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Neural interrelationships of autonomic ganglia from the pelvic region of male rats

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ABSTRACT

The aims of the present study were to describe, in male rats, the anatomical organization of the major and accessory pelvic ganglia (MPG, AG; respectively), the interrelationship of the pelvic plexus components, and the morphometry of the pelvic postganglionic neurons. Anatomical, histochemical and histological studies were performed in anesthetized adult Wistar male rats. We found that the pelvic plexus consists of intricate neural circuits composed of two MPG, and three pairs of AG (AGI, AGII, AGIII) anatomically interrelated through ipsilateral and contralateral commissural nerves. Around 30 nerves emerge from each MPG and 17 from AGI and AGII. The MPG efferent nerves spread out preganglionic information to several pelvic organs controlling urinary, bowel, reproductive and sexual functions, while AG innervation is more regional, and it is confined to reproductive organs located in the rostral region of the urogenital tract. Both MPG and AG contain nerve fascicles, blood vessels, small intensely fluorescent cells, satellite cells and oval neuronal somata with one to three nucleoli. The soma area of AG neurons is larger than those of MPG neurons ($p < 0.005$). The MPG contains about 75% of the total pelvic postganglionic neurons. Our findings corroborated previous reports about MPG inputs, and add new information regarding pelvic ganglia efferent branches, AG neurons (number and morphometry), and neural interrelationship between the pelvic plexus components. This information will be useful in designing future studies about the role of pelvic innervation in the physiology and pathophysiology of pelvic functions.

1. Introduction

The pelvic area is the anatomical substrate for urinary, fecal, sexual and reproductive functions, which are controlled by autonomic innervation provided by the pelvic plexus, which contains sympathetic and parasympathetic postganglionic neurons (Carlstedt et al., 1988, 1989; Luckensmeyer and Keast, 1998; Keast, 2006; de Groat et al., 2015).

Although an ample body of literature exist on the biochemical characteristics of the autonomic postganglionic neurons of the urogenital and bowel organs (Keast, 1995; Luckensmeyer and Keast, 1995) many pelvic innervation features remain to be discovered. For example, the role of pelvic autonomic innervation in pelvic dysfunctions such as overactive bladder, bowel disorders and pelvic cancer. Recently, it has been postulated that autonomic hyperactivity may be related to prostate cancer (Magnon et al., 2013).

One limiting factor to study pelvic innervation is the anatomical complexity of the pelvic plexus in large animals. In humans, dogs, rabbits and cats, the pelvic plexus is made-up of a diffusally distributed pelvic ganglia (Cruz et al., 2017; Keast, 2006; Langley and Anderson, 1896; Li and Masuko, 2001). In contrast, in rats most of the pelvic postganglionic neurons are hosted in a couple of pelvic ganglia, each one has been called major pelvic ganglion (MPG; Greenwood et al., 1985; Purinton et al., 1973). Thus, the rat model can be considered as the most appropriate animal for performing neurourological and bowel studies (Hehemann et al., 2018; Keast, 1999; Luckensmeyer and Keast, 1998).

In male rats, the MPG innervates the prostate, seminal vesicles, bulbourethral glands, vas deferens, testis, penis, distal colon, rectum and urinary bladder (Dail et al., 1989; Keast et al., 1989; Keast, 1992; Kepper and Keast, 1995; Kepper and Keast, 1997; Kolbeck and Steers, 1993; Rauchenwald et al., 1995; Luckensmeyer and Keast, 1994;

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Luckensmeyer and Keast, 1995). Two nerve inputs to the MPG are provided by the hypogastric (HG) and the pelvic nerves (PN), which carry axons from sensory and preganglionic lumbosacral neurons (Purinton et al., 1973).

The sensory and autonomic information is spread-out from the MPG to pelvic organs through several processes. However, with exception of the cavernous nerve (also known as penile nerve, or main penile nerve (Dail et al., 1989; Purinton et al., 1973; Steers et al., 1988) and rectal nerves (Luckensmeyer and Keast, 1998), the MPG efferent nerves have been poorly described, which unable researchers to investigate the effect of specific pelvic organs denervation, for example prostate or bladder.

In addition to limited studies about MPG nerve branches, little is known about the nomenclature and organization of the small ganglia localized near the base of the vas deferens and anatomically related with the MPG. These small ganglia have been called accessory ganglia (AG; Keast et al., 1989; Purinton et al., 1973), peripheral ganglia (Langworthy, 1965) or hypogastric ganglion (Melvin et al., 1988) and contain a significant percentage of the autonomic postganglionic neurons in pelvic organs (Keast et al., 1989).

Furthermore, the inconsistencies in the nomenclature of the pelvic plexus components make it difficult to have comparative studies. The aims of the present study were to describe, in male rats, the anatomical organization of the major and accessory pelvic ganglia, the interrelationship of pelvic plexus components, and the morphometry of the pelvic postganglionic neurons.

2. Material and methods

Sixteen adult male Wistar rats (250–300 g) were housed in plastic cages and maintained in a light/dark (12/12) cycle. Food and water were provided ad libitum. All animal care and the experimental protocol were approved by the Tlaxcala University Committee on Laboratory Animals, in accordance with the guidelines of the Mexican Council on Laboratory Animals' Care (NOM-062-Z00-1999) and the Guide for the Care and Use of Laboratory Animals (National Research Council, USA).

2.1. Experimental design

The anatomical characteristics, topography and organization of the neural circuitry of the AG and the MPG were determined by gross anatomy (n = 10) and acetylcholinesterase histochemistry (n = 3). Histological studies were performed to determine the number of neurons in both AG and MPG ganglia, as well as the morphometry of the somata (n = 3).

2.2. Gross anatomy

The animals were anesthetized with urethane (1.5 g/kg i. p.) and placed in supine position. A laparotomy was performed and the pubis bone resected. The lower urinary tract and the left lateral prostate lobe were exposed. Using a Leica M80 stereomicroscope the left MPG and the AG were dissected using a 30× amplification. By means of a 40–46× amplification the ganglia branches were dissected, counted and followed to their organ targets.

Nerve counting started on the first MPG-AG connecting nerve, and followed in a clockwise direction. We wanted to start the nerve counting with the cavernous nerve due its large size, which makes it easy to be localized, however, considering that we were going to count AG efferent nerves too, we decided to start with a conspicuous nerve connecting both ganglia. In this way, the MPG and AG connecting nerves would have the same number when counting efferent nerves for each ganglia. Photographs of the neural circuitry were taken and drawings of the distribution of the ganglia branches elaborated. The animals were euthanized with an overdose of the anesthetic.

2.3. Acetylcholinesterase histochemistry

The animals were transcardially perfused with phosphate-buffered saline (PBS) and the rostral region of the genitourinary tract dissected in a block, from the distal portion of the vas deferens to the pelvic urethra. The associated fat tissue was removed as much as possible. The tissue was immersed in 10% neutral formalin for 24 h at 4 °C, then washed in PBS and processed for Acetylcholinesterase histochemistry as previously described (Karnovsky and Roots, 1964). Briefly, the genitourinary tissue was incubated for 2.5–3 h at room temperature with a solution consisting of substrate acetylthiocholine iodide dissolved in 0.1 M sodium hydrogen maleate buffer, pH 6.0, 0.1 M sodium citrate, 30 mM $CuSO_4$, water, 5 mM potassium ferricyanide and 0.1% Triton X-100.The tissue was then rinsed in distilled water, dehydrated with ascending alcohols, cleared with xylene, and observed with a stereoscopic microscope (Leica M80). Photographs were taken.

2.4. Histology and morphometric neuronal analysis

The left AG and MPG were fresh excised and immersed in 10% formalin for at least 48 h. Then, the tissue was embedded in paraffin (Paraplast Xtra, Sigma-Aldrich), and longitudinally sectioned (7 μm thick) with a microtome (Leica RM2125RT). The sections were stained with a Masson's trichrome technique and observed with a light microscope (Zeiss Axiostar Plus model). Photographs were taken with a digital camera (Cannon PowerShot S50).

We used a two steps Cavalieri-Disector combination method to estimate the number of neurons, as described elsewhere (Coggeshall, 1992; Kaplan et al., 2012; Sterio, 1984). The Cavalieri principle was used to calculate the volume of each ganglia. In a second step, a physical disector was used to determine the numerical density value. Briefly, to calculate the ganglion volume a section was randomly chosen, and then 10 additional sections were selected at fixed intervals as a representative sample of the total slides. From the photomicrographs, the area of each ganglion was determined using AxioVision 4.8. The area of the ganglion was then averaged and multiplied by the total number of sections. The obtained number was multiplied by the section's thickness to estimate the ganglion volume.

To determine the numerical density, the diameter of neurons with clear nucleus was determined in 10 sections of the ganglion. Binucleated neurons were scarce and they were not counted. Ten disectors at fixed intervals were analyzed per ganglion, and 1/3 of the smallest diameter neuron was used as the height of the disector. From the sections at fixed intervals, the reference and look up sections were determined. Neurons with clear nucleus localized in the reference sections but not found in the look up sections were counted. The area of the ganglion containing the counted neurons was determined, and the volume of the disectors of reference calculated. Then, the neuronal density was estimated by dividing the total disector neuronal number by the total disector volume.

The total numbers of neurons per ganglion was estimated by multiplying the numerical density by the total volume of the ganglion (it was the reference volume obtained in step 1). The morphometry of the neurons was measured in the sections used to determine the ganglia volume. The soma area, as well as major and minor axis of the somata, were measured in ~500 MPG and 200 AG neurons with a clearly delimitated membrane and a visible nucleus.

2.5. Statistical analysis

Values of soma area, major axis and minor axis of MPG and AGI neurons were analyzed using student's *t*-test (AG neurons versus MPG neurons) and presented in results as means ± standard error of the mean (SEM). A $p < 0.05$ was considered to indicate a significant difference. Data of soma area, major axis, and minor axis were also ranged at intervals of 200 μm^2. Percentage per range was calculated per rat,

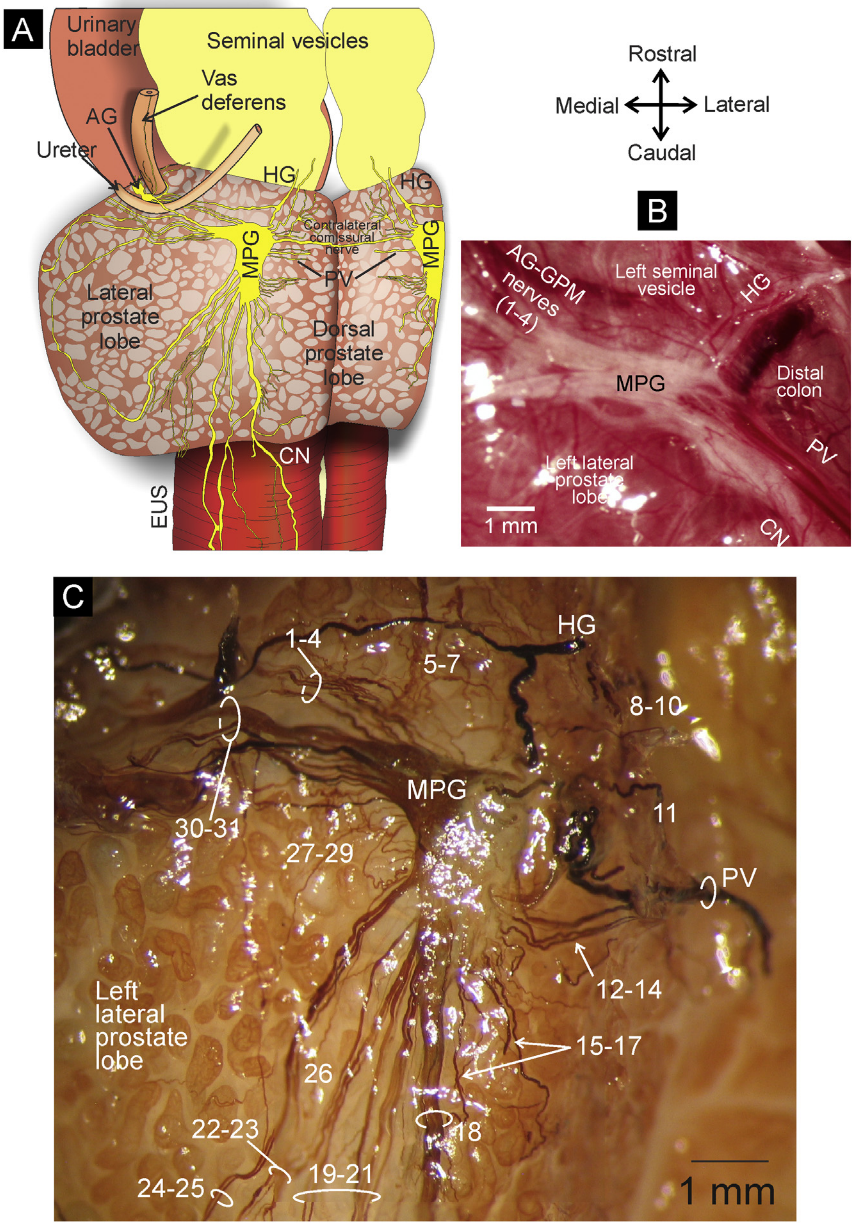

Fig. 1. Schematic representation of the lower urinary tract of the male rat and its innervation. A, diagrammatic representation of the anatomic interrelation between the major pelvic ganglion (MPG) and the accessory ganglia (AG). B, Photomicrograph of a MPG in gross anatomy. C, the MPG and their efferent branches are shown in pelvic tissue treated with acetylcholinesterase. Numbering indicate: 1–4, communicating nerves between MPG and accessory ganglia (AG); 5–7, nerves to left seminal vesicle; 8–10, nerves to dorsal prostate lobe; 11, MPG contralateral commissural nerve; 12–14, nerves to distal colon and rectum; 15–17, nerves to lateral prostate lobe; 18, cavernous nerve; 19–21, nerves that anastomoses to plexus in sensory branch of pudendal nerve; 22, 23, nerves to the cranial segment of the external urethral sphincter; 24, 25, nerves to the ventral prostate lobes; 26–29 nerves of the lateral prostate lobe; 30, 31, nerves to the urinary bladder. HG, hypogastric nerve; PV, pelvic nerve.

and then averaged to build the histograms.

3. Results

3.1. Anatomical interrelationships of the pelvic autonomic ganglia

In the rostral region of the urogenital tract we found an intricate network of neural tissue anatomically interrelated (Fig. 1A). The following description of the left MPG of an animal in supine position is presented in a clockwise direction.

Table 1
Variability in nerve numbers of the left MPG of male rats.

No. of nerves	No. of animals
26	2
28	1
30	1
31	3
33	2
34	1

3.1.1. MPG neural circuitry

In 100% of the animals, the MPG was localized on the lateral lobe of the prostate (Fig. 1A–C). MPG has a triangle shape and from this structure (Fig. 1C) were observed 26 to 34 nerves (Table 1).

From the cranial region of the MPG emerged seven to eight nerves; three to five of them run craniomedially to connect with the AG-I, (numbers 1–4 in Figs. 1B–C, 2C) and the others run toward the seminal vesicles (numbers 5–7 in Fig. 1C). Lateral to them arrives the HG (Fig. 1B–C).

In the lateral region of the MPG, caudal to the HG, arose six to eight nerves; three to four of them run to the dorsal lobe of the prostate (numbers 8–10 in Fig. 1C). The rest run along the dorsal lobe of the prostate and decussate to the contralateral MPG (contralateral commissural nerve in Fig. 1A; number 11, Fig. 1C), and another two to four nerves run to the distal colon and rectum (numbers 12–14 in Fig. 1C). Over these last nerves passes the pelvic nerve (PN in Fig. 1C), which

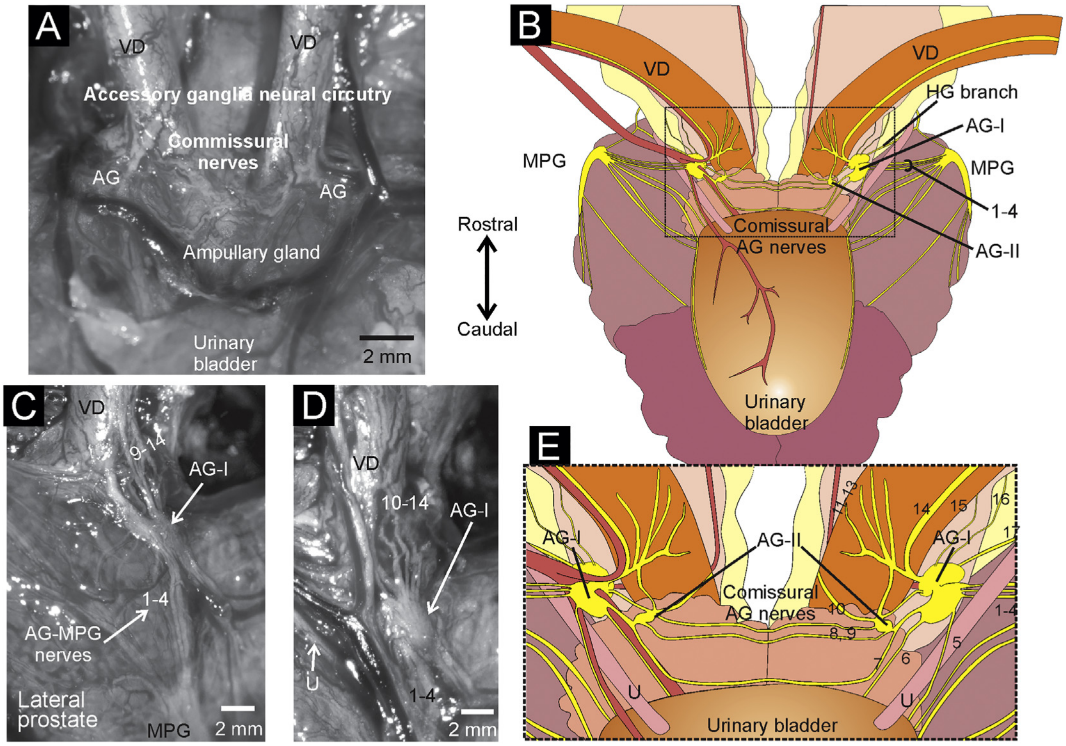

Fig. 2. Description and organization of the pelvic neural circuitry of a male rat. A, B ventral view of the vas deferens (VD) and accessory ganglia (AG) neural circuitry. Note communicating nerves between AG localized over the ampullary gland. B shows the rostral region of the urogenital tract in a ventral view. The bladder has been pulled down to appreciate the nerves dorsally to it. In the left side, blood vessels were omitted for better appreciation of the AGI and their ipsilateral connection with the MPG (nerves 1–4). C and D, Photomicrographs of the ventrolateral region of the left vas deferens and the neural circuitry localized nearby. In C the blood vessels were withdrawn for better appreciation of the AG-MPG commissural nerves (1–4). E, an amplification of the neural circuitry showed in B. 1–4, AG-MPG nerves, 5, 6 nerves going to the dorsal base of the urinary bladder; 7–9, contralateral commissural AG nerves; 10–13, deferential nerves; 14, 15 and 16 indicate innervation toward the epididymis, seminal vesicle and coagulating gland, respectively; 17, branch of the hypogastric nerve (HG).

contains three to four nerve bundles running below the internal iliac vein that attach to the MPG (Fig. 1C).

In the caudal region of the MPG were seen nine to eleven nerves that run caudally; four of them (numbers 15–17, 26 in Fig. 1C) entered into the lateral prostate, another two form the cavernous nerve (number 18 in Fig. 1C), and the other three go down over the ventrolateral wall of the external urethral sphincter and anastomoses to a small plexus attached to the dorsal nerve of the penis (numbers 19–21 in Fig. 1C).

The cavernous nerve was followed caudally to identify its targets. The nerve divided, one branch passed through the levatorani muscle fibers, but no innervation to this muscle was observed. The other two branches run caudally to the distal rectum, anus and perineal skin. No anatomical evidence of innervation of the penis through the cavernous nerve was observed.

From the medial region of the MPG emerged six nerves that were distributed into the rostral region of the urethra (numbers 22–23 in Fig. 1C), the ventral prostate lobe (numbers 24–25 in Fig. 1C) and the lateral prostate lobe (numbers 27–29 in Fig. 1C). The next three nerves run craniomedially and ramify to innervate the lateral and ventral wall of the urinary bladder (numbers 30–31 in Fig. 1C).

3.1.2. AG neural circuitry

Dorsal to the bladder and ventral to the base of the vas deferens was an intricate neural circuitry composed of two small ganglia (accessory ganglia I and II; AG I, AGII) and their nerves (Fig. 2A–E). Three to four commissural nerves run on the ampullary gland and connect the left and right AG (Fig. 2A, D, E). In each side AG have two components, the AG-I and the AG-II (Fig. 2A, B, E).

AG-I (~2–3 mm) has an elongated shape, and is embedded in blood vessels adjacent to the ventrolateral wall of the vas deferens (Fig. 2B–E). In 70% of the animals, a portion of the AG-I was localized on the venous plexus that arises from the umbilical artery, and the remaining ganglion portion was beneath the blood vessels. In the other 30% of animals, the AG-I was found under the ureter, just before entering into the urinary bladder.

Around 17 nerves were attached to each AG-I. Laterally we identified three to four nerves passing under the ureter and joining AG-I, these AG-MPG connecting nerves were numbered as branches 1–4 of the MPG in the previous section (Figs. 1C and 2B, C, E).

From the ventromedial region of the AG-I emerged five to seven branches, two of them are short (number 5, 6 in Fig. 2E), and run with a vein toward the dorso-caudal region of the urinary bladder. The other nerves (numbers 7–9 in Fig. 2E) are commissural communicating the AG contralaterally (Fig. 2A, B, E).

From the rostral region of the AG-I arose seven to ten nerves (numbers 10–16 in Fig. 2E); ~ six of them were distributed in the vas deferens (number 10–14 in Fig. 2D, E), and the others ran to the ventral wall of the coagulating gland and seminal vesicles (numbers 15, 16 in Fig. 2E). In 70% of animals a branch of the HG joined to the AG-I laterally (number 17 in Fig. 2E).

AG-II ($\leq$1 mm) have a round shape, are localized ventromedially to the base of the VD, on the surface of the ampullary gland, (Fig. 2A).

3.1.3. Intra-subject variability

As mentioned above, in 70% of the animals the HG and its main branch entered to the MPG, while the other branch joined the AGI. In the remaining 30% of animals, the HG entered to the MPG without a division. Other intra-subject variability observed was in the number of MPG efferent nerves to its targets, for example, the dorsal prostate lobe may be innervated by 3 to 5 MPG nerves, however, the position of origin of the nerves in the MPG was fixed. For instance, nerves innervating rectal targets and the dorsal prostate always emerged in the

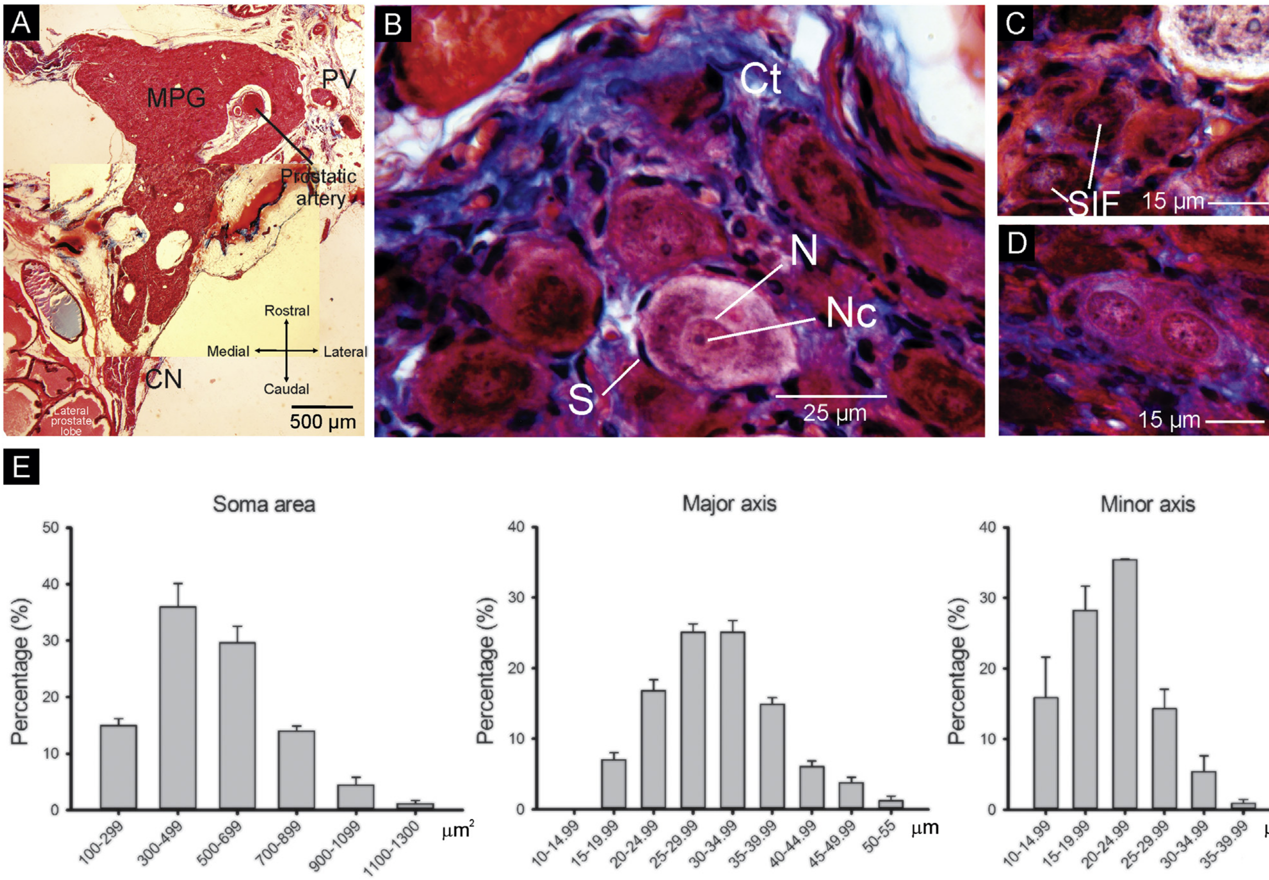

Fig. 3. Morphometry of the major pelvic ganglion (MPG) in the male rat. A, shows a composite image to exhibit the neurons localized in the MPG and in the origin of the cavernous nerve (CN). B–D, histological characteristics of MPG neurons, note a binucleated ganglia neuron in D. The tissue was stained with Masson's trichrome technique. E, morphometric measurements for MPG neurons. Values represent mean ± SEM, n = 3. HG, hypogastric nerve; PV, pelvic nerve; CN, cavernous nerve; Ct, connective tissue; nucleus (N), nucleolus (Nc), and small intensely fluorescent cells (SIF).

dorsolateral region of the MPG.

3.2. Morphometry of pelvic neurons

3.2.1. MPG neurons

The MPG is ~3500 µm on its major axis, and 2200 µm on the minor axis (Figs. 1B, 3A). It constitutes a lobulated structure (Fig. 3A) that contains 11,900 ± 2300 neurons (means ± SEM), nerve fascicles and blood vessels between the neuronal somata, as well as small intensely fluorescent cells (SIF) and satellite cells surrounding ganglionic neurons (Fig. 3B–D). A main cluster of neurons is localized medially to the prostatic artery that crosses the MPG, and small clusters are present along the first ~1.5 cm of the cavernous nerve, into the fascicles connecting to the AG and lateral to the prostatic artery (Fig. 3A). The MPG neuronal bodies are mostly oval in shape, with one to two nucleoli (Fig. 3C, D). A few of them are binucleated (Fig. 3D). The somata area ranges from 100 to 1300 μm^2, with an average of 513 ± 23.5 μm^2. The neuronal major and minor axis ranges from 10 to 50 µm (Fig. 3E), with a mean value of 30.4 ± 0.22 µm and 20.6 ± 1.1 µm, respectively.

3.2.2. AG neurons

The AG-I contains 3300 ± 250 neurons (means ± SEM), which are mainly clustered in the cranial and caudal regions of the ganglion, a thin portion with few neurons unites these regions (Fig. 4A–B). The neuronal somata are mostly elongated in shape, monopolar, with one to three nucleoli, a few of them are binucleated (Fig. 4C–E). This ganglion also contains satellite and scattered SIF cells (Fig. 4C). The area of the somata ranges from 100 to 1300 μm^2 (Fig. 4F) with an average of 665.8 ± 18 μm^2. The major and minor neuronal axis ranges from 10 to 55 µm (Fig. 4F), with an average of 34.8 ± 0.5 µm and 24 ± 0.5 µm, respectively. The neurons from the AG were larger than those from the MPG neurons (soma area p = 0.007; major axis p = 0.001, minor axis p = 0.004). The AG-II was constituted by 3 to 4 small clusters that all together contain about 30 neurons.

4. Discussion

The present study provides a detailed description of the peripheral neural circuitry in the pelvic plexus of the male rat, the main laboratory animal used for urological and bowel studies. Our findings corroborate previous reports about MPG inputs (Keast, 1995; Langworthy, 1965; Purinton et al., 1973), and add new information regarding the efferent branches, neural interrelationship between the pelvic plexus components, and AG neuronal characteristics.

We corroborate that inputs to MPG arrives from the HG and PN nerves, which contains afferents and efferent preganglionic fibers, HG also contains postganglionic sympathetic axons from inferior mesenteric ganglion and the PN also contains axons from caudal sympathetic chain (de Groat et al., 2015; Keast, 1995; Langworthy, 1965; Purinton et al., 1973).

Our data agree with previous reports about individual variations in the HG (Keast et al., 1989; Kihara and de Groat, 1997b), with a main pattern (70% of the animals) having a dividing branch that joins the AGI. In the other 30% of the animals no nerve bifurcation was observed, probably because in this pattern the axons from the HG reach the AGI through the MPG-AG connecting nerves.

Regarding the PN (Langworthy, 1965; Purinton et al., 1973), in female rats this innervation has been called viscerocutaneous branch of

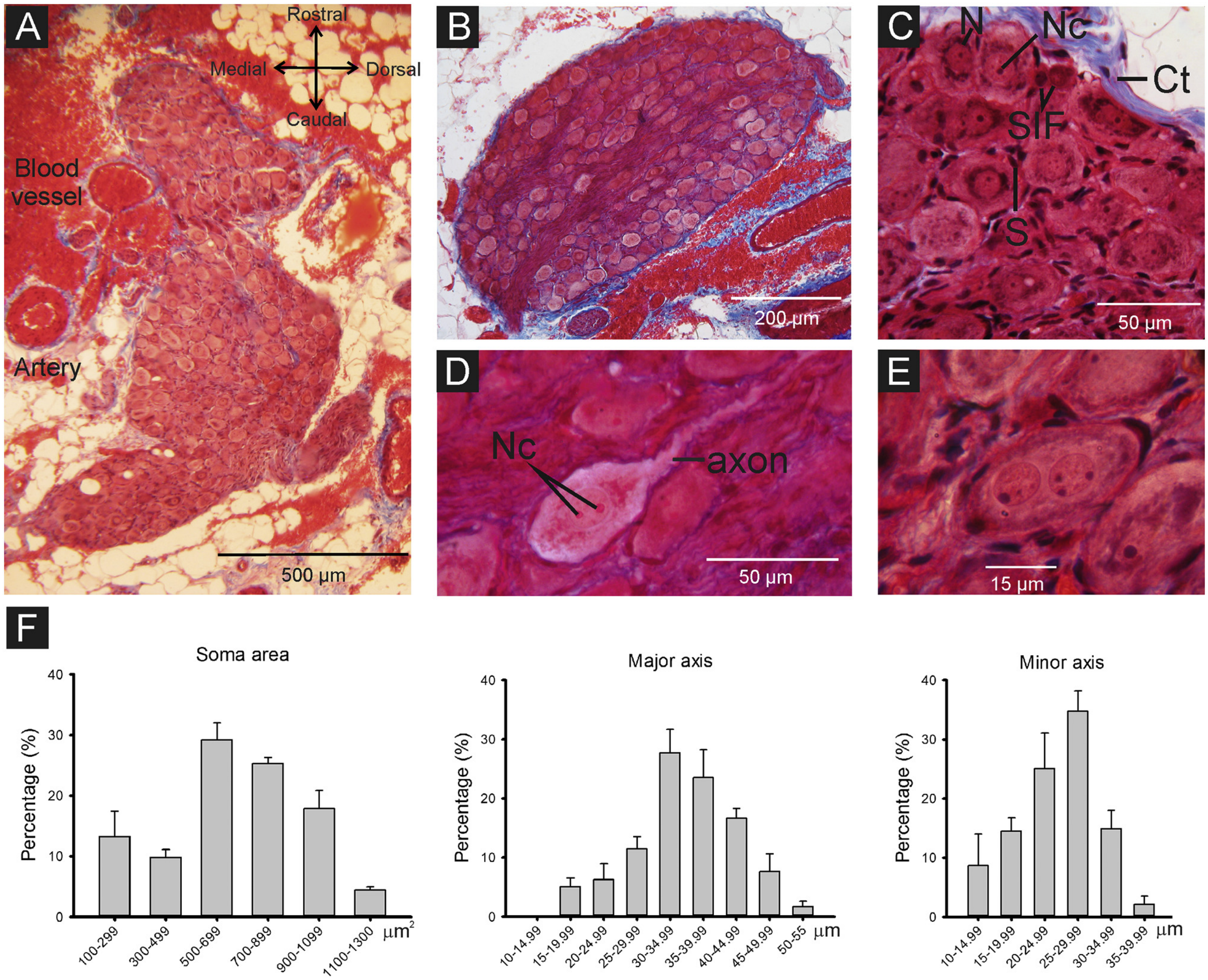

Fig. 4. Histological characteristics of the AGs in the male rat. A–E show the left accessory ganglion (AGl) stained with Masson's trichrome technique, note in A that AGl has two lobulated regions, with the rostral portion embedded in blood vessels. B, Cranial portion of AGl, note that nerve fascicles are seen between the ganglion cell bodies. As shown in C–E, the AG cells consist of satellite cells (S), small intensely fluorescent cells (SIF) and unipolar neurons with either a single nucleus (N) or binucleated (not common). Neurons have one to three nucleoli (Nc). F, morphometric measurements for neurons from the left AGl. Values represent mean ± SEM.

the pelvic nerve because this nerve carries information from the perineal skin (Pacheco et al., 1989). In male rats, it has been reported that the penile nerve carries sensory information from the perineal skin (Steers et al., 1988), which could reach dorsal root ganglia via the PN. If this is thrue, it is important to reconsider the name of this nerve as viscerocutaneous instead of PN.

Regarding MPG nerve outputs, there are very few studies focused on analyzing the total number of MPG processes and their anatomical targets. Our anatomical evaluation agrees with some previous results, indicating that there are processes for the prostate, bladder, urethra, penis, colon and rectum, as well as communicating nerves to other neural structures (Keast et al., 1989; Keast and de Groat, 1989; Kepper and Keast, 1995; Kihara and de Groat, 1997b; Langworthy, 1965; Luckensmeyer and Keast, 1998; Purinton et al., 1973). Our results also showed that the nerves originate close to their targets. Intra-subject variability in the number of processes running to a target was found, but not in the position of origin within the MPG.

A fixed origin of MPG efferent processes agree with evidence of a topographical organization of MPG postganglionic neurons innervating some pelvic organs (Dail et al., 1989; Kepper and Keast, 1995; Keast et al., 1989). According to the origin of the MPG nerve bundles, we propose that the dorsolateral region of the MPG contains the innervation for the dorsal lobe of the prostate, colon and rectum. The caudal region innervates the urethra, penis, and anorectal region (in this region also cross afferent axons to the perineal skin). Finally, the medial region of the MPG innervates bladder, ventral prostate lobe and the rostral region of the seminal glands. These topographical organization of the MPG processes indicate that regional lesion of the ganglion would impair the function of specific pelvic organs. Call the attention that bladder efferent innervation arose only from the medial region of the MPG, despite that their neurons are distributed throughout the whole ganglion (Keast et al., 1989).

Innervation to the penis present an intricate neural network, including an anatomical relation of MPG nerves with a small ganglia (AG III) localized at the base of the penis (Pastelín et al., 2011). Our results partially agree with previous studies describing that three MPG nerves innervate the penis, being the main one the cavernous nerve (Dail et al., 1989; Purinton et al., 1973). We did not find a nerve going directly to the penis, but there are three MPG nerves that anastomose to a small plexus (AG III) in the sensory branch of the pudendal nerve (SBPDn), which suggest that the MPG innervates the penis through these pathways. Other innervation paths to the penis arrive from the PN via the SBPDn (Pastelín et al., 2011). Thus the dorsal nerve of the penis is a complex nerve carrying autonomic and somatomotor components

(Pastelín et al., 2011; Galindo et al., 1997) with axonal components from the SBPDn, AG III postganglionic neurons and the MPG postganglionic neurons.

The precise axonal composition and role of the cavernous nerve in autonomic innervation of the penis requires further studies since we did not found an anatomical connection of this nerve with the penis. Although it has been described that electrical stimulation of this nerve increased intracavernous pressure, authors of such study did not indicate whether the stimulation was applied in the distal region of a transected nerve to stimulate postganglionic axons, otherwise cavernous pressure increase could be reflexively induced (Steers et al., 1988). In addition, it has been described that transection of the main penile nerve (cavernous nerve) reduced, but did not eliminate, retrograde labelling of penile neurons in the MPG and only modestly decreased NADPH-d+ fibers in the penis, which indicates that this nerve is not the main vasodilator pathway to the penis (Dail et al., 1999). Which seem to be clear is that this nerve innervates the rectum, in addition to the rectal nerves (Luckensmeyer and Keast, 1998).

Rodents have been considered a good model to investigate the anatomical and physiological characteristics of postganglionic neurons innervating pelvic viscera, mainly because in these species most of the neurons are hosted in a pair of compact ganglia, the MPG (Keast, 2006; Purinton et al., 1973). However, the findings here described, and complemented with data previously published (Keast et al., 1989; Keast and de Groat, 1989; Kihara and de Groat, 1997a, 1997b; Langworthy, 1965; Pastelín et al., 2008; Pastelín et al., 2011; Purinton et al., 1973), demonstrate that even rats have a complex pelvic plexus and the autonomic innervation of the pelvic viscera is provided by a couple of MPG and three pairs of small ganglia anatomically interrelated through ipsilateral and contralateral commissural nerves (Fig. 5).

In previous studies, it has been reported the presence of 2 to 4 AG (Keast et al., 1989; Melvin et al., 1988; Purinton et al., 1973). However, we only found two AG in the mentioned region. The ganglia can be looked-up following the commissural branches of MPG-AG or following the AG-AG commissural nerves. We noticed that AGI is a large structure whose lateral region is positioned lateral to the vas deferens wall, and deeper than the medial region. Considering that blood vessels cover part of AGI, it is extremely difficult to characterize the complete ganglia if blood vessels are not withdrawn, so that their visible components could be considered as different structures. On the other hand, we agree with other authors describing AG localized below the ureters (Keast et al., 1989; Kolbeck and Steers, 1993), or closer to the base of the vas deferens (Melvin et al., 1988) since we found that the position of the AG varied between animals. In some of them (30%) were closer to the ureter and in others (70%) near the base of the vas deferens. We believe that the shape, characteristic position of AG and the site from which it is examined could explain the variability described in previous studies.

The small ganglia localized close to the vas deferens or ureters have been named accessory ganglia (Keast et al., 1989; Keast and de Groat, 1989; Kepper and Keast, 1995; Purinton et al., 1973), peripheral ganglia (Langworthy, 1965) or hypogastric ganglion (Melvin et al., 1988). Another pair of small ganglia localized in the plexus of the DNP has been called minor pelvic ganglia (Pastelín et al., 2011). The name of AG is arbitrary and confusing, thus a nomenclature is required and here we have named them as AGI, AGII and AGIII. If more ganglia appear they may be numbered consecutively.

The high interrelationship between the pelvic plexus components may underlie the characteristic high plasticity of the pelvic plexus and neuronal reorganization after injury, for example, sexual function is recovered after bilateral transection of HG or PN nerves (Lucio et al., 1994), which suggest a relevant and functional crosstalk between the pelvic neurons. The AG commissural nerves carry preganglionic sympathetic axons that innervate the bladder neck, urethra and vas deferens contralaterally (Kihara and de Groat, 1997a, 1997b), and are also present in men (Taguchi et al., 1999).

While contralateral commissural AG-AG and MPG-MPG nerves (Keast, 2006; Kihara and de Groat, 1997b; Purinton et al., 1973) may be a protective mechanism for neural damage (Kihara and de Groat, 1997b), this kind of neural organization could represent the action of a coordinated activation of paired contralateral organs, for example

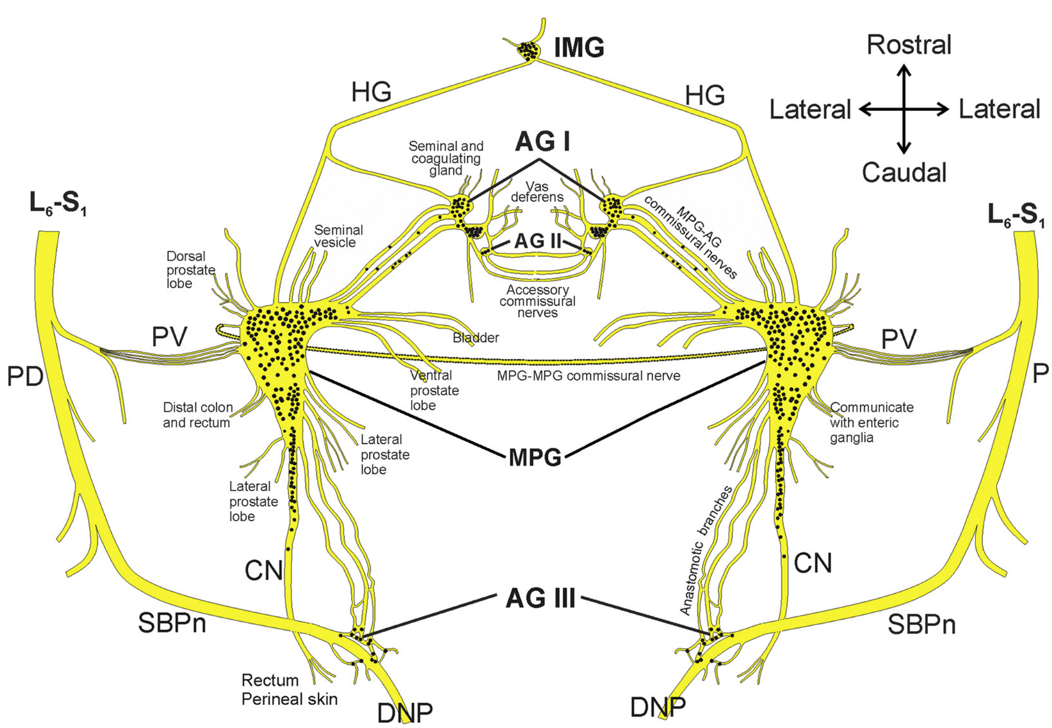

Fig. 5. Drawing representing the neural components of the pelvic plexus in the male rat. Note that the plexus is composed by 4 bilateral ganglia (major pelvic ganglia, MPG and three accessory ganglia, AG I, II and III), two input nerves, ipsilateral and contralateral commissural nerves and efferent nerves. IMG, inferior mesenteric ganglion; HG, hypogastric nerve; PN, pelvic nerve; PD, pudendal nerve; SBPd, sensory branch of the pudendal nerve, DNP, dorsal nerve of the penis, CN, cavernous nerve. Dots represent soma neurons within the pelvic plexus.

during seminal emission, when left and right accessory glands contract synchronically to expel its content.

The autonomic pelvic innervation can be explained by a two neuron pathway, the preganglionic and the postganglionic (de Groat et al., 2015). However, a role for intraganglionic interneurons and afferent neurons in autonomic ganglia has been postulated (Keast, 2006), although solid physiological evidence for this function is still lacking.

Regarding the pelvic neurons, Melvin et al. described that the hypogastric ganglion, which match with the anatomical localization of the AG, contains 3078 ± 193 (mean ± SEM) neurons (Melvin and Hamill, 1986; Melvin et al., 1988).This number is very close to our neuronal count for the AG (3300 ± 250) and represent approximately 21.5% of the total number of pelvic postganglionic neurons. However, it is important to note the fact that many neuronal cell bodies are found along several millimeters of the cavernous nerve and embedded in the accessory nerves, which makes it difficult to determine absolute neuronal numbers in the entire pelvic ganglion network.

Our data demonstrate that AG neurons are larger that MPG neurons. This finding suggest that AG neurons are likely to comprise primarily noradrenergic neurons since it has been described that MPG noradrenergic neurons are significantly larger than cholinergic pelvic ganglion neurons (Keast and de Groat, 1989). This is also consistent with the fact that AG neurons project to reproductive organs, which have a strong noradrenergic innervation (Kepper and Keast, 1997), in contrast to other pelvic organs such as bladder, where non-vascular noradrenergic innervation is sparse (Keast and de Groat, 1989).

The fact that the MPG innervates several pelvic viscera (bladder, urethra, prostate, penis, seminal glands, vas deferens, distal colon and rectum), corroborates the idea that this ganglion is a divergence center for information from spinal preganglionic and some prevertebral neurons controlling urinary, reproductive, bowel and sexual functions. In addition, according to the number of neurons spreading out from the MPG, it stands as the main pelvic ganglion of the pelvic plexus, containing 77.5% of the total neurons hosted in the pelvic plexus. The AG seems to be essential for regional innervation and reproductive function. This idea is supported with the evidence that 70% of vas deferens neurons are hosted in the AG (Kolbeck and Steers, 1993).

We conclude that the pelvic plexus of the male rat is assembled of anatomically interrelated ganglia such as two MPGs and three paired AGs (AGI, AGII, AGIII), with efferent nerves presenting a uniform topographical organization. The detailed description of the pelvic plexus further the knowledge of the autonomic innervation of the pelvic viscera in the male rat, and will be useful in designing studies about the role of pelvic innervation in the physiology and pathophysiology of pelvic functions.

Declarations of interest

None.

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