Ganglionectomy in the adult male rat increases neuronal size and synaptic density in remaining contralateral major pelvic ganglion

Abdullah Aldahmash and Muhammad Atteya

Department of Anatomy and Stem Cell Unit, College of Medicine, King Saud University, Riyadh 11461, P.O. Box 2925, Saudi Arabia

Abstract

This study aimed to characterize ganglion hypertrophy in the major pelvic ganglion (MPG) of male rats after contralateral ganglionectomy. Eighteen adult male Sprague-Dawley rats were used in this study. Nine of them were subjected to long-term (6 weeks) contralateral ganglionectomy, and nine were used as a control group. Six weeks postoperatively, the animals were euthanized and the MPG was identified and removed completely with its accessory ganglia and the associated nerves. The ganglia were then studied at the light microscope level using acetylcholinesterase histochemistry and synaptophysin immunohistochemistry, and at the ultrastructural level using transmission electron microscopy. To assess the size of ganglionic neurons, morphometric analysis was done at the light microscope level of sections of resin-embedded pelvic ganglia. No shift was found in location of remaining ganglion after contralateral ganglionectomy. The remaining ganglion was larger and more elongated dorso-ventrally. Morphometric analysis done using semi-thin and thin sections revealed an increase in the neuronal size and expansion of synaptic connections of the contralateral ganglionectomy over the control, but there was no evidence of increased neuronal number following contralateral ganglionectomy, despite the fact that it was found that after few weeks post contralateral ganglionectomy the remaining ganglion extended its innervations to cover the opposite side which was innervated by the excised ganglion. We demonstrated the occurrence of hypertrophy in the neurons of the pelvic ganglion in the adult male rats, and that this hyperatrophy was a result of increase in neuronal size rather than number. This study has paved the way for investigating the mechanisms underlying neuronal hypertrophy, such as the role of cellular signaling processes and neurotrophic factors involved in pelvic neuron plasticity.

Keywords: Ganglionectomy, major pelvic ganglion, neuronal hypertrophy, neuronal plasticity

Accepted

Introduction

The major pelvic ganglion (MPG) is the largest of the neuronal masses in the pelvic plexus, which is the part of the autonomic nervous system innervating the pelvic organs and contains both sympathetic and parasympathetic components [1,2]. Other smaller neuronal masses in the pelvic plexus are known as accessory ganglia (AC) [3].

The number of neurons in MPG varies between adult male and female rat. In the male rat, the MPG has 12,000 neurons on average, while in the female, the MPG has 7,000 neurons on average [4]. These neurons vary from 20 μm to 40 μm in diameter [5]. The average neuronal size in the MPG in adult male rat has been reported to be larger than in adult female rats [6].

Neurons of the MPG are mixture of sympathetic and parasympathetic types, receiving synapses from preganglionic fibers travelling in the hypogastric and pelvic nerves [5,7]. Each MPG gives rise to postganglionic nerve fibers which travel to their target organs by way of separate and discrete nerve bundles [3].

Several studies have reported hypertrophy of ganglion neurons in various pathological conditions, including diabetes [8], hypertension [9] and as an age-related change [10]. However, other studies have reported age-associated attrition of the sympathetic but not the parasympathetic population of the pelvic ganglion [11-13]. Hypertrophy of ganglion neurons has been induced in laboratory animals by different methods, including partial obstruction of hollow organs, e.g. intestine [14] and urethra [15], pelvic
nerve transection [16] and administration of nerve growth factor (NGF) which is normally synthesized by the target tissues of sympathetic and sensory ganglia and retrogradely transported to the ganglion cells [17,18]. NGF has been found to play a crucial role in survival and differentiation during early development [19]. Levi-Montalcini and Aloe [20] have induced ganglion neuron hypertrophy in animals treated with exogenous NGF, while ganglion neuron atrophy resulted when the animals were treated with an antiserum to NGF.

During pelvic surgical procedures (such as prostatectomy and hysterectomy), there is always a high risk of injuring pelvic ganglion neurons and their connections to pelvic organs. Metabolic disorders, such as diabetes, can also greatly affect the integrity of pelvic ganglion neurons [21]. However, strategies need yet to be developed for prevention or reversal of structural and functional pelvic neuronal loss after injury. Advances in this area depend on better understanding of the effects of injury on the cellular signaling process in pelvic neurons and also the role of gonadal steroids and neurotrophic factors in the development, plasticity and regenerative processes of pelvic neurons [21,22].

Most of the previous work on pelvic ganglion hypertrophy was done only in female rats, for practical reasons. MPG neuronal hypertrophy in female rats was reported after long-term urethral obstruction [23] in the remaining (contralateral) ganglion after unilateral ganglionectomy [24] and in MPG neurons innervating the urinary bladder and colon in streptozotocin-diabetic rats [25].

Data on pelvic ganglion hypertrophy in male rats are lacking in the literature. We, therefore, were curious whether pelvic ganglion hypertrophy in response to contralateral ganglionectomy occurs also in the male rats, for various reasons; one of which is that the pelvic ganglia show remarkable sensitivity to androgens and estrogens, which impacts on their development into sexually dimorphic structures [22]. Secondly, the organs innervated are different, and they probably have different contents of trophic factors. So our aim was to find out whether this hypertrophy also occurs in the male rat pelvic ganglion, to compare structural features of the hypertrophied neurons in the male against those reported for the female MPG and to quantify the amount of hypertrophy.

Methods

Animals

Eighteen (18) adult male Sprague-Dawley rats were used in this study. Nine of them had long-term (6 weeks) contralateral ganglionectomy, and nine were used as a control group. The age of the rats was between 8 to 10 weeks.

Ganglionectomy

The rats were anaesthetized with 0.03 ml/kg fentanyl/fluanisone (hypnorm, VetaPharma) and 0.08 ml/kg Midazolam (hypnoval, Sandoz) administered intramuscularly. A midline incision was made along the pelvic region and a small area at the left side of the pelvic cavity was exposed. The MPG was identified and then removed completely with its accessory ganglia and the associated nerves. The animals were euthanized six weeks post-operatively by an overdose of anesthetic, which was pentobarbitone (Sagatal™), injected intra-peritoneally. The volume used for over-anesthesia was 60 mg/100 g (0.2 ml/100 g) rat body weight.

Sample preparation

Krebs solution contains (their molarity) NaCl (58.45), KC1 (74.55), NaHCO₃ (84.00), CaCl₂ (110.99), MgCl₂.6H₂O (203.33), NaH₂PO₄·2H₂O (156.01), and Glucose (180.16). Sodium nitrite (0.1%) and heparin (1%) were also added. The perfusion was done by gravitational pressure, thorough a cut at the left ventricle was cut. The perfusion usually lasted about 3.5 minutes with 150-200 ml of Krebs solution depending on the size of the animal. This was then followed by perfusion with 1% paraformaldehyde and 3% glutaraldehyde in 100 mM sodium cacodylate for samples intended for morphometry. Alternatively, samples that were going to be processed for immunohistochemistry were excided with the pelvic organs as a block and immersed in cold 10% buffered formalin. Then, either the block was used for whole mount preparation of acetylcholinesterase staining, or the ganglion was dissected (as described below) for cryostat sections.

Dissection

Under the dissection microscope, the bladder was immobilized at the midline, the vas deferens pulled upwards. The MPG was identified in the area under the vas deferens on the lateral side of the posterior lobe of the prostate gland, and was dissected out with its nerves. The ganglia were then pinned on a Sylgard plate under the dissection microscope. Some of the associated blood vessels and connective tissue was not removed from the ganglion to allow the cutting of sections of known orientation. Then the ganglion was immersed in fixative (the same as that used in perfusion), while still pinned on a Sylgard.

Acetylcholinesterase Histochemistry

The whole block including the pelvic organs was taken as described before, then fixed in cold 10% buffered formalin. Next, the tissue was washed in Krebs solution and left overnight in the refrigerator in a solution of Hyaluronidase (0.33 mg/100 ml; Sigma Ltd, UK), and tetraisopro- pylpyrophosphoramide (OMPA). The solution was then...
changed to the acetylcholinesterase incubating solution: 5 mg acetylthiocholine iodide (Sigma), 6.5 ml acetate buffer (0.1), 0.5 ml sodium citrate (0.15 M), 1 ml potassium ferric cyanide (5 mM). The incubating solution was changed every 4 h, 3 times, then overnight.

**Embedding in resin**
The ganglia were taken out of the fixative (1% paraformaldehyde and 3% glutaraldehyde in 100 mM sodium cacodylate, followed by post-fixation in osmium tetroxide), briefly washed in sodium cacodylate buffer for five minutes, followed by standard EM sample preparation protocols [26].

**Sectioning and staining procedures for resin-embedded ganglia:**

I- Semi-thin sections: Glass knives were used for cutting semi-thin serial sections of 1 μm thickness, on Reichert OMU2 microtome. On average, 2500 sections were cut from each ganglion. All sections were collected on glass slides, dried on a hot plate at 55°C for 25 minutes. Sections were stained with 1% alcoholic toluidine blue for 30-80 seconds. 50% ethanol was used to remove excess stain and bring about staining contrast.

II- Thin sections: Smaller blocks were made, and thin sections about 100 nm thickness were cut with glass knives, on Reichert OMU2 microtome. The ribbon was collected on copper grids. The sections were stained by 3% uranylacetate (in 50% ethanol) for 2 minutes, followed by 2 minutes brief wash in distilled water, then 2 minutes in Petri dish on lead citrate, in the presence of sodium hydroxide pellets.

**Synaptophysin Immunohistochemistry**
Synaptophysin immunohistochemistry was used to study the changes in the synaptic activity within the hypertrophied ganglion at the acute and chronic stages. The cryostat sections were cut from the ganglion at a thickness of 10 μm. The sections were picked up on poly-lysine coated slides and left to dry at room temperature. Sections were then incubated over night with rabbit polyclonal synaptophysin antibody (Abcam, ab68851) (1:1000), at room temperature in a moist chamber. The next day, they were washed in PBS for 30 minutes three times and were left for one hour with Donkey-Anti-rabbit-Ig-biotin (1:250) in moist chamber, at room temperature. The slides were washed by PBS for 30 minutes, then incubated in fluorescent reagent (Amersham RPN1232) for one hour. Finally, they were washed in PBS, and mounted using Citifluor, and covered by glass coverslip.

**Morphometry**
Serial sections of approximately 50 μm length were photographed; section's thickness was 1 μm. On the photographed neurons, were followed individually. The largest profile, of each neuron, was identified and marked. This profile was then traced in pencil on paper from the original negative, by using De Vere 504 enlarger, the final magnification was x1186. Profile area was measured with a digitizing tablet connected to a personal computer. A neuron whose nucleus appeared in the first section was excluded, while a neuron whose nucleus appeared in the last section was included, in the measurement. In addition, only neurons were measured if they were within the chosen area, which was surrounded by a natural outline, either the capsule of the ganglion or septa of connective tissue that separated groups of neurons within the ganglion (Fig. 1). In some cases (in two animals) photographs were showing neurons from the midmost of the ganglion, with no natural outline at two ends. In those cases, boundary lines were drawn at the ends of the photographs. Neurons that touched one line were excluded, while neurons that touched the opposite line were included.

**Results**

**Acetylcholinesterase Histochemistry**
In control rats, the ganglion was 2 mm dorso-ventrally and 3 mm caudal-rostrally, and it had a pyramidal shape. The base of the ganglion was on the dorso-lateral lobe of the prostate gland, under the vas deferens, which was pulled upward during staining to expose the ganglion (Fig. 2A). The majority of nerves leaving the ganglion supplied the prostate gland. A relatively small nerve left the ganglion through the rostral border, proximal to the rostral-ventral corner and supplied the vas deferens (Fig. 2B).

In unilaterally ganglionectomized rats, the contralateral ganglion was 4 mm dorso-ventrally and 2 mm caudal-rostrally, and it was more elongated dorso-ventrally than the control. There was a marked increase in the size of the pelvic nerve and the hypogastric nerve, which entered the ganglion through the dorsal-caudal border and rostral border, respectively. Postganglionic nerves of the hypertrophic ganglion were larger, and their number seemed more than those of the control ganglion. Accessory ganglia were more prominent than in controls, especially the one close to the vas deferens (data not shown).

**Light microscopy**
In control rats, transverse sections through the ganglion showed that the ganglion has an irregular shape. The profile of the ganglion was larger in the sections near the ventral border then in sections near the dorsal border. Most of the ganglion neurons profiles were either circular or ovoid and smooth-surfaced, and many of them exhibited a nuclear profile which was pale in appearance and
Figure 1. Light micrographs of major pelvic ganglion, showing a sample of semithin serial sections from pelvic ganglion (A-D) of approximately 4 μm length. The arrows point to profiles of a small neuron serially sectioned, its largest profile can be seen at B (arrowhead). Magnification x160. Scale bar = 100 μm.

Figure 2. (A) Acetylcholinesterase histochemistry whole mount preparation of the pelvic organs for control adult male rat (right side view). MPG, Major Pelvic Ganglion; R, Rectum; E, Enteric Plexus; S, Seminal Vesicle; P, Prostate Gland; V, Vas Deferens; PN, Pelvic Nerve; HN, Hypogastric Nerve (cut loose). Magnification x9.09. Scale bar = 1250 μm. (B) Acetylcholinesterase histochemistry staining for the ganglion, showing the pre- and postganglionic nerves. GN, Genital Nerve; BN, Bladder Nerve. Magnification x22.2. Scale bar = 500 μm.

generally centrally positioned (Fig. 3A); some of the larger neurons were binucleate (Fig. 4E). The ganglion showed a thin capsule of connective tissue, neurons, blood vessels and an extensive neuropil with predominantly unmyelinated, but also some myelinated, fibers. In between the ganglion neurons there were small blood vessels, running mostly in a ventro-dorsal direction; generally the profiles of the blood vessels were circular or ovoid. Profiles of blood vessels present in close proximity to the anterior border of the capsule were often observed to enter the ganglion when followed in serial sections (Fig. 4C). In unilaterally ganglionectomized rats, transverse sections through the contralateral ganglion showed an increase in the size of the ganglion profiles, a substantial increase in the size of neurons and an increase in the number of blood vessels, when compared to sections of
Figure 3. Light micrographs of major pelvic ganglion: control ganglion (A) and operated (hypertrophic) ganglion (B), showing various cell types: BV, Blood Vessels; N, ganglion Neurons (many of which display pale nuclear profiles); C, ganglion Capsule; G, nucleus of satellite Glia cell; E, Endothelial cell. Magnification x800. Scale bar = 25 μm.

Figure 4. (A) The major pelvic ganglion and small accessory ganglia (AC). The accessory ganglion is similar to the MPG, but smaller. The capsule (c) is clearly visible around the entire ganglion. Nerve fibers (f) (possibly axons, some of which are myelinated) are seen in between the ganglion neurons (n), many of which display pale nuclear profiles. Magnification x320. Scale bar = 50 μm. (B) Cluster of veins surrounded by connective tissue. Magnification x320. Scale bar = 50 μm. (C) Blood vessel in close proximity to the anterior border. Magnification x320. Scale bar = 50 μm. (D) Small group (3 cells) of SIF cells. Magnification x500. Scale bar = 50 μm. (E) Binucleate neuron from hypertrophic ganglion.
Ganglionectomy in the adult male rat

Magnification x500. Scale bar = 50 μm. (F) Binucleate neuron from normal ganglion. Magnification x500. Scale bar = 50 μm.

similar level from a control ganglion (Fig. 10A,B). The hypertrophic ganglion neurons were similar to those of the controls; most of their profiles were either circular or ovoid and smooth-surfaced, and some of the larger neurons were binucleate (Figs. 3B and 6E). However, there were more blood vessels in the hypertrophic ganglion than in the control; they were running in similar direction (a ventro-dorsal direction), and they had similar shape, and their profiles were, in general, circular or ovoid. Vacuolated neurons seemed to be more common in the hypertrophic ganglion then in the control, but no accurate counts were performed on these cells (Fig. 4B).

Electron Microscopy

The ganglion neurons were surrounded with glial cells, the nuclei of which were only hardly identified under the light microscope (Fig. 5). In accordance with the light microscopic observations, nerve fibers were predominantly unmyelinated and only few myelinated fibers were seen (Fig. 6). Some blood vessels with their endothelial cells were observed in normal and hypertrophic ganglion (Fig. 6A).

Neurons morphometry

The cell size, in terms of the area of the largest profile of a cell serially sectioned at 1 μm, was measured in about 200 neurons from each of 3 control and 3 operated (unilateral ganglionectomy) rats. In total, about 1200 neurons were measured (Fig. 7). In control rats, neurons ranged in size from 103.51 μm² to 1218.96 μm². The average neuronal size was 370 μm². Most of the neurons were in the range of 250-650 μm². In unilaterally ganglionectomized rats, neurons ranged in size from 128.9 μm² to 2657.84 μm². The average neuronal size was 679.77 μm². Most of the hypertrophic ganglion neurons were in the range of 400 μm² to 1100 μm².

![Figure 5. Electron micrograph from normal rat pelvic ganglion showing the neuron of the ganglion surrounded with glial cell (g). The neuron nucleus (n) is clear. Magnification x15000. Scale bar = 1 μm.](image)
Synaptophysin Immunohistochemistry

In control ganglion, synaptophysin immunofluorescence in their cell bodies was very weak. The nucleus appeared as a dark small circle in the centre (i.e. completely negative to synaptophysin immunohistochemistry). The most conspicuous fluorescent structures were small spots, about 1 µm across, distributed around the surface of every ganglion neuron, forming almost a basket around the neuronal cell bodies and interpreted as the synapsing nerve endings packed with vesicles (Fig. 8A). Some cells were intensely fluorescent, arranged in small cluster and very close to each other. Immunofluorescence for synaptophysin was found in various axons running along the larger vessels. A few small accessory ganglia were observed close to the major ganglion. The number of neurons in them was in average about 14 neurons. The immunoreactivity to synaptophysin in the accessory ganglion was similar compared to the major ganglion. In unilaterally ganglionectomized rats, the immunoreactivity to synaptophysin in the hypertrophic ganglion showed larger cell bodies, and increase in the fluorescence intensity over the control. The nuclei of the neurons were relatively larger.

Figure 6. (A) Electron micrograph of pelvic ganglion showing a blood vessel (bv), an endothelial (E) cell lining the blood vessel, identified by its dense nucleus (EN), and unmyelinated nerve fibers (Un), with Schwann cells (Sn) seen around some of them. (B) Electron micrograph of pelvic ganglion showing predominantly unmyelinated fibers (Un) and one myelinated fiber (M). The myelin is seen as a dark black ring around the fiber. Magnification x20000. Scale bar = 1 µm.
Figure 7. Distribution of ganglion neuronal size (the area of the largest profile of a cell serially sectioned at 1 μm) in 3 control (in total 600 neurons) and 3 operated (in total 600 neurons) rats.

Figure 8. Micrographs showing synaptophysin immunofluorescent staining in adult male rats: (A) In the normal ganglion immunofluorescence for synaptophysin can be seen around the cell bodies of the ganglion neurons as small fluorescent spots. The fluorescence in the cell bodies is very weak. (B) In the hypertrophic ganglion, immunofluorescence for synaptophysin can be seen around the cell bodies, which are larger than the control, as fluorescent spots. The intensity of these spots and the fluorescence in the cell bodies are higher in the hypertrophic than the normal ganglion. The nuclei of the neurons can be seen as small dark circle mostly at the center. Magnification x252. Scale bar = 100 μm.

Figure 9. (9A and 9B) Fluorescent micrographs showing synaptophysin staining in hypertrophic ganglion of adult male rats. The major pelvic ganglion and small accessory ganglion (AC). The accessory ganglion is similar to the MPG, but smaller. Immunofluorescence for synaptophysin, in AC, can be seen around the cell bodies as small spots, the intensity of which is similar to the hypertrophic MPG. The nuclei of the neurons can be seen as small dark circle mostly at the centre. Binuculate neurons (N) can be seen but they were few in number. V, vessel; SI, synaptophysin immunofluorescence. Magnification x126. Scale bar = 100 μm.
than the controls but, similar to the controls, they were completely negative, and appeared mostly at the centre of the cell bodies as small dark circles (Fig. 8B). A few of the large neurons were binucleate, with their nuclei located at the centre of the cells bodies as two dark circles (Fig. 9B). Highly fluorescent spots, forming almost a basket around the neuronal cell bodies, were interpreted as the synapsing nerve endings packed with vesicles, with higher intensity and larger size than the controls, while their number seemed similar to the controls. Accessory ganglia with approximately similar neuron number to the control were found near the major ganglion (Fig. 9A). Neurons in accessory ganglia were larger than in the controls, but similar in their immunoreactivity to the neurons in the hypertrophic major ganglion. Similar to control ganglia, synaptophysin immunofluorescence was also found in various axons running along the larger vessels within the ganglion (Fig. 9B).

Discussion

Effect of unilateral ganglionectomy

Unilateral ganglionectomy in female rats showed hypertrophy in the remaining ganglion; the surviving ganglion was found to take over and innervate the whole bladder [23,24]. In this study, hypertrophy of the pelvic ganglion in male rat was observed 6 weeks after contralateral ganglionectomy; the increase in the size of the ganglion was obvious under the dissection microscope during dissection. The hypertrophy at the ganglion profile, in sections from the hypertrophic ganglion compared with sections at the similar level from control ganglion, was clear under the light microscope. The number of blood vessels in the hypertrophic ganglion was more than in the control. This suggests that the blood supply to the ganglion increases with the increased volume of the ganglion and it is probably maintained to a similar level to the control (Fig. 10A,B). This observation also indicates that during ganglion hypertrophy there is also new angiogenesis in the ganglion. New blood vessels are formed by mechanisms that are still unknown. Hypoxia, increased neural function and synaptic activity and release of neurotrophic factors are among the proposed mechanisms underlying neoangiogenesis [27]. Previous studies have not reported occurrence of hypertrophy in accessory ganglia in the female. However, this study has shown that there is an increase in the size of the accessory ganglia of the hypertrophic major pelvic ganglion in the male rat.

Hypertrophic neurons

The extent of the hypertrophy in the major pelvic ganglion in male rat was investigated quantitatively. Morphometry has shown an increase in neuronal size in the hypertrophic ganglion over the control. The neuronal size ranged from 129 μm² to 2658 μm² in the hypertrophic ganglion while in control ganglion neurons ranged in size from 104 μm² to 1219 μm². Therefore, there was an average size increase of 306 μm² in the operated over the control rats, and the size range was considerably wider in the hypertrophic than in the control ganglia. The largest class of neurons was that of 400-500 μm² in the control but that of 700-800 μm² in the operated rats. The percentage of neurons in the classes smaller than 500 μm² was 65% in control but it was only 18% in the operated animals. The percentage of neurons in the classes between 600-1200 μm² was 30% in control but 60% in the operated and almost 10% of the neurons in the hypertrophic ganglia belonged to classes which were beyond the maximum size found in controls. Therefore, there was an overall shift of neurons with smaller sizes towards the largest sizes. The difference in neuronal size between control and operated ganglion was also apparent by synaptophysin immunohistochemistry, where the number of fluorescent spots associated with each neuronal profile was either similar or higher in hypertrophic ganglion than in the control. Although the evidence is still preliminary and not yet based on quantitation, it seems that in the hypertrophic ganglion there is an expansion of the synaptic connections between preganglionic fibers and ganglion neurons, as compared to control. Hypertrophic pelvic ganglion in the male basically showed similar general features to the hypertrophic pelvic ganglion in the female [15]. However, it was possible to make only a preliminary comparison, on the basis
of the data available, because of the large number of variables.

**Binucleate neurons**

Some binucleate ganglion neurons were encountered in this study. They appeared not to differ from the mononucleate neurons and, therefore, were included in the measurements. Although binucleate neurons are known to exist, the knowledge of their functional significance is far from complete. Ninomiya [28] claimed they are sensory neurons based on the presence of anti-substance P immunostaining in the absence of tyrosine hydroxylase immunostaining.

In conclusion, the present study has shown that following long-term removal of one of the major pelvic ganglia in the adult male rat, the contralateral MPG showed an overall marked increase in size and an alteration in shape. Further analysis revealed that the ganglion neurons themselves were increased in size and the synaptic connections were expanded. These findings have demonstrated the occurrence of hypertrophy in the neurons of the pelvic ganglion in the adult male rats, and have paved the way for investigating the mechanisms underlying neuronal hypertrophy, like the role of cellular signaling processes and neurotrophic factors involved in pelvic neuron plasticity. In this study the pelvic ganglion neurons were treated as a single population, and the average changes were calculated. However, these neurons project to several different organs and they are part of sympathetic and parasympathetic pathways. It will, therefore, be important to establish whether the hypertrophy response is different in these different groups of neurons. Injury to the pelvic ganglion occurs frequently during pelvic surgeries, and strategies for its repair need yet to be developed. Moving forward in this area depends on better understanding of the effects of injury on the plasticity of pelvic neurons and the role played by cellular signaling processes and neurotrophic factors during compensatory hypertrophy of surviving neurons.

**References**


19. Martin DP, Schmidt RE, DiStefano PS, Lowry OH, Carter JG, Johnson EM. Inhibitors of protein synthesis and RNA synthesis prevent neuronal death caused by...
Ganglionectomy in the adult male rat ..... 


Correspondence to:
Abdullah Aldahmash
Department of Anatomy and Stem Cell Unit
College of Medicine, King Saud University
Riyadh 11461, P.O. Box 2925
Saudi Arabia
Phone: +966(1) 4670809
Fax: +966(1) 4671300
Email: dahmash@ksu.edu.sa or a.aldahmash@gmail.com
M. Atteya: mhasan1@ksu.edu.sa or atteya.m@gmail.com