mole-rats, which provides orientation and valuable information regarding object localization (Crish et al., 2003). Furthermore, hair follicle cells and facial whiskers have previously described functions in sensing environmental cues (Kazmierczak & Müller, 2012; Petreanu et al., 2012; Ebner & Kaas, 2015). Therefore, in this study we analyzed skin from different regions to identify possible structural differences and evaluated whether the scrub sensory organ in *Nannospalax xanthodon* has potentially evolved to provide sensory perception.

There are no studies in the literature investigating the morphological and cytological structures of the scrub sensory organ and no histological or histochemical analysis has been performed. Thus, this study provides the first data on the structure of adult *Nannospalax* skin tissue, nasal pad and the scrub sensory organ, providing evidence towards identification of this specialized skin patch as an additional sensory organ.

Materials and Methods

Animals

Wildtype *Nannospalax* rats (n=6) were obtained from Niğde/Turkey. Without any treatment, animals were sacrificed via ether inhalation.

After sacrifice, the animal was weighed, and the height was measured. Skin from the neck, nasal pad and a 3 cm-wide specialized patch on both sides of the nose (scrub sensory organ) (Figure 1) were removed and prepared for histologic analysis.

Figure 1. A representative picture of a blind mole rat, *Nannospalax xanthodon.* Locations where the different skin tissue samples were derived are indicated.

Histological procedures

Harvested tissues were fixed in 10% formaldehyde for a week. During the preparation of paraffin embedded blocks through routine procedures, neck skin and nasal pads were processed directly due to their homogenous structure. However, the scrub sensory organ had different thickness as end-points being thin and the middle being thicker; therefore, the tissue for each animal was systematically cut into five equal pieces using a ruler and processed. 5 micron sections were cut using Leica Biosystems RM 2245 Microtome (Germany) and stained with Hematoxylin Harris (Product Code: HEMH-OT-100, Biognost) & Eosin Y (Product Code: HST-EOQ-0500, Histoplus) (H&E) and cresyl violet (acetate, Product Code: 912.D01, Isolab). From each animal, three sections from the neck skin and nasal pad were analyzed. For the scrub sensory organ, three sections from each piece were evaluated from each animal.

Measuring skin thickness

5 micron sections of skin, nasal pad and scrub sensory organ were stained, and their thickness was measured using a light microscope (Olympus, BX53, Japan) equipped with a digital camera (DP 80, Olympus, Japan), using *Arbitrary Line* drawing function of the Cellsens standard program (version1.17) at x2 magnification. For all samples, the skin thickness was measured from the top of the epidermis to the bottom of the dermis layers. The hypodermis layer was not taken into consideration as it is mostly composed of fat and cannot be properly preserved after histological procedures.

Statistical Analyses

Statistical analyses were performed using Statistical Package for the Social Sciences (SPSS) (22.0 USA) software. Using Shapiro-Wilk test and histogram graphs, the data was determined to be normally distributed and therefore parametric tests were employed. ANOVA with PostHoc tests (Tukey) was used for group comparisons. Values were calculated as mean±standard deviation (mean±SD). For all comparisons $p < 0.05$ was considered statistically significant.

Results

Measurements for the animal's body

The body weight, height, foot and arm lengths are given in Table 1.

Table 1. Measurements for the animals' body and skin thickness.

Measured parameters			
Blind mole rats (n=6)	**Mean**	**Min**	**Max**
Weight	203.7±56 g	134.0 g	274.0 g
Height	201.6±48 mm	103.0 mm	235.0 mm
Arm length	0.0 mm	0.0 mm	0.0 mm
Foot length	26.9±1.2 mm	25.0 mm	28.0 mm
Skin thickness (micrometer)			
Neck skin	1472.3±115.7	776.0	1087.0
Scrub sensory organ	5796.7±1256.0	3427.0	12870.0
Nasal pad	6348.5±1700.6	4417.0	7983.0

Measurements for the skin thickness

The first finding of comparatively analyzing the different regions of skin from *Nannospalax xanthodon* was that the nasal pad and the scrub sensory organ (SSO) were thicker than the neck skin. In order to quantify and statistically analyze this difference, we evaluated the H&E stained sections from each animal and measured skin thickness. The neck skin had a mean thickness of 1472.3±115.7 micrometers, while the nasal pad and the SSO were 6348.5±1700.6 and 5796.7±1256.0 micrometers thick, respectively (Table 1). The difference in mean thickness between neck skin and nasal pad as well as the SSO was statistically significant ($p<0.001$) although there was no statistically significant difference between the nasal pad and the SSO in thickness ($p=0.719$) (Figure 2 and Figure 3, upper panel).

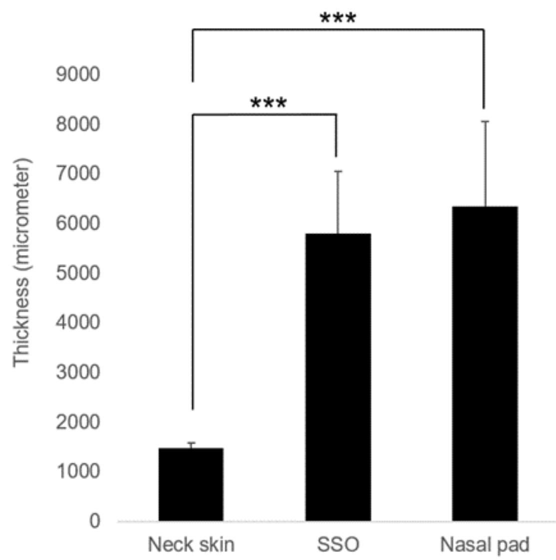

Figure 2. Mean skin thickness in the tissue samples derived from neck, nasal pad and Scrub Sensory Organ (SSO), measured using Cellsens software (n=6). The error bars represent the standard deviation within each group of samples. *** indicates p<0.001.

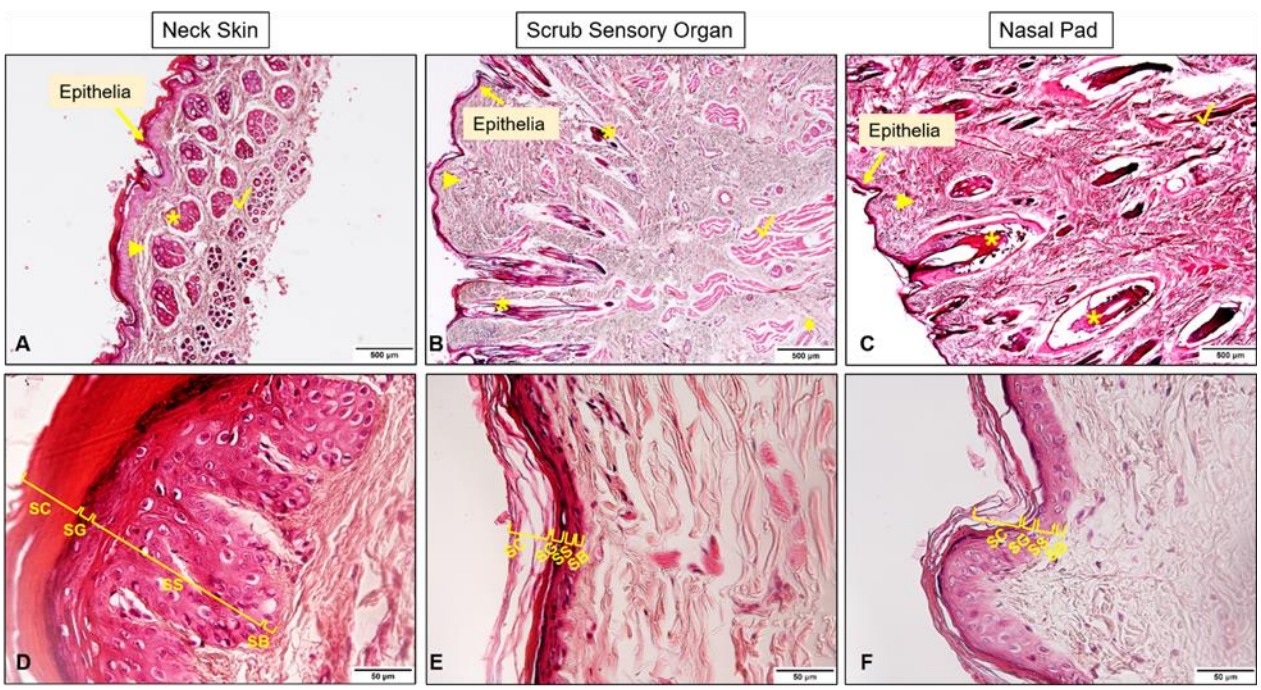

Figure 3. Panoramic images (A, B, C) and epithelial layers (D, E, F) of the neck skin, scrub sensory organ and the nasal pad, respectively. Hair follicles (*), muscle layers (✓), connective tissue (►) and blood vessels (♦) are indicated (x4), H&E. SC: stratum corneum, SG: stratum granulosum, SS: stratum spinosum, SB: stratum basale (x40), H&E.

Histopathological evaluation

The difference in skin thickness was further evaluated by histopathological analyses, which indicated that the epithelial layer was thicker in the neck skin compared to other skin samples. In particular, the stratum spinosum and stratum corneum layers of the neck skin epithelia was enlarged (Figure 3, lower panel). In the nasal pad and the SSO, the connective tissue and the muscle layers were more pronounced. The histological organization of all three skin samples appeared normal and showed several hair follicles.

In order to have additional evidence that could suggest a sensory role for the SSO, we performed Cresyl Violet staining, which selectively stains neurons (Adeli et al., 2019; Kaplan et al., 2018; İkinci et al., 2013), on all skin sections. We observed the presence of structures that are similar to pyramidal cells within the SSO, which are normally found in the brain including the cerebral cortex, the hippocampus, and the amygdala; and is absent in the skin (Spruston, 2008) (Figure 4).

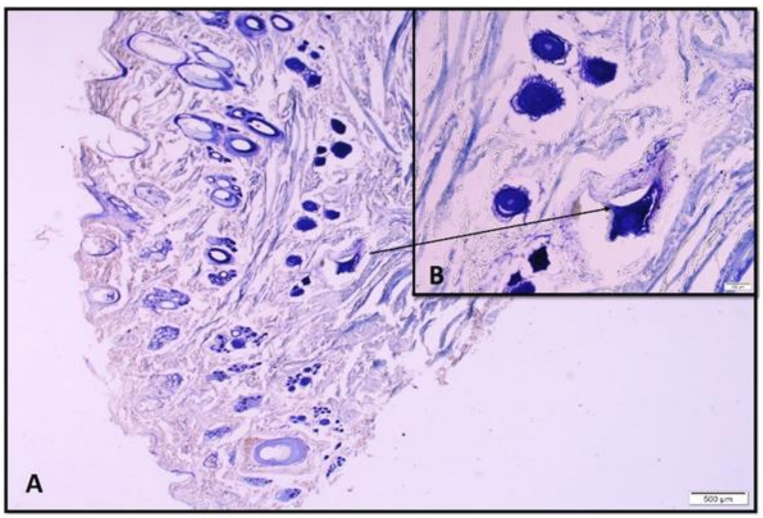

Figure 4. Panoramic images of the A) Scrub Sensory Organ (x2) and the B) pyramidal cell (x10), Cresyl violet.

Discussion

Skin is a sensory organ, which possibly presents an even greater significance for the blind mole rats as they are devoid of visual stimulus and have to rely on other modes of perception. Here in this study, we comparatively analyzed different regions of the skin in Nannospalax. We found that the specialized skin patch located on both sides of its nose, as we named Scrub Sensory Organ (SSO), was significantly thicker compared to the skin derived from the neck, despite having a thinner epithelial layer. The SSO thickness varied greatly between animals and different pieces obtained from the same animal. This is in part due to its unique structure, as end-points being thin and the middle being thicker, as well as due to the overall size of the animal. Nonetheless, even the thinnest SSO section was thicker than the thickest section obtained from the neck skin; pointing towards the validity of our results.

The SSO is covered by regularly arranged thick and long scrub-like hair follicle cells, which we assumed could play a role in somatosensation. The sensory function of hair follicles has previously been addressed by researchers and it was suggested that animals could use acoustic signals through activating hair follicle cells in order to communicate and to obtain information on their surroundings (Kazmierczak & Müller, 2012). Similarly, some studies propose that the lack of a functional visual system in the blind mole rat could be compensated by an auditory system that is directed by vibrational stimulus (Bronchti et al., 2002). It is possible to consider that hair cells inside the ear are involved in the perception of these afore mentioned vibrational stimulus. Having established the sensory role of hair follicle cells, we could speculate that the hair on the SSO provides the first contact with the surrounding objects and guides the blind mole rat in the absence of visual information. This hypothesis is further supported by our finding that pyramidal cells, which are considered as the 'movers and shakers' of the brain as they are responsible for carrying signals along to drive muscle function (Bekkers, 2011), exist within the blind mole rat's SSO. Similarly, pyramidal neurons have been implicated in whisker sensing as part of somatosensation in rats and mice (Manns et al., 2004; Petreanu et al., 2012; Ebner & Kaas, 2015).

There are more than 250 rodent species across the globe that adopted a subterranean lifestyle (Begall et al., 2007). From an evolutionary perspective, the extreme conditions of the underground world are considered as driving forces for developing adaptations. Different animal groups have independently developed similar traits over time, such as enhancements for efficient digging as well as navigating in the dark (Lacey et al., 2000). In line with this, animals that dig with their teeth such as the blind mole rats have more developed incisor teeth while their limbs are usually regressed (Partha et al., 2017). Due to the lack of light stimulus underground, vision is limited in most subterranean animals, although at differing degrees. Blind mole rats have completely subcutaneous eyes, which points towards their strictly subterranean lifestyle (Sanyal et al., 1990). Loss of vision is often compensated by the subterranean rodents via enhanced non-visual sensory systems (Partha et al., 2017). For instance, naked moles and star-nosed moles have developed sensory hairs and improved snouts respectively, to provide sensory perception (Catania,1999; Hill et al., 2009). Some of the anatomical and molecular adaptations developed by Nannospalax to underground are previously described (Sanyal et al., 1990; Nevo et al., 1995; Nevo, 2013). Furthermore, compensation of the visual system function by auditory adaptations have been reported in the blind mole rats (Bronchti et al., 2002; Burda, 2006). In addition to these established evolutionary adaptations of Nannospalax, here in this paper we identified a specialized skin patch, namely the scrub sensory organ (SSO) and defined it as an additional sensory organ with novel somatosensory adaptations.

In conclusion, our histopathological analysis on the blind mole rat's neck skin, nasal pad and the scrub sensory organ revealed significant findings such as the presence of structures that are similar to pyramidal cells in the SSO, which have not been reported elsewhere. These findings are important in having a better understanding of their evolution and adaptation to subterranean life.

Conflict of Interest: The authors declare that they have no conflict of interest.

Ethical approval: All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All animal procedures were approved by the Institutional Ethics Committee at Niğde Ömer Halisdemir University (Date: 10.09.2019, Protocol number: 2019/23) 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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