Effects of brainstem and spinal cord electrical stimulation and sub-total spinal cord lesions on bladder and external urethral sphincter activity in the decerebrate cat.

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by

Brent A. Fedirchuk

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EFFECTS OF BRAINSTEM AND SPINAL CORD ELECTRICAL STIMULATION AND SUB-TOTAL SPINAL CORD LESIONS ON BLADDER AND EXTERNAL URETHRAL SPHINCTER ACTIVITY IN THE DECEREBRATE CAT

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ABSTRACT

Micturition is mediated by a central neural organization with both supraspinal and spinal components which are connected via a spinobulbospinal loop. Previous studies have shown the ability of electrical and chemical stimulation in the mesencephalon to evoke bladder responses and micturition. There remains debate as to the anatomical localization both of the supraspinal centres and spinal pathways involved. The purpose of this study was to examine the central nervous system levels at which electrical stimulation could access the central neural circuitry capable of producing micturition and to examine the spinal pathways mediating the effects utilizing sub-total spinal cord lesions.

All experiments were conducted on precollicular/postmammillary decerebrate male cats which were paralysed with Flaxidil and maintained on a respirator. The bladder was cannulated near its apex through the detrusor muscle and bladder wall to allow the infusion of saline and the monitor of bladder pressure. External anal sphincter, external urethral sphincter (EUS), and sensory branches of the pudendal nerve as well as additional hindlimb muscle nerves were dissected and made available for recording of electroneurographic activity on bipolar electrodes. A laminectomy removing the dorsum of the T9-T12 (and occasionally the C1-C2 or L5-S1) vertebrae allowed access to the spinal cord for electrical stimulation and/or sub-total lesions. Electrical stimulation of brainstem and spinal cord sites was accomplished using monopolar cathode electrodes. Current spread was assessed by moving the electrode and observing the response and minimized by keeping stimulus currents as low as possible. Brainstem stimulation sites were deemed to be the pontine micturition centre (PMC) if their stimulation produced micturition characterized by

contraction of the bladder coordinated with a selective reduction in EUS electroneurographic activity.

Coordinated micturition was evoked in this study by electrical stimulation of the PMC (which were localized to; periaqueductal gray, locus coeruleus, and parabrachial regions of the dorsolateral tegmentum in the mesencephalon) as well as from the ventrolateral aspect of the cervical and dorsolateral funiculus (DLF) of the thoracic and lumbar spinal cord. Bilateral lesions of the thoracic DLF were able to block micturition from more caudal thoracic DLF stimulation sites, but not from more rostral thoracic DLF or brainstem PMC sites. Thoracic DLF stimulation caudal to the DLF lesions was able produce a reduction in pudendal electroneurographic activity which was unrelated to the micturition cycle and which was abolished by an additional DLF lesion caudal to the stimulation site.

The PMC sites located in this study show fairly dispersed anatomical localizations and are in general agreement with the regions previously described to influence the bladder. These sites were able to exert their effects via pathways located outside the DLF of the spinal cord. Spinal cord stimulation was able to produce a similar pattern of coordinated micturition and it is proposed that this is mediated by activation of an ascending pathway travelling in the DLF. In addition, a descending pathway in the DLF was shown to have inhibitory actions on pudendal electroneurographic activity not linked to the micturition cycle. This pathway could be analogous to the DLF descending inhibitory pathways demonstrated to modulate sensory and segmental motor reflex pathways and may serve to "gate" sacral reflex function.

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ABBREVIATIONS

CNS	-	Central Nervous System
PMC	-	Pontine Micturition Centre
DLF	-	Dorsolateral Funiculus
EUS	-	External Urethral Sphincter
ENG	-	Electroneurograph
C	-	Cervical
T	-	Thoracic
L	-	Lumbar
S	-	Sacral

CONTENTS

INTRODUCTION

A: GENERAL OVERVIEW

Micturition is the process by which urine stored in the urinary bladder during continence is expelled from the body. Normally, urine accumulation takes place, distending the bladder and activating stretch receptors in the bladder wall (for review see Kuru 1965, DeGroat 1975). This distention continues until the afferent activity produced is sufficient to activate a micturition reflex. The micturition reflex can be described as the contraction of the detrusor smooth muscle of the bladder and simultaneous relaxation of the external urethral sphincter striated muscle such that there is complete expulsion of urine from the bladder through the urethra and out of the body. The volume of fluid in the bladder required to initiate the micturition reflex is referred to as the "micturition threshold" or "reflex threshold". As this reflex involves both smooth and striated musculature, it requires the coordination of activity between both autonomic and somatic elements of the nervous system respectively.

The urinary bladder is innervated by the sympathetic nervous system from the thoracic spinal cord levels via the hypogastric nerve and lumbar sympathetic chain (Langley and Anderson 1896, Stewart 1899, Downie et al. 1984). This sympathetic innervation subserves urine storage by causing relaxation of the bladder body and increased tone of the bladder neck. The sympathetic system may also inhibit transmission through parasympathetic ganglia and thus regulate the relative contributions of sympathetic and parasympathetic innervation to bladder motility (DeGroat and Saum 1971). Parasympathetic innervation originates centrally from preganglionic

neurons located in the sacral spinal cord and passing to the parasympathetic ganglia of the bladder detrusor muscle via the pelvic nerve (for reviews see; Kuru 1965, DeGroat 1975, DeGroat et al. 1981). This parasympathetic innervation causes bladder detrusor contraction and facilitates bladder emptying. The external urethral sphincter is innervated by pudendal motoneurons in the sacral spinal cord via the external urethral sphincter branch of the pudendal nerve (Langley and Anderson 1896, Rampal and Mignard 1975).

Afferent information from the pelvic viscera travels to the CNS in both the pelvic and hypogastric nerves. The afferent fibres from the external urethral sphincter and external anal sphincter travel in the pudendal nerve supplying input to the lumbosacral spinal cord. Cutaneous input from the genital and perianal region reaches the sacral spinal cord through the superficial perineal nerve and caudal cutaneous femoral nerve which travel to sacral segments (Martin et al. 1974).

Even though afferent input from bladder wall stretch receptors is a prerequisite for the production of distention-evoked voids, Barrington's description (1921, 1925) of a mesencephalic area essential for a normal micturition reflex indicated that there exists a more complex neural circuitry producing this behavior than a series of segmental spinal reflex arcs alone. Barrington (1921,1933) and others (Nathan and Smith 1958, Kuru et al. 1961, Bradley and Conway 1966, DeGroat 1975) have shown that the micturition reflex involves a spinobulbospinal loop. This neural network is then not only sensitive to segmental afferent input, but also allows for descending input from higher brain centres onto brainstem areas involved in micturition.

When supraspinal influences on micturition are not present, such as during neonatal development or after spinal cord injury, the fact that some form of the adult micturition reflex can often occur shows that spinal circuitry alone can mediate some aspects of the behavior (Denny-Brown and Robertson 1933, DeGroat et al. 1982). Neither the relative contribution of the supraspinal and spinal components to the behavior nor the details of the neural organization which comprise this system are known.

B: BRAINSTEM ORGANIZATION

Barrington (1921, 1925) demonstrated the necessity of an intact mesencephalon to elicit voiding characterized by complete bladder emptying. The initial localization of Barrington's mesencephalic bladder centre was vague, owing both to the poor resolution of the gross lesions and the limited observational evaluation of the behavior's nature. Langworthy and Kolb (1933) and later Tang (1955) utilized both total and subtotal lesions of the mesencephalon and hypothesized that there exists a discrete mesencephalic area mediating a normal reflex micturition threshold and complete bladder emptying. Complimenting the lesion studies, Kabat et al. (1936) and Wang and Ranson (1939) produced bladder contraction by discrete electrical stimulation of an area within the dorsolateral tegmentum at the level of the inferior colliculi. This area correlated well with the previously described "Barrington's point". Tang and Ruch (1956) described two additional brainstem areas outside the region Barrington described, the posterior hypothalamic micturition facilitatory area in the mamilliary region and the midbrain micturition inhibitory area in the upper tegmentum at the level of caudal superior

colliculi, which they postulated to be involved in micturition. These investigators were unable to histologically identify and attribute the observed responses to distinct nuclei within their stimulation areas, but were able to generate reproducible bladder responses by stimulation of the specific brainstem areas.

Later research expanded on the premise of functional rather than strict anatomical tegmental areas and attempted to further elucidate the anatomical correlates of these functionally defined areas as well as their connections to other areas of the central nervous system. Kuru et al. (1961) explored the connections between the mesencephalic micturition facilitatory area (Barrington's point) and other bulbar vesico-motor centres. A mesencephalic micturition facilitatory area was located in the dorsolateral tegmentum and in the adjacent tectal and tegmental regions at the level of, and slightly below, the superior colliculi. A vesico-relaxer area was described as being located slightly caudal to the micturition facilitatory area. Further, Kuru et al. (1961) described the facilitatory area as being composed of two distinct regions. One region is located at the lateral border of the central gray matter at the level of the superior colliculus and extending to dorsal and ventral neighbouring areas. This cell group was found to send fibres to bulbar vesico-relaxer and vesico-constrictor areas as well as to the pontine lateral reticular formation. The other mesencephalic region related to bladder contraction was more ventral, lateral and caudal, being located in the ventrolateral tegmentum at an intercollicular level. This cell group connects to an ipsilateral medullary vesico-constrictor centre but may also be related to the previously described more medial facilitatory area via pontine lateral

reticular formation fibres.

The interconnections of the more medial mesencephalic micturition facilitatory area with the other brainstem areas known to be important in bladder function suggests this region's importance in the coordination of activity between the various centres involved in influencing bladder contractility. The consistent bladder contraction produced by electrical stimulation of the medial mesencephalic micturition facilitatory area lead investigators to refer to it as the "pontine detrusor nucleus" (Kuru and Yamamoto 1964), an updated name for the previously described "Barrington's point". Further, Kuru and Yamamoto (1964) suggest that during micturition, the pontine detrusor nucleus transmits impulses both to bulbar vesico-constrictor and sacral vesico-motor centres bilaterally so that its output is multiplied.

The pontine detrusor nucleus may also have connections to other pontine regions which influence the external urethral sphincter and facilitate coordinated activity between detrusor and sphincter during micturition (Kuru and Iwanaga 1966). Koyama et al. (1966) described a pontine urine-storage nucleus in the ventrolateral reticular formation of the rostral pons and examined the connections of this nucleus and the facilitatory pontine detrusor nucleus. They found both ipsilateral and contralateral projections to the pontine-urine storage nuclei from the pontine detrusor nucleus. Also, the pontine-urine storage nucleus projects to its contralateral counterpart as well as the ipsilateral pontine detrusor nucleus. Stimulation of the pontine detrusor nucleus resulted in a decreased output from the pontine external urethral sphincter area and decreased electromyographic activity of the external urethral sphincter

muscle such that its relaxation was synergistic to detrusor contraction. This coordinated voiding could be disrupted by stimulation of the pontine sphincter area, a region which produces external urethral sphincter and pelvic floor activation. Mackel (1979) described an electrophysiological connection between this pontine sphincter area and pudendal sphincter motoneurons in the sacral spinal cord.

Further description of the anatomical organization of both the brainstem and descending aspects of the supraspinal control of micturition has been done using electrical stimulation to examine the brainstem effects on bladder contractility assessed with bladder pressure recordings and pelvic electromyographic activity (McMahon and Spillane 1982, Holstege et al. 1986). From these studies the pathway of the fibres from the pontine detrusor nucleus and lateral sphincter area could be traced. Holstege et al. (1986) described an "M region" as a dorsomedial area which when stimulated produced bladder contraction. Further, Holstege et al. (1986) labelled cells with ^{3}H-leucine in the pontine tegmentum and found that the M region projected to intermediomedial and intermediolateral spinal cell groups. From their description of the location of the M region, this area could overlap the previously described pontine detrusor nucleus (Kuru and Yamamoto 1964). In addition, Holstege et al. (1986) described a more ventrolateral "L region" which produced increased sphincter activity with stimulation. The L region projected fibres to the nucleus of Onuf, the somatic nucleus containing pelvic floor and external urethral sphincter motoneurons. This L region was more ventral and lateral than any brainstem area previously described as involved in micturition. It may be that the effects of the L region sites on somatic motoneurons

are not restricted to the pudendal motoneuron population and micturition, a possibility not assessed by Holstege et al. (1986).

Koyama et al. (1966) speculated that a region including part of the locus coeruleus may comprise the pontine detrusor nucleus. Sugaya et. al (1987) demonstrated that injection of carbachol into the locus coeruleus was able to induce micturition of the cat, but they did not assess whether the cell bodies that were stimulated were mediating the effects themselves or through connections to other cell groups comprising the activated descending system. The ability to evoke micturition with chemical activation of cell bodies in this region of the mesencephalon with a cholinomimetic agent is, nonetheless important. Satoh et al. (1978a) showed that while bilateral locus coeruleus lesions did not cause urinary incontinence in the rat, destruction of a region in close association to the locus coeruleus, the dorsolateral tegmentum, did. Satoh et al. (1978a) postulated that the dorsolateral tegmentum with its diverse connections to adjoining areas and other tegmental fields may be synonymous with, or at least was an important component of Barrington's point. Loewy et al. (1979), confirmed that in the rat, the pontine micturition centre corresponded to the nucleus tegmentalis laterodorsalis and anatomically showed the projection of dorsolateral tegmentum neurons to sacral preganglionic neurons. Loewy et al. (1979) speculated that the dorsolateral tegmentum neurons exert a direct excitatory effect on sacral preganglionic neurons as well as exerting indirect effects on lumbar, pelvic floor and sympathetic preganglionic neurons through lateral medullar reticular formation or solitary nucleus connections.

Another brainstem nucleus in this mesencephalic area, the Kolliker-

Fuse nucleus, has been proposed as the straining centre for defecation and micturition in dogs (Fukuda and Fukai 1986). Its location, slightly ventral to the locus coeruleus, could allow for its participation with nearby centres in influencing micturition.

C. SPINAL PATHWAYS

The location within the spinal cord of the pathways involved in the micturition reflex has been investigated since Budge (1841) first electrically stimulated the exposed spinal cord in the dog and produced a bladder contraction. Dorsal hemisection did not abolish the evoked bladder response so Budge hypothesized that the pathway responsible travelled in the ventral half of the spinal cord. Contrary to Budge (1841), Mosso and Pellacani (1882) found dorsal hemisection abolished the bladder response while ventral hemisection did not. While it appeared that this data supported a more dorsal pathway mediating bladder responses, Mosso and Pellacani (1882) used noxious forepaw pressure to evoke bladder responses, so they may have been activating a very different behaviorial system as compared to Budge (1841) in their attempts to produce voiding. Stewart (1899) stimulated subdivided lateral funiculi and localized the descending pathway for bladder contractility to be in the most dorsal aspect of the lateral column in the cat.

Barrington (1933) examined the effects of subtotal spinal cord lesions at the lower thoracic or upper lumbar levels on the cat's ability to control micturition. He found that discrete bilateral lesions involving the dorsal half of the lateral columns abolished normal micturition. Massive spinal cord lesions sparing only these dorsolateral areas resulted in no permanent micturition abnormalities. Despite Barrington's

observations, the location within the spinal cord of the descending pathway for bladder control remained in debate. Wang and Ranson (1939) reported that from their studies of partial spinal cord lesion at the cervical level, bladder impulses descend in the ventrolateral column of the spinal cord. More recently, McMahon and Morrison (1982c) were able to evoke efferent discharge in vesicular parasympathetic preganglionic neurons by electrical stimulation of the ventrolateral quadrant at cervical segments.

The location of the pathway for micturition within the spinal cord was also examined in humans. This literature also contains contradicting results. McMicheal (1945) gave evidence for a pathway in the dorsolateral funiculus in the voluntary control and perception of bladder sensation. Nathan and Smith (1958) examined bladder characteristics of patients in whom surgical spinal lesions had been made at cervical or thoracic levels in order to alleviate intractable pain. These authors found the most pronounced effects on the bladder were obtained from lesions made in ventrolateral white matter. The discrepancy in results might be attributed to a change in course of the fibres of interest between cervical and thoracic levels. Barrington's observations in the cat were made with lesions at the thoracic spinal level, while some of the studies indicating a more ventral passage examined cervical segments. The possibility exists that the fibres important in micturition have different funicular trajectories at different levels of the spinal cord, travelling in ventral or ventral/lateral funiculus at cervical segments and DLF at lower spinal segments. It is also possible that some of the contradiction in results is due to inability to assess whether the subtotal lesion disrupted the

ascending or descending limb of the spinobulbospinal loop, or both.

D. ASCENDING PATHWAYS INVOLVED IN MICTURITION

Further characterization of the ascending limb of the spinobulbospinal micturition reflex is an important step in the process of understanding of the neural organization of bladder function. Presently, however, relatively little is known about the pathway(s). Yamamato et al. (1956) described sensory fibres in the posterior funiculus of the spinal cord which responded to passive bladder distention. Kuru (1965) postulated this dorsal column system (the "pelvic sensory vagus") to be a component of the ascending portion of the spinobulbospinal loop. Kuru (1965) also described a "sacral-bulbar" tract ascending in the lateral funiculus. Kamikawa et al. (1962) described the response of ascending fibres in the dorsal portion of the lateral funiculus at L2-L4 levels to bladder distention, presumed to be sacral-bulbar ascending fibres. More recently, McMahon and Morrison (1982a) described ascending fibres in the lateral funiculus at thoracic levels and ventrolateral funiculus at cervical levels which responded to both electrical and physiological visceral stimulation.

The ability of DLF lesions to disrupt normal micturition or DLF sparing to allow normal micturition (as mentioned earlier) implicates the DLF as having a significant role in mediating micturition. Lamina 1 cells have been shown to have ascending projections which travel through the DLF both in rat, cat and monkey (Yamamoto et al. 1956, Kumazawa et al.1975, McMahon and Wall 1983, Jones et al. 1985, Apkarian et al. 1985). More importantly, these ascending lamina 1 neurons have been shown to have projections to mesencephalic nuclei both in rat (McMahon and Wall 1985)

and cat (Hylden et al. 1985). Though function was not assessed, the possibility that these fibres are involved in ascending sensory processing which may include visceral afferent pathways remains to be determined.

E. DESCENDING PATHWAYS INVOLVED IN MICTURITION

Rigorous exploration of the brainstem for micturition specific centres (ie. the pontine micturition centre) using more sensitive anatomical techniques has allowed for studies of spinal trajectories of micturition specific pathways. Holstege et al. (1969) used brainstem injections of ^{3}H leucine into the nucleus subcoeruleus and neighboring pontine tegmentum and documented direct projections to somatic motoneurons as well as bladder preganglionic and pudendal motoneuron populations. The spinal cord trajectory of these fibres was described as including both the ventral and lateral funiculi. More specifically, Holstege et al. (1969) describe the fibre course as being in the ventral funiculus of cervical and thoracic spinal cord and in the DLF of more caudal levels. Direct anatomical projections from brainstem areas important in micturition have also been demonstrated by Kuru and Yamamoto (1964) in cat and by Satoh et al. (1978b) and Loewy et al. (1979) in rat. While Kuru and Yamamoto (1964) and Satoh et al. (1978b) did not fully examine the spinal cord trajectory of the fibres, Loewy et al. (1979) showed the descending fibres to be travelling primarily in the ipsilateral lateral funiculus. This trajectory is supported by the description of descending fibres in cats responding to electrical and physiological visceral stimulation in the lateral funiculus of thoracic levels and ventrolateral funiculus at cervical levels (McMahon and Morrison 1982a).

F. SUMMARY

Since Barrington's description of a pontine micturition centre (Barrington 1921, 1925), the importance of this area as well as its relays to both supraspinal and spinal centres involved in micturition has been investigated. Initially there was the documentation of several mesencephalic facilitatory and inhibitory areas, but with experimental refinements, the pontine detrusor nucleus and the pontine sphincter area emerged as the most important centres. Unfortunately, because many of the previous studies have largely focused only on bladder contractility changes or only assessed the responses directly related to micturition, the degree of specificity of many of the brainstem-evoked effects to micturition is uncertain. Also, the mechanism of control of the external urethral sphincter by supraspinal or spinal segmental systems is an aspect of micturition often overlooked by previous studies. Anatomically, both the brainstem centres and ascending and descending spinal pathways involved in micturition have resisted definitive anatomical classification. This results in many regions considered as physiologically defined areas which likely encompass several anatomical systems of cell bodies and fibres of passage. In addition, the relative importance of supraspinal centres and spinal segmental systems in producing micturition remains unknown. It is the goal of this thesis research to examine the pontine and spinal locations from which micturition characterized by appropriate coordination of bladder pressure and pudendal electroneurographic activity during actual voiding can be evoked with electrical stimulation. By using acute sub-total spinal cord lesions, statements about the ascending or descending nature of the spinal pathways

involved can be made. The documentation of the effects and degree of specificity to micturition of supraspinal systems being activated will be a step in the process of determining the relative importance of supraspinal pathways and sacral spinal reflex activity both to micturition and pudendal motor control. This may clarify understanding of previous micturition literature and will extend our understanding of the mechanisms of control of the pudendal motor systems important in micturition.

MATERIALS AND METHODS

SURGICAL TECHNIQUES

The data for this study was obtained from 35 male cats ranging in weight from 2.2 to 4.3 Kg. The animals were fasted for a 12-18 hour period before surgery. During surgery, animals were anaesthetized with a nitrous oxide, oxygen and vaporized halothane mixture (NO_2 flow 700 ml/min.; O_2 flow 300 ml/min.). Initial induction was done with 5% halothane in a customized transport box, while maintenance levels (1.0-2.5% halothane) were delivered by mask and later by a tracheal tube inserted after tracheotomy. The animal was kept warm by a heating pad fastened under the surgery table and with occasional use of a 250 Watt brooder lamp.

A cannula filled with 0.9% saline was used to catheterize the left carotid artery. The catheter was then attached to a Sensym pressure transducer which was connected to an analogue voltmeter calibrated for millimetres of mercury pressure and provided a monitor for blood pressure throughout the experiment. A peristaltic infusion pump was attached to this arterial line via a T-connector and a bicarbonate/glucose buffer (0.84 g $NaHCO_3$ and 5 g glucose in 100 ml water) infused at 4-5 ml/hour to keep the line free of blood. A tracheotomy was performed and the anaesthetic administered through a tube inserted into the trachea. A loose loop was placed around the right carotid artery to allow its temporary occlusion during the decerebration. The right external jugular vein was isolated and cannulated and used for the intravenous administration of drugs and fluids for the remainder of the experiment. A second venous line was placed in the left forelimb cephalic vein. A 2 mg dose of dexamethasone was given at this time and an equal dose was given later in

the experiment in an effort to reduce tissue edema.

The urinary bladder was exposed via a midline abdominal incision through the skin and abdominal wall. During exposure the bladder was moistened with 37 ^{o}C saline and rinsed any time urine contacted the outer bladder wall. The bladder was cannulated by inserting a size 5 french feeding tube through the detrusor muscle of the bladder fundus. After suturing around the cannula with 3-0 silk and tying it in place, the ability to infuse and withdraw warm saline through the cannula was tested and the integrity of the cannula/detrusor junction assessed. The abdominal wall was sutured closed, taking care to retain the position of both the cannula and the bladder itself. The abdominal incision was fastened closed with wound clips. The bladder catheter would later be attached via a T-connector to a syringe pump for the infusion of warm saline (rate; 2ml/min.) and to a pressure transducer with output displayed in millimetres of mercury pressure on a voltmeter.

The animal was then turned over to allow access to its dorsal aspect for dissection of the right side peripheral nerves. A longitudinal incision extending over the gluteus muscles lateral to the sacral plate and following the dorsal aspect of the hindlimb was used to expose the following nerves on the right side of the cat; the external urethral sphincter branch, external anal sphincter branch and the sensory branch of the pudendal nerve. In addition the following right hindlimb muscle nerves were exposed; semimembranosus/anterior biceps nerves, posterior biceps/semitendinosus nerves. In several experiments the medial gastrocnemius nerve, lateral gastrocnemius nerve, and common tibial nerve were also dissected. The nerves were carefully dissected free from

surrounding tissue with glass rods, cut and ligated, so as to allow their later placement on bipolar hook electrodes. Once the nerve dissection was complete, the nerves were covered with cotton moistened with saline and the wound temporarily closed with removable clips.

The spinal cord was exposed via a laminectomy removing the thoracic vertebrae T9-T12 and sometimes lumbar vertebrae L5-S1 or cervical vertebrae C1-C2. Bleeding was controlled by applying bone wax to the cut surfaces of the vertebrae and cautery to the surrounding muscles as required. When the laminectomy was complete, cotton moistened with saline was placed on the exposed dura matter. Clamps for later mechanical support of the animal were attached to the exposed lateral surface of an upper lumbar vertebra and the spinous processes of upper thoracic vertebrae.

The animal was placed in a frame supported by the vertebral clamps as well as by steel pins placed under the iliac crest. The animal's head was securely placed in a stereotaxic apparatus and the skull exposed through a midline dorsal incision extending from the interocular level to the base of the skull. The temporalis muscle was released from its origin on the skull and retracted to allow for the craniotomy. Sufficient parietal cranium was removed caudal to the coronal suture to allow the retraction and removal of the cerebral cortices and visualization of the dorsal tectum. Bleeding was reduced during the procedure by applying tension on the loop around the right carotid artery to temporarily occlude the vessel. The decerebration was then completed by sectioning the mesencephalon with a spatula at a precollicular postmammillary level at which time the anaesthetic was discontinued. The base of the skull was packed with Avitene (microfibrillar collagen hemostat) and Surgicel

(oxidized regenerated cellulose absorbable hemostat) to control bleeding and the cranium filled with agar until brainstem stimulation was to be done.

The wound from the peripheral nerve dissection and laminectomy were then opened and the skin retracted to form a holding pool for 37 °C mineral oil. The nerves were placed on bipolar hook electrodes which were introduced into the mineral oil pool. The temperature of the mineral oil and the animal was maintained by the use of thermistor regulated heating lamps with thermistors placed in a mineral pool as well as in the animal's stomach via the esophagus. The animal was then paralysed with 2-3 mg/Kg Flaxidil (gallamine triethiodide) and maintained on a respirator adjusted to maintain expired CO_2 at 3-5% (ventilation: rate; 20-35 per min. flow; 1.0-1.8 L/min.) for the duration of the experiment. Subsequent doses of Flaxidil (1-2 mg/Kg/hour) and 6% dextran were administered intravenously during the experiment as required.

ELECTRICAL STIMULATION TECHNIQUES

Brainstem stimulation was performed with monopolar cathode steel electrodes manipulated with a Narashigi stereotaxic device. Stimulation parameters were 20-250 uA, 20, 50 or 100 Hz, .2 or .5 msec square wave pulses. Spinal cord stimulation was accomplished with monopolar tungsten cathode electrodes. These were etched and varnished such that 60-200 μm of tip was exposed. Their impedance ranged from 100K-1.5M ohms. Stimulus parameters were 20-500 uA, 100 Hz, .2 msec square wave pulses. The discreteness of both brainstem and spinal cord stimulation sites was assessed by observing the loss of effect with movement of the electrode

<0.5 mm. Both brainstem and spinal cord stimulation sites were electrolytically lesioned at the end of the experiment (2.2 mA, 1000 Hz, .5 msec square pulses) and the brainstem and/or spinal cord placed in a buffered sucrose formalin fixative (10% formaldehyde, 20% sucrose, phosphate buffered). Once adequately fixed, ~48 hours post experiment, 60 uM frozen sections were cut on a cryostat, mounted on slides, and stained with 0.5% cresyl violet. The sections with the electrolytic lesions were then examined microscopically and drawn using either a camera lucida or a photographic enlarger.

SPINAL CORD LESION TECHNIQUES

The spinal cord was cooled for at least 10 minutes with crushed, frozen 0.9% saline prior to any lesion. All lesions were made at thoracic levels with fine scissors, jeweller's forceps and a dissecting microscope and verified at the time of the lesion with a fine glass rod. The cold saline was then suctioned out of the mineral oil pool and the spinal cord was warmed with fresh 37 °C mineral oil and the careful use of a thermistor regulated heat lamp. This procedure was repeated for subsequent lesions in the same experiment. Histological verification of subtotal lesions (as described for electrolytic lesions) was performed when there was doubt as to the extent of the lesion.

DATA ACQUISITION AND ANALYSIS TECHNIQUES

Electroneurograms were amplified, filtered (high pass of 30-100 Hz; low pass of 1 or 3 KHz) and then digitized (1 or 2 KHz sampling rate) using a Masscomp 5400 computer. The bladder pressure and stimulus marker

records were also digitized (333-500 Hz and 1 or 2 KHz respectively). Data acquisition and subsequent display and analysis was done using an Info West software package.

EXPERIMENTAL PROTOCOLS

The ability of a brainstem stimulation site to evoke coordinated micturition with a volume in the bladder less than the micturition threshold (<60% micturition threshold) and with the lowest currents was used to define the site as the "Pontine Micturition Centre" (PMC) within that animal. Care was taken to omit regions which produced more global somatic or autonomic effects rather than micturition alone. Once a PMC was found, responses from stimulation of that site could be compared to responses evoked from stimulation of other levels of the CNS (as well as to test the integrity of the brainstem and descending micturition pathways during the course of the experiment).

After spinal cord sites capable of producing responses similar to those of the PMC had been located and their evoked responses documented, subtotal lesions of the spinal cord were used in an attempt to determine the spinal location of the pathway that was being activated both by the spinal cord and PMC stimulation. Once the effects of a given spinal cord subtotal lesion were documented, additional lesions were often made and their effects documented to determine which spinal areas were mediating the effects evoked by the stimulus. In order to maximize the information obtained during an experiment, subtotal spinal cord lesions were often made between two thoracic spinal cord stimulation electrodes which were

displaced rostro/caudally. In this way the effectiveness of the subtotal lesion of ablating the response from both more rostral and more caudal spinal cord stimulation sites could be assessed.

RESULTS

A. STIMULUS EVOKED VOIDS

DISTENTION EVOKED VOIDS

Distention evoked voids were elicited by infusion of warm saline into the bladder at a rate of 2 ml/minute. The volume of saline required to elicit a void, the micturition threshold, ranged from 10-46 ml in the decerebrate cat. Voids typically had a duration of 10-35 seconds and a peak pressure of 20-45 mmHg (mean ~25 mmHg) with no or very little residual volume in the bladder following the void. A typical distention-evoked void is shown in Figure 1. These distention-evoked voids were evoked repeatedly in several experiments to allow assessment of the capability of the system to produce coordinated micturition as well as to determine which subtotal spinal cord lesions disrupted the ascending arm of the spinobulbospinal loop mediating the micturition.

BRAINSTEM ELECTRICAL STIMULATION EVOKED VOIDS

In 25 cats voids were evoked by electrical stimulation of the brainstem in an area of the mesencephalon at the level of, or slightly caudal to, the inferior colliculus. The sites stimulated were within the area of P 1 to 3, L 1 to 5, H 2 to -3 (Berman 1968). Within each animal, moving the stimulating electrode 0.5 mm in any direction altered the response. As shown in Figure 2, collectively the sites were not limited to a distinct, identifiable nucleus but rather, were dispersed in close proximity to the locus coeruleus, periaqueductal gray matter, the parabrachial nucleus and the cuneiform nucleus. All sites produced a

change in bladder pressure and EUS afferent activity at a 1-2 second latency from stimulus onset. The voiding had similar bladder pressure characteristics (10-35 second duration, 20-45 mmHg peak pressure) and the same coordination of EUS efferent activity as the distention-evoked control voids in the same cat (see Figure 1). The presence, if any, of residual volume in the bladder after the void was also comparable to the distention-evoked voids in any one cat. Other brainstem sites producing voiding were found but deemed to be suboptimal because they produced a reduction of EUS efferent activity that was stimulus-locked rather than coordinated to the bladder response. The ability of the PMC to evoke micturition was tested at regular intervals throughout the experiment to ensure that the brainstem circuitry remained viable. PMC-evoked micturition often remained consistent for several hours.

Lateral hemisection of the thoracic spinal cord (n=9 cats) did not disrupt voiding evoked from either the ipsilateral or contralateral PMC site (Figure 3 B,E). Continuation of the spinal cord lesion to include the dorsal aspect of the contralateral spinal cord (Figure 3 C,F) diminished the bladder contraction evoked from both PMC sites such that voiding was not induced. The rebound increase in EUS efferent activity following the bladder contraction (as seen in Figure 3 A,B,D,E) was retained after the lesions and followed the diminished bladder response evoked from the PMC site ipsilateral to the electroneurograms and remaining intact ventral quadrant (Figure 3 F).

SPINAL CORD ELECTRICAL STIMULATION EVOKED VOIDS

Figure 4 illustrates the coordinated voids produced by PMC

stimulation as well as those evoked by electrical stimulation of the superficial aspect of the dorsolateral funiculus (DLF) at both the thoracic (n=19 cats) and lower lumbar (n=2 cats) spinal cord levels. The DLF-evoked voids had the same 1-2 second latency from stimulus onset to bladder response, 10-35 second duration, 20-45 mmHg peak pressure and residual volumes as the PMC and distention evoked voids. To ensure that the voiding produced from the thoracic DLF was not mediated by a segmental reflex, the mixed roots one segment rostral and caudal to the stimulation site were ligated and/or cut bilaterally (n=3 cats) with no effect on the DLF-evoked voiding. In an additional 2 cats cutting the L7-S3 dorsal roots bilaterally did not affect the DLF evoked voiding. The DLF stimulation sites were just lateral to the dorsal root entry zone and within 100 uM under the surface of the spinal cord. Electrolytic lesions of two thoracic DLF spinal cord stimulation sites are shown in Figure 5. Tracking with electrical stimulation throughout the thoracic spinal cord (n=4 cats) revealed that only the superficial DLF site was capable of producing coordinated micturition at this level. Small bladder contractions were observed during stimulation of thoracic ventrolateral white matter, but these contractions were weak and insufficient to produce voiding and no decrease in the EUS efferent activity was observed in these cases. Other stimulation sites, which were ventral or ventrolateral to the micturition-producing superficial DLF site, produced solely somatic responses which were seen in the pudendal and hindlimb electroneurograms as increased efferent firing.

At the cervical level, coordinated micturition was elicited by bipolar electrical stimulation (n=3 cats) and discrete monopolar

stimulation (n=1 cat) of the ventrolateral spinal cord (see Figure 6). Stimulation of the DLF at this level did not produce micturition. Electrical stimulation at the cervical level was not pursued in later experiments because of the volatility of blood pressure responses associated with electrical stimulation and the difficulties associated with subtotal or total lesions of the spinal cord at this level. The spinal cord stimulation sites often produced blood pressure responses in addition to micturition, though the magnitude of these blood pressure responses was smaller at the micturition producing sites than those of immediately adjacent spinal cord areas.

B. EFFECTS OF SPINAL CORD LESIONS

DORSAL COLUMN LESIONS

As shown in Figure 5D, bilateral lesions of the dorsal columns at a thoracic level did not disrupt either the bladder contraction or the EUS ENG coordination of voids evoked from thoracic DLF spinal cord stimulation sites (n=9 cats) which were either rostral or caudal to the lesions. Distention and PMC evoked voiding also remained unaffected after bilateral dorsal column lesion.

DORSOLATERAL FUNICULUS LESIONS

In 7 cats, bilateral lesion of the DLF as well as the dorsal columns at a thoracic level did not affect coordinated voiding evoked from the PMC site or the thoracic DLF spinal cord stimulation site rostral to the lesion (see Figure 7 A,B). Thoracic DLF stimulation caudal to the dorsal columns/DLF lesion (Figure 7C) could no longer elicit voiding nor could

distention-evoked voids be elicited. Despite the loss of ability to produce a bladder contraction, stimulation of the more caudal spinal cord site was able to repeatably reduce EUS and anal ENG activity (n=4 cats). As shown in Figure 8, this reduction of pudendal ENG activity was abolished by a second bilateral dorsal column/DLF lesion caudal to the stimulation site. Caudal lesions limited to the ipsilateral DLF were also able to abolish the reduction in pudendal ENG activity.

FIGURE 1 Reflex and PMC-evoked voids in the decerebrate cat.

Panel A shows a distention-evoked void (micturition threshold 17cc) with the coordinated reduction in external urethral sphincter (EUS) electroneurographic activity during the bladder contraction. Panel B shows the bladder pressure and EUS electroneurographic response to brainstem PMC stimulation (200 uA, 100 Hz, .5 ms) with a volume of 8 cc in the bladder (<50 % micturition threshold). The first stimulation period (indicated by thickened bar below EUS ENG) produced a void which emptied the bladder. Subsequent electrical stimulation of the site with the bladder empty caused a small deflection in the bladder pressure and a short decrease in activity in the external urethral sphincter electroneurographic activity but no urine was expelled.

FIGURE 1

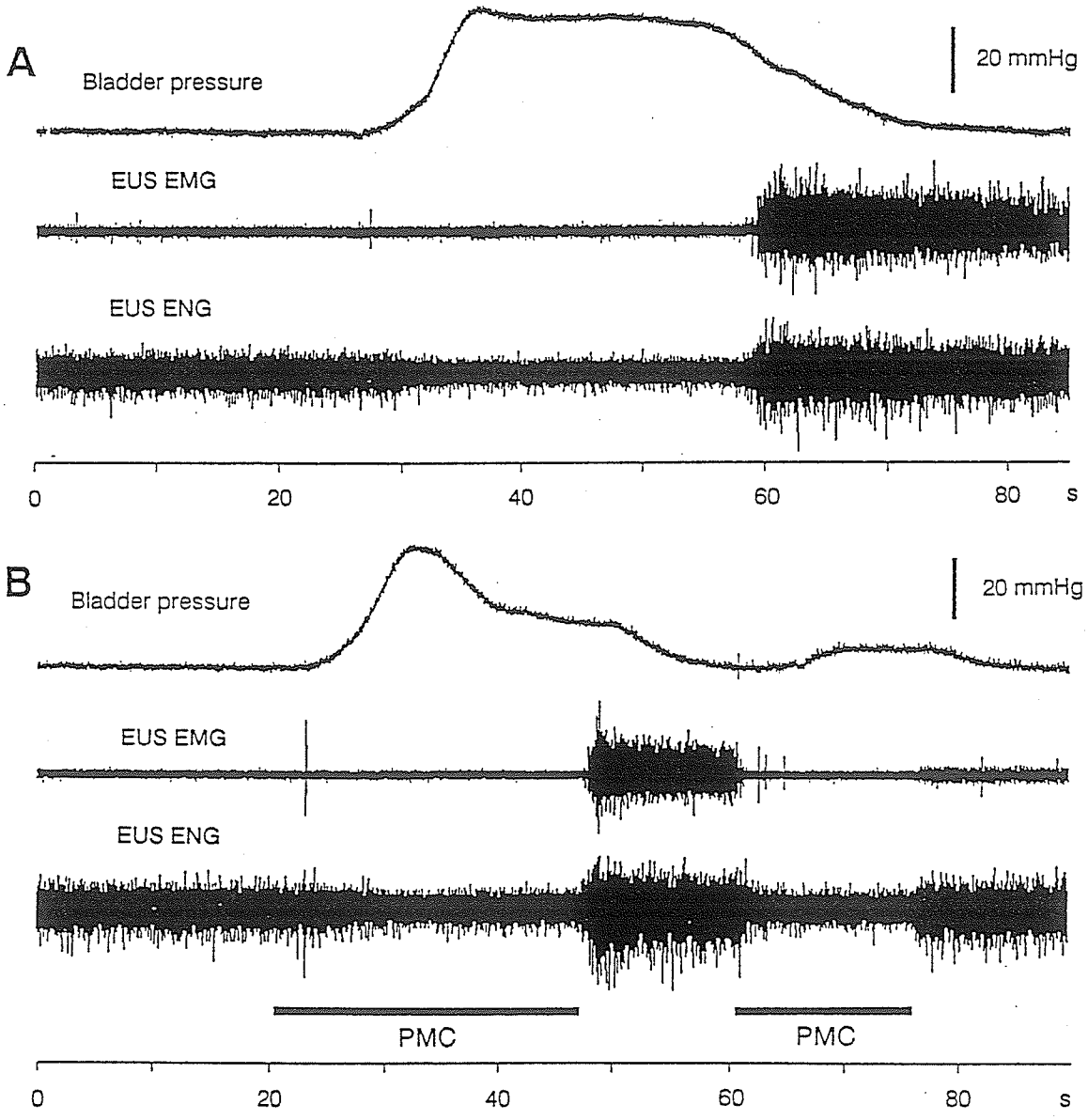

A
Bladder pressure
20 mmHg
EUS EMG
EUS ENG
0
20
40
60
80
s
B
Bladder pressure
20 mmHg
EUS EMG
EUS ENG
PMC
PMC
0
20
40
60
80
s

FIGURE 2

Figure 2 represents a diagrammatic representation of histologically verified brainstem Pontine Micturition Centre (PMC) in 21 cats. All sites evoked repeatable voiding with a coordinated reduction in external urethral sphincter (EUS) electroneurographic activity. Stimulus strengths ranged from 20-200 uA, square pulse duration was .2 or .5 milliseconds delivered at a frequency of 20, 50 or 100 Hz. Note that these sites ranged from P2-P3 (Berman coordinates) but have been drawn on one section (P2); their position is relative to each other, and shown as accurately as possible on this section.

FIGURE 2

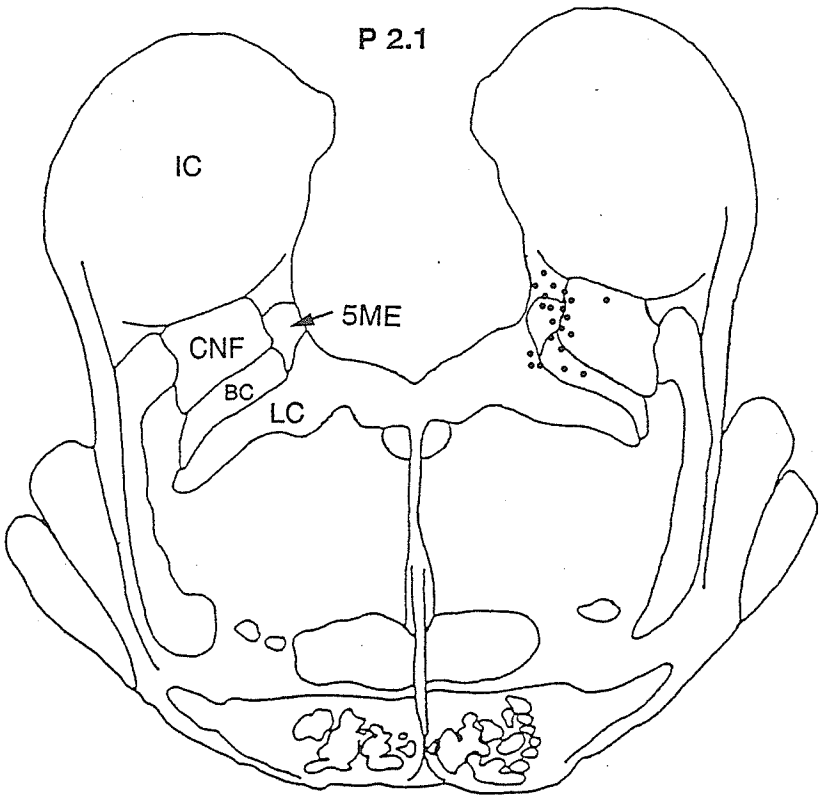


FIGURE 3

A,D; Stimulation of the left or right PMC sites (100 uA, 100 Hz, .2ms) produced coordinated micturition.

B,E; Voiding was elicited from both PMC sites after left T9 hemisection (left PMC 150 uA; right PMC 100 uA) indicating the ability of left PMC stimulation to exert influences descending in the right half of the spinal cord.

C,F; An additional lesion of the right DLF at T10 reduced the bladder response evoked from both sides (left PMC stimulation at 150, 200. 250 uA; right PMC stimulation at 200 uA), The EUS neurographic activity showed an increase in efferent discharge following the stimulation of the PMC site ipsilateral to the electroneuorograms (F).

FIGURE 3

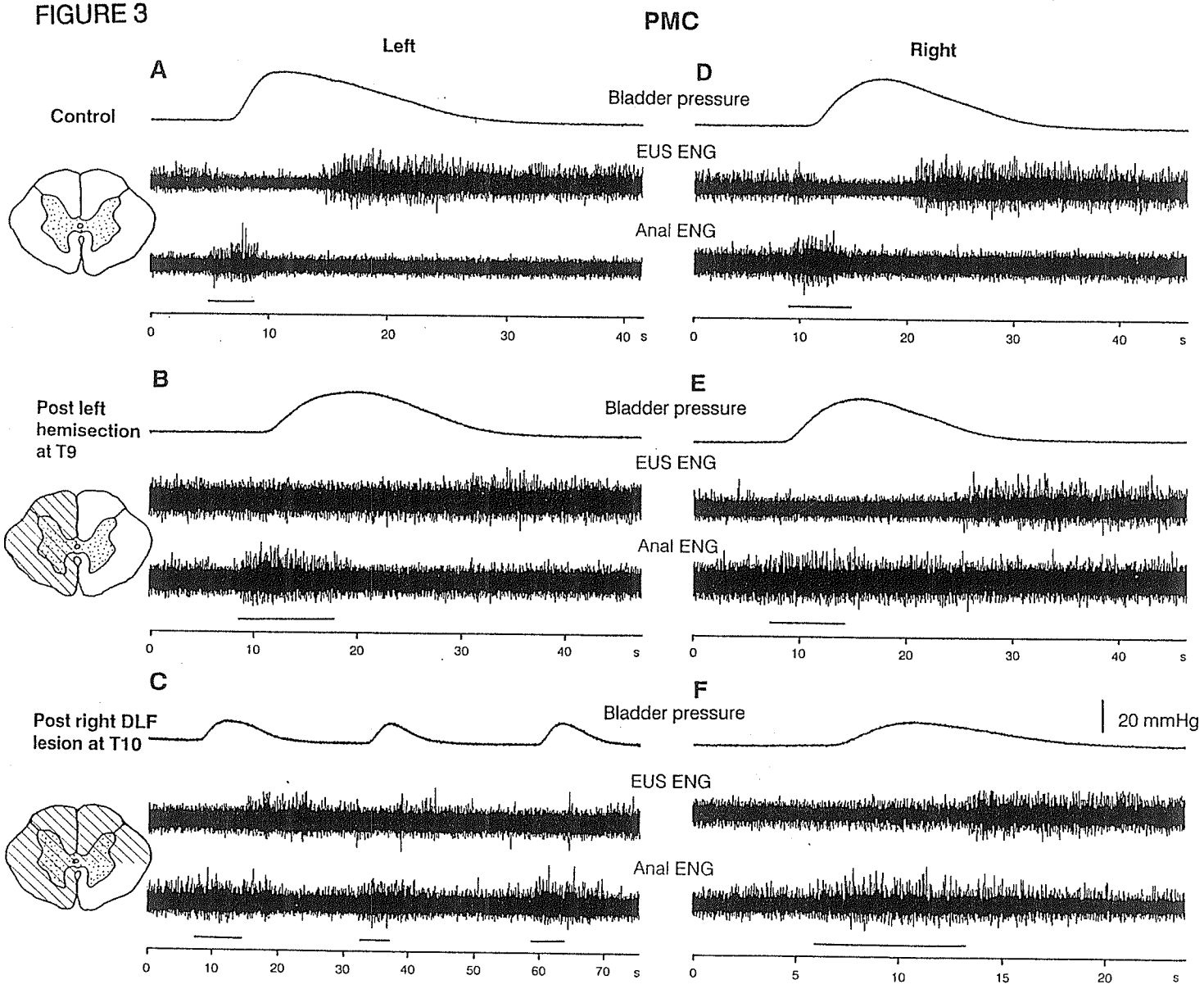

PMC
Left
Right
A
D
Control
Bladder pressure
EUS ENG
Anal ENG
0
10
20
30
40
s
B
E
Post left
hemisection
at T9
Bladder pressure
EUS ENG
Anal ENG
0
10
20
30
40
s
C
F
Post right DLF
lesion at T10
Bladder pressure
20 mmHg
EUS ENG
Anal ENG
0
10
20
30
40
50
60
70
s
0
5
10
15
20
s

FIGURE 4 PMC and spinal cord evoked micturition.

Panel A illustrates the bladder pressure increase and coordinated decrease in external urethral sphincter (EUS) electroneurographic activity evoked by electrical stimulation of the PMC (100 uA, 50 Hz .2ms), as well as from the dorsolateral funiculus of the thoracic (panel B) and lumbar (panel C) spinal cord (50 uA, 50 Hz, .2 ms).

FIGURE 4

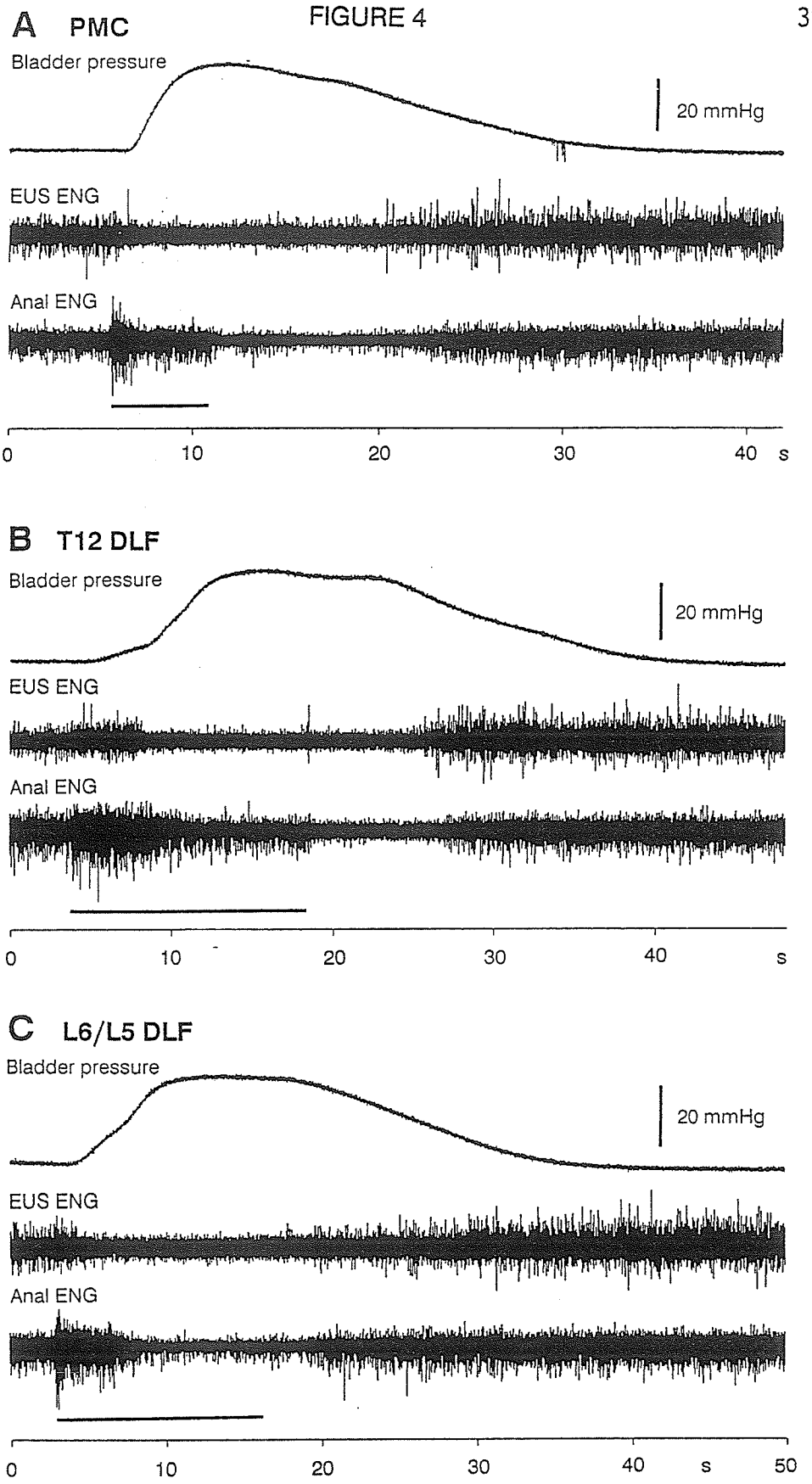

A PMC
Bladder pressure
20 mmHg
EUS ENG
Anal ENG
0
10
20
30
40
s
B T12 DLF
Bladder pressure
20 mmHg
EUS ENG
Anal ENG
0
10
20
30
40
s
C L6/L5 DLF
Bladder pressure
20 mmHg
EUS ENG
Anal ENG
0
10
20
30
40
s
50

FIGURE 5 Anatomical localization of PMC and thoracic spinal cord sites, and their evoked responses before and after lesion of the dorsal columns.

Panel A shows the PMC site which produced the coordinated voiding illustrated (150 μA). Panels B and C show the evoked responses (50 and 100 μA respectively) and the electrolytic lesion made at thoracic DLF stimulation sites. Panel D illustrates the evoked response produced by stimulation rostral and caudal to a bilateral dorsal column lesion at T11 (lesion histology shown).

FIGURE 5

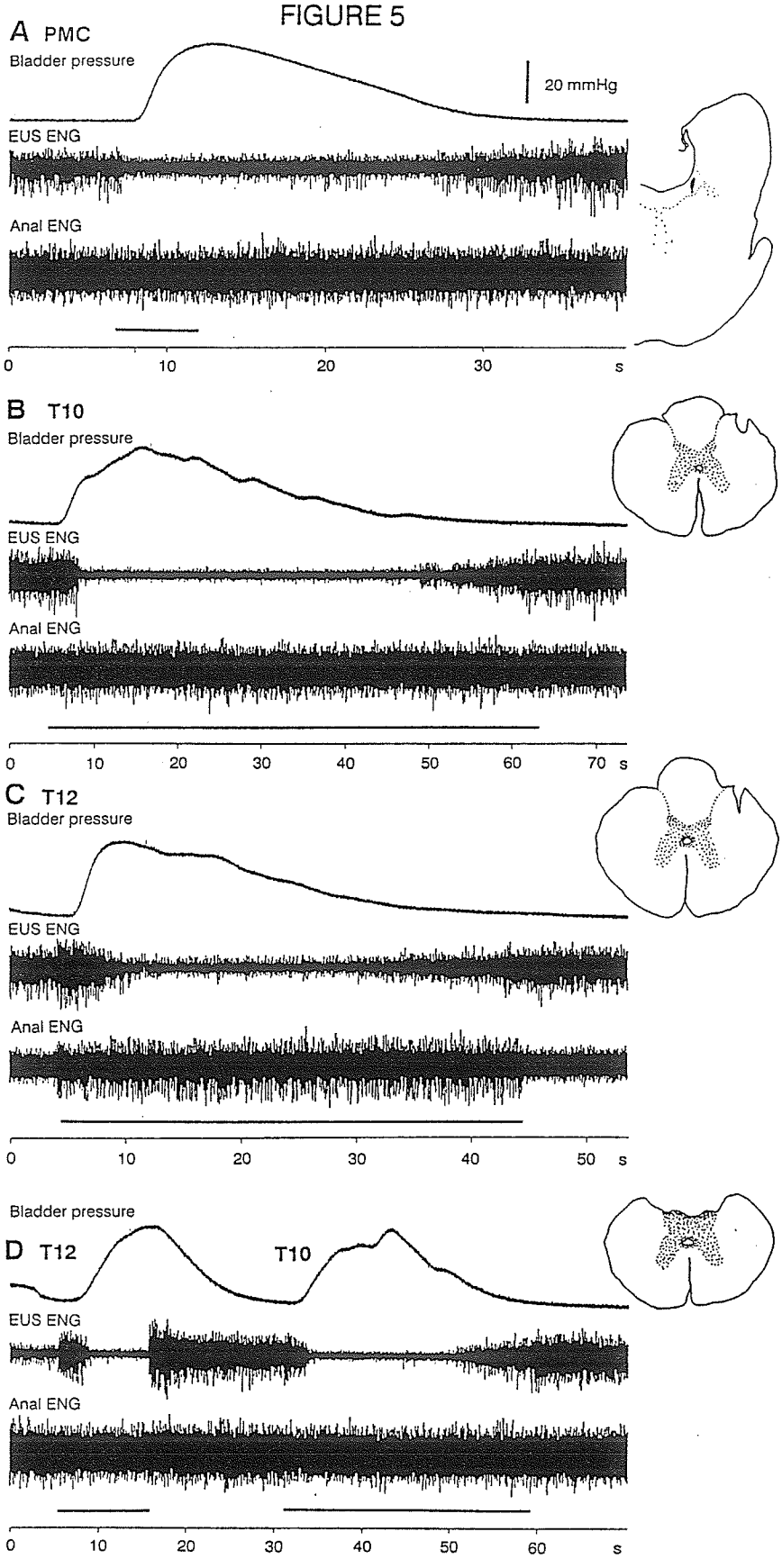

A PMC
Bladder pressure
20 mmHg
EUS ENG
Anal ENG
0
10
20
30
s
B T10
Bladder pressure
EUS ENG
Anal ENG
0
10
20
30
40
50
60
70
s
C T12
Bladder pressure
EUS ENG
Anal ENG
0
10
20
30
40
50
s
Bladder pressure
D T12
T10
EUS ENG
Anal ENG
0
10
20
30
40
50
60
s

FIGURE 6 Cervical spinal cord evoked micturition.

Stimulation of the ventrolateral cervical (C2) spinal cord (bipolar stimulation 500 uA, 100 Hz, .5 ms) evoked voiding with a coordinated reduction of external urethral sphincter (EUS) electroneurographic activity. A stimulus-evoked increase in the anal sphincter pudendal activity was also observed. Stimulation of the dorsal cervical white matter failed to evoke coordinated voiding.

FIGURE 6

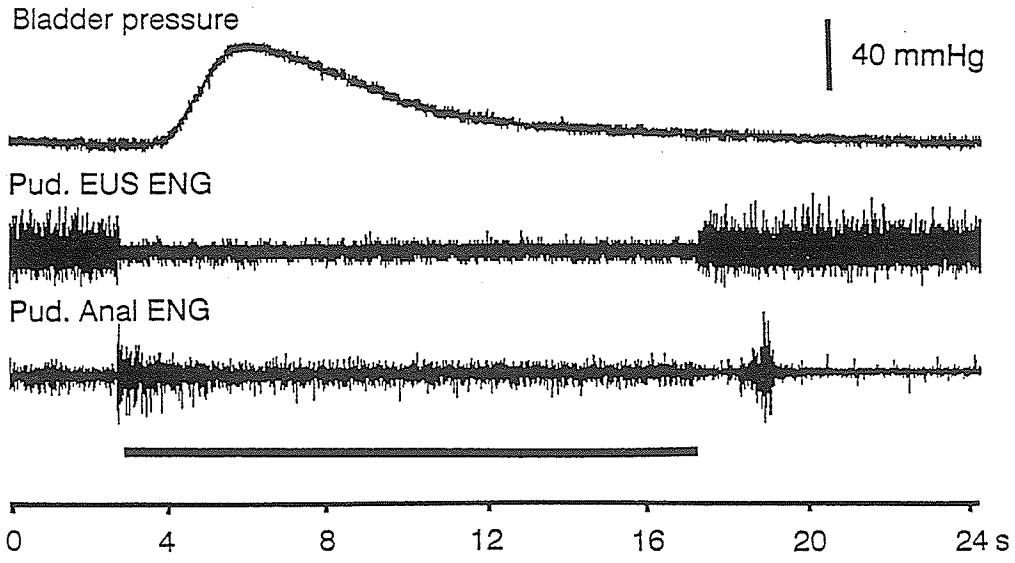

Bladder pressure
40 mmHg
Pud. EUS ENG
Pud. Anal ENG
0
4
8
12
16
20
24 s

FIGURE 7 Effects of bilateral dorsal column and dorsolateral funiculi lesions of evoked micturition.

Left panels (A-C) show voids evoked from the PMC (100 uA) and the thoracic spinal cord DLF at T9 and T11 (75 uA) prior to the spinal cord lesion. Panels D-F illustrate the responses seen following the lesion (shown in E) at the T10 level. Micturition was evoked with PMC stimulation and T9 DLF stimulation. Electrical stimulation caudal to the lesion at T11 did not produce a bladder response although a decrease in the EUS efferent activity was observed.

FIGURE 7

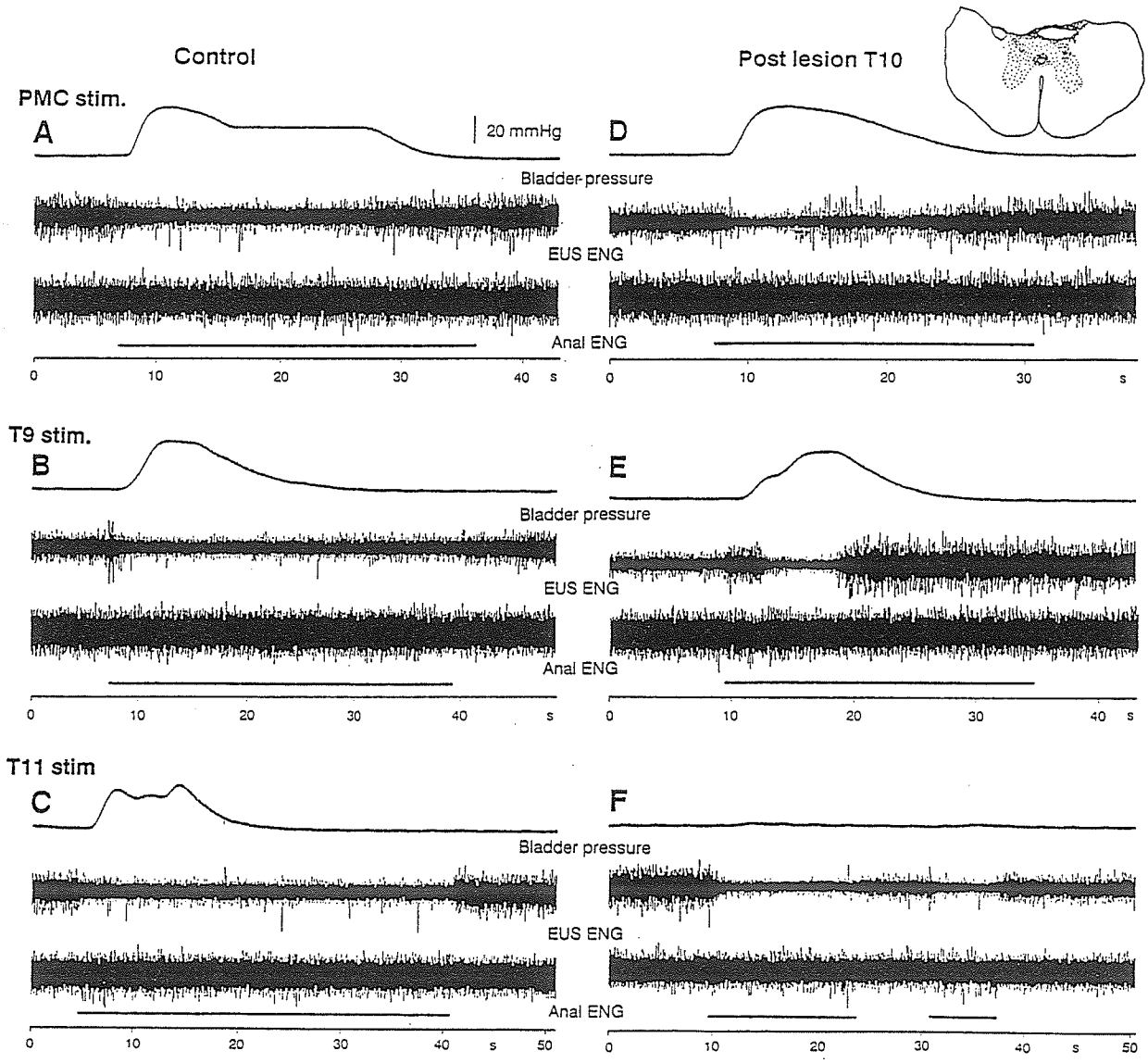
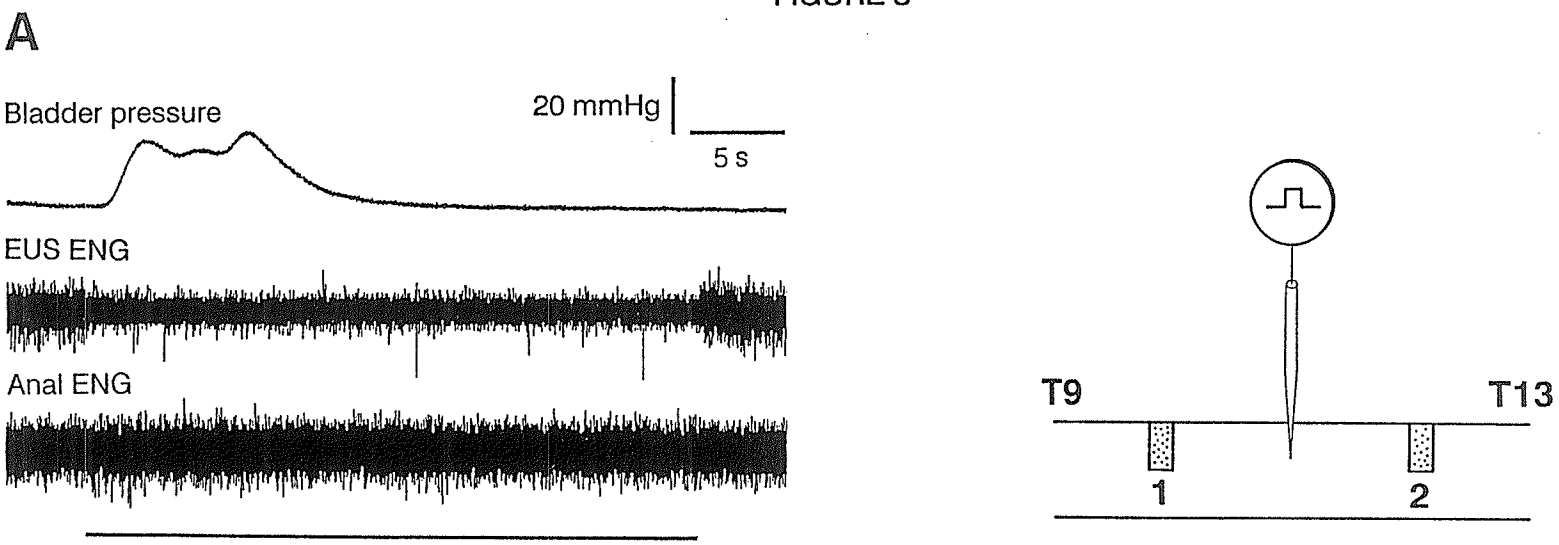

Control
Post lesion T10
PMC stim.
A
20 mmHg
D
Bladder pressure
EUS ENG
Anal ENG
0
10
20
30
40
s
T9 stim.
B
E
C
F
T11 stim
50

FIGURE 8 **Effects of caudal cord lesions on DLF produced reduction in EUS efferent activity.**

Panel A shows a coordinated void evoked by stimulation of the T11 DLF (75 μA). This same example is in Figure 9. After a bilateral dorsal column and DLF lesion (panel B), the T11 DLF stimulation (100 then 200 μA) could no longer produce a bladder response but did cause a reduction in the EUS neurographic activity. After a second bilateral dorsal column and DLF lesion caudal to the stimulation site (lesion 2), the stimulus (200, 500 μA) no longer decreased EUS electroneurographic activity.

FIGURE 8


A
Bladder pressure
20 mmHg
5 s
EUS ENG
Anal ENG
T9
T13
1
2
B
Post lesion 1
Bladder pressure
EUS ENG
Anal ENG
C
Post lesion 1 + 2
Bladder pressure
EUS ENG
Anal ENG

DISCUSSION

In this study electrical stimulation was capable of activating the central neural circuitry mediating micturition at brainstem and several spinal cord levels. The spinal cord stimulation sites eliciting voiding were shown to be activating an ascending portion of the spinobulbospinal loop. This route of stimulation is presumed to activate the same bulbar circuitry as the PMC stimulus sites.

The brainstem PMC sites in this study (Figure 2) show a fairly broad distribution rather than one anatomically identifiable cell group. The sites overlap or encompass a portion of the periaqueductal gray matter, the locus coeruleus and immediately neighbouring areas. As mentioned in the introduction, this region of the rostral pons has long been postulated to be important in micturition because lesions of this area disrupts micturition and more recently because chemical activation of neurons in this area was capable of evoking voiding (Sugaya et al. 1987). In addition, projections from this area to the sacral cord in the rat and diverse connections between this brainstem area and other neighboring mesencephalic areas which may also be involved in micturition have been demonstrated (Koyama et al. 1966, Satoh et al. 1978a,b, Loewy et al. 1979).

The locus coeruleus has been implicated as being the major cell group involved in the supraspinal control of micturition (Koyama et al. 1966, Yoshimura et al. 1988), both because of its descending projections to the spinal cord and because it is in the rostral pons region known to be important to bladder function. Besides being a discrete brainstem

centre exerting direct descending effects onto the spinal cord, the locus coeruleus gives diverse projections to higher centres and other brainstem areas (Wiklund et al. 1981). Most notable of its brainstem connections are those to the raphe nucleus (Wiklund et al. 1981, Katayama et al. 1986, Mokha et al. 1986). The raphe system, which also receive inputs from the periaqueductal gray, has been shown to be an important system in producing nociceptive suppression (Geisler and Liebeskind 1976, Eisenhart et al. 1983, Katayama et al. 1986) and able to exert inhibitory actions on sympathetic preganglionic neurons (Cabot et al. 1979). In addition to the ability of the periaqueductal gray matter to alter nociception, it has been implicated as an important integrating centre for autonomic and somatic elements and afferent and efferent processing in the defense reaction (Bandler and Tork 1987). It is this type of autonomic and somatic integration that would be required in the neural control of micturition to produce the appropriate brainstem output for a given ascending visceral input. The fact that brainstem PMC sites in this study included sites in the periaqueductal gray suggests that this area could activate other mesencephalic structures and/or converge with other structures descending onto the raphe system, with the total output mediating micturition.

The notion of a supraspinal neural network important in micturition and involving interconnections of brainstem areas rather than one area specific to micturition has been previously postulated (Koyama et al. 1966, Satoh et al. 1978a) and is supported by the dispersal of PMC stimulation sites in this study. While it has been shown that chemical activation of cell bodies in the region of the locus coeruleus can produce micturition (Sugaya et al. 1987), and that a similar stimulus in the

dorsolateral tegmentum can modify bladder reflexes (Roppolo et al. 1988), it is also possible that the electrical brainstem stimulation activates a cell group or fibres of passage which in turn activate components of the supraspinal network involved in micturition not localized within the locus coeruleus.

While the supraspinal stimulation sites in the present study were not limited to one anatomical location, the fact that some sites produced voiding with stimulus-locked inhibition of the external urethral sphincter activity rather than inhibition related to the micturition cycle suggests that these stimulation locations may have activated a portion of other pathway(s) in addition to the micturition neural network. The ability to find PMC sites in the same animal in which sites producing stimulus-locked inhibition were also described suggests that these latter stimulation sites may have been sub-optimal because of activation of other fibres or cell bodies not related to micturition. The inhibition from the other pathway(s) was maintained throughout the stimulation period regardless of bladder pressure response. This characteristic allowed this type of inhibition of the EUS electroneurographic activity to be readily identified.

Studies involving the tracing of pathways with immunohistochemistry have allowed the spinal localization of descending projections from specific brainstem areas. A direct noradrenergic spinal projection from a brainstem area in or around the locus coeruleus has been postulated to be important in bladder function (Kuru and Yamamoto 1964, Yoshimura et al. 1988). A search for catecholamine containing neurons in the cat dorsolateral pontine tegmentum revealed a spinal projection with a

primarily ventrolateral spinal trajectory (Chu and Bloom 1974, Westlund et al. 1982). While this described projection arises from the locus coeruleus, noradrenergic systems from the subcoeruleus and Kolliker-Fuse nuclei have been shown to descend in the thoracic DLF of the cat (Stevens et al. 1985). Later studies (Satoh et al. 1977, Nygren and Olson 1977, Commissiong et al. 1978, Karoum et al. 1980) describing noradrenergic spinal projections from locus coeruleus and neighbouring nucleus tegmentalis laterodorsalis have been conducted almost exclusively in rat. These studies have established a strong noradrenergic projection, crossed and uncrossed, travelling both ventrally and dorsally in the rat spinal cord. Further, this noradrenergic nucleus tegmentalis laterodorsalis projection has been shown to have terminations in the intermediolateral cell column important in central autonomic function (Loewy et al. 1979). Although the description of such a descending pathway is important, the presence of a micturition specific descending noradrenergic pathway has yet to be documented in the cat. Sillen et. al (1979) observed the effects of a dopa-decarboxylase inhibitor as well as L-dopa injection on bladder motility in rat, and described an adrenergic (presumed dopaminergic) central system mediating hyperactive vesicular motility. It has been shown that noradrenergic depletion or bilateral lesion of the locus coeruleus does not to ablate micturition in the rat (Satoh et al. 1978a). In contrast, Yoshimura et al. (1990) found that catacholaminergic depletion of the locus coeruleus did cause an impairment of the micturition reflex in cats. Thus, although there is evidence to suggest anatomical projections catacholiminergic systems, the functional contributions of these systems to bladder function remain inconclusive.

The fact that lateral hemisection of the thoracic spinal cord does not disrupt the voiding evoked from the PMC either ipsilateral or contralateral to the lesion shows the ability of the PMC to exert its effects bilaterally. This ability may be due to the PMC giving rise to bilateral descending projections or the ability of a unilaterally descending PMC to activate the contralateral supraspinal micturition circuitry either by projecting to the contralateral PMC or some other portion of the contralateral brainstem network. It is also possible that both ipsi- and contralateral PMC sites share portions of the "micturition network" somewhere in the brainstem such that activation of either site causes bilateral output to the spinal cord. This observation supports the previous report by Holstege et. al (1986) that stimulation of either left or right "M-region" causes the same characteristic bladder response, and that destruction of the M-region bilaterally is required to ablate the response. Since they traced the descending projections from this area and found primarily an ipsilateral projection to the sacral intermediolateral nucleus, it seems more probable that the bilateral effects are due to activation of the contralateral system somewhere in the brainstem rather than by bilateral spinal projections from each brainstem site.

Subtotal spinal cord lesions conducted in this study have assisted in localizing the spinal cord trajectory of fibres descending from the supraspinal centres involved in micturition. The fact that bilateral thoracic dorsal column and DLF lesions does not ablate micturition evoked from the PMC site (Figure 7), shows that an important portion of the descending influences from the PMC must be travelling in the ventral or ventrolateral aspect of the thoracic spinal cord. When these bilateral

dorsal column and DLF lesions were extended more ventrally, the weak bladder contraction with the coordinated reduction of EUS electroneurographic activity observed suggests that the descending pathway is dispersed enough that only some of the fibres were damaged and a portion remained to mediate the effects, through a weaker drive to the bladder parasympathetic preganglionic neurons. Previous studies (Holstege and Kuypers 1987) have shown spinal cord projections from this mesencephalic region having this type of lateral/ventrolateral dispersal. It is also possible that there is a non-specific descending pathway in the dorsolateral aspect of the spinal cord which acts to "prime" the sacral spinal cord circuitry and facilitate sacral reflexes. Lesion of such a pathway might allow descending influences from the PMC to exert effects on a less excitable pool of sacral neurons and produce the reduced effects observed.

The spinal cord stimulation used in the present study was able to reproducibly activate the central neural circuitry controlling micturition. The fact that bilateral lesions of the DLF abolished voiding evoked from the more caudal superficial thoracic DLF stimulation sites, but not from DLF stimulation sites rostral to the lesion, suggests that an ascending portion of the spinobulbospinal loop was activated. The possibility that a thoracic sympathetic spinal segmental system was responsible for the observed voiding was negated by the ligation and lesion of the thoracic mixed roots in the area of the stimulation (n=3 cats). The fact that these procedures had no effect on the voiding evoked is in agreement with Ingersoll et al. (1961), who were not able to elicit voiding with sympathetic stimulation.

Previous reports have described an ascending spinothalamic tract pathway responding to visceral stimulation (Hancock et al. 1975) and stimulation of bladder afferents (Milne et. al 1981). McMahon and Morrison (1982a) showed the funicular trajectory of the ascending cells responding to bladder distention to be in the dorsal aspect of the lateral funiculus at the thoracic spinal cord while having a more ventral trajectory in cervical segments. This type of funicular trajectory is in agreement with the spinal cord stimulation sites able to evoke micturition in this study. A significant portion of dorsal spinothalamic tract neurons have been reported to have collateral projections to the mesencephalon as well as to the thalamus (Price et al. 1978, Willis et al. 1979). Such neurons could comprise the ascending pathway important in micturition. The possibility that spinocervical tract neurons were also involved in such an ascending visceral convergent system was addressed by Cervero and Iggo (1978), who demonstrated that physiological bladder distention does not affect the firing properties of these tract cells.

Another ascending pathway which may mediate the passage of bladder afferent information is the spinomesencephalic tract. Axons of spinomesencephalic neurons have been shown to ascend in the ipsilateral ventrolateral aspect of the cervical spinal cord (Bjorkeland and Boivie 1984, Yezierski and Schwartz 1986, Yezierski 1990) and then project to contralateral brainstem structures (Yezierski and Schwartz 1986). The ventrolateral funicular course of spinomesencephalic axons has not been confirmed in more caudal segments and it is possible that the spinal cord trajectory of the spinomesencephalic axons has a dorsolateral location in thoracic segments which changes to a ventrolateral location in cervical

segments, as does the distribution of spinal cord stimulation sites able to produce voiding in this study.

Brainstem terminations of the spinomesencephalic tract have been demonstrated to periaqueductal gray matter, intercollicular nucleus, posterior pretectal nucleus, nucleus of Darkschewitsh, cuneiform nucleus, anterior pretectal nucleus, Edinger-Westphal nucleus and mesencephalic reticular formation (Wiberg and Blomqvist 1984, Bjorkeland and Boivie 1984, Yezierski 1988). The studies of the spinomesencephalic tract have largely been focused on brainstem nociception suppression effects (Yezierski and Schwartz 1986, Yezierski and Schwartz 1984, Yezierski 1990), though the value of such a spinobulbospinal system producing both inhibitory and excitatory effects in visceral regulation has also been discussed (Yezierski and Schwartz 1986, Yezierski 1990). Recent studies on the spinomesencephalic tract and its role in mesencephalic processing of nociceptive sensory information have shed light on possible general mechanisms underlying supraspinal control over spinal activity (Yezierski and Schwartz 1986, Yezierski and Schwartz 1984, Yezierski 1990).

In addition to supporting the interpretation that the DLF-evoked voiding was elicited by ascending fibres, the bilateral DLF lesions in the present study allowed the subsequent stimulation of a descending pathway within the DLF which produced a decrease in pudendal electroneurographic activity in the absence of a bladder response. As illustrated in Figure 8, the stimulus locked reduction in pudendal electroneurographic activity evoked by stimulation of the superficial thoracic DLF caudal to a bilateral dorsal column/DLF lesion was abolished by a second DLF lesion caudal to the stimulation site, indicating that the effects were mediated

by a descending pathway restricted to the DLF. This DLF descending inhibitory pathway affects activity in both the anal sphincter and EUS branches of the pudendal nerve, and should therefore be considered separate from the pathway mediating inhibition of EUS efferent activity during micturition. The specific reduction in EUS pudendal efferent activity during micturition is retained after bilateral lesion of the DLF (Figure 7B). This separation of descending inhibitory pathways is significant in that it will allow for their selective activation in future studies and assessment of their individual effects on pudendal systems.

Description of inhibitory pathways descending in the DLF is not new. A variety of studies have been conducted regarding the nature of DLF descending inhibition, both with regard to spinal nociception suppression mechanisms, and spinal reflex control mechanisms. Descending inhibitory pathways can mediate inhibition of a system by inhibiting the system's afferent input, the motoneuron (ie. output), or the system's interneuronal pool (reviewed in Lundberg 1982). Of these three possible sites of inhibition, Lundberg (1982) suggested that only inhibition directed at the interneuronal pool of a motor system can influence a pathway selectively (ie. allowing the system to still respond to different afferent inputs). Such descending inhibition of interneurons has been demonstrated by Engberg et al. (1968) and is known to be most easily observed in a decerebrate preparation where the brainstem centres producing the inhibition are themselves no longer inhibited by higher centres (Holmqvist and Lundberg 1959).

Two of the descending systems which are thought to mediate descending inhibitory effects are the serotonergic and adrenergic systems.

The adrenergic system is comprised of fibres descending from the nucleus raphe magnus and neighbouring areas of the medulla and directly from mesencephalic centres (Basbaum and Fields 1979, Basbaum et al. 1988, Yezierski 1990). These raphe centres receive inputs from mesencephalic centres which overlap areas which receive afferent input from spinomesencephalic neurons (Yezierski 1990) and this suggests that such a system could form a functional spinobulbospinal loop. The ability of such a loop to not only inhibit certain spinal reflexes but also exert excitatory effects on dorsal horn neurons (Yezierski 1990) would allow supraspinal control of reflexes based on ascending sensory information. Such a system may be postulated as the supraspinal component of the neural organization of micturition.

Descending inhibitory pathway(s) modifying the response of spinal motor reflexes could be analogous to the descending DLF mediated inhibition of pudendal electroneurographic activity described in the present study. In fact, preliminary observations indicate that this descending DLF pathway may inhibit polysynaptic pudendal/pudendal reflexes. This inhibition is demonstrable as the release of activity seen after bilateral lesion of the dorsal spinal cord. There has been no previous description of such an inhibitory pathway with respect to pudendal reflex activity, but one may speculate how bladder/sphincter dyssynergia, a common occurrence after spinal cord lesion, could be induced by the loss of such a tonic reflex inhibitory pathway. The interneurons described by McMahon and Morrison (1982b) and the suggestion by Floyd et. al (1982) of an interneuron-mediated "gating" mechanism controlling reflex activity between anal and urethral sphincter pudendal

populations could provide the substrate through which a descending tonic inhibitory pathway could mediate its effects. Neither this possibility nor the possibility of a direct action of the inhibitory pathway on the pudendal motoneurons could be assessed in the present study. Such inhibition of motoneurons was documented by Hounsgaard et. al (1988), who described a serotonergic system able to modify excitability of motoneurons through modulation of membrane properties (ie. plateau potentials). It seems quite possible that such a system could be important in micturition through modulation of the spinal neurons involved. In fact, Loewy and McKellar (1981) and Bowker et al. (1981) have described the anatomical projection of a descending serotonergic system in the rat having terminations in the intermediolateral cell column and ventral horn of the spinal cord. Though the importance of this pathway with respect to bladder function was not assessed, the demonstration of such a serotonergic system descending to the region of preganglionic spinal neurons is itself noteworthy.

As micturition involves coordination between somatic and autonomic elements, one might expect any descending system involved to also exert effects on the autonomic portions of the micturition circuitry. Downman and Hussain (1958) examined the descending effects from supraspinal centres on visceromotor reflexes. Although they did not look at brainstem-evoked voiding, they examined the effects of subtotal spinal cord lesions on intercostal segmental spinal reflexes evoked by splanchnic and intercostal nerve stimulation. Lesion of the dorsolateral aspects of the spinal cord produced the most consistent and predominant effect, a marked release of this thoracic visceral spinal reflex. Thus, Downman and Hussain

(1958) discussed a tonically active inhibitory pathway travelling in the dorsolateral funiculus (DLF) influencing visceromotor responses.

In summary, this study has shown CNS sites from which electrical stimulation is able to selectively evoke micturition characterized by coordinated activity between the bladder and EUS. Assessment of this coordination of activity is important, as not all sites producing a bladder response elicit coordinated micturition. The PMC sites in this study have a regional but not anatomically distinct localization. They correspond with sites previously described to have effects on bladder activity and overlap with brainstem areas now postulated to be involved in the integration and suppression of ascending afferent information. A significant portion of the ascending limb of the spinobulbospinal loop mediating the micturition reflex has been localized to the DLF in the thoracic spinal cord, and electrical stimulation of this region can repeatably evoke coordinated micturition. Although its funicular trajectory at the thoracic levels has not yet been confirmed, the spinomesencephalic tract, with its brainstem terminations and involvement in processing of ascending afferent information seems a strong candidate for the identity of this pathway, though the possibility of participation by dorsal spinothalamic tract neurons cannot be discounted. In addition, the present study has described a descending pathway in the DLF having effects on pudendal neurons similar to the DLF mediated tonic inhibition previously described for other spinal reflex systems. The overlap in brainstem areas thought to be important in bladder control with those associated with descending inhibitory pathways suggests that these systems may both be involved in micturition. Possibly the PMC triggers the correct

spinal circuitry while the descending inhibitory system(s) sets the gain of the spinal circuitry, "priming" it for the behavior.

The observations from this study are consistent with a spinobulbospinal system capable of integrating both somatic and visceral information and exerting both inhibitory and excitatory effects on the neurons involved in various sacral spinal reflexes. Further investigation will lead to insight as to the exclusiveness of the neural organization controlling micturition as well as the relative contribution of supraspinal influences and sacral reflex activity in producing micturition.

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