



Firing properties of medullary expiratory neurons during fictive straining in cats

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Abstract

Expiratory (E) neurons in the caudal nucleus retroambigualis extend descending spinal axons to the lumbar and sacral spinal cord. Discharge rates of single E neurons were recorded to examine differences in activity of E neurons projecting to the lumbar or sacral spinal cord during fictive straining induced by distention of the colon with a balloon. Firing frequencies of E neurons with descending axons in the thoracic and lumbar spinal cord increased during the repetitive rise of rectum pressure, whereas those of E neurons with descending axons in the sacral spinal cord decreased. E neurons with descending axons in the thoracic/lumbar and sacral spinal cord exhibit different firing characteristics during the repetitive rise of rectum pressure when straining during defecation. The activity of abdominal nerves during fictive straining is in phase with changes in rectum pressure, but out of phase with the activity of E neurons.

Keywords Brainstem · Expiratory neuron · Spinal cord · Straining

Introduction

Expiratory (E) neurons in the caudal nucleus retroambigualis send descending spinal axons to the contralateral spinal cord [1, 2] and distribute axon collaterals in the thoracic and upper lumbar spinal cord [3–5]. E neurons exert respiratory synaptic effects on internal intercostal motoneurons [6–8] and abdominal (Abd) motoneurons monosynaptically and/or via interneurons [4, 9–13]. The majority of E neurons extend their descending spinal axons to the lower lumbar and sacral spinal cord [14]. In our previous study, the trajectory of collaterals was reconstructed based on the location of low-threshold foci and latency of antidromic spikes to investigate the intraspinal distribution of axon collaterals in the lumbar and sacral spinal gray matter of single E neurons

[5]. Many axon collaterals of E neurons were observed in the upper lumbar spinal cord. Abd muscles receive motor innervation from the upper lumbar segments [15] and are important for respiration, especially the control of expiration [16, 17], vomiting [18], and defecation [19, 20]. Axon collaterals in the sacral gray matter were also observed in one-third of E neurons, and collaterals were observed within the nucleus of Onuf, the region dorsal to the nucleus of Onuf, and the intermediate region [5].

Several studies have indicated functional connections between E neurons and Abd muscles in vomiting [18] and vocalization [21]. Cross-correlation histograms of neural discharge between E neurons and motoneurons innervating L1 Abd muscles exhibited several peaks [12]. Defecation is normally assisted by contraction of the diaphragm and Abd muscles. We recently discovered that Abd muscles and the diaphragm are co-activated to raise intra-abdominal pressure when defecation is induced by expansion of the colon (induced defecation) [20]. Thus, the diaphragm (which is related to the control of inspiration) and Abd muscles (which are related to the control of expiration) function simultaneously during induced defecation, especially during straining. Thus, we hypothesized that, since E neurons of the caudal nucleus retroambigualis extend their axons to both the lumbar and sacral spinal cord, they may play a role in

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co-ordinating the activities of neurons that regulate both respiration and autonomic functions.

The purpose of this study was to examine the possible roles of E neurons in both respiration and defecation, especially straining. We hypothesized that different activity would be observed during straining between E neurons projecting to the lumbar or sacral spinal cord. Therefore, we recorded the discharge of single E neurons in the caudal nucleus retroambigualis, identified the spinal segmental level of their descending axons, and analyzed the firing properties of E neurons during straining induced by distension of the colon with a balloon.

Methods

General procedures

Experiments were performed on ten adult cats, weighing 2.4–4.7 kg. In a preparatory surgical procedure, anesthesia was induced and maintained by 2–4% halothane delivered in a mixture of oxygen and room air in an anesthesia induction box. A tracheal tube was inserted into the trachea, and tracheal pressure was monitored through the intubated tube. Cats were maintained on 1–2% halothane through the intubated tube. The femoral artery and cephalic vein were cannulated to monitor blood pressure and administer drugs, respectively. Arterial blood pressure was maintained at 100–180 mmHg. Subcutaneous temperature was maintained at 37–38 °C with a heating pad. Special attention was paid to general conditions by observing the electrocardiogram, arterial blood pressure, body temperature, and pain-free state by monitoring narrow pupil size and stable arterial blood throughout the experiments.

The nerves innervating the Abd muscles (L1–L2) were dissected free, cut distally, and placed on bipolar cuff electrodes to record nerve discharges. We recorded from the lateral branch of the lumbar nerve innervating the external oblique muscle, and medial branch of the lumbar nerve innervating the internal oblique, the transverse abdominal, and rectus abdominal muscles. We used the medial branch for analysis due to obvious firing at the time of expiration. The phrenic (Ph) nerve (C5 and C6 branches) innervating the diaphragm was placed on bipolar cuff electrodes. In one experiment, after removal of the gluteal muscles, the bilateral pudendal nerves were dissected free and cut to remove the effect of mechanoreceptors in the rectal wall. The animals were mounted in a stereotaxic frame with a 20° nose-down orientation. The brainstem was exposed with a caudal craniotomy. Typically, no cerebellar tissue was removed. The spinal cord was exposed by a laminectomy at the C1–Th1 and Th13–S3 spinal levels, and spinal clamps were placed at Th12 and the sacral vertebra. The dura mater was opened,

and mineral oil pools were formed over the exposed spinal cords, with the temperature maintained at 36–37 °C.

After surgery, a mixture of urethane (100 mg/kg) and α -chloralose (50 mg/kg) was administered intravenously, while gaseous anesthesia was gradually removed. Animals were immobilized by the intravenous administration of pancronium bromide (0.4 mg/h, Mioblock; Sankyo, Organon) and were maintained on artificial ventilation. End-tidal CO₂ was monitored (CAPSTAR-100.CWE. Inc) and maintained between 4 and 6%. All experimental procedures were approved by the Animal Ethics Committee of Ibaraki Prefectural University of Health Sciences and were conducted in accordance with the guiding principles for care and use of animals in the field of physiological sciences of the Physiological Society of Japan.

Recording and stimulating procedures

Glass micropipettes filled with 2 M NaCl solution saturated with Fast green FCF dye (1–2 M Ω resistance) were used for extracellular recording of single E neurons. Extracellular spikes of E neurons and the activity of each nerve were recorded after amplification and band-pass filtering (150 Hz to 3 kHz) (AB-651J, Nihon Kohden, Japan). The pressure within the balloon was also recorded after amplification (AP-601G, Nihon Kohden, Japan). All signals were saved on a digital recorder (PC-208Ax, Sony, Japan). After the experiment, data were digitized at 10 kHz (Micro1401mkII, ADC12, Cambridge Electronic Design, Cambridge, UK) for subsequent analysis. Nerve discharges were full-wave rectified and integrated using a smoothing function with a time constant of 0.01 s (Spike 2, Cambridge Electronic Design, Cambridge, UK).

The rise in intra-abdominal pressure is achieved by co-contraction of the Abd muscles and the diaphragm [20]. In the present study, the balloon was inserted into the colon and kept in place by closing the anus. The balloon was made by tying a condom over a silicon tube. Defecation was induced by infusing warm water into the balloon. Once water was poured into the balloon, the activity of Abd and Ph nerves increased. We considered the co-activation of Abd and Ph nerves as fictive straining. The pressure in the rectum and activity of each nerve discharge were recorded during fictive straining.

Spinal projections of E neurons were tested by monopolar stimulation through a small Ag–AgCl ball electrode placed on the surface of the ventrolateral funiculus of Th13. Rectangular cathodal electrical pulses of 0.15 ms were applied. To estimate the most caudal spinal projection level of each E neuron, the surface of the ventrolateral funiculus was stimulated with another Ag–AgCl ball electrode; this stimulating electrode was shifted in a caudal direction along the lumbar and sacral spinal cord until antidromic spikes could no

longer be elicited. When stimulus voltage increased sharply and the latency of antidromic spikes became almost constant, electrical stimulation was terminated, and this location was considered as the lowest spinal level of the descending axon (Fig. 1a) [5].

E neurons with descending axons in the lumbar and sacral spinal cord were selected for further analysis. Recording sites of E neurons in the brainstem were marked with Fast green FCF dye injected electrophoretically through the recording microelectrode.

To measure firing properties of E neurons, the instantaneous frequency (IF), plotting the inverse of the time interval between a spike of and the previous spike as firing frequencies, interspike interval, plotting the time interval between a spike of and the previous spike (ISI), and coefficient of variation, the ratio of the standard deviation to the mean (CV) and to document covariation of activity of E neurons and the Abd nerve, cross-correlation between the instantaneous frequencies of E neuron discharge and integrated Abd nerve discharge (CC) were calculated (Spike 2, Cambridge

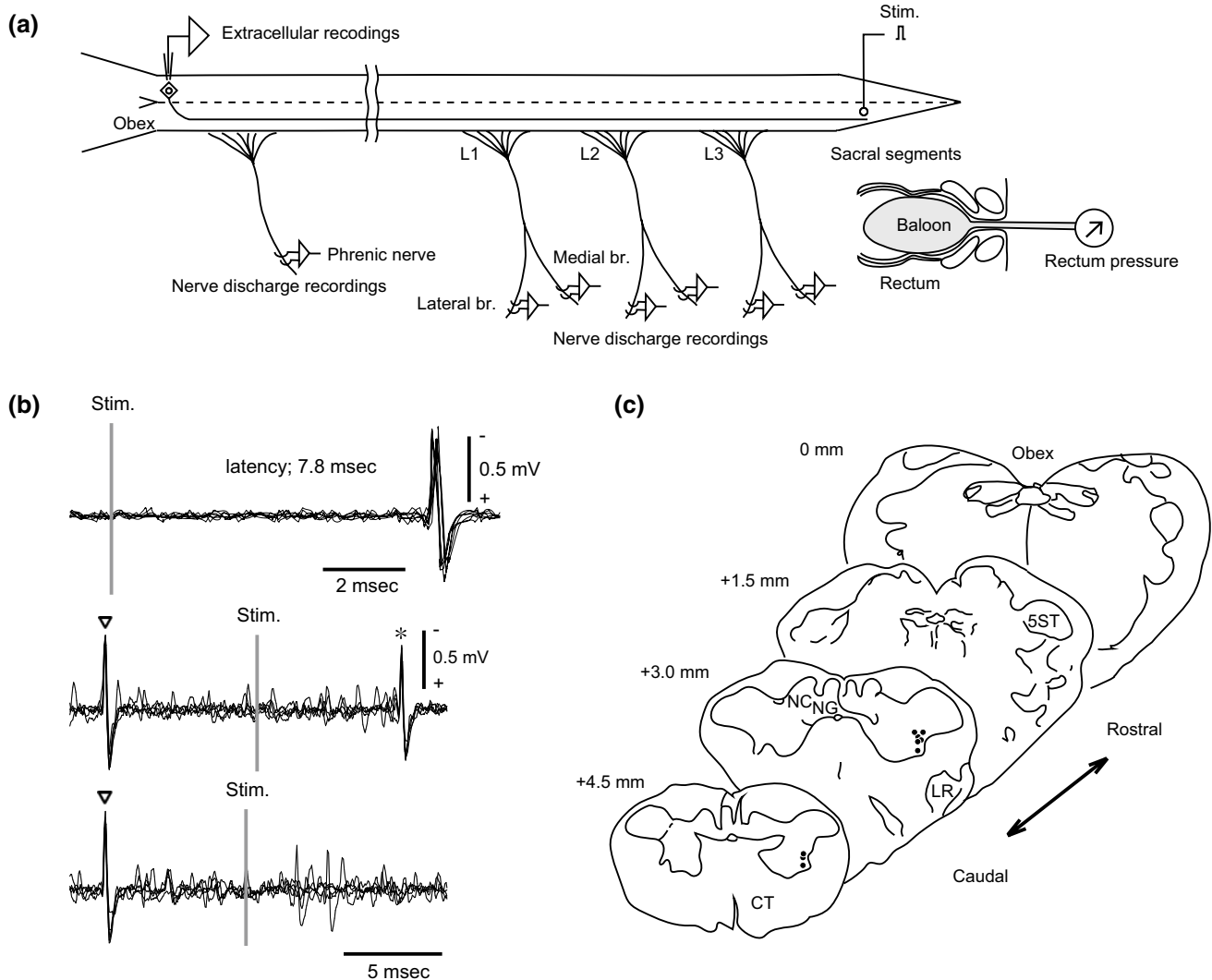


Fig. 1 Experimental arrangements and identification of antidromic spikes. **a** Experimental arrangements. Extracellular recordings were obtained from E neurons in the caudal nucleus retroambigialis. Nerve discharge recordings were obtained from the lateral and medial branch of the L1, L2 and L3 lumbar nerve and Phrenic nerve. Balloon was inserted into the rectum and measured the pressure. *Stim* electrical stimulation, *lateral br* and *medial br* lateral branch and medial branch. **b** Identification of antidromic spikes. Antidromic activation following stimulation in S3 segment (top). Antidromic activation was confirmed by a collision test triggered by spontaneous spikes (mid-

dle, bottom). Spontaneous spikes are indicated with open triangles (∇) and evoked spikes with asterisks (*). The stimulator was triggered 7.7 ms (middle) or 7.0 ms (bottom) after spontaneous spikes. Note that stimulation failed to evoke spikes (7.0 ms) in contrast to the full activation with slightly delayed stimulation (7.7 ms). **c** Recording sites of E neurons reconstructed on four transverse sections of the lower brainstem. E neurons were located in the vicinity of the nucleus retroambigialis. They were distributed 3.0–4.5 mm caudal to the obex. *NG* gracile nucleus, *NC* cuneate nucleus, *CT* corticospinal tract, *5ST* spinal trigeminal nucleus, *LR* lateral reticular nucleus

Electronic Design, Cambridge, UK). Data are expressed as mean \pm standard deviation. Differences were considered statistically significant at $p < 0.05$ using a Student's t test.

Histological procedures

At the end of each experiment, animals were killed by intravenous administration of a lethal dose of sodium pentobarbital (50–60 mg/kg). Then, the animals were perfused with Ringer's solution followed by 10% formalin solution through the aorta. The brainstem and spinal cord were removed, and spinal levels were identified. The brainstem was sectioned serially (100 μ m) using a freezing microtome in the transverse plane. Serial sections of the brainstem were stained with cresyl violet.

Results

Identification of spinal projections

The spinal projections of E neurons were examined by antidromic activation with the ball electrode (Fig. 1a). The most caudal level of spinal projections in 12 of 17 E neurons was examined in 7 animals. Figure 1b shows antidromic spikes with a latency of 7.8 ms, which were induced in one such neuron by stimulation of the caudal end of the descending axon in S3. Antidromic activation was confirmed with collision tests using the preceding spontaneous spikes (Fig. 1b). Seven E neurons descended to the thirteenth thoracic and lumbar spinal cord and 5 to the sacral spinal cord. All spinal axons except one descended to the contralateral spinal cord with respect to the cell body of E neurons, and one neuron descended in the ipsilateral spinal cord. The latencies of antidromic spikes evoked from the Th13 and lumbar spinal cord were 8.79 ± 4.50 ms (4.36–19.01 ms, $n = 7$), and the latencies of those from the sacral spinal cord were 10.46 ± 2.50 ms (7.81–14.23 ms, $n = 5$). We were able to confirm antidromic activation in 10 of 12 E neurons.

Recording sites for 9 of 17 E neurons were reconstructed on four transverse sections of the lower brainstem (Fig. 1c). These nine E neurons were located in the vicinity of the nucleus retroambigualis, which is associated with the caudal ventral respiratory group, and were distributed 3.0–4.5 mm caudal from the obex ($n = 9$).

Firing properties

In total, extracellular recordings were made from 17 E neurons in the caudal nucleus retroambigualis. Seven of these had axons descending to the Th13 and lumbar spinal cord. Five had axons extending to the sacral spinal cord. The

most caudal spinal projections of 5 of 17 E neurons were not tested.

Typical examples of the firing patterns of an E neuron are shown in Fig. 2. Figure 2a shows an E neuron with an axon descending to the Th13 spinal cord. Figure 2b shows an E neuron with an axon descending to the S3 spinal cord. Generally, E neurons discharged action potentials during the expiratory phases of Ph nerve activity (Fig. 2a, left, b, left), in phase with Abd nerves (Fig. 2b, left). During the repetitive rise of rectum pressure caused by infusing warm water into the balloon, the discharges of the Abd and Ph nerves increased (Fig. 2a, right, b, right). Firing frequencies of E neurons projecting to the thoracic and lumbar spinal cord increased during the repetitive rise of rectum pressure (Fig. 2a, right). Conversely, firing frequencies of E neurons projecting to the sacral spinal cord decreased (Fig. 2b, right). Firing frequencies of 17 E neurons were measured; these included 7 neurons projecting to the thoracic and lumbar spinal cord, 4 projecting to the sacral spinal cord, 5 for which descending axons were not identified, and one projecting to the sacral spinal cord after cutting the bilateral pudendal nerves to eliminate the effects from mechanoreceptors in the rectal wall. Table 1 shows the firing properties of the seven E neurons projecting to the thoracic and lumbar spinal cord and of the four E neurons with descending axons in the sacral spinal cord. Of the seven E neurons projecting to the thoracic and lumbar spinal cord, five exhibited higher IF during rectum pressure “on” than that during rectum pressure “off” ($p < 0.05$). Four neurons exhibited an ISI that was lower during rectum pressure “on” than “off”, while two demonstrated a higher ISI ($p < 0.05$), and one showed no change ($p > 0.05$). Four neurons exhibited increased CV, and three exhibited decreased CV during rectum pressure “on”.

All four E neurons projecting to the sacral spinal cord exhibited lower IF and higher ISI during rectum pressure “on” than “off” ($p < 0.05$ for both), as well as lower CV. The E neuron projecting to the sacral spinal cord upon cutting the bilateral pudendal nerves showed little change in either IF, ISI, or CV.

The firing patterns of E neurons exhibited a respiratory rhythm during the expiratory phases, in accordance with the activities of Abd nerves during rectum pressure “off”. However, the activity of Abd nerves came in phase with changes in rectum pressure and out of phase with the firing pattern of E neurons during rectum pressure “on” (Fig. 3a). To document the covariation of the firing patterns of E neurons and Abd nerves during rectum pressure on/off, cross-correlations (CC) between IF of E neuron discharges and integrated Abd nerve discharges were calculated. The time periods of rectum pressure “off” and “on” lasting 20–30 s were analyzed. To calculate CC, we required activity to be present in Abd nerves even during rectum pressure “off”. We were able to analyze 13 such pairs. Representative CCs during the rectum pressure “off” and

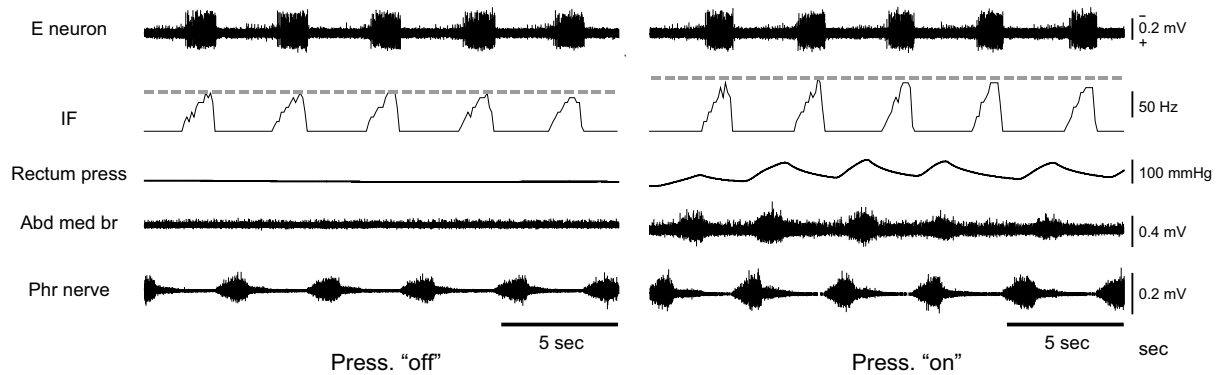
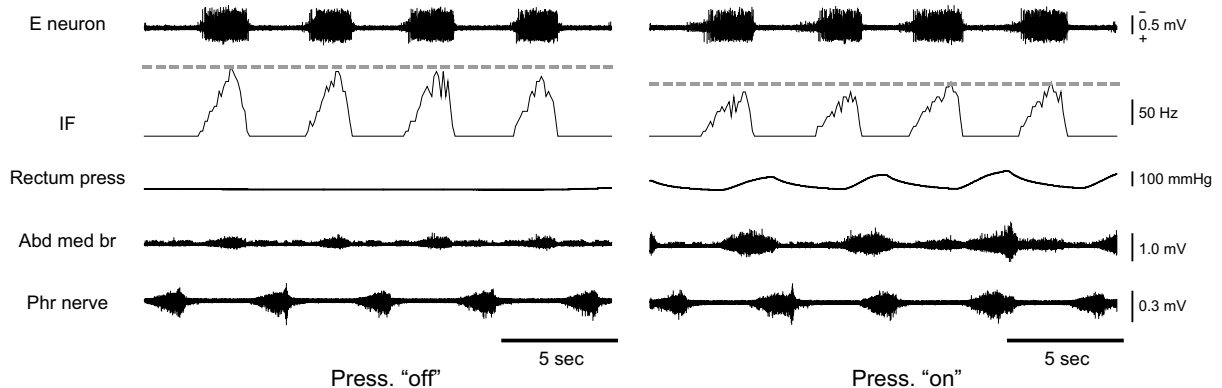
(a) E neuron with axon to Th13**(b)** E neuron with axon to S3

Fig. 2 Typical examples of the firing patterns of E neurons. **a** E neuron with descending axons to the T13. Firing frequencies of E neurons increased during the repetitive rise of rectum pressure. **b** E neuron with descending axons in the S3. Firing frequencies of E neurons

decreased during the repetitive rise of rectum pressure. *IF* instantaneous frequency, *Rectum press* rectum pressure, *Abd med br* abdominal medial branch, *Phr nerve* Phrenic nerve, *Press. “off”* and *Press. “on”* rectum pressure “off” and “on”

“on” are shown in Fig. 3b. The peak value demonstrated a high correlation during rectum pressure “off”, which was reduced during rectum pressure “on”. The peak value was 0.61 ± 0.03 (0.43–0.94, $n = 13$) during the rectum pressure “off” and 0.27 ± 0.04 (0.01–0.38, $n = 13$) during the rectum pressure “on”. This difference in peak correlation between rectum pressure “off” and “on” periods was significant ($p < 0.05$). The lag of peak correlation was 0.10 ± 0.01 s (–0.01 to 0.24 s, $n = 13$) during rectum pressure “off” and -0.31 ± 1.12 s (–1.38 to 2.61 s, $n = 13$) during the rectum pressure “on”. There was no significant difference in lag of peak correlation between rectum pressure “off” and “on” periods ($p > 0.05$).

Discussion

An anatomical study reported a projection from the caudal nucleus retroambigualis to the nucleus of Onuf using autoradiographic techniques [22]. Axon collaterals of E neurons were observed within the nucleus Onuf, in the region dorsal to the nucleus of Onuf and in the intermediate region, but collateral arborizations were not extensive [5]. Thus, E neurons seem to project to the pudendal motoneurons mainly via interneurons. Inspiratory neurons in the nucleus retroambigualis distribute collaterals in the

Table 1 Firing properties of E neurons projecting to the thoracic and lumbar spinal cord, and E neurons in the sacral spinal cord during rectum pressure “on” and “off”

Units	Anti	Press. “off”			Press. “on”		
		IF	ISI	CV	IF	ISI	CV
		Ave	Ave		Ave	Ave	
1	L2	45.68 ± 16.74	0.026 ± 0.014	0.54	36.63 ± 12.54*	0.033 ± 0.018*	0.54
2	T13	71.17 ± 23.43	0.017 ± 0.011	0.65	76.41 ± 25.52*	0.015 ± 0.009*	0.56
3	L6	55.22 ± 22.84	0.022 ± 0.017	0.71	72.56 ± 34.86*	0.019 ± 0.015*	0.78
4	L7	44.88 ± 13.16	0.025 ± 0.012	0.46	47.79 ± 15.08*	0.024 ± 0.012	0.50
5	L4	7.78 ± 1.21	0.131 ± 0.020	0.15	8.49 ± 1.38*	0.121 ± 0.022*	0.19
6	L6	10.53 ± 3.48	0.106 ± 0.036	0.34	10.53 ± 5.10	0.120 ± 0.036*	0.30
7	L1	14.12 ± 3.67	0.077 ± 0.027	0.35	19.86 ± 5.20*	0.056 ± 0.022*	0.34
8	S3	98.13 ± 38.58	0.013 ± 0.009	0.69	86.58 ± 34.34*	0.014 ± 0.010*	0.66
9	S1	31.74 ± 10.15	0.037 ± 0.021	0.56	18.26 ± 9.51*	0.070 ± 0.034*	0.49
10	S1	53.41 ± 31.79	0.026 ± 0.019	0.73	36.18 ± 32.17*	0.042 ± 0.026*	0.62
11	S1	10.73 ± 5.72	0.109 ± 0.039	0.36	7.05 ± 2.29*	0.160 ± 0.063*	0.39

Anti antidromic activation, *Press. “off”* rectum pressure “off”, *Press. “on”* rectum pressure “on”, *IF* instantaneous frequency (Hz) (plotting the inverse of the time interval between a spike of and the previous spike), *ISI* interspike interval (sec) (plotting the time interval between a spike of and the previous spike), *Ave* average (sec), *CV* coefficient of variation (the ratio of the standard deviation to the mean)

*Significantly different compared with *Press. “off”* values at $p < 0.05$ with a Student’s *t* test

phrenic nucleus and many descending stem axons end between T10 and T13 [23]. It is unknown whether inspiratory neurons in the brainstem other than in the nucleus retroambigualis project to the sacral spinal cord. It is suggested that E neurons are only one source to the nucleus of Onuf which carries information on the respiratory rhythm and motoneurons in the nucleus of Onuf receive information about respiratory rhythm from E neurons during fictive straining [5]. The firing pattern of E neurons normally exhibits respiratory activity in the phase coinciding with Abd nerves, and spike discharges cease in the phase of Ph nerve inspiratory activity [4], but during vomiting, E neurons are active in periods of Abd and Ph nerve co-activation or between every burst of such co-activation, and during normal or increased levels of activity [18].

Our previous study demonstrated that Abd muscles raise intra-abdominal pressure during induced defecation, and that both Abd muscles and the diaphragm are simultaneously active to raise this pressure [20]. In the present study, we recorded the discharges of Abd nerves and E neurons during fictive straining induced by distention of the colon with a balloon to emphasize the activity of straining during defecation. The discharges of Abd nerves increased as rectal pressure increased. Even during the repetitive rise of rectum pressure, the respiratory rhythm of E neurons was not affected. To document the covariation of the firing patterns of E neurons and Abd nerves during rectum pressure “on”/“off” periods, cross-correlations between IF of E neuron discharge and integrated Abd nerve discharge was calculated. The peak correlation was high during rectum pressure “off”, but was reduced during rectum pressure “on”.

Thus, the rhythmic discharges of E neurons do not seem to be influenced by altered Abd muscle activity during fictive straining.

Firing frequencies of E neurons with descending axons in the thoracic and lumbar spinal cord increased during fictive straining, whereas those of E neurons with descending axons in the sacral spinal cord decreased. The firing properties of the latter neurons are more similar to those of expiratory units that cease firing during fictive straining [24]. These neurons may be related to their enhanced activation during coughing or vomiting when maintenance of continence is required to prevent defecation [25]. During the repetitive rise of rectum pressure, IF of E neurons with thoracic and lumbar axons was higher during rectum pressure “on” than “off”. The IF of E neurons with sacral axons was instead lower. The IF, ISI, and CV of E neuron discharge did not change when the bilateral pudendal nerves were cut. These findings suggest that E neurons unlikely receive signals from the muscle spindle, similar to mechanoreceptors in the rectal wall via the pudendal nerves [26]. As the present study was conducted with the anus closed during fictive straining, a phenomenon similar to that of maintaining rectum continence such as coughing or vomiting according to increased activity of Abd muscles may occur. During fictive straining, some target neurons of E neurons in the sacral spinal cord may be inhibitory interneurons, and activities of such interneurons in the sacral spinal cord decrease to facilitate motoneurons in Onuf nucleus and maintain rectum continence.

Lesion studies have demonstrated a degree of anatomical separation among the descending projections that serve

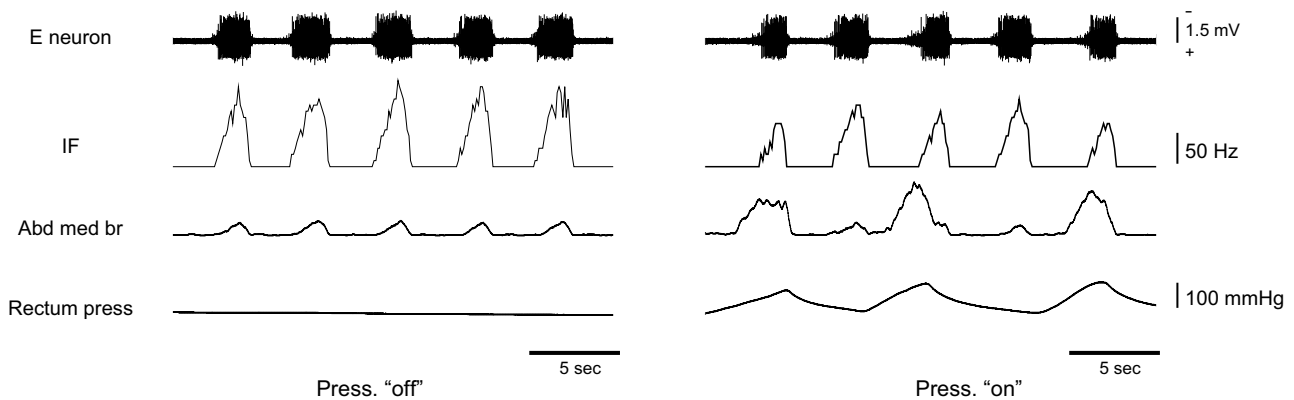
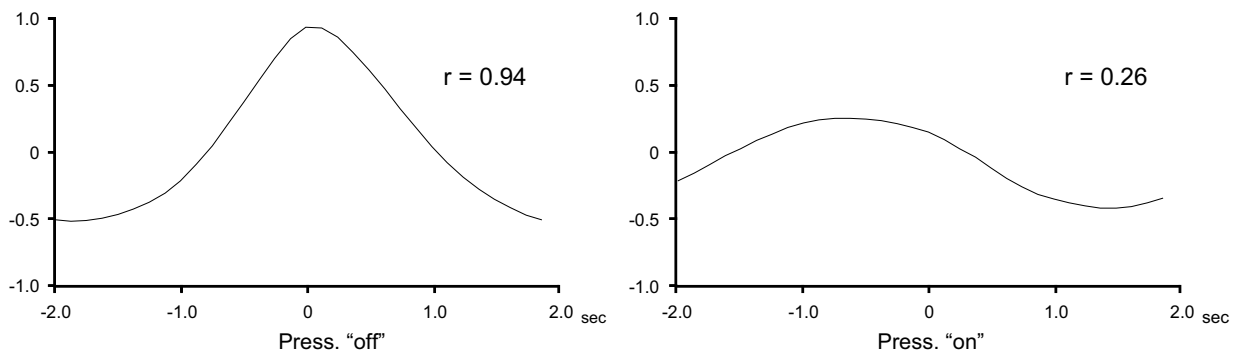
(a) E neuron & Abd nerve**(b)** Cross-correlations between E neuron & Abd nerve

Fig. 3 Cross-correlations between E neuron and Abd nerve. **a** Expiratory (E) neuron discharges and integrated abdominal (Abd) nerve discharges. **b** cross-correlations between IF of E neuron discharges and integrated Abd nerve discharges. Cross-correlations during the

rectum pressure “off” (Left) and “on” (Right). The peak correlation shows a high value during the rectum pressure “off” and a lower value during the rectum pressure “on”

the functions of respiration, coughing, and defecation [27]. When recombinant isogenic strains of pseudorabies virus were injected into the diaphragm and rectus abdominis muscles in ferrets, dual-infected neurons were predominantly found in the magnocellular part of the medullary reticular formation. The co-activation of inspiratory and expiratory muscles during vomiting and postural adjustments could be elicited through collateral projections from neurons in the magnocellular part of the medullary reticular formation [28]. When Abd muscles and the diaphragm are active simultaneously to raise intra-abdominal pressure, a switch to these neurons may occur in the brainstem, although it is not known if there are functional connections between the medullary reticular formation and E neurons with lumbar axons and those with sacral axons. The aforementioned neurons in the reticular formation may exert their influence on E neurons to alter frequencies of discharge.

Conclusions

This study highlights how E neurons with descending axons in the thoracic and lumbar spinal cord and those with descending axons in the sacral spinal cord exhibit different firing characteristics during fictive straining induced by distention of the colon with a balloon, thus emphasizing the activity of straining during defecation. This is one of many functions of E neurons. However, the synaptic connections between E neurons and the nucleus of Onuf remain unclear; further research is needed to clarify this.

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Author contributions All co-authors participated in data collection and analysis. All co-authors have read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All experimental procedures were approved by the Animal Ethics Committee of Ibaraki Prefectural University of Health Sciences and were in accordance with the guiding principles for care and use of animals in the field of physiological sciences outlined by the Physiological Society of Japan.

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