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## Title:

Internal anal sphincter nerves – a macroanatomical and microscopic description of the extrinsic autonomic nerve supply of the internal anal sphincter

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## ABSTRACT

#### Background:

The internal anal sphincter (IAS) contributes substantially to anorectal functions. While its autonomic nerve supply has been studied at the microscopic level, little information is available concerning the macroscopic topography of extrinsic nerve fibres. This study was designed to identify neural connections between the pelvic plexus and the IAS, provide a detailed topographical description, and give histological proof of autonomic nerve tissue.

### Methods:

Macroscopic dissection of pelvic autonomic nerves was performed under magnification in seven (5 males, 2 females) hemipelvises obtained from body donors (67-92 years). Candidate structures were investigated by histological and immunohistochemical staining protocols to visualize nerve tissue.

## **Results:**

Nerve fibres could be traced from the anteroinferior edge of the pelvic plexus to the anorectal junction running along the neurovascular bundle anterolaterally to the rectum and posterolaterally to the prostate/vagina. Nerve fibres penetrated the longitudinal rectal muscle layer just above the fusion with the levator ani muscle (conjoint longitudinal muscle) and entered the intersphincteric space to reach the IAS. Histological and immunohistochemical findings confirmed the presence of nerve tissue.

#### Conclusions:

Autonomic nerve fibres supplying the IAS emerge from the pelvic plexus and are distinct to nerves entering the rectum via the lateral pedicles. Thus, they should be

classified as internal anal sphincter nerves. The identification and precise topographical location described provides a basis for nerve-sparing rectal resection procedures and helps to prevent postoperative functional anorectal disorders.

## Key Words:

internal anal sphincter internal anal sphincter nerves pelvic autonomic nerves autonomic nerve preservation sphincter-sparing anterior rectal resection

# What does this paper add to the literature?

The topography of the extrinsic IAS nerve supply has not yet been described. This paper gives a detailed macroscopic description of nervous connections between the pelvic plexus and the IAS in formalin-fixed adult specimens with microscopic confirmation of findings. The data provide an anatomical basis for preservation of these nerves.

#### INTRODUCTION

Anorectal dysfunction is a common ailment after sphincter-sparing low anterior rectal resection and intersphincteric rectal resection. The set of symptoms that may occur is summarised under the term low anterior resection syndrome (LARS) [1]. Amongst these symptoms, anal incontinence and urgency are well recognized as factors that negatively impact on quality of life [2,3] and are functionally related to autonomic pelvic nerves. Therefore, new techniques have been developed to locate and better protect autonomic pelvic nerves during rectal surgery, such as nerve-oriented mesorectal excision (NOME), pelvic intraoperative nerve monitoring (pIONM) or transanal total mesorectal excision (TaTME) [4-6].

These novel approaches have stimulated and revived the interest in the autonomic nerve supply of the lower rectum and internal anal sphincter (IAS) [7,8]. In fact, autonomic nerve preservation has always been an integral part of total mesorectal excision (TME) and has prompted several macroscopic studies to describe the course of autonomic nerves in the small pelvis [9,10]. However, while much emphasis was laid on the nerve supply of urogenital organs [11], innervation of the IAS remained unclear. It was postulated by Goetze in 1951 that inferior fibres of the pelvic plexus were responsible for IAS nerve supply [12]. Only recently, microscopic immunohistochemical studies have proven autonomic nerve fibres between the longitudinal and the circular rectal muscle layer entering the IAS [13-16].

The aim of the present study was to clearly identify at a macroscopic level those nerve fibre connections extending between the pelvic (inferior hypogastric) plexus and the smooth muscles of the anorectal junction and to give a detailed topographical description of their course in adults. Moreover, microscopic studies

including histological and immunohistochemical staining were carried out to confirm the nerve-specific nature of the macroscopically dissected structures.

### METHODS

#### **Body donors**

Body donors (five males, two females, median age: 76 years, range: 67-92 years,) who had given written consent were recruited from the body donation program of the Institute of Anatomy, Christian-Albrechts-University of Kiel. Preservation of body donors was achieved by formalin (3%) perfusion fixation via femoral arteries and postfixation in ethanol (70%). From each body donor an intact hemipelvis was removed for macroscopic and microscopic studies including three left and two right male hemipelvises, and a left and a right female hemipelvis. Specimens showed no evidence of previous pelvic or perineal surgery except for one body donor with a prior hysterectomy.

### **Macroscopic studies**

Macroscopic dissection was performed under direct vision with a Zeiss KS 3.2x magnification glass (focal distance: 500 mm). The relevant findings were photodocumented with a full HD camera (Olympus E 620 with digital ED lens, 50 mm, 1:2 Macro) coupled to a computer using the Olympus Studio 2 software program (Version 2.3).

#### TME plane

Dissection adhered to the principles of TME with separation of the mesorectum from the parietal pelvic fascia. Initially, the parietal pelvic fascia was left intact to follow the autonomic nerve fibre bundles embedded between its inner and outer lamella. The mesorectum was mobilized along the posterior and lateral aspects until the lateral rectal pedicles were reached.

Hypogastric nerve and lateral rectal pedicles

The hypogastric nerve was released from the parietal pelvic fascia and followed towards the pelvic plexus. The rectal pedicle was properly identified by gentle traction and countertraction to expose the neural connections between the pelvic plexus and the lateral rectal wall ("T-junction").

#### Pelvic plexus and pelvic splanchnic nerves

The lateral rectal pedicle was cut to gain access to the lower part of the rectum and the pelvic sidewall. The inner lamella of the parietal pelvic fascia was dissected off the pelvic plexus and the pelvic splanchnic nerves. Connective and adipose tissues were removed to allow optimal tracing of the branching pattern of the pelvic plexus. Nerve branches were followed towards the urogenital pelvic organs, i. e. distal ureter and urinary bladder, vas deferens, seminal vesicles, prostate and penile bulb in males, and vagina and clitoris in females. However, their course beyond the rectogenital septum was only pursued as far as necessary for the identification of their final destination.

#### Nerve branches to the IAS

Main emphasis was given to those nerve fibres extending from the lower aspect of the pelvic plexus towards the distal rectum possibly penetrating the longitudinal rectal muscle layer at the level of the anorectal junction. The anorectal junction was defined as the conjunction of the levator ani muscle, represented by the pubococcygeal muscle, with the longitudinal rectal muscle layer corresponding to the upper border of the anal canal. The presence and number of these nerve fibres were photodocumented as was their relationship to the neurovascular bundle, in particular the cavernous nerve. Finally, the intersphincteric space was opened from below to estimate the distance between the entrance of the nerve fibres into the longitudinal rectal muscle layer and the IAS.

## **Microscopic studies**

After macroscopic dissection, tissue samples (approximately 5 mm x 5 mm) were taken from selected branches traveling towards the IAS and processed for microscopic studies to confirm the presence of nerve fibres. Furthermore, representative tissue samples were also collected from the hypogastric nerve, pelvic splanchnic nerves and pelvic plexus. The samples were dehydrated, embedded in paraffin wax and cut into sections (6 µm thickness) perpendicular to the nerve fibre axis. After histological and immunohistochemical staining findings were assessed with a light optical microscope (Nikon 6000, Nikon, Tokyo) coupled to a digital camera (Digital Sight, Nikon, Tokyo). Data acquisition was performed with NIS-Elements BR 3.2 software (Nikon, Tokyo).

## Histology

Masson's trichrome staining was applied to depict the histological details producing effective labeling of connective and perineural tissue (green), neuronal and glial cell nuclei (dark brown) and neuronal somata (light red).

#### Immunohistochemistry

For unequivocal confirmation of even small-sized nerve fibres immunohistochemical visualization of the peripheral glial cell marker S-100 was performed. After pretreatment with 3% hydrogen peroxide and citrate buffer (pH 6.0, 95 °C water bath) for 20 min, sections were incubated overnight with a rabbit polyclonal antibody against S100 (1:20.000, Dako, Glostrup, Denmark) diluted in antibody diluent (Zymed, Invitrogen, CA) followed by incubation with biotinylated secondary antibodies (goat anti-rabbit IgG, 1:200, Dako) for 30 min and treatment with avidin-biotin-complex (Vectastain ABC Standard, Vector Laboratories, Burlingame, CA)

conjugated with horseradish peroxidase for 1 h. Antibody binding was visualized with 3,3'-diaminobenzidine (DAB, DakoCytomation) followed by hematoxylin counterstain.

### RESULTS

#### **Macroscopic findings**

The complete autonomic pelvic network comprising the hypogastric nerve, pelvic splanchnic nerves and pelvic plexus was thoroughly explored in five specimens down to the IAS. In the two remaining specimens dissection was limited to the region of interest between the lower part of the pelvic plexus and the connections to the IAS to confirm the presence of nerve bundles emanating towards the anorectal junction.

#### Hypogastric nerve and pelvic splanchnic nerves

The hypogastric nerve consisted of 2-4 parallel running bundles connected by short communicating branches. Before entering into the pelvic plexus, the hypogastric nerve ramified into several distal branches at the level of the second sacral vertebra (4 out of 5 cases). In the remaining case many branches derived directly from the superior hypogastric plexus. From the third and fourth ventral sacral foramina, 2-3 branches of pelvic splanchnic nerves entered the pelvic plexus at a short distance. They intermingled with the ramifications of the hypogastric nerve to form a large neural network extending from the ventral sacral foramina to the anterior border of the posterior pelvic compartment (Fig. 1).

## Pelvic plexus and lateral rectal pedicle

The pelvic plexus displayed a triangular shape with its base orientated towards the pelvic floor in 3 of 5 specimens, whereas it exhibited a rectangular form in the two other specimens. Abundant nerve fibres emerged from the pelvic plexus and fanned out towards the distal ureter, urinary bladder, vas deferens, seminal vesicles and prostate in males or to the vagina in females. Embedded within the parietal pelvic fascia, the pelvic plexus resembled a thin plate of nerve fibre meshes that could be

lifted from the lateral pelvic wall like a cloth. From the pelvic plexus, 2-4 rectal nerve branches diverged towards the lateral rectal wall entering the mesorectum in a row (Fig. 2). The middle rectal artery was present in 2 of 5 hemipelves, the middle rectal veins in 3 of 5 specimens.

#### Internal anal sphincter nerves

From the most inferior part of the pelvic plexus, distinct nerve bundles could be followed that ran anterolaterally to the rectum towards the apex of the prostate in males or towards the lateral distal vagina in females (Fig. 3). The nerve bundles diverged from the urogenital neurovascular bundle and approached the anorectal junction. The length, branching pattern, and the distinctiveness of these nerve bundles differed among specimens. A varying number of nerve fascicles (2-6) could be identified that entered the longitudinal rectal muscle layer anterolaterally just above the conjunction with the levator ani muscle (Fig. 4, 5). In two cases the levator ani muscle was dissected from the anorectal junction and the longitudinal and circular rectal muscle layers separated to follow the intramural course of these nerve fibres. They extended within the space between the longitudinal muscle layer forming the conjoint muscle and the circular muscle layer that continued to form the IAS (Figure 6, 7). Since these terminal nerve fibre branches reached the internal anal sphincter muscle, they were designated as internal anal sphincter nerves.

The remainder of the neurovascular bundle continued towards the urogenital organs. In particular, the cavernous nerve corresponding to the most caudal branch of the neurovascular bundle could be clearly identified running just above and lateral to the perineal body towards the penile cavernous bodies in males or to the clitoris in females (Fig. 3, 5).

## **Microscopic findings**

#### Hypogastric nerve, pelvic splanchnic nerves and pelvic plexus

Both Masson's trichrome and immunohistochemical staining confirmed the presence of nerve tissue in representative tissue samples. The hypogastric and pelvic splanchnic nerves were composed of several nerve fibre bundles embedded by a fascial envelope and surrounded by perineural connective tissue (Fig. 8 a-d). Blood vessels were found adjacent to and within nerve fibre bundles (vasa nervorum). The pelvic plexus showed a similar histological architecture, but additionally displayed ganglia densely packed with nerve cells (Fig. 8 e, f). The histological features of these nerve fibres served as reference for the histological assessment of the internal anal sphincter nerves.

### Internal anal sphincter nerves

Selected tissue samples from 3 of 5 specimens revealed nerve fibre bundles resembling the histological features as documented for the hypogastric and pelvic splanchnic nerves. The nerve fibre bundles were surrounded by perineural tissue and accompanied by small blood vessels embedded in loosely arranged connective tissue (Fig. 8 g, h).

#### DISCUSSION

The present study aimed to close the gap in our understanding of the topographical organization of the pelvic autonomic nerves from the pelvic plexus down to the autonomic extrinsic nerve supply of the IAS. In fact, we could demonstrate consistently that nerve branches from the anteroinferior aspect of the pelvic plexus run underneath the inner lamella of the parietal pelvic fascia close to the anterolateral aspect of the rectum down to the area where the mesorectum tapers and eventually disappears. At the anorectal junction the nerve branches penetrate the longitudinal rectal muscle layer and travel within the fibromuscular layer of the conjoint longitudinal muscle towards the IAS. They diverge from the urogenital neurovascular bundle and, thus, do not correspond to the rectal nerve branches that enter the mesorectum more cranially at the lateral rectal pedicles ("T-junctions", Fig. 9). The presence of nerve tissue was confirmed by microscopic studies revealing autonomic nerve fibre bundles in most of the investigated tissue samples.

The topographical anatomy of autonomic nerves supplying the IAS was investigated by Goetz and Goetze back in 1951 [12,17]. They already demonstrated rectal branches from the pelvic plexus and supposed that the innervation of the IAS is mediated by intramural nervous connections. Although the corresponding nerve fibres were described by schematic drawings, their exact course was not evidenced by photographic documentation.

At the microscopic level, nerve fibres to the IAS have been traced by Japanese researchers [14,15]. They could demonstrate nerve branches from the pelvic plexus running on the inner surface of the levator ani and penetrating the longitudinal rectal muscle layer at the level of the anorectal junction. They gave evidence that these nerves join the intrinsic myenteric plexus (Auerbach) and named the extrinsic

nervous connection either inferior rectal branches of the pelvic plexus or nerves serving the IAS. Our macroscopic description is in accordance with these microscopic findings. However, since both the origin and course of the nerve branches to the IAS are distinct from rectal nerve branches approaching the rectal wall via the lateral rectal pedicles, we propose the term internal anal sphincter nerves to provide an appropriate terminology.

Moszkowicz et al. analyzed the extrinsic nerve supply of the IAS in human fetuses using digitized serial histological sections for three-dimensional reconstruction. Contrary to our findings, they described the course of the nerves from the posterior rather than the anterior aspect of the inferior pelvic plexus to the lower rectum [16]. This discrepancy may be attributed to topographical changes during fetal growth and postnatal life resulting in a ventral shift of the internal anal sphincter nerves. In another study on human fetuses, Fritsch was able to localize the entire pelvic plexus anterolateral to the rectum and also emphasized that its position may be subjected to changes in the postnatal period [18].

Recently, an electrophysiologically supported study during TaTME located the nerve supply of the IAS posterolaterally [19,20]. This is not necessarily in conflict to our results, because at the anorectal junction the anorectum bends about 90°-100°. Thus, the perspective gained by transanal approach may suggest a more posterior location of the autonomic nerve supply. Further anatomical studies simulating the down-to-up technique will clarify the specific topographical relationship of these nerves to the anorectum [20].

The present findings indicate that the internal anal sphincter nerves run in the triangle between the anterolateral aspect of the rectum, the posterolateral border of either the prostate or the vagina and the inferiormedial part of the levator ani muscle. The latter

part corresponds in its anterolateral portion to the pubococcygeal muscle which merges with the longitudinal muscle layer of the rectum to form the conjoint longitudinal muscle. Whereas the mesorectal fascia and the parietal pelvic fascia are distinguishable at higher levels of the small pelvis, they become incomplete and condensed at the anorectal junction and eventually merge towards the conjoint longitudinal muscle and the perineal body [18,21]. Thus, preservation of the internal anal sphincter nerves is a challenging task due to absence of clearly identifiable planes and easy-to-open interfaces

Whenever possible, transection of the rectum during TME should be done proximal to the conjunction of the pubococcygeal and longitudinal rectal muscle just at the lowermost end of the mesorectum (Fig. 9). In intersphincteric resections preservation of these nerves is as a rule not possible, because the region of their penetration into the anorectal muscle tube is part of the resected specimen. Nevertheless, residues of nerve function have been found in spite of proctectomy including mucosectomy [22]. This might be either because these patients suffered from benign disease and a cuff of the lower rectum containing these nerve branches was preserved or nerve fibers from the pudendal nerve [13] are able to compensate to some degree the loss of the connection to the pelvic plexus.

Functionally, the IAS contributes to the majority of the anal resting pressure and plays the most important role in maintaining anal continence [23,24]. While its myogenic tone is intrinsically generated [25-27], relaxation and modulation depend on autonomic nerve activity. Physiologic relaxation during defecation is based on the rectoanal inhibitory reflex (RAIR) [28,29] which is mainly mediated by neural nitric oxide synthase (nNOS) containing intrinsic ganglion cells in the lower rectal wall projecting to the IAS [30]. For the extrinsic innervation, both sympathetic and

parasympathetic nerve fibres have been found to supply the IAS [13,15,16]. While parasympathetic fibres also release nitric oxide supporting sphincter relaxation and additionally transmit afferent stimuli from the anorectum [31], the role of sympathetic supply to the IAS is more variable, although muscle tone enhancing properties prevail [32-36]. Thus, relaxation of the IAS is primarily mediated by parasympathetic fibres, whereas reinforcement of anal resting pressure is of a sympathetic nature [36]. Subsequently, damage of the internal anal sphincter nerves may result in (1) disturbed afferent functions contributing to a loss of sensory control and urgency, (2) impaired RAIR leading to uncoordinated rectal evacuation during defecation, and (3) loss of sympathetic reinforcement of the anal resting tone leading to incontinence for flatus or liquid stool. These considerations need further clinical validation, e.g. documentation of the level of rectal transection and possible nerve injury in one or even both sides and subsequent correlation with functional scores to quantify the sequelae of damage.

The strength of the present study is the clear topographical depiction of nerve branches running from the pelvic plexus to the IAS, both in males and females. The findings were consistent in all specimens and give evidence that the IAS is supplied by nerves distinct from other nerve ramifications derived from the pelvic plexus. Limitations of the study include the small number of specimens and the advanced age of the body donors (67-92 years). However, the data retrieved from 7 specimens were sufficient to confirm the presence and illustrate the course of internal anal sphincter nerves with respect to topographic landmarks. Concerning specimen age, it is possible that in younger individuals the internal anal sphincter nerves are even larger in size and exhibit a network-like pattern. Furthermore, age-related degenerative processes may have taken place, so that histological confirmation of

nerve tissue was not achieved in all specimens. However, as samples for histological studies were not taken systematically to at least partly preserve the dissected specimens for further skills laboratory demonstrations, we deemed the macroscopic dissections valid when a continuous connection between the pelvic plexus and the anal sphincter region was found. Moreover, no information in regards to the anorectal functional status of the body donors was available, once again underlining the uncertainty of the extent of neurodegenerative alterations.

#### Conclusion

In summary, the present study describes at a macroscopic level those nerves that extrinsically supply the IAS (Fig. 9). They derive from the anteroinferior edge of the pelvic plexus, travel within the neurovascular bundle close to the posterolateral wall of the prostate or vagina and enter the anorectal junction anterolaterally to the rectum between the longitudinal rectal muscle layer and the innermost part of the levator ani muscle. According to their distinct course and function, they should be termed internal anal sphincter nerves.

Because of their peculiar topographical anatomy internal anal sphincter nerves are endangered in all surgical procedures carried out in the deep pelvis and/or pelvic floor, e.g. prostatectomy, hysterectomy, ventral rectopexy and low anterior rectal resection. The awareness of their origin and course is an essential precondition for nerve-sparing surgical interventions and will help to reduce postoperative functional compromises (LARS). In particular, novel surgical approaches (e.g. TaTME) and nerve-tracing techniques (e.g. pIONM) appear to be promising tools for optimized identification and preservation of the autonomic extrinsic nerve supply of the IAS.

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## FIGURE LEGENDS

### Figure 1:

Overview of the topographical architecture of pelvic autonomic nerves. The rectum is mobilized according to the principles of TME and turned caudally to the opposite side, the inner lamella of the parietal pelvic fascia is removed to expose the autonomic nerve network. The hypogastric nerve exhibits several nerve fibre strands which enter the pelvic plexus together with the pelvic splanchnic nerves. From the pelvic plexus emerge rectal nerve branches to supply the rectum. Female right hemipelvis after hysterectomy, medial view. Green plastic strips = pelvic autonomic nerves, yellow plastic strip = ureter.

### Figure 2:

Rectal nerve branches from the pelvic plexus to the rectum. The rectum is mobilized according to the principles of TME and turned medially to the opposite side, the inner lamella of the parietal pelvic fascia is partly removed. The rectal branches (n=4) enter the mesorectum via the lateral rectal pedicles, the cranial branch is accompanied by a middle rectal vein. Male left hemipelvis, craniomedial view. Green plastic strips = hypogastric nerve and rectal nerve branches.

#### Figure 3:

Neurovascular bundle running in the triangle between levator ani muscle (lateral), rectum (dorsal) and prostate (ventral). The rectum is shifted dorsally, the intersphincteric plane is opened. From the posteroinferior portion of the neurovascular bundle several nerve fibres enter the anterolateral rectal wall just

above the anorectal junction that is detached ventrally from the perineal body (dotted white line). The cavernous nerve and other terminal nerve branches of the neurovascular bundle head towards the apex of the prostate and cavernous bodies. Male left hemipelvis, medial view. Green plastic strip = neurovascular bundle, yellow tube = urethra.

#### Figure 4:

Entrance of internal anal sphincter nerve branches into the rectal wall. The rectum is mobilzed and turned caudally to the opposite side, the anorectal junction is detached dorsally (dotted white line). Nerve fibres emerge from the anteroinferior aspect of the pelvic plexus and penetrate the conjoint longitudinal muscle at the anorectal junction. The longitudinal rectal muscle layer (asterisk) is clearly discernible at the caudal end of the mesorectum. Male left hemipelvis, medial view. Green plastic strip = internal anal sphincter nerves

### Figure 5:

Neurovascular bundle, rectal nerve branches and internal anal sphincter nerves. The anterior rectal wall is partly detached from the upper perineal body and shifted dorsally. The anal canal is opened, while the anal verge is circumferentially preserved. The internal anal sphincter nerves diverge from the neurovascular bundle and enter the anorectal region in a network-like pattern. The cavernous nerve is well discernible at the lowermost posterolateral border of the vagina. Female right hemipelvis, medial view, same specimen as in Fig. 1.

## Figure 6:

Entrance of the internal sphincter nerves into the conjoint longitudinal muscle. The rectum is mobilized and turned caudally to the opposite side. The penetration of the conjoint longitudinal muscle by internal anal sphincter nerves takes place at the anterolateral side of the rectal wall, posterolateral to the vagina and medial to the levator ani muscle. Female right hemipelvis, craniomedial view, same specimen as in Figure 1.

## Figure 7:

Internal anal sphincter nerves and anal sphincter complex. The anterior rectal wall is shifted dorsally, the anorectal junction is detached from the perineal body (asterisk), the internal anal sphincter is separated from the conjoint longitudinal muscle. The internal anal sphincter nerves approach the anorectal junction and run between the longitudinal and circular rectal muscle layer. Note the sectioned haemorrhoidal plexus. Male left hemipelvis after parasagittal section, medial view. Green plastic strip = neurovascular bundle.

## Figure 8:

Histological (left column) and immunohistochemical (right column) characteristics of pelvic autonomic nerves. a: the hypogastric nerve is composed of several nerve bundles (empty asterisks) accompanied by blood vessels (black asterisk) and ensheathed by the inner and outer lamella (black arrowheads) of the parietal pelvic fascia. b: each hypogastric nerve bundle is surrounded by a perineural sheath (black arrowhead) and frequently displays intrafascicular blood vessels (vasa nervorum,

white arrowhead). c-d: pelvic splanchnic nerves (white asterisks) are surrounded by a perineural sheath (black arrowheads) and accompanied by blood vessels (black asterisk). e-f: the pelvic plexus is composed of multiple nerve bundles (white asterisks) and ganglia abundantly filled with nerve cells (black arrowheads). Several blood vessels (black asterisks) accompany both nerve bundles and ganglia. g-h: internal anal sphincter nerves display microscopic features similar to those of other autonomic pelvic nerves (a-f) exhibiting nerve bundles surrounded by a perineural sheath (black arrowheads) and accompanied by blood vessels (black asterisks). Left column: Masson's trichrome staining with labeling of connective and perineural tissue (green), neuronal and glial cell nuclei (dark brown) and neuronal somata (light red). Right column: S100-immunohistochemistry with specific labeling of peripheral autonomic nerve fibres/glial cells (dark brown), counterstain with hematoxylin. a, c, e: magnification 10x; b, d: magnification 20x; f, g, h: magnification 40x.

## Figure 9:

Schematic presentation of the topographical organization of the internal anal sphincter nerves (male right hemipelvis, parasagittal section). a: right-sided pelvic autonomic nerves after removal of the rectum and internal anal sphincter. The hypogastric nerve (HN) and pelvic splanchnic nerves (PSN) join to form the pelvic plexus (PP). While rectal nerve (RN) branches diverge from the center of the pelvic plexus (dotted white circle) to reach the rectum, the internal anal sphincter nerves (IASN) emerge from the anteroinferior edge of the pelvic plexus. They travel within the neurovascular bundle along the inner aspect of the levator ani (LAM) muscle postero-laterally to the urogenital organs and anterolaterally to the rectum to penetrate the anorectal junction at 2 (and 10) o'clock position. B, urinary bladder; P,

prostate; SV, seminal vesicles; U, urethra, PB, perineal body; EAS, external anal sphincter. b: left-sided pelvic autonomic nerves with the anorectum left in place. While the cavernous nerves (CN) emerge from the neurovascular bundle (NVB) in anterocaudal direction crossing the perineal body towards the cavernous bodies, the internal anal sphincter nerves (IASN) bend caudally to reach the anorectal junction. The red dotted line indicates the lowermost level where a transection of the rectum is possible without causing damage of the internal anal sphincter nerves. For details see insert (blue rectangle). c: at the anorectal junction the internal anal sphincter nerves (IASN) penetrate the longitudinal rectal muscle (LM) layer just at the fusion line with the pubococcygeal muscle (PCM) forming the conjoint longitudinal muscle (CLM). They enter the intersphincteric space to reach the internal anal sphincter (IAS). CM, circular rectal muscle layer; EAS, external anal sphincter (deep portion); HP, haemorrhoidal plexus.