External urethral sphincter motor unit recruitment patterns during micturition in the spinally intact and transected adult rat

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D’Amico SC, Collins WF 3rd. External urethral sphincter motor unit recruitment patterns during micturition in the spinally intact and transected adult rat. J Neurophysiol 108: 2554–2567, 2012. First published July 25, 2012; doi:10.1152/jn.00927.2011.—In the rat, external urethral sphincter (EUS) activation during micturition consists of three sequential phases: 1) an increase in tonic EUS activity during passive filling and active contraction of the bladder (guarding reflex), 2) synchronized phasic activity (EUS bursting) associated with voiding, and 3) sustained tonic EUS activity that persists after bladder contraction. These phases are perturbed following spinal cord injury. The purpose of the present study was to characterize individual EUS motor unit (MU) patterns during micturition in the spinally intact and transected adult rat. EUS MU activity was recorded from either the L5 or L6 ventral root (intact) or EUS muscle (transected) during continuous flow cystometry in urethane-anesthetized adult female Sprague-Dawley rats. With the use of bladder pressure threshold and timing of activation, four distinct patterns of EUS MU activity were identified in the intact rat: low threshold, medium/high threshold sustained, medium/high threshold not sustained, and burst only. In general, these MUs displayed little frequency modulation during active contraction, generated high-frequency bursts of action potentials during EUS bursting, and varied in terms of the duration of sustained tonic activity. In contrast, three general patterns of EUS MU activity were identified in the transected rat: low threshold, medium threshold, and high threshold. These MUs exhibited considerable frequency modulation during active contraction of the bladder, no bursting behavior and little to no sustained firing. The prominent frequency modulation of EUS MUs is likely due to the enhanced guarding reflex seen in EUS whole muscle electromyogram recordings in transected rats (D’Amico SC, Schuster IP, Collins WF 3rd. Exp Neurol 228: 59–68, 2011). In addition, EUS MU recruitment in transected rats more closely followed predictions by the size principle than in intact rats. This may reflect the influence of local synaptic circuits or intrinsic properties of EUS motoneurons that are active in intact rats but attenuated or absent in transected rats.

rhabdosphincter; motoneuron; EUS bursting; guarding reflex; spinal cord injury

The pattern of activation of the external urethral sphincter (EUS) during micturition in the adult rat is well established (de Groot et al. 1998; Fraser 2011; Kruse et al. 1993; Maggi et al. 1986; Mersdorf et al. 1993; Streng et al. 2004; Van Asselt et al. 1995) and consists of three distinct phases. 1) Prior to voiding, passive filling and active contraction of the bladder result in tonic activation of the EUS muscle (i.e., the guarding reflex) that increases with bladder pressure and functions to maintain continence. 2) At the peak of bladder contraction, the emergence of highly synchronized phasic EUS activity (i.e., EUS bursting) coincides with and promotes efficient voiding. 3) Following EUS bursting, there is a period of extended EUS activity (i.e., sustained tonic EUS activity) that persists past complete relaxation of the bladder (D’Amico et al. 2011).

As a model for studying spinal cord injury (SCI) and its impact on the lower urinary tract, many studies have examined changes in bladder and EUS activity following midthoracic SCI in the adult rat (Kruse et al. 1993; Leung et al. 2007; Pikov and Wrathall 2001, 2002; Wrathall and Emch 2006; Yoshiyama et al. 2000). Extensive focus has been given to addressing the deleterious effects of SCI on bladder function, primarily through assessment of relevant cystometric variables (e.g., bladder capacity, voiding volume, residual volume, and voiding efficiency) during micturition. Because these variables are highly dependent on the efficacy of EUS bursting, more recent studies have addressed the process of EUS activity using whole EUS muscle electromyography (EMG) in the spinally intact and injured rat. However, other aspects of EUS activity associated with micturition such as the guarding reflex and sustained tonic activity have received much less attention. Previous work from this laboratory quantified EUS activity in the intact rat and demonstrated that chronic spinal cord transection increased the guarding reflex and inhibited sustained tonic EUS activity (D’Amico et al. 2011).

Most of our understanding of EUS activation during micturition in the adult rat is based on whole muscle EMG recordings in combination with continuous flow cystometry. Although basal discharge frequencies of single EUS and external anal sphincter (EAS) motor units (MU) in urethane-anesthetized rats have been reported (Buffini et al. 2012), there has been no attempt to characterize EUS MU recruitment patterns during micturition before or after SCI. Knowledge of EUS MU recruitment in the rat is of paramount importance to fully understand the role of the EUS in micturition and any pathological changes associated with SCI. It is expected that recruitment patterns will vary across EUS MUs, since combinations of histochemical and in vitro electrophysiological analyses indicate that the rat EUS is composed of different types of muscle fibers. Immunohistochemical detection of myosin heavy chain (MHC) in the female rat EUS confirmed the presence of both slow and fast muscle fibers, the preponderance of which were of the fast MHC type (Praud et al. 2003). In a more detailed analysis, Buffini et al. (2010) found the rat EUS to be largely oxidative as a whole and, at the single muscle fiber level, determined it to be a mixed muscle composed of fast, fatigue-resistant (type 2A myosin), fast-fatiguing (type 2B myosin), and slow oxidative (type 1 myosin) fibers. In...
vitro intracellular recordings from EUS motoneurons in the adult rat (Carp et al. 2010) revealed EUS motoneurons with long- or short-duration afterhyperpolarizations (AHP) indicative of type S or type F MUs, respectively (Gardiner 1993; Zengel et al. 1985).

The purpose of the present study was to characterize the recruitment patterns of individual EUS MUs during micturition in the spinally intact and transected adult rat so as to better understand the impact of SCI on the process of EUS activation. Consistent with the observation that the EUS is composed of different muscle fiber types, diverse patterns of EUS MU activity were identified in the intact rat on the basis of bladder pressure threshold (i.e., the bladder pressure at which EUS MUs became activated), instantaneous firing frequency, and sustained tonic activity. In contrast, EUS MU activity patterns in the transected rat were less distinct, with no EUS bursting and little to no sustained tonic activity. As such, individual EUS MUs were classified almost exclusively on the basis of bladder pressure threshold. However, these MUs exhibited greater spike frequency modulation during the guarding reflex. Results of this study have been presented in abstract form (D’Amico and Collins 2010).

MATERIALS AND METHODS

Chronic Spinal Cord Transection and Animal Care

All terminal experiments and survival surgeries necessitating animal care were done with adherence to the policies of and approved by the Stony Brook University IACUC and Office of Research Compliance. Of the 28 adult female Sprague-Dawley rats (Taconic; 200–300 g) used in this study, 15 underwent complete spinal cord transection as previously described (D’Amico et al. 2011). Briefly, under isoflurane anesthesia (5% induction, 2% maintenance) and with a dorsal approach, the spinal cord was transected after laminectomy at L3/L4 level of the spinal cord and retracted laterally. Under mineral oil, the dura was cut down the midline of the spinal cord and retracted laterally. With the use of glass nerve hooks, the L5 or L6 VR was placed in continuity on bipolar platinum electrodes and identified by antidromic stimulation of the ipsilateral PMN. Action potential conduction distance was determined by measuring the length of a suture placed between the PMN and VR electrodes to calculate conduction velocity.

Under mineral oil, the L5 or L6 VR was split multiple times in continuity by using Dumont forceps (no. 5; Fine Science Tools) to produce fine root filaments for recording single EUS motor axon activity. Simultaneous recordings of bladder pressure, EUS EMG, left PMN ENG, and left L5 or L6 VR ENG were digitized and sampled at 20 kHz with a DAQ analog-to-digital converter card (M-series; National Instruments) during repeated micturition events and stored using the software Igor Pro 6.1 (WaveMetrics). The EUS EMG, PMN ENG, and VR ENG signals were amplified (EUS EMG gain ×1,000, PMN and VR ENG gain ×10,000) through an AC differential preamplifier (A-M Systems) with high- and low-pass filters set to 0.1 and 10 kHz, respectively. The bladder pressure signal was amplified through a PM-1000 transducer amplifier (CWE) and filtered DC to 0.5 kHz.

Identification of EUS MUs in the Spinally Intact Rat

Confidence in the positive identification of EUS MUs in VR filament recordings was dependent on several criteria. 1) Phasic activity in the VR was time-locked to bursting activity in both the PMN and EUS. 2) In 19 of the 64 EUS MUs, it was possible to positively identify via spike-triggered-averaging orthodromic MU action potential propagation through the distal PMN, a site that only contains motor axons that innervate the EUS and EAS in the female rat (Bremer et al. 2003; McKenna and Nadelhaft 1986). Unpublished preliminary data from this laboratory indicate that EAS MUs do not exhibit phasic activity during micturition (however, see Pezzone et al. 2005; Thor and de Groat 2010). 3) Of the 19 EUS MUs resolved in the PMN, all 4 EUS MU patterns reported in this study were represented. 4) The initial attempt to directly record from the EUS in the intact rat (see Different Recording Techniques) corroborated the existence of the same general EUS MU patterns identified from the VR. Together, these factors firmly indicate that most, if not all, MUs identified from the L5 or L6 VR were EUS specific.

Single-MU Recording in the Spinally Transected Rat

Rats with chronic spinal cord transection (6- to 10-wk survival; n = 15) were anesthetized with urethane and set up for continuous flow cystometry as described above with the exception that wire electrodes were not placed into the EUS. Rather, with the animal supine, a midline abdominal incision was made, and the pubic symphysis was removed to expose the full rostrocaudal extent of the EUS. Single EUS MU activity during rhythmic bladder contractions was recorded.
with a tungsten microelectrode (A-M Systems; 1 MU) advanced into the EUS from the ventrolateral surface using a manual micromanipulator (David Kopf Instruments). The bladder pressure and EUS EMG signals were amplified as described for the intact rat. However, data were sampled at 40 kHz, and the EUS EMG signal was low-pass filtered at 20 kHz.

**Different Recording Techniques**

As outlined in the previous sections, two different methods were used in the present study to record single EUS MU activity: 1) extracellular recording of L5 or L6 VR filaments using bipolar platinum hook electrodes, and 2) direct recording from the EUS muscle using a tungsten microelectrode. However, this decision was not made arbitrarily. Initially, attempts were made to record MU activity directly from the EUS muscle to ensure the identity of the MU. However, in spinal intact rats, the movement of the muscle during phasic activity associated with voiding had a tendency to dislodge the electrode from the surface of the muscle, making it difficult to cleanly resolve MU activity during EUS bursting and to stabilize the amplitude of the signal long enough to accurately measure sustained tonic activity. Consequently, this approach was abandoned in the intact rat in favor of simultaneous extracellular recordings of the L5 or L6 VR, PMN, and EUS. This technique proved advantageous because it was possible to use the phasic activity recorded in the VR filament to identify putative EUS MUs. Additionally, in some cases, conduction velocity could be calculated. In transected rats, direct recording from the EUS yielded stable single-unit recordings since the absence of EUS bursting mitigated the instability problem. Thus both of these techniques were attempted in intact and transected rats; however, the recording of single fibers in the VR was more successful in the intact animal, whereas directly recording from the EUS muscle was better suited toward rats with chronic spinal transection.

**Data Analysis**

In spinal intact and transected rats, the timing of action potentials generated by individual EUS MUs was detected with the use of software amplitude/window discrimination (custom program in Igor Pro 6.1) and expressed as instantaneous frequency. For intact rats, MU action potentials recorded from VR filaments were used to resolve single-MU action potentials in the ipsilateral PMN and the EUS muscle via spike-triggered-averaging. In addition to verifying propagation through the PMN to the EUS, this allowed calculation of MU latency in the PMN for conduction velocity measurements ($n = 19$). Conduction latency measurements were not possible in transected rats. Individual MUs in the intact ($n = 64$) and spinaly transected rat ($n = 83$) were grouped according to their bladder pressure threshold and pattern of activity. Data from EUS MUs of the same group were pooled, averaged, and expressed as means (SD) ($n$), with $n$ denoting the number of EUS MUs. Statistical significance ($P < 0.05$) was determined using one-way ANOVA and post hoc tests in SigmaPlot 11.

**RESULTS**

**EUS MU Patterns in the Intact Rat**

The first goal of this study was to determine the organization of EUS MU recruitment in the spinaly intact rat during micturition. Motor axon activity of individual EUS MUs ($n = 64$) was recorded from small L5 or L6 VR filaments isolated in continuity. Each MU exhibited phasic activity during voiding that was time-locked to the bursting activity recorded simultaneously in the PMN and EUS. However, there was considerable variability between MUs in the bladder pressure threshold (i.e., the bladder pressure at the onset of MU activity) and the duration of MU activity following complete bladder relaxation (i.e., sustained tonic activity). On the basis of bladder pressure threshold and duration of sustained activity, EUS MUs in the intact rat were divided into four groups: 1) low threshold sustained (LS; $n = 15/64, 24\%$), 2) medium/high threshold sustained (MHS; $n = 38/64, 59\%$), 3) medium/high threshold not sustained (MHNS; $n = 5/64, 8\%$), and 4) burst only (BO; $n = 6/64, 9\%$).

LS MUs were always recruited during passive filling before active bladder contraction and remained active after complete bladder relaxation (i.e., exhibited sustained tonic activity) (Fig. 1A). MHS MUs were recruited during active bladder contraction before or at the onset of EUS bursting and exhibited sustained tonic activity (Fig. 1B). MHNS MUs were recruited during active bladder contraction before or at the onset of EUS bursting and exhibited only brief tonic firing after EUS bursting that was not sustained past complete bladder relaxation (Fig. 1C). Finally, BO MUs were recruited immediately at the onset of EUS bursting and exhibited no sustained tonic activity after EUS bursting (Fig. 1D).

**Duration of MU activity.** In the intact rat, each group of EUS MUs exhibited a systematic difference with respect to duration of tonic activity before and after EUS bursting (Table 1). LS MUs exhibited the longest total duration of neuronal activity, as well as the longest duration of activity before EUS bursting. However, the difference in duration of sustained tonic activity after EUS bursting was not found to be statistically significant between LS and MHS MUs. In contrast, MHNS MUs exhibited brief neuronal activity immediately before and after bursting. By definition, BO MUs exhibited no tonic EUS activity before or after EUS bursting. As such, MHNS and BO MUs exhibited the shortest durations of neuronal activity.

**Frequency of MU activity.** In the intact rat, most EUS MUs exhibited little frequency modulation during the guarding reflex but reached high instantaneous frequencies during EUS bursting. EUS MU instantaneous frequency was measured 1) at the time of MU activation (i.e., onset frequency), 2) immediately before, during, and immediately after EUS bursting, and 3) at the time of MU inactivation (i.e., off frequency) (see Table 1). To get a sense of the contribution that each MU had on the guarding reflex (i.e., during passive bladder filling and active bladder contraction), we compared the instantaneous frequency at activation (onset firing frequency) with the frequency just before the start of EUS bursting. LS MUs ($n = 6/6$) were activated during passive bladder filling and showed moderate to large increases in instantaneous frequency with increasing bladder pressure before EUS bursting. Upon activation, LS MUs initially exhibited stochastic low-frequency firing ($1.7 \pm 1.6$ Hz) that transitioned into rhythmic firing ($8.3 \pm 2.2$ Hz) during passive bladder filling and continued to increase in frequency during active bladder contraction (see Fig. 1A). In contrast, MHS and MHNS MUs were activated during active contraction of the bladder, and only a very small number of MHS MUs ($n = 4/30$) and no MHNS MU ($n = 0/5$) exhibited significant increases in firing frequency during the period of active bladder contraction (Fig. 2A). Since BO MUs exhibited no tonic activity before bursting, this measurement was undefined and they were excluded from this analysis.

In whole muscle EMG recordings, EUS bursting in the rat is characterized by phasic bursts of activity (i.e., burst events)
alternating with periods of complete silence during which voiding occurs. Since EUS bursting is essential for efficient voiding in the adult rat (Conte et al. 1991; Kruse et al. 1993; Peng et al. 2006, 2008; Yoshiyama et al. 2000), it was imperative to examine MU behavior during this time. In the present study, many EUS MUs exhibited a gradual transition from tonic firing to phasic activity before EUS bursting. This was typically seen in low-threshold EUS MUs (e.g., all LS and some MHS MUs) that developed a burstlike pattern of activity that progressively became more synchronized, leading to full synchronization of EUS MU activity at the onset of EUS bursting (example in Fig. 3).

During EUS bursting, each MU fired a series of individual burst events. Each burst event was composed of one to five action potentials at two or three dominant frequency ranges (Figs. 4 and 5). The first action potential invariably had the lowest instantaneous frequency and reflected the frequency of the burst event (6–8 Hz). Almost universally, the second action potential occurred at the shortest interval corresponding to the upper range of dominant frequencies (75–90 Hz), but

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**Fig. 1.** Classification of external urethral sphincter (EUS) motor unit (MU) activity patterns in the spinally intact rat. A–D each illustrate simultaneous recordings of bladder pressure (top, arrows signify beginning of active bladder contraction) and single EUS MU activity in a L5 or L6 ventral root (VR) filament (electroneurogram, ENG; bottom) during a micturition event. EUS MUs were detected (circles) using amplitude/window discrimination. A: an example of a low-threshold sustained (LS) MU. All LS MUs were recruited before the beginning of active bladder contraction and exhibited sustained tonic activity following complete relaxation of the bladder. B: an example of a medium/high-threshold sustained (MHS) MU. MHS MUs were recruited during active bladder contraction or just before EUS bursting and exhibited sustained tonic activity following complete relaxation of the bladder. C: an example of a medium/high-threshold not sustained (MHNS) MU. Similar to MHS MUs, MHNS MUs were recruited during active bladder contraction or just before EUS bursting; however, they exhibited tonic activity following EUS bursting that did not persist past complete bladder relaxation (i.e., not sustained). D: an example of a burst-only (BO) MU. BO MUs were recruited immediately at the onset of EUS bursting and, by definition, exhibited no tonic activity after EUS bursting.
Table 1. Quantification of EUS MU activity in the spinally intact rat

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Low Threshold Sustained</th>
<th>Medium/High Threshold Sustained</th>
<th>Medium/High Threshold Not Sustained</th>
<th>Burst Only</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bladder pressure threshold, mmHg</td>
<td>7.0 (SD 1.2)‡† (14)</td>
<td>12.0 (SD 3.1)† (34)</td>
<td>15.3 (SD 4.2)† (5)</td>
<td>17.3 (SD 2.3)† (6)</td>
</tr>
<tr>
<td>Total duration of MU activity, s</td>
<td>106.0 (SD 88.9)† (11)</td>
<td>40.9 (SD 24.6)† (37)</td>
<td>10.0 (SD 2.3)† (5)</td>
<td>5.9 (SD 2.4)† (6)</td>
</tr>
<tr>
<td>Conduction velocity, m/s</td>
<td>22.5 (SD 9.2) (4)</td>
<td>16.7 (SD 6.0) (11)</td>
<td>22.5; 24.0 (2)</td>
<td>19.0; 25.0 (2)</td>
</tr>
<tr>
<td>Before EUS bursting</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duration of MU activity, s</td>
<td>47.1 (SD 55.5)§ (11)</td>
<td>2.5 (SD 2.5) (37)</td>
<td>1.5 (SD 1.4) (5)</td>
<td>N/A</td>
</tr>
<tr>
<td>Onset frequency, Hz</td>
<td>1.7 (SD 1.6)* (11)</td>
<td>6.0 (SD 2.4) (37)</td>
<td>5.8 (SD 3.4) (5)</td>
<td>5.7 (SD 1.9) (6)</td>
</tr>
<tr>
<td>Frequency immediately before burst, Hz</td>
<td>32.3 (SD 12.5)* (6)</td>
<td>9.8 (SD 8.8) (29)</td>
<td>7.8 (SD 1.1) (5)</td>
<td>N/A</td>
</tr>
<tr>
<td>During EUS bursting</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of burst events</td>
<td>30.4 (SD 25.6) (5)</td>
<td>34.3 (SD 20.8) (27)</td>
<td>36 (SD 4.6) (5)</td>
<td>25.8 (SD 10.4) (6)</td>
</tr>
<tr>
<td>No. of action potentials per burst event</td>
<td>3.4 (SD 0.9) (5)</td>
<td>3.0 (SD 0.6) (24)</td>
<td>2.6 (SD 0.5) (5)</td>
<td>2.2 (SD 0.3)* (6)</td>
</tr>
<tr>
<td>Total no. of action potentials</td>
<td>107.0 (SD 74) (3)</td>
<td>102.9 (SD 75.8) (24)</td>
<td>95 (SD 24.8) (5)</td>
<td>58.1 (SD 30.7) (6)</td>
</tr>
<tr>
<td>Burst event frequency, Hz</td>
<td>7.7 (SD 1.7) (4)</td>
<td>8.5 (SD 1.4) (27)</td>
<td>7.9 (SD 1.0) (5)</td>
<td>6.9 (SD 0.9) (6)</td>
</tr>
<tr>
<td>Middle dominant frequency, Hz</td>
<td>44.6 (SD 51.7) (4)</td>
<td>47.5 (SD 60.6) (23)</td>
<td>53.6 (SD 3.4) (5)</td>
<td>43.3 (SD 4.9) (6)</td>
</tr>
<tr>
<td>Upper dominant frequency, Hz</td>
<td>91.3 (SD 20.4) (3)</td>
<td>81.5 (SD 13.5) (17)</td>
<td>88.1 (SD 9.1) (3)</td>
<td>67 (1)</td>
</tr>
<tr>
<td>Maximum instantaneous frequency, Hz</td>
<td>169.0 (SD 51.3) (3)</td>
<td>126.5 (SD 48.4) (20)</td>
<td>148.5 (SD 31.8) (5)</td>
<td>63.5 (SD 13.3)* (6)</td>
</tr>
<tr>
<td>After EUS bursting</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duration of MU activity, s</td>
<td>42.0 (SD 37.8) (11)</td>
<td>33.5 (SD 23.3) (38)</td>
<td>2.4 (SD 1.1)* (5)</td>
<td>N/A</td>
</tr>
<tr>
<td>Frequency immediately after burst, Hz</td>
<td>37.4 (SD 2.9) (5)</td>
<td>42.3 (SD 9.0) (26)</td>
<td>34.6 (SD 12.3) (5)</td>
<td>N/A</td>
</tr>
<tr>
<td>Off frequency, Hz</td>
<td>3.9 (SD 2.6) (11)</td>
<td>3.4 (SD 1.7) (37)</td>
<td>3.7 (SD 1.2) (5)</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Values are means (SD) (n), where n is the no. of external urethral sphincter (EUS) motor units (MUs). For each parameter (within same row): *P < 0.05, mean value is statistically different from the other group means; †P < 0.05 or ‡P < 0.05, mean values with same symbols are statistically different from one another.

with wide variability in terms of individual instantaneous frequencies (as high as 150 Hz). Subsequent action potentials generally occurred at an intermediate frequency comprising the middle dominant frequency range (40–55 Hz). LS, MHS, and MHNS MUs fired three to five action potentials per burst event (example in Fig. 4A) and exhibited three dominant frequencies (burst event, middle, upper; Fig. 5, A–C). In contrast, BO MUs fired only one to two action potentials per burst event (example in Fig. 4B) and consistently exhibited only two dominant frequencies (burst event and middle; Fig. 5D). The highest maximum instantaneous frequencies were seen in LS MUs (upper dominant frequency range), whereas BO MUs fired at the lowest maximum frequencies (middle dominant frequency range).

Finally, for LS, MHS, and MHNS MUs, the instantaneous frequency of tonic activity immediately after EUS bursting was compared with the frequency of tonic firing immediately before bursting (Table 1). LS MUs fired at similar instantaneous frequencies immediately before and after EUS bursting. In contrast, the instantaneous frequency immediately after EUS bursting in MHS and MHNS MUs was significantly higher than the instantaneous frequency immediately before bursting. In addition, the average tonic firing frequency after EUS bursting was similar among LS, MHS, and MHNS MUs (35–45 Hz). Since, by definition, BO MUs exhibited no tonic activity before or immediately after EUS bursting, they were excluded from this analysis.

**MU conduction velocity.** For 19 EUS MUs in the intact rat, it was possible to clearly resolve the MU action potential propagating through both the VR filament and the PMN. In these cases, MU conduction velocity was calculated by measuring the conduction distance and MU action potential latency between the VR and PMN recording electrodes (Fig. 6A). Unfortunately, it was not possible to calculate a conduction velocity for every MU. This was likely the result of a conduction block distal to the VR recording electrodes that interrupted propagation of the MU action potential to the periphery. Although a wide range of conduction velocities was observed among EUS MUs (8–31 m/s), there was no statistical difference in the average conduction velocity between LS, MHS, MHNS, and BO MUs (Table 1). However, the duration of sustained tonic activity appeared to vary inversely with conduction velocity, particularly MHS MUs, such that MUs with slower conduction velocities exhibited longer durations of sustained activity (Fig. 6B).

**EUS MU Patterns in the Spinally Transected Rat**

The second goal of this study was to examine individual EUS MU recruitment patterns during micturition in the spinally transected rat and to compare their organization with MUs in the intact rat. Recordings from single EUS MUs (n = 83) were made directly from the ventral surface of the EUS muscle using tungsten microelectrodes. In contrast to the intact rat, no bursting and little to no sustained tonic activity was observed in these MUs. Consequently, bladder pressure threshold was the primary criterion used to group individual EUS MU activity in the transected rat. Three general patterns of EUS MU activity were identified: 1) low threshold (LT; n = 22/83, 26%), 2) medium threshold (MT; n = 47/83, 57%), and 3) high threshold (HT; n = 14/83, 17%). LT MUs were always recruited before the beginning of active bladder contraction (Fig. 7A), with some (n = 6/22) being tonically active at small bladder volumes and low bladder pressures. In addition, a subset of LT MUs (n = 9/22) remained active past bladder relaxation (i.e., exhibited short-duration sustained tonic activity, 1–15 s). MT MUs were recruited during active bladder contraction, and HT MUs were recruited near the peak of bladder contraction (Fig. 7, B and C). MT and HT MUs never exhibited sustained activity and always ceased firing before complete bladder relaxation.

**Duration of MU activity.** An inverse relationship was noted between bladder pressure threshold and activity duration such that MUs with higher bladder pressure thresholds tended to...
in the transected rat was measured) 1) at the time of activation (i.e., onset frequency), 2) at or near the peak of bladder contraction (maximum instantaneous frequency of tonic firing), and 3) at the time of inactivation (i.e., off frequency). To assess the contribution that each EUS MU had on the guarding reflex in the transected rat, the onset frequency was compared with the maximum tonic firing frequency, the latter of which occurred at or just before peak bladder pressure. The increase in frequency from activation to peak bladder contraction was large in LT MUs, moderate in MT MUs, and small in HT MUs (Fig. 2B). In addition, an inverse relationship was noted between bladder pressure threshold and maximum instantaneous frequency such that MUs of lower bladder pressure threshold typically reached higher maximum tonic firing frequencies (see Fig. 7. A–C). The average maximum frequency between different EUS MU groups in the transected rat was found to be statistically significant (Table 2). It should be noted that the vast majority of MUs (n = 73/83) in the transected rat reached maximum tonic firing before peak bladder pressure (Table 2). Among the 10 MUs that did not, 9 fired maximally at the peak of bladder pressure and 1 immediately after.

**DISCUSSION**

In the present study, individual EUS MUs could be differentiated on the basis of bladder pressure threshold, firing frequency, and duration of activity following micturition. In the intact rat, four EUS MU patterns of activity were identified: low threshold sustained (LS), medium/high threshold sustained (MHS), medium/high threshold not sustained (MHNS), and burst only (BO). During EUS bursting, each of these MUs fired multiple action potentials (1 to 5) within each burst event.
reaching high instantaneous frequencies. However, they displayed little frequency modulation during active bladder contraction and differed mainly with regard to their bladder pressure threshold and degree of sustained tonic activity. In contrast, three general MU activity patterns were identified in the transected rat based on bladder pressure threshold: low threshold (LT), medium threshold (MT), and high threshold (HT). No bursting behavior and little to no sustained firing were observed in transected rats. During active bladder contraction, EUS MU activity increased irrespective of bladder pressure threshold and reached only low to moderate frequencies.

Guarding Reflex

To maintain continence, there is an increase in EUS activity during bladder filling. Referred to as the guarding reflex, this response is initiated through activation of afferents in both the pelvic and pudendal nerves and organized segmentally in the lumbosacral spinal cord (Fowler 2008; Thor and de Groat 2010). Whereas the guarding reflex is prominent in humans (Park et al. 1997), its importance in the rat is less appreciated (McMurray et al. 2006). In the rat, the guarding reflex consists of two distinct phases: 1) a small increase in EUS activity as the bladder passively fills and 2) a robust EUS response during active bladder contraction or events associated with rapid rises in bladder pressure (D’Amico et al. 2011). Therefore, the guarding reflex is somewhat limited during passive bladder filling but quite pronounced when the bladder actively contracts during the initial phase of a micturition event.

In the present study, only 16% of EUS MUs in intact rats exhibited significant frequency modulation as bladder pressure increased. This observation was unexpected, since the EMG activity recorded from the whole EUS has been shown to increase with bladder pressure during active bladder contraction before EUS bursting (D’Amico et al. 2011). It was predominately the LS MUs recruited well before active bladder contraction that exhibited frequency modulation. In contrast, most MHS and MHNS MUs recruited during active bladder contraction did not significantly increase their firing rate before EUS bursting and voiding. Because MHS and MHNS MUs together constitute the majority of EUS MUs (~67%), this strongly suggests that the guarding reflex in the intact rat is largely mediated by the recruitment of additional MUs during active bladder contraction as opposed to increased temporal summation at the level of individual MUs. Nevertheless, one should not discount the role of frequency-modulated LS MUs on the guarding reflex. Because BO MUs were activated immediately at the onset of EUS bursting, it is unlikely that they contribute in any way to the guarding reflex.

In contrast to those in the intact rat, the majority of EUS MUs in the transected rat exhibited marked increases in spike frequency during active bladder contraction. This was particularly the case for LT and MT MUs. Furthermore, nearly all of these MUs reached their maximum firing frequency before peak bladder pressure. A previous study from this laboratory demonstrated that the gain of the guarding reflex is augmented in rats with chronic spinal cord transection (D’Amico et al. 2011). The present data now indicate that this enhanced guarding reflex in the transected rat is largely determined by frequency-modulated LT and MT MUs that accelerate and achieve maximal tonic firing during the period of active bladder contraction. Although HT MUs in the transected rat do share similar characteristics with LT and MT MUs (e.g., frequency modulation), their high bladder pressure threshold, short duration of activity, and low maximum frequency indicate they contribute little to the guarding reflex.
EUS Bursting

EUS bursting in the spinally intact adult rat is characterized by highly synchronized whole muscle phasic activity composed of individual burst events that alternate with periods of total quiescence and is essential for efficient voiding (Conte et al. 1991; Kruse et al. 1993; Peng et al. 2006, 2008; Yoshiyama et al. 2000). SCI disrupts this activity, resulting in urine retention and increased bladder capacity. Because EUS bursting is essential for efficient voiding in the spinally intact rat, it was of interest to observe single-MU behavior during this phase of micturition. In the intact rat, all EUS MUs exhibited synchronized phasic activity during EUS bursting; however, a subset of individual EUS MUs developed burstlike patterns of activity before the onset of EUS bursting and voiding. This occurred primarily in LS and MHS MUs and was evident as a progressive transition to phasic activity during the period of active bladder contraction leading up to EUS bursting. The timing of this transition depended on when the individual MU was recruited. For instance, LS MUs became tonically active during passive bladder filling and exhibited a gradual transition to phasic activity. In contrast, MHS and MHNS MUs became active during active bladder contraction prior to EUS bursting, but exhibited a transition that was much more abrupt.

Fig. 5. EUS MUs in the spinally intact rat exhibit 2–3 dominant firing frequencies (burst event, middle, and upper) during EUS bursting. A–D each illustrate single EUS MU activity in a L5 or L6 VR filament (bottom) expressed as instantaneous frequency (top) during a micturition event. Horizontal bars indicate the timing of EUS bursting. A–C: examples of LS (A), MHS (B), and MHNS MUs (C). Each of these MU groups exhibited 3 dominant frequencies during EUS bursting. D: an example of a BO MU. BO MUs consistently exhibited only 2 dominant frequencies during EUS bursting (burst event and middle).
The results of this study indicate the possibility of two separate excitatory synaptic inputs (potentially mediated by 2 independent interneuron sources) to EUS motoneurons that are simultaneously active and produce different patterns of excitation. The first could be a slow excitatory input that produces smooth depolarization of EUS motoneurons driving them to fire tonically. Superimposed on this slow excitation is a second excitatory input consisting of rapid phasic depolarizations that grow in amplitude and drive the motoneurons to fire in high-frequency phasic bursts. These patterns of simultaneous slow tonic and rapid phasic excitation have been observed in in vivo intracellular rat EUS motoneuron recordings during micturition (Collins 2010). In the present study, low-threshold MUs in intact rats (e.g., LS and some MHS) exhibited tonic firing upon initial activation and gradually transitioned into phasic activity. Higher threshold MUs (e.g., MHS and MHNS) exhibited less tonic firing before abruptly switching to phasic activity, and the highest threshold MUs (e.g., BO) exhibited only phasic activity. A possible explanation is that lower threshold EUS MUs are highly susceptible to both tonic and phasic excitatory inputs and exhibit tonic firing followed by a smooth transition into phasic activity. Conversely, high-threshold MUs are more heavily influenced by phasic excitatory input and consequently exhibit less tonic firing and a more abrupt transition into phasic bursting.

Some investigators have posited a central pattern generator (CPG) in the lumbosacral spinal cord that mediates EUS bursting (Chang et al. 2007; Dolber et al. 2007), so it is tempting to speculate that activity of this CPG is responsible for the increasing phasic excitatory drive to the EUS motoneurons. Once phasic excitation reaches its maximum and EUS
motoneurons become fully synchronized, one could envision complete EUS bursting during voiding as recurring pulsatile discharges of phasic excitation superimposed on tonic inhibition in EUS motoneurons. However, following EUS bursting, the transition back to tonic activity is more abrupt. This was evident not only in single fibers but also in whole muscle EMG recordings, where the termination of EUS bursting was much more rapid than its initiation. However, it is not clear from these observations what mechanism may be responsible for turning off phasic activity, but it is possible that it involves the simultaneous removal of tonic inhibition of EUS motoneurons and direct inhibition of the bursting CPG.

During EUS bursting, all MUs contracted simultaneously firing multiple burst events. However, within a single burst event, the number of MU action potentials varied between one and five and exhibited up to three dominant/preferred frequency ranges (7–8 Hz, 40–55 Hz, and 75–90 Hz) with individual instantaneous frequencies reaching >150 Hz. From previous work that established the EF50 (i.e., the frequency at half-maximum force generation, 29.1 ± 0.8 Hz) and half-relaxation time (27.5 ± 1.1 ms) of the rat EUS (Buffini et al. 2010), it is evident that the high firing frequencies of individual MUs during EUS bursting are well above those needed for temporal summation of muscle contractions and would most likely produce tetanic contractions and maximum sphincteric tension. Also, since all individual EUS MUs fired together during EUS bursting, the idea that the whole EUS muscle bursts as a single symmetrical unit during voiding is undoubtedly correct (Thor and de Groat 2010).

The complete inhibition of EUS bursting has been reported in rats with chronic SCI (Kakizaki et al. 1997; Kruse et al. 1993). However, more recent studies have documented that a spinal mechanism mediating EUS bursting can still become active, albeit less frequently, in the lightly anesthetized or awake rat with incomplete or complete SCI (Cheng and de Groat 2004; Leung et al. 2007). These observations indicate that a EUS bursting mechanism is functional after SCI, yet sensitive to anesthesia. Leung et al. (2007) in particular make the distinction between two groups of rats, “tonic” and “phasic,” where the former does not exhibit EUS bursting and efficient voiding. This is also consistent with a previous study from this laboratory (D’Amico et al. 2011), where very few (~10%) urethane-anesthetized rats with complete spinal cord transection exhibited “burst-transients,” described as the brief (~1 s) appearance of phasic activity that did not necessarily coincide with voiding. It is apparent that EUS bursting with

![Fig. 7. Classification of EUS MU activity patterns in the spinally transected rat.](http://jn.physiology.org/)

**A**–C each illustrate simultaneous recordings of bladder pressure (*top*, arrows signify beginning of active bladder contraction) and single-MU activity recorded directly from the EUS muscle (electromyogram, EMG; *bottom*) during a micturition event. EUS MUs were detected (circles) using amplitude/window discrimination and expressed as instantaneous frequency (*middle*). **A**: an example of a low-threshold (LT) MU. LT MUs were recruited before the beginning of active bladder contraction, fired at moderate maximum instantaneous frequencies, and exhibited little to no sustained tonic activity following complete relaxation of the bladder. **B**: an example of a medium-threshold (MT) MU. MT MUs were recruited during active bladder contraction, fired at low to moderate maximum instantaneous frequencies, and exhibited no sustained activity. **C**: an example of a high-threshold (HT) MU. HT MUs were recruited near or at the peak of bladder contraction, fired at low maximum instantaneous frequencies, and exhibited very short durations of activity.
efficient voiding after complete SCI is undoubtedly a significant component of the micturition response, and the neural substrate mediating this activity is functional in the lumbosacral spinal cord distal to the site of injury. However, it is not ubiquitously expressed under all experimental conditions. In the present study, no transected animal exhibited EUS bursting (i.e., all rats were tonic). Therefore, analysis and quantification in the transected rat was focused on single EUS MU activity associated with rhythmic bladder contractions.

Like EUS bursting, this pattern of bladder-EUS activation has been shown to be present in a significant percentage of rats with SCI and is also thought to be an important component of the micturition response (D’Amico et al. 2011; Kruse et al. 1993; Leung et al. 2007). Since EUS MUs did not exhibit bursting, EUS MUs in the transected rat did not attain maximum instantaneous frequencies comparable to those seen during EUS bursting in the intact rat. Although it is true that some spinaly injured rats can exhibit EUS bursting, one could hypothesize that burst events in the transected rat would consist of a reduced number of MU action potentials with lower maximum instantaneous frequencies than the intact rat. Additionally, because the EUS in transected rats did not burst, there was also no evidence for a progressive transition from tonic to phasic activity during bladder contraction. However, the highest average tonic firing frequency in the transected rat (LT MUs, ~40 Hz) was very close to the instantaneous frequency (35–45 Hz) immediately after EUS bursting of LS, MHS, and MHNS MUs in the intact rat, suggesting an upper frequency limit for tonic firing. These observations indicate that SCI may result in changes to local circuitry in the lumbosacral spinal cord. It is entirely possible that, following SCI, the slow excitatory tonic synaptic input to EUS motoneurons remains functional and is even potentially enhanced (i.e., the guarding reflex) while the phasic excitatory and tonic inhibitory synaptic inputs alluded to earlier are inhibited.

**Sustained Tonic EUS Activity**

In the intact rat, EUS motoneurons display sustained tonic activity following EUS bursting that can persist for up to a minute or longer. However, this activity is largely inhibited following spinal cord transection (D’Amico et al. 2011). In the present study, MUs from the intact rat varied with respect to derecruitment and sustained tonic activity. More than 80% of these MUs (LS and MHS) exhibited significant sustained tonic activity following phasic bursting. Intrinsic persistent inward currents (PICs) are likely candidates for mediating sustained activity in EUS MUs. Ca2+ and Na+ PICs are well known to increase the excitability of spinal motoneurons by producing sustained plateau potentials, repetitive self-sustained firing, and bistable behavior in the turtle, cat, and rat (Heckman et al. 2005, 2008; Li et al. 2007; Murray et al. 2011; Rank et al. 2011). Recording intracellularly from rat EUS motoneurons, Carp et al. (2010) found that a large population of cells (~40%) exhibited spontaneous tonic firing in the absence of stimulation. Another study investigating membrane properties of EUS motoneurons in the cat revealed that these neurons displayed repetitive firing after afferent pudendal nerve stimulation that could be quenched by injection of hyperpolarizing current (Paroschy and Shefchyk 2000). These data support a possible role of PICs in EUS motoneuron activation; however, their existence has not been conclusively demonstrated in adult rat EUS motoneurons. In the present study, it was not possible to identify a specific mechanism for sustained tonic activity; however, it is likely that the putative mechanism is distributed systematically across the EUS motoneuron pool. For instance, sustained tonic activity of EUS MUs was found to vary inversely as a function of conduction velocity such that the longest durations of repetitive firing were generally seen in MUs with the slowest conduction velocities.

![Fig. 8. Total duration of individual EUS MU activity during micturition plotted as a function of bladder pressure threshold for MU activation. A: in spinally intact rats, total duration of MU activity varied widely, reflecting the duration of sustained activity exhibited in many EUS MUs following micturition events. B: in contrast, sustained activity was largely absent in EUS MUs in spinally transected rats, revealing an inverse relationship between MU activity duration and activation threshold.](http://jn.physiology.org/doi/fig/10.1152/jn.00927.2011)
Table 2. Quantification of EUS MU activity in the spinally transected rat

<table>
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<tr>
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<th>Low Threshold</th>
<th>Medium Threshold</th>
<th>High Threshold</th>
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<tbody>
<tr>
<td>Bladder pressure threshold, mmHg</td>
<td>5.8 (SD 1.7)† (16)</td>
<td>12.1 (SD 3.3)† (47)</td>
<td>23.1 (SD 4.5)† (14)</td>
</tr>
<tr>
<td>Onset frequency, Hz</td>
<td>3.4 (SD 2.0) (10)</td>
<td>6.1 (SD 3.3) (43)</td>
<td>6.2 (SD 4.8) (13)</td>
</tr>
<tr>
<td>Bladder pressure off, mmHg</td>
<td>8.0 (SD 3.8) (15)</td>
<td>17.0 (SD 5.2) (47)</td>
<td>25.2 (SD 5.5) (14)</td>
</tr>
<tr>
<td>Off frequency, Hz</td>
<td>5.3 (SD 3.5) (10)</td>
<td>5.0 (SD 3.4) (43)</td>
<td>6.1 (SD 4.8) (13)</td>
</tr>
<tr>
<td>Maximum instantaneous frequency, Hz</td>
<td>40.2 (SD 6.8) (12)</td>
<td>27.8 (SD 8.5) (42)</td>
<td>17.8 (SD 10.1)† (13)</td>
</tr>
<tr>
<td>Peak bladder pressure, mmHg</td>
<td>21 (SD 8.0) (11)</td>
<td>25.3 (SD 5.6) (47)</td>
<td>28.6 (SD 5.0) (14)</td>
</tr>
<tr>
<td>Bladder pressure at maximum frequency, mmHg</td>
<td>17.8 (SD 7.6) (9)</td>
<td>22.4 (SD 5.6) (42)</td>
<td>26.5 (SD 5.6) (13)</td>
</tr>
<tr>
<td>Duration of MU activity, s</td>
<td>39.0 (SD 25.0) (14)</td>
<td>19.8 (SD 8.3) (47)</td>
<td>5.7 (SD 2.9) (14)</td>
</tr>
<tr>
<td>Duration of bladder contraction, s</td>
<td>30.0 (SD 14.0) (16)</td>
<td>29.8 (SD 13.6) (47)</td>
<td>22.2 (SD 4.0) (14)</td>
</tr>
<tr>
<td>Normalized duration of activity, %</td>
<td>126.0 (SD 28)† (11)</td>
<td>68.3 (SD 17.5)† (47)</td>
<td>25.5 (SD 12.7)† (14)</td>
</tr>
<tr>
<td>Sustained activity, s</td>
<td>3.6 (SD 4.6) (9)</td>
<td>0</td>
<td>0</td>
</tr>
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</table>

Values are means (SD) (n). For each parameter row (within same row): †P < 0.05, mean values are statistically different from one another. Normalized duration of activity was calculated by dividing the duration of MU activity by the duration of bladder contraction and multiplying by 100.

In a previous study (D’Amico et al. 2011), sustained tonic EUS activity in the transected rat was rarely seen, ultimately leading to the expectation that little if any sustained firing would be seen at the level of individual EUS MUs. This turned out to be correct. Although some LT MUs (n = 9/22) continued to fire after complete bladder relaxation, the duration was short-lived (1–15 s). Therefore, EUS stimulation (i.e., bladder stretch and the rise in bladder pressure during active contraction) in the transected rat does not consistently generate prolonged sustained firing in EUS MUs. Although this in agreement with previous data from this laboratory, it is nonetheless surprising since it stands in stark contrast to work by others who have reported the presence of PICs, plateau potentials, and repetitive firing in briefly stimulated sacrocaudal motoneurons in a chronic SCI rat model of spasticity (Bennett et al. 2001, 2004; Li et al. 2003, 2007; Li and Bennett 2003; Murray et al. 2011; Rank et al. 2011). Taken together, these findings indicate that PICs may be active in EUS motoneurons before, but not after, spinal cord transection. Furthermore, the EUS may not exhibit classic signs of spasticity (e.g., sustained activity and rigidity following stimulation) that are regularly seen in tail and hindlimb muscle after chronic spinal injury.

Comparison Between MUs in the Intact and Transected Rat

It is tempting to draw parallels between groups of MUs in the intact and transected rat. Although the putative mechanisms that govern the guarding reflex (i.e., MU recruitment), EUS bursting (i.e., CPG), and sustained tonic EUS activity (i.e., PICs) may be perturbed following spinal cord transection, it is likely that the specific type of MU is preserved. On the basis of bladder pressure threshold, instantaneous frequency, duration of activity, and their similar proportions, one could make the argument that LS, MHS, and BO MUs in the intact rat are correspondingly analogous to LT, MT, and HT MUs in the transected rat. In support of this view, LS MUs (24%) in the intact and LT MUs (26%) in the transected rat were both tonically active at low bladder pressures, became activated in advance of active bladder contraction, and exhibited the longest durations of activation and highest maximum instantaneous frequencies among their respective groups. Moreover, LT MUs were the only group in the transected rat to exhibit any level of sustained activity after bladder relaxation, while LS MUs in the intact rat always displayed sustained firing. In further support of this view, MHS (59%) in the intact rat and MT MUs (57%) in the transected rat all became activated during active bladder contraction and represented the majority of MUs. Finally, BO MUs in the intact and HT MUs in the transected rat shared similar attributes. Both became activated either at or close to the peak in bladder pressure, were active for the shortest periods of time, and exhibited the lowest maximum instantaneous firing frequencies among their respective groups. However, not all EUS MUs fit comfortably into distinct niches due to similar behaviors. For instance, it is ambiguous whether the small population of MHNS MUs in the intact rat is simply a variation of MHS or BO MUs, and whether they are analogous to MT or HT MUs in the transected rat.

Size Principle and Muscle Fiber Type

The classic model for MU recruitment was developed from seminal work on the stretch reflex in decerebrate cats. It describes an orderly recruitment pattern beginning with MUs of smaller size and slower conduction velocity, followed by larger and faster MUs that generate stronger force (i.e., the size principle) (Clamann 1993; Henneman 1957; Henneman et al. 1965a, 1965b; Hodson-Tole and Wakeling 2009). For example, in the cat gastrocnemius, MU recruitment begins with slow oxidative (type S) followed by fast fatigue-resistant (type FR) and, lastly, fast-fatigable (type FF) MUs (Cope and Clark 1991; Henneman and Mendell 1981). In the intact rat, EUS MU recruitment deviated from the classic size principle in three majors ways. First, bladder pressure threshold was independent of MU conduction velocity (Table 1). Second, most EUS MUs (e.g., MHS and MHNS units) exhibited little or no frequency modulation with increasing bladder pressure (Fig. 2A). Third, bladder pressure threshold was not predictive of the duration of EUS MU activity (see Fig. 8A). In contrast, EUS MU recruitment in the spinally transected rat largely followed predictions by the size principle. For instance, once activated, most EUS MUs exhibited progressive increases in firing rate with bladder pressure (Fig. 2B). Additionally, EUS MUs recruited first, LT MUs, had the longest durations of neural activity (i.e., they were the last to be derecruited), followed by MT and HT MUs, respectively (see Fig. 8B). It is tempting to speculate that the different EUS MU recruitment patterns in intact and transected rats reflect SCI-induced changes in spinal circuits. For example, recruitment and frequency modulation prior to EUS bursting in intact rats may be dictated by synaptic inputs (e.g., phasic excitatory inputs underlying EUS bursting or inhibitory inputs activated during voiding) that are attenuated or absent in the transected rat.
Similarly, the sustained EUS MU activity following micturition characteristic of the intact rat may be dependent on descending neuromodulation that is absent in the transected rat.

The EUS in the adult female rat is a heterogeneous muscle. While highly oxidative and fatigue resistant, it is a mixed muscle consisting of both slow-twitch (type 1) and fast-twitch (type 2) muscle fibers (Buffini et al. 2010; Praud et al. 2003). Specifically, the highest proportion of muscle fibers is the fast, fatigue-resistant type (type 2A, ~37%), followed by fast-fatigable (type 2B, ~16%) and slow oxidative (type 1, ~4%). The remaining 30% remain uncharacterized (Buffini et al. 2010). Furthermore, EUS motoneurons exhibit either short- or long-duration AHPs (Carp et al. 2010) characteristic of type S and type F MUs, respectively (Gardiner 1993; Zengel et al. 1985). In the present study, EUS MU activity patterns in the intact rat were also found to be heterogeneous with respect to bladder pressure threshold, instantaneous frequency, and sustained tonic activity. LS MUs constituted 24% of those MUs identified in the intact rat. MHS and MHNS MUs together represented the majority of MUs at 67%, and BO MUs made up the remaining 9%. Given the distribution of muscle fiber types in the EUS and the individual activity patterns of MUs in this study, by analogy with hindlimb muscle, one might expect 

1) LS MUs to innervate oxidative, slow-twitch muscle fibers, 
2) MHS MUs to innervate fast, fatigue-resistant muscle fibers, and 
3) MHNS and BO MUs to innervate fast-fatigable muscle fibers. If one compares the results of the present study with the work carried out by Buffini et al. (2010), it is apparent that the absolute percentages of MU recruitment pattern and muscle fiber type do not match. However, this discrepancy can be reconciled by acknowledging that specific types of MUs differ with respect to the number of muscle fibers innervated. Slow oxidative MUs are generally composed of a small number of individual muscle fibers, and as recruitment progresses, higher threshold MUs with a greater number of muscle fibers are recruited (Clamann 1993; Hodson-Tole and Wakeling 2009).

Conclusions

This is the first comprehensive study of single EUS MU activity during micturition in the spinally intact adult female rat and the first to reveal the heterogeneous nature of EUS MU recruitment consistent with the presence of different muscle fiber types. Furthermore, this is the first documentation of single EUS MU activity in the adult rat following SCI. Although EUS MUs exhibited a broad continuum of bladder pressure thresholds, once activated, MUs in intact and transected rats exhibited very different recruitment patterns. All EUS MUs in the intact rat displayed marked high-frequency phasic activity during EUS bursting, with most exhibiting sustained tonic activity following bladder relaxation but little frequency modulation during active contraction of the bladder. These activity patterns facilitated the categorization of EUS MUs into distinct groups. In contrast, EUS MUs in the transected rat were less distinctive, with no EUS bursting and little to no sustained tonic activity. However, these MUs exhibited a greater acceleration in tonic firing during active contraction of the bladder. The increased frequency modulation of MUs in the transected rat is hypothesized to account for the enhanced guarding reflex seen at the level of the whole muscle.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS


REFERENCES


