Novel technique for monitoring micturition and sexual function in male rats using telemetry

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Nout YS, Bresnahan JC, Culp E, Tovar CA, Beattie MS, Schmidt MH. Novel technique for monitoring micturition and sexual function in male rats using telemetry. Am J Physiol Regul Integr Comp Physiol 292: R1359–R1367, 2007. First published November 9, 2006; doi:10.1152/ajpregu.00532.2006.—We developed a novel technique to simultaneously monitor micturitions and erections in rats by using pressure monitoring within the corpus spongiosum of the penis (CSP). We present data validating this technique and report pressure waveform characteristics of micturition and erectile events during four different behavioral contexts in 10 awake, freely-moving male rats. Telemetric pressure transducers were implanted in the bulb of the CSP. CSP pressure was monitored while the animals were simultaneously recorded on video for determination of presence and volume (n = 7) of micturitions and while the animals underwent behavioral tests for determination of erections. Observed micturitions and CSP pressure waveform characteristics of micturitions occurred simultaneously (r = 0.98) at a frequency of 32 ± 4 micturitions per 24 h and with a volume of 0.95 ± 0.12 mL/urination. Micturition duration recorded by CSP pressure and volume determined by urine weight were highly correlated (r = 0.82). We found that 100% of visually confirmed erectile events occurred simultaneously with CSP pressure waveform characteristics of erections during ex copula reflex erection tests. During noncontact erection and mating tests more erections were identified by telemetry than by observation alone. Erections during mating tests had a different appearance than those seen in other contexts; they were shorter in duration (P < 0.05) and typically were characterized by a single suprasystolic CSP pressure peak, highlighting the context-specificity of erections. Quality of recordings remained stable in three of four rats we followed for 8 wk. We demonstrate that telemetric recording of CSP pressure provides a quantitative and qualitative assessment of penile erections and micturition in freely behaving rats.

Numerous techniques have been developed to monitor either penile erections or micturition events, but there are no published data validating the use of a single technique capable of recording both penile erections and micturitions simultaneously. We report on a new technique involving telemetric monitoring of pressure within the bulb of the corpus spongiosum of the penis (CSP) in freely moving rats and its ability to identify micturition and erectile events.

Existing methods to examine micturition function in rats include cystometric and electromyographic (EMG) techniques (7, 14, 19, 20) and monitoring animals in metabolic cages (7, 24, 25, 26). Limitations of these techniques are: 1) use of anesthesia or heavy sedation and placement of catheters in bladder or urethra, both of which are known to alter the micturition reflex (6, 7, 34); 2) previous techniques are generally suitable only for a single experiment that includes instrumentation and recording, limiting its use to an acute study rather than allowing chronic follow up; 3) compromise of animal well being when animals are housed in metabolic cages for extended periods of time, particularly when animals are paraplegic; and 4) nonphysiological testing situations. Existing methods to study sexual function in rats include visual observation in either copulatory (10) or noncopulatory (24) settings and ex copula reflex erection tests (12, 22). Limitations of these tests include the long-known difficulty and even inability to directly observe erections and the nonphysiological testing situations. A method without these limitations and capable of recording both erections and micturitions would have numerous scientific applications, for example, in animal models of spinal cord injury (SCI) and for monitoring sleep-related erections.

Impairment of lower urinary tract and erectile function following SCI in humans and the subsequent complications are highly prevalent and clinically very important (1, 16), necessitating continued research with respect to the pathophysiology and treatments to improve recovery. Telemetric monitoring of CSP pressure has previously been applied in studies of sleep-related erections (30, 32), ex copula penile erections (31), and SCI (18), but its validation with respect to micturition has been lacking. A recent comprehensive review of urologic and sexual dysfunction (15) makes no mention of this technique, highlighting the importance of validation of the technique as performed in this study.

Investigators of penile erection physiology have reported two different techniques to monitor erectile events in awake rats by continuously recording pressures within either the corpus cavernosum of the penis (CCP; 8) or the CSP (33). During erections, blood enters the cavernous sinuses of the CCP and CSP, causing expansion of the spongy erectile tissue. Thisfilling of the erectile tissues is associated with an increase in baseline CCP and CSP pressures (5, 31). Brief pressure peaks generated in the CCP during the erection in the rat result in the occurrence of flips of the penile body, whereas pressure peaks in the CSP result in engorgement of the glans (31). Pressure peaks that occur in the CCP are secondary to ischio cavernous (IC) muscle contractions (2, 4, 21), whereas pres-

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sure peaks in the CSP occur secondary to bulbospongiousus (BS) muscle contractions (3, 29, 31, 33). The IC and BS muscles display robust EMG activity during visually confirmed erections (2, 3, 11, 13, 31). Contraction of the IC muscles are considered critical for the augmentation of penile rigidity, whereas contractions of the BS muscles are considered essential for augmenting glans engorgement and may aid in ejaculation by generating pressure waves along the urethra to the glans during expulsion of semen (29). These two perineal muscles of the pelvic floor surround and insert onto the CCP and CSP, respectively, and are anatomically positioned to increase CCP and CSP pressures by squeezing or compressing the proximal portions of these erectile tissue systems.

Traditionally, monitoring of CCP pressure is considered the standard method for evaluating erectile activity since the BS muscles and CSP pressures have traditionally been associated with ejaculatory function. However, erections also appear to be consistently associated with CSP pressure changes (31 –33), suggesting that monitoring of CSP pressure is also a valid method of assessing erectile events. Moreover, monitoring CSP instead of CCP pressure carries two distinct advantages. First, the bulb of the CSP is more vascular and contains less dense fibrous tissue than the CCP, allowing easier implantation and longer recording times. Second, our data suggest that CSP recording allows for simultaneous monitoring of erectile (31) and micturition events (18, 28).

For recording penile erections using the telemetric technique described by Giuliano et al. (8) and Schmidt et al. (31 –33), a pressure catheter is implanted in the CCP or bulb of the CSP, respectively. Pressure changes are detected by the transducer and transmitted to a receiver unit through AM radio waves. The receiver unit collects and transmits these signals to a computer system with software that processes and stores the collected data.

The goals of this study were to verify that CSP pressure waveforms obtained by telemetry were consistently associated with micturitions or penile erections and to perform a detailed qualitative analysis of these waveforms. We hypothesized that a positive relationship exists between the duration of the micturition pressure waveform and volume of urine expelled. Furthermore, we hypothesized that CSP pressure waveform characteristics during erections differ depending on the behavioral context during which erections are studied. Here we demonstrate the use of CSP pressure monitoring in the assessment of micturition and sexual function in normal, freely-moving male rats.

MATERIALS AND METHODS

Subjects

Ten adult, male Long-Evans hooded rats (Simonsen Laboratories, Gilroy, CA) age 70 ± 3 days (average ± SE) were used in this study. Rats were housed individually in plastic cages, maintained on a 12:12-h light-dark cycle, and had free access to food and water. All animal experiments were conducted after approval by the Institutional Laboratory Animal Care and Use Committee of The Ohio State University and were performed in compliance with National Institutes of Health guidelines and recommendations.

Surgical Procedures and Postoperative Care

Transducer implantation was carried out aseptically under deep anesthesia induced by intraperitoneal administration of xylazine (10 mg/kg TranquiVed; Vedco, St. Joseph, MO) and ketamine (80 mg/kg ketamine HCl; Abbott Laboratories, N. Chicago, IL). Anesthetic plane was determined by withdrawal to foot pinch. A preoperative dose of cefazolin (50 mg/kg Ancef; Abbott Laboratories) was administered subcutaneously. Lactrilube ophthalmic ointment (Allergan Pharmaceuticals, Irvine, CA) was applied to the eyes prior to surgery, and body temperature was maintained at 37.5 ± 0.5°C using a rectal thermal probe and heating pad.

The rat was placed in dorsal recumbence and surgical sites were shaved and cleaned with Betadine. A 3-cm skin incision was made on midline on the raphe scroti and a 3–4 cm skin incision was made parallel to midline in the right inguinal area. A telemetric pressure transducer catheter (TA11PA-C40; Data Sciences International, St. Paul, MN) was implanted in the bulb of the CSP as described previously (31). Briefly, the battery of the transducer was placed subcutaneously in the lower right abdomen and secured to the external oblique abdominal muscle with two sutures. The catheter was tunneled subcutaneously to the perineal area. Testes were retracted, and BS muscles and CSP were exposed. With minimal retraction of the BS muscles, a guide hole was made into the distal bulb of the CSP with a 21-gauge needle. The open tip of the catheter was placed into the bulb of the CSP and secured with biological glue (Vetbond; World Precision Instruments, Sarasota, FL) and one suture (Fig. 1). This surgical approach is similar to the one used to implant pressure transducers into the CCP (5), with the exception that for implantation in the CCP the open tip of the catheter is placed in the proximal shaft of the right CCP, and here the open tip is placed into the bulb of the CSP on midline. The scrotal incision was then closed in one layer and the inguinal incision was closed in two layers. The rat was allowed to recover, and telemetric studies were commenced 4–5 days postoperatively.

CSP Pressure Recording

Physiological telemetry data were recorded on a personal computer using a Spike2 data acquisition program (version 3.1; Cambridge Electronic Design, Science Park, Milton Road, Cambridge UK). Saturation level of our system is 400–500 mmHg. Animals underwent 2 × 24-h CSP pressure recordings while housed in a plastic cage with a wire mesh floor. Colored paper was mounted on a scale underneath the mesh floor of the cage and was recorded on video, making it possible to see when a micturition event occurred. The volume was based on change in scale readout (1 ml = 1 mg). The receiver unit of the telemetry system was placed on top of the cage. To avoid time discrepancies, the camera time was recorded in a simultaneously running event channel of the acquisition program and used as the start time. In this way, the time and approximate volume of each micturition could be determined and correlated to the pressure wave characteristics. During the dark hours, a red light was placed over the colored paper to facilitate detection of micturition events. For all rats,
Scoring Criteria for Micturitions and Erections

were performed while animals were housed in their regular cages. 2 mo to determine quality of chronic recordings. These recordings the scale readout. Four rats were recorded for a 24-h period weekly for directly compared with the volume of that micturition obtained from forms for one (2 rats) or two (5 rats) 24-h time periods and was the duration of each micturition event was obtained from CSP waveforms for one (2 rats) or two (5 rats) 24-h time periods and was directly compared with the volume of that micturition obtained from the scale readout. Four rats were recorded for a 24-h period weekly for 2 mo to determine quality of chronic recordings. These recordings were performed while animals were housed in their regular cages.

Scoring Criteria for Micturitions and Erections

Viewing window. Scoring of penile erections and micturition was performed manually with respect to CSP pressures and thus required a “viewing window” to allow adequate visualization of the pertinent pressure details, in particular of the lower-level CSP pressures. To score the presence or absence of micturition and erectile events, the CSP pressure range to be displayed on screen (y-axis) was kept to no more than 0–200 mmHg. The time axis (x-axis) was kept <45 s in duration on a typical computer monitor.

Flaccid Baseline (FB). The flacid baseline (FB) was determined for each recording prior to scoring erectile or micturition events. The FB is best determined when the animal is resting (immobile) during either quiet wakefulness or sleep. The FB is characterized by a stable low pressure of 5–15 mmHg that possesses periodic or transient increases of 5–10 mmHg every 15–30 s. The FB is defined as the consistent nadir of the CSP pressure. A horizontal cursor was placed on the CSP pressure recording throughout the scoring session to demarcate the FB (Fig. 2).

Micturition events. Micturition events must consist of the following CSP pressure changes: 1) a rapid series of short duration pressure peaks (~55 ms) with peak pressures generally exceeding 40 mmHg and occurring at a highly conserved frequency of 8–11 Hz (duration of event is typically 3–5 s); 2) several “after peaks” (generally 2–4) occurring every 1–5 s are typically present immediately following the rapid series of pressure peaks; and 3) the troughs of all pressure peaks (during urine flow and after peaks) remain at or only slightly above the FB, and should not exceed the tumescence threshold [FB +30; see Tumescence Threshold (FB +30)].

Urine flow analyses. A vertical cursor was placed immediately preceding the onset of the rapid series of short duration and low amplitude pressure peaks, and a second vertical cursor was placed following the last short duration pressure peak of the series. A detailed analysis was then performed. This included the duration of each urine flow event as defined by the two vertical cursors (micturition event duration). The number of pressure peaks between the two cursors was manually counted to determine the frequency (Hz) of the pressure peaks.

After-peak analyses. The individual after peaks immediately following each micturition event were analyzed. A vertical cursor was placed at the onset of the after peak, defined as the moment when the CSP pressure first demonstrated the sharp rise above the background FB. A second vertical cursor was placed at the end of the individual after peak, defined when the pressure returned to the FB according to the criteria noted above.

Tumescence Threshold (FB +30). The tumescence threshold is defined as the FB + 30 mmHg (FB +30). This tumescence threshold represents the minimum pressure required to be considered a “tumescence baseline” pressure. A horizontal cursor was placed over the CSP pressure recording at a position corresponding to the FB +30 to represent the tumescence threshold (Fig. 2).

Peak Threshold (FB +130). A third horizontal cursor was placed at a position corresponding to the FB + 130 mmHg (FB +130). This peak threshold is used to define full erectile events (see below). This peak threshold of FB +130 was chosen for two reasons. First, a pressure peak of 130 mmHg above the FB is ~1.5 standard deviations below the weakest glans engorgement seen on ex-copula reflex tests (mean E1 CSP peak pressures=223.5 ± 14.5 mmHg; 31). Second, 130 mmHg above the FB represents a pressure peak that is most likely to be suprasystolic and correspond to our definition of a full erection, whereas a pressure <130 mmHg above the FB is more likely to be subsystolic and represent our definition of a partial erection (see Defining full erections and partial erections and Fig. 2).

FB+15 mmHg (FB +15). A fourth horizontal cursor was placed exactly between the FB and the tumescence threshold at a position corresponding to FB +15 mmHg (FB +15). This final cursor was used to help define the end of the erectile event and to differentiate multiple events when in close temporal association [see Defining the end of the erectile event (including the “5–15” rule) and Fig. 2].

Defining full erections and partial erections. Full erectile events must consist of the following CSP pressure changes: 1) an increase in CSP pressure to or above the tumescence threshold (FB +30); 2) at least one suprasystolic CSP pressure peak at or above the peak threshold (FB +130); 3) a pulse pattern in the CSP pressure recording indicating blood flow should be present at some point during the erectile event.

Partial erectile events are generally 5–30 s in duration. These events generally do not have CSP pressure peaks; however, low-level CSP pressure peaks may be present. Partial erectile events must consist of the following CSP pressure changes: 1) an increase in CSP pressure to or above the tumescence threshold (FB +30); 2) a pulse pattern in the CSP pressure recording indicating blood flow should be present at some point during the erectile event; 3) any CSP pressure peaks, if present, must be below the peak threshold (FB +130).

Defining the beginning of the erectile event. The beginning of the erectile event was defined as the moment when the CSP pressure first increases to or above the tumescence threshold (FB +30). A CSP pressure peak of any magnitude greater than the tumescence threshold (FB +30) that precedes the eventual rise in baseline pressure was considered to be part of the erectile event, and thus would define the onset of the erection, as long as the end of the pressure peak occurred no more than 5 s before the CSP pressure raised above the tumescence threshold (FB +30) as part of the remainder of the erectile event (Fig. 2).

Fig. 2. Viewing window of a full erectile event. Vertical cursor lines mark the start and end of the event and horizontal cursor lines mark the flaccid baseline (FB) pressure, the FB+15 mmHg pressure, the tumescence threshold (FB+30 mmHg) pressure, and the peak threshold (FB+130 mmHg) pressure.
Defining the end of the erectile event (including the “5–15” rule). The end of most erectile events was easily defined as the moment when the CSP pressure falls below the tumescence threshold (FB+30). However, during some erectile events, there may be some fluctuation in the CSP pressure during maximal tumescence (such as the transient and immediate drop in pressure below the tumescence threshold following a typical suprasystolic pressure peak or in the event of a rapid series of multiple erectile events), and the “5–15” rule was used to define the end of any single erectile event: 1) if the CSP pressure falls below the FB+15 cursor for >5 s, then any subsequent increase in erectile tissue pressure meeting criteria for an erectile event was considered a separate erectile event; 2) if the CSP pressure does not fall below the FB+15 threshold but remains below the tumescence threshold (FB+30) for >15 s, then any subsequent increase in erectile tissue pressure meeting criteria for an erectile event was also considered a separate erectile event.

Behavioral Tests

CSP pressure recording during behavioral tests was performed to verify simultaneous occurrence of observed erectile events and waveform characteristics of erections on the computer readout. The receiver unit was placed underneath either the restraining cylinder during ex copula erection tests or underneath the cage in which noncontact erection and mating tests were performed. During erection tests, observed erectile events were recorded on the event channel during simultaneous data acquisition.

Ex copula reflex erection tests. Ex copula reflex erection tests were performed twice in 10 awake animals as previously described (10, 12, 31). Briefly, rats were placed on a board in dorsal recumbence with the head and anterior torso in a loose-fitting restraining cylinder. The abdomen was secured to the board with masking tape. The head and anterior torso could move freely in the cylinder. The telemetric receiver unit was placed directly under the board. The preputial sheath was retracted (the stimulus to elicit reflex erections) and maintained in retracted position by placing the glans penis through a hole in a small piece of masking tape fastened to the abdomen. Once the sheath was retracted, the reflex test lasted for 20 min. Rats were habituated to this procedure prior to surgery (2 × 10 min and 2 × 20 min), and the animals quickly adapted to the testing situation. Events were visually scored according to previously described criteria (31): E1, weak glans engorgement; E2, moderate glans engorgement involving some dilation of the distal glans; E3, intense flaring or cup of the distal glans; F1, dorsiflexion of the penile body; F2, dorsiflexion or flip of the penile body greater than 90° with respect to the body of the rat. These reflexes were scored with a numerical keypad as events 1–5, respectively, and were recorded on the event channel during simultaneous data acquisition.

Noncontact erection tests. Noncontact erection tests were performed similarly as described by Sachs et al. (24). Animals were tested twice in a glass aquarium (51 × 30 × 29), which was divided in half by a sheet of wire mesh. The male rat was allowed 5 min to adjust to the new environment prior to the start of the test. An estrous female rat was placed in one compartment and the male in the other. The female rat had been administrated estradiol cypionate (200 µg = 0.1 ml sc) 24 h prior to the test and progesterone (500 µg = 0.1 ml sc) 6 h before the test to ensure receptive behavior. Before the test, female receptivity was verified with nonexperimental male rats. The rats were observed for 30 min. Events (1 = visible erections, 2 = grooming of body parts, 3 = grooming of genital area) were recorded in the event channel as described above. This test was performed with the telemetric receiver unit placed directly under the aquarium.

Mating tests. Mating tests were performed twice in a glass aquarium (51 × 30 × 29). The male rat was allowed a 5-min adjustment period prior to introduction of a female rat in estrous. After the male and female rats were placed together, they were observed for 30 min. The female rat had received the same hormonal treatment as described for the noncontact erection test. Events (1 = visible erections, 2 = grooming of body parts, 3 = grooming of genital area, 4 = mounts, 5 = intromissions, 6 = ejaculations) were recorded in the event channel as described above. This test was performed with the telemetric receiver unit placed directly under the aquarium.

Statistics

Data are presented as means ± SE. To compare the number of micturitions detected by telemetry with those detected by video and to determine the relationship between duration and volume of micturition, regression analysis was performed. Analysis of variance (ANOVA) was used to identify significant differences in CSP waveform characteristics of erectile events recorded in the three different behavioral contexts. The statistical computations were performed with software packages (SigmaStat 3.0 and SPSS 12.0; SPSS, Chicago, IL).

RESULTS

Rats were 70 ± 3 days old and weighed 300 ± 10 g for transducer implantation. Complications that occurred around the transducer implantation site were minor and included mild swelling, loss of hair, and scabbing, all of which healed with time.

For all 10 rats, micturition and erectile events were easily identified from computer readouts, and the characteristics of three types of events are described below. Quality of chronic recordings remained stable in three of the four rats we followed weekly for an 8-wk period. Explanting of the transducer of the 4th rat revealed that the catheter tip was no longer in the CSP.

Micturition

Telemetrically obtained CSP pressure waveforms during a micturition event are characterized by a smooth FB pressure on top of which a rapid series of short duration CSP pressure peaks occurs with a highly conserved frequency of 9.1 ± 0.3 Hz and a mean maximum pressure of 93.8 ± 8.9 mmHg. Following the expulsion of urine, a series of “after peaks” (3.2 ± 0.3) are typically seen occurring every 1–5 s (Fig. 3). The phase during which urine is expelled and the after peaks are illustrated in Fig. 3. During micturition events, we do not observe a vascular component in the CSP pressure waveforms since the troughs of the pressure peaks always return to or near the FB pressure. However, a muscular component is suggested in the form of CSP pressure peaks, likely resulting from BS muscle contractions. Detailed characteristics of CSP pressures during micturition events are summarized in Table 1. The mean number of micturitions per 24 h that were seen on video was 30 ± 3 and recorded by telemetry was 32 ± 4 in rats.
housed on the wire mesh floor. Linear regression analysis between telemetry and visually confirmed micturition events on video resulted in $r = 0.98$ ($P < 0.001$).

The mean volume per micturition was 0.95 ± 0.12 ml. The relationship between micturition pressure waveform duration and volume of urine expelled is shown in Fig. 4. The formula for the positive relationship between the two parameters is also shown after combination of all data. Linear regression analysis revealed $r = 0.82$ ($P < 0.001$).

### Sexual Function

Penile erections were consistently associated with an increase in baseline erectile tissue pressure from ~10–15 mmHg in the flaccid state to a tumescence pressure of 50–70 mmHg. Because of the low pressure in the FB condition, movement of the animal and body positions placing pressure directly on the perineum, FB pressures were found to have some fluctuation corresponding to the activity of the animal. For this reason and the need to eliminate background artifact, the beginning of an erectile event was defined by a CSP pressure increase to at least 30 mmHg above the FB pressure. Moreover, all visually confirmed erectile events demonstrated an increase of at least 30 mmHg above the FB.

During ex copula reflex erection tests 100% of visually confirmed erectile events corresponded with CSP pressure waveform patterns characteristic of erectile events (Fig. 5). Following an increase in baseline CSP pressure, glans engorgement (E1–E3 events) was systematically associated with CSP pressure peaks often in excess of 300 mmHg and with a mean maximum pressure of 333 ± 26 mmHg (Table 2). The average duration of an ex copula full erection was 54 ± 1.3 s and comprised an average of 6.2 ± 1.2 CSP pressure peaks. These pressure peaks demonstrated an average duration of 0.82 ± 0.05 s (Fig. 5A, Table 2). Partial erections occurred infrequently and were associated with flushing of the glans base but not with dilatation of the glans. These partial events were characterized by an increase in baseline erectile tissue pressure above the tumescence threshold, but generally CSP pressure peaks were absent. If a pressure peak was present, it was a low level event and did not exceed 130 mmHg above the FB. A pulsatile pattern was seen in the CSP pressure recording during these partial erectile events (Fig. 5, B and C).

### Table 1. Micturition waveform characteristics in the adult Long-Evans rat. Analysis of 24-h micturition events

<table>
<thead>
<tr>
<th>Event Analysis</th>
<th>After-Peak Analysis</th>
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<tr>
<td>Duration, s</td>
<td>AUC, mmHg × s</td>
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<tr>
<td>3.6±0.2</td>
<td>119±11.5</td>
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</table>

Values are mean ± SE. AUC, area under the curve.

Fig. 4. Relationship between duration and volume of micturition.

Fig. 5. CSP pressure waveform obtained by telemetry during a full erectile (A) and partial erectile event (B and C).
CSP pressure waveforms characteristic of full erectile events were seen at a frequency of 53 ± 4 per 24 h and waveforms characteristic of partial erectile events were seen at a frequency of 8 ± 2 per 24 h during home-cage monitoring. Virtually identically appearing CSP pressure waveforms characteristic of full erectile events were observed during noncontact and mating conditions. Significant differences in CSP waveform characteristics of erectile events recorded during the four different contexts of erection tests (24-h home cage, ex-copula, noncontact, and mating) were identified and are illustrated in Fig. 6 and Table 2.

During noncontact erection tests, CSP pressure waveforms characteristic of full erectile events were observed during visible erections associated with grooming of the genital area. Furthermore, identical-looking CSP pressure waveforms were seen while the male rat was engaged in grooming or exploratory behavior in spite of no visually confirmed observation of erections. Inability to see the penis was due to the genital area being obscured. During mating tests, suprasystolic peaks were seen associated with various components of mating, such as mounting, copulation, and intromission. However, these erectile events were of significantly shorter duration (P < 0.05) and typically involved a single suprasystolic CSP pressure peak (Fig. 6 and Table 2). A vascular component was generally observed as an increase in baseline CSP pressure either immediately prior to or following the suprasystolic pressure peak. Although partial erectile events were also recorded during mating tests, only full erectile events were seen during intromission.

Table 2 shows that the duration and, as a result, the area under the curve (AUC) of erectile events varied significantly among contexts, in that the erections observed during ex copula tests were of the longest duration (54 ± 1.3 s), whereas mating erections had the shortest durations (4.2 ± 1.1 s). The duration of the noncontact erection events was shorter than the ex copula erections and with fewer pressure peaks. Furthermore, pressure peak analyses revealed that the number, duration, and AUC of the pressure peaks also were significantly different among contexts. For example, as seen in Table 2, pressure peaks associated with ex copula events were longer in duration (0.82 ± 0.05 s) compared with mating erections (0.54 ± 0.05 s).

<table>
<thead>
<tr>
<th>Context</th>
<th>Duration, s*</th>
<th>AUC, mmHg × s†</th>
<th>Mean Pressure, mmHg‡</th>
<th>Maximum Pressure, mmHg‡</th>
<th>No. Peaks*</th>
<th>Mean Pressure, mmHg‡</th>
<th>Maximum Pressure, mmHg‡</th>
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<tr>
<td>24 h</td>
<td>25±2.5</td>
<td>1401±154</td>
<td>54±1.1</td>
<td>320±14</td>
<td>4.2±0.2</td>
<td>0.73±0.03</td>
<td>76±3.6</td>
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<tr>
<td>Reflex</td>
<td>54±1.3</td>
<td>2855±584</td>
<td>55±3.1</td>
<td>333±26</td>
<td>6.2±1.2</td>
<td>0.82±0.05</td>
<td>86±10</td>
</tr>
<tr>
<td>Noncontact</td>
<td>13±2.3</td>
<td>622±165</td>
<td>45±5.1</td>
<td>235±41</td>
<td>2.8±0.9</td>
<td>0.50±0.09</td>
<td>49±8.1</td>
</tr>
<tr>
<td>Mating</td>
<td>4.2±1.1</td>
<td>227±54</td>
<td>56±3.4</td>
<td>274±22</td>
<td>1.7±0.5</td>
<td>0.54±0.05</td>
<td>55±5.7</td>
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<td>108±3.1</td>
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<td>106±5.2</td>
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Values are means ± SE. Significance levels: *P < 0.05; †P < 0.01. ‡Not significant.

Fig. 6. Erectile CSP pressure waveforms recorded during reflex (A), noncontact (B), and intromission (C) behavioral contexts.
**DISCUSSION**

In this study, we demonstrate the accuracy of CSP pressure recording as a modality for measuring frequency and quality of both micturition and erectile events in awake freely moving male rats. Furthermore, duration of micturition appears to be predictive of micturition volume, and significant differences were observed in the CSP pressures during erections with respect to the context in which the erection was generated. Transducer implantation is a minimally invasive surgery with no major complications encountered during our study period of 2 mo. Analysis of pressure waveforms that were recorded by telemetry is straightforward and the three types of events (micturition, full erectile, and partial erectile events) are easily distinguished and recorded.

Telemetric monitoring of CSP pressure for assessment of micturition and erectile events has clear benefits over traditional methods of examining these functions in freely moving animals. CSP pressure monitoring allows simultaneous assessment of micturition and erectile function, allowing for more than one autonomic functional outcome. This method allows examination of animals housed in a natural habitat and determination of events that are in general very hard to observe by eye or video recordings. Furthermore, recordings obtained by telemetry not only provide us with quantifiable data, such as number of events per 24 h, but also allows us to determine other parameters of importance in assessment of these events, such as duration and amplitude of pressures that occur during these events. Since the bulb of the CSP is more vascular than the CCP, implantation of transducers is easier and failure rate is less in our experience. Furthermore, the ability to perform long-term measurements is an advantage of CSP monitoring vs. CCP monitoring. Finally, CCP pressure monitoring only allows detection of erectile events and is not capable of simultaneously monitoring micturition events (Schmidt MH, unpublished observations).

Previous studies, in which BS EMG and CSP pressure recordings were made simultaneously during ex copula reflex erection tests have shown that BS muscle activity is directly associated with glans engorgement (Fig. 7, Ref. 31). BS muscle bursts, thus, generate the suprasystolic CSP pressure peaks, i.e., the muscular component of the glans erection. Simultaneous acquisition of BS EMG and CSP pressure recordings during micturition events has shown that CSP pressure peaks during micturition are associated with a rhythmic BS muscle bursting activity (Fig. 8, Ref. 27). BS muscle contractions during micturition occur in a rapid bursting manner and result in pressure peaks within the CSP characterized by a rapid series of pressure peaks (Figs. 3 and 8). The troughs of the pressure peaks during micturition remain at the FB level. These data suggest that micturition, therefore, is generated primarily by the muscular component without vascular engorgement or prefilling of the erectile tissues.

The potential role of the BS muscles in micturition in the rat represents a new finding, since traditionally the BS muscle has only been associated with ejaculation and its role in micturition has not been investigated. Indeed, coordinated activation of the external urethral sphincter (EUS) and contraction of the detrusor muscles are considered the main processes involved in micturition (20). Urine from the rat is expelled in a pulsatile pattern, unlike many other mammalian species in which urine flow is continuous or nonpulsatile, and we suggest that, in addition to the role the BS muscle plays in expelling seminal fluids, it likely also plays a role in expelling urine in the rat. We propose that the BS muscle and the EUS contract simultaneously in rodents. Thus, EUS muscle contractions may prevent backflow of urine into the bladder while simultaneous BS muscle contractions assist in expelling urine. The fact that BS muscle activity is important during both micturition and erectile events is also suggested by the finding that, after SCI in rats, it is common to see micturition events directly precede erectile events (Nout YS and Schmidt MH, unpublished observation). Following SCI, removal of supraspinal inhibition may allow for erections to be easily triggered (17), such as after pelvic floor muscle activation during micturition. Perhaps BS muscle firing during micturition enables the triggering of subsequent reflex erectile events following SCI.

Simultaneous recording of CSP pressures and videotaping of animals demonstrated a consistent agreement between visually confirmed urine flow during micturition events and the occurrence of a CSP pressure waveform characteristic for micturition. Slightly more micturitions were detected by telemetry vs. video observation resulting in an $r$ of 0.98 instead of 1. We
speculate this is due to the fact that some micturition events were missed on video either due to changing of the videotape while recording or due to poor lighting during the dark phase resulting in inability to see urine fall on the colored paper.

While animals were housed on wire mesh we found an average frequency of $32 \pm 4$ micturition events per 24 h. One other study that used metabolic cages to determine frequency of micturition in male Sprague-Dawley rats found that this species urinated $\sim 21$ times per 24 h (26). However, while the rats in our study had not been accustomed to the study condition, the rats in that study had been acclimatized to the metabolic cages during a 7-day period prior to testing. When we examined micturition characteristics of male rats housed in their regular cages we found a micturition frequency of $26 \pm 2$ per 24 h (18), which is closer to what Schmidt et al. (26) reported. In the present study, we found a mean volume per micturition of $0.95 \pm 0.12$ ml. This is slightly less than the 1.3 ml reported by Schmidt et al. (26). However, when combining these results, the total volume of urine expelled per 24 h is similar between the two studies. The rats in our study apparently urinated smaller volumes more frequently, perhaps related to the different housing environment, strain differences, or display of more pronounced territorial marking behavior. Another study reported a smaller volume (0.6 ml) per micturition in male Wistar rats examined in metabolic cages (9). These rats had a smaller body weight (mean: 250 g) compared with the rats we used (mean: 300 g) and the rats used by Schmidt et al. (26) (mean: 399 g). It is likely that smaller rats produce a smaller volume per micturition. Since the number of micturition events per 24 h and the volume of micturitions in this study compared well to prior data gathered using metabolic cages, transducer implantation apparently does not affect the micturition reflex.

Telemetrically obtained CSP pressure waveforms during full erectile events are characterized by development of a tumescence pressure of at least 30 mmHg above the FB on top of which one or many suprasystolic pressure peaks occur, similar to what has previously been described for CCP telemetric pressure recordings (5). We defined a “full erection” as an event with at least one peak that reached a pressure of 130 mmHg above the FB pressure (Fig. 5A) based on analyses of prior published data (31) showing that a pressure of 130 mmHg above the tumescence pressure is $\sim 1.5$ standard deviations below the average maximum pressures during weak E1 glans erections (200 mmHg). Events that reach pressures of at least 130 mmHg above the FB are thus considered full erectile events. Partial erections are similar to full erectile events with the exception that peaks never reach 130 mmHg over the FB pressure (Fig. 5, B and C). Partial erectile events tend not to have pressure peaks and generally only have an increase in baseline erectile tissue pressure. In addition, pressure peaks of these partial events, when present, tend to be shorter in duration. These partial events clearly demonstrate the vascular subsystolic phase of erections that is due to cardiogenic vasodilatation of the erectile tissues in that a distinct pulse pattern can typically be seen with the same frequency as the QRS complex seen in electrocardiograms (Schmidt MH, unpublished observation). In contrast, full erectile events have characteristics of both the vascular subsystolic component (tumescence with a pulse pattern) and the muscular suprasystolic component (pressure peaks).

Telemetric monitoring of erectile events appears to be a consistent and accurate method for detecting these events in freely moving rats and in awake rats during behavioral tests. In this study, we reconfirm the well-understood problem of detecting rodent erectile events by visual observation during noncontact erection and particularly during mating tests. Typically in behavioral testing conditions, we infer intromission from a specific behavioral pattern, although we observe neither intromission nor erection. We suggest that the use of CSP pressure analysis is more sensitive and less likely to miss events; indeed, there was a one-to-one relationship between visually confirmed erections during ex copula reflex erection tests and characteristic CSP pressure changes. Moreover, we never observed erectile events without CSP pressure changes.

Significant differences were present in CSP pressure waveforms during erectile events recorded in different behavioral contexts. This highlights the importance of context specificity of erectile events and suggests interpretation of the various erectile events by telemetry should occur within the context of
specific experimental conditions as other investigators have suggested as well (5, 23). The CSP pressure characteristics may differ among the four behavioral contexts studied here likely due to differences in erectile neurophysiology that occur during these different circumstances. Perhaps this reflects the paradigm that reflex erections are likely to be more spinaly mediated, and erections that occur during intromission likely have more supraspinal input. In addition, sensory feedback during erections in these different contexts is different. Further study of the characteristics of CSP waveforms during erectile events in noncontact erection and/or mating tests and their relationship with observed behavior may improve our understanding of context specificity of erectile events. Future research involving pressure monitoring in both CSP and CCP is required to further elucidate the roles of both cavernous systems and of the muscles controlling them during erectile events.

In conclusion, changes of CSP pressure waveform characteristics detectable by telemetry are a valuable tool for detecting micturition and erectile events in freely moving rats. Micturition and erectile function are predominantly regulated by the autonomic nervous system, and damage to these components following SCI results in alterations of these important physiological functions (18). We anticipate the use of telemetric recording of CSP pressure will aid in future studies examining changes in autonomic outcome following SCI.

GRANTS
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REFERENCES