Urol Res (1998) 26:291–297 

ORIGINAL PAPER

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# The distribution of intramural nerves in urinary bladder after partial denervation in the female rat

Received: 2 October 1997 / Accepted: 2 January 1998

**Abstract** We evaluated the degree of neuronal plasticity following a partial denervation of the rat urinary bladder. Using acetylcholinesterase staining we found that the postganglionic nerves from the pelvic ganglion reach the intact bladder as 1–4 nerve trunks on each side, slightly ventral and caudal to the ureteral orifices. Normally a few thinner nerves also reach the bladder posterolateral to the ureterovesical junction. The nerves ventral to the ureters run in the ventral longitudinal muscle layer as well-defined trunks with a pattern that does not differ much from one animal to another. The nerves reaching the bladder dorsolaterally innervate the dorsolateral aspects in a more irregular fashion. Some anastomoses are found across the midline between nerves from either side. This nerve pattern is already in place in newborn rats. After removal of the pelvic ganglion on one side in the adult rat the ipsilateral ventral nerves rapidly degenerate, whereas some dorsolateral nerves usually survive. Axons from the intact ventral nerves can be seen crossing over to the denervated side in the anastomoses. After 13 weeks the surviving ventral nerves, which normally run at some distance from the ventral midline, now run in the midline with equal amounts of ventral longitudinal muscle on either side, and with their branches evenly distributed to both sides. The same pattern is seen after 27 weeks. Unilateral ganglionectomy in 3-week-old rats leads to the same changes in nerve distribution as in the adult rat. We conclude that there is a high degree of plasticity in the bladder innervation following a partial denervation, and that this plasticity includes the distribution of its main intramural nerve trunks.

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**Key words** Urinary bladder · Rat · Pelvic ganglion · Innervation · Denervation · Plasticity · Age

## Introduction

In the rat the parasympathetic nerve fibres for the urinary bladder originate from neurons in the pelvic ganglion [2, 11]. Normally no intramural neurons are found [7], although in pathological conditions and early in development some neurons can be demonstrated [1, 14]. Most of the postganglionic nerves reach the bladder hemisphere from the ipsilateral ganglion [2, 11]. Some nerves, however, cross over to the other side behind the bladder [2, 11] and there are also some anastomoses between the nerves on the ventral side of the bladder [2]. This would suggest some degree of cross-innervation from the right and left ganglion.

Carpenter and Rubin [5] found that stimulation of one pelvic nerve produced a pressure that amounted to about 50% of the response when both nerves were stimulated. We have not found any reference to stimulation of one pelvic nerve contracting either the entire bladder or only the ipsilateral half.

Carpenter and Rubin [5] did not find any difference in number of silver-stained intramural nerve trunks in the right and left bladder hemispheres after unilateral pelvic ganglionectomy. This would suggest a high degree of cross-innervation but the time chosen was 2 weeks after the ganglionectomy so it is possible that some sprouting could have occurred. Ekström et al. [6], on the other hand, found that unilateral ganglionectomy induced in the bladder an ipsilateral decrease in choline acetyltransferase activity to about 25% of the initial, whereas no significant change was detected in the contralateral bladder half. They suggested that 75% of a ganglion's postganglionic nerves went to the ipsilateral and 25% to the contralateral bladder half. Nothing is known regarding possible regional differences in cross-innervation in the bladder.

The present study had two aims. First, to describe in more detail the arrangement of the intramural nerves in the rat bladder, and to provide new anatomical data regarding possible cross-innervation. Second, to study the degree of anatomical plasticity of the bladder innervation following unilateral pelvic ganglionectomy in the young and the adult rat.

## Materials and methods

### Animals

Sixteen 15-week old female Sprague-Dawley rats underwent unilateral destruction of the pelvic ganglion (see below). They were then killed 12 days ($n = 8$), 13 weeks ($n = 4$) or 27 weeks later ($n = 4$). Nine unoperated litter mates served as controls. Five newborn control rats and five control rats 3 weeks of age were used in a separate experiment to study postnatal development of bladder innervation. Four other rats underwent unilateral destruction of the pelvic ganglion at age 3 weeks, and were killed 16–22 weeks later.

### Surgery

The animals were anesthetized with ketamine (100 mg/kg i.m.; Ketalar, Parke Davis, Barcelona, Spain) and xylasin (15 mg/kg i.m.; Rompun, Bayer, Leverkusen, Germany). The lower abdomen was opened and the left pelvic ganglion was visualized (for anatomical details see ref. 1). A $5 \times 7$-mm area between the distal ureter, the proximal urethra and the vaginal–rectal border was then frozen several times with a metal probe cooled in and containing liquid nitrogen, providing complete destruction of the ganglion [8] without any bleeding from the blood vessels through the ganglion or in its vicinity. The abdomen was then closed and the animals recovered.

### Histology

#### *Wholemounts*

At the appropriate time the animals were anesthetized as described above, the thorax was opened and a catheter was inserted through the left ventricle into the aorta and the right atrium was cut open. The blood was washed out with saline. The bladder was then dissected out together with the proximal urethra, distended with 0.7–1.0 ml saline (adult rats), 0.1 ml (newborn) or 0.5 ml (3-week-old), and transferred to 4% phosphate-buffered (pH 7.4) formaldehyde solution for fixation. After 15 min. the bladders were opened by a dorsal (ventral in a few bladders) longitudinal midline incision. After 1 h total fixation time the bladders were transferred for at least 4 h to Krebs solution containing hyaluronidase (0.33 mg/100 ml) and ISO-OMPA ($10^{-4}$ M). The bladders were then stained for acetylcholinesterase according to the variation of the Karnovsky and Roots [9] method proposed by Baker et al. [3]. At the end of the incubation period (usually about 8 h) the bladders were opened by radial cuts allowing them to be flattened with minimal folds. After dehydration the bladders were mounted in DPX on slides. The width of the main ventral nerve trunks was measured under a light microscope equipped with an ocular scale. Photographs were taken in a dissection microscope, in a light microscope, and by placing the slides directly in an enlarger.

#### *Phase-contrast microscopy of nerve bundles*

The bladders were dissected out and distended as described above, and were then fixed in 5% glutaraldehyde in 100 mM Na cacodylate buffer (pH 7.4), dehydrated and embedded in Araldite. Sections were then cut from the lower ventral portion of the bladder and examined by phase-contrast microscopy.

### Statistics

Quantitative measurements of nerve widths are given as mean value ± standard error (SE). Student's *t*-test (two-tailed) was used for testing of significance.

## Results

### Normal pattern of innervation

Wholemount preparations consisted of the entire extent of the bladder opened and flattened out and of the entire thickness of its wall. The acetylcholinesterase reaction stained all the nerves and the nerve trunks in the wall, including the small trunks close to the muscle bundles. Small nerve bundles and individual nerve fibres (of whose course we know through electron microscopy and histochemistry for synaptophysin) were not stained. There was also a weak staining of the musculature, including that of blood vessels.

On the lateral and dorsal aspects of the bladder, muscle was arranged in bundles with a criss-cross pattern and no clearcut organization in layers of different directions. On the ventral aspect the bladder muscle was arranged in longitudinal bundles, mostly well defined and separated from each other by loose connective tissue. Nearby bundles often anastomosed. This ventral longitudinal muscle layer was more stained than the more randomly orientated muscle of the rest of the bladder.

The main nerves from the pelvic ganglia reached the bladder on each side as 1–4 nerve trunks. The nerves usually went the shortest way from the ganglion to the bladder, ventrally and caudally to the uretero-vesical junction. A few thinner nerves reached the bladder posterolateral to the uretero-vesical junction. These nerves had a longer course, going at first cranially from the ganglion, and then medially to the bladder. Often such nerves could be traced over the ventral surface of the uterus, just above the cervix and reaching the other side of the posterior aspect of the bladder.

The nerves ventral to the ureters were mainly distributed to the ventral part of the bladder with its longitudinal muscle, whereas the nerves reaching the bladder dorsolaterally to the ureters innervate the dorsolateral aspects. There was a marked bilateral symmetry in innervation pattern, but a complete asymmetry between the innervation of the ventral and dorsal side of the bladder. The nerves to the bladder will in the text be referred to as the right and left ventral and the right and left dorsolateral nerves.

The innervation pattern of the anterior longitudinal muscle was more regular than that of the dorsolateral

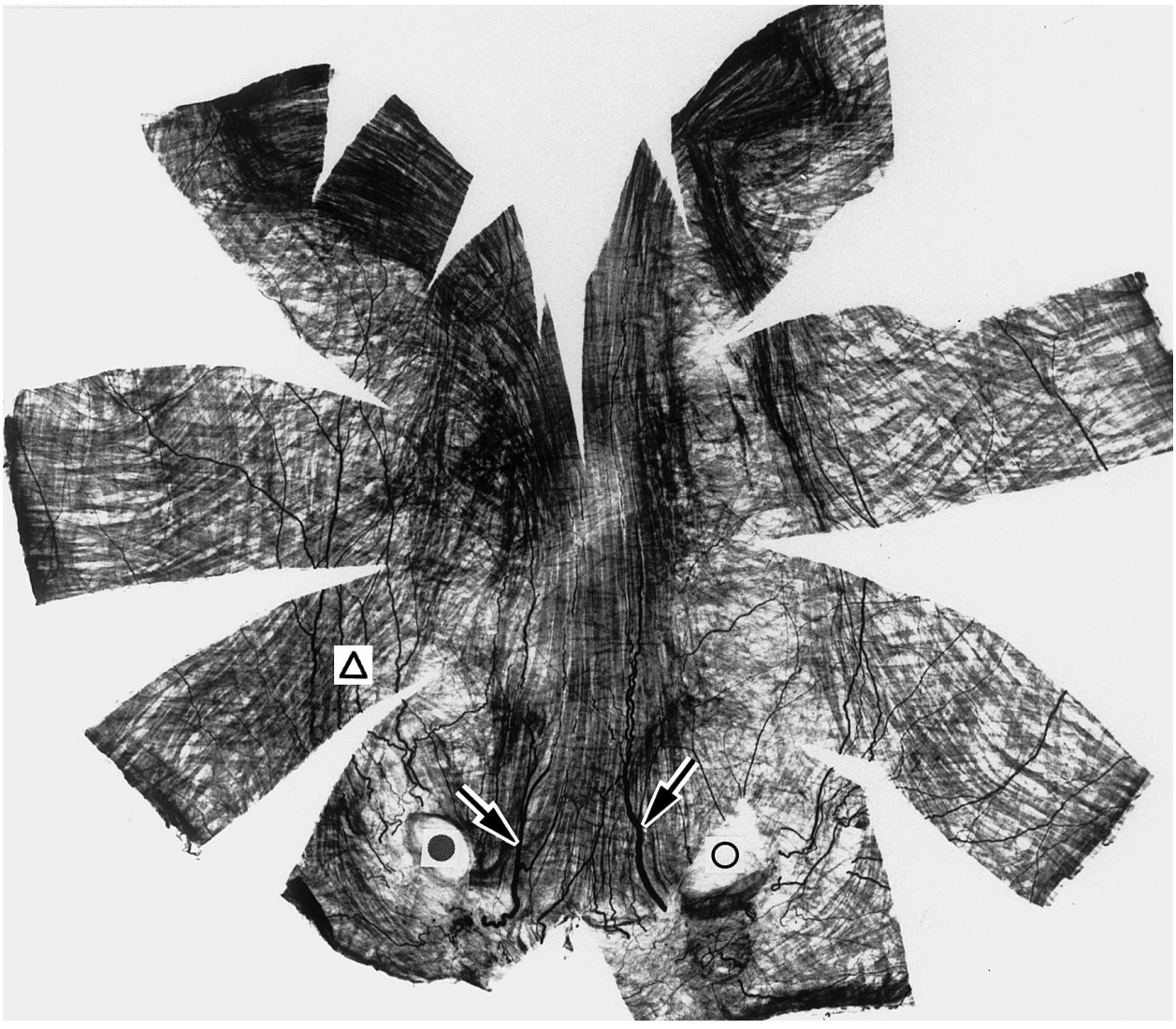


**Fig. 1** Wholemount of control bladder. Acetylcholinesterase staining. The bladder was cut open on the dorsal side. The right and left ureters are marked with *filled* and *open circles*, respectively. The right and left ventral nerves (*arrows*) are seen running in the ventral longitudinal muscle layer. The right dorsolateral nerves are marked with a triangle. Magnification ×5.9

aspects of the bladder (Fig. 1). The ventral nerves of the left and right side run approximately parallel to each other and to the longitudinal muscle bundles, giving off smaller nerves that also run in parallel. Some anastomoses are easily observed across the midline.

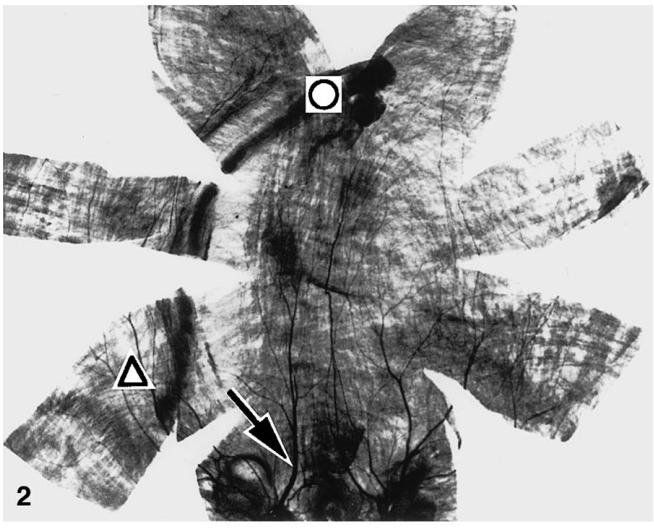
The anastomoses between the right and left ventral nerves were either almost at right angles to the long axis of the nerves or are formed by coalescence of small branches into one. Both patterns can be found in nerves of all sizes.

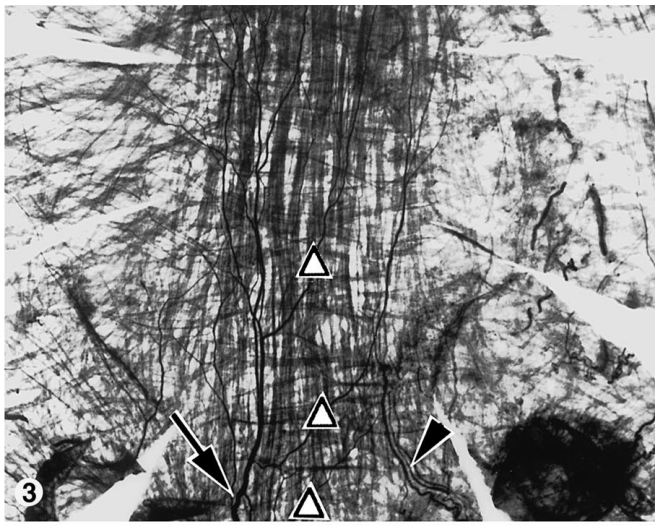
The dorsolateral nerves radiated out from where they reached the bladder. The nerves were thinner than the ventral nerves, and ramified early in their course to produce a pattern less dense and more irregular than that of the ventral nerves. Also on the dorsal aspect of the bladder some anastomoses between nerves from the right and left side could be observed but the pattern was less regular than on the ventral side. Some bladders were almost devoid of anastomoses on the dorsal side.

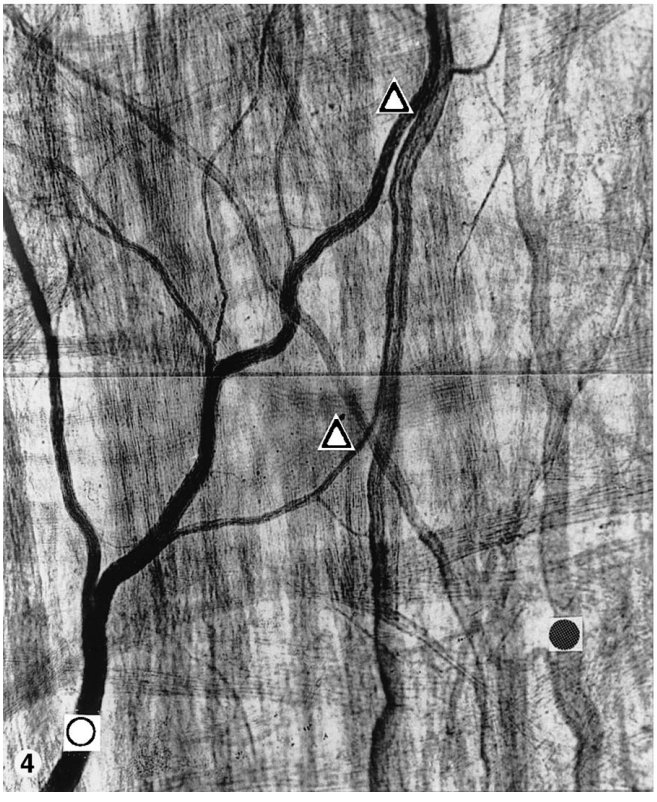
## Postnatal development of bladder innervation

The staining of the smooth muscle in the newborn was less intense than in the adult (Fig. 2). On the ventral side most muscle seemed to have a longitudinal course although the arrangement in bundles was less well defined than in the adult (Figs. 1 and 2). On the dorsal side the arrangement of the muscle was less regular than on the ventral side.

Already at birth there was a clear bilateral symmetry in innervation (Fig. 2). The ventral nerves were identified, running between the ventral longitudinal muscle bundles. Also, the dorsolateral nerves had the same distribution as in the adult rat. There was little difference in innervation pattern between the newborn rats.



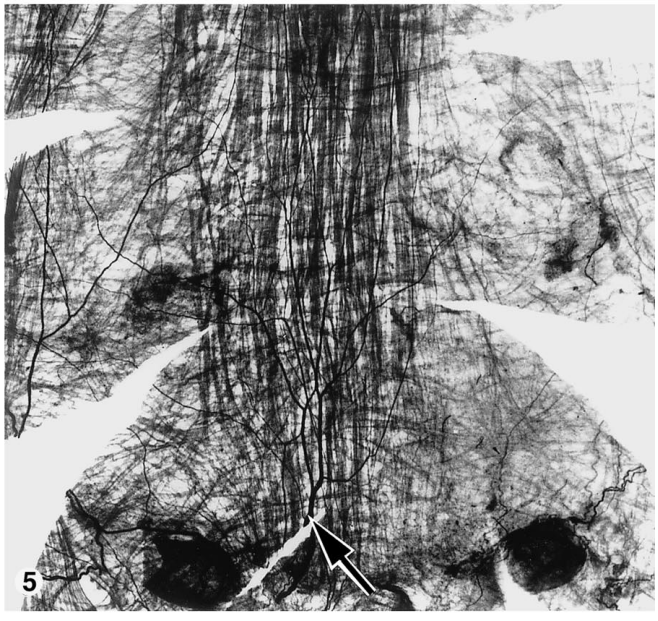




**Fig. 2** Wholemount, newborn control. The right ventral nerve is marked with an *arrow*. The *triangle* denotes the area of the right dorsolateral nerves. The *open circle* marks the umbilical artery which is found normally on the right side. The nerve pattern in the newborn rat is similar to that in the adult. Magnification ×6

**Fig. 4** Ventral aspect of wholemount, adult bladder 12 days after unilateral ganglionectomy (left side). Montage showing anastomosing nerves (*triangles*) from right (intact) and left (denervated) side. The direction of the specimen is as in Fig. 1. The heavily stained right ventral nerve is marked with an *open circle*, and the left, faintly stained, with a *filled circle*. Magnification ×63

**Fig. 3** Wholemount, adult bladder 12 days after unilateral ganglionectomy (left side). Note the well-preserved right ventral nerve (*arrow*) and the medium-sized nerves (*triangles*) that can be traced going over to the left side. The left ventral nerve (*arrow head*) is only faintly stained. Magnification ×6

**Fig. 5** Wholemount, adult bladder 27 weeks after unilateral ganglionectomy (left side). The right ventral nerve (*arrow*) is now situated in the ventral midline of the bladder with an equal amount of ventral longitudinal muscle on either side. The pattern of branching is symmetric. There is no staining of any left ventral nerve. Magnification ×5.3

At the age of 3 weeks the innervation pattern, the staining properties of the smooth muscle and its arrangement were similar to the adults. Apart from their smaller size, such bladders could not be distinguished from adult bladders.

## Effect of unilateral ganglionectomy in the adult

A marked decrease in number and staining intensity of the ventral nerves could be noted on the ipsilateral side of the bladder 12 days after cryo-ablation of the area of

the left pelvic ganglion (Fig. 3). Phase-contrast microscopy of sections from Araldite-embedded proximal segments of the ventral nerves showed that virtually no axons remained on the operated side.

At this stage the arrangement of the contralateral ventral nerves seemed unaffected by the ganglionectomy, the course of the nerve was well away from the ventral midline of the bladder (Fig. 3) and no degeneration could be demonstrated by phase-contrast microscopy. Along the whole length of the intact ventral nerve anastomoses with the corresponding "empty" ramifications from the side of the lesion could be seen (Figs. 3 and 4). As a consequence of this partial cross-innervation there remained some visible nerves in the longitudinal muscle at the side of the ganglionectomy, despite the total lack of staining of axons in the proximal portion of the ventral nerve.

Regularly some remaining dorsolateral nerves could be demonstrated on the side of the lesion. Also, often some branches from the intact side could be followed over to the side of lesion.

In bladders 27 weeks after ganglionectomy there was a shift in the main course of the remaining ventral nerve, which now ran in the ventral midline of the bladder, with an equal amount of longitudinal smooth muscle on either side (Fig. 5). The branching off of nerves to the right and left side was symmetrical. No remains of the proximal parts of the ventral nerve on the side of the lesion were apparent.

Bladders from animals 13 weeks after ganglionectomy showed an intermediate pattern in nerve distribution as compared with the 12-day and 27-week bladders.

The width of proximal segments of the right and left ventral nerves was measured using an ocular scale. In the controls the widths on the right and left sides were similar (Fig. 6). The corresponding measurements in the 13- and 27-week ganglionectomized bladders revealed a significant increase in width of the intact ventral nerves, and almost no trace of ventral nerves on the side of the lesion.

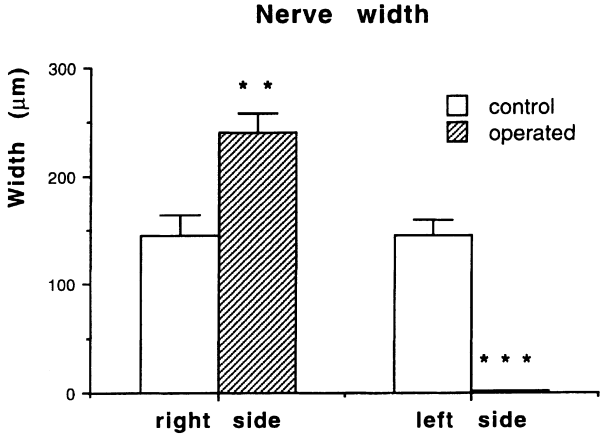



**Fig. 6** Histograms showing the width of the right and left ventral nerves in adult rats. In the control group ($n = 9$) the left and right nerves had similar widths. 13–27 weeks ($n = 8$) after removal of the left pelvic ganglion there was no staining of any left ventral nerve but the width of the contralateral, intact, nerve had increased significantly

### Effect of unilateral ganglionectomy in the young rat

In all bladders ganglionectomized at age 3 weeks and stained for acetylcholinesterase 16–22 weeks later, we found some surviving dorsolateral nerves on the side of the lesion. The ipsilateral ventral nerves were barely visible. Branches from the intact ventral nerve could be traced crossing over the midline to run between longitudinal muscle bundles on the side of the lesion. There was a shift of the contralateral ventral nerve towards the side of the lesion in a manner similar to that described above for the adult rats at corresponding times. The changes in innervation pattern were thus similar to those found for the adult rat.

## Discussion

The smooth muscle of the urinary bladder can grow or shrink in response to changed functional demands, i.e., it has a high degree of plasticity. For example, an increased outlet resistance leads to smooth muscle cell hypertrophy and to a 10-fold increase in the detrusor weight within 6 weeks [7].

The innervation of the bladder also has a marked plasticity. The total choline acetyltransferase (CAT) activity per bladder increased in the female rat by 250% following a 6-week obstruction, which increased the bladder weight by 800% [12]. Also, the average volume of pelvic ganglion neurons increased by more than 80% following bladder outlet obstruction [8, 13].

After removal of one pelvic ganglion in the male rat, the bladder CAT activity falls by about 50% within 3 days [4]. The cholinergic innervation seems to recover rapidly and there is no significant difference in CAT activity in denervated and control bladders between 25 and 60 days postoperatively [4]. In the female rat, the average neuronal volume in the surviving ganglion [8] increases by 50% 7 weeks after contralateral ganglionectomy.

Information regarding the anatomy of the innervation in the bladder wall is limited. According to Baljet and Drukker [2] the ventral side of the female rat bladder is innervated by nerves running directly from the pelvic ganglia, whereas the dorsal side is reached by nerves that run via a nerve plexus on the uterine surface, close to the distal ureter.

We found that the innervation of the ventral part of the bladders differs little from one animal to another. Generally, one (sometimes two) ventral nerve originating from the area just ventral and medial to the ureteral orifice, runs on each side in the ventral longitudinal muscle layer, giving off branches to the surrounding tissue. In contrast, there are several dorsolateral nerves reaching the bladder just cranial and lateral to the

ureteric orifice. These nerves are thinner than the ventral ones, and spread and arborize over the lateral and dorsal aspects of the bladder.

The pattern of the intramural bladder nerves is already established in newborn animals, although the finer branches are absent. At age 3 weeks the innervation pattern is indistinguishable from that of adult animals. We have not found any functional studies on fetal rat bladder, but fetal bovine bladder contracts to field stimulation in vitro at mid-term indicating functional nerve–muscle transmission [10]. The typical arrangement of the smooth muscle in the bladder with anterior longitudinal muscle, and the criss-cross pattern on the lateral and dorsal aspects was discernible in the newborn. Presumably the course of the nerve trunks is influenced by the direction of the smooth muscle bundles.

It is not precisely known how extensively the nerves from each side of the bladder extend across the midline. Carpenter and Rubin [5] found no difference in number of silver-stained nerve trunks in the right and left hemispheres 2 weeks after unilateral pelvic ganglionectomy in the male rat, a result which is hard to understand in the light of subsequent studies, including the present one. Ekström et al. [6] suggested on the basis of CAT measurements 3 days after unilateral ganglionectomy, that a 25% cross-innervation existed. Whether the cross-innervation is evenly distributed, or only found close to the midline remained unknown.

We found that there are anastomoses across the midline between the branches of the right and left ventral nerves. Often medium-sized nerves anastomose but anastomoses can be found also between thin branches far out in the periphery. It is common that two nerves coalesce and form a single nerve. On the dorsal side of the bladder the pattern is more irregular. In some bladders the dorsolateral nerves from the right and left sides have little contact, in others nerves can be found to cross the midline to arborize in the contralateral side.

The removal of one pelvic ganglion affects the ipsilateral ventral nerve(s) more than the dorsolateral ones. This may suggest that, even when the cryoganglionectomy was extensive, some ganglion cells survived close to the bladder in the nerves passing behind the ureter. An alternative is that intact axons from the contralateral pelvic ganglion reach the dorsal aspect of the denervated side via nerves crossing the midline on the ventral surface of the uterus, as described in the Results section.

The proximal segments of the ipsilateral ventral nerves were almost unstained in both short- and long-term ganglionectomy bladders, a sign of complete axonal degeneration. This shows that the nerves from the contralateral side that reach the ipsilateral side in the normal bladder do this more distally. It also shows that the axons from the contralateral side that reinnervate the ventral aspect of the denervated side (see below) do this also via more peripherally located nerves. Furthermore, it shows that axons of contralateral origin regenerating within degenerated nerve trunks grow exclusively in a distal direction.

After ganglionectomy, the width of the proximal ventral nerves doubled on the intact side. We have not performed the morphometry necessary to elucidate whether this is due to an increase in diameter of the individual axons or an increased number of axons.

While we provide evidence that the recovery of the innervation on the side of the bladder which had the ganglionectomy is due to axons of contralateral origin, we could not ascertain whether these axons grow exclusively along pre-existing trunks, normal and degenerated, or whether they also form completely new nerve trunks. The first alternative seems the most probable, as the medium-sized nerves, partly emptied of axons (Fig. 4), that were so typical of the earlier stages after ganglionectomy, are absent after longer periods.

In the long-term postganglionectomy bladders a shift of the remaining ventral nerve to the midline is observed. This is not due to atrophy of the longitudinal muscle on the denervated side, as the muscle maintains a complete left to right symmetry. It is not clear how this shift of the main nerve trunk is attained. The results suggest that the plasticity of the bladder innervation is so marked that the course of major nerve trunks can shift when the area of innervation changes.

We found the same changes in nerve pattern in bladders from animals that had undergone ganglionectomy at age 3 weeks (final age 19–25 weeks) and 15 weeks (final age 42 weeks). This would suggest that the plasticity of bladder innervation is similar if the lesion had occurred in young and middle-aged rats. An argument against this interpretation of the results would be that part of the reinnervation of the denervated side might have not have taken place until the young animals had become adult. The plasticity of the bladder innervation seems, however, to be a fairly fast process, bladder choline acetyltransferase activity in adult rats is almost back to normal [4] 25 days after a unilateral ganglionectomy.

**Acknowledgements** The study was supported by grants from the Swedish Medical Research Council (14x-9479) and the Wellcome Trust. We thank Peter Trigg for excellent technical support.

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