



## Award Winning Paper — Tanagho Prize

# Significant physiological roles of ancillary penile nerves on increase in intracavernous pressure in rats: experiments using electrical stimulation of the medial preoptic area

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The objectives of this work were to evaluate the contributions of the ancillary penile nerves to penile erection in male rats *in vivo*. We investigated the effects of unilateral and bilateral transection of the cavernous nerve (main penile nerve) on the increase in intracavernous pressure (ICP) following electrical stimulation of the medial preoptic area (MPOA) in male rats *in vivo*. After unilateral or bilateral transection of the cavernous nerve (main penile nerve), the ICP responses showed decreases of 28% and 55%, respectively compared to those ICP responses before transection. In other words, even after bilateral transection of the cavernous nerve, significant increases in the ICP response following central stimulation were observed. In contrast to these findings, the ICP response was completely eliminated following bilateral pelvic nerve transection. These data suggested that the ancillary penile nerves, which originate from the major pelvic ganglia, have a complementary role to the cavernous nerves in the autonomic motor innervation of the penis. *International Journal of Impotence Research* (2001) 13, 82–88.

**Keywords:** prostate cancer; penile erection; cavernous nerves

## Introduction

Many recent advances in our understanding of the mechanisms and modulation of penile erection can be traced to the development of various animal models of human disease.<sup>1</sup> Currently the rat is the most frequently used animal model of rectile physiology and dysfunction. However, the integrative peripheral and central neural control of penile erection in those animals is still unclear.<sup>1,2</sup> In particular, it is very important to clarify the anatomical distribution and physiological roles of the penile nerves, which are considered as the final common pathways of penile erection. From an anatomical standpoint, the cavernous nerve is usually considered the main neural projection from the major pelvic ganglion (MPG) to the erectile tissue in male rats.<sup>3–6</sup> Recently Dail *et al*<sup>3</sup> demon-

strated that not only the cavernous nerve (ie main penile nerve) but also ancillary penile nerves contain nicotinamide adenine dinucleotide phosphate diaphorase (NADPH-d) fibers, indicative of their role as a supplier of nitric oxide (NO).

Given the critical role of NO-mediated corporal smooth muscle relaxations in the erectile process,<sup>1</sup> such data are consistent with a potentially significant role for ancillary penile nerves in the modulation of erectile capacity. However, the physiological contribution of such ancillary penile nerves on penile erection is unknown. As far as we are aware, only one study in the rat model has even been suggested a significant role of ancillary penile nerves to penile erection as determined by measuring intracavernous pressure (ICP).<sup>7</sup> However, other reports showed that bilateral transection of the main penile nerve (cavernous nerve) eliminated visible penile repose in those animals.<sup>8,9</sup>

As such, the goal of this study was to evaluate the physiological contribution of ancillary penile nerves to penile erection in a well-established rat model *in vivo*. To this end, we used electrical stimulation of the medial preoptic area (MPOA), and the normal evoked erectile response to that stimulus, to evaluate the effects of transection of the cavernous nerves (main

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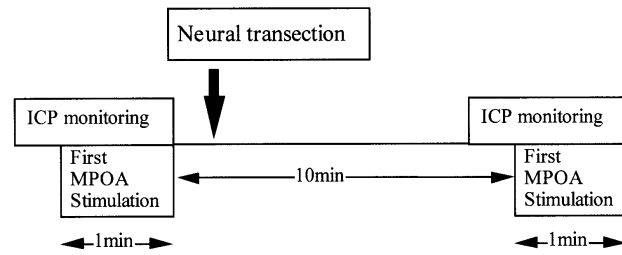
penile nerves) on the intracavernous pressure (ICP) response.<sup>10</sup> The use of a central stimulation method seems a reasonable scientific approach for evaluating this issue, as central stimulation of this nucleus is thought to elicit penile erection through the sum of activation of the individual penile nerves.<sup>10,11</sup>

Of further potential clinical importance is the fact that ancillary penile nerves on the ventral side of the human prostate have also been reported.<sup>7</sup> Thus, clarification of the physiological role of the ancillary penile nerves in the rat could lead to a better understanding of the role of those same nerves in erection in humans, and moreover, the expected physiological effects of damage to such, following major pelvic surgery such as radical prostatectomy or cysto-prostatectomy.<sup>12,13</sup>

## Materials and methods

### Animal preparation and pretreatment

A total of 22 male rats (Taconic Farms, Germantown, NY), ranging in age from 12 to 16 weeks of age and



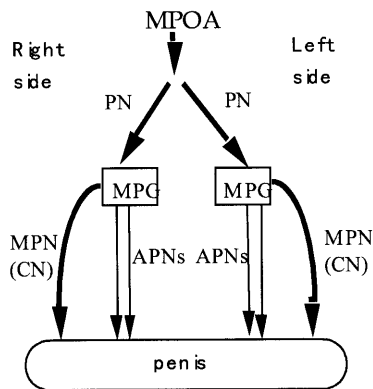
**Figure 1** Stimulation protocol: All animals were stimulated twice, with a 10-min interval between stimulations. Immediately after the first stimulation, rats were subdivided into distinct experimental groups according to the nerve transections that were performed on one or both of the cavernous or pelvic nerves.

337–425 g were used in these studies. The animals were housed under a 12:12 h light:dark cycle. Food and water were available *ad libitum*.

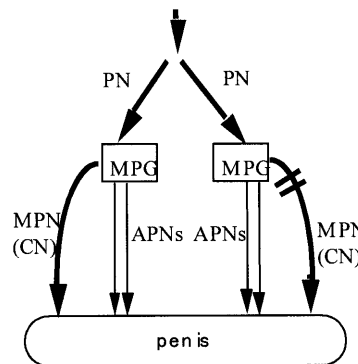
### Experimental design

All animals were stimulated twice each with 10-min intervals between stimulations. First, a control response to electrical stimulation of the MPOA was

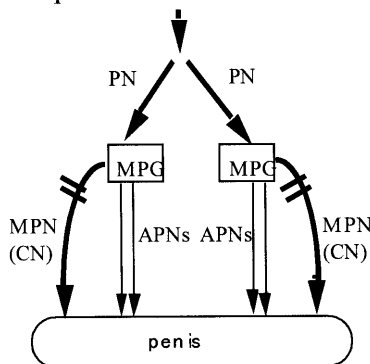
#### Group 1 Control (No neuronal transection)



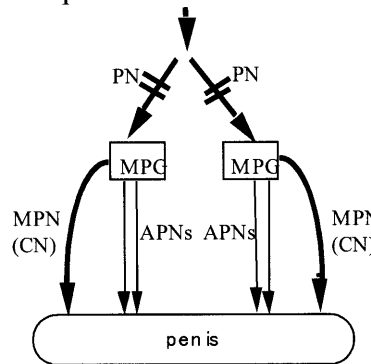
#### Group 2 Unilateral MPN transection



#### Group 3 Bilateral MPN transection



#### Group 4 Bilateral Pelvic N transection



**Figure 2** Schematic depiction of experimental groups. Note that the crossed double bars represent nerve transection. In Group 2, unilateral cavernous nerve transection was performed on the left side in three and on the right side in three animals (see Methods). CN: cavernous nerve; (MPN: main penile nerve), MPG: major pelvic ganglion, PNs: penile nerves.

performed in all animals prior to nerve transection (Figure 1). Immediately after the first stimulation, the animals were placed into one of the following four experimental groups (see Figure 2). Group 1: control animals ( $n=4$ ), not subjected to any nerve transection; Group 2: ( $n=6$ ), unilateral cavernous nerve transection (main penile nerve) (three on left side, three on right side); Group 3: ( $n=6$ ), and Group 4: ( $n=6$ ), were subjected to either bilateral transection of the cavernous or pelvic nerves, respectively. The cavernous and pelvic nerves were transected next to the major pelvic ganglion.

#### *Surgical technique for in vivo pressure monitoring and placement of MPOA electrode*

Anesthesia was induced by an intraperitoneal injection (35 mg/kg) of pentobarbital sodium (Abbott, North Chicago, IL). Anesthesia was maintained during the course of the experimental protocol (2–3 h) by a subsequent injection of pentobarbital sodium (5–10 mg/kg) every 45–60 min as a required for maintenance of anesthesia.

The rats were placed in a supine position, and the bladder and prostate exposed through a midline abdominal incision. The major pelvic ganglia, hypogastric nerves, pelvic nerves, cavernous nerves (main penile nerves) and several adjacent fine branches, including the so-called lateral and ventral branches<sup>6</sup> were identified posterolateral to the prostate on both side. The pelvic nerves and cavernous nerves were isolated bilaterally in all animals (including control animals) before the first electrical stimulation. Mean arterial blood pressure was monitored via a 20-gauge cannula placed in the left carotid artery. The striated penile muscles were exposed. The rats were fixed to a stereotaxic headholder (Kopf 900, David Kopf instruments, Tujunga, CA) and the electrode was placed in the MPOA. The stereotaxic coordinates for the tip of the electrode were 0.1–0.25 mm posterior, 0.4–0.6 mm lateral (right) and 8.6–8.8 mm ventral.<sup>10</sup> The tip of the electrode was confirmed to be located in the MPOA by histological examination following completion of the experiments. The lower part of the body was rotated and a 20-gauge needle was inserted into the crus of the corpus cavernosum. Systemic blood pressure and intracavernous pressure lines were connected to a pressure transducer, which was connected via a transducer amplifier to a data-acquisition board (MacLab/8e7, ADI Instrument, Milford, MA). Real-time display and recording of pressure measurements were performed on a Macintosh computer (MacLab software V3.4, ADI instruments). Other details of surgical procedures have been described in previous reports.<sup>10,14,15</sup>

#### *Brain stimulation protocol*

Electrical stimulation of the MPOA was performed with a stainless-steel bipolar concentric electrode (SNE-100, Rhodes medical instruments, Woodlands Hills, CA). MPOA stimulation was applied with square wave pulses delivered as a 2-ms duration pulse, 150  $\mu$ A, 30 Hz for 1 min using a Grass S88 stimulator coupled to a constant-current isolation unit (PSIU6, Grass, West Warwick, RI).<sup>10</sup>

#### *Histological examination of the brain*

After completion of experiment, electrical coagulation of the stimulation area was performed. The brains were removed and fixed in 10% formaldehyde-saline for at least 48 h. Frozen sections, 30  $\mu$ m thick, were stained by Toluidine-Blue O (Sigma, St Louis, MO) to confirm the location of electrical stimulation. Only animals in which electrode placement was verified to be in the area between –0.3 mm and –0.5 posterior to bregma and beneath the anterior commissura were utilized in these studies.<sup>10</sup>

#### *Histological examination of the human prostate following radical prostatectomy*

To confirm existence of nerve bundles in the ventral and dorsal surfaces of human prostate (ie as human ancillary penile nerves), additional (to the standard pathological evaluation) histological examination was performed on three human prostates following nerve sparing radical perineal prostatectomies. The prostate specimens were cut in a standard manner, and the tissues were fixed in 10% formalin and routinely processed. They were stained with either hematoxylin and eosin or S-100 keratin binding protein (a marker of Schwann cells in axons). Dr Belur Bhagavan, Professor of Pathology at our medical center, independently performed examination and interpretation of the specimens for ancillary nerve fibers.

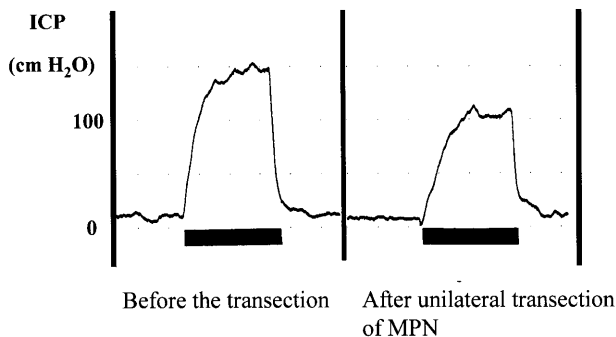
#### *Data analysis*

Changes in ICP were evaluated as the ratio of the ICP/BP (mean arterial blood pressure). Data were expressed as mean  $\pm$  s.e. Repeated measure ANOVAs (four groups  $\times$  two stimulations) with post hoc multiple comparisons (Fisher PLSD) was utilized as appropriate for comparison the ICP response between the control group and neuronal transection group. A  $P$ -value of  $<0.05$  was considered significant.

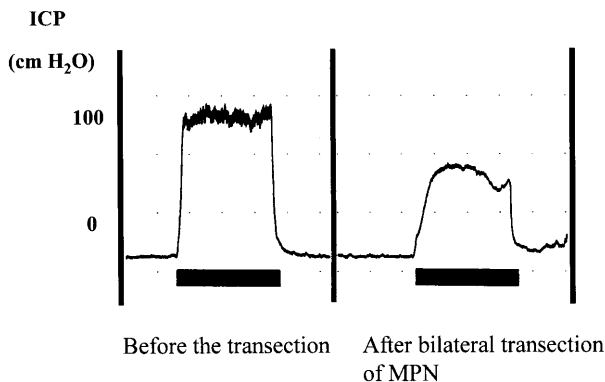
## Results

### The ICP response in each experimental group

As shown in the representative example displayed in Figure 3, unilateral transection of the cavernous nerves significantly reduced the amplitude of the observed increase in the ICP response, although more than half of the amplitude of the control response still remained. In addition, even bilateral transection of the cavernous nerves did not completely ablate the ICP response. That is, as illu-

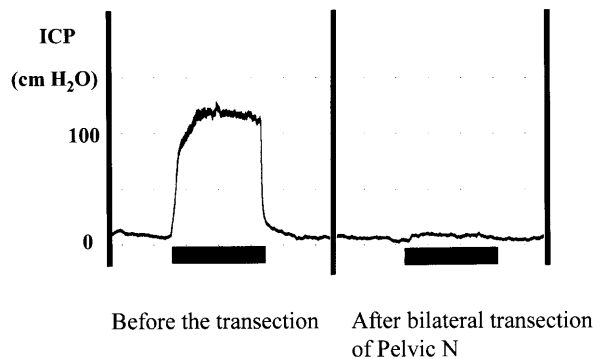


**Figure 3** The effects of the unilateral cavernous nerve transection on the MPOA-stimulated ICP response (Group 2). Shown are representative examples of the ICP response before and after unilateral transection of the cavernous nerve, respectively, *on the same animal*. Although unilateral transection of the cavernous nerve did reduce the amplitude of the ICP response by more than 50%, a demonstrable ICP response still remained. The 1 min period of stimulation is denoted by the solid black bar underlying the ICP response.

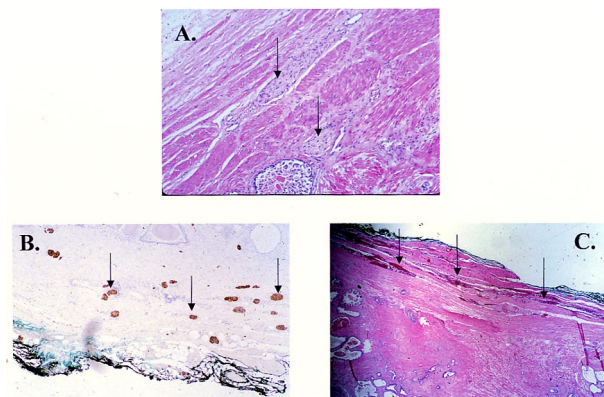


**Figure 4** The effects of bilateral cavernous nerve transection on the MPOA stimulated ICP response (Group 3). Representative examples of ICP responses before and after bilateral transection of cavernous nerves, respectively, *on the same animals*. As shown, bilateral transection did not totally ablate the ICP response. A significant response still remained after cavernous nerve ablation. The 1 min period of stimulation is once again shown by the solid black bar underlying the ICP response.

strated in Figure 4, an obvious increase in the ICP response to MPOA stimulation was still observed after bilateral transection of cavernous nerve. In stark contrast to the effects of bilateral cavernous nerve transection, the ICP response to MPOA stimulation was completely eliminated following bilateral transection of the pelvic nerve (Figure 5). Note that there was no detectable significant difference between the mean ICP/BP ratios during two consecutive electrical stimulations of the MPOA in the control animals. The mean data and corresponding statistical analyses/comparisons for all experimental treatment groups are expressed as the ICP/BP ratio and displayed in Table 1.



**Figure 5** The effects of the bilateral pelvic nerve transection (Group 4). Shown are representative example of the MPOA-stimulated ICP responses before and after bilateral transection of the pelvic nerve. In contrast to the other transection groups, the MPOA-stimulated ICP response was completely eliminated following bilateral transection of the pelvic nerves.



**Figure 6** (A) Photomicrograph of ventral surface of the human prostate stained with H&E after radical prostatectomy showing nerve bundles just beneath the prostatic capsule ( $\times 400$ ), (B, C) photomicrograph of the dorsal surface of the prostate capsule showing the nerve bundles stained with S-100 A and H&E. ( $\times 200$ , arrows point to the various nerve fibers).

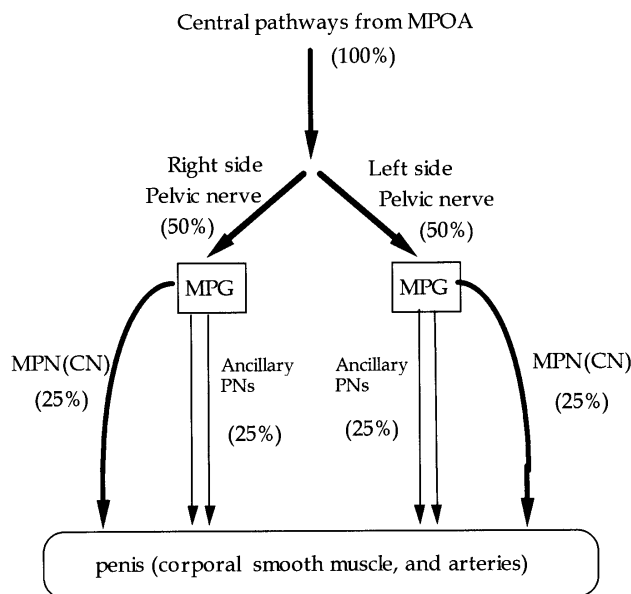
**Table 1** Erectile response to MPOA stimulation

Group	ICP/BP ratio		
	Before transection	After transection	
Control (n = 4)	0.78 ± 0.03	0.79 ± 0.04	] P < 0.01*
Unilateral MPN (n = 6)	0.77 ± 0.04	0.559 ± 0.02	
Bilateral MPN (n = 6)	0.78 ± 0.05	0.34 ± 0.09†	] P < 0.01*
Bilateral Pelvic N (n = 6)	0.76 ± 0.05	0.08 ± 0.01†	

Values are means ± s.e. Two-way ANOVA for repeated measures revealed significant differences among various groups (ANOVA F (3, 17) = 22.137, P < 0.0001). \*Significantly different from corresponding values in different groups by post hoc Fisher's PLSD. †Without any nerve transection (see materials & methods). MPN: main penile nerve (cavernous nerve), Pelvic N: Pelvic nerve

### Histological analysis of human prostatic tissue obtained from patients undergoing radical prostatectomy

Representative examples of the histological examination from the three specimens are shown in Figure 6. Staining of the tissue with standard hematoxylin and eosin (H & E) and neuron specific S-100 keratin binding protein show a dense innervation of nerve



**Figure 7** Schematic diagram of the relative contributions of the various neural pathways to the MPOA-stimulated ICP response. Shown in parentheses are the approximate per cent contribution of each penile nerve branch to the MPOA-stimulated increase in ICP, where the ICP response elicited by the MPOA stimulation in the nerve intact condition is depicted as 100%.

fibers beneath the surface of the capsule. The neurons were observed with both stains and were present in *both* the dorsal and ventral surface of the prostate in each of the three radical prostatectomy specimens that were examined.

## Discussion

The cavernous nerve is a main branch of the penile nerve that originates from the major pelvic ganglion and is considered as the final common effector of penile erection.<sup>3-6,8,16-21</sup> Dail *et al*,<sup>3</sup> clearly documented that in the rat, the cavernous nerve and ancillary nerves (ie ventral and lateral penile nerves) all contain NADPH-d positive fibers derived from the major pelvic ganglion, and moreover, that they innervate the corpus cavernosum and penile arteries. As additional proof of the potential physiological relevance of the ancillary nerve pathway, it was also shown that significant NADPH-d positive fibers remained in the penile tissue after transection of the cavernous nerves.<sup>3</sup> These important anatomical findings are consistent with the supposition that the ancillary penile nerves are important modulators of penile erection/capacity.

In the current study we utilized electrical stimulation of the MPOA following various neuronal transection protocols in order to more directly evaluate the contribution of the ancillary penile nerves to the ICP response *in vivo*. The studies are summarized in Figure 7. In that regard, the rationale for utilizing central neural stimulation of the MPOA is related to its widely known role as an important integrative brain center for modulating penile erection and sexual behavior.<sup>10,22</sup> In fact, previous studies have demonstrated<sup>10,11</sup> that the MPOA stimulated ICP response elicits corporal and arterial smooth muscle relaxation through the major pelvic ganglia and pelvic nerves. Moreover, there is no striated penile muscle contraction associated with MPOA stimulation.

In short, our current observations document that while unilateral and bilateral transection of the cavernous nerve produce statistically significant step-wise decrements in the amplitude of the MPOA stimulated ICP response ( $0.55 \pm 0.02$  and  $0.34 \pm 0.09$ , respectively; see Table 1 and Figures 3 and 4), neither unilateral *nor* bilateral transection of the cavernous nerve(s) was sufficient to completely ablate the MPOA-stimulated ICP response. In sharp contrast, bilateral transection of the pelvic nerve completely ablated the MPOA-stimulated ICP response (Figure 5). A cogent interpretation of these findings is that the ancillary penile nerves contribute 40–50% of the ICP response elicited by the MPOA stimulation. More specifically, the MPOA-stimulated ICP response remaining after ablation of the cavernous nerves reflects the physiological

contribution of the ancillary penile nerves, as predicted by the initial rat neural anatomic studies.<sup>3–6</sup>

The conclusion then, is that a large portion of the penile vasodilator outflow from the major pelvic ganglion derives from those ancillary pathways (ie the average number of neurons through ancillary nerves may be about one half of the total neurons in the major pelvic ganglion).<sup>3,4</sup> Zvara *et al*,<sup>7</sup> have also shown an  $\approx 50\%$  reduction of ICP response following unilateral peripheral nerve stimulation after transection of branches of the cavernous nerve. While their stimulation protocol differed from ours (peripherally nerve stimulation vs central stimulation with the MPOA), the authors also concluded that there was a large physiological contribution of ancillary branches of cavernous nerve on penile erection. In another functional study, in which penile erection was induced either by apomorphine or pelvic nerve stimulation, the ICP response also remained after chronic cavernous nerve ablation.<sup>23</sup>

Prior to the aforementioned reports, and our more recent data reported herein, it had been shown that bilateral cavernous nerve ablation almost totally eliminated *visible* penile erection,<sup>8,9</sup> leading to the mistaken impression that the cavernous nerve made the most physiologically relevant contribution to penile erection. Our data are not inconsistent with these previous observations, as we have noted that an ICP/BP ratio of greater than 0.5:0.6 ratio is required for a visible penile erection; smaller increases in ICP/BP produce only penile tumescence, but not obvious penile erection (unpublished data). Thus, in light of the relative contributions of these the ancillary (40–50%) and cavernous nerves (50–60%) to the ICP response following MPOA stimulation, one would in fact predict that ablation of the cavernous nerves would eliminate the visible penile erection in the continuing presence of a measurable and considerable ICP response. Thus, our current study confirms and extends previous observations.<sup>7–9,23</sup> Therefore, it seems prudent that the physiological role of these ancillary penile nerves should be considered in various rat models when evaluating both normal neural physiology and the pathophysiology of erectile dysfunction.

To further validate the potential implications of data derived from our rat model to the equivalent human condition, we conducted histological studies on prostatic tissue excised from patients undergoing nerve-sparing radical prostatectomies. In this regard, prior evaluation of the perineural structure of the human prostate gland has shown that well-recognized NOS containing fibers are present on the dorsolateral surface of the prostatic capsule within the neurovascular bundle.<sup>19</sup> However, it had also been reported that NADPH-d fibers were located on the lateral and ventral surfaces of the human prostate similar to that found in the rat.<sup>7</sup> The result of the present histological examination of three

prostate glands removed during nerve sparing radical prostatectomies confirms these previous observations, and documents that many neural bundles are also present within both the ventral and dorsal surface of the prostate (Figure 6). As such, it is clear that nerve-sparing radical prostatectomies may still be removing physiologically important nerve fibers. Such a hypothesis is consistent with the observation that erectile capacity is reported to have been lost or compromised in significant percentages of the patients even when nerve-sparing surgery has been done.<sup>12,13,25–30</sup> In fact, even after nerve sparing radical prostatectomy or cystoprostatectomy, the time to recover erectile potency may range from several months up to one year or more.<sup>24,25</sup> The cause of that variation from various reporting centers has been unclear. Patient age and surgical technique, as well as possible injury to accessory pudendal arteries have all been suggested as potential explanations.<sup>25,32,33</sup> The results of the present physiological observations in the rat model, when taken in conjunction with the human histological data, suggest the possibility that the ancillary penile nerves are physiologically relevant and that injury to them during radical extirpative surgery may further impair penile erection. Clearly, additional study is needed to confirm the importance of these ancillary branches of human penile nerves.

## Conclusions

This study has clearly demonstrated that the ancillary penile nerve as well as the cavernous nerve plays an important physiological role in penile erection in the rat. The physiological roles of these ancillary neuronal pathways should be given important consideration in rat models of erection and erectile dysfunction. The finding of ancillary nerve fibers in the capsule of the human prostate glands suggests that further investigation is needed to determine the modulatory role that these ancillary branches have in men.

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