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Research report

Descending modulation of visceral nociceptive transmission from the locus coeruleus/subcoeruleus in the rat

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ABSTRACT

The purpose of the present investigation was to examine whether electrical stimulation in the locus coeruleus/subcoeruleus (LC/SC) could modulate visceral pain evoked by noxious colorectal distention (CRD). Experiments were performed on 40 pentobarbital anesthetized male Sprague–Dawley rats. Extracellular potentials of single L_6–S_2 spinal neuron were recorded with a carbon filament electrode. CRD (80 mmHg) was produced by inflating a balloon inside the descending colon and rectum. Electrical stimulation of the LC/SC (30, 50 and 70 μA, 100 Hz, 0.1 ms pulses) was delivered either ipsilaterally or contralaterally. Results showed that for 42/62 (68%) short-latency abrupt (SL-A) neurons, all of the short-latency sustained (SL-S) and long-latency (LL) neurons, LC/SC stimulation produced intensity-dependent attenuation of the CRD-evoked discharge. For 10/62 (16%) SL-A neurons, 6/8 (75%) inhibited (INHIB) neurons LC/SC stimulation increased the evoked discharge, for 10/62 (16%) SL-A neurons and 2/8 (25%) INHIB neurons, the evoked discharges were unaffected by the LC/SC stimulation. LC/SC stimulation also had different effects on the spontaneous activities of these neurons. The effects of LC/SC stimulation were the same both ipsilaterally and contralaterally either for the evoked discharges or for spontaneous activities. Following LC/SC lesions, LC/SC stimulation did not inhibit nociceptive responses, whereas inhibitory effects were observed by stimulation of the intact LC/SC contralateral to the recording site. These data suggest that the transmission of visceral pain was under the control of the centrifugal pathways from the LC/SC.

1. Introduction

The nucleus locus coeruleus (LC), the A6 cell group, located in the dorsolateral pons, is the largest cluster of noradrenergic neurons in the brain [12,49]. Anatomically, the axons of the neurons in the LC are highly branched and have extensive projections throughout the brain, the LC has been considered to play an important role in such functions as attention, vigilance [3], brain plasticity [25,41] learning and memory [47]. The LC also innervates the spinal cord via descending pathways, it sends fibers into all segments of the spinal cord [7,15,40]. Electrophysiological experiments have shown that activation of the LC region, including the nucleus subcoeruleus (SC), either electrically or chemically can inhibit nociceptive activity in dorsal horn neurons [17,21–24,28], and produce profound antinociception [14,20,26,42,48,50]. The coeruleospinal inhibitory system appears to play a significant role in spinal nociceptive processing.

Most studies of the coeruleospinal nociceptive control system have employed mechanical or thermal cutaneous stimuli. For example, Hodge et al. [17] have reported that the LC can modulate dorsal horn neuron responses to innocuous hair movement or noxious heating stimulation. Mokha et al. [28] reported that unilateral electrical stimulation of LC/SC markedly inhibits the responses of spinal dorsal horn neurons to noxious pinch cutaneous stimulation. Tsuruoka and Willis [46] have revealed that bilateral lesions of the LC significantly decrease paw withdrawal latency (PWL) to thermal cutaneous stimuli during carrageenan-induced inflammation. All these data suggest that the LC/SC is involved in the descending modulation of nociception arising from cutaneous structure.

Visceral pain is different from cutaneous pain in the pathways and mechanisms, it is generally diffused and difficult to localize, often refers to superficial regions of the body [16]. The mechanisms of visceral pain have not been investigated as widely, in part because of the difficulty in defining a noxious visceral stimulus. Recently CRD stimulation has been considered to be a useful method for evoking acute visceral pain, because this method can mimic lower bowel obstruction, it can produces pain in humans and evokes avoidance behavior and pseudoaffective reflexes in awake

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rats, it has the advantage of being more natural, reliable, reversible and reproducible than other methods [29,32].

In the present study, the CRD-evoked visceral pain models were used to explore whether LC/SC participated in the descending modulation of visceral nociception. Electrophysiological evidence has demonstrated that CRD stimulation can activate spinal cord neurons [31,34]. As the first central nervous system relay station, the spinal cord can modulate, process and transmit these noxious visceral inputs. It is expected that if LC/SC is involved in the descending modulation of visceral pain, focal electric stimulation in the LC/SC perhaps can modulate the CRD-evoked activities of spinal cord neurons.

2. Materials and methods

2.1. Surgical preparation

Experiments were performed on 40 male Sprague–Dawley rats weighing 240–320 g. Animals were housed in groups of 3–4 in a cage containing sawdust bedding, with free access to rat chow and water in a laboratory equipped with a 12:12 h (08:00–20:00 h) light/dark cycle. Room temperature and humidity were maintained at 23 ± 0.5 °C and 60%, respectively. All procedures were reviewed and approved by the University Animal Care and Use Committee and are consistent with the guidelines of the International Association for the Study of Pain [52] and the NIH Guidelines for the Care and Use of Laboratory Animals.

Animals were anesthetized and paralyzed with sodium pentobarbital (50 mg/kg i.p.) and sodium pancuronium (3 mg/kg i.v.). After tracheotomy, animals were artificially ventilated to maintain an end-tidal CO_2 level between 3.5% and 4.5%. Anesthesia and paralysis were maintained during the experiment by infusing a mixture of 50 mg of sodium pentobarbital and 5 mg of sodium pancuronium in 44 ml of 0.9% NaCl at a rate of 0.04 ml/min given i.v. The core body temperature was regulated at approximately 37.5 °C using a thermostatically controlled heating blanket. A laminectomy was performed to expose the lower lumbar/upper sacral spinal cord (vertebrae L_1–L_4). The head and the vertebral column were fixed rigidly in a stereotaxic frame.

The skull was exposed and two holes (bilateral) were drilled for stereotaxic placement of a stimulating electrode into the LC/SC. At the end of surgical procedures, a balloon attached to tygon tubing was inserted through the anus into the descending colon and rectum, the lumbosacral dura was cut and retracted and the exposed spinal cord was covered with warm paraffin oil.

2.2. Stimulation

The visceral stimulus used was CRD which was produced by inflating a balloon inside the descending colon and rectum. The balloon was made from the finger of a latex glove with a length approximately 5 cm and attached to polyethylene tubing. The balloon was inserted into the rectum and the descending colon through the anus and was held in place by taping the tubing to the tail. CRD stimuli having an intensity above 40 mmHg are considered noxious in rats and painful in humans [32,35]. During each experimental situation, the distentions (80 mmHg, 10 s for short-latency abrupt (SL-A) neurons, short-latency sustained (SL-S) and inhibited (INHIB) neurons; 80 mmHg, 30 s for long-latency (LL) neurons) were repeated three times at 1 min intervals, the interval between each experimental situation is 10 min.

In central stimulation, a monopolar Teflon-coated stainless-steel electrode (0.1 mm in diameter), insulated except for 0.5 mm at the tip, was inserted into the LC/SC. Stereotaxic coordinates have been defined by Paxinos and Watson [38] (9.6 mm caudal to bregma; 1.15–1.20 mm lateral to the midline; 2.5 mm above the interaural axis). Electrical stimulation (30, 50 and 70 μA, 100 Hz, 0.1 ms pulses) of the LC/SC either ipsilateral or contralateral to the site of the recorded neurons was initiated 5 s before and continued throughout the second application of CRD. At this stimulus intensity, there was no trouble with the electrical artifact in relation to single-unit recording during LC/SC stimulation. In lesion experiments, after the effect of LC/SC stimulation on a single neuron was examined, the rats received electrolytic lesions of the LC/SC ipsilateral to the recording sites, then effects of LC/SC stimulation contralateral on the same neurons response to CRD were recorded 30 min later. Lesions were induced with a cathodal current (1 mA, 20 s).

2.3. Electrophysiological recordings

Carbon filament electrodes (4–6 MΩ) were used for single-unit recording in the L_6–S_2 spinal segment from 0.00 to 1.00 mm lateral to the midline and 0.1–1.3 mm from spinal cord dorsum. CRD (80 mmHg intraluminal pressure) was used as a search stimulus and all neurons responding to this stimulus were characterized. The experiment on each neuron started with mapping of the receptive field, and determination of the control mechanically evoked activity using innocuous and noxious mechanical stimuli. Innocuous brush stimuli were delivered by repeated brushing in a stereotyped manner with a camel's hair brush. Pinch stimuli were applied with an arterial clip. The pinch was distinctly painful with a force of 613 g/mm^2 when applied on human skin. Nociceptive spinal cord neurons were classified as wide dynamic range (WDR) or high threshold (HT) according to their responses to brush or pinch stimuli [5], HT neurons responded almost exclusively to noxious mechanical stimuli, and WDR neurons responded to both innocuous and noxious mechanical stimuli. Twenty minutes after determination of the receptive field, the CRD-evoked discharge of the neuron was tested. Its activity was fed into a computer data collection system (CED 1401 in acquisition software, Pentium PC), which constructed peristimulus histograms.

2.4. Histology

At the end of each experiment, cathodal electrolytic lesions (1 mA, DC for 15 s) were made to mark the site of electrical stimulation in the intact LC/SC, and then the animals were deeply anesthetized with sodium pentobarbital and perfused intracardially with a 10% formalin solution. Brains were removed, sectioned at a thickness of 50 um, and stained with cresyl violet for histological verification of electrode placement and reconstruction of the lesion.

2.5. Data collection and analysis

The spontaneous activity of a neuron was counted for 10 s immediately before the onset of CRD, and response to CRD is defined as the increase in activity during CRD above spontaneous activity. Responses of neurons are presented as total number of impulses/10 s (30 s for LL neurons) or normalized to a percentage of the response to 80 mmHg CRD. Responses to two distensions before and after LC/SC stimulation were averaged to define the control response. Responses of neurons to LC/SC stimulation are also defined as the increase in activity above spontaneous activity. Because electrical stimulation was started 5 s before the onset of CRD, the effects of stimulation on spontaneous activity was assessed during these 5 s and compared with spontaneous activity in the immediately preceding 5 s before the onset of electrical stimulation, spontaneous activity of neurons are presented as total number of impulses/5 s. A unit was considered to be affected by LC/SC stimulation if its CRD-evoked activity or spontaneous activities changed at least ± 10% from its pre-LC/SC stimulation activity.

Data were presented as means ± S.E. Statistical analysis was carried out using analysis of variance (ANOVA) (Newman–Keuls test for post hoc comparisons). In all cases, $P < 0.05$ was considered significant.

3. Results

3.1. Unit sample

A total of 92 neurons in 40 rats responsive to CRD were isolated in the L_6–S_2 segments of the spinal cord. The neurons were divided into four groups based on their responses to CRD [31]: 62 SL-A neurons, 15 SL-S neurons, 7 LL neurons and 8 INHIB neurons. SL-A neurons were excited at short-latency (<1 s), rapidly reached the peak following the onset of CRD and rapidly returned to the baseline level within 1 or 2 s at stimulus offset; SL-S neurons were characterized by the presence of a sustained afterdischarge for 5–40 s following termination of CRD, SL-S neurons were also excited at short-latency (<1 s), but they reached a peak near or at the time of stimulus termination; LL neurons were excited at relatively long-latencies (mean, 6.1 ± 0.5 s); INHIB neurons were spontaneously active and were inhibited by CRD. Examples of representative responses of these four different types of neurons are given in Fig. 1.

Eighty-eight (96%) neurons had convergent cutaneous receptive fields located in the caudal part of the body. Four (4%) SL-S neurons responded only to CRD, and a convergent somatic receptive field could not be localized by innocuous or noxious cutaneous stimulation. Among the 88 units for which convergent receptive fields were found, the receptive fields of 71 units were localized in the tail, scrotum, lower back or hip regions, they responded to both innocuous and noxious mechanical stimuli and were WDR neurons. The remaining 17 units responded only to noxious pinch of the tail or hip regions, they were HT neurons (Table 1). The recording sites of these neurons were located from 0.00 to 1.00 mm lateral to the midline and 0.1–1.3 mm from spinal cord dorsum, suggesting that

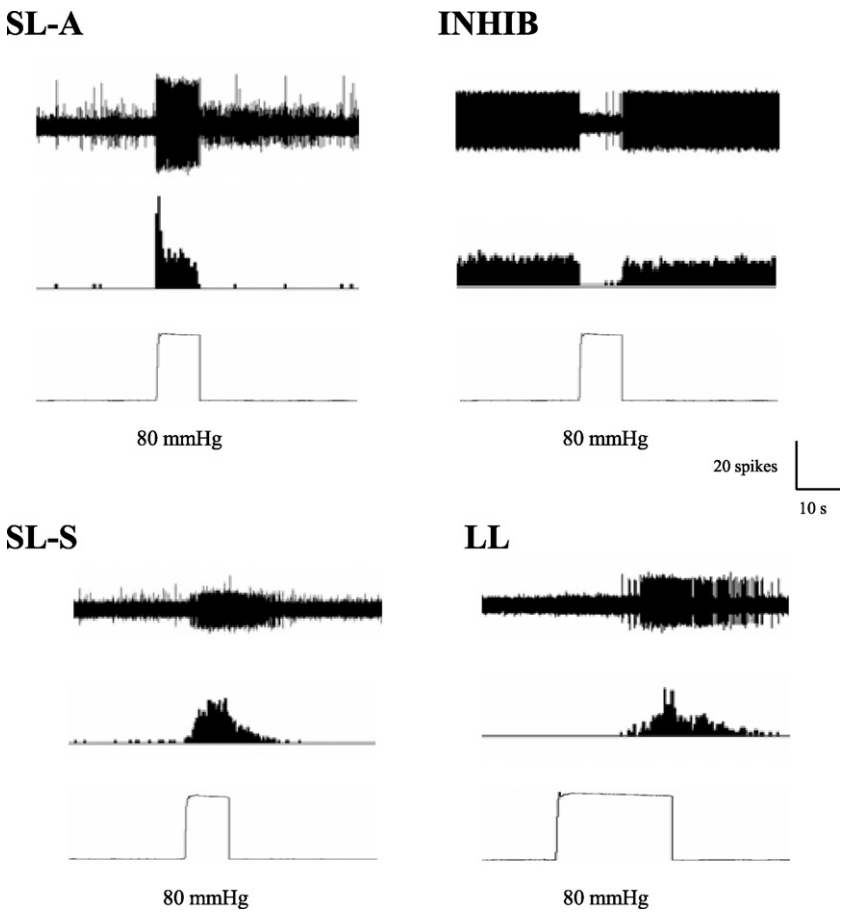

Fig. 1. Responses of 4 types of spinal cord neurons to noxious colorectal distension (CRD). For each kind of neuron, the *1st trace* shows the extracellular recording and the *2nd trace* shows the peristimulus time histogram (0.5-s bins). Duration of the CRD is shown as the bottom trace.

the cell bodies were located in the laminae I–VI and laminae X of the spinal cord [38]. There were no differences in the depths of recording sites for the different groups of spinal neurons.

The effects of electrical stimulation in the LC/SC on CRD-evoked discharge and spontaneous activities of these four types of neurons are described below.

3.2. Effects of LC/SC stimulation on the CRD-evoked discharge of the spinal cord neurons

In this type of experiment, stimulus intensity was altered in 30, 50 and 70 μA. Focal electric stimulation of the LC/SC had different effects on the CRD-evoked discharge of these four types of spinal cord neurons (Table 2). For 42/62 (68%) SL-A neurons, all of the 15 SL-S neurons and 7 LL neurons, electric stimulation in the LC/SC produced statistically significant intensity-dependent attenuation of the evoked discharge. For 10/62 (16%) SL-A neurons and 6/8 (75%) INHIB neurons, electric stimulation facilitated the evoked discharge. For the remaining 10/62 (16%) SL-A and 2/8 (25%) INHIB neurons, electrical stimulation did not affect the responses to CRD. LC/SC stimulation had the same effects both ipsilaterally and contralaterally (data not shown).

3.2.1. Inhibitory modulation

For 42/62 (68%) SL-A neurons, 15 SL-S neurons and 7 LL neurons, electrical stimulation in the LC/SC decreased the evoked discharge (Table 2). Prior to LC/SC stimulation, 80 mmHg CRD resulted in an average increase in neuronal activity of 10.62 ± 1.15 spikes/s in SL-A neurons, 12.21 ± 2.75 spikes/s in SL-S neurons and 9.64 ± 4.15 spikes/s in LL neurons. During LC/SC stimulation the average responses to CRD decreased to 36.31%, 10.54% and 4.21% in SL-A neurons, 48.93 %, 16.49% and 9.85% in SL-S neurons and 65.73%, 9.78%, 4.50% in LL neurons at intensities of 30,

Table 1
Characterization of four types of spinal cord neurons to cutaneous stimulation

	WDR	HT	Total
SL-A			
Inhibited by CRD	37	5	42
Excited by CRD	10	0	10
No change	10	0	10
SL-S	6	5	11
LL	0	7	7
INHIB	8	0	8

WDR: wide dynamic range neurons that respond to both noxious and innocuous mechanical stimuli. HT: high threshold neurons that exclusively respond to noxious mechanical stimuli. SL-A: short-latency abrupt neurons; SL-S: short-latency sustained neurons; LL: long-latency neurons; INHIB: inhibited neurons.

Table 2
Effects of LC/SC stimulation on the response of spinal cord neurons evoked by CRD

Effects of LC/SC stimulation				
	Inhibition	Facilitation	No effect	Total
SL-A	42	10	10	62
SL-S	15	0	0	15
LL	7	0	0	7
INHIB	6	0	2	8

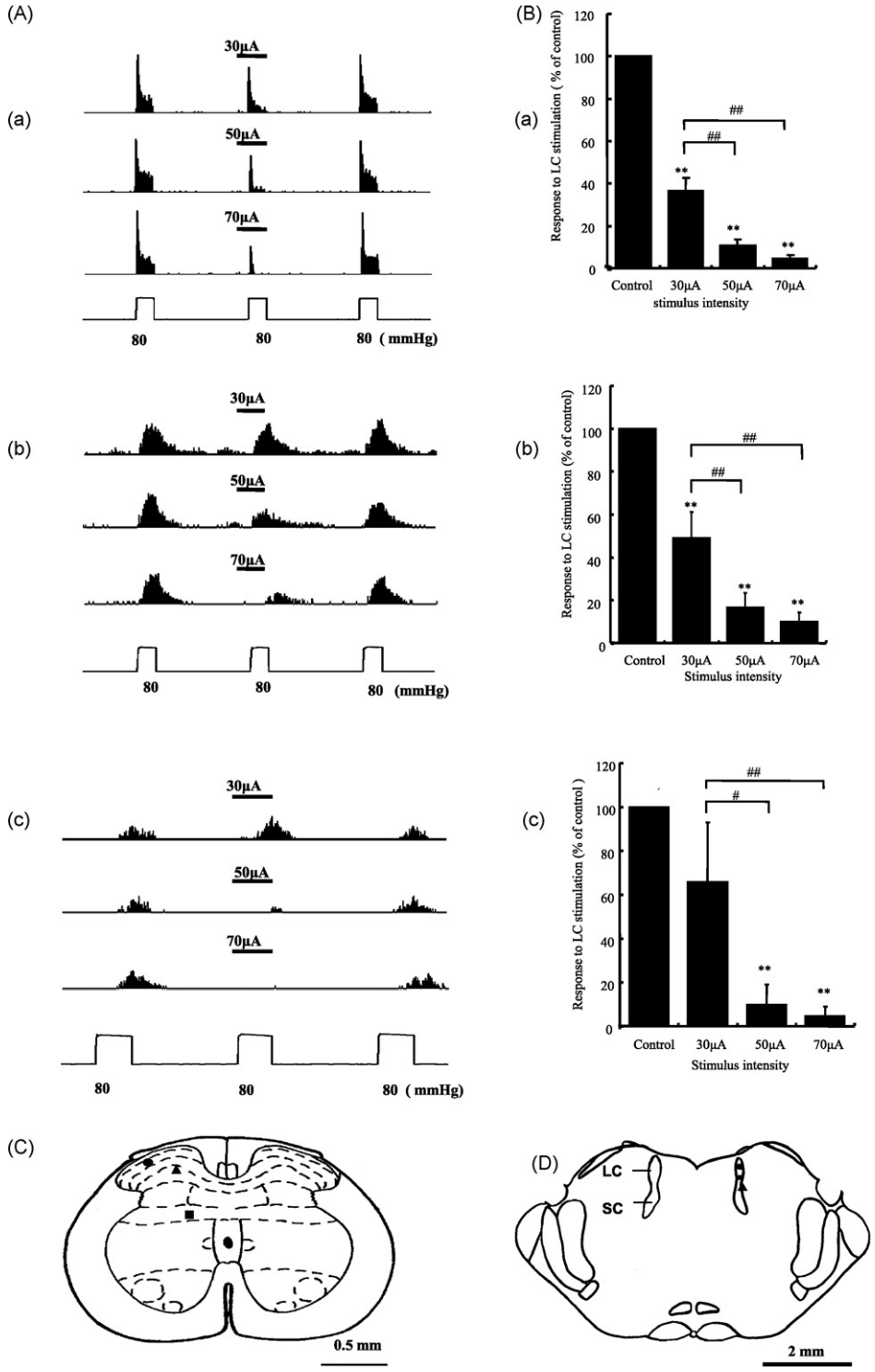

Fig. 2. Effects of LC/SC stimulation on CRD-evoked responses of different types of spinal cord neurons. (A) Peristimulus time histograms (0.5-s bin width) showing the inhibitory effects of LC/SC stimulation with an intensity ranging from 30 to 70 μA. Horizontal lines above histograms represent times of application of LC/SC stimulation. The duration of CRD is shown as the bottom trace. (a) SL-A neurons; (b) SL-S neurons; (c) LL neurons. (B) Graphs summarize the inhibitory effects of LC/SC stimulation at various intensities on CRD-evoked responses. Effects of LC/SC stimulation (ordinate) are expressed as a percentage of the control (without LC/SC stimulation). (a) SL-A neurons ($n = 42$); (b) SL-S neurons ($n = 15$); (c) LL neurons ($n = 7$). $^{**}P < 0.01$, significantly different from the control. $^{\#\#}P < 0.01$, significantly different between two groups of stimulus intensity. (C) The recording site in the S1 spinal cord for the data presented in A. Filled circle, SL-A neuron; filled triangle, SL-S neuron, filled squares, LL neuron. (D) Localization of the LC/SC stimulation site for the data presented in A. Filled circle, SL-A neuron; filled triangle, SL-S neuron, filled squares, LL neuron.

50, and 70 μA, respectively, compared with the control. Fig. 2 shows the inhibitory effects of LC/SC stimulation on the evoked discharge in the SL-A, SL-S and LL neurons. For SL-A and SL-S neurons, ANOVA analysis revealed that the changes induced by LC/SC stimulation were statistically significant between the different stimulus intensities and the control ($P < 0.01$), the change is also significant between 30 and 50 μA as well as 70 μA intensity ($P < 0.01$). For LL neurons, although the change induced by

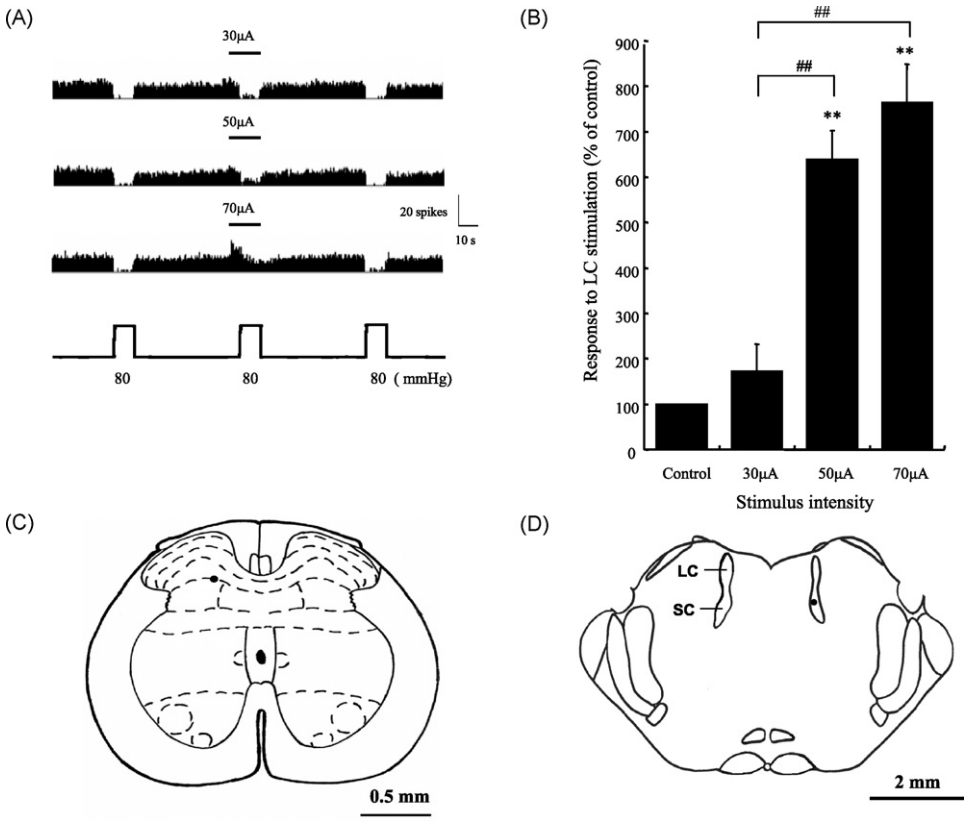

Fig. 3. Effects of LC/SC stimulation on CRD-evoked responses of INHIB neurons. (A) Peristimulus time histograms (0.5-s bin width) showing the effects of LC/SC stimulation with an intensity ranging from 30 to 70 μA. Horizontal lines above histograms represent times of application of LC/SC stimulation. The duration of CRD is shown as the bottom trace. (B) Graphs summarize the effects of LC/SC stimulation at various intensities on CRD-evoked responses ($n = 6$). Effects of LC/SC stimulation (ordinate) are expressed as a percentage of the control (without LC/SC stimulation). $^{**}P < 0.01$, significantly different from the control. $^{\#\#}P < 0.01$, significantly different between two groups of stimulus intensity. (C) The filled circles indicate the recording site in the S1 spinal cord for the data presented in A. (D) Localization of the LC/SC stimulation site for the data presented in A.

30 μA is not significant compared with the control, the difference was statistically significant for 50 and 70 μA as compared with the control. The electrical threshold for these inhibitory effects was usually between 5 and 30 μA for SL-A neurons, 5 and 40 μA for SL-S neurons and 10 and 30 μA for LL neurons.

Inhibitory effects had also been observed in 6 of the 8 CRD-inhibited neurons (Fig. 3). Prior to LC/SC stimulation, 80 mmHg CRD resulted in the mean spontaneous activity reduced to 1.03 ± 0.39 spikes/s in these 6 neurons. The average responses to CRD during LC/SC stimulation at intensities of 30, 50, and 70 μA increased to 175.9%, 242.6% and 348.5% at 30, 50 and 70 μA, respectively, compared with pre-LC/SC stimulation values. ANOVA analysis revealed the difference was statistically significant for 50 and 70 μA ($P < 0.01$) as compared with the control, there is also a significant difference between 30 and 50 μA, as well as 70 μA intensity ($P < 0.01$). For all of these neurons, the CRD-evoked activities returned to pre-stimulation levels of spontaneous activity at 1 min following the termination of stimulation. The electrical threshold for the inhibitory effects was usually between 20 and 30 μA.

3.2.2. Facilitatory modulation

For 10/62 (16%) SL-A neurons, electrical stimulation in the LC/SC increased the evoked discharge, prior to LC/SC stimulation, 80 mmHg CRD resulted in an average increase in neuronal activity of 9.88 ± 0.76 spikes/s in these neurons. During LC/SC stimulation the average responses to CRD at intensities of 30, 50, and 70 μA increased to 175.9%, 242.6% and 348.5%, respectively, compared with the control. Fig. 4 shows the facilitatory effects of LC/SC stimulation on the evoked discharge in these neurons. The changes were statistically significant for 50 ($P < 0.05$) and 70 μA ($P < 0.01$) as compared with the control, there is also a significant difference between 30 and 70 μA ($P < 0.01$). The electrical threshold for the facilitatory effects was usually between 10 and 30 μA.

3.3. Effects of LC/SC stimulation on the spontaneous activities

Thirty-eight of the 62 (61%) SL-A neurons, 8/15 (53%) SL-S neurons, all of the 8 INHIB neurons were spontaneously active (mean: SL-A, 6.54 ± 1.26 spikes/s; SL-S, 9.65 ± 2.84 spikes/s; INHIB, 8.63 ± 1.48 spikes/s) (Table 3). Focal electric stimulation of the LC/SC produced intensity-dependent reduction of the spontaneous activities of 12/38 (32%) SL-A neurons (Fig. 5A) and all the 8 SL-S neurons (Fig. 5C). The changes were statistically significant for 50 and 70 μA as compared with the control, but there were not statistically significant between the different stimulus intensities. For 18/38 (47%) SL-A neurons, 4/8 (50%) INHIB neurons, LC/SC stimulation increased the spontaneous activities, ANOVA analysis demonstrated that for SL-A neurons the changes were statistically significant between 70 μA and the control (Fig. 5B), for INHIB neurons there was no significant difference between the different stimulus intensities and the pre-LC/SC stimulation values (Fig. 5D). LC/SC stimulation did not alter the spontaneous activities of the remaining 8/38 (21%) SL-A neurons and 4/8 (50%) INHIB neurons.

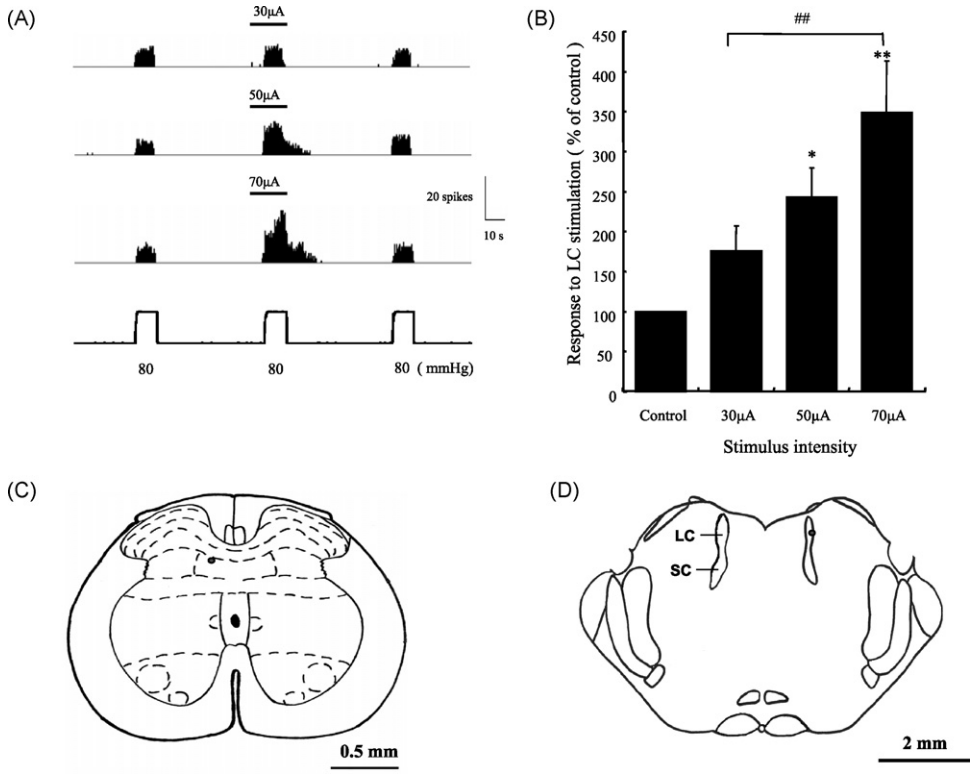

Fig. 4. Effects of LC/SC stimulation on CRD-evoked responses of SL-A neurons. (A) Peristimulus time histograms (0.5-s bin width) showing the facilitatory effects of LC/SC stimulation with an intensity ranging from 30 to 70 μA. Horizontal lines above histograms represent times of application of LC/SC stimulation. The duration of CRD is shown as the bottom trace. (B) Graphs summarize the facilitatory effects of LC/SC stimulation at various intensities on CRD-evoked responses ($n = 10$). Effects of LC/SC stimulation (ordinate) are expressed as a percentage of the control (without LC/SC stimulation). $^{**}P < 0.01$, significantly different from the control. $^{\#\#}P < 0.01$, significantly different between two groups of stimulus intensity. (C) The filled circles indicate the recording site in the S1 spinal cord for the data presented in A. (D) Localization of the LC/SC stimulation site for the data presented in A.

3.4. Effects of LC/SC stimulation on the CRD-evoked discharge after ipsilateral LC/SC lesions

In this type of experiments, lesions of the LC/SC ipsilateral to the recording site were induced after recordings of the modulatory effects of LC/SC stimulation. Thirty minutes later, the modulatory effects were examined again by LC/SC stimulation ipsilaterally and contralaterally. A total of 5 neurons in 5 rats were selected on the basis of histological results which showed that the ipsilateral LC/SC was almost completely destroyed. The rostrocaudal extension was between 0.8 and 1.5 mm, and the LC/SC was always completely destroyed ventrodorsally throughout its rostrocaudal extension. All the 5 neurons were SL-A neurons and the CRD-evoked discharges were inhibited by LC/SC stimulation. The effects of LC/SC stimulation on the CRD-evoked responses in LC/SC lesioned rats are shown in Fig. 6. LC/SC stimulation did not reduce the responses when LC/SC stimulation was applied to the ipsilateral LC/SC after the lesions, whereas reductions were observed by stimulation of the intact LC/SC contralateral to the site of recordings. No significant difference in the modulatory effects was observed between LC/SC stimulation ipsilateral and contralateral. The 5 neurons had very low or no spontaneous activities and were not affected by the LC/SC lesions.

4. Discussion

The coeruleospinal system has been demonstrated to be one of the endogenous analgesia systems. Focal electrical stimulation of the LC/SC produces profound antinociception [20,21,50] and increases significantly the spinal content of norepinephrine metabolites of cutaneous pain models [9]. For example, activation of the LC/SC either electrically or chemically can inhibit nociceptive activity in dorsal horn neurons to skin heating [22–24], the descending pathways from the LC/SC are activated during inflammation of peripheral tissues [46]. All these results indicated that LC/SC appears to play an important role in cutaneous nociceptive processing, while relatively few studies have focused on descending

Table 3
Effects of LC/SC stimulation on the Spontaneous activities of spinal cord neurons

Effects of LC/SC stimulation						
	Number of Spontaneously active units				Number of not Spontaneously active units	Total
	Inhibition	Facilitation	No effect	Total		
SL-A	12	18	8	38	24	62
SL-S	8	0	0	8	7	15
LL	0	0	0	0	7	7
INHIB	0	4	4	8	0	8

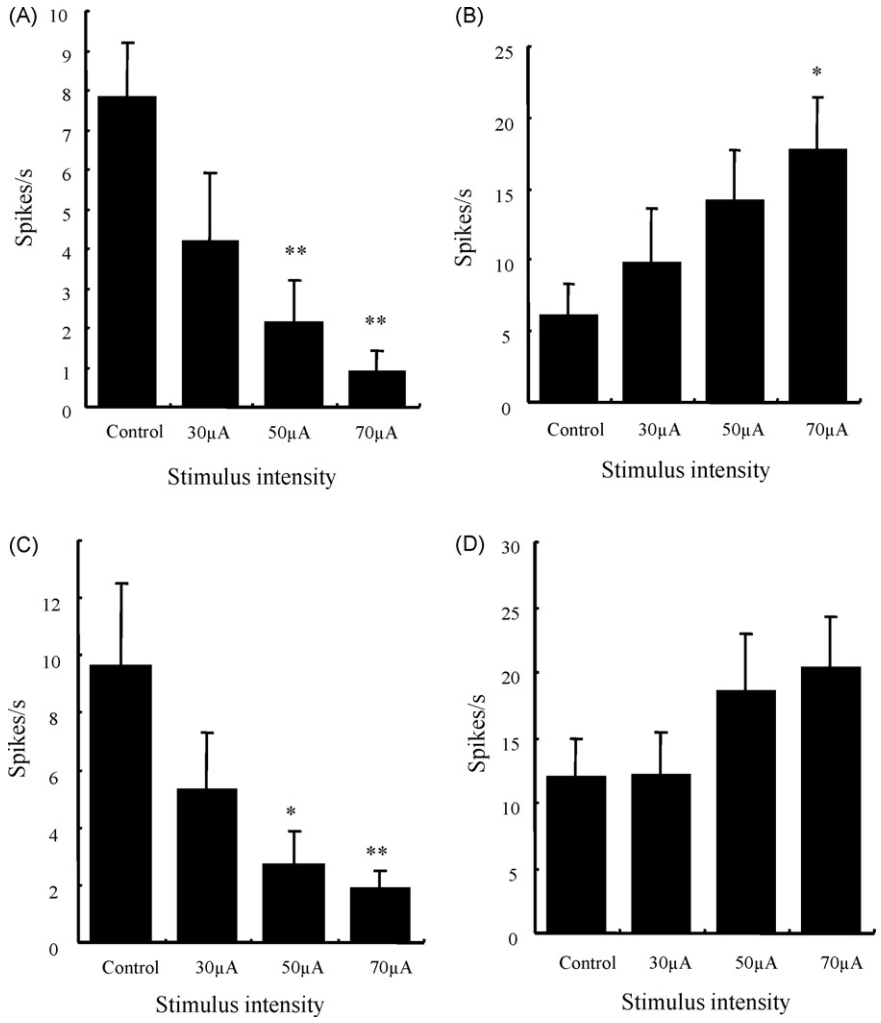

Fig. 5. Bar graphs summarizing the effects of LC/SC stimulation on spontaneous activities of different types of neurons. (A) LC/SC stimulation decreased the spontaneous activities of 12 SL-A neurons. (B) LC/SC stimulation increased the spontaneous activities of 18 SL-A neurons. (C) LC/SC stimulation decreased the spontaneous activities of 8 SL-S neurons. (D) LC/SC stimulation increased the spontaneous activities of 4 INHIB neurons. $^{*}P < 0.05$, $^{**}P < 0.01$, significantly different from the control.

modulatory influences on visceral nociceptive processing. The present study revealed that visceral nociceptive transmission evoked by CRD in the spinal cord is also under the control of the centrifugal pathways from the LC/SC. Focal electrical stimulation of the LC/SC produced intensity-dependent changes of the CRD-evoked discharge of most neurons. The effect of LC/SC stimulation on neuronal activity to CRD was confirmed by two experimental conditions. One was the site of LC/SC stimulation, only data from rats in which the tip of the stimulating electrode was located within the LC/SC were adopted; the second condition was the lesion of the LC/SC, following LC/SC lesions, LC/SC stimulation failed to modulate the neuronal activity to CRD. This result indicates that LC/SC stimulation did not spread to nuclei or axons outside the LC/SC throughout the experiments. Judging from these findings, it could be considered that modulation of the neuronal activity to CRD is due to activation of the LC/SC.

It has been confirmed that visceral nociceptive information ascends in the spinal cord by both the spinothalamic tract (STT) and the dorsal column (DC) pathways [30,36]. STT neurons excited by visceral nociceptive stimuli are located in the dorsal horn, and postsynaptic dorsal column (PSDC) neurons which conduct visceral nociceptive signals in the DC pathway are located near the central canal (lamina X) of the spinal cord [1,2,37]. In the present study, LC/SC stimulation modulated visceral nociceptive responses of neurons located not only in the dorsal horn but also in the vicinity of the central canal. Although we do not test the characteristics of these neurons, it seems reasonable to think that descending LC/SC neurons modulate visceral nociceptive responses of both STT neurons and PSDC neurons.

The present study demonstrated that electrical stimulation in both the ipsilateral and contralateral LC/SC was effective in significantly modulating CRD-evoked spinal neurons activity, these results are in agreement with previous studies. In 1983, Hodge et al. [18] revealed that the heat-evoked dorsal horn unit activity could be bilaterally inhibited by LC stimulation in cats. In 2005, Tsuruoka et al. [45] indicated that visceromotor responses to CRD could be bilaterally inhibited by LC stimulation in the rat. Other anatomical and electrophysiological evidence also demonstrated that the LC/SC sends bilateral projections to the spinal cord, the axons of the neurons in LC/SC descend the dorsolateral or ventrolateral funiculus to terminate in the ipsilateral or contralateral dorsal horn [6–8,23,40,43,44], although the roles of these pathways remain unclear, it may reflect the importance of the LC/SC in modulating nociceptive processing in the spinal cord.

Quantitative neurophysiological studies in rats have demonstrated the existence of four CRD responsive spinal neuron populations (SL-A, SL-S, LL and INHIB neurons) [31]. The present study shows that LC/SC stimulation has different effects on these

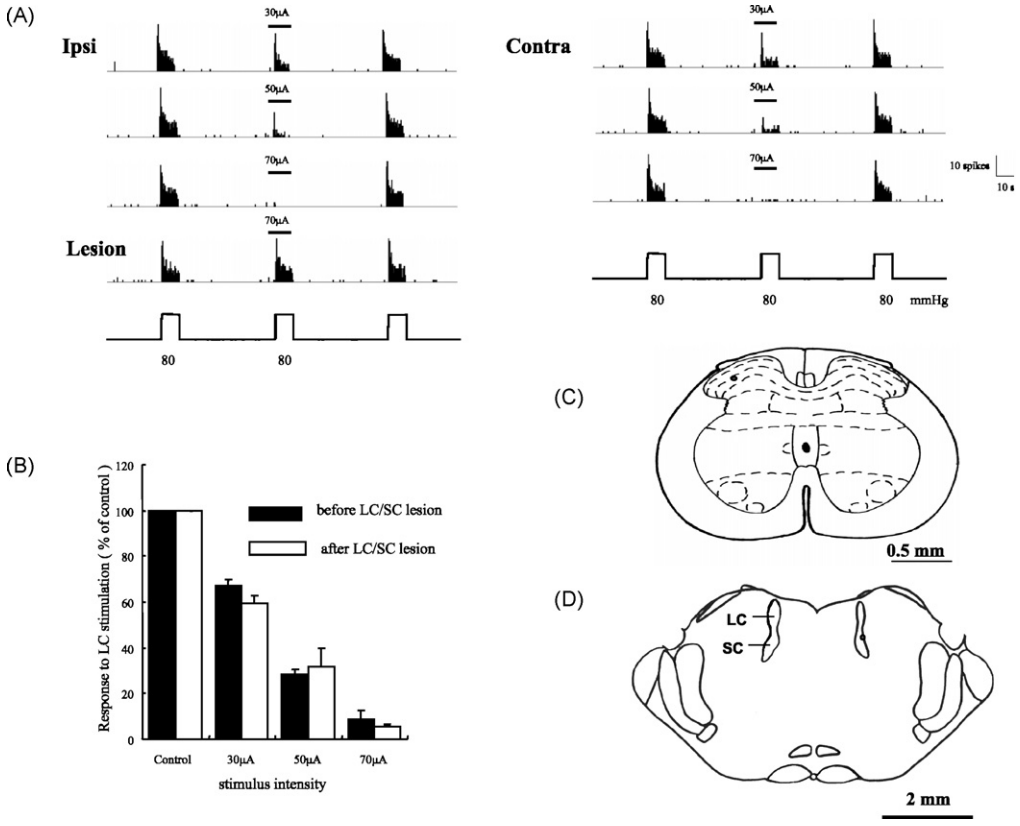

Fig. 6. Effects of LC/SC stimulation on CRD-evoked responses of SL-A neurons before and after lesions of the LC/SC ipsilateral to the recording site. (A) Representative example demonstrating that stimulation of the LC/SC ipsilateral to the recording site was effective in suppressing CRD-evoked responses at various intensities (Ipsi). Stimulation of the lesioned LC/SC failed to suppress CRD-evoked responses (Lesion). Stimulation of the intact LC/SC contralateral to the recordings site was effective in suppressing CRD-evoked responses (Contra). Horizontal lines above histograms represent times of application of LC/SC stimulation. The duration of CRD is shown as the bottom trace. (B) Comparison of the inhibitory effects of LC/SC stimulation ipsilaterally and contralaterally to the recording site on CRD-evoked responses before and after ipsilateral LC/SC lesion ($n = 5$). Effects of LC/SC stimulation (ordinate) are expressed as a percentage of the control (without LC/SC stimulation). No significant difference was observed between LC/SC stimulation of both sides. (C) The filled circles indicate the recording site in the S1 spinal cord for the data presented in A. (D) Localization of the LC/SC stimulation site for the data presented in A.

spinal neurons. For SL-S, LL and INHIB neurons the effects are inhibitory, for SL-A neurons the response is different, with 68% inhibitory, 16% excitatory and 16% not responding. The mechanism of the various effects is unclear, perhaps the transmitters released by the fibers or the receptors activated are different. Although it has been reported that LC/SC stimulation predominantly releases noradrenaline from coeruleospinal terminals, however, a great deal of evidence also shows that LC/SC neurons contain a variety of other transmitters, such as substance P [39], enkephalin [4], and 5-HT [51], therefore, electrical stimulation in the LC/SC perhaps can lead to the release of more than one transmitter in the spinal cord from descending fibers. These transmitters can be expected to produce a variety of actions on the spinal cord neurons by acting directly or indirectly via interneurons. An individual transmitter also may exert multiple actions via different receptors or interneurons. In SL-S and LL neurons, these various effects cannot be found, it is likely that the fewer neurons we have recorded compared with SL-A neurons.

The neurons evoked by CRD are implicated in visceral nociception because they exhibit many of the characteristics of nociceptive neurons, such as convergent nociceptive cutaneous receptive fields and high thresholds for activation [32]. In our experiment 96% CRD responsive neurons were characterized as having convergent cutaneous receptive fields located in the tail, perineal, lower back or hip regions. This finding is in agreement with many other studies [10,19,33] but differs from the study of Ness and Gebhart who demonstrated only 62.3% of the CRD excited neurons in the L_6–S_1 spinal segment had excitatory cutaneous receptive fields [31], the difference may be due to differences in preparation and/or recording site. They focused selectively on medial spinal cord, whereas our study examined not only the medial but also the lateral extent of the spinal cord. These multireceptive neurons are most likely responsible for viscerosomatic referred pain from the colon. The modulatory action on these neurons may, therefore, represent an important neuronal mechanism contributing to the generation of analgesia from LC/SC.

It has been reported that CRD can evoke cardiovascular and visceromotor reactions, these reactions can be eliminated by cold block the cervical spinal cord but unaffected by decerebration [32], this suggests that a spino-bulbo-spino pathway may exist between the spinal cord and the brain stem. According to previous studies, in the four types of CRD responsive spinal cord neurons, except LL neurons, the other kinds have long ascending axonal projections to the brain [31]. It can be expected that stimulation of peripheral nociceptors by CRD lead to the activation of these neurons, and the information ascends by projection pathways (spinocervical, spinoreticular tract or spinothalamic) [27] to the brain stem and activates the LC/SC neurons, which in turn lead to the increase of descending modulation onto second order spinal cord interneurons. A previous study has found profound activation of brain NE neurons in the LC in association with passive distension of the distal colon [11]. An abundance of evidence also demonstrates that noxious peripheral stimuli can activate descending modulation systems and increase noradrenergic metabolite levels in the rat spinal

cord [9], but whether there is a loop between the spinal cord and the LC/SC remains to be explored.

In summary the data reported in this study clearly indicate a role for the LC/SC in modulation of spinal visceral nociceptive inputs. However, the function of the LC/SC in sensory processing must be considered carefully, because the axons of the neurons in the LC/SC are highly branched and have extensive projections throughout the CNS. The LC/SC also receives wide and divergent sources of inputs from the spinal cord, brain stem and forebrain. The role of the LC/SC control of sensory transmission is almost certainly broader than just an analgesic mechanism. In fact, the LC/SC is involved in many higher biological functions such as orientation [13], vigilance [3], brain plasticity [25,41], stress, learning and memory [47].

Conflict of interest

There are no conflicts of interest.

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References

[1] E.D. Al-Chaer, Y. Feng, W.D. Willis, Comparative study of viscerosomatic input onto postsynaptic dorsal column and spinothalamic tract neurons in the primate, J. Neurophysiol. 82 (1999) 1876–1882.
[2] E.D. Al-Chaer, N.B. Lawand, K.N. Westlund, W.D. Willis, Pelvic visceral input into the nucleus gracilis is largely mediated by the postsynaptic dorsal column pathway, J. Neurophysiol. 76 (1996) 2675–2690.
[3] G. Aston-Jones, C. Chiang, T. Alexinsky, Discharge of noradrenergic locus coeruleus neurons in behaving rats and monkeys suggests a role in vigilance, Prog. Brain Res. 88 (1991) 501–520.
[4] Y. Charnay, L. Leger, F. Dray, A. Berod, M. Jouvet, J.E. Pujol, P.M. Dubois, Evidence for the presence of enkephalin in catecholaminergic neurons of cat locus coeruleus, Neurosci. Lett. 30 (1982) 147–151.
[5] J.M. Chung, D.J. Surmeier, K.H. Lee, L.S. Sorkin, C.N. Honda, Y. Tsong, D. Willis, Classification of primate spinothalamic and somatosensory thalamic neurons based on cluster analysis, J. Neurophysiol. 56 (1986) 308–327.
[6] F.M. Clark, D.C. Yeomans, H.K. Proudfit, The noradrenergic innervation of the spinal cord: differences between two substrains of Sprague–Dawley rats determined using retrograde tracers combined with immunocytochemistry, Neurosci. Lett. 125 (1991) 155–158.
[7] F.M. Clark, H.K. Proudfit, The projection of locus coeruleus neurons to the spinal cord in the rat determined by anterograde tracing combined with immunocytochemistry, Brain Res. 538 (1991) 231–245.
[8] F.M. Clark, H.K. Proudfit, Anatomical evidence for genetic differences in the innervation of the rat spinal cord by noradrenergic locus coeruleus neurons, Brain Res. 591 (1992) 44–53.
[9] J.N. Crawley, R.H.M.J.W. Roth, Locus coeruleus stimulation increased noradrenergic metabolite levels in rat spinal cord, Brain Res. 166 (1979) 180–184.
[10] W.C. Degroat, I. Nadelhaft, R. Milne, A. Booth, C. Morgan, K. Thor, Organization of the sacral parasympathetic reflex pathways to the urinary bladder and large intestine, J. Physiol. Lond. 306 (1980) 519–536.
[11] M. Elam, P. Thoren, T. Svensson, Locus coeruleus neurons and sympathetic nerves: activation by visceral afferents, Brain Res. 375 (1986) 117–125.
[12] S.L. Foote, F.E. Bloom, G. Aston-Jones, Nucleus locus ceruleus: new evidence of anatomical and physiological specificity, Physiol. Rev. 63 (1983) 844–914.
[13] S.L. Foote, C.W. Berridge, L.M. Adams, J.A. Pineda, Electrophysiological evidence for the involvement of the locus coeruleus in alerting, orienting and attending, Prog. Brain Res. 88 (1991) 521–532.
[14] R.L. Freitas, C.M.R. Ferreira, S.J. Ribeiro, A.D. Carvalho, D.H. Elias-Filho, N. Garcia-Cairasco, N.C. Coimbra, Intrinsic neural circuits between dorsal midbrain neurons that control fear-induced responses and seizure activity and nuclei of the pain inhibitory system elaborating postictal antinociceptive processes: a functional neuroanatomical and neuropharmacological study, Exp. Neurol. 191 (2005) 225–242.
[15] J.-M. Fritschy, R. Grzanna, Demonstration of two separate descending noradrenergic pathways to the rat spinal cord: evidence for an intragriseal trajectory of locus coeruleus axons in the superficial layers of the dorsal horn, J. Comp. Neurol. 291 (1990) 553–582.
[16] G.F. Gebhart, J.N. Sengupta, Evaluation of visceral pain, in: T.S. Gaginella, F.L. Boca Raton (Eds.), Handbook of Methods in Gastrointestinal Pharmacology, CRC, 1996, pp. 359–373.
[17] C.J. Hodge Jr., A.V. Apkarian, R. Stevens, G. Vogelsang, H.J. Wisnicki, Locus coeruleus modulation of dorsal horn unit responses to cutaneous stimulation, Brain Res. 204 (1981) 415–420.
[18] HodgeF C.J. Jr., A.V. Apkarian, R. Stevens, G. Vogelsang, O. Brown, J.I. Franck, Dorsolateral pontine inhibition of dorsal horn cell responses to cutaneous stimulation: lack of dependences on catecholaminergic systems in cat, J. Neurophysiol. 50 (1983) 1220–1235.
[19] C.N. Honda, Visceral and somatic afferent convergence onto neurons near the central canal in the sacral spinal cord of the cat, J. Neurophysiol. 53 (1985) 1059–1078.
[20] S.L. Jones, Descending noradrenergic influences on pain, Prog. Brain Res. 88 (1991) 381–394.
[21] S.L. Jones, G.F. Gebhart, Characterization of coeruleospinal inhibition of the nociceptive tail-flick reflex in the rat: mediation by spinal a_2-adrenoceptors, Brain Res. 364 (1986) 315–330.
[22] S.L. Jones, G.F. Gebhart, Quantitative characterization of coeruleospinal inhibition of nociceptive transmission in the rat, J. Neurophysiol. 56 (1986) 397–410.
[23] S.L. Jones, G.F. Gebhart, Spinal pathways mediating tonic, coeruleospinal, and raphe-spinal descending inhibition in the rat, J. Neurophysiol. 58 (1987) 138–159.
[24] S.L. Jones, G.F. Gebhart, Inhibition of spinal nociceptive transmission from the midbrain, pons and medulla in the rat: activation of descending inhibition by morphine, glutamate and electrical stimulation, Brain Res. 460 (1988) 281–296.
[25] S. Kasamatsu, Adrenergic regulation of visuocortical plasticity: a role of the locus coeruleus system, Prog. Brain Res. 88 (1991) 599–616.
[26] D. Margalit, M. Segal, A pharmacologic study of analgesia produced by stimulation of the nucleus locus coeruleus, Psychopharmacology 62 (1979) 169–183.
[27] D.B. McCreery, J.R. Bloedel, E.G. Hames, Effect of stimulation of raphe nuclei and in reticular formation on responses of spinothalamic neurons to mechanical stimuli, J. Neurophysiol. 42 (1979) 166–182.
[28] S.S. Mokha, J.A. McMillan, A. Iggo, Descending control of spinal nociceptive transmission: actions produced on spinal multireceptive neurons from the nuclei locus coeruleus (LC) and raphe agnus (NRM), Exp. Brain Res. 58 (1985) 213–225.
[29] T.J. Ness, Models of visceral nociception, ILAR J. 40 (1999) 119–128.
[30] T.J. Ness, Evidence for ascending visceral nociceptive information in the dorsal midline and lateral spinal cord, Pain 87 (2000) 83–88.
[31] T.J. Ness, G.F. Gebhart, Characterization of neuronal responses to noxious visceral and somatic stimuli in the medial lumbosacral spinal cord of the rat, J. Neurophysiol. 57 (1987) 1867–1892.
[32] T.J. Ness, G.F. Gebhart, Colorectal distension as a noxious visceral stimulus: physiologic and pharmacologic characterization of pseudoaffective reflexes in the rat, Brain Res. 450 (1988) 153–169.
[33] T.J. Ness, G.F. Gebhart, Characterization of neurons responsive to noxious colorectal distension in the T13-L2 spinal cord of the rat, J. Neurophysiol. 60 (1988) 1419–1438.
[34] T.J. Ness, G.F. Gebhart, Characterization of superficial T13-L2 dorsal horn neurons encoding for colorectal distension in the rat: Comparison with neurons in deep laminae, Brain Res. 486 (1989) 301–309.
[35] T.J. Ness, A.M. Metcalf, G.F. Gebhart, A psychophysiological study in humans using phasic colonic distention as a noxious visceral stimulus, Pain 43 (1990) 377–386.
[36] J. Palecek, V. Paleckova, W.D. Willis, The role of pathways in the spinal cord lateral and dorsal funiculi in signaling nociceptive somatic and visceral stimuli in rats, Pain 96 (2002) 297–307.
[37] J. Palecek, V. Paleckova, W.D. Willis, Fos expression in spinothalamic and postsynaptic dorsal column neurons following noxious visceral and cutaneous stimuli, Pain 104 (2003) 249–257.
[38] G. Paxinos, C. Watson, The Rat Brain in Stereotaxic Coordinates, Academic Press, New York, 1998.
[39] V.M. Pickel, T.H. John, D.J. Reis, S.E. Leeman, R.J. Miller, Electron microscopic localization of substance P and enkephalin in axon terminals related to dendrites of catecholaminergic neurons, Brain Res. 160 (1979) 387–400.
[40] H.K. Proudfit, F.M. Clark, The projections of locus coeruleus neurons to the spinal cord, Prog. Brain Res. 88 (1991) 123–141.
[41] S.J. Sara, M. Segal, Plasticity of sensory responses of locus coeruleus neurons in the behaving rat: implications for cognition, Prog. Brain Res. 88 (1991) 571–585.
[42] M. Segal, D. Sandberg, Analgesia produced by electrical stimulation of catecholamine nuclei in the rat brain, Brain Res. 123 (1977) 369–372.
[43] K.A. Sluka, K.N. Westlund, Spinal projections of the locus coeruleus and the nucleus subcoeruleus in the Harlan and the Sasco Sprague–Dawley rat, Brain Res. 579 (1992) 67–73.
[44] M. Tsuruoka, M. Maeda, I. Nagasawa, T. Inoue, Spinal pathways mediating coeruleospinal antinociception in the rat, Neurosci. Lett. 362 (2004) 236–239.
[45] M. Tsuruoka, M. Maeda, T. Inoue, Stimulation of the nucleus locus coeruleus/subcoeruleus suppresses visceromotor responses to colorectal distention in the rat, Neurosci. Lett. 381 (2005) 97–101.
[46] M. Tsuruoka, W.D. Willis, Bilateral lesions in the area of the nucleus locus coeruleus affect the development of hyperalgesia during carrageenan-induced inflammation, Brain Res. 726 (1996) 233–236.
[47] L. Velley, B. Cardo, E. Kempf, P. Mormede, S. Nassif-Caudarella, J. Velly, Facilitation of learning consecutive to electrical stimulation of the locus coeruleus: cognitive alteration or stress-reduction? Prog. Brain Res. 88 (1991) 555–569.

[48] D.L. Voisin, N. Guy, M. Chalus, R. Dallel, Nociceptive stimulation activates locus coeruleus neurons projecting to the somatosensory thalamus in the rat, J. Physiol. 566 (2005) 929–937.
[49] B.D. Waterhouse, B. Border, L. Wahl, G.A. Mihailoff, Topographic organization of rat locus coeruleus and dorsal raphe nuclei: distribution of cells projecting to visual system structures, J. Comp. Neurol. 336 (1993) 345–361.
[50] W.L. West, D.C. Yeomans, H.K. Proudfit, The function of noradrenergic neurons in mediating antinociception induced by electrical stimulation of the locus coeruleus in two different sources of Sprague–Dawley rats, Brain Res. 626 (1993) 127–135.
[51] L. Wiklund, L. Lenger, M. Persson, Monoamine cell distribution in the cat brain stem. A fluorescence histochemical study with quantification of indolaminergic and locus coeruleus cell groups, J. Comp. Neurol. 203 (1981) 613–647.
[52] M. Zimmermann, Ethical guidelines for investigation of experimental pain in conscious animals, Pain 16 (1983) 109–110.