Identification of penile inputs to the rat gracile nucleus

Kyle J. Cothron,1 James M. Massey,1,2 Stephen M. Onifer,3 and Charles H. Hubscher1,2
1Department of Anatomical Sciences and Neurobiology, 2Kentucky Spinal Cord Injury Research Center, University of Louisville School of Medicine, Louisville, Kentucky; and 3Spinal Cord and Brain Injury Research Center, University of Kentucky, Lexington, Kentucky

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Identification of penile inputs to the rat gracile nucleus. Am J Physiol Regul Integr Comp Physiol 294: R1015–R1023, 2008. First published January 2, 2008; doi:10.1152/ajpregu.00656.2007—Neurons in the medullary reticular formation (MRF) of the rat receive a vast array of urogenital inputs. Using select acute and chronic spinal cord lesions to identify the location of the ascending neural circuitries providing either direct or indirect inputs to MRF from the penis, our previous studies demonstrated that the dorsal columns and dorsal half of the lateral funiculus convey low- and high-threshold inputs, respectively. In the present study, the gracile nucleus was targeted as one of the likely sources of low-threshold information from the penis to MRF. Both electrophysiological recordings and neuroanatomical tracing (injection of cholera toxin B subunit (CTB) into a dorsal nerve of the penis) were used. After discrimination of a single neuron responding to penile stimulation, testing for somatovisceral convergence was done (mechanical stimulation of the distal colon and the skin over the entire hindquarters). In 12 rats, a limited number of neurons (43 in total) responded to penile stimulation. Many of these neurons also responded to scrotal stimulation (53.5%, dorsal and/or ventral scrotum) and/or prepuce stimulation (46.5%). Histological reconstruction of the electrode tracks showed that the majority of neurons responding to penile stimulation were located ventrally within the medial one-third of the gracile nucleus surrounding obex. This location corresponds to sparse innervation by CTB-immunoreactive primary afferent terminals. These results indicate that neurons in the gracile nucleus are likely part of the pathway that provides low-threshold penile inputs to MRF, a region known to play an important role in mating processes.

dorsal columns; pudendal; pelvic; convergence; cholera toxin b subunit

SPINAL CORD INJURY RESULTS not only in significant sensory and motor dysfunctions of the hind limbs but in disruption of pathways responsible for normal sexual function. To devise effective treatments and therapies for these patients, a more thorough understanding of the spinal circuitries mediating sexual function is needed. In the normal spinal cord, the dorsal columns are organized with sensory information from the lower half of the body traveling more medially as it ascends in the fasciculus gracilis and from the upper body further laterally in the fasciculus cuneatus (35, 60). These somatotopically organized projections terminate into the dorsal column nuclei of the caudal medulla, which comprises the nucleus gracilis (Gr) (input from the lower body) and both the nucleus cuneatus (Cu) and external cuneate nucleus (input from the upper body). In the rat Gr, more caudal structures, such as the tail and perineum are represented medially vs. structures such as the leg and foot, which are found to be located more laterally in the nucleus (36). Maslany et al. (31) demonstrated that the representation of the dorsal foot is located ventral to the toes and foot pads, the latter being the most dorsal. In addition, densely innervated areas of the body, such as the forepaw and hind paw, receive a disproportionately larger representative area in the dorsal column nuclei compared with other regions of the body. This topographical organization in the Gr is maintained all the way to the cortex, where inputs from the lower body terminate most medially (for a review, see Ref. 54).

Examination of the early human cortical maps, such as those by Penfield and Rasmussen (44), raises the question as to the cortical and subcortical CNS location of inputs that are responsible for a wide spectrum of sensations from the genitalia and internal reproductive and other pelvic organs. Several studies using somatosensory evoked potentials have assessed the cortical location of neurons responsive to stimulation of various pelvic regions, including the male and female genitalia, bladder, and rectum (5, 15, 29, 30, 63). Human cerebrocortical potentials have also been used to determine the extent of cortical penile representation. In these experiments, it was found that the responsive area for the penis in humans was much larger and located more laterally along the superior somatosensory cortex than what was previously thought, suggesting that there should be a modification to the homunculus that was previously proposed (9).

The central regions processing and relaying information from the reproductive organs to higher cortical centers are under investigation in a number of laboratories. Studies in our laboratory using techniques similar to those in the present study indicate a vast array of urogenital and colon inputs to the medullary reticular formation (MRF) and thalamus in both male and female rats (19, 20, 22, 26, 46). Furthermore, we have demonstrated in female rats that there is a substantial amount of input from the uterus, cervix, and vaginal canal into both the nucleus tractus solitarius and Gr (6, 18). Several neuroanatomical tracing studies have shown inputs to Gr from nerves innervating the internal reproductive organs and external genitalia. For example, Fluorogold or horseradish peroxidase (HRP)-injected into the pelvic or pudendal nerve labels axons within Gr medially near the level of obex (12, 55, 56). However, the pudendal nerve studies by Ueyama et al. (55, 56) indicated that there was more labeling in response to the application of HRP to the cut end of the perineal nerve branch than to the dorsal nerve of the penis (DNP). It is interesting to note, however, that although a study in cats found penile...
projections to Gr, the inputs from the clitoris in females were not in an analogous position (26a).

Using both acute and chronic midthoracic spinal cord lesions, we have previously reported that the ascending projections conveying low (touch) and high (pinch) threshold information originating from the penis to neurons in the MRF are located within the sensory dorsal columns and dorsolateral funiculus, respectively (21, 23). Because none of the axons within the dorsal columns have been shown to project directly to MRF, the purpose of the present set of experiments was to determine the extent and location of neurons within Gr that were responsive to penile stimulation. Electrophysiological recordings were used in male rats to determine the location of neurons in the Gr responsive to penile stimulation and the degree of convergence with other hindquarter regions. We have previously demonstrated somatovisceral and viscerovisceral convergence within Gr in female rats (6). On the basis of retroviral tracing studies of the pudendal nerve (55) and previous mapping studies of the body, it was hypothesized that most responses will be localized to the medial portions of the nucleus near obex. Here, we describe electrophysiological findings that reveal limited convergence and potential contralateral/bilateral responses. Also, using a unilateral injection of a neuroanatomical tracer directly into the DNP of an additional group of rats, we demonstrate the location and laterality of the primary afferent terminals within Gr.

MATERIALS AND METHODS

Seventeen adult male Wistar rats were used in this study, with ages ranging between 60 and 70 days old and weights varying between 250 and 300 g. Animals were subjected to a 12:12-h light-dark cycle, with lights on at 6 A.M. and off at 6 P.M. Each animal was handled daily for 2 wk before experiments were conducted. All animal procedures were reviewed and approved by the Institutional Animal Use and Care Committee at the University of Louisville School of Medicine.

Electrophysiology Surgical Preparation

Twelve rats were used for terminal electrophysiology studies. Each rat was anesthetized with an intraperitoneal injection of a solution of 50% urethane (1.2 g/kg). The left jugular vein and trachea were cannulated and intubated to provide an intravenous infusion route for anesthesia (5% urethane supplements, as needed, based on withdrawal and corneal reflexes) and to monitor respiratory rate/end-expired PCO2. Body temperature was monitored and maintained at 37°C by using an esophageal thermistor in conjunction with a heating pad and body coil containing circulating, heated water. To access the penis for stimulation, the suspensory ligaments were exposed and cut using microdissection scissors via a ventral midline incision (20).

The head of the rat was secured in a stereotaxic device at approximately a 30° angle posterior-to-anterior. A dorsal midline incision was made beginning between parietal bones and extending to the approximate a 30° angle posterior-to-anterior. A dorsal midline incision was made beginning between parietal bones and extending to the

At the end of the electrophysiological experiment, the animals were euthanized and perfused transcardially with a 0.9% normal saline solution at pH 7.2 followed by a 4% paraformaldehyde solution. The
caudal brain stem was removed and cryoprotected at 4°C in a 30% sucrose solution for at least 24 h. The tissue was then sectioned at 30-μm thickness in the transverse plane on a cryostat. The tissue sections were placed sequentially on charged microscope slides and stored at −80°C. The sections were then thawed and viewed under a light microscope (Nikon Eclipse E-400) with an EXFO X-CITE fluorescent illumination unit and a Nikon D-FL EPI Fluorescence attachment filter. Images were taken using a Spot Insight camera (Diagnostic Instruments, Sterling Heights, MI) attached to the microscope. Fluorescent images of the electrode tracks were overlaid on brightfield images for confirmation of their locations (33). Measurements were taken using a digital micrometer overlaid onto the composite images to assess the position of the tracks in relation to the measurements recorded from the stereotaxic device. These images and measurements were used for the reconstruction of tracks and unit locations.

Surgical Preparation for Tracer Injection

Five rats were used for a neuroanatomical study involving cholera toxin B subunit (CTB) as an anterograde tracer of primary afferent terminals. The animals were anesthetized with a mixture of ketamine (80 mg/kg) and xylazine (10 mg/kg). Supplements were given as needed. A dorsal incision, through the left gluteus superficialis and left biceps femoris muscles, was made in each animal to expose the left DNP. The nerve was identified (20, 40) and crushed to compromise its integrity and facilitate retrograde transport of the tracer. A 5-μl suspension of 1% CTB (39) was injected into the left DNP rostral to the crush site using a 33-gauge round-nose needle (Hamilton, 7762–06; Reno, NV) (33, 39). The same amount was injected subcutaneously into the second digit on the right forepaw (33, 39) to provide a positive control (Cu is positioned lateral to Gr) for the label itself, as well as for laterality (forepaw primary afferent projection is known to be unilateral). The incision was sutured, and the rat was kept at 37°C on a heating pad until it regained consciousness.

Immunohistochemistry

Four days after tracer injection, the rats were deeply anesthetized by an intraperitoneal injection of 50% urethane and perfused transcardially as described above. The segment of the brain stem that included the obex was removed and cryoprotected. The tissue was then sectioned with the cryostat as described above and placed on charged slides for immunohistochemistry.

A double immunohistochemical technique (for CTB and MAP2) was used, as previously described (33). The CTB labels terminals, and the monoclonal anti-MAP2 allowed us to visualize the second-order Gr neurons and their dendrites (MAP2, combination of mouse anti-microtubule-associated peptide 2a and 2b; see Ref. 33). The tissue was thawed to room temperature briefly, after which a border was created around the sections using a hydrophobic slide marker (Research Products International, Mount Prospect, IL). Tris-buffered saline (TBS) at pH 7.4 was used to hydrate the sections in three 20-min rinses. Blocking consisting of 10% normal donkey serum (NDS) and 0.25% TritonX-100 (Tx) to total volume was added to each section, and they were left for 1 h in darkness at room temperature. The primary antibodies, monoclonal anti-MAP2 (1:500, Sigma, St. Louis, MO) and goat anti-choleragenoid (1:1,000, List Biological Laboratories, Campbell, CA), were mixed with 10% NDS and 0.25% Tx-TBS. The secondary antibody solution was added to the sections, and they were incubated at room temperature in darkness for 1 h. A final series of three 20-min rinses with TBS were done prior to coverslipping with Fluormount (SouthernBiotech Associates, Birmingham, AL).

Quantification of CTB-Labeled Primary Afferent Terminals

The immunostained sections were examined on a light microscope (Nikon Eclipse E-400) equipped with an EXFO X-Cite fluorescent illumination unit and a Nikon D-FL Epi Fluorescence attachment initially to locate CTB labeling. The same sections were then examined on an Olympus Optical (Melville, NY) 3 Laser Fluoview 500 scanning confocal microscope. Composite ×20 confocal images were obtained by performing a sequential scan for each fluorochrome in 1-μm increments throughout a 30-μm-deep section to surround the area of greatest labeling. Scanning was done at three different levels surrounding the obex (100 μm rostral, at the level of the obex, and 300 μm caudal), as well as ipsilaterally and contralaterally to the injected nerve to compare the extent of bilateral labeling in each respective area. The composite images were used to quantify the extent of unilateral/bilateral labeling (33) of penile inputs to the Gr and unilateral labeling from the forepaw digit to Cu (control injections). Code identifiers were assigned to each image, which were then analyzed by a blind experimenter using Matlab (ver. 6.5; MathWorks, Natick, MA) and its corresponding Image Processing Toolbox (33). To eliminate any contribution of ambient background fluorescence in the quantification of CTB-immunoreactive terminals, background tissue intensities were sampled in areas outside the dorsal column nuclei from sections taken from each animal and averaged using Adobe Photoshop ver. 7.1. To reliably distinguish positive labeling from background fluorescence, this averaged intensity was used as a threshold value. All pixels with intensities above this threshold value were included in the quantification. Final values from these (counted) positive pixels representing CTB-immunoreactive terminals from the Gr on each side were then calculated as a percentage of total CTB-traced terminals from both sides.

RESULTS

Electrophysiology

A total of 319 electrode tracks in 12 rats were made within the narrow medullary search area (see shaded areas in Figs. 1 and 2). This includes areas ranging from 300 μm rostral to obex to 500 μm caudally and 500 μm laterally on each side of midline. The percentage of electrode tracks containing neurons with responses to touch/stroke/gentle pressure of various regions on the hindquarters (from highest to lowest) was 35.7% scrotum, 30.7% perineum, 27.6% hind paw, 12.9% penis, and 12.9% prepuce. The electrode tracks covered a maximum depth of 800 μm beginning from the dorsal surface of the medulla.

Gracile neurons responsive to stimulation of the penis.

Forty-three units within 41 of the 319 tracks surveyed in the search region (12.9%) responded to low-threshold (touch/ stroke/gentle pressure) penile stimulation (Fig. 1). All of these neurons were found in the medial 1/3 of the Gr and were limited to the area surrounding the obex. The majority were confined to the ventromedial region of the Gr. This penile-responsive zone was surrounded rostrally, laterally, and caudally with electrode tracks that contained neurons responsive to other regions of the body. Although most of these neurons responded to unilateral stimulation of the penis (ipsilateral to the recording), seven neurons responding to pressure along the midline seemed to respond to contralateral stimulation as well, leading to the
unilateral tracer injections of the DNP (see Cholera Toxin B Tracing below). No neurons were found to be responsive to high-threshold (pinch) levels of penile stimulation.

NG neurons responsive to other regions innervated by the pudendal nerve. The scrotum, prepuce, and perineum are, like the penis, innervated by branches of the pudendal nerve (40). There were 202 units across 114 tracks (35.7%) responding to dorsal and ventral scrotal (touch/stroke/gentle pressure) stimulation (Fig. 2). All of the responses were to stimulation of the ipsilateral hind paw. None of these penile-responsive neurons responded to touch/stroke/gentle pressure of the hind paw [which is innervated by branches of the sciatic nerve (57)]. In addition, none of the 43 penile-responsive Gr neurons responded to distention of the distal colon [which is innervated by the pelvic nerve (51)]. Also, 16 of 58 electrode tracks through the Gr area just medial to the region containing penile-responsive units had neurons responding to touch/stroke/gentle pressure of the inner thigh (n = 11) and the nonglabrous portion of the tail (n = 9; most medial responses in Gr).

CTB Tracing Results

CTB was injected unilaterally into the left DNP (sensory branch of the pudendal nerve), as well as into the right forepaw digit 2 (positive control). A typical example of CTB-labeled terminals in Gr and Cu, respectively, of the caudal medulla at the level of obex is presented in Fig. 4. Bilateral CTB terminal labeling with an ipsilateral predominance can be seen within Gr, vs. only ipsilateral labeling within Cu. The labeled region of the Cu, which served as our positive control, corresponded with previous tracing studies showing the restricted location of ipsilateral terminal primary afferents originating from digit 2 of the forepaw (31–33).

CTB labeling was seen in the midline of the dorsal columns ipsilateral to the DNP injection site (Fig. 5). In the caudal Gr, clusters of fiber terminals occupied medial locations and tended to be located ventrally. In sections located further rostrally, closer to the obex, some of the fiber terminal labeling was visible on the contralateral side just beyond midline. At the level of the obex, labeling was located bilaterally within Gr with an ipsilateral predominance. In contrast to more caudal areas of the Gr in which CTB labeling was densest along the midline ventrally, CTB-labeled terminals at obex were located in more lateral and superficial locations. In the sections just rostral to obex, contralateral labeling was sparse, and ipsilateral labeling extended only about 100 µm rostral to obex. A representative example of images taken from one rat at three different levels of the caudal medulla, including the level of the obex, 300 µm caudal to the obex, and 100 µm rostral to the obex are shown in Fig. 5.

Confocal images obtained for this area were used to quantify CTB labeling in the Gr and Cu on both sides of the brain stem. Seventy percent of terminal labeling from traced axons in the Gr was observed ipsilateral to the DNP injection site (Fig. 6) compared with 30% of the labeling found in the contralateral Gr. In conjunction with the electrophysiology data, many terminals were identified in the ventromedial region of the Gr. The quantification of the positive control showed that virtually
all (99%) of the Cu labeling occurred ipsilateral to the injection site (Fig. 6).

**DISCUSSION**

In the present electrophysiological study, a limited number of Gr neurons (43 in total) responded to penile stimulation. Many of these neurons also responded to scrotal stimulation (53.5%, dorsal and/or ventral scrotum) and/or outer prepuce stimulation (46.5%). Histological reconstruction of the electrode tracks showed that the majority of neurons responding to penile stimulation were located ventrally within the medial third of Gr at and immediately surrounding the level of obex. This location corresponded to some innervation by CTB-immunoreactive primary afferent terminals labeled by CTB injected into the DNP unilaterally. There was evidence of sparse bilateral input to Gr from the penis, both from the electrophysiology and anatomy experiments. These findings are consistent with the hypothesis that the loss of low-threshold penile inputs to the MRF with dorsal column lesions is due to interruption of a relay through Gr.

**Penile Inputs to Nucleus Gracilis**

One of our previous electrophysiological studies (20) found that the majority of DNP-responsive MRF neurons in the rostral medulla responded to high threshold (noxious) levels of penile stimulation in male rats. Only 26 of 165 DNP-responsive MRF neurons responded to low-threshold (touch/stroke/gentle pressure) levels of stimulation. A subsequent study (21) using select acute and chronic spinal lesions at the mid-thoracic level demonstrated that the high-threshold nociceptive inputs from the penis ascended to the MRF (either directly or indirectly) within the white matter of the dorsolateral quadrant bilaterally, whereas the low-threshold innocuous penile inputs ascended via the dorsal columns. In the present study, only low-threshold penile-responsive neurons were found in Gr. In addition, the number of penile responses was relatively small compared with other territories, such as the scrotum, which is also innervated by the pudendal nerve. This small number of responses in Gr is consistent with the proportionately small number of low-threshold penile responses within the MRF. Importantly, a few studies have shown that the MRF is interconnected with Gr (37, 53), suggesting that the Gr is a likely source of low-threshold inputs from the penis directly to the MRF. An alternate route could be an indirect circuit to first a more rostral target(s), such as within the diencephalon, which would ultimately project back to (either directly or indirectly) the MRF, a region known for its multiple array of inputs/outputs (e.g., 25).

In the first part of the present study, an initial survey of Gr narrowed the search for penile-responsive neurons to an area 300 μm rostral to obex, 500 μm caudal to obex, and 500 μm lateral to the midline. Recordings done along 319 electrode tracks in 12 animals within this narrow region revealed only 43 single units responding to penile stimulation. The penile-responsive neurons, which were surrounded in all directions by CTB-immunoreactive primary afferent terminals labeled by CTB injected into the DNP unilaterally. There was evidence of sparse bilateral input to Gr from the penis, both from the electrophysiology and anatomy experiments. These findings are consistent with the hypothesis that the loss of low-threshold penile inputs to the MRF with dorsal column lesions is due to interruption of a relay through Gr.
Several units in the present study were seen to possibly have contralateral responses as well. It was technically difficult to stimulate the midline region of the glans unilaterally. Thus, to address the potential bilaterality of these pathways, CTB tracing was used to map them. After injecting only the left dorsal nerve of the penis, CTB was located within the medial half of the Gr almost throughout its entire rostrocaudal extent. Bilateral labeling was limited to the level of obex and just caudal to it. Results of the tracing study confirmed the bilaterality of these pathways suggested by the electrophysiological recordings, a finding consistent with two previous tracing studies (55, 56), in which labeling also was seen to move bilaterally near the level of obex and no Gr labeling occurred by injecting just the motor branch of the perineal nerve (57).

A study by Kitchell et al. (27) determined that the dorsal nerve of the penis in the rat contained slowly and rapidly adapting mechanoreceptor afferents, both of which have small peripheral receptive fields on the glans. This finding makes the possibility that a bilateral response in the electrophysiology experiment might be indicative of a peripheral receptive field extending bilaterally unlikely. Since primary afferents were seen to terminate bilaterally through the tracer studies with an ipsilateral predominance in Gr, unilateral lesions superior to Gr would not eliminate sensation entirely from the contralateral side.

With regard to fiber type, CTB is known to be transported by myelinated axons with both small- and large-diameter fibers (32, 47, 48), whereas a tracer such as wheat-germ agglutinin conjugated to horseradish peroxidase (WGA-HRP) selectively labels unmyelinated and small-diameter fibers (28, 52). Cholagenoid-HRP has also been shown to selectively label large dorsal root ganglion cells (30–50 μm), while WGA-HRP has a propensity for smaller dorsal root ganglion cells (15–25 μm) (28). The DNP consists of unmyelinated C-fibers and small-diameter myelinated axons (24). In a previous study using a direct current polarization technique to selectively block peripheral myelinated nerve fibers, some C-fiber-mediated responses to cutaneous stimulation were found in Gr (45). In addition, the presence of type C fibers has been demonstrated by several other studies (10, 14, 41, 42, 59). LaMotte et al. (28) also found both myelinated (A) and unmyelinated (C) fibers within the Gr. They demonstrated that the small unmyelinated fibers occupied a focused area within the area of myelinated fibers. However, taking both the electrophysiological and tracking results into consideration, the penile inputs to Gr are likely from small-diameter myelinated fibers. Unmyelinated fiber types convey nociceptive information, and no penile neurons responded to pinch in the present study (just touch/stroke/gentle pressure).

Additionally, Gr receives inputs from multiple sources, including, for example, postsynaptic dorsal column fiber input (13). Some of the responses to stimulation of the penis, therefore, could be indirect and not via a primary afferent projection. However, given the small number of responsive neurons in combination with the tracing results, the possibility of the inputs determined electrophysiologically being indirect is not likely.

Somatovisceral Convergence

Several investigators have now shown that visceral nociceptive information ascends along with the general touch and conscious proprioception sensory modalities that are known to travel within the dorsal columns. Berkley and Hubscher (6), for example, first demonstrated inputs to Gr, originating from the...
female reproductive organs and showed the near absence of nociceptive input from the uterus following a dorsal column lesion. Similarly, Al-Chaer and colleagues (2) then used electrophysiological recordings from the ventroposterolateral nucleus (VPL) to identify its visceral inputs. Lesions of the Gr attenuated the responses of the VPL to innocuous cutaneous stimulation and colorectal distention. These findings were confirmed in a different study, whereby labeling in the Gr corresponded to the area representing the colon/rectum (12).

In our previous MRF recordings in dorsal column-lesioned rats, responses still remained for colon distention (on the order of about 30%) despite the loss of low-threshold penile inputs, suggesting these inputs ascend to MRF via different pathways (23). In the present study, none of the penile-responsive neurons in Gr responded to colon distention, a finding consistent with different input sources for at least some of the convergent information being relayed through to the MRF. The lack of responses to distention of the distal colon is likely related to the narrowed region in Gr around the obex that receives penile input (those were the only units tested for convergence). In a previous electrophysiological study of 43 (out of 212) Gr neurons responding to colorectal distention (50), the convergent somatic receptive fields were distributed differently than ours; that is, along the outside of the leg from the hip to toes. Note that in their study as well as in the experiments by Al-Chaer and colleagues (1, 2), both the distal colon and rectum are distended, therefore the Gr neurons may have responded due to indirect activation of the perianal region (our balloon was positioned too deep to have such an effect, even upon distention).

The cutaneous regions of the body with convergent input onto the penile-responsive Gr neurons originated from territories innervated by the same nerve (pudendal, albeit different branches; see Ref. 40). This finding differs from our previous MRF recording, in which convergent inputs came from widespread territories, many of which are from the entire body (19, 20). This finding also differs from previous Gr recordings in female rats, in which the most common somatic region with convergent input from the pelvic reproductive organs was the foot/toes, followed by the leg/ankle (6). The difference between these studies is once again likely a consequence of the sources of inputs, as these female reproductive organ inputs are likely indirect (for example, via postsynaptic dorsal column input, as suggested for the colon inputs to Gr; see Ref. 1). In previous spinal cord recordings (6a), cervix-responsive neurons were found to respond to stimulation of the hind paws. Note that the focus of the studies in the female rats was convergence with the internal pelvic organs and not the external genitalia (i.e., clitoris, innervated by the dorsal nerve of the clitoris, was not tested). Thus, different projections (direct vs. indirect) originating from a variety of various sources likely accounts for the differences.

Functional Considerations

Penile hypersensitivity has been demonstrated in patients with primary premature ejaculation (62), which was further shown to be correlated with significantly shorter latencies for

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Fig. 5. Representative confocal images of three areas surrounding obex. Red immunofluorescence represents primary afferent terminals labeled by CTB injected into the dorsal nerve of the penis (seen as clusters at lower magnification). Note the bilateral terminal labeling in Gr at the level of obex. The inset at obex (~300 μm in length) is a magnification of the area within which contralateral labeling was located. Green immunofluorescence represents MAP2 (combination of mouse anti-microtubule-associated peptide 2a + 2b) immunostaining and is used to illustrate the proximity of the CTB-labeled terminals to the second-order gracile neuron dendrites. Cu labeling is not included in the images. Anatomical locations are equivalent to the plate drawings shown in Fig. 1. Magnification factor ×100.

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Fig. 6. Graphic representation of the density of CTB-immunoreactive (IR) within the Gr, ipsilateral and contralateral to the injected dorsal nerve of the penis. Also shown are the results from control injections of CTB into digit 2 of the forepaw on the opposite side. The minimal percentage of CTB-IR pixels in the contralateral cuneate nucleus (Cu) represents the level of background labeling.
somatosensory evoked potentials (61). The penile inputs to Gr demonstrated in the present study are therefore a potential target for the treatment of premature ejaculation. A number of pharmacological agents and their use to reduce penile sensitivity are discussed by Giuliano in a recent interview article (34).

The presence/absence of genital sensation has been shown to be a predictor of the likelihood of experiencing orgasm post spinal cord injury (3). Most men with spinal cord injuries experience sexual dysfunction, which is associated with erectile and ejaculatory dysfunctions, poor semen quality, diminished sexual arousal, and decreased fertility (4, 8, 11). Male spinal cord injury patients report the loss of normal sexual function to be of the highest priority (3), and the most common reasons cited are for sexual pleasure and intimacy (4). As we have previously shown, the dorsal column is one of several pathways conveying ascending information to the brain from the penis. The loss of low-threshold tactile sensory input that relays through the Gr likely contributes to sexual dysfunction due to diminished genital sensation, although spinal cord injuries in a given individual affect multiple ascending and descending pathways that can influence different aspects of sexual function. Note that in spinal cord-injured patients, the spinal ejaculatory reflex is possible with strong vibratory stimuli applied to the ventral midline of the penis, which is likely effective enough to recruit all sensory modalities to elicit the spinal ejaculatory reflex (7).

**Perspectives and Significance**

The results of the present study demonstrate, using anatomical and electrophysiological techniques, bilateral penile inputs to a limited region of the nucleus gracilis around the obex. The dorsal column nucleus in the caudal medulla is a region traditionally known to receive and process information from small ipsilateral cutaneous areas. The list of pelvic/visceral structures with inputs to gracilis neurons also includes the female reproductive organs (uterus, cervix, vagina; see Ref. 6), colon/rectum (1) and pancreas (58). The information gained from this study furthers our continued investigation of the ascending spinal pathways and target supraspinal nuclei involved in the central processing of information originating from pelvic/visceral organs (for a review, see Ref. 16). Evidence to date points to multiple spinal pathways conveying innocuous and noxious pelvic/visceral information to multiple supraspinal regions, which are involved in different aspects of sensation, as well as autonomic integration and motor control. Thus, characterization of not just the peripheral but also the central neural mechanisms involved will likely lead to novel clinical interventions for reducing sexual, pelvic, and visceral organ dysfunctions and alleviation of chronic pain.

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