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# Quantitative analysis of development and aging of genital corpuscles in glans penis of the rat

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ABSTRACT

The aim of the present postnatal developmental study was to determine densities of unique genital corpuscles (GCs) in glans penis of developing and aged rats. GCs were identified as corpuscular endings consisting of highly branched and coiled axons with many varicosities, which were immunoreactive for protein gene product 9.5. In addition, GCs were immunoreactive for calcitonin gene-related peptide and substance P, but not for vasoactive intestinal polypeptide and neuropeptide Y. GCs were not found in the glans penis of 1 week old rats. Densities of GCs were low at 3 weeks, significantly increased at 5 and 10 weeks, reached the peak of density at 40 weeks, and tended to decrease at 70 and 100 weeks. Sizes of GCs were small in 3 weeks old rats, increased at 5 and 10 weeks, reached the peak-size at 40 weeks and reduced in size at 70 and 100 weeks. Considering sexual maturation of the rat, the results reveal that GCs of the rat begins to develop postnatal and reaches to the peak of their development after puberty and continues to exist until old age, in contrast to prenatal and early postnatal development of other sensory receptors of glabrous skin.



## 1. Introduction

A unique organization of sensory receptors in the glans penis has been reported in a variety of mammalian species including human (Halata and Munger, 1986), goat (Halata et al., 1988), and rat (Johnson and Halata, 1991). In contrast to the typical glabrous skin, the glans penis contains a predominance of free nerve endings and numerous corpuscular endings unique to the glans penis, but lacks Merckel cell endings and Meissner corpuscles with only a few Pacinian and Ruffini corpuscles (Johnson and Halata, 1991). The corpuscular endings in the glans penis have a characteristic appearance consisting of highly branched and coiled axons with many varicosities and have been given different names, such as end bulb (Krause, 1859, 1881), mucocutaneous end organ (Winkelmann, 1957), genital end bulb (Munger and Hershey, 1971; Munger and Ide, 1988), encapsulated nerve ending (Patrizi and Munger, 1965), lamellated corpuscle (Johnson and Halata, 1991), and we hereafter refer to this studies as genital corpuscles (GCs) the term originally used by Retzius (1890) as well as other investigators (Dogiel, 1893; Ohmori, 1924).

Previous studies (Ohmori, 1924; Winkelmann, 1957) have qualitatively evaluated the human GCs in various development stages and shown that they develope mainly in postnatal period reaching the peak of their development after puberty. Other sensory mechanoreceptors of the glabrous skin, including Merkel cell endings, Meissner corpuscles, and Pacinian corpuscles develop in prenatal and early postnatal periods (Cauna, 1965; Malinovský and Sommerová, 1972; Zelená, 1978; Holbrook, 1983; Zelená and Jirmanová, 1988; Mills et al., 1989).

In rats, testosterone-dependent penile mechanoreceptors have been found (Johnson and Murray, 1990), and an electrophysiological study demonstrated that the sensitivity of these penile mechanoreceptors of old animals is significantly lower than that of mature adults (Johnson and Murray, 1992). These findings strongly suggest that number and/or sensitivity of GCs is correlated with male sexual maturation.

Somatosensory information from penile mechanoreceptors that encode a variety of penile skin movement is very important for driving male sexual behaviors, especially penile erection and ejaculation. Knowledge about GC development is thus necessary for understanding the mechanisms underlying the development and aging of male sexual behaviors. The development of GCs in human has previously been described by using classical histological methods such as methylenblue staining or silver impregnation (Ohmori,

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1924; Winkelmann, 1957). However, other species, including rats, have not been examined and a quantitative study of GC development is also lacking. Here we report a quantitative evaluation of the development and aging of GCs in the rat glans penis using protein gene product 9.5 (PGP9.5) immunoreacivity as a neuronal marker (Day et al., 1990). The results revealed that GCs in the rat develop postnatally, reach the peak of development after puberty, and continue to exist until old age. In addition, neural elements in the glans penis were studied by immunohistochemical staining for calcitonin gene-related peptide (CGRP), substance P (SP), vasoactive intestinal polypeptide (VIP), and neuropeptide Y (NPY).

## 2. Materials and methods

The research protocols were approved by Toho University Animal Care and Use Committee (No. 14-53-181) and conformed to the National Institutes of Health animal use guidelines.

A total of 38 male rats of SD-strain (Spraque-Dawley rat strain, CLEA Japan, Inc.) were examined at different developmental stages; postnatal 1, 3, 5 10, 40, 70, and 100 weeks. The animals were anesthetized intraperitoneally with chloral hydrate (3.5 mg/100 g body weight), and perfused through the heart with 0.1 M phosphate buffer (PB) containing 0.2% heparin, followed by 4% paraformaldehyde in 0.1 M PB. The penises were removed, postfixed for 5–12 h, cryoprotected in 20% sucrose in PB, and sectioned coronally with a freezing microtome set at 60 μm.

The sections were immersed in 0.1 M phosphate buffered saline containing 4% normal goat serum for 30 min, and incubated with following antibodies for 2 days at 4 °C; anti-rat PGP 9.5 raised in rabbit (1:4000; Ultra Clone Ltd., Wellow, UK), anti-rat CGRP raised in rabbit (1:3000; Cambridge Research Biochemicals, Billingham, UK), anti-rat SP raised in rabbit (1:5000; UCB-Bioproduct Inc., Brussels, Belgium), anti-rat VIP raised in rabbit (1:5000; INCSTAR Co., Stillwater, MN, USA), and anti-rat NPY raised in rabbit (1:5000; INCSTAR Co., Stillwater, MN, USA). Then, they were incubated with biotinylated anti-rabbit IgG, followed by immersion in a solution containing the avidin–biotin–peroxidase complex (Vector Lab., Burlingame, CA) for 90 min. The localization of peroxidase activity was visualized by incubation of the sections with 0.02% 3–3′ diaminobenzidine (DAB, Sigma Chemical Co, St. Louis, USA) and 0.007% $H_2O_2$ at room temperature. After the reaction, sections were mounted on slides. For histological observation, penises of 1, 3, 5, and 10 weeks old rats were dehydrated through a graded series of ethanol, embedded in paraffin, sectioned coronally and sagittally at 10 μm, and stained with hematoxilin–eosin.

PGP 9.5-immunostained sections through the fibrous cartilages of the os penis (Hebel and Stromberg, 1986) of four or five rats at each stage were analyzed (Fig. 1). The density of GCs was caluculated as the number per 1 $mm^3$ of the dermal layer. The borders between the epidermis and dermis and between the dermis and sinus cavernosum glandis capsule were drawn using a camera lucida (Olympus BX51, Olympus Co., Tokyo, Japan) with a 20× objective as shown in Fig. 2D and the area was measured using Cosmosome (Cosmosome, Nikon Co., Tokyo, Japan). The volume was calculated by multiplying the area by the thickness of sections. Criteria for the identification of GCs are mainly based on previous descriptions on GCs vitally stained with methlene blue (Retzius, 1890; Dogiel, 1893; Ohmori, 1924), or silver impregnation (Ohmori, 1924; Winkelmann, 1957; Patrizi and Munger, 1965). as follows: (1) various-shaped corpuscular endings, into which one to several PGP 9.5-positive nerve fibers entered. (2) These nerve fibers repeatedly branch into fine and coiled axons within the corpuscle. (3) The fine and coiled axons exhibit. The long and short axes of GCs and penile spines were measured in each stage of development. Statistical significance of an overall difference between groups was determined by one-way ANOVA. If one way ANOVA was significant, differences between individual groups were estimated using the Turkey–Kramer's multiple comparison method.

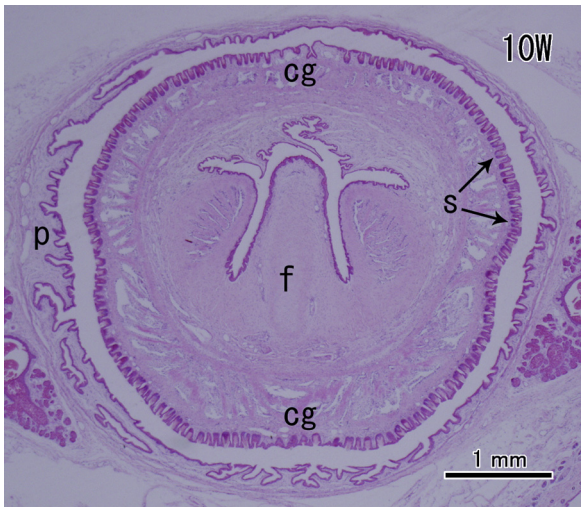


**Fig. 1.** A cross section of glans penis at the level of fibrous cartriage of os penis using H–E stain at 10 weeks in age. P: pariental leaf of the prepuce. f: fibrous cartiage of the os penis. cg: corpus cavernousum glandis. S: keratinized penile spine.

## 3. Results

### 3.1. Postnatal development and aging of glans penis and genital corpuscles

At 1 week after birth, the epidermis of the glans penis was in close contact with the epidermis of the preputium, and was not endowed with penile spines (Fig. 2A). The dermis of the glans penis was a flat layer without dermal papilla. The sinus cavernosum glandis was poorly developed. PGP 9.5-, CGRP-, and SP-positive nerve fibers were found in the dermis of the glans penis and the preputium. However, corpuscular structures were not observed in the glans penis (Fig. 3A).

At 3 weeks, immature penile spines were found in the glans penis and the epidermis of the glans penis was still contact with the epidermal of the preputium. The dermal papillae invaded in the center of penile spines forming a fibrous core or underneath the epithelium between neighboring penile spines (Fig. 2B). Small GCs were only rarely found in the dermis of glans penis (Fig. 3B). CGRP- and SP-positive nerve fibers were found in the area between the dermis and the sinus cavernous glandis capusules.

At 5 weeks, keratinized penile spines were covered by the epithelium of the preputium (Fig. 2C). PGP 9.5-positive GCs were occasionally found in the dermal papillae between neighboring penile spines and in the deep part of the dermis (Fig. 3C). There were few CGRP-and SP-positive GCs in the dermis.

At 10 weeks, the epidermis of the glans penis with many keratinized penile spines were completely separated from the epidermis of the preputium (Fig. 2D). Many PGP 9.5-positive GCs were located in the dermal papillae between two neighboring penile spines and in the deep part of the dermis (Fig. 3D). However, GCs were not found in the fibrous core of penile spines, nor in the trabeculae of the sinus cavernosum glandis. Free nerve endings were found throughout the glans penis and penile spines and their dermal papillae were not innervated by free nerve endings or any other neural structure. PGP 9.5-, CGRP-, and SP-positive nerve fibers were found in the dermis and in the trabeculae of the sinus cavernosum glandis. VIP-, and NPY-positive nerve fibers were found around the