Effects of Acute and Chronic Midthoracic Spinal Cord Injury on Neural Circuits for Male Sexual Function. I. Ascending Pathways

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Hubscher, Charles H. and Richard D. Johnson. Effects of acute and chronic midthoracic spinal cord injury on neural circuits for male sexual function. I. Ascending pathways. J. Neurophysiol. 82: 1381–1389, 1999. Normal male reproductive function, particularly ejaculation, requires the integrity of urogenital sensory input and its ascending spinal projections. After midthoracic chronic spinal cord injury, sexual dysfunction occurs in both rats and humans. Neurons in the medullary reticular formation (MRF) are involved in the processing of bilaterally convergent sensory inputs from multiple cutaneous, mucocutaneous, and visceral regions of the body, including the penis and male urogenital tract. A variety of acute and chronic lesions were used to determine the midthoracic location of ascending spinal pathways conveying sensory input from the penis and male urogenital tract to MRF. A total of 371 single neurons were recorded in the MRF of 34 urethan-anesthetized mature male rats. Twenty-seven rats received a chronic T8 dorsal (DHx) or lateral (LHx) hemisection or contusion (Cx) injury 30 days before the terminal electrophysiological experiments. In addition, nine dorsal nerve of the penis (DNP)-responsive MRF neurons in seven intact control animals were tested completely both before and after various acute spinal cord lesions. The chronic lesion data indicate that low and high threshold input from the penis (mucocutaneous) and male urogenital tract (visceral) ascend bilaterally within the dorsal quadrant at T8 as opposed to high threshold input from the hindpaws (cutaneous), which ascends unilaterally in the ventrolateral quadrant (VLQ). The acute lesion data indicate that the low-threshold information conveyed from the penis and male urogenital tract ascends in the dorsal columns, as opposed to the high-threshold nociceptive inputs that ascend bilaterally in the dorsolateral quadrant (DLQ). These results, as well as previous data on ascending projections from female reproductive organs within the dorsal columns and DLQ to other caudal brain stem nuclei, provide evidence for ascending pathways conveying nociceptive information centrally via the DLQ. This spinal gray-DLQ pathway(s) conveying information from mucocutaneous/pelvic/visceral territories therefore differs from the traditionally recognized spinal gray-VLQ pathway(s), which is known to convey nociceptive information from cutaneous regions of the body.

INTRODUCTION

The peripheral sensory limb of the segmental reflexes for erection and ejaculation is carried primarily by afferents in the dorsal nerve of the penis (DNP) (Johnson 1988; McKenna and Nadelhaft 1986; Rampin et al. 1994). Desensitization of the penis, produced by local anesthesia or dorsal nerve section, causes an impairment of reflexogenic erection, intromission, and ejaculation (reviewed in Sachs and Meisel 1988). The primary afferent population in the DNP has been characterized (Johnson and Halata 1991; Johnson and Murray 1992; McKenna and Nadelhaft 1986; Nunez et al. 1986; Steers et al. 1988). Electrophysiological techniques have been used to investigate single spinal cord interneurons in the dorsal horn and intermediate zone of primarily L2–S1 that receive input from these DNP afferents (Johnson 1989). All of the penile interneurons exhibit receptive fields on the penis that are significantly larger than the receptive fields for single primary afferent neurons, thereby demonstrating a central convergence of penile sensory input. Almost all of the penile interneurons have receptive fields on both sides of the body, and their electrical characteristics strongly suggest a monosynaptic input from both ipsilateral and contralateral DNP fibers (Johnson 1989).

DNP afferents produce bilateral (crossed and uncrossed) reflex facilitation of pudendal motoneurons. The synaptic efficacy of DNP afferents and associated interneurons onto pudendal motoneurons is reduced progressively over time in rats with chronic spinal cord injuries (Johnson 1994). The enhanced erectile and depressed ejaculatory reflexes in rats after midthoracic transection and 30 days recovery (Hart and Odell 1981; Mas et al. 1987; Sachs and Garinello 1979) is likely due to a loss of supraspinal influences. Thus the pudendal afferent facilitation of ejaculation may require a brain stem loop, much like the control of micturition (deGroat et al. 1981).

Previous studies show that the nucleus reticularis gigantocellularis and surrounding nuclei, located in the medullary reticular formation (MRF), are likely involved in this supraspinal loop (Marson and McKenna 1990; Marson et al. 1993; Tanaka and Arnold 1993; Yells et al. 1992). The MRF contains many neurons responsive to bilateral DNP stimulation (Hubscher and Johnson 1996a). The majority of these neurons are responsive to mechanical stimulation (majority high-threshold) of the penis, especially the distal glans/cup region. Almost half of the neurons also respond to bilateral pelvic nerve (PN) stimulation and receive convergent inputs from several cutaneous regions of the body outside the pelvic/pudendal nerve territory, including the hindfeet, forefeet, and ears. Furthermore bilateral electrical stimulation of specific DNP-responsive MRF subregions suppress DNP-mediated pudendal motoneuron reflex discharges (Johnson and Hubscher 1998) and activate sympathetic fibers in the pudendal nerve (Johnson and Hubscher 1997).

The location of the ascending and descending spinal projections comprising the supraspinal loop between the lumbosacral ejaculatory circuitry and the MRF is unknown. In the present study, the spinal cord location of ascending projections within

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METHODS

Chronic spinal cord lesions
A total of 27 male Wistar rats (age of ~90 days) received one of three types of spinal cord injury at T8: DHx, LHx, or Cx. The T8 level of spinal cord was chosen because it is rostral to the spinal reflex centers for erection and ejaculation and it is caudal enough to ensure stability and survivability of all groups of animals for the duration of the experiment. The recovery period for each animal was 30 days. At the end of the recovery period, each animal was anesthetized and the terminal electrophysiological experiment performed according to the protocol outlined in following text. Lesion surgeries were performed under aseptic conditions. Each animal (~90 days of age) was anesthetized with a mixture of ketamine (80 mg/kg ip) and xylazine (10 mg/kg ip). A long-acting antibiotic (Flo-Cillin: 0.5 ml; Fort Dodge Laboratories, Fort Dodge, IA) was administered before surgery. The spinal cord was exposed at the T8 level via removal of the overlying T8 vertebral lamina. Hemisections (DHx or LHx) were made through a longitudinal dural incision using a pair of microdissecting scissors. The dura was closed with a pair of 10–0 monofilament sutures. Contusion of the spinal cord was performed using a rapid compression of the T8 cord with a concave probe having a radius and size matching the overlying lamina. The probe was attached to a displacement-controlled mechanostimulator (Chubbuck 1966) driven with a trapezoid waveform using severe contusion parameters (1.9–2.0 mm, 5 s) modified from previously described protocols (Bresnahan et al. 1987; Theriault and Tator 1994). Thrombin-soaked absorbable gelatin sponge (Gelfoam) was placed into the vertebral defect. The surrounding musculature and subcutaneous tissue were sutured in layers with 4–0 monofilament. The skin was closed with Michel clips.

The animals recovered in a temperature-controlled environment while housed singly in plastic cages with wood chips. An analgesic (Buprenex) was administered as needed to alleviate postoperative discomfort. During the first postoperative day, the animals were encouraged to eat with apple slices. Throughout the recovery period the animals were tended to three times daily, 7 days a week. At these times, the animals were exercised, washed, and observed for evidence of infection or other complications, and the bladder expressed until reflex voiding function returned (6–12 days).

Surgical preparation for terminal electrophysiological experiments
Seven uninjured male Wistar rats at ~120 days of age were used in the acute lesion experiments. The remaining 27 rats had a chronic injury for 30 days before the terminal experiment. Using our previously described protocol (Hubscher and Johnson 1996a), each animal was anesthetized with urethan (1.2 g/kg ip). Supplements were given as needed. The common carotid artery, jugular vein, and trachea were intubated for the purposes of blood pressure monitoring, intravenous infusion route, and facilitation of respiration, respectively. Body temperature was maintained at 37°C through an esophageal thermistor and circulating water heating pad/body coils. The ventilatory state was monitored with an end tidal pCO2 monitor designed for rodents. Mean blood pressure was maintained at ~75 mmHg throughout the experiment.

The head was clamped in a stereotaxic holder and the brain stem exposed as previously described (Hubscher and Johnson 1996a). The ventral aspect of the pelvic region was swung caudally by pivoting the hindquarters around the axis through the hip pins and tying the tail in an upward direction. This positioning allowed the penis, ventral abdomen, and perineum to be exposed for stimulation. Specially fabricated bipolar silicon-cuff microelectrodes were placed bilaterally around the dorsal nerve of the penis (DNP), pelvic nerve (PN), and the proximal cut end of the deep perineal (motor branch of the pudendal) nerve (see experimental setup in Fig. 1 of Hubscher and Johnson 1996a).

Electrophysiological recordings
Glass-coated platinum-plated tungsten microelectrodes with a 20-μm exposed tip (Merrill and Ainsworth 1972) attached to a stepping microdrive were used as previously described (Berkley et al. 1993a; Hubscher and Berkley 1994; Hubscher and Johnson 1996a). Two microelectrodes were set for bilateral penetration of the MRF, in the same anterior/posterior plane and equidistant to the midline. Four stereotaxic equivalent tracks in the “hottest” DNP/PN responsive MRF region (see Fig. 1 A in Hubscher and Johnson 1999) was searched for neurons responsive bilateral stimulation of the DNP, as well as pinching of the ears. Ear pinch (unilateral; alternating between left and right sides) was applied as infrequently as possible (every 200 μm and/or when a spontaneously active neuron was encountered) to reduce potential sensitization/tissue damage. Note that the search protocol was altered from being just bilateral DNP stimulation (Hubscher and Johnson 1996a) due to the loss (or potential loss) of DNP inputs below the level of lesion after chronic spinal cord injury. The ear was chosen because the input pathway enters the CNS above T8 (lesion level) and, in intact controls, almost all DNP-responsive MRF neurons respond to pinching of the ears (Hubscher and Johnson 1996a).

Acute spinal cord lesions
After the characterization of the last DNP-responsive MRF neuron in each of the seven uninjured animals, a partial T7–T8 spinal cord lesion was made with microscissors, and the response characteristics were redetermined (Berkley and Hubscher 1995). This process was repeated with each of three additional lesions, all placed at different rostrocaudal sites in the T7–T8 spinal cord to facilitate postmortem acute lesion reconstruction. A typical sequence is illustrated in Fig. 1. In two animals, simultaneous unit recordings from both electrodes allowed for study of two units through the lesioning process.

Histology
At the end of the experiment, the animal was euthanized with an anesthetic overdose and perfused transcardially with 0.9% saline followed by 10% formalin. The block of brain stem tissue containing the recording sites was removed and stored overnight in a 10% formalin/30% sucrose solution. Recording sites were visualized in 50-μm vibratome sections stained with cresyl violet and reconstructed under light and dark field illumination (Paxinos and Watson 1997). The perfused spinal cord was analyzed histologically (paraffin sections) for confirmation of acute or chronic lesion extent. Spinal cord tissue sections were stained with both luxol fast blue and cresyl violet (Klüver-Barrera stain).

RESULTS

Acute spinal cord lesions
A total of nine DNP-responsive neurons were tested in the uninjured rats (n = 7) before and after acute midthoracic spinal
lesions. Before a lesion was made, three of the MRF neurons tested had excitatory responses to gentle stroking [a low-threshold (LT) stimulus] and pinching [high-threshold (HT) stimulus] of the glans penis. After an acute lesion, which included the dorsal columns (see Fig. 2, left), LT responses were eliminated; high-threshold (HT) responses remained. Bilateral lesions, which included the dorsal lateral quadrant (DLQ; see Fig. 2, right) were necessary for elimination of the HT DNP responses (see example in Fig. 1). However, MRF neuronal responses remained for bilateral pinching of the toes (except in a few cases where the lesion encroached on the ventrolateral quadrant—VLQ: see left side of lesion and responses in Fig. 1) as well as bilateral pinching of the ears and forepaws.

Chronic spinal cord lesions

A total of 27 male rats received a midthoracic spinal cord lesion 30 days before terminal electrophysiological experiments. The results of these experiments relative to the presence or absence of responses from convergent territories originating from regions below the level of injury onto single MRF neurons are summarized in Table 1 and described in more detail in the following text. A total of 362 single neurons responded to bilateral DNP and/or ear stimulation. Neurons responding to bilateral DNP stimulation were found among the LHx (83%), DHx (30%), and moderate Cx (83%) groups. The effects of these chronic lesions on the responses of cutaneous convergent territories located at or above the level of injury are presented elsewhere (Hubscher and Johnson 1999).

CHRONIC CONTUSION INJURIES. The MRF of 10 rats was searched for neurons responsive to bilateral electrical stimulation of the DNP and gentle pressure of the ears 30 days after Cx injury. Histological examination of the spinal cords at the epicenter of the Cx injury revealed severe damage in 6 of the 10 cases studied (see typical example in photomicrograph Fig. 3A). In the six rats with severe Cxs, no DNP-responsive neurons were found. However, many neuronal responses were found for stimulation of the ears (n = 66), and the majority responded to more gentle levels of stimulation than had been observed in intact controls (Hubscher and Johnson 1996a). A typical example of responses is shown in Fig. 4. No responses were found for bilateral PN stimulation or the toes of the hindfeet (pinch), which were tested frequently in addition to the search stimuli (i.e., bilateral DNP and ears).

In the four remaining rats, histological examination of the spinal cords at the epicenter of the injury revealed intact white matter. In one case, only the gray matter was damaged, whereas in the other three cases, only a portion of the white matter remained (unilateral for 1 case and bilateral for the other 2 cases—see example in Fig. 3B). In the four cases with more moderate Cxs, significantly more (χ², P < 0.01) DNP-responsive neurons were found per track than after severe Cx (as was the case for intact controls). In general, no significant differ-

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**FIG. 1.** Example of the changes in receptive field characteristics after successive acute spinal cord lesions. Records show the responses of a single neuron in right nucleus reticularis gigantocellularis pars alpha to bilateral stimulation of the penis, toes (forepaw and hindpaw) and ears before and after various T7/T8 acute spinal cord lesions. Illustration shows the change in receptive field characteristics after 3 consecutive spinal cord lesions (cumulative lesion extent in black). Note the loss of low-threshold responses with the bilateral dorsal column lesion and the reduced response to high-intensity stimulation of the penis after a unilateral dorso-lateral quadrant (DLQ) lesion (i.e., stronger pinch necessary to get an equivalent magnitude of response).

**FIG. 2.** Summary of acute midthoracic lesion data from 7 animals. Histological reconstructions of lesion extent were made with camera lucida drawings. Left: lesions resulted in the loss of medullary reticular formation (MRF) neuronal responses to stroking the glans penis but not to pinching of the glans/cup/inner prepuce. Right: lesions resulted in the loss of the neuronal response to pinching of the penis.
ences were found for MRF neuronal response properties between animals with a moderate Cx injury and intact controls (see summary in Table 1).

CHRONIC DORSAL HEMISECTIONS. The MRF of nine rats was searched for neurons responsive to bilateral electrical stimulation of the DNP and gentle pressure of the ears 30 days after T8 DHx. Histological examination of the spinal cord sections at the epicenter of the lesion revealed five of nine cases where the DHx was complete bilaterally; i.e., the lesion extended down to or just beyond the dorsal limit of the ventral quadrant on both sides of the cord. In the five animals with complete DHxs (see typical example in Fig. 3C), many MRF neuronal responses were found for stimulation of the ears (n = 79) but only a few responses remained (7%) for bilateral DNP and bilateral PN stimulation and their respective peripheral territories. Unlike the chronic contusion injuries, however, responses remained for pinching of the toes of the hindfeet bilaterally (see example in Fig. 5). In the four cases with incomplete DHxs, many ear responsive single MRF neurons also were found (n = 43); however, the majority of these (70%) responded to bDNP stimulation, which is why the overall value shown in Table 1 is so high.

CHRONIC LATERAL HEMISECTIONS. The MRF of eight rats was searched for neurons responsive to bilateral electrical stimulation of the DNP and gentle pressure of the ears 30 days after lateral T8 hemisection. Histological examination of the spinal cord sections at the epicenter of the lesion revealed three cases where the lesion was complete unilaterally and did not encroach on the contralateral dorsal columns or spinal gray matter. In two other cases, the lesion was complete unilaterally but in addition impinged on the contralateral dorsal columns and spinal gray. For the remaining three cases, which also were complete unilaterally, the lesion again impinged on the contralateral dorsal columns and spinal gray, but in addition a portion of the contralateral white matter was damaged (1 typical example is shown in Fig. 3D). However, regardless of

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<th>TABLE 1. Effects of chronic midthoracic spinal cord lesions on MRF response properties</th>
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<td>Number of DNP-responsive neurons</td>
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<td>Intact Controls (14)</td>
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<td>Mean number of DNP neuronal responses per animal (4 equivalent tracks)</td>
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<td>12</td>
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<td>Percentage of neurons responsive to DNP + PN</td>
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<td>Percentage of neurons exhibiting excitatory responses</td>
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<td>Percentage of neurons with low threshold penile fields</td>
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Numbers of animals is in parentheses. Values in lower 3 rows are derived from the percentage (±SEM) of the total number of dorsal nerve of the penis (DNP)-responsive neurons given in row 1. Data for Intact Controls was obtained from animals used in our previous study (Hubscher and Johnson 1996). LHx and DHx, chronic lateral or dorsal hemisection; Cx, contusion; PN, pelvic nerve. * Statistically significant difference from intact control group (χ², P < 0.01).

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**FIG. 3.** Postmortem histological verification of 4 types of chronic lesions at the midthoracic level of spinal cord. Sections were taken near the lesion epicenter. Each image was digitized with a high resolution scanner. Magnification factors: ×33 (A), ×40 (B), ×48 (C), and ×36 (D).
these histological differences, all eight animals with complete or over lateral chronic hemisections had many MRF neurons 30 days postinjury that responded to both ipsilateral and contralateral stimulation of the DNP and PN. These MRF neuronal responses occurred regardless of whether their brain stem location was ipsilateral or contralateral to the side of the lesion.

Overall, both the number of DNP-responsive neurons and their response properties did not differ significantly from intact controls throughout equivalent tracks (see Table 1 and example in Fig. 6). However, differences in degree of convergence and magnitude of responses were observed between LHx animals and intact controls. For example, DNP-responsive MRF neurons did not respond to pinching the toes of the hindpaw that was contralateral to the side of the lesion, regardless of MRF neuronal location (i.e., ipsilateral or contralateral to the lesion). Also, the magnitude and duration of MRF neuronal responses was greatest for bilateral peripheral nerve stimulation (of the DNP and PN), as was the case for intact controls (Hubscher and Johnson 1996a). However, for the majority of neurons tested in the LHx group of animals, stimulation of the DNP ipsilateral to the side of the lesion had a greater effect (i.e., on the magnitude and/or duration of the neuronal response) than the DNP on the side contralateral to the lesion, again regardless of brain stem location (i.e., left or right MRF). In addition, stimulation of the PN ipsilateral to the side of the lesion had a much greater effect on the responses of most of the MRF neurons tested than did the PN on the side contralateral to the lesion (again, regardless of MRF location). In fact, frequently no response would be detected from stimulating the contralateral-lesion PN. However, it often was observed that the contralateral-lesion PN had an additive effect with the ipsilateral-lesion PN on MRF neuronal responses, despite being ineffective on its own.

There were also differences in MRF neuronal response properties observed between LHx and DHx groups of animals, as was the case between DHx and intact control animals. Some of these differences are shown in Table 1 and will be discussed further in the DISCUSSION.

![FIG. 4. Example of responses from a multunit recording in right nucleus reticularis gigantocellularis to bilateral (b) stimulation of the dorsal nerve of the penis (DNP), toes (hindpaw), and ear after complete bilateral dorsal hemisection. Responses from some, but not all, of the regions tested below the level of the lesion were present. However, all responses from regions above the level of lesion (including the toes of the forepaw, and face—not shown) were present. Symbols as in Fig. 4.](http://jn.physiology.org/)

**FIG. 5.** Example of responses of a single neuron located in left nucleus reticularis gigantocellularis to bilateral (b) stimulation of the dorsal nerve of the penis (DNP), toes (hindpaw), and ear after complete bilateral dorsal hemisection. Responses from some, but not all, of the regions tested below the level of the lesion were present. However, all responses from regions above the level of lesion (including the toes of the forepaw, and face—not shown) were present. Symbols as in Fig. 4.

**DISCUSSION**

The results of the present study demonstrate the presence of MRF neuronal responses to inputs from the male genitalia after either a chronic LHx or a moderate Cx injury (i.e., with at least 1 spared lateral rim of white matter) and the absence of these responses after either a severe chronic Cx injury or a complete chronic DHx. When taken together, the results suggest that the ascending spinal projections originating from the male genitalia are bilateral and are located within the dorsal quadrant at the midthoracic level of the spinal cord. The results of these chronic lesion experiments were confirmed with select acute lesions, which showed the small number of LT penile inputs to the MRF projected rostrally via the dorsal columns versus the more numerous HT inputs that projected bilaterally within the DLQ.

The present study also demonstrates the presence of bilateral inputs from cutaneous/mucocutaneous/visceral territories innervated by either the DNP or PN to MRF neurons on both sides of the brain stem after an LHx. Whereas cutaneous inputs from previously responsive territories caudal to the spinal cord injury were only maintained from the side ipsilateral to the lesion (i.e., for toes of the hindfoot), mucocutaneous/visceral inputs were maintained from both sides of the body, although there was a predominance from the side ipsilateral to the lesion. When taken together, the results indicate a truly bilateral system from the penis and male urogenital tract in which information about noxious stimuli applied to mucocutaneous and visceral tissues is conveyed via spinal dorsal horn neurons on both sides of the spinal cord and ascends bilaterally through the DLQ at the midthoracic level of the spinal cord to synapse, either directly or indirectly, onto MRF neurons on both sides of the brain stem (see summary diagram in Fig. 7 and further discussion in the following text). In addition, these results confirm and expand on a previous report investigating central projections originating from female reproductive organs (Berkley and Hubscher 1995; Hubscher and Berkley 1995); i.e., that the classical “pain pathways” within the VLQ are not the only neurons through which nociceptive information can be conveyed centrally.
Responses of MRF neurons to inputs from the male genitalia via the DNP were eliminated after chronic DHx and after acute dorsal quadrant lesions. The MRF responses from both the left and right DNP were not eliminated after an acute unilateral lesion of the dorsal quadrant at the midthoracic level of spinal cord or after chronic LHx, although the magnitude and/or duration of the majority of MRF neuronal responses was greater from the DNP that was located ipsilateral to the LHx. These results indicate that the projections from the lumbosacral spinal cord originating from the DNP are bilateral with a contralateral predominance.

Pelvic nerve-responsive MRF neurons

Chronic bilateral DHx eliminated MRF neuronal responses to bilateral electrical stimulation of the PN. In contrast, chronic LHx failed to eliminate MRF neuronal responses on either side of the brain stem. Although these neurons responded to stimulation of the PN’s innervating both sides of the body, there was a definite contralateral predominance; i.e., MRF neurons (on either side of the brain stem) responding mostly to the PN ipsilateral to the LHx, although the largest magnitude and/or duration of response was to bilateral PN. These results, when taken together, indicate that unilateral spinal projections in the DLQ at midthoracic spinal cord conveys information from both PNs (with a contralateral predominance) and that the projections are crossed and uncrossed above the lesion.

MRF inputs from skin

Chronic severe Cx was the only injury to eliminate bilateral responses to noxious mechanical stimulation of the skin of the hindlimb (such as pinching the toes of the hindfoot). Responses to cutaneous territories were lost unilaterally after either a chronic LHx or cases where only a lateral rim of white matter remained (i.e., after a chronic moderate Cx injury). In addition, unlike the loss of responses to stimulation of the male urogenital tract after a chronic DHx, MRF neuronal responses remained for stimulation of the distal hindlimb skin (i.e., for regions that responded in intact controls). These results, when taken together, indicate that unilateral spinal-MRF projections in the VLQ at the midthoracic level convey information primarily from contralateral distal hindlimb cutaneous regions.

Location of ascending pathways

DORSAL NERVE OF THE PENIS. Although the majority of MRF responses to stimulation of the penis were elicited by firm pressure or pinch, a small number of neurons was excited by both stroking and pinching the penis. An acute bilateral lesion of the dorsal columns eliminated responses to stroking the penis, whereas the responses to pinching were maintained (and only eliminated after a subsequent bilateral DLQ lesion). These results suggest that the LT-DNP inputs are conveyed centrally via the dorsal columns, whereas the HT-DNP inputs are conveyed bilaterally within the DLQ. The results from the acute lesion experiments are consistent with those of the chronic ones, where a complete DHx eliminated all MRF neuronal responses to DNP stimulation, further evidence that these projections ascend bilaterally within the DLQ.

PELVIC NERVE. Because the chronic DHx lesions included both the DLQ and dorsal columns, it is uncertain which of these pathways contains rostral projections from the PNs. Previous studies suggest that the central projections of nociceptive inputs from visceral territories ascend via both pathways. These findings are different from an independent LT and HT pathway (dorsal column and DLQ, respectively), as determined for the penis, i.e., mucocutaneous tissue (Johnson and Halata 1991) innervated by the DNP. The existence of ascending projections conveying visceral PN inputs within one or both pathways is likely dependent on one or more factors. Possibilities include the type of stimulus (mechanical, thermal, and chemical), the type of tissue and its function, the tissues embryonic origin, the location of the dorsal horn interneurons (laminae and/or spinal level), and the location of the target nucleus in the brain stem. In support of the importance of both dorsal column and DLQ pathways, neurons in the nucleus gracilis failed to respond to uterine distention and colorectal distension [conveyed centrally primarily via hypogastric and pelvic nerves, respectively (Berkley et al. 1993b)] after an acute lesion of the dorsal columns (Al-Chaer et al. 1996; Berkley and Hubscher 1995). However, the same lesions did not eliminate noxious inputs from the cervix and vaginal canal (Berkley and Hubscher 1995) [i.e., those organs whose DRG supply is located primarily at the lumbosacral spinal level (Berkley et al. 1993b)]. Similar to inputs from the male genitalia, noxious inputs from the cervix and vaginal canal to the caudal brain stem in female rats were eliminated with an acute bilateral DLQ lesion, although the neuronal response properties were affected by a prior lesion of the dorsal columns (Berkley and Hubscher 1995).

SKIN. The presence of cutaneous receptive fields (e.g., on both hindfeet) after a chronic DHx confirms previous work on acute-lesioned animals (reviewed by Willis and Coggeshall 1991), indicating that mechanical nociceptive information conveyed from the skin ascends within the VLQ to MRF (either directly or indirectly). The data obtained from the chronic LHx...
group of animals also confirms that the ascending pathways from skin are primarily from the contralateral side of the body, once again being consistent with early studies demonstrating a predominantly unilateral pathway composed of dorsal horn cells that project across the midline and then ascend rostrally. However, because neurons on both sides of the MRF respond to unilateral inputs from the skin (as was the case after LHx), there is both a crossed and uncrossed projection above the level of injury. The point at which the information crosses the midline (for inputs from skin and the male urogenital tract) is not yet known (Fig. 7).

**Ascending pathways: direct or indirect?**

The results of the present study indicate that central projections originating from the male genitalia, conveyed rostrally from lumbosacral dorsal horn cells to MRF, are located in the DLQ. At the present time, it is unclear whether these projections reach the MRF directly or indirectly via one or more synaptic contacts in other regions of the brain. There is little evidence in the literature that would strongly support or refute either of the two possibilities. For example, the location of direct spinoreticular projections ascending in the white matter of the rat spinal cord is unknown, with the exception of one anatomic study that used large bulbar injections of horseradish peroxidase to demonstrate the loss of retrogradely labeled L₅-L₆ cells around the central canal after a VLQ lesion at T₁₂ (Nahin et al. 1986). The evidence from that study relative to the present one in terms of consistency or the lack of is inconclusive because the lesions appeared to encroach on the ventral portion of the DLQ, the lesions were at T₁₂ (T₇/T₈ in the present study) and there is evidence for a dorsal shift of axons as they ascend rostrally (Willis and Coggeshall 1991), and the study focused on cells adjacent to the central canal, which are some of many dorsal horn neurons to project directly on neurons in the MRF (Chaouch et al. 1983; Menetrey et al. 1980) and respond to bilateral DNP (Johnson 1989). There is also evidence for the existence of several different ascending pathways within the DLQ of the rat, any of which could provide indirect projections to MRF and thus may convey both mucocutaneous and visceral information. Such pathways include a spinomesencephalic pathway (McMahon and Wall 1983; Zemlan et al. 1978) and a spinohypothalamic pathway (Kostarczyk et al. 1997).

**Chronic versus acute data**

The acute multilesion data yielded valuable information regarding immediate changes in MRF neuronal response status to a variety of peripheral inputs. Such information included ascending penile inputs in both the dorsal columns and DLQ (i.e., due to the loss of LT penile responses after dorsal column lesions and HT responses after DLQ lesions) and the need for a bilateral lesion to eliminate mucocutaneous and visceral responses. The chronic lesion data yielded valuable information regarding long-term changes in responses of MRF neurons to peripheral inputs after different kinds/extents of spinal cord injury. The results from these studies, also at the midthoracic level of spinal cord, were consistent with those after acute lesions, with one exception being that a slightly deeper DLQ lesion was necessary to eliminate MRF responses to stimulation of the DNP bilaterally. This chronic lesion depth, as depicted in Fig. 7, is likely to be more accurate because a large number of neurons were sampled post lesion (vs. 1 neuron being followed pre- and postacute lesion). A second exception is the presence of LT penile fields after chronic moderate Cx, which damages the dorsal columns (see Table 1; not depicted in Fig. 7).

**Functional implications**

The MRF is a multifunctional zone, with multiple ascending and descending inputs and outputs. In general, a nociceptive pathway from the male genitalia ascending via the DLQ to MRF may on one hand be part of a control mechanism for reflexive reactions to noxious stimuli (including modulation of ejaculatory reflexes) and/or be a relay for the ascending control of cortical...
arousal leading to perceptions of pain (Jones 1995; Peterson 1979). Similar studies are now under way in the thalamus, which contains many neurons responsive to DNP stimulation (Hubscher and Johnson 1998). A loss of thalamic responses after a bilateral DLQ lesion would be more indicative of a role in the neural mechanisms that underlie the perception of noxious stimuli to mucocutaneous and visceral tissues, such as those that occur under a variety of pathological conditions (for example, see review by Wesselmann et al. 1997).

Relative to spinal cord injury and its effects on ejaculatory responses, the presence of DNP-responsive neurons in the MRF, which contains neurons that project to and modulate the lumbosacral circuits for ejaculatory responses (Johnson and Hubscher 1998), suggests that these sensory neurons are part of a spino-bulbo-spinal circuit important for proper coordination of perineal muscle contractions. Bilateral disruption of this ascending sensory pathway in the midthoracic spinal cord likely contributes to the loss of ejaculatory ability after chronic spinal cord injury in animals (Mas et al. 1987) and humans (Seftel et al. 1991), although the chronic lesions in the present study also disrupted the descending projections from MRF to the lumbosacral spinal cord, which are also contained in the DLQ at the T8 spinal level (Hubscher and Johnson 1996b).

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REFERENCES


