

Convergence of multiple pelvic organ inputs in the rat rostral medulla

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Electrophysiological recordings were used to investigate the degree of pelvic/visceral convergent inputs onto single medullary reticular formation (MRF) neurons. A total of 94 MRF neurons responsive to bilateral electrical stimulation of the pelvic nerve (PN) in 12 urethane-anaesthetized male rats were tested for responses to mechanical stimulation of the urinary bladder, urethra, colon and penis, and electrical stimulation of the dorsal nerve of the penis (DNP) and abdominal branches of the vagus. Responses to distension of the bladder were found for 51% ($n = 48$) of the MRF neurons tested. Of these 48, 71% responded to urethral infusion, 81% responded to colon distension, 100% responded to penile stimulation (and DNP), and 85% responded to vagal stimulation, with 62% responding to stimulation of all four of these territories. This high degree of visceral convergence (i.e. 62%) in a subset of PN-responsive MRF neurons is significantly greater than for the subset of PN-responsive MRF neurons that did not respond to urinary bladder distension (i.e. out of the 46 remaining neurons, none responded to all four of the other pelvic/visceral stimuli combined). These results suggest that the neurons processing information from the urinary bladder at this level of the neural axis are likely to be important for mediating interactions between different visceral organs for the coordination of multiple pelvic/visceral functions.

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Normal functions related to the male pelvic/visceral organs, such as urination, defaecation, and ejaculation, involve coordination between the different organ systems. One example of a process in which coordination is important is ejaculation, which consists of emission of the seminal fluid from the ejaculatory ducts to the proximal urethra, bladder neck closure and antegrade ejaculation out of the proximal urethra (Seftel *et al.* 1991; Truitt & Coolen, 2002). Disruption of this coordination, such as following spinal cord injury, results in the failure of bladder neck closure and thus a subsequent retrograde ejaculation leading to infertility (Heruti *et al.* 2001).

Another example in which coordination between the pelvic organs is important is during voiding of the urinary bladder, which requires contraction of the external anal sphincter, thereby preventing defaecation. In humans, distension of the urinary bladder produces contractions of the anal sphincter (Basinski *et al.* 2003). Experimental studies using cats have shown that distension of the urinary bladder both inhibits colonic contractions (Bouvier *et al.* 1990) and produces simultaneous contraction of the anal sphincter (Bouvier & Grimaud, 1984). The reverse also occurs, i.e. the urinary system is inhibited during defaecation. In humans, functional stimulation of the anal

sphincter inhibits detrusor muscle contraction (Cheng *et al.* 2002). In animal studies, distension of the rectum has been shown to inhibit urinary bladder activity in female rats (Sugaya *et al.* 1998), and stimulation of colonic branches of the pelvic nerve in cats has been shown to have an inhibitory effect on micturition (Floyd *et al.* 1978).

Interactions and functional coordination between various organ systems creates a situation where a pathological condition in one pelvic/visceral organ can affect the normal function of another organ. For example, some patients who suffer from irritable bowel syndrome have micturition problems, such as voiding incontinence (Whorwell *et al.* 1986a). In addition, 46% of female patients who suffer from urinary tract problems have sexual dysfunction, such as decreased sexual activity, disorders in sexual arousal, and dyspareunia (pain with intercourse) (Salonia *et al.* 2004). In an experimental study using female rats, urinary bladder inflammation was shown to decrease uterine contractions (Dmitrieva *et al.* 2001), thereby demonstrating that the pathology of one organ, such as the urinary bladder, can affect the function of another.

The neural mechanisms underlying the interactions between the various pelvic/visceral organs are likely to

be mediated by both the peripheral and central nervous systems. For the reflex circuitries to be efficient in coordinating pelvic/visceral functions there must be some sort of viscerovisceral convergence within the CNS, both in the spinal cord itself, as well as supraspinally. In the spinal cord, various neuroanatomical studies have shown innervation of different visceral organs by the same set of neurons (Russo & Conte, 1996; Nadelhaft & Vera, 2001). Also, various electrophysiological studies have shown that individual neurons in the spinal cord of rats (Berkley *et al.* 1993; Qin & Foreman, 2004), cats (Foreman *et al.* 1984) and primates (Milne *et al.* 1981) respond to different pelvic/visceral and somatic stimuli. At the brainstem level, numerous regions, including Barrington's nucleus, the solitary nucleus and the nucleus gracilis, have been shown to receive viscerovisceral convergent inputs (Hubscher & Berkley, 1994; Berkley & Hubscher, 1995; Rouzade-Dominguez *et al.* 2003b).

The medullary reticular formation (MRF) is another brainstem region that receives multiple pelvic/visceral organ inputs. The extensive connections of MRF neurons with the forebrain, limbic system, brainstem nuclei, cerebellum and spinal cord, are an indication of the potential importance of these neurons in the functional coordination between different systems (Brodal *et al.* 1980; Van Bockstaele *et al.* 1993; Tavares & Lima, 1994; Cobos *et al.* 2003; Sun & Panneton, 2005). MRF neurons, for example, that are responsive to electrical stimulation of dorsal nerve of penis (DNP) and mechanical stimulation of penis (low- and high-threshold stimuli) also respond to electrical stimulation of the pelvic nerve (PN), as well as to mechanical stimulation of the perineum and limbs (Hubscher & Johnson, 1996).

Previous neuroanatomical and electrophysiological studies have, however, focused primarily on either inputs to MRF from territories involved with one pelvic organ system (such as from either the bladder and urethra or from the colon and rectum or from the genitalia) or on somatovisceral convergence (Roy *et al.* 1992; Hubscher & Johnson, 1996, 2004; Almeida & Lima, 1997; Nadelhaft & Vera, 2001; Hubscher *et al.* 2004). Given the vast array of inputs that have been shown to go to the MRF from the pelvic viscera, as well as convergent inputs from widespread somatic territories, it is hypothesized that there is a significant degree of overlap from different pelvic territories onto individual MRF neurons. Using electrophysiological studies, a defined region of the MRF was searched for neurons responsive to PN stimulation. Each PN-responsive neuron was then tested for responsiveness to urinary bladder (UB) distension and urethral stimulation, electrical stimulation of the DNP and abdominal branches of the vagus, mechanical stimulation of the genitalia, and distension of the distal colon.

Methods

Animal preparation

A total of 12 male Wistar rats (120 days of age) were used. Each animal was anaesthetized with 50% urethane (1.2 g kg^{-1}). The jugular vein, carotid artery and trachea were exposed and intubated for anaesthetic supplement (5% urethane, as needed, based on assessing withdrawal reflexes), blood pressure monitoring, and respiratory rate/end-expired P_{CO_2} level monitoring, respectively. Using an oesophageal heat sensor probe connected to a thermometer, the animal temperature was monitored throughout the experiment, and maintained at around 37°C using a circulating water-heating pad.

Each animal was mounted onto a stereotaxic device. A dorsal incision was made to gain access to the brainstem. The dorsal surface of rostral medulla was exposed by removing some occipital bone and suctioning the caudal midline portion of the cerebellum (Hubscher & Johnson, 1996, 1999).

Electrical nerve stimulation

A dorsal incision, through the gluteus superficialis and biceps femoris muscles, was made in each animal to expose the PN and DNP bilaterally. The somato-motor branch of the PN was cut and a portion removed to avoid undesirable contractions; only the viscerocutaneous branch was prepared for stimulation. The nerves were separated from the connective tissues and placed on specially fabricated bipolar electrodes (Hubscher & Johnson, 1996). The stimulus intensity, for both nerves, was set at $30\text{--}50 \mu\text{A}$, 0.1 ms duration, with trains of 14 pulses at 70 pulses s^{-1} , 100 ms train duration, 1 train s^{-1} (Hubscher & Johnson, 1996).

A bipolar electrode was introduced alongside the oesophageal thermometer probe, just caudal to the oesophageal hiatus, in order to stimulate the abdominal branches of the vagus nerve that are situated on the external wall of the oesophagus (Hubscher *et al.* 2004). The stimulus intensity was set at 8 mA for 2 ms duration. This stimulus intensity produces compound action potentials in the vagus nerve, as recorded with bipolar hook electrodes in the neck where the vagus runs along the common carotid artery (Hubscher *et al.* 2004).

Mechanical stimulation of pelvic/visceral organs

A midline abdominal incision was made to expose the UB and proximal urethra. UB and urethral catheters, comprising PE 60 tubing attached to a syringe, were implanted through an incision in the proximal urethra, with the UB catheter directed rostrally toward the UB, and the urethral catheter directed caudally toward the

distal urethra (see Fig. 1). The ureters were tied close to the UB and were cut proximal to the suture and drained externally using PE 10 tubing (Kakizaki & de Groat, 1997). The abdominal incision was then closed.

A 10-mm-long balloon, made from condom latex material and attached to a 25 g × 3/4 in catheter (Terumo Corporation, Tokyo, Japan), was inserted intra-anally for distal colon distension (Berkley *et al.* 1993). The UB and colon catheters were connected to a pressure monitor (World Precision Instruments). Pressures were recorded on videotape and analysed offline using Data Wave software (www.dwavetech.com). Once fully distended (2–3 s), the UB and colon remained distended until a response was obtained up to a maximum of 10 s before deflation.

Electrophysiological recordings

An epoxyite-insulated tungsten microelectrode with standard tip (Frederick Haer and Co., Bowdoinham, ME, USA) was lowered from the dorsal surface with a motorized drive (Frederick Haer and Co.) into the MRF. Stereotaxic coordinates were 3400 μm rostral to obex and 400 μm and 800 μm lateral to midline on both sides of the brainstem (four tracks per animal, in total). The search area for each dorso-ventral track covered a length of 2800–3000 μm , which penetrated the rostral part of the nucleus reticularis gigantocellularis (Gi), the Gi pars alpha (GiA) and the medial part of lateral paragigantocellular nucleus (LPGi) (Hubscher & Johnson, 1999).

Bilateral electrical stimulation of the PN (bPN) was used as the search stimulus. Once a neuron was found to be responsive to bPN (twice- or half-background activity for excitatory and inhibitory responses, respectively, and at least three spikes for non-spontaneous units; Hubscher & Johnson, 2003, 2004), responses of that neuron to distension of the UB and colon were tested, as well as infusion of the urethra. Responses to electrical stimulation of the DNP and the abdominal branches of the vagus nerve were then tested. In addition, the responses to mechanical (stroke and pinch) stimulation of different somatic regions, such as the penis, scrotum, trunk, upper and lower limbs, ears, eyelids and face, were tested. All of the spike recordings made while testing the PN-responsive MRF neurons were recorded to videotape and analysed offline using Data Wave software.

Histology

At the end of the experiment, each animal was perfused using 0.9% normal saline followed by 4% paraformaldehyde introduced through the left ventricle. The brainstem was extracted from each animal and sectioned at 100 μm thickness on a vibratome, and then

processed histologically and stained with cresyl violet. The electrophysiological tracks were visualized in these sections under the light microscope, thereby confirming the location of each electrode track (Hubscher & Johnson, 1999). Stereotaxic depth measurements obtained relative to the dorsal surface were used to plot the location of each PN-responsive neuron that was recorded along each of the electrode tracks.

Data analysis

Data was analysed for significance using Student's *t* test and the χ^2 test. Results were considered significant when $P < 0.05$. Spike histograms were generated using the Data Wave program. The study was performed in accordance with guidelines of the Animal Care and Use Committee (IACUC) of the University of Louisville School of Medicine, and the Guide for the Care and Use of Laboratory Animals (National Academy of Sciences, publication no. 0-309-05377-3).

Results

A total of 94 MRF neurons in 24 tracks responded to bPN. About half of these neurons responded to distention of the UB. A comparison between the characteristics of PN-responsive MRF neurons that did and did not respond to UB distension is presented in Table 1. There were no significant differences ($P > 0.05$) in background activity, neuronal responses and nerve response latencies between these two groups of MRF neurons. Although the majority of responses were excitatory, inhibitory and complex (mixed excitatory and inhibitory) neuronal responses were also observed. The percentage of inhibitory PN-responsive MRF neurons was significantly higher for those responding to UB distension (77% of the total number of inhibitory responses).

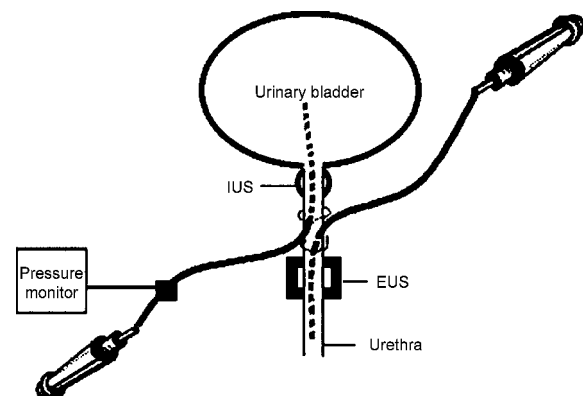


Figure 1. Diagram illustrating the insertion location of urinary bladder (UB) and urethral catheters into the proximal urethra

Table 1. Characteristics of medullary reticular formation (MRF) neurons

	Neurons responsive to PN and UB distension (<i>n</i> = 48)	Neurons responsive to PN but NOT UB distension (<i>n</i> = 46)
Background activity		
Neurons with spontaneous background activity	37.5%	26.1%
Mean spontaneous background activity (spikes s ⁻¹)	14.7 ± 1.9	18.2 ± 3.7
Neuronal responses		
Percentage of neurons excitatory	77.1%	87.0%
Percentage of neurons inhibitory	20.8%*	6.5%
Percentage of neurons complex	2.1%	6.5%
Response latencies		
PN	141.1 ± 11.6 ms	140.3 ± 12.5 ms
DNP	152.7 ± 13.1 ms	160.9 ± 18.1 ms
Vagus	196.8 ± 25.8 ms	195.2 ± 19.6 ms
UB distension	3.8 ± 0.6 s	NA
Colon distension	3.7 ± 0.6 s	2.5 ± 0.5 s

*Significantly different (χ^2 , $P < 0.05$) from those MRF neurons not responding to urinary bladder (UB) distension. PN, pelvic nerve; DNP, dorsal nerve of the penis; NA, not applicable.

Table 2. Distribution of PN-responsive neurons within the MRF

	Neurons responsive to PN and UB distension (<i>n</i> = 48)	Neurons responsive to PN but NOT UB distension (<i>n</i> = 46)
Gi neuronal responses		
No. excitatory	27	28
No. inhibitory	3	1
No. complex	0	1
DPGi neuronal responses		
No. excitatory	4	4
No. inhibitory	0	0
No. complex	1	0
GiA + RMg neuronal responses		
No. excitatory	6	8
No. inhibitory	7*	2
No. complex	0	2

*Significantly different (χ^2 , $P < 0.05$) from those MRF neurons not responding to UB distension. DPGi, dorsal paragigantocellular nucleus; Gi, gigantocellular reticular nucleus; GiA, Gi pars alpha; RMg, raphe magnus nucleus.

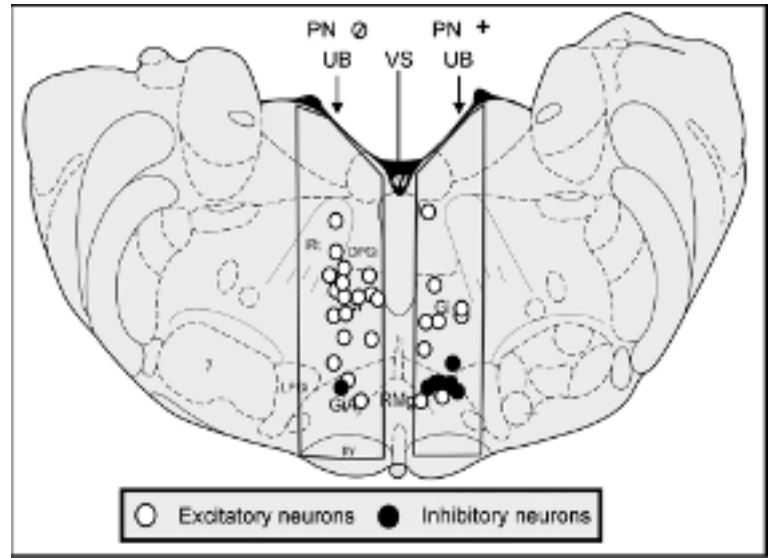
The distribution of the excitatory, inhibitory and complex neurons differed from one MRF subregion to another (Table 2). There were, however, no significant differences in the pattern of distribution of PN-responsive neurons that did and did not respond to UB distension. The location of a sample of neurons from four animals (selected at random) is illustrated in Fig. 2. Note the ventral location of neurons that were inhibited by PN and bladder stimulation. Examples from electrophysiological recordings are provided for individual neurons with excitatory (Fig. 3) and inhibitory (Fig. 4) responses to both PN and UB distension. An example of a PN-responsive MRF neuron that did not respond to UB distension is provided in Fig. 5.

Viscero-visceral convergence

Many of the PN-responsive MRF neurons studied that were excited or inhibited by UB distension also responded to stimulation of other organs/nerves (urethral infusion, colon distension, penis stimulation, DNP and abdominal branches of the vagus stimulation – 62% responded to all of these stimuli) as well as numerous cutaneous territories (face, eyelid and trunk). A summary comparing the convergent responses of PN-responsive MRF neurons that did and did not respond to UB distension is presented in Table 3. Note that the degree of convergence from multiple pelvic/visceral territories was significantly greater for those neurons responding to UB distension. In fact, the amount of convergence for those responding to all the

Figure 2. Distribution of PN-responsive MRF neurons that did and did not respond to UB distension

Example showing the distribution of pelvic nerve (PN)-responsive medullary reticular formation (MRF) neurons (recorded from 4 animals) that did and did not respond to UB distension (right and left side, respectively). Note the distribution of excitatory *versus* inhibitory responses of neurons in the outlined area under investigation. Also note that the left/right distribution is for illustrative purposes only. Neurons were found in the indicated regions on both sides of the MRF for both groups. The cross section is adapted from atlas Fig. 65 (Paxinos & Watson, 1998). 4V, 4th ventricle; 7, facial nucleus; DPGi, dorsal paragigantocellular nucleus; Gi, gigantocellular reticular nucleus; GiA, Gi pars alpha; IRt, intermediate reticular nucleus; LPGi, lateral paragigantocellular nucleus; py, pyramidal tract; RMg, raphe magnus nucleus.



other pelvic/visceral territories tested was quite different for the subsets of neurons responding and not responding to UB distension (62 *versus* 0%, respectively, responded to urethra as well as colon and vagal stimulation).

Of the total number of PN-responsive MRF neurons, 66 (70.2%) had convergent inputs from one or more of the pelvic/visceral stimuli examined. All the neuronal responses of the MRF neurons to UB and colon distension were in the noxious range (70 mmHg pressure). The

average response latency (from the beginning of distension to the beginning of the response) for the MRF neurons to UB and colon distension is provided in Table 1. Note, though, that the time until the balloon was fully distended was approximately 2–3 s. For a few MRF neurons, the response delay exceeded the 10 s stimulus duration (i.e. responded after deflation – as for the example shown in Fig. 3). In addition, all the responses to UB, colon and urethra had after discharges that lasted anywhere

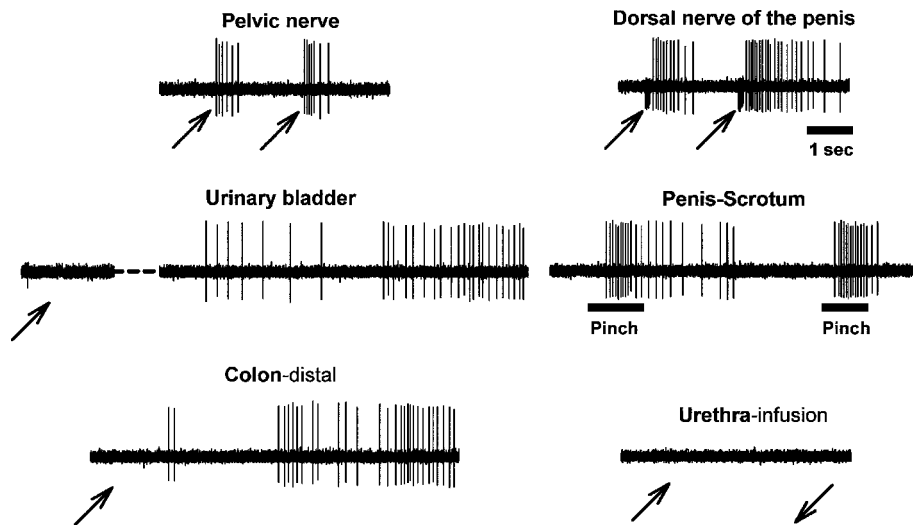


Figure 3. Sample raw record of convergent excitatory inputs to MRF

Example of a single neuron recording in the left nucleus reticularis pars alpha obtained during testing of electrical stimulation of PN and dorsal nerve of the penis (DNP), UB and distal colon distension, urethral infusion and pinching of the glans penis and scrotum. As shown, this neuron responded to UB and distal colon distension, but not to urethral infusion. This neuron also responded to pinching the ear, eyelid, face, forepaw and hindpaw (not shown). There was no response to electrical stimulation of the abdominal branches of the vagus (not shown). Note the stimulus artifact in the DNP record and that the urinary bladder had a 14 s response delay (partial record shown). The upward arrows indicate the beginning of the stimulus, the downward arrow indicates the end of the stimulus, and bars indicate the onset and duration of the mechanical stimulus. The response discharge to UB and colon distension lasted for 46 and 20 s, respectively. The time scale for the DNP record applies to all traces.

from several seconds, to on occasion several minutes (see examples in Figs 3 and 4). The mean MRF neuronal responses to UB and colon distension lasted 10.4 ± 1.3 and 9.7 ± 1.4 s, respectively (i.e. total response duration – see response criteria in Methods).

Most MRF neurons that responded to the stimulation of one visceral organ responded to stimulation of other visceral organs (i.e. viscerovisceral convergence). The degree of viscerovisceral convergence in the MRF is illustrated graphically in Fig. 6. Note that many responses were found for electrical stimulation of the abdominal branches of the vagus nerve, which is known to innervate a number of pelvic/visceral organs, including the stomach, liver, pancreas, colon and the female reproductive organs (Sauter *et al.* 1983; Precht & Powley, 1985; Schoenen *et al.* 1992; Altschuler *et al.* 1993; Hubscher & Berkley, 1995). Examples illustrating varying degrees of viscerovisceral convergence onto individual PN-responsive MRF neurons can be seen by comparing responses shown in Figs 3, 4, 5 and 7.

Note that about 8.5% of the neurons responding to UB and colon distension did not respond to urethral infusion (see example in Fig. 3). Also, only 5.6% of the neurons responding to UB distension and urethral infusion did not respond to colon distension. In addition some of the neurons not responding to UB distension responded to stimulation of one of the other pelvic viscera (urethra only (8.5%), see example in Fig. 5; colon only (2.8%)). All

neurons responding to both urethra and colon responded to UB distension.

Somato-visceral convergence into MRF

All of the PN-responsive MRF neurons had convergent input from somatic territories (bilateral). Of the 94 PN-responsive MRF neurons, 27.7% responded to both low- and high-threshold stimulation of somatic receptive fields, whereas 72.3% responded to high-threshold levels of stimulation only. The subregion distribution of PN-responsive MRF neurons with responses to both low- and high-threshold levels of stimulation was as follows: 73.0% located in Gi, 11.5% located in DPGi, and 15.4% located in GiA and raphe magnus nucleus; these levels were not significantly different from the distribution of the PN-responsive MRF neurons that only had responses to high-threshold levels of stimulation. The PN-responsive MRF neurons that responded to UB distension were more responsive to low-threshold stimulation of several different somatic territories (see Fig. 8) compared with the neurons that did not respond to UB distension (43.8 *versus* 10.9%, respectively).

Discussion

The results of the present study indicate that there is a high degree of convergence from the pelvic viscera

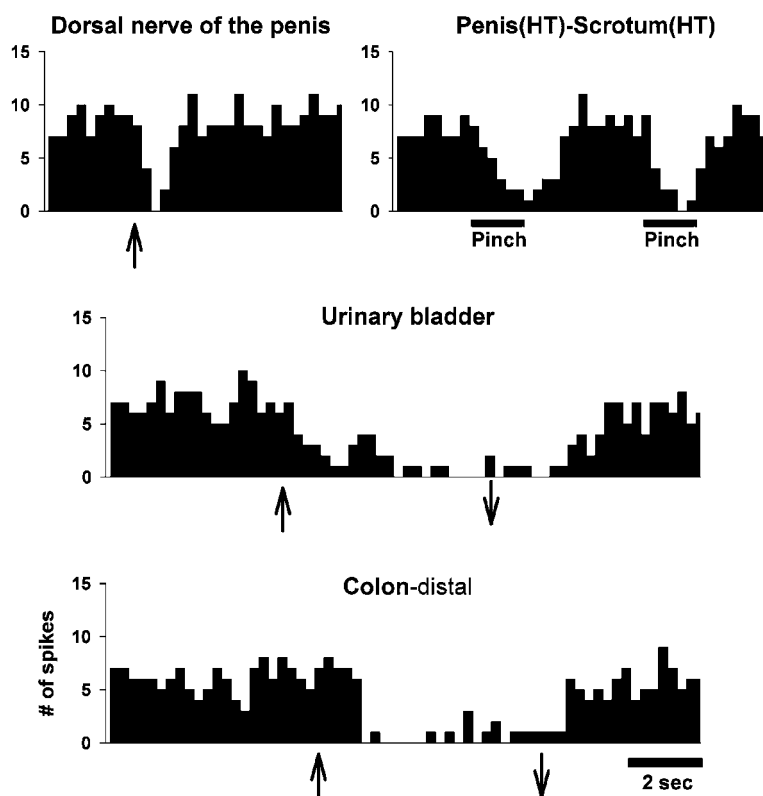


Figure 4. Sample histogram of convergent inhibitory inputs to MRF

Histogram showing an example of inhibitory responses of a single neuron, located in the left nucleus reticularis gigantocellularis pars alpha, to bilateral electrical stimulation of the DNP, pinching of the glans penis and scrotum, and distension of the UB and distal colon. The neuron also responded to pinching the dorsal trunk, eyelid, forepaw, hindpaw, and electrical stimulation of PN and abdominal branches of vagus (not shown). The response latency for UB and distal colon distension was about 2 s, and it had a response discharge of about 10 and 7 s, respectively. The bin width is 0.25 sec. Symbols as in Fig. 3.

(UB, colon and urethra) to single MRF neurons that are responsive to electrical stimulation of the PN. All of these neurons were responsive to electrical stimulation of the DNP and mechanical stimulation of the glans penis (low-threshold and/or high-threshold), and many responded to stimulation of the abdominal branches of the vagus, consistent with previous results (Hubscher & Johnson, 1996; Hubscher *et al.* 2004). The same region also has somatic convergent inputs from different parts of the body (limbs, perineum, face and trunk), a finding also consistent with previous findings (Hubscher & Johnson, 1996). A significant amount of viscerovisceral convergence, in addition to a high degree of somato-visceral convergence, indicate that these areas of the MRF (e.g. Gi, GiA) are likely to play a role in coordinating the eliminative functions of different pelvic viscera (micturition, defaecation and ejaculation). It is possible, though, that at least some of the visceral inputs to MRF result from ascending inputs that have already converged at lower spinal cord levels, as seen for spinal neurons that have been shown to receive either both UB and colon inputs (Qin & Foreman, 2004), or both UB and urethral inputs (Russo & Conte, 1996).

UB/urethral input to MRF

More than half of the PN-responsive MRF neurons responded to distension of the UB. Most of these neurons were also responsive to urethral stimulation. The high degree of convergence from UB and urethra to single MRF neurons indicates that these neurons may play a role in coordinating or modulating the micturition process. Neuroanatomical tracing studies using transsynaptic tracers are consistent with these electrophysiological findings. For example, pseudorabies virus transfection studies (using two histologically distinguished modified types of pseudorabies virus) show double labelling of many MRF neurons following injection of the UB and external urethral sphincter (Nadelhaft & Vera, 2001). Only a very small number of double-labelled neurons are found, however, in the pontine micturition centre (Nadelhaft & Vera, 2001), which is known to be involved in the control of urine voiding and storage through its facilitatory and inhibitory projections, respectively, to lumbosacral spinal neurons that contribute to the functioning of the UB and the external urethral sphincter (Holstege *et al.* 1986; Noto *et al.* 1989; Kruse *et al.* 1990; Yoshimura, 1999; Griffiths, 2002).

A few studies have demonstrated the ability of MRF neurons to modulate the function of the pelvic organs involved in micturition. In one study using cats, MRF neurons were shown to be activated during voiding (Sugaya *et al.* 2003). In the same study, MRF neurons were shown to have reciprocal connections with the pontine micturition centre and to project axons to the spinal

cord, suggesting that two descending spinal pathways from the pontine micturition centre play important roles in micturition, one of which projects by way of the MRF (Sugaya *et al.* 2003). In another study using cats, electrical stimulation of the MRF inhibited UB contractions (McMahon & Spillane, 1982).

In the present study, most of the inhibitory neurons in the MRF region examined were located in ventral areas (GiA and raphe magnus), which is consistent with previous results (Hubscher & Johnson, 1996). This segregation of the inhibitory and excitatory neurons coincides with segregation between the areas that have inhibitory *versus* excitatory descending influences on spinal reflexes, as shown in a study examining the effect of electrical stimulation of MRF subregions on the nociceptive tail-flick reflex (Zhuo & Gebhart, 1990).

Convergence of UB and colon inputs in the MRF

Most of the UB-responsive neurons responded to colon distension. Other electrophysiological studies

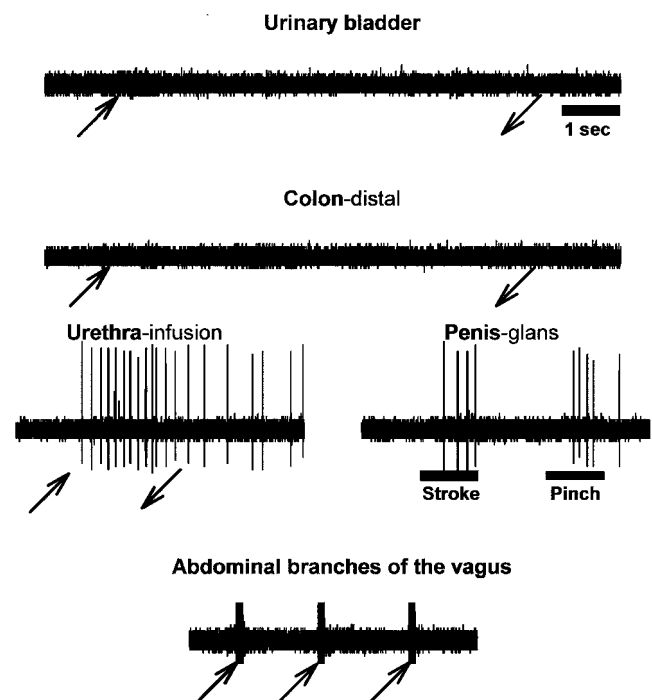


Figure 5. Example of an MRF neuron not responsive to bladder distension

Record showing an example of the responses of a single neuron located in the left nucleus reticularis gigantocellularis to UB and distal colon distension, urethral infusion, low- and high-threshold stimulation of the glans penis, and electrical stimulation of the abdominal branches of the vagus. As shown, this neuron responded to stimulation of one pelvic/visceral organ (urethra), but didn't respond to the other pelvic/visceral organs (UB and distal colon) or to electrical stimulation of abdominal branches of the vagus (stimulus artifact can be seen in record). Symbols as in Fig. 3.

Table 3. Summary of MRF visero-visceral and somato-visceral convergence in PN-responsive MRF neurons that did and did not respond to UB distension

Region stimulated	Neurons responsive to PN and UB distension	Neurons responsive to PN but NOT UB distension
DNP	100%	100%
Vagus (abdominal branches)	85.4%*	52.2%
Urethra (infusion)	70.8%*	15.2%
Distal colon (distension)	81.3%*	13.0%
Glans penis		
LT + HT	27.8%	12.1%
HT only	72.2%	87.9%
Face		
LT + HT	6.3%	0.0%
HT only	45.8%	28.3%
Eyelid		
LT + HT	35.4%*	10.9%
HT only	56.3%	67.4%
Dorsal trunk		
LT + HT	20.8%*	2.2%
HT only	68.8%*	43.5%

*Significantly different (χ^2 , $P < 0.05$) from those not responding to UB distension. LT, low threshold, HT, high threshold.

also demonstrate the convergence of UB and colon inputs to single neurons in the brainstem, including studies of neurons within Barrington's nucleus (Rouzade-Dominguez *et al.* 2003b), the lateral medulla (Robbins *et al.* 2005) and rostral medulla (Baez *et al.* 2005), although the latter study tested UB voiding and not distension. Neurons in Barrington's nucleus that have been implicated to play a functional role in the micturition process are double labelled following pseudorabies virus transfection of the UB and colon (Rouzade-Dominguez *et al.* 2003a),

MRF neurons are likely to be part of the neural circuitry involved in coordinating eliminative processes, including micturition and defaecation. The underlying

mechanisms for this control are not known. Lumbosacral spinal neurons have recently been shown in the rat to receive visero-visceral convergent inputs from the bladder and colon (Qin & Foreman, 2004). These spinal neurons are obvious targets for brainstem modulation of the cross talk that is likely to be occurring between the various visceral organs.

The responses of the MRF neurons to UB and colon distension were in the noxious range (70 mmHg pressure). These results may indicate a role of MRF neurons in visceral nociception as well. It has been shown that the MRF can modulate nociceptive processing by its descending (excitatory and inhibitory) inputs (McCreery *et al.* 1979; Haber *et al.* 1980; Gebhart *et al.* 1983; Zhuo & Gebhart,

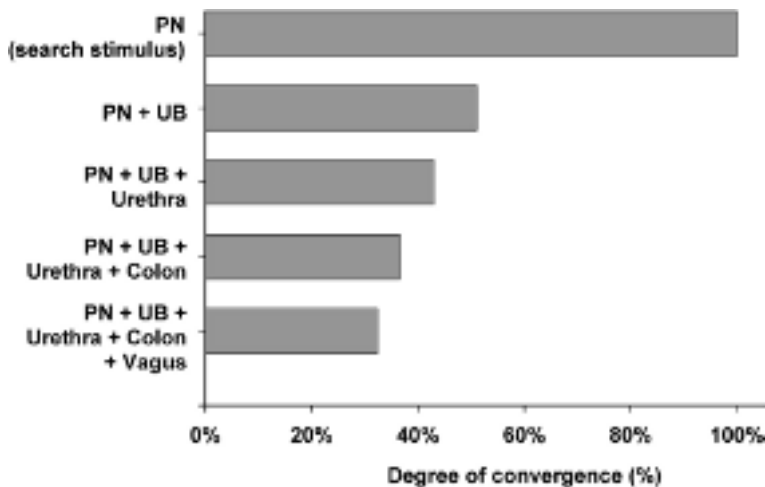


Figure 6. Visero-visceral convergence in MRF Illustration of the degree of visero-visceral convergence to MRF neurons responsive to bilateral electrical stimulation of the PN (the search stimulus). Most of the neurons that are responsive to one pelvic/visceral organ also respond to others.

1990, 2002*a,b*), and work as relay nuclei to higher centres for nociceptive processing and ultimately pain perception (Foreman *et al.* 1984).

Convergence of UB and colon with genital inputs in the MRF

All the PN-responsive MRF neurons (thus all UB and colon-responsive MRF neurons) were responsive to electrical stimulation of dorsal nerve of the penis and mechanical stimulation of penis. These results indicate a role of the MRF neurons in the coordination of urogenital functions. For example, a subpopulation of MRF neurons may inhibit both voiding and defaecation circuitries during ejaculation. The brainstem neurons may also facilitate other aspects that are necessary for normal

ejaculation, such as excitation of neurons important for closure of the internal urethral sphincter, which is important in order to avoid retrograde ejaculation.

The MRF has been shown to have a modulatory effect over sexual functions through its descending projections (Marson & McKenna, 1990; Johnson & Hubscher, 1998, 2000). For example, recordings from the motor branch of the pudendal nerve to MRF stimulation demonstrate that MRF neurons (mainly within the LPGi) have an inhibitory effect on the pudendal reflex (Johnson & Hubscher, 1998) and an excitatory effect on sympathetic fibres in the pudendal nerve (Johnson & Hubscher, 2000). Also, injection of an anterograde neuronal tracer (Fluoro-Ruby) into Gi and LPGi (Hermann *et al.* 2003) and raphe obscurus nucleus (Hermann *et al.* 1998) shows direct projections from these nuclei to motor neurons labelled

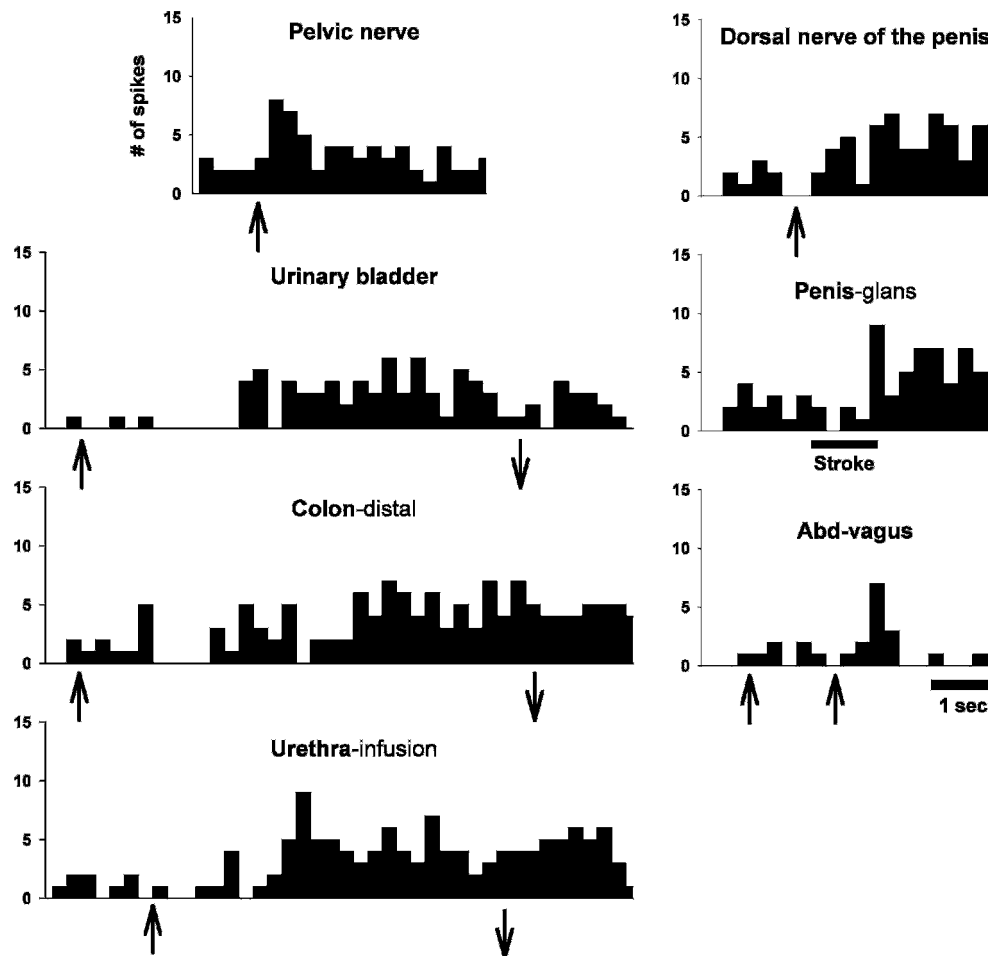


Figure 7. Example showing extent of visceral convergence in MRF

Histogram showing an example of excitatory responses for a single neuron located in the left nucleus reticularis gigantocellularis to bilateral stimulation of PN and dorsal nerve of the penis, UB and distal colon distension, urethral infusion, glans penis stroke (low-threshold (LT) stimulus) and electrical stimulation of the abdominal branches of the vagus. The same neuron responded also to stroking (LT) the dorsal trunk, stretching (LT) the eyelid, and pinching the face, forepaw and hindpaw (not shown). This neuron had convergent inputs from all the pelvic/visceral organs tested, as well as responses to stimulation of the abdominal branches of the vagus. The response discharge to UB and colon distension lasted for 15 and 17 s, respectively. The bin width is 0.25 sec. Symbols as in Fig. 3.

with a retrograde neuronal tracer (Fluoro-Gold) injected into the external urethral sphincter, ischiocavernosus and bulbospongiosus muscles. On the other hand, other studies indicate that there are descending inputs from MRF to the spinal cord motor neurons that innervate different pelvic organs. For example, transneuronal-tracing studies with pseudorabies virus injected into UB (Marson, 1997; Sugaya *et al.* 1997; Zermann, 2002), prostate (Zermann, 2002), penis (Marson *et al.* 1993) and ischiocavernosus and bulbospongiosus muscles (Marson & McKenna, 1996) labels neurons in different brainstem nuclei (including the MRF nuclei under investigation in this study).

Convergence of vagal inputs into MRF

The PN-responsive MRF neurons that responded to UB distension have significantly higher convergent inputs from abdominal branches of vagus than neurons that did not respond to UB distension. The high convergence of pelvic/visceral inputs with the input resulting from vagus nerve stimulation could indicate a dual innervation of the pelvic/visceral organs. Other studies have demonstrated vagal innervation to pelvic/visceral organs. For example, bilateral vagotomy, in female rats, eliminates the responses of solitary nucleus neurons to uterine horn distension, and affects the response to cervical and vaginal stimuli (Hubscher & Berkley, 1995). Neurons in the dorsal motor nucleus of the vagus and the solitary nucleus have been labelled with neuroanatomical tracers from all areas of the colon, with the exception of rectum (Altschuler *et al.* 1993). In addition, tracers injected into the urinary bladder labels some nodose ganglion neurons. Capsaicin treatment

doesn't affect this pattern of labelling in the nodose ganglion (Jancso & Maggi, 1987), indicating that the UB may be innervated by myelinated fibres in the vagus nerve.

Somato-visceral convergence in the MRF

All the PN-responsive MRF neurons have convergent inputs from cutaneous regions all over the body. The convergence of somatic inputs from all over the body to the same neurons that are responsive to the pelvic/visceral organs may indicate a role of MRF in controlling the body posture during micturition and/or preventing unwanted reflexes that may interrupt micturition (Baez *et al.* 2005). The significance for an increase in the amount of low-threshold sensory inputs to the MRF for neurons responding to urinary bladder distension (as indicated in Fig. 8) is not known, but perhaps relates to the maintenance of body posture during micturition.

The high degree of pelvic/visceral and somatic convergence onto single MRF neurons could also explain the diffuse and overlapping perception of pelvic visceral pain in some pelvic/visceral pathologies (Whorwell *et al.* 1986b; Longstreth, 1994; Cervero & Laird, 1999). In the present study, some of the PN-responsive MRF neurons had complex response properties, having opposite responses from stimulation of different areas (usually stimulation of the face evoked an inhibitory response with the remaining convergent responsive territories evoking excitatory responses). This may indicate the ability of MRF neurons to modulate cutaneous and/or visceral pain through somatic stimulation of other areas (see Fig. 3 in Hubscher & Johnson, 2004).

Viscero-visceral convergence in the MRF

The high degree of convergence of afferent inputs from multiple visceral organs into various regions of the CNS (including the MRF) provides a way for the CNS to control and coordinate functions between various pelvic/visceral organs, and may also explain how a pathology associated with one organ can affect the physiology/functioning of another organ (Qin *et al.* 2005; Berkley, 2005). The importance of regions located supraspinally in viscero-visceral interactions is apparent from the interruption of the physiological functions of the pelvic/visceral organs after spinal cord injury (Heruti *et al.* 2001). The MRF is one of the supraspinal regions that can play a key role in modulating these pelvic/visceral functions.

The ability of MRF neurons to modulate and/or coordinate multiple pelvic/visceral functions, as discussed in the present paper, necessitates the convergence of sensory inputs from different internal organs onto the same neurons. The results of this study indicate that the PN-responsive MRF neurons that respond to UB distension show a high degree of viscero-visceral convergence, which may indicate a role for a subset

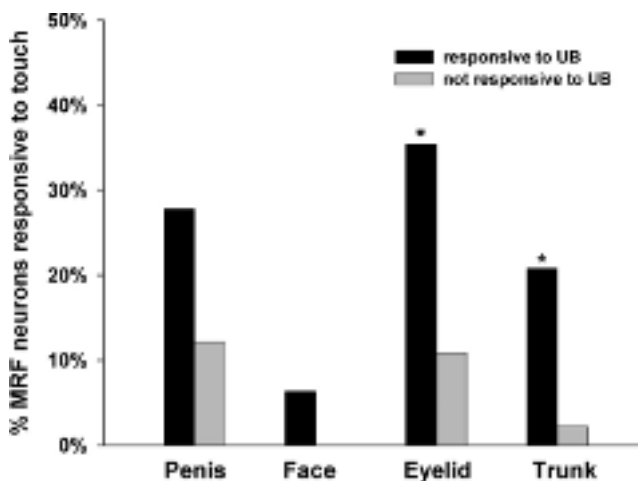


Figure 8. Summary of MRF responses to low threshold levels of stimulation

Diagram comparing the percentage of responses to LT stimulation of different somatic receptive fields, for those PN-responsive MRF neurons that did, versus those that did not, respond to UB distension. As shown, PN-responsive MRF neurons that respond to UB distension have more LT responses. *Significant difference (χ^2 , $P < 0.05$).

of neurons at this level of the neural axis in the modulation of spinal reflexes for the coordination of functions associated with these organs, such as elimination (urination, defaecation, ejaculation).

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