Penile erection and micturition events triggered by electrical stimulation of the mesopontine tegmental area

Juan Carlos Toledo Salas,1 Hiroshi Iwasaki,2 Eiichi Jodo,1 Markus H. Schmidt,3 Akihiro Kawauchi,4 Tsuneharu Miki,4 Yukihiko Kayama,1 Manabu Otsuki,5 and Yoshimasa Koyama6

1Department of Physiology, Fukushima Medical University, Fukushima; 2Osaka Minami Hospital, Osaka; 3Department of Urology, Kyoto Prefectural University of Medicine, Kyoto; 4Osaki Sleep Clinic, Fukushima; and 5Department of Science and Technology, Fukushima University, Fukushima, Japan; and 6Ohio Sleep Medicine and Neuroscience Institute, Dublin, Ohio

Submitted 4 April 2007; accepted in final form 18 October 2007

Toledo Salas JC, Iwasaki H, Jodo E, Schmidt MIH, Kawauchi A, Miki T, Kayama Y, Otsuki M, Koyama Y. Penile erection and micturition events triggered by electrical stimulation of the mesopontine tegmental area. Am J Physiol Regul Integr Comp Physiol 294: R102–R111, 2008. First published October 31, 2007; doi:10.1152/ajpregu.00226.2007.—The cholinergic neurons in the laterodorsal tegmental nucleus (LDT) play a crucial role in the regulation of penile erection and micturition. Moreover, different afferent structures may play a role in erectile control, we electrically stimulated the mesopontine tegmental area in and around the LDT of awake (unanesthetized) head-restrained rats. To detect penile erection, corpus spongiosum of the penis (CSP) pressure was measured through a telemetric device with simultaneous bulbo-pontine tegmental area.

Penile erections occur in varying and specific contexts, as in the presence of an accessible female (copulatory penile erections) or nonaccessible female (noncontact penile erections) or as a reflex after the retraction of the preputial sheath (25, 41, 49). Penile erections are also observed during sleep in both human males (39) and rats (51).

It is well known that the medial part of the preoptic area plays a critical role in penile erections generated in sexual contexts (25), and the electrical stimulation of the medial preoptic area elicits erectile events (13). Recently, Schmidt et al. (50) have shown that the lateral preoptic area (LPOA) is involved in the regulation of penile erections during rapid eye movement (REM) sleep. It has also been reported that injection of carbachol into the LPOA induces penile erections (48), suggesting that the cholinergic afferents to the LPOA have a crucial role in inducing penile erections. In the mesopontine tegmentum, there are two populations of the cholinergic neurons, the laterodorsal tegmental nucleus (LDT) and the pedunculopontine tegmental nucleus. These cholinergic nuclei are critically involved in the generation of REM sleep (19, 30, 45, 55). It has been reported that some cholinergic neurons are active both during REM sleep and waking, whereas others are active only during REM sleep (10, 21, 55). Recently, we have found that a group of cholinergic neurons in the LDT fire in close temporal relation with penile erections during REM sleep (23). Several anatomical studies revealed an ascending cholinergic projection to the LPOA from the LDT (48, 52, 61). These findings raise the possibility that the REM-related penile erections are regulated by cholinergic neurons in the LDT.

To test the hypothesis that the LDT or surrounding cholinergic structures may play a role in erectile control, we electrically stimulated the mesopontine tegmental area in and around the LDT of awake (unanesthetized) head-restrained rats and examined the effect on penile erections while monitoring erectile tissue pressure using a telemetric monitored technique developed by Nout et al. (37) and Schmidt and colleagues (51).

MATERIALS AND METHODS

The present study was carried out under the control of the Animal Research Committee in accordance with the Guidelines on Animal Experiments of Fukushima Medical University and the Animal Protection and Management Law of the Japanese Government (No. 105).

Surgical procedure. Fourteen male Sprague-Dawley rats (350–450 g body wt) were used. Pentobarbital sodium (50 mg/kg) was administered intraperitoneally before surgery, and additional doses of the same anesthetic were given to maintain them anesthetized during the surgery. The abdomen and perineal regions were shaved and cleaned. The surgical instruments and the telemetric pressure transducer (TA11PA-C40; Data Sciences International, St. Paul, MN) were sterilized with Hibitane solution before the surgery. Incisions were made over the skull, at the back of the neck, and over the abdomen and the perineum. The body of the telemetric transducer was fixed subcutaneously to the abdominal wall. The distal portion of the bulb of the corpus spongiosum of the penis (CSP) was gently exposed, and the tip of the transducer catheter was inserted in the bulb through a slit in the skin.

Address for reprint requests and other correspondence: Y. Koyama, Dept. of Science and Technology, Fukushima Univ., 1 Kanaya gawa, Fukushima, 960-1296 Japan (e-mail: koyamay@sss.fukushima-u.ac.jp).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
previously made using a needle as described by Nout et al. (37) and Schmidt and colleagues (51). The catheter was secured using a biological glue (Vetbond; 3M Animal Care Products) at the point of entrance of the catheter and was sutured by a thin thread to Buck’s fasia overlying the penile shaft. To record electromyographic (EMG) activity, a pair of stainless wires were inserted in the bulbospongious (BS) muscle using a needle as described by Nout et al. (37) and Schmidt and colleagues (49) and were passed subcutaneously to the incision over the skull. To record sleep-waking cycles, screw electrodes for cortical electroencephalogram (EEG) and wire electrodes for neck muscle EMG were implanted. A U-shaped plastic plate was attached to the skull using dental acrylic cement so that the cranium could be painlessly attached to a stereotaxic frame. Gentamicin ointment (0.1%) was applied at the incision sites, and, after the operation, penicillin (60 mg) was subcutaneously injected for 4 or 5 days. The rats were given 1 wk of recovery.

Experimental procedure. Before the experiment (1 day), rats were attached to the stereotaxic frame under ketamine anesthesia (50 mg/kg) using the U-shaped plate on their pedestal, and a small hole was made through the occipital bone using a dental drill to expose the surface of the cerebellum. The rats were later sleep deprived for 12 h using a rotating wheel with food and water available ad libitum. On the day of the experiment, they were then fixed to the stereotaxic frame using the U-shaped plate on their pedestal. The stimulating electrode consisted of a glass pipette filled with Woods metal, the tip of which was replaced by a carbon fiber (10 μm in diameter; see Ref. 57). To avoid penetration of the venous sinus, the electrode was angled posteriorly at 30° and was lowered through the cerebellum. To scan the most effective sites, stimulation was applied usually at 0.2-mm intervals by moving the electrode with an oil microdrive manipulator. Each site was stimulated with 0.5-ms rectangular pulses repeated at 50 Hz for 3 s. The stimulus was given during waking, slow wave sleep and REM sleep. However, in the present experiment, the results were analyzed regardless of the sleep waking state. The most effective site in each track was marked by passing 15–20 μA positive current (direct current) for 30 s.

At the end of the experiment, the rats were deeply anesthetized with pentobarbital sodium and were perfused transcardially with 300 ml of heparinized saline followed by 300 ml of 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). The brain was then removed, postfixed in the same fixative, soaked in 30% sucrose, and sectioned in the heparinized saline followed by 300 ml of 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). The brain was then removed, postfixed in the same fixative, soaked in 30% sucrose, and sectioned in the coronal plane at a thickness of 50 μm. To identify the stimulation site relative to the LDT and the locus coeruleus, the sections were processed for NADPH-diaphorase histochemistry (60) and were counterstained with neutral red.

Definition of responses. As shown in Fig. 1A, an erectile event is defined by a slow increase in CSP pressure (vascular component) and one or more sharp superimposed CSP pressure peaks (muscular component). In addition, bursts of the BS muscles are simultaneously observed with these peaks. A “spontaneous erection” was defined as an erectile event that occurred naturally in the absence of brain stem electrical stimulation. According to Nout et al. (37) and Schmidt and colleagues (49), naturally occurring erections also refer to as full erections are defined by the simultaneous occurrence of vascular subystolic and muscular suprasystolic components. The vascular component in the rat is associated with an increase in the baseline erectile tissue pressure from ~10 to 15 mmHg in the flaccid state (flaccid baseline, FB) to a tumescence pressure of ~50–70 mmHg. The muscular component is easily identified by the sharp, suprasystolic CSP pressure peaks occurring on top of the tumescence pressure. By convention, a full erection must have at least a 30-mmHg increase in the tumescence pressure above the FB and at least one CSP pressure peak 130 mmHg greater than the FB (Fig. 1A and see Ref. 37). The start of the erection is defined as the moment the CSP pressure first exceeds the level of FB + 30 mmHg (Fig. 1A). As previously published (37), the end of the erection is defined when the pressure dropped below the level of FB + 30 mmHg (Fig. 1A). To
distinguish between two erections occurring in close temporal association, a new erection is considered to occur when the pressure drops below the level of FB + 30 mmHg for >15 s (Fig. 1A) or when the pressure drops below the level of FB + 15 mmHg for >5 s (Fig. 1B).

For statistical purposes, CSP pressure peaks greater than FB + 80 mmHg (Fig. 1A) were evaluated. When the CSP peak is composed of several peaks in rapid succession (Fig. 1C), the end of any peak is defined when the trough of the peak falls to within 15 mmHg of the base of the peak’s initial pressure rise (Fig. 1C, a–b and b–d). Finally, a partial erection is defined by a primarily vascular event in that there is an increase in baseline erectile tissue pressure to the FB + 30 mmHg but lacking the CSP pressure peaks typical of a normal or full erection and were thus termed “vascular events” for this study. These partial erections typically demonstrate a pulse pattern during the tumescence pressure that has the same frequency with the heart rate.

Micturition events in the rat are associated with a distinct pulsatile pattern in the CSP pressure recording involving a rapid series of short duration (~55 ms), low amplitude pressure peaks (generally <80 mmHg), and a highly conserved frequency of 10–11 Hz (37). Urine flow in the rat does not occur in a steady stream but in a pulsatile manner with the same frequency seen in the pressure recording. At the end of the urine flow, three to five “after peaks” occur in the CSP recording, with each peak clearly isolated and easily identifiable. Micturition events are distinctly characterized by the lack of a vascular component in that the baseline CSP pressure remains at or near the FB level, i.e., there is a lack of tumescence phase. In addition to these typical micturition events, we also identified muscular-type events characterized by a series or cluster of CSP pressure peaks but lacking both the typical micturition pattern or a vascular component. CSP pressure peaks in these muscular-type events often were <80 mmHg. As a result, all peaks were analyzed for statistical purposes.

Data analysis. The CSP pressure was analyzed using Spike 2 data software (Cambridge Electronic Design, Cambridge, UK). Peak frequencies were obtained by dividing the number of peaks by the time from the first peak to the last peak. The vascular latency was measured from the start of the stimulus to the start of erection (Fig. 1A), whereas the peak latency was from the start of the stimulus to the first CSP pressure peak.

Because the maximum value measured by the transducer was 400 mmHg, the CSP peaks sometimes exceeded this value. A CSP pressure >400 mmHg was counted as 400 mmHg for the statistical analysis. Mann-Whitney U-test was performed to compare the median of each response using Statistica software. Differences were considered to be significant at P < 0.05.

RESULTS

After 2,190 stimuli in 1,069 sites in the brain stem, four different types of CSP pressure responses were evoked: full erections, muscular-type events (CSP pressure peaks without an increase in baseline pressure), complex-type, and typical micturition events. The stronger stimuli often caused body or leg movement, so in most cases the stimuli were limited to <100 μA.

Full erections. An evoked full erection had similar CSP pressure and BS muscle activity patterns as the spontaneous erection. As shown in Fig. 2, the stimulus of 50 μA to the boundary of LDT and dorsal tegmental nucleus (DT) induced a slow CSP increase and several sharp peaks without any observable movements or neck EMG activation. The CSP peak amplitude was in correlation with the BS muscle activity. The frequency and the highest amplitude of CSP pressure peaks were similar to those of spontaneous erections (Table 1). The latency of the response ranged from 4.8 to 176 s. Although the erectile events were often observed several hundreds seconds after the stimulus, it was difficult to judge them as stimulus evoked. So, in the present experiment, the erections observed within 180 s were considered to be stimulus evoked (see DISCUSSION).

Muscular-type response. The response in Fig. 3 shows a series of sharp increases in CSP pressure with an absent or minimal vascular component. Sharp CSP pressure peaks occurred simultaneously with BS muscle contractions and were associated with only a small vascular component (<30 mmHg increase in baseline CSP pressure). These events were referred to as muscular-type response. The muscular-type response had higher frequency peaks than the spontaneous (P < 0.001) and evoked (P < 0.001) full erections. The interval of the peaks was regular and shorter at the early phase of the response and gradually increased as the response proceeded. The highest peak amplitude was smaller than the evoked (P < 0.01) and spontaneous full erection (P < 0.01). The peak latency was shorter than that found in the evoked full erections (P < 0.01). The amplitude of CSP peak pressure did not always correlate with BS muscle activity.

The CSP pressure of the muscular-type response exhibited a steeper rise, or slope, than CSP pressure peaks seen during the typical full erections (Figs. 2 and 3), and the descending phase exhibited a curve similar to an exponential decline as the CSP pressure returned to the baseline. See Fig. 7 for the relation of the peak amplitude with the rate of rise (slope) of the peak, where it is shown that, in the muscular-type response, the slope of the peaks was positively correlated with peak amplitude, whereas, in the spontaneous and evoked full erections, the slope did not increase in relation with the amplitude increase.

Mixed-type response. As shown in Fig. 4, the mixed-type response begins with a vascular component similar to that of the spontaneous and evoked full erections. However, on this vascular component, two types of sharp CSP pressure peaks occur sequentially; the first is a high-frequency (1.09–3.81 Hz) series of pressure peaks, named phase A, while the latter had low-frequency (0.06–1.66 Hz) series of pressure peaks resembling pressure peaks seen in normal full erections and was named phase B.

In phase A, the peak frequency was significantly higher than that of phase B, spontaneous erection, evoked full erections, or evoked muscular-type responses (P < 0.001 in all cases). The vascular and peak latencies were similar to those of the evoked or full erections. Three main types of CSP pressure peaks were
Table 1. Parameters in spontaneous erection and stimulus-evoked responses

<table>
<thead>
<tr>
<th>Type</th>
<th>n</th>
<th>Median Latency, s</th>
<th>Mean ± SD</th>
<th>Median Vascular Duration, s</th>
<th>Mean ± SD</th>
<th>Median Peak Amplitude, mmHg</th>
<th>Mean ± SD</th>
<th>Median Peak Frequency, Hz</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spontaneous</td>
<td>48</td>
<td>4.8 ± 70.9</td>
<td>31.1</td>
<td>50.3 ± 46.4</td>
<td>6.8 ± 170.9</td>
<td>53.6 ± 46.9</td>
<td>9.0 ± 122.5</td>
<td>191 ± 3.8</td>
</tr>
<tr>
<td>Full erection</td>
<td>81</td>
<td>4.8 ± 130.9</td>
<td>20.5</td>
<td>33.6 ± 36.1</td>
<td>10.0 ± 156.1</td>
<td>21.7 ± 35.5</td>
<td>35.5 ± 236.2</td>
<td>245 ± 106</td>
</tr>
<tr>
<td>Muscular (phase A)</td>
<td>42</td>
<td>3.3 ± 103.9</td>
<td>20.5</td>
<td>33.6 ± 36.1</td>
<td>10.0 ± 156.1</td>
<td>21.7 ± 35.5</td>
<td>35.5 ± 236.2</td>
<td>245 ± 106</td>
</tr>
<tr>
<td>Micturition</td>
<td>69</td>
<td>0.2 ± 170.9</td>
<td>3.9</td>
<td>36.8 ± 50.3</td>
<td>117</td>
<td>0.2 ± 170.9</td>
<td>36.8 ± 50.3</td>
<td>117</td>
</tr>
</tbody>
</table>

Sometimes in full erection and micturition responses after one stimulus more than one response was evoked, hence the difference in n values. Ranges are in parentheses. *P* < 0.01 and **P** < 0.001 vs. spontaneous erection.

Fig. 3. Muscular-type response. After the electrical stimulation (100 μA) to point "b" in Fig. 6, a CSP pressure pattern was evoked without a vascular component but accompanied by steep periodical CSP peaks.

Observed in phase A. In one of the extreme cases, the CSP pressure peak slopes were similar to that of muscular-type responses (Fig. 4Ba). In this case, the CSP pressure peak amplitude gradually increased, whereas the peak interval remained almost constant. Similar to the muscular-type response, there was a positive correlation between the peak amplitude of the CSP peak and the slope of the peak's rise in pressure (see Fig. 7). In a second type of CSP pressure pattern found in phase A, sharp CSP peaks were superimposed on more broad CSP peaks, constituting multiple broad CSP pressure peaks (Fig. 4Bb). The interval of these sharp peaks was similar to that of Fig. 4Ba, or muscular-type response. Finally, a third type of CSP pressure peak pattern in phase A was defined by many sharp peaks occurring at irregular intervals riding on the vascular component (Fig. 4Bc).

As noted above, all mixed-type responses had a second component of CSP pressure peaks occurring at a slower frequency, phase B, and occurring immediately after the faster frequency phase A pattern. Phase B CSP pressure peaks had similar characteristics to spontaneous full erection responses. The amplitude and frequency of peaks in phase B demonstrated no significant differences from these two kinds of full erection. The mixed-type response occurred only during wakefulness but not during REM sleep.

**Micturition-type response.** The micturition-type response had regular, high-frequency, and small-amplitude CSP peaks (Fig. 5). The peak frequency was from 5.89 to 16.87 Hz, which covers the range of the discharge frequency of the external urethral sphincter muscle during micturition (6–8 Hz; see Ref. 62) and the micturition frequency reported by Nout et al. (10–11 Hz; see Ref. 37). The peak frequency of the micturition-type events was the highest of all other responses (*P* < 0.001). After one train of stimulus, the response was often evoked several times. In >85% of the responses (99/117), the maximum peak amplitude was smaller than 30 mmHg (ranging from 2.6 to 28.8 mmHg), whereas that of the remaining (18/117) ranged from 31.3 to 92.5 mmHg. The peak latency following stimulation was shorter than that of full erection (*P* < 0.001) and the mixed-type response (*P* < 0.01). The high-frequency peaks were in synchronous with BS muscle bursting; however, when the response was evoked during stimulation (with the latency of <3 s), the BS muscle bursting was largely suppressed.

**Localization of responses.** In total, 1,069 sites were stimulated through 175 tracks. Of these, 125 sites were effective in
type events were evoked. Sites (Fig. 6). In another six sites, mixed-type and micturition-full erection and mixed-type responses were evoked in eight one type of response was evoked (Fig. 6). Among them, both stimulation.

Data from Fig. 6 and is composed of high-frequency CSP pressure peaks (10 Hz)

studies (plates 2–7). Most of the responses were located in or peripheral to the LDT (plates 3–5). At plates 3 and 4, they were intermingled with those for full erection responses, whereas at plate 5, the sites for these two responses were separated. Micturition events were evoked from the DR of plate 1 and caudally scattering around the locus coeruleus, LDT, and the CG in and around the Barrington’s nucleus (plates 2–7). Most of the responses obtained from the DR of plate 1 (73 of 99) were of small amplitude (<30 mmHg), whereas in the caudal area (plates 2–7), most of the responses had amplitudes >30 mmHg. In some stimulus sites, more than one type of response was evoked (Fig. 6). Among them, both full erection and mixed-type responses were evoked in eight sites (Fig. 6). In another six sites, mixed-type and micturition-type events were evoked.

DISCUSSION

Brain stem stimulation generated four types of CSP pressure changes that closely resembled erection and micturition patterns. Effective stimulation sites were anatomically segregated within the dorsal pontine tegmentum with erection responses resulting from stimulation of the LDT and the surrounding area, whereas micturition responses generated from stimulation in and around the Barrington’s nucleus and the dorsal raphe. Stimulation of other sites in the dorsal pons resulted in a mixed pattern of vascular autonomic and muscular responses. These results suggest that the mechanisms generating penile erection and micturition reside in the brain stem.

The erectile events were often observed >180 s after the stimulus. Because the spontaneous erection occurred with a mean interval of 1,076 s, the erectile events observed with a latency of ~200–300 s looks to be stimulus evoked. However, the latency varied when the responses were obtained repeatedly after stimulating the same points. So, we disregarded the events observed >180 s after the stimulus. Even though we consider the response with latency of <180 s as stimulus evoked, such latency responses could not be simply explained by neuronal events or neural circuits. It would be possible that some humoral factors are involved in such long latency responses. For example, after the LDT stimulation, nitric oxide (NO), a strong facilitatory molecule of penile erection (5), might be released in the brain, remaining elevated for 5–10 min, in a similar way as is in the thalamus (35). Because the LDT neurons send ascending projections to the hypothalamic areas, including paraventricular nucleus (PVN; see Ref. 52), the LDT stimulation might have induced NO release in the PVN with a long latency, resulting in penile erections as previously shown by the injection of NO donors in the PVN (31, 34). Alternatively, the oxytocin released in the posterior pituitary may have some effects. Because it is known that oxytocin has a facilitatory role in penile erection (59), it is still possible that the activation of ascending LDT efferents induced circulating oxytocin through activation of the PVN-pituitary axis, which would continue for several minutes (4, 8, 17).

We have observed that the electrical stimulation to the LDT causes an increase of the systemic blood pressure (unpublished observation). However, compared with the latency of evoked erection, that of the blood pressure change was very short (within a few seconds) and was largely constant. Although the
LDT activation directly causes a change of blood pressure, it would return to the baseline level when the erection is evoked. So, the stimulus-evoked erection would not be affected by blood pressure change but would be a consequence of activation of the erection-inducing system.

**Full erections.** The most commonly observed evoked response was an erection pattern that was similar to the normal spontaneous erection (nonevoked responses). The CSP pressures demonstrated a slow increase (vascular component) and sharp peaks concurrent with BS muscle bursting (muscular component). The amplitude and frequency of CSP peaks and duration of the vascular component for the evoked erectile events were within the ranges reported previously for full or normal erections (3, 49). Although we did not directly observe the erectile events during CSP pressure changes, correspondence of the sharp CSP peaks and erectile events have already been reported (3, 37, 49).

**Muscular-type response.** In muscular-type responses, a vascular component was absent in that the baseline CSP pressure remained at or near the FB level. However, sharp CSP pressure peaks were observed and had a steeper slope (rate of rise) with smaller amplitude than in the normal full erections and did not always occur in association with BS muscle activity. According to Bernabe et al. (3), intracavernous pressure shows sharper pressure peaks during flps than during other erectile events. Schmidt et al. (49) reported that the small-amplitude CSP peaks (<80 mmHg) that synchronized with ischiocavernous (IC) muscle activity corresponded to flps of the penile body. Hart and Melese-D’Hospital (16) showed that, after surgical removal of the IC muscles, the flps virtually disappeared. It is unclear if the sharp CSP peaks of muscular-type responses may correlate with IC muscle activity rather than just BS muscle bursts since the IC muscles were not recorded during this study. One of the characteristics of the muscular-type response was a lack of vascular component. Bernabe et al. (3) reported that, during copulation, the sharp intracavernous pressure (ICP) peaks appeared in the absence of a vascular component, casting doubt whether the vascular component was required for the generation of peaks. However, Nout et al. (37) report that, when the pressure was recorded from the CSP, a vascular component accompanied by suprasystolic peaks is present during copulatory erections. This vascular component is more difficult to appreciate in the ICP recording from the more dense and fibrous corpus cavernosum (Schmidt, unpublished data). Sato and Christ (46) reported that stimulation of the preoptic area induced a vascular component and sharp ICP peaks. After the transection of cavernous nerves, the same stimulus failed to generate the vascular component, but the sharp peaks remained. Considering these results, we can suppose that, in some cases, only the BS/IC muscle contraction can evoke sharp CSP peaks. Moreover, our data demonstrate that, without the vascular component, or prefilling of the erectile tissues, the slope or rate of rise of the CSP pressure peaks are quantifiably different from the CSP peaks generated in the presence of a vascular filling.

**Mixed-type response.** Mixed-type responses demonstrate a vascular phase with an increase in baseline CSP pressure but also were comprised of two types of CSP pressure peaks. These mixed-type responses began with a phase of high-frequency CSP peaks (phase A) that was a mixture of several components and followed by low-frequency CSP peaks (phase B) that resembled spontaneous full erections or normal type responses. This result suggests that two separate neural systems may be activated, one that generates high-frequency BS muscle activity and the other that generates the lower-frequency CSP peaks similar to those observed in full erections. In most cases, the higher-frequency phase A preceded the lower-frequency phase B series of peaks.

The phase A of mixed-type response was composed of several peak patterns. The response in Fig. 4B was similar to the muscular-type response, although, considering the interval of steep peaks, the response in Fig. 4B more closely resembles a mixture of muscular-type response and normal-type responses. These findings suggest that phase A is produced by simultaneous activation of several neural substrates, including those that regulate the muscular-type response, normal-type response, or others. Mixed-type response was observed only during waking, and the frequency of sharp peaks of phase A

---

**Fig. 6.** Coronal sections showing the location of effective sites that induced each type of response. Red circles, evoked full erections; yellow circles, muscular-type response; blue circles, mixed-type response; green circles, micturition-type response; circles with multiple colors, sites from where multiple responses were evoked. Small dots, cholinergic neurons; Bar, Barrington’s nucleus; CER, cerebellum; CG, central gray; DR, dorsal raphe nucleus; DT, dorsal tegmental nucleus; LDT, laterodorsal tegmental nucleus; LPB, lateral parabrachial area; RF, reticular formation. LDT is surrounded by thin lines, and other areas are specified by the boundaries drawn on the brain map. Letters a, b, c, and d show the sites where full erection, muscular, mixed, and micturition responses were evoked and shown in Figs. 2, 3, 4A, and 5, respectively.
(1.09–3.95 Hz) roughly corresponds to that of reflex erection (~1.3 Hz, calculated from Fig. 6A in Ref. 37), suggesting that phase A is partly composed of reflex erection. If so, occurrence of mixed-type response only during waking would reflect the activation of reflex mechanisms only during waking.

**Micturition-type response.** Micturition-type responses had a higher frequency of CSP pressure oscillation than all other responses and were evoked from the limited area in the DR (plate 1 in Fig. 6) and the scattered area in and around the LDT and the Barrington’s nucleus. Because the frequency of the CSP peaks (5.8–16.8 Hz) covered the frequency of external sphincter muscle discharge during micturition, and the latencies overlapped with those of bladder contraction induced by stimulation of the micturition center, the micturition-type response evoked in the present study appears to resemble a micturition event (37, 62). In some cases, we observed voiding when the high-frequency CSP fluctuation occurred (unpublished observation). Nout et al. (37, 38) also visually confirmed that the urine flowed out in a pulsatile manner during the high-frequency CSP fluctuation. Even during small-amplitude CSP fluctuation that is observed after spinal cord injury, micturition was observed (38). Hence, it is possible that the small-amplitude (<30 mmHg) micturition-type response obtained from the DR would be accompanied by micturition, although it will be required to visually clarify the micturition after the DR stimulation. In rats, prior data show that stimulation to the limited area close to the micturition center (Barrington’s nucleus) induces bladder contraction and micturition or external urethral sphincter activity that corresponds to micturition (24, 36, 62). The present study indicates that the wide areas in the caudal CG in and around the Barrington’s nucleus may also be involved in micturition-type responses. The difference in findings may be because of the use of anesthesia (urethane) in the previous studies. Urethane suppresses the glutamatergic (N-methyl-D-aspartate) receptors, which are closely involved in urinary bladder activity (63, 64). The Barrington’s nucleus receives afferents from wide areas, including the brain stem, from where micturition responses were evoked in the present experiment (58). In the previous studies, which were done under urethane anesthesia, activation of such areas would not have been enough to induce micturition. Another possibility remains that stimulation

### Table 2. Proportion of effective sites to total stimulation sites for each response in each anatomical area of Fig. 6

<table>
<thead>
<tr>
<th>Stimulus Sites</th>
<th>Positive Sites/Total Sites</th>
</tr>
</thead>
<tbody>
<tr>
<td>Full erection</td>
<td></td>
</tr>
<tr>
<td>CER</td>
<td>0/5 0/1 0/2 0/17 0/0 1/32 0/73</td>
</tr>
<tr>
<td>CG</td>
<td>3/67 0/65 0/28 1/48 6/30 1/36 1/29 12/303</td>
</tr>
<tr>
<td>DR</td>
<td>0/35 0/0 0/3 0/0 0/0 0/0 0/0 0/50</td>
</tr>
<tr>
<td>DT</td>
<td>0/0 0/0 0/0 2/14 4/25 1/8 1/5 8/52</td>
</tr>
<tr>
<td>LDT</td>
<td>0/0 1/94 7/99 6/60 1/38 0/0 0/0 15/291</td>
</tr>
<tr>
<td>RF</td>
<td>0/17 1/49 0/21 3/52 8/62 2/24 2/43 16/268</td>
</tr>
<tr>
<td>LPB</td>
<td>0/0 0/0 0/0 0/32 0/0 0/0 0/0 0/32</td>
</tr>
<tr>
<td>Mixed type</td>
<td></td>
</tr>
<tr>
<td>CER</td>
<td>0/5 0/1 0/2 0/17 0/0 1/32 0/73</td>
</tr>
<tr>
<td>CG</td>
<td>2/67 0/65 0/28 2/48 3/40 1/36 1/29 9/303</td>
</tr>
<tr>
<td>DR</td>
<td>3/35 0/10 0/2 0/3 0/0 0/0 0/0 3/50</td>
</tr>
<tr>
<td>DT</td>
<td>0/0 0/0 0/0 0/14 3/25 1/8 0/5 4/52</td>
</tr>
<tr>
<td>LDT</td>
<td>0/0 0/94 8/99 4/25 1/38 0/0 0/0 15/291</td>
</tr>
<tr>
<td>RF</td>
<td>1/17 0/49 2/21 3/52 2/62 1/24 0/43 8/268</td>
</tr>
<tr>
<td>LPB</td>
<td>0/0 0/0 0/0 0/32 0/0 0/0 0/0 0/32</td>
</tr>
<tr>
<td>Total</td>
<td>5/124 2/219 10/152 9/226 10/169 9/70 0/109</td>
</tr>
<tr>
<td>Muscular type</td>
<td></td>
</tr>
<tr>
<td>CER</td>
<td>0/5 0/1 0/2 0/17 0/0 1/32 0/73</td>
</tr>
<tr>
<td>CG</td>
<td>1/67 0/65 0/28 2/48 3/30 0/36 0/29 4/503</td>
</tr>
<tr>
<td>DR</td>
<td>1/35 0/10 0/2 0/3 0/0 0/0 0/0 1/50</td>
</tr>
<tr>
<td>DT</td>
<td>0/0 0/0 0/0 0/14 0/25 0/8 0/5 0/52</td>
</tr>
<tr>
<td>LDT</td>
<td>0/0 1/94 0/99 0/25 0/62 0/12 0/43 1/268</td>
</tr>
<tr>
<td>RF</td>
<td>0/17 0/49 0/21 0/52 0/62 1/24 0/43 1/268</td>
</tr>
<tr>
<td>LPB</td>
<td>0/0 0/0 0/0 1/32 0/0 0/0 0/0 1/32</td>
</tr>
<tr>
<td>Micturition type</td>
<td></td>
</tr>
<tr>
<td>CER</td>
<td>0/5 0/1 0/2 1/17 0/14 0/2 1/32 2/73</td>
</tr>
<tr>
<td>CG</td>
<td>4/67 0/65 0/28 3/48 0/30 0/36 0/29 8/303</td>
</tr>
<tr>
<td>DR</td>
<td>16/35 0/10 0/2 0/3 0/0 0/0 0/0 16/50</td>
</tr>
<tr>
<td>DT</td>
<td>0/0 0/0 0/0 0/14 0/25 0/8 0/5 1/52</td>
</tr>
<tr>
<td>LDT</td>
<td>0/0 2/94 2/99 6/60 4/38 0/0 0/0 14/291</td>
</tr>
<tr>
<td>RF</td>
<td>0/17 1/49 0/21 2/52 1/62 1/24 2/43 7/268</td>
</tr>
<tr>
<td>LPB</td>
<td>0/0 0/0 0/0 2/32 0/0 0/0 0/0 2/32</td>
</tr>
</tbody>
</table>

Table 2. Proportion of effective sites to total stimulation sites for each response in each anatomical area of Fig. 6

A higher proportion of evoked full erections was evoked from plate 4 (DT and LDT) and plate 5 (CG, LD, LDT and RF). See Fig. 6 for abbreviations.
Brain stem and penile erection. Numerous studies have reported the involvement of the brain stem in several aspects of sexual behavior (11, 14, 15, 26, 27, 44). However, only a few studies suggest the involvement of the LDT in sexual function; in monkeys, penile erection was evoked by electrical stimulation to the pontine CG, the area corresponding to the caudal edge of the LDT (26). In rats, BS muscle activation induced by the medial preoptic area (MPOA) stimulation was disrupted after lesion of the CG (27). Also, electrical and ibotenic acid lesions to the CG decreased ejaculation latency, ejaculation interval, and intromission frequency (15). In the latter two studies, the lesioned sites overlap only with the anterior part of the LDT, but the central part of the LDT has scarcely been examined. The present study is the first to implicate the involvement of the LDT in penile erection.

It is well known that the LDT has a crucial role in the regulation of REM sleep (19, 30, 45, 55). The cholinergic neurons in the LDT display specific firing pattern during sleep waking cycles; some are active both during REM sleep and waking, whereas others are specifically active during REM sleep and are called REM-on or paradoxical sleep (PS-on) neurons (10, 21, 55). Recently, we have found that a group of REM-on (PS-on) neurons discharge in close relation with penile erection during REM sleep; one of them shows a burst firing pattern in synchronous with each erectile event, whereas another increases its tonic firing activity in advance of the penile erection. The firing changes of these types of neurons were specific for erection during REM sleep, suggesting that the cholinergic neurons in the LDT are involved in the regulation of penile erection specifically during REM sleep (23). We hypothesize that the erection evoked after the LDT stimulation is a consequence of activation of the cholinergic neurons in the LDT. It remains to be determined whether the effect of the stimulation on erection differs during waking and during REM sleep.

Penile erection was also evoked from the medial part of the dorsal pontine area (DT and the surrounding areas). The DT sends efferents to the hypothalamic areas, including the dorsomedial hypothalamus, posterior hypothalamus, or mammillary nucleus (2, 53), areas that have been reported to induce penile erection after electrical stimulation (40, 48, 53). Stimulation of the DT and the surrounding areas would have activated these ascending systems that project to the hypothalamus.

It has been reported that the nucleus paragigantcellaris and the nucleus raphe obscurus have an inhibitory influences on penile erection through the serotonergic system (20, 29), whereas stimulation to the raphe magnus induces penile erection (56). Anatomical studies have revealed a descending projection from the LDT to the raphe magnus (18). If the descending LDT neurons are involved in penile erection, the influence could be mediated through the relay structures such as the raphe magnus.

Preoptic/hypothalamic areas and penile erection. The preoptic/anterior hypothalamic area, mainly the medial preoptic area, is known to play a crucial role in the regulation of a variety of sexual functions (7, 9). Among them, several authors have reported the involvement of the preoptic area, bed nucleus of stria terminalis, or the PVN in the regulation of penile erection (1, 12, 25, 47, 50). Anatomical studies revealed the descending projections from the preoptic area to the brain stem area in and around the LDT (28, 43, 54). Ascending projections from the spinal cord pass in close proximity or through the LDT (42). Because we used an electrical stimulation method, it is possible that the erection evoked from the LDT is caused by activating the passing fibers that originate from the preoptic area or the spinal cord as well as by activating some of the LDT neurons.

It has been reported that the LDT neurons send ascending projections to the PVN or the LPOA (52), areas that may play a role in penile erection generation. Penile erection was evoked when several substances were injected in the PVN, including apomorphine (33), oxytocin (32), or glutamate (6, 65). FOS immunohistochemical study in the PVN suggested the increase of PVN neuronal activity during penile erection (22). Because the PVN projects directly to the intermediolateral region of the spinal cord (42), stimulation of the LDT could have resulted in the activation of the PVN and, through its direct descending projection, triggered a penile erection generator in the spinal cord. Schmidt et al. (50) have reported that the chemical lesion of the LPOA disrupts penile erection specifically during REM sleep while leaving waking-state erections intact. MPOA lesions, on the other hand, disrupt copulatory behavior. It has also been reported that the injections of carbachol in the LPOA induce penile erections and that the source of these cholinergic afferents is the LDT (48). Because we have recorded the cholinergic LDT neurons that fire in close relation with penile erection during REM sleep (23), we could hypothesize that the cholinergic neurons in the LDT, through ascending projections to the LPOA, may have a role in the regulation of penile erection during REM sleep.

Perspectives and Significance

Our brain stem stimulation data suggest a role for the dorsal pontine tegmentum in the erectile control, an area that deserves further elucidation in penile erection neurophysiology, and an implication of the LDT in broader aspects of REM sleep.
related events, in addition to EEG desynchronization, muscular atonia, or blood pressure fluctuation. Because the LDT is abundant in cholinergic neurons, the results further suggest the possibility of the cholinergic treatment for the therapy of erection dysfunction.

REFERENCES