Convergence and cross talk in urogenital neural circuitries

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Studies targeting the interactions between male and female urogenital neural circuitries have been limited, despite the very high incidence of sexual disorders. The neural circuitry controlling ejaculation and voiding are very complex (Allard et al. 2005; Calabro et al. 2011). Ejaculation is dependent on myelinated penile afferents in the pudendal nerve [dorsal nerve of the penis (DNP)] to the lumbosacral cord in rats (Hart and Leedy 1985; Johnson 2006; Wieder et al. 2000). Current views propose that a spinal center for ejaculation in the lumbar cord is somewhat capable of mediating the emission and expulsion of semen after acute (minutes to a few hours) but not chronic (30–60 days) midthoracic cord transection (Allard et al. 2005; Coolen et al. 2004; Truitt and Coolen 2002). The loss of inhibitory brain stem control of coordinated pelvic striated muscle contractions via pudendal motoneurons results in loss of ejaculatory ability (Coolen et al. 2004; Giuliano and Rampin 2004; Johnson 2006). Similarly, bladder function involves lumbar sympathetics, sacral parasympathetics, and a somatic supply to the external sphincter (Fowler et al. 2008; Sengupta and Gebhart 1995). Bladder afferents travel to the spinal cord via the pelvic (primarily parasympathetic) and hypogastric (primarily sympathetic) nerves (Jancso and Maggi 1987; Pacual et al. 1993). Various techniques have characterized bladder afferents, spinal cord interneurons, and intermediate zone receiving bladder input in rats (see, e.g., Cadden and Morrison 1991; Hwang et al. 2005; Iijima et al. 2009; Wang et al. 1998). The spino-bulbospinal control of micturition, involving the pontine micturition center, has also been characterized (Blok and Holstege 2000; Matsumoto et al. 1995; Nuding and Neldehaft 1998).

The medullary reticular formation (MRF) is involved in the control of sexual function in males and females (Daniels et al. 1999; Marson and McKenna 1990; Modianos and Pfaff 1979; Schwartz-Giblin et al. 1996; Tanaka and Arnold 1993). With microstimulation, a discrete ventrolateral MRF area in the gigantocellularis pars alpha (GiA) and lateral paragigantocellularis (LPGi) nuclei produces an inhibitory effect on pudendal motoneuron reflex discharges (Johnson and Hubscher 1998) and produces microstimulation-elicted field potentials in pudendal motor pools (Tanaka and Arnold 1993). Bilateral lesioning of this MRF area has been shown to affect ejaculation (Marson and McKenna 1990; Yells et al. 1992), and this area receives connections from the mPOA and PAG (Murphy and Hoffman 2001) and sends bulbospinal axons to the pudendal motor spinal circuitry (Hermann et al. 2003; Marson and McKenna 1996). Neurons in this MRF region are labeled bilaterally after unilateral injection of the transsynaptic pseudorabies virus tracer into the bulbospongiousus muscle, which contributes to erection, ejaculation, and micturition (Johnson et al. 2011).

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Sexual dysfunction is a common public health concern that affects approximately one-third of men and two-fifths of women (Dunn et al. 1998; Laumann et al. 1999). Sexual dysfunction takes on many forms with varying degrees of prevalence; for example, erectile dysfunction increases in prevalence with age (Beutel et al. 2006). Importantly, a substantial number of published studies have related comorbidity of sexual dysfunctions with other diseases/conditions, such as obstructive bladder (Heidler et al. 2010), chronic kidney disease (Hofmann and Schmidt 2010), voiding symptoms and nocturia (Ponholzer et al. 2004), benign prostatic hyperplasia (Rosen et al. 2005), and other conditions like diabetes, hypertension, stroke, and psychiatric disorders (Beutel et al. 2006). The purpose of the present study is to assess the interactions between neural circuitries mediating sexual and bladder function, as a therapy directed toward one of these organ systems could likely affect the other.
MRF neurons also show high convergence of a high variety of pelvic structures. Extracellular recordings of single MRF neurons have demonstrated widespread convergent somato-visceral ascending inputs, including the lower urinary tract and external genitalia (Hubscher and Johnson 1996; Hubscher et al. 2004; Kaddumi and Hubscher 2006). Differential inhibition and excitation may lead to coordinated responses, such as inhibition of voiding and defecation circuits during ejaculation (Kaddumi and Hubscher 2006), and cross talk between pelvic organ systems may aid normal visceral functioning. For example, distension of the urinary bladder leads to contractions of the external anal sphincter, preventing defecation and allowing for micturition (Basinski et al. 2003) and vice versa. The convergence of inputs from the lower urinary tract on MRF circuitries mediating arousal (Wu et al. 2007) could relate to the inhibition of micturition such as during low levels (sleep) and high levels (sex) of activity. Injury or trauma leading to disruption of the coordination of any of these mechanisms can cause the injured organ to affect the functionality of the noninjured organ (Kaddumi and Hubscher 2006; Malykhina et al. 2006; Pezzone et al. 2005). Both bladder-to-colon and colon-to-bladder cross-sensitization has been linked with painful sensations (Alagiri et al. 1997; Brumovsky and Gebhart 2010; Malykhina et al. 2013; Nickel et al. 2010; Pezzone et al. 2005; Qin et al. 2005; Theoharides et al. 2008; Ustinova et al. 2006, 2010).

Electrical stimulation of various nerves has been used to inhibit and activate bladder reflexes of animals (Snellings and Grill 2012; Su et al. 2012a; Woock et al. 2010) and as a therapy area that has been designed for use with both idiopathic and neurogenic bladder disorders (Chartier-Kastler 2008; Kennelly et al. 2011; Light 1993; Middleton and Keast 2004; Schmidt et al. 1999; Thompson et al. 2010). Electrical stimulation of the DNP or the dorsal nerve of the clitoris (DNC) can also be used to activate MRF neurons to mimic sexual sensory inputs (Hubscher 2006; Hubscher and Johnson 1996). Given this high level of convergence, the present study examined amplitude- and frequency-response curves of individual MRF neurons to DNP/DNC stimulation in an in vivo urethane-anesthetized rat preparation. These effects were compared to neurostimulation responses evoked by a more proximal stimulation site of the L7/S1 trunk. Finally, the effects of DNP neurostimulation on voiding were determined, followed by the examination of DNP/DNC-responsive MRF neuronal responsiveness to bladder distension with varying levels of neurostimulation. Further characterization of interactions between urogenital circuitries both peripherally and centrally would further our understanding of the underlying interactions, which will help in the development of potential therapies for related disorders.

METHODS

Animals. A total of 36 Wistar rats (24 males and 12 females, Harlan Sprague Dawley) 60-70 days old and weighing 220-250 g were used in this study. Each rat was housed individually in an animal room with a 12:12-h light-dark cycle. All animal procedures were reviewed and approved by the Institutional Animal Use and Care Committee at the University of Louisville, School of Medicine. For the female rats, vaginal smears were taken just prior to the start of the experiments to ensure that no experiments were performed during proestrus, as MRF neuronal response properties have been shown to vary during proestrus as a consequence of elevated serum levels of estradiol (Hubscher 2006). All rats were anesthetized with 50% urethane (1.2 g/kg ip). A catheter (PE-50) was placed in the jugular vein for anesthetic supplement (5% urethane; 0.1 ml of 0.05 g/ml urethane, as needed). The trachea was exposed and intubated for monitoring respiratory rate/ end-expiratory Pco2 level (to monitor anesthesia depth in addition to standard reflex testing). Body temperature was monitored throughout the experiment by an esophageal probe connected to a thermometer and maintained at 37°C with a circulating-water heating pad.

Surgical preparation. For the transvesical bladder catheter (Maggi et al. 1986), the bladder was exposed via a midline abdominal incision through skin and musculature. A purse-string suture (4-0 Ethilon) was placed in the urothelium of the bladder dome. The tip of the PE-60 tubing was heated to form a collar ~2 mm from the end. The catheter was inserted through the bladder dome within the suture limits, secured, and connected to an infusion pump and pressure transducer. After the skin was closed with wound clips, a dorsal incision through the gluteus superficialis and biceps femoris muscles was made in each animal to expose the DNP/DNC (males/females, respectively) bilaterally. The nerve was separated from the connective tissues and placed on specially fabricated bipolar electrodes (Hubscher and Johnson 1996). In a separate group of 12 male rats, the L7/S1 trunk on one side was also exposed proximal to the DNP where the pelvic and pudendal branches merge (see Fig. 1 in Pastelin et al. 2008). The rat was then mounted onto a stereotactic device. A dorsal incision was made to gain access to the brain stem, and the dorsal surface of rostral medulla was exposed by removing part of the occipital bone and suctioning the caudal midline portion of the overlying cerebellum (Hubscher and Johnson 1996, 1999b; Kaddumi and Hubscher 2006).

DNP/DNC and L7/S1 trunk neurostimulation. With two Grass Photoelectric Stimulus Isolation Units (model PS1U6; 1 per nerve) and a Grass S88 stimulator (Astro-Med, West Warwick, RI), the stimulus intensity was initially set at 30–50 μA, 0.1-ms duration with trains of 14 pulses at 50pps, 100-ms train duration, 1 train/s per our previous studies (Hubscher 2006; Hubscher et al. 2010; Hubscher and Johnson 1996, 1999b; Kaddumi and Hubscher 2006). To test the effect of neurostimulation on voiding in a subset of male rats prior to electrophysiological recordings (first 5 of the 12 used), normal saline was infused into the bladder at a rate of 0.25 ml/min to evoke voiding contractions (Maggi et al. 1986). Once the voiding cycles were consistent (at least 5 consecutive voiding events with consistent time intervals in between), the effects of bilateral DNP neurostimulation were assessed with varying intensities to determine amplitude response threshold. Voiding and nonvoiding events were easily visualized, as the animal was mounted in a Kopf small-animal spinal unit (model 900 with 980 base) with hip pins, with the tail elevated. Bladder pressure readings were displayed on a calibrated BP-1 Pressure Monitor from World Precision Instruments (Sarasota, FL). Pressures and time (Fisher Scientific timer) were logged into the experimenter notes every 15 s as whenever there was a pressure change > 2 mmHg. Voids were logged as well (stream, single drop, or multiple drops). Pressures were recorded with a VR-10B Digital Data Recorder from Instrutech (Port Washington, NY) to videotape (Sony VCR) for off-line playback with DataWave software (www.dwavetechn.com). Voiding cycle length and pressure changes were calculated from the tabulated log entries.

MRF recordings. A tungsten microelectrode with ~7 ± 1 MΩ impedance (Fridrich Haer, Bowdoinham, ME), coated with fluorescent dye (Dil, Invitrogen, Carlsbad, CA) (Chadha and Hubscher 2008; Massey et al. 2006), was lowered from the dorsal surface of the brain stem with a motorized drive (Fridrich Haer) into the MRF. Stereotoxic coordinates were 2,700–3,300 μm rostral to obex and 300–1,400 μm lateral to midline on both sides of the brain stem. The search area for each dorso-ventral track was 2,800–3,000 μm in length, which included the rostral part of the nucleus reticularis gigantocellularis (Gi), GiA, and the medial part of the LPG (Hubscher and Johnson 1999a).
Recording of a single neuron was established by monitoring the action potential on an oscilloscope with a spike-triggered analog delay module for discrimination of somato-dendritic neuron profiles from nerve fiber spikes as described previously (Hubscher and Johnson 1996, 1999b, 2006). Once a single neuron responsive to bilateral DNP or DNC was found, stimulus frequency and amplitude were varied to assess intensity threshold as well as threshold and optimal frequency. Each stimulus parameter was tested multiple successive times to account for any response variations due to potential wind-up. Responses to bladder distension were then tested (Kaddumi and Hubscher 2006, 2007) with the transvesical PE-60 catheter attached to a syringe (manual inflation at a steady rate of ~4 ml/min). In addition, for the 12 female rats, MRF responses to cervix pressure were also evaluated as previously described with a glass probe assembly device connected to a pressure transducer and monitor (Hubscher 2006). In a separate group of 12 male rats, responses to L_o/S, trunk stimulation were also tested. MRF neuronal responses were recorded to videotape and analyzed off-line with DataWave software (www.dwavetech.com).

Tissue processing. After completion of the terminal electrophysiology experiment, each animal was euthanized with an anesthetic overdose of urethane. Perfusions were performed transecturally with 0.9% saline followed by 4% paraformaldehyde. The portion of brain containing the MRF (292.2 ± 13.0 μm) was removed, stored in a 10% formalin-30% sucrose solution, and subsequently cut on a vibratome in 50-μm sections. The dye-traced electrode tracks were visualized and reconstructed as previously described (Chadha and Hubscher 2008; Massey et al. 2006).

Analysis. For the effects of neurostimulation on voiding, the data were averaged within each animal (for the different parameters tested) and then the mean values were calculated across all five rats. For the MRF recordings, group means for male and female rats were calculated based on animal averages. Comparisons were made between groups by analysis of variance (ANOVA) with a significance level of P < 0.05. All values reported in the manuscript are means ± SE.

RESULTS

DNP-evoked MRF responses. Single-unit extracellular recordings were done in the rostral medulla under urethane anesthesia in 12 adult male Wistar rats. In total, 102 MRF neurons responded to DNP stimulation (n = 12). DNP response threshold was significantly higher for neurons located within the GiA subregion of the MRF (21.5 ± 2.5 μA) relative to the other MRF subregions [Gi: 15.4 ± 0.8 μA; intermediate reticular nucleus: 11.7 ± 1.1 μA; dorsal para-Gi (DPGi): 13.0 ± 1.1 μA]. An example showing the response threshold for a typical Gi neuron is presented in Fig. 1. Response latency was very short (<20 ms) for 23.5% of the neurons tested, with no differences based on subregion location (16.7% in DPGi vs. 26.0% in Gi; no significant difference, P > 0.05). Response latencies ≥ 20 ms were significantly longer for the DPGi subregion of the MRF (29.2 ± 27.5 ms) relative to the other subregions (Gi: 179.2 ± 16.1 ms; GiA: 195.0 ± 33.0 ms). Neuronal locations based on histological reconstructions were made per previous studies (Hubscher 2006; Hubscher and Johnson 1996, 1999a, 1999b, 2002, 2004; Hubscher et al. 2010; Kaddumi and Hubscher 2006) with a standard rat atlas for subregion specifications (Paxinos and Watson 1998).

The majority of MRF neurons responsive to DNP neurostimulation were excited with stimulation (64.7%), whereas 30.4% were inhibited. No significant differences were found between MRF subregions. The remaining five neurons (of 102) were characterized as “mixed” (see Hubscher 2006). Two of the MRF neurons, for example, had no background activity and were unresponsive to DNP stimulation. However, when excited by stimulation of the face, the DNP inhibited the response (when stimulated simultaneously). This was the case when other parts of the body, such as the hindpaws, were stimulated (so effect not exclusive to DNP—may relate to a general attention/orientation or arousal type of response). A total of 45.8% of the DNP-responsive MRF neurons had spontaneous background activity. The mean activity of those neurons was 10.9 ± 1.0 impulses/s. No significant differences were found between the various MRF subregions.

DNC-evoked MRF responses. Single-unit extracellular recordings were also done under urethane anesthesia in 12 adult female Wistar rats. A total of 64 MRF neurons responded to DNC stimulation. DNC response threshold was significantly higher for the Gi subregion of the MRF (10.5 ± 0.7 μA) relative to the DPGi subregion (7.9 ± 0.8 μA), with an average threshold frequency of 20 Hz in both regions. Response latency was very short (<20 ms) for 32.7% of the neurons tested, with a greater proportion located in the Gi (17.6% DPGi vs. 45.5% Gi; significant difference, P < 0.01). The longer-latency responses had a mean latency of 186.0 ± 11.7 ms and did not differ between MRF subregions. A typical example of a neuronal response within the Gi subregion of female rats is provided in Fig. 2. An example showing the lower DNC response threshold in DPGi is provided in Fig. 3.

Of the DNC-responsive MRF neurons tested for input from the cervix (pressure), 74.5% (38 of 51) responded with a mean response pressure of 34.3 ± 2.7 mmHg (equivalent to 46.6 g-force/cm²). This force is known to elevate mean arterial blood pressure by ~15 mmHg and heart rate by ~10 beats/min in fully conscious rats (Catelli et al. 1987). The low response pressures are consistent with the rats’ estrous phase (vaginal smears taken just prior to the start of the electrophysiological recordings) (Hubscher 2006). Elevated threshold during proestrus due to increased serum estradiol has been related to aspects of mating (see discussion in Hubscher 2006).

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Sex differences in MRF responses. Compared with MRF recordings in male rats \((n = 12)\), those in females had significantly lower response thresholds for stimulation of the DNC versus the DNP in male rats \((P < 0.001)\), a significant difference that was found in both Gi and DPGi MRF subregions. Note that no DNC-responsive neurons were found in the GiA subregion, versus 7% in males (DNP). In addition, maximal response frequency was similar for stimulation of the DNC in female and the DNP in male rats \((30–40 \text{ Hz})\). An example from each is provided in Figs. 4 and 5, respectively. Although no overall significant differences in MRF response latencies were found for DNP versus DNC stimulation \((P > 0.05)\), the response latencies for the DPGi subregion differed significantly from Gi but in different ways. In female rats a smaller proportion of short-latency DPGi neuronal responses were found relative to Gi, whereas in the DPGi of male rats latencies \(> 20 \text{ ms} \) were significantly longer relative to Gi.

**L6/S1-evoked MRF responses.** Single-unit extracellular recordings were done in the rostral medulla under urethane anesthesia in 12 additional adult male Wistar rats. In total, 70 MRF neurons responded to unilateral (left side) stimulation of the L6/S1 trunk \((n = 12)\). Mean response threshold to trunk stimulus was 54.2 \(\pm 4.6 \mu \text{A} \) (range from 16 to 400 \(\mu \text{A} \)), which was significantly higher than the threshold for stimulation of the DNP \((P < 0.001)\). An example showing the response threshold of a typical MRF neuron for stimulation of the left L6/S1 trunk and the left DNP in the same rat is presented in Fig. 6. Similar to DNP/DNC, stimulation above threshold resulted in increased MRF neuronal firing, less wind-up, and longer afterdischarges. Maximal frequency reached when the neuronal response (impulses/s) would plateau was 30 Hz, although response thresholds occurred with settings as low as 10 Hz. MRF response latencies to L6/S1 trunk neurostimulation were very short \(< 20 \text{ ms} \) for 23% of the neurons tested (no significant difference with respect to DNP, \(P > 0.05\), in terms of subregion location). The longer-latency responses \(77\%\) of those tested) had a mean latency of 229.0 \(\pm 18.8 \text{ ms} \), with a range between 54 and 732 ms (no significant difference with respect to DNP, \(P > 0.05\)). Similar to DNP neurostimulation (first group of 12 male rats), the majority of trunk-responsive MRF neurons were excitatory \(73.0\%\), whereas 20.0\% were inhibitory. The remaining 7% were characterized as "mixed." Also, a total of 48.6% of the trunk-responsive MRF neurons had background activity. The mean activity of those neurons was 11.1 \(\pm 1.4 \text{ impulses/s} \). Because the L6/S1 trunk is a mixture of afferent and efferent projections, muscle contractions were observed with stimulation. The mean threshold for muscle contraction in the perineum/scrotum/hindquarter region with L6/S1 trunk stimulation was 36.8 \(\pm 6.6 \mu \text{A} \) range from 6 to 80 \(\mu \text{A} \); \(n = 12\), suggesting that stimulation intensity was rarely, if ever, greater than two times motor threshold.

**Effects of DNP stimulation on voiding.** Voiding was evaluated with and without neurostimulation in five adult male urethane-anesthetized Wistar rats. The mean bladder pressure at onset of voiding was 25.4 \(\pm 2.7 \text{ mmHg} \). Filling/voiding cycles were lengthened with DNP neurostimulation at amplitude of 11.0 \(\pm 0.9 \mu \text{A} \), with a maximal effective frequency of 30 Hz. During stimulation at or just above threshold, bladder pressures remained elevated (near void pressure) with slow increases and then slight decreases with the release of one or two drops of urine. An example is provided in Fig. 7. The fill/void cycle interval would then return to prestimulation patterns at \(~ 2–3 \text{ min} \) after stimulus offset (at stimulus threshold; larger effects were seen with suprathreshold levels of stimulation, data not shown).

**MRF neuronal responses to bladder filling.** Centrally, many DNP/DNC-responsive MRF neurons responded with either excitation or inhibition to bladder filling \(35.0\% \) DNP and 68.3\% DNC) at proportions consistent with our previous experiments in male rats \(51.1\% \); not significantly different from either value) (Kaddumi and Hubscher 2006, 2007). A typical example from a male rat is provided in Fig. 8. Evaluation of
almost all neurons tested in 12 female rats (n = 60) revealed that the MRF responses to bladder filling occurred either just before (43%) or right at (57%) the initiation of the voiding reflex. In addition, most bladder-responsive neurons in the DPGi subregion (94%) responded at the time of voiding, whereas those in the Gi subregion were a mix of just before and at void response times, adding to the complexity of the central neural circuitries for the control of micturition. Note that an example of a before void response is shown in Fig. 2.

**Histology.** Neurons from all 36 rats combined (236 in total) were distributed across various MRF subregions, the majority of which were located within the Gi (n = 171; 72.5%). Significant differences (P < 0.05) based on location were found related to sex for the DPGi subregion and for GiA for DNP neurostimulation (Table 1), which could reflect differences in neurostimulation activation thresholds and/or differences in circuitries.

**DISCUSSION**

*Summary.* The results of this study demonstrate that many MRF neurons are responsive to electrical stimulation of several nerves innervating the pelvic region. This was true when targeting a distal nerve branch in both males (DNP stimulation) and females (DNC stimulation) as well as a more proximal nerve target (L6/S1 trunk). At all stimulation sites, the majority of MRF neuronal responses were excitatory, though both inhibitory and “mixed” cellular responses were also seen. Stimulation amplitudes to evoke MRF responses differed across nerve targets and sex (L6/S1 trunk > DNP > DNC) and also showed some variation across different subregions within the MRF. Stimulation frequencies to induce MRF responses were similar at all sites and generally plateaued (e.g., maximum response) at 30–40 Hz. This frequency (30 Hz) also effectively increased voiding cycles during filling, and many MRF neurons altered their firing near or at the time of voiding during bladder filling experiments.

**MRF responses to neurostimulation at DNP, DNC, and L6/S1.** MRF neuronal response properties to DNP and L6/S1 trunk neurostimulation were excitatory (majority; firing at least twice the threshold relative to background), inhibitory (firing at a rate at least half of background), and mixed (responding with excitation or inhibition, depending on the convergent territory being stimulated).

Sex differences in the MRF responses to stimulation of the sensory branch of the pudendal nerve (DNP/DNC) were found, likely reflecting differences in their respective roles for mating and reproduction. There were several differences between male and female rats.
and female rat MRF responsiveness to neurostimulation, including the amplitude threshold, response latency, and cellular properties (ratio of excitatory to inhibitory to mixed), which likely reflects the composition of afferent types in the nerves relating to their roles in various aspects of urogenital functions. The lower amplitude threshold for DNC stimulation could also relate to the presence of ovarian steroids (low serum concentrations present, as female rats were not tested if they were in or entering the proestrus stage of their cycle). In previous studies using female rats, neuronal responses to pelvic re-productive organ stimulation were found to vary with stage of estrus as a consequence of levels of estradiol and not progesterone. These findings were demonstrated for neurons present at different regions of the neural axis, including the preoptic area of the hypothalamus and bed nucleus of the stria terminals (Chadha and Hubscher 2008), the thalamus (Reed et al. 2009), and the MRF (Hubscher 2006). Responses to cervix stimulation at low pressures were consistent with low and not high serum levels of ovarian hormones in the present study (Chadha and Hubscher 2008; Hubscher 2006; Reed et al. 2009).

The location of the electrodes revealed a significantly lower MRF response threshold for stimulation of the DNP relative to the L1/S1 trunk, a finding consistent with previous cat studies (Snellings and Grill 2012) and a likely consequence of directly stimulating the appropriate axons and also avoiding unwanted muscle contractions that would occur with the higher stimulus amplitudes that would be needed for activation of the L4/S1 trunk.

**MRF responses to bladder filling and voiding.** The present data revealed that many MRF neurons respond to bladder infusion. Two distinct responses were seen in MRF, one involving responses prior to voiding and the other at the time of void. These two types of responses could participate in multiple aspects of eliminative functions including continence and micturition. For example, the reciprocal activation and inhibition may result in rhythmic activity such as the fill and void cycle of the bladder. A previous study in cat demonstrated two ascending and two descending pathways for micturition, with direct projections between the pontine micturition center and spinal cord and indirect projections via the MRF (Sugaya et al. 2003). That study demonstrated MRF neurons involved in storage and voiding of urine (Sugaya et al. 2003), findings consistent with the present study in rats as well as previous work that also demonstrated neurons in the ventromedial medulla responding before and during micturition (Baez et al. 2005). Those responses of MRF neurons just prior to voiding have been related to a preparatory role for micturition, as demonstrated with a variety of noxious stimuli that delay the onset of voiding (Baez et al. 2005). Given the established role of MRF neurons in sexual function, it is possible that MRF neurons may also participate in the cross-inhibition of different behaviors (e.g., urination is inhibited during sexual activity).

**DNP stimulation effects on voiding.** DNP stimulation increased the voiding cycle during filling at relatively low amplitudes and frequencies, with a lasting aftereffect of 2 min on average after stimulation. This finding is consistent with clinical neuromodulation studies showing that conditional and continuous DNP stimulation significantly increases bladder capacity (Kirkham et al. 2001). The stimulus parameters to evoke the response are also consistent with animal studies showing that low-frequency DNP stimulation inhibits bladder contractions (Su et al. 2012a; Woock et al. 2008). Studies in adult cats also indicate that optimal inhibition of bladder contractions has a wider effective range of frequencies and amplitudes via DNP stimulation than other targets (pudendal, S1) (Snellings and Grill 2012).

In contrast, stimulation of DNC afferents in cats has also been shown to promote bladder contractions. In multiple studies, stimulation frequencies in the 20–40 Hz range evoked bladder contractions, but even lower frequencies (10 Hz) could promote contractions, which was hypothesized to be related to stimulation of multiple pudendal branches and/or afferent fiber diameter (Boggs et al. 2005; Snellings and Grill 2012; Yoo and Grill 2007). Note that pudendal sensory afferent discharges with similar ranges of frequencies have been shown to be related to a rate of fluid flow along the urethra (Todd 1964). The above data are also consistent with the low-amplitude stimuli

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**Table 1. Summary of responsive MRF neurons by subregion locations based on electrode track reconstructions**

<table>
<thead>
<tr>
<th>Neurostimulation</th>
<th>Site</th>
<th>No. of Units</th>
<th>Units per Track</th>
<th>% DPgi</th>
<th>% Gi</th>
<th>% GiA</th>
<th>% IRt</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNP</td>
<td>L1/S1</td>
<td>102</td>
<td>2.9</td>
<td>14.7</td>
<td>71.6</td>
<td>7.8*</td>
<td>5.9</td>
</tr>
<tr>
<td>DNC</td>
<td></td>
<td>64</td>
<td>2.1</td>
<td>29.7*</td>
<td>67.2</td>
<td>0</td>
<td>3.1</td>
</tr>
</tbody>
</table>

MRF, medullary reticular formation; Gi, nucleus reticularis gigantocellularis; GiA, Gi pars alpha; DPgi, dorsal-Gi; IRt, intermediate reticular nucleus; DNP, dorsal nerve of the penis; DNC, dorsal nerve of the clitoris.

*Significantly different from other groups (P < 0.05).

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Fig. 7. Bladder pressure recording (0.25 ml/min infusion rate; raw record shown) in a urethane-anesthetized male rat illustrating the effect of bilateral DNP neurostimulation on voiding. Pressures ranged from a baseline of 10 mmHg (13.6 cmH2O) to a peak of ~35 mmHg (47.6 cmH2O). Note the slight rise in pressure at the offset of the 3-min DNP stimulus. Single drops of urine would release every 20–30 s during the stimulation as well as after it until voiding resumed (between 2 and 3 min after stimulus offset for stimulation intensities at the response threshold). The interruption in the voiding cycle in the example shown lasted 38 min until voiding cycles resumed to the pre-stimulation pattern (suprathreshold intensity of 20 μA at a frequency of 30 Hz was used for example shown).

Fig. 8. Typical MRF neuronal recording illustrating the onset of an excitatory response at the time of voiding. Note that the bladder was not emptied prior to infusion of saline (thus a relatively short response time).
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REFERENCES


