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Brain Research 1033 (2005) 41–50
BRAIN RESEARCH
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Research report

Properties of Barrington's neurones in cats: units that fire inversely with micturition contraction

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Accepted 16 November 2004
Available online 11 January 2005

Abstract

Barrington's nucleus contains neurones that decrease their firing during micturition contraction, as well as neurones that increase their firing during this phase. These neurones are commonly termed *inverse neurones* and *direct neurones*, respectively. The aims of the present study were to determine whether inverse neurones send descending axons to the spinal cord and to clarify how these neurones regulate bladder contractility. Forty-five single neurones were recorded from the dorsolateral pontine tegmentum. Spinal-projecting neurones were identified by antidromic stimulation of the spinal cord. More than half of inverse neurones were located outside Barrington's nucleus. Only three were spinal-projecting neurones. The results were in marked contrast with direct neurones that we studied previously: the majority of them were located within Barrington's nucleus, and 56% were spinal-projecting neurones. The firing frequency of inverse neurones ranged between 6 and 37 Hz during the relaxation phase of the micturition contraction–relaxation rhythm. The firing of all neurones began to decrease within 8 s after the onset of micturition contraction. During micturition contraction, neurones displayed little firing, being virtually silent ($n = 29$) or displayed weak tonic firing (3–11 Hz; $n = 16$). All neurones began to increase their firing within 8 seconds after the onset of bladder relaxation. These results suggest that inverse neurones do not trigger bladder contraction or relaxation, despite the finding that a few of them send descending axons to the spinal cord. One possible role of the inverse neurone is to regulate firing activities of direct neurones in Barrington's nucleus.

Theme: Endocrine and autonomic regulation
Topic: Gastrointestinal and urogenital regulation

Keywords: Micturition; Bladder; Pons; Barrington's nucleus

1. Introduction

Previous anatomical, electrophysiological, and pharmacological studies have revealed that the micturition reflex centre of several species is located in the dorsolateral pontine tegmentum (for a review, see Ref. [19]). A distinct population of neurones localized within a limited area of the dorsolateral pontine tegmentum projects directly to the sacral parasympathetic nucleus [1–3,8]. This same area has been retrogradely labelled via transneuronal transport of pseudorabies virus (PRV) following injections into the bladder wall [5]. Electrical stimulation of this pontine area causes bladder contraction, inhibition of the external urethral sphincter, and relaxation of the urethra [7,9], indicating that this specific region of the pontine tegmentum is a central component of the micturition circuit. Hence, this region has been described as the pontine micturition centre, the M-region, or Barrington's nucleus.

Barrington's nucleus contains a large number of neurones that increase their discharge during micturition contraction (*direct neurones*) (rats: [15,18], cats: [5]). Fifty-six percent of direct neurones in this area sent descending axons to the spinal cord: most of them to the sacral spinal cord [13,14]. In addition to *direct neurones*, Barrington's nucleus also contains neurones that decrease their discharge during micturition contraction (*inverse neurones*) (rats: [15,18], cats: [5]). The terms direct neurones and inverse neurones

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doi:10.1016/j.brainres.2004.11.016

were coined by de Groat and colleagues [5]. In contrast to direct neurones, it is unknown whether inverse neurones project to the spinal cord. The lumbar sympathetic nucleus inhibits bladder contraction when urine collects in the bladder, and the sacral parasympathetic nucleus activates micturition contraction. Determining whether inverse neurones project to the spinal cord is necessary to clarify their role. One would expect that neurones directly associated with bladder contractility would send descending axons to the spinal cord (i.e., spinal-projecting neurones).

The present study aimed to elucidate the role of inverse neurones in controlling bladder contractility. We sought to record inverse neurones in the dorsolateral pontine tegmentum, specifically aiming for Barrington's nucleus. Firing properties such as onset of firing decrease and onset of firing recovery were examined. Descending axonal projections to the spinal cord were identified antidromically.

2. Materials and methods

2.1. General procedures

Experiments were performed on 18 adult cats of both sexes weighing between 2.3 and 5.1 kg. All experimental procedures were approved by the Tokyo Medical University Institutional Animal Care and Use Committee in accordance with the *NIH Guidelines for the Care and Use of Laboratory Animals*. Cats were initially injected with ketamine (100–150 mg; i.m.). After tracheotomy, animals were anaesthetised with halothane–N_2O (0.8–1.2% halothane in a 33% N_2O and 67% O_2 inhalation mixture) before recording. A femoral artery and vein were cannulated for blood pressure recording and drug administration. The urethra was cannulated for bladder pressure recording. Animals were then mounted in a spinal cord recording frame and a stereotaxic frame. Preparation included making a craniotomy in the interparietal bone, partially aspirating the anterior cerebellum to facilitate insertion of a recording electrode, and performing a laminectomy to gain access to the spinal cord for electrical stimulation. Throughout surgery, the cats were artificially ventilated in the absence of immobilizing drugs. Corneal and pedal withdrawal reflexes were monitored continually to assess depth of anaesthesia; anaesthesia was adjusted accordingly. Halothane was then discontinued, and the animals were anaesthetised throughout the remainder of the experiment with α-chloralose (initial dose: 35–40 mg/kg, i.v.). Following this anaesthetic change, the animals remained immobile for 1–2 h. Additional doses of α-chloralose (3–5 mg/kg; i.v.) were injected every one or 2 h; the total dosage over the course of the experiment was 60–100 mg/kg. Depth of anaesthesia was monitored continually throughout the experiment.

To prepare for recording and stimulation, the dura was opened, and the exposed spinal cord was covered with mineral oil to prevent desiccation. Equipment for recording single units and for stimulating the spinal cord was setup and adjusted. During recording, the animals were paralyzed by pancuronium bromide (Mioblock, Organon, The Netherlands) and were artificially ventilated. Rectal temperature was maintained around 38 °C with a thermo-regulated heating blanket. Blood pressure was recorded with a chart recorder (RTA1200, Nihon Kohden, Japan) throughout the experiments. The blood pressure of all animals remained stable. The animals' pupils were checked several times each hour. Their pupils remained constricted throughout the experiment, indicating that anaesthesia was maintained at a level at which no indication of pain could be detected in intact animals. To ensure that the neuromuscular blocking agent was not masking signs of discomfort and to confirm that the level of anaesthesia was sufficient, we discontinued the pancuronium bromide once or twice for 1–2 h during the experiments.

At the end of the experiment, animals were deeply anaesthetised and were perfused with 20% formalin. For histological identification of recording and stimulating sites, the brainstem was removed and sectioned serially (100 μm) in the transverse plane. The tissue sections were subsequently stained with Cresyl violet.

2.2. Preparation of the bladder

A catheter (size, Fr 5) was inserted through the urethral orifice in 13 male cats. In 5 female cats, the urethra was exposed via an abdominal incision, and a catheter was inserted through a small puncture made in the urethra about 1–2 cm distal to the bladder neck. The catheter was connected via a three-way stopcock to a low-pressure transducer (LPU-0.1, Nihon Kohden) for measurement of the intravesical pressure, or to a syringe for infusion or withdrawal of the intravesical fluid. The micturition contraction–relaxation rhythm under this isovolumetric condition was displayed on the chart recorder.

2.3. Stimulation

In 15 animals, microstimulation of the spinal cord was done using a carbon monopolar electrode insulated with a glass pipette, except for the tip (diameter of the carbon fibre, 8 μm; uninsulated tip length, 10–20 μm). Prior to each experiment, the optimal electrode position in the cord for evoking bladder contraction was systematically determined by stimulating the cord with 30–50 train pulses (200 Hz, 50–200 μA, 150 μs cathodal square pulses). Bladder contractions with 0.25–0.5 s latencies were effectively evoked from the surface of the dorsolateral funiculus (DLF) of the spinal cord. For eliciting antidromic spikes in the pontine tegmentum, one or two carbon electrodes were placed on the surface of the DLF at the upper lumbar cord (the border between the thoracic [T13] and lumbar cord [L1], indicated as T/L hereafter) or between L1 and L2 (L1/2; 5 animals), at the middle lumbar cord (L3/4 or L4/5; 6 animals), and/or at the

lower lumbar cord (L6/7 or L7/S1; 9 animals). Microstimulation of the spinal cord was not done in 3 animals. In one of these animals, the gluteus muscle was removed to expose the pudendal nerve at the level of the sciatic nerve, where it branches and courses towards the deep part of ischiorectal cavity. The cutaneous branch of the pudendal nerve was dissected free and was stimulated orthodromically using bipolar platinum hook electrodes.

2.4. Recording of single units

Glass micropipettes filled with 2 M NaCl saturated with fast green FCF dye (resistance 2–5 MΩ) were used for extracellular recording of single units. Recordings were made with a differential amplifier (AB-651J, Nihon Kohden; low cut off: 50 Hz). Spikes of single units were counted with a pulse-counting window discriminator (QC-111J and ET-612J, Nihon Kohden). Recording electrodes were angled 30–45° in the rostral direction from the Horsely-Clarke frontal plane. Exploration for units was restricted to the region around the dorsolateral pontine tegmentum 1.5–3.5 mm lateral to the midline, 0–4 mm caudal to the level of the trochlear decussation, and up to 3 mm deep from the surface of the midline. When bladder-contractility-related units were encountered, they were examined for antidromic activation from the spinal cord. Antidromic spikes evoked from the stimulating electrode were tested for positive collisions with spontaneous spikes generated by the single unit to verify that they were being emitted from the same neurone. Neuronal firing, firing rate, and bladder contractility were displayed on the chart recorder, and firing of selected units was also recorded with a DAT recorder (PC-108 M, Sony, Japan) for off-line analysis. Digitized waveforms of antidromic

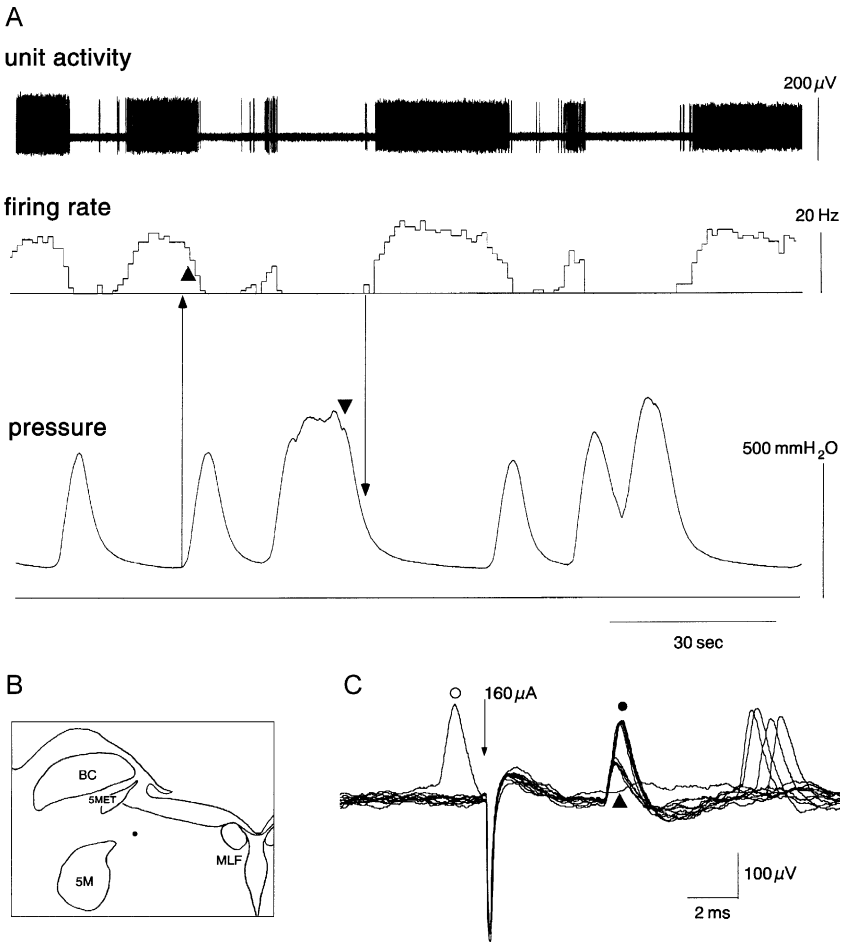

Fig. 1. An inverse neurone recorded in the dorsolateral pontine tegmentum that ceases firing during micturition contraction. This neurone sent a descending axon to the spinal cord. (A) Raw voltage traces of a single unit recorded in the dorsolateral pontine tegmentum (upper trace), firing rates (pulses/s) (middle histogram), and bladder contractility (lower trace). Upward arrow, onset of micturition contraction; arrowhead, start of firing decrease; downward arrowhead, onset of bladder relaxation; downward arrow, start of firing increase. (B) A recording site of this single unit (●). For abbreviations, see the legend of Fig. 4. (C) Identification of antidromic spikes. Antidromic spikes (●) were evoked after stimulation (arrow; 160 μA) of the dorsolateral funiculus (DLF) between L4 and L5 (L4/5). Stimulation failed to evoke an antidromic spike (arrowhead) when a spontaneous spike (○) precedes antidromic stimulation. The amplitudes of three antidromic spikes were reduced consistently by about one-half, suggesting that soma-dendritic spikes were blocked. This implies that the neurone was recorded from the cell body, not from the axon.

spikes were stored on disk with a conventional computer system. In each experiment, the location of a recorded single unit was marked with iontophoretically deposited fast green FCF dye by passing negative current (30–40 μA, 10–30 min) before changing recording electrodes. The recording sites of single units were reconstructed with reference to the dye marks and the depths of the microelectrodes noted during the experiment.

3. Results

The present study recorded inverse neurones in the dorsolateral pontine tegmentum. We determined whether these neurones send axonal projections to the spinal cord by antidromic stimulation tests. We also recorded from a few bladder contractility-related neurones that displayed complex firing patterns and a number of other single units whose firing was uncorrelated with micturition contraction. The recording data of these neurones are described separately in the last subsection.

3.1. Inverse neurones

Fig. 1A shows the typical firing pattern of an inverse neurone in the dorsolateral pontine tegmentum (filled dot in Panel B). The neurone displayed spontaneous firing with a frequency of around 23 Hz. Firing began to decrease 1 s (upward arrowhead) after the onset of micturition contraction (upward arrow). Thereafter, the neurone rapidly became silent. This complete inhibition of firing continued throughout the contraction phase. The neurone started to fire again (downward arrow) 4–6 s after the onset of bladder relaxation (downward arrowhead). During this time, bladder pressure declined, falling close to base line pressure. Firing frequency gradually increased and reached constant levels 9–11 s after the onset of bladder relaxation.

Stimulation of the DLF at the L4/5 level elicited fixed latency, antidromic spikes in this neurone (Fig. 1C; indicated by filled dot (●)). One antidromic spike collided with a preceding spontaneous spike (Fig. 1C; trace indicated by open circle (○) and arrowhead), indicating that both antidromic and spontaneous spikes were evoked

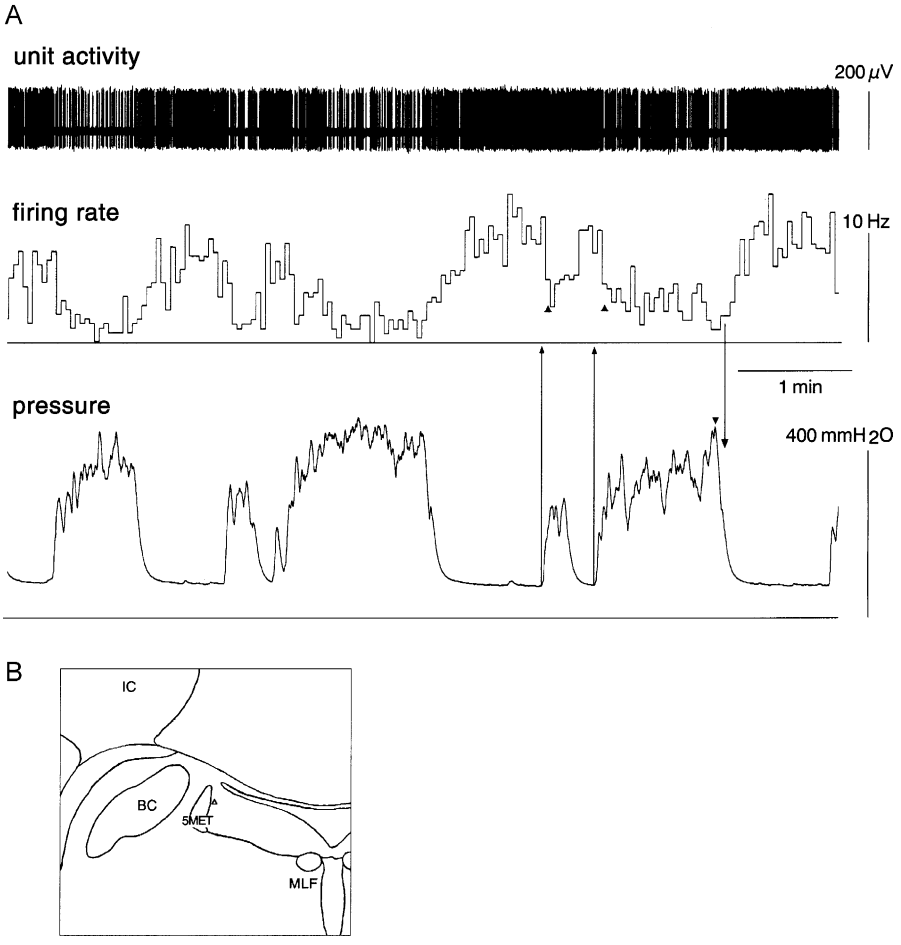

Fig. 2. A single unit that decreased firing during micturition contraction. (A) Arrangement and symbols are the same as in Fig. 1A. (B) A recording site of this single unit (△).

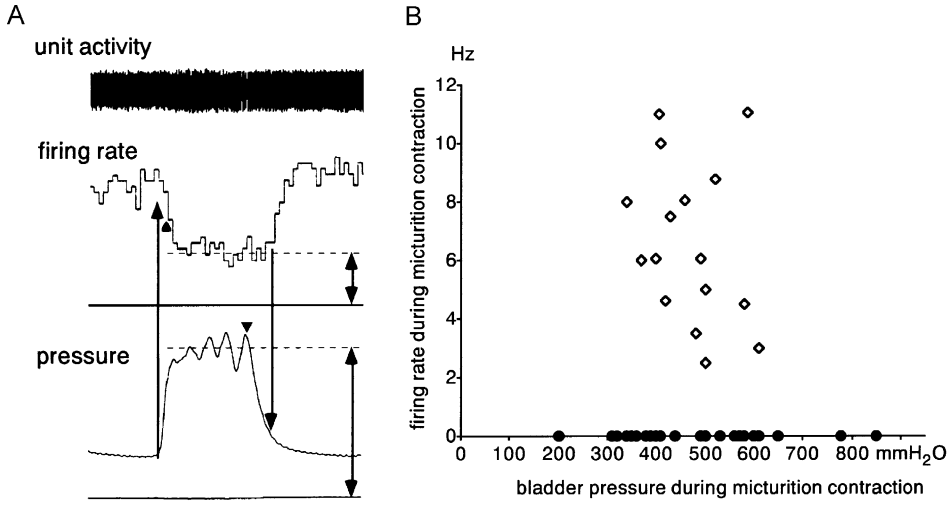

Fig. 3. Firing rate relationship of inverse neurones to bladder pressure during micturition contraction. (A) Schematic representation of the analyses. Example is taken from partially depressed neurones. Arrangement is the same as in Fig. 1A. Upward- and downward-pointing arrows indicate the firing rate (middle trace) and the bladder pressure during micturition contraction (lower trace). Upward arrow indicates onset of micturition contraction; upward arrowhead indicates start of firing decrease; downward arrowhead indicates onset of bladder relaxation; downward arrow indicates start of firing increase. (B) Lack of relationship between bladder pressure and firing rate during micturition contraction. Method for calculating values is shown in Panel A. Filled dots (●) represent completely depressed neurones; diamonds (◇) represent partially depressed neurones.

in the same neurone. From these observations, we concluded that this neurone was an inverse neurone that projected to the spinal cord.

Fig. 2A shows the firing pattern of another inverse neurone in the dorsolateral pontine tegmentum (triangle (△) in Panel B). As with the firing pattern of the neurone described above (Fig. 1), this neurone decreased firing within 2–4 s after the onset of micturition contraction. In contrast to the first neurone, however, this neurone continued to fire at low frequencies during micturition contraction. Firing increased 2–4 s after the onset of bladder relaxation, reaching constant levels 12–20 s after the onset of bladder relaxation. Antidromic stimulation (1 mA) delivered at the border between T13 and L1 (T/L) of the spinal cord failed to antidromically activate this neurone.

In total, 45 inverse neurones were recorded from the dorsolateral pontine tegmentum. The firing rate of neurones during the relaxation phase of the micturition contraction–relaxation rhythm was between 6 and 37 Hz (mean ± SD: 17.3 ±1 1.2 Hz). During micturition contraction, firing of 29 neurones was depressed completely (cf. Fig. 1). For 16 neurones, firing was only partially depressed (3–11 Hz; mean ± SD: 6.6 ± 2.7 Hz) during micturition contraction (cf. Fig. 2).

We examined the possibility that firing is depressed completely when the bladder pressure during micturition contraction is high. Fig. 3B shows the relationship between bladder pressure during micturition contraction and firing rate in 45 neurones. Partially depressed neurones (diamonds (◇)) clearly had a different firing rate distribution from that of completely depressed neurones (●). No tendency emerged suggesting that the firing rate of partially-depressed neurones was lower at higher pressure ranges during micturition contraction (correlation coefficient: $r = -0.25$, $P > 0.1$). In addition, 6 neurones were silent, even when the bladder pressures were lower than those observed during recordings of partially depressed neurones (340–610 mm H_2O). These results indicate that some inverse neurones exist that are not completely depressed during micturition contraction.

Table 1
Spinal levels of antidromic stimulation examined for 45 inverse neurones

Type	Antidromic spike	Number	Spinal level of antidromic stimulation		
			T/L-L1/2	L3-5	L6/7-L/S
Completely depressed	+	3	2	1	0
	–	23	10	6	13
	NID	3			
Partially depressed	+	0	0	0	0
	–	13	8	4	5
	NID	3			

Completely depressed: neurones that were completely depressed during micturition contraction. Partially depressed: neurones that were partially depressed during micturition contraction. +: neurones antidromically activated; –: neurones not activated. NID: neurones that were not tested by antidromic activation. The sum of neurones (T/L-L1/2 + L3-5 + L6/7-L/S) is larger that the number of neurones in the third row, because one or two different level(s) were stimulated in each neurone.

Antidromic tests were performed in the upper lumbar (T/L or L1/2), mid lumbar (L4/5), and/or lumbosacral segments (L6/7 or L/S) for 39 neurones (see Table 1). Most of the neurones (n = 36) were not activated antidromically from the spinal cord with stimulus intensities ranging between 1–5 mA; we termed these ANTI (−) neurones. Only three neurones were activated antidromically from the T/L (n = 2) or L4/5 (n = 1) with stimulus intensities ranging between 50 and 400 μA; we termed these spinal-projecting neurones. In two of these neurones, we performed antidromic collision tests. (We did not test the neurone shown in Fig. 1.) Antidromic spikes evoked from the stimulating electrode collided with spontaneous spikes generated in the single unit, verifying that they were being emitted from the same neurone. The conduction velocity of the descending axon was estimated using the measured antidromic latency and the distance between the stimulating and the recording sites; 0.2 ms was subtracted to account for the latent period of spike initiation at the stimulating site [10]. The conduction velocity was 67.5 m/s for the neurone shown in Fig. 1, and 12.5 and 23.1 m/s for the remaining two neurones. All spinal-projecting neurones were completely depressed during micturition contraction.

Recording sites of 39 inverse neurones were successfully reconstructed from the histological sections. Recording sites were located in the rostral part of the dorsolateral pontine tegmentum. They extended from the level of the inferior colliculus rostrally, and to the rostral edge of the motor trigeminal nucleus caudally (Figs. 4A–C). Neurones were recorded at sites ventral to the mesencephalic tract of the trigeminal nerve (5 MET) at rostral levels, and at sites medial and ventral to the 5 MET at middle and caudal levels. Stippled areas indicate the location of direct neurones that send descending axons to the spinal cord [14]. Both the completely depressed (circles) and partially depressed (triangles) neurone types were distributed within (n = 18) or around (n = 21) the stippled area. One spinal-projecting neurone was located within the stippled area (Fig. 4A: closed circles (●)). The remaining two spinal-projecting neurones were located lateral to (Fig. 4B) or ventral to this area (Fig. 4C).

As shown in Figs. 1A, 2A, and 3A, firing rates began to decrease shortly after the onset of micturition contraction (between upward arrow and upward arrowhead in Fig. 3A). The decreased firing rates persisted for a while after the onset of bladder relaxation (between the downward arrowhead and downward arrow). The possible existence of this firing pattern was examined in 45 neurones. Two to four micturition cycles of the rhythm were evaluated. In most neurones (n = 36), the firing rate began to decrease after the onset of micturition contraction (Fig. 5A). The remaining 9 neurones started to decrease their firing at approximately the onset of micturition contraction. The latency of the decrease ranged

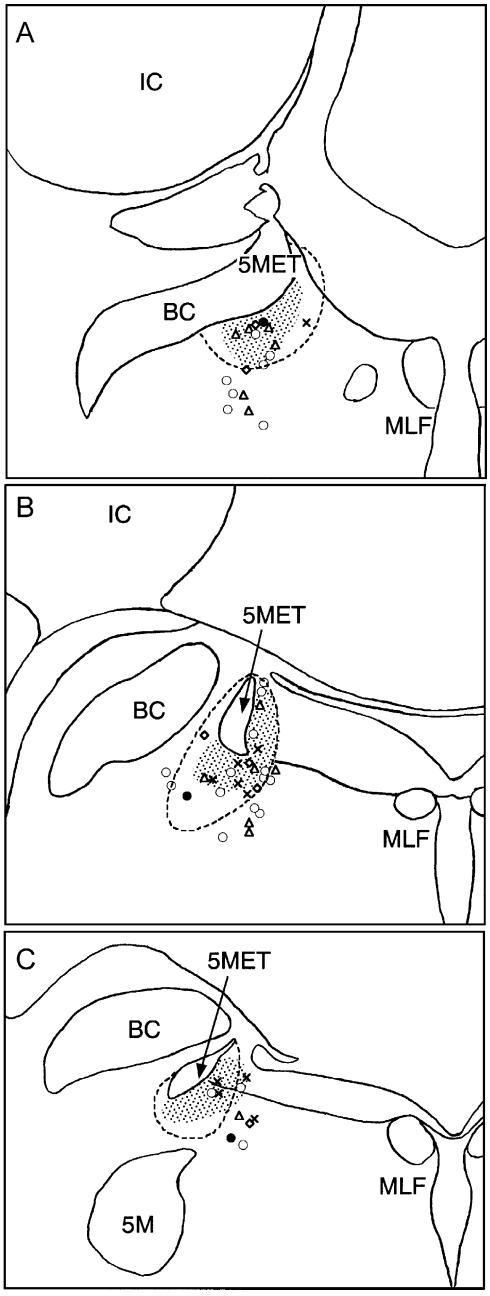

Fig. 4. Recording sites of 55 single units in the dorsolateral pons. (A–C) Rostral (A), mid-rostrocaudal (B), and caudal (C) levels of the dorsolateral pontine tegmentum. Circles represent inverse neurones whose firing was completely depressed during micturition contraction; triangles represent inverse neurones whose firing was partially depressed during contraction; and diamonds represent neurones that displayed complex firing. The crosses represent spinal-projecting neurones whose firing was uncorrelated with micturition contraction. Filled circles represent spinal-projecting neurones, and open symbols represent neurones that could not be activated antidromically from the spinal cord. Stippled areas indicate the locations of direct neurones that issue descending axons to the spinal cord [14]. Areas surrounded by broken lines indicate the locations of the locus coeruleus. To enhance the clarity of this illustration, neurones whose spinal projections were not identified (177 neurones whose firing was uncorrelated with micturition contraction and 6 inverse neurones including a unit in Fig. 6C) were not incorporated into this figure. Abbreviations: 5MET, mesencephalic tract of the trigeminal nerve; 5 M, trigeminal motor nucleus; BC, brachium conjunctivum; CNF, cuneiform nucleus; MLF, medial longitudinal fasciculus; IC, inferior colliculus; TD, dorsal tegmental nucleus; TV, ventral tegmental nucleus.

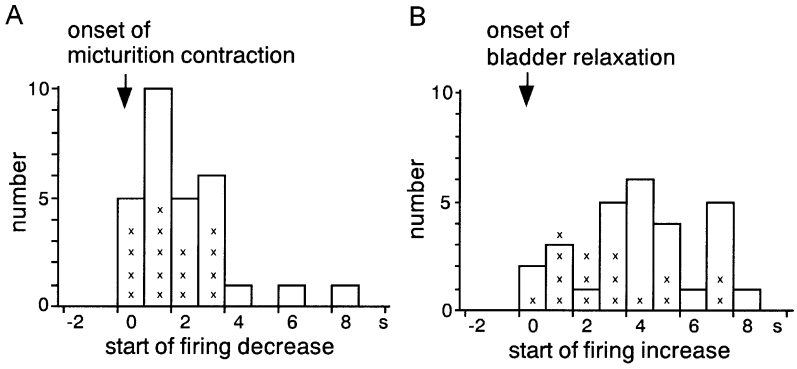

Fig. 5. Firing properties of inverse neurones in relation to micturition contraction. (A) Frequency histogram for 45 neurones showing the onsets of firing decrease (upward arrowhead in Fig. 3A) after the onset of micturition contraction (downward arrow; see upward arrow in Fig. 3A). (B) Frequency histogram for 45 neurones showing the onsets of firing increase (downward arrow in Fig. 3A) after the onset of bladder relaxation (downward arrow; see downward arrowhead in Fig. 3A). (A) and (B) Open bars represent neurones that were completely depressed during contraction; crosses represent neurones that were partially depressed during contraction.

between 0 and 7 s (2.1 ± 1.8 s) in the completely depressed type of neurones, and between 0 and 4 s (1.6 ± 1.2 s) in the partially depressed type. The firing rates decreased further with time and became silent in the completely depressed type within 10 s (4.4 ± 3.4 s) from the onset of contraction, or in the partially depressed type, reached a plateau within 8 s (4.0 ± 2.4 s). Depression of firing frequency was maintained for 0–8 s after the onset of bladder relaxation (4.5 ± 2.8 s in completely depressed neurones; 3.1 ± 2.0 s in partially depressed neurones) (Fig. 5B). We found no significant differences among the measured parameters of Fig. 5 for comparisons between the completely depressed neurone and the partially depressed neurone types ($P > 0.1$, Student's two tailed t test).

Inverse neurones displayed tonic firing during the relaxation phase of the micturition contraction–relaxation rhythm. We next examined whether this characteristic is inherent in the inverse type of neurone. Three neurones displayed a gradual firing decrease when we lowered the base bladder pressure below the micturition threshold by withdrawing intravesical fluid. The average firing rates of the neurone shown in Fig. 6A (the same neurone shown in Fig. 2) decreased from 23 Hz to 3.5 Hz, 12 min after lowering the pressure. In the other neurone, firing rates decreased to 5% and 26%, 5 and 24 min after emptying the intravesical fluid, respectively. On the other hand, two neurones displayed a gradual firing decrease when the relaxation phase of the micturition contraction–relaxation rhythm was prolonged (see Fig. 6B). When compared to firing in the relaxation phase of the regularly occurring micturition contraction–relaxation rhythm, firing rates in this prolonged period decreased to 8% (Fig. 6B; between upward arrows) or 31% (the remaining one). Interestingly, the neurone shown in Figs. 6B and C began to fire robustly when the cutaneous branch of the pudendal nerve was electrically stimulated (downward arrow). Although the bladder began to display small contractions (arrowheads; 2–3 mm H_2O), the base pressure was virtually the same as that before stimulation.

3.2. Other types of micturition-related neurones in the dorsolateral pontine tegmentum

Six neurones with complex firing patterns were recorded. All these neurones fired tonically during the relaxation phase of the micturition contraction–relaxation rhythm. Four neurones displayed a firing increase before the onset of micturition contraction. We observed several patterns. The firing rate was completely depressed just after the onset of micturition contraction ($n = 1$), or the firing rate continued to increase during the rising phase of micturition contraction, but was depressed at even higher pressure levels of micturition contraction ($n = 3$). The remaining 2 neurones displayed a firing decrease before the onset of micturition contraction. Firing continued to decrease during the rising phase of micturition contraction, but at even higher pressure levels of contraction, firing was almost identical to that during relaxation phase. None of these neurones could be activated antidromically from the spinal cord with stimulus intensities between 1 and 5 mA. They were located in or around the stippled area (Fig. 4, diamonds).

We also recorded 187 neurones whose firing was not correlated with bladder contractility. These were encountered in all areas of the dorsolateral pontine tegmentum where we recorded. Antidromic tests were performed for some of these neurones, most of which were recorded simultaneously with, or very close to, the bladder contractility-related neurones. Ten neurones were activated antidromically from the spinal cord with stimulus strengths ranging between 60 and 500 μA. Seven spinal-projecting neurones were located in the stippled area (Fig. 4, crosses).

4. Discussion

In the present study, 45 inverse neurones were recorded from the dorsolateral pontine tegmentum. During micturition contraction, firing of the majority of inverse neurones was depressed completely. The firing of a minority of inverse

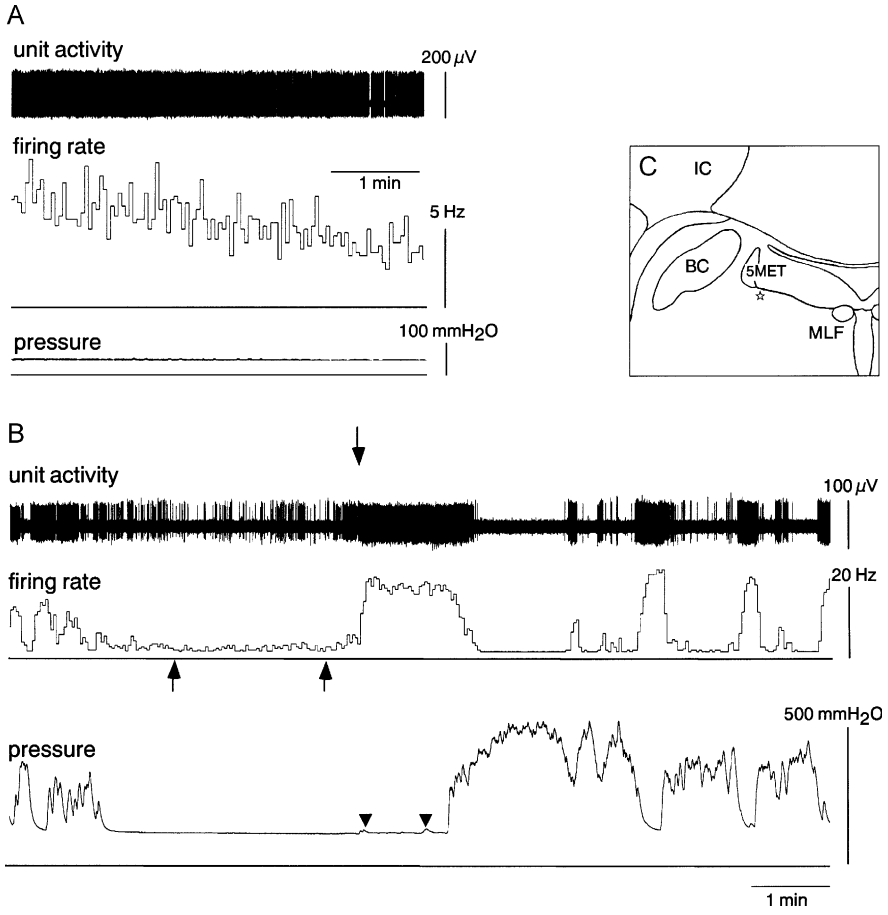

Fig. 6. Firing activity decreases gradually after lowering the bladder pressure below the micturition threshold (A) or with prolonged relaxation phase (B). (A) and (B) Arrangement is the same as Fig. 1A. (A) This unit is the same one as shown in Fig. 2. Firing activity 8–12 min after lowering bladder pressure by withdrawal of intravesical fluid. (B) A relaxation period of the micturition cycle prolonged unintentionally; the firing rate of this unit gradually decreased (compare with firing rates displayed in the other relaxation phase). Downward arrow in the upper trace indicates electrical stimulation of a cutaneous branch of the pudendal nerve (2V, single shock): the unit began to fire vigorously after stimulation. Decreased firing rate was measured at the period between the upward arrows. Arrowheads in the lower trace indicate small contractions with pressure amplitudes of 2–3 mm H_2O. (C) A recording site of the unit shown in B (☆).

neurones, however, was not completely depressed during micturition contraction. Thirty-nine inverse neurones were tested for antidromic activation from the spinal cord; three inverse neurones (7%) were identified as spinal-projecting neurones; the remaining 36 neurones failed to produce antidromic spikes. The proportion of spinal-projecting neurones is in marked contrast with that of direct neurones: 56% of them sent descending axons to the spinal cord [14]. Anatomical studies show that descending axons from Barrington's nucleus are packed tightly as they pass through the DLF [9]. We stimulated the surface of the DLF with strong stimuli, varying in intensity between 1 mA and 5 mA (typically 1 mA). Failure to elicit antidromic spikes was not overcome even when stimulating two different levels of the spinal cord (n = 11).

Inverse neurones were located in the rostral part of the dorsolateral pontine tegmentum, medial to the ventral part of the 5MET (Fig. 4). Their distribution extended from the level of the inferior colliculus to the rostral edge of the motor trigeminal nucleus. The stippled area in Fig. 4 indicates the region where spinally projecting direct neurones were compactly located [14]. This region corresponds to Barrington's nucleus [1,3,5]. More than half of inverse neurones were located outside the stippled area. This distribution contrasts with the previously examined direct neurones: the majority of them were located within the stippled area [14].

Very few spinally projecting, inverse neurones were located within (n = 1), or in close vicinity to (n = 2), the stippled area. ANTI (−) inverse neurones distributed in a somewhat broader region (beyond the stippled area of Fig. 4). A part of direct neurones that do not send a descending axon to the spinal cord are also distributed more broadly than the stippled area [14]. Thus, the present and previous studies suggest that a somewhat broad region of the dorsolateral pontine tegmentum deals with neural processes related to micturition function, but that final outputs to the spinal cord originate exclusively from a circumscribed area (i.e., Barrington's nucleus).

Antidromic tests were performed by stimulating three different parts of the spinal cord: upper lumbar, middle lumbar, and lumbosacral segments. Neurones that were not activated antidromically from the upper lumbar segments

were thought to be those that do not project to the spinal cord. Three inverse neurones were activated antidromically from the upper or middle lumbar spinal cord. Unfortunately, we could not determine whether they projected to the lumbosacral segments. Instead, antidromic tests were performed at the lumbosacral segments for 18 neurones (Table 1). These tests showed that no neurone sent descending axons to the lumbosacral segments. This implies that a few inverse neurones indeed project to the lumbar cord, and very few or none project to the sacral parasympathetic nucleus. This is in marked contrast with direct neurones in Barrington's nucleus, in which almost all spinal-projecting neurones sent axons to sacral segments [13]. Even if a few inverse neurones project to the sacral parasympathetic nucleus, they may not trigger bladder relaxation, since virtually no neurone increased firing before the onset of bladder relaxation (Fig. 5B). The present study thus supports the idea that an inhibitory mechanism of micturition contraction (i.e., "off-switch") does not exist in Barrington's nucleus [14]. A possible projection site of a few spinal-projecting neurones might be the bladder sympathetic nucleus or lumbar motor neurones that cooperate during micturition. But their role may be minor considering the very small number of spinal-projecting neurones that we encountered.

Antidromic tests performed in the upper lumbar segments suggested that most inverse neurones do not project to the spinal cord (Table 1). Instead, they may project to some other neural substrate of micturition in the brain. It is postulated that inverse neurones may be involved in local inhibitory circuits that modulate the switching mechanism in Barrington's nucleus and regulate the volume threshold for inducing micturition [5]. One possible target of inverse neurones might be the direct neurones of Barrington's nucleus that produce micturition contraction. In this case, inverse neurones would prevent excessive firing of direct neurones before micturition contraction, and would enhance increased firing of direct neurones during micturition contraction. The former would result from GABA- or glycinergic inhibition by inverse neurones, and the latter would result from inverse neurone-mediated disinhibition of direct neurons.

The firing rate of 3 inverse neurones decreased when bladder pressure was lowered below the micturition threshold (Fig. 6A). One possible explanation for this observation is that these neurones may receive excitatory inputs from bladder afferents. Indeed, electrical stimulation of afferent fibres in the pelvic nerve has been shown to activate inverse neurones [5]. Thus, the increase in the bladder pressure may exert opposite influences on the inverse neurones: that is, the pressure increase induces their firing via excitatory inputs from bladder afferents, but further pressure increase (micturition contraction) depresses their firing. In addition, the firing decreases of these inverse neurones were gradual, either after lowering the bladder pressure ($n = 3$) or after prolonging the relaxation phase ($n = 2$) (Fig. 6). The firing activity may not exclusively depend on bladder pressure. Rather, some activating mechanism appears to operate before micturition contraction, in at least part of the inverse neurones. One neurone displayed abrupt firing increase after the stimulation of the pudendal nerve, even when the bladder pressure was kept at virtually the same low level (Fig. 6B), which seems to support the existence of some activating mechanism. Activating mechanisms are also suggested to exist for directing firing patterns in direct neurones [14].

Recent neurone tracing studies have identified direct projections from the lumbosacral spinal cord to the caudal part of the lateral periaqueductal grey (PAG) and Barrington's nucleus: projections to the former are much larger than those to the latter [4,16]. The PAG relays afferent information from the bladder to Barrington's nucleus [4]. Thus, the PAG may be a candidate for a component that processes pattern formation. Whether the activating mechanisms, that are proposed in the previous and the present studies, exist in the PAG, or whether this "higher level" has a reciprocal effect on Barrington's neurones (excitatory on direct neurones, and inhibitory on inverse neurones) will be clarified in future study by recording the PAG neurones.

Consistent with previous studies [5,15], a number of neurones that fire independently of micturition contraction (independent neurones) were also encountered within and around Barrington's nucleus. This nucleus overlaps to some extent with the region of the locus coeruleus (Fig. 4; the area surrounded by broken line), which contains neurones that regulate sleep and wakefulness [12]. Most sleep/wakefulness-related neurones (noradrenergic and cholinergic neurones) in this area have been shown to be independent neurones [15]. A part of independent neurones, including those encountered in Barrington's nucleus, might also regulate certain pelvic organs. Transneuronal tracing has shown that pseudorabies virus injected into various pelvic organs (bladder, urethra, external urethral sphincter, penis, clitoris, colon, uterus, and ovary, see [6,11,17]) retrogradely labels neurones in Barrington's nucleus. This is consistent with the findings of the present study showing the existence of independent neurones that project to the spinal cord (Fig. 4, crosses). Whether these spinal-projecting neurones synapse with pelvic organs other than the bladder will be clarified by simultaneously recording spinal-projecting neurones and monitoring contractility of target organs. Such studies will advance our understanding of the functional role of the Barrington's nucleus in the control of pelvic organs.

Acknowledgments

The author would like to thank Professor Y. Uchino for constant encouragement and valuable advice throughout these experiments. This work was supported by the Grant-in Aid for Scientific Research (C) from the Ministry of Education, Science, Sports, and Culture of Japan (11680810).

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