

Inhibition of electroacupuncture on nociceptive responses of dorsal horn neurons evoked by noxious colorectal distention in an intensity-dependent manner

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Background: The transmission of visceral nociception can be inhibited by electroacupuncture (EA) at the spinal level. However, relationships between current intensity and EA-induced analgesia are still lacking. This study compares the effects of different intensities of EA at local acupoints and heterotopic acupoints on nociceptive responses of spinal wide dynamic range (WDR) neurons induced by noxious colorectal distention (CRD).

Materials and methods: Experiments were conducted on 40 Sprague Dawley rats anesthetized with 10% urethane. Discharges of WDR neurons in the L1–L3 segments of the dorsal horn of the spinal cord were recorded extracellularly by glass micropipettes. Different intensities of EA (0.5, 1, 2, 4, 6, and 8 mA, 0.5 ms, 2 Hz) were applied to contralateral “Zusanli” (ST 36) or “Neiguan” (PC 6), with either the same or different segmental innervation of the colon.

Results: In local acupoints, the increased discharges of WDR neurons evoked by CRD were significantly inhibited by EA at 0.5–8 mA. A positive relationship between current intensity and the inhibiting rate was observed within 0.5–4 mA, but the inhibiting rate reached a plateau when EA exceeded 4 mA. In heterotopic acupoints, the increased discharges of WDR neurons evoked by CRD were significantly inhibited by EA at 2–8 mA. A positive relationship between current intensity and the inhibiting rate was observed within 2–6 mA. Further increase in the current beyond 6 mA also resulted in a plateau effect.

Conclusion: Within a certain range, the nociceptive responses of dorsal horn neurons induced by CRD could be inhibited by EA in an intensity-dependent manner.

Keywords: electroacupuncture, current intensity, wide dynamic range, colorectal distention

Introduction

Visceral pain is one of the most common forms of pain caused by stress or injury arising from the internal organs.^{1,2} Electroacupuncture (EA) has been widely used for analgesia by passing electrical current to acupoints via acupuncture needles connected to an electrical stimulator. Accumulating evidence suggests that EA is an efficacious treatment for alleviating acute and chronic visceral pain.^{3–5} Interestingly, such an antinociceptive effect was not seen in sham EA without electrical stimulation, even if sham EA is applied to the same acupoints as used in the real EA group.⁶ Consequently, we speculate that current intensity is important for effective analgesia. In fact, many human and animal studies have shown that EA with different intensities can excite different types of peripheral afferent fibers and produce different extents of analgesia.^{7–9} However, a comprehensive

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understanding of the relationship between current intensity and EA-induced analgesia on visceral pain is still unknown.

Electrophysiological studies can directly examine the mechanism of EA-induced analgesia. It is generally accepted that EA achieves analgesic effects on visceral pain via somato-visceral interactions on convergent neurons at different levels of the central nervous system.¹⁰ The wide dynamic range (WDR) neurons of the spinal dorsal horn could play an important role in the management of visceral nociception.¹¹ These neurons receive a convergence of inputs from the external environment (the skin) and the internal milieu (the viscera, muscles, etc.).¹² We have previously confirmed that WDR neurons could be activated by EA and manual acupuncture in normal rats.¹³ Interestingly, in rats with noxious colorectal distension (CRD), the nociceptive response of WDR neurons could be inhibited by manual acupuncture.¹⁴ These observations indicate the involvement of WDR neurons in the antinociceptive effects of EA on visceral pain. It is worth noting that WDR neurons can encode the strength of mechanical stimuli in a certain range. The discharges of WDR neurons linearly increase during graded electrical or thermal stimuli.^{15,16} As such, it is evident that these neurons may respond to graded intensities of EA.

Therefore in this study, we observe the responses of WDR neurons to different intensities of EA in rats with noxious CRD stimulation and we aim to explain the relationships between current intensity and the effects of EA on inhibiting nociceptive visceral afferents.

Materials and methods

Animals

Experiments were performed on 40 male Sprague Dawley rats weighing between 220 and 280 g that were purchased from the Laboratory Animal Center of China Academy of Military Medical Sciences (production license number: SCXK-(Military)-2012-0004; Beijing, China). After arrival, the rats were given food and water ad libitum and habituated to the experimental environment for at least 1 week. All animals were housed in standard conditions at an ambient temperature of $22^{\circ}\text{C}\pm 0.5^{\circ}\text{C}$ and under artificial 12 hours light/dark cycle. All manipulations and procedures had been approved by Animal Ethics Committee of China Academy of Chinese Medical Science (no. 20160218) and were conducted according to the *Guideline on the Humane Care and Use of Laboratory Animals* issued by the Ministry of Science and Technology of the People's Republic of China in 2006.

Noxious visceral stimulus

Previous studies have shown that CRD stimuli with an intensity of >40 mmHg were recognized as noxious visceral stim-

uli.¹⁷ In this experiment, 60 mmHg CRD was administered as noxious visceral stimuli. In brief, a 6 cm balloon was gently inserted into the descending colon and rectum through the anus. During the recording sessions, the balloon was inflated with air, and the intracolonic pressure was monitored using a pressure transducer. The pressure of CRD stimulation applied to rats was 60 mmHg, lasting 50 seconds. To prevent possible sensitization triggered by frequent stimulation of colorectum, the interval between two bouts of CRD stimulation was at least 10 minutes.

EA stimulation

A pair of noninsulated needles (0.22 mm \times 25 mm, Beijing Zhongyan Taihe Medical Instrument Co. Ltd., Beijing, China) were inserted into acupoint ST 36 (on the anterolateral side of the hind limb near the anterior crest of the tibia below the knee under the tibialis anterior muscle) or PC 6 (on the medial of forelimb and 3 mm above the wrist) \sim 0.3 cm apart from each other. After insertion, acupoints were stimulated with different intensities of EA via the inserted needles connected to an electrical stimulator (88–102 G; Nihon Kohden, Tokyo, Japan). During a 30-second EA session, intensities of 0.5, 1, 2, 4, 6, and 8 mA were applied in a random order. The duration and frequency of electrical stimulation were set at 0.5 ms and 2 Hz, respectively.

Extracellular recording

After 12 hours of fasting, unit discharges of WDR neurons in anesthetized rats were recorded. Following intraperitoneal injection of 100 μg of atropine sulfate, the rats were deeply anesthetized with 10% urethane (1.0–1.2 g/kg) and were then artificially ventilated through an endotracheal tube. Body temperature was maintained at $37.5^{\circ}\text{C}\pm 0.5^{\circ}\text{C}$ using a feedback-controlled heating blanket.

A laminectomy was performed at the lumbar spinal to expose the L1–L3 segments of the spinal cord, and the corresponding vertebrae were fixed in a rigid frame. Unitary extracellular recordings were made using glass micropipettes (8–12 M Ω) filled with a mixture of 2% Pontamine Sky Blue dye and 0.1 M of sodium ascorbic acid. According to the three-dimensional coordinates of WDR neurons, 0.5–1.5 mm lateral to the midline and 500–1500 μm beneath the surface, micropipettes were then inserted into the right-hand side of the spinal cord. Neuronal discharges were routed to a window discriminator and displayed on an oscilloscope. Outputs of the window discriminator and amplifier were fed into a data collection system (PowerLab) and a personal computer-based data acquisition system (Chart 5.0) to compile histograms or watermark files.

Experimental procedure

1. After finding a neuron with stable discharges, noxious (pinch) and innocuous (brush) skin stimuli were used to identify the targeted neurons. As reported in our previous study,¹³ WDR neurons were excited by both the noxious and innocuous stimulation applied to their skin receptive fields. However, WDR neurons gave no response to stimulation applied to non-receptive fields. When a WDR neuron was identified, the responses of WDR neurons to pinch and brush skin stimuli were recorded, and the skin receptive fields of WDR neurons were mapped.
2. Second, the responses of WDR neurons to CRD stimulation were recorded. The responses of WDR neurons to different intensities of EA stimulation without CRD were then recorded.
3. Third, the responses of WDR neurons to different intensities of EA stimulation during CRD were recorded. A standard conditioned recording procedure was administered for 60 seconds: 1) The background discharge was recorded for 5 seconds. 2) 60 mmHg CRD stimulation was applied to rats for 50 seconds. In the first 10 seconds, the response of neuronal discharges to a single CRD stimulation was observed. 3) During CRD, different intensities of EA were applied at the contralateral ST 36 or PC 6 for 30 seconds. The effects of EA on CRD-induced neuronal discharges were observed. 4) After stopping EA, CRD stimulation was continued for 10 seconds. 5) After stopping CRD, the recovery of neuronal discharge was recorded for 5 seconds.
4. After single-unit recordings, the recording sites were marked by electrophoretic deposition of Pontamine Sky Blue and checked by H&E coloration. Locations of the recording sites were then determined using the rat brain atlas.

Data collection and statistical analysis

The average frequency of neuronal discharges (spikes/s) and the inhibitory percentage of EA on CRD-induced neuronal discharges were calculated and analyzed by PowerLab, Chart 5.0, and SPSS 13.0. All data are presented as mean \pm standard error of the mean (SEM). Paired *t*-test analyses were used for statistical analysis. $P < 0.05$ was deemed statistically significant.

Results

General characteristics of WDR neurons in the dorsal horn of the spinal cord

A total of 132 neurons were recorded in the L1–L3 segments of the dorsal horn of the spinal cord, among which 115 neurons were identified as WDR neurons. The receptive field of

these neurons was distributed in the ipsilateral caudal parts of the body, including the hip region, tail root, hind limb, and hind paw (Figure 1A). The activity of WDR neurons was excited by both innocuous (brush) and noxious (pinch) stimulation of their peripheral receptive field (Figure 1B). Examination of the rat spinal slices verified that the majority of recording sites were located in laminae IV and V, and a few in laminae I and VI of the gray matter (Figure 1C). These sites were marked by electrophoresis with the Pontamine Sky Blue dye at the end of the experiment (Figure 1D).

The response of WDR neurons to 60 mmHg CRD stimulation

If neurons were identified as WDR neurons, their response to CRD stimulation was tested. Among 115 WDR neurons, 106 (92.17%) neurons were excited by CRD, 3 (2.61%) were inhibited, and 6 (5.22%) showed no response to CRD. We observed the reaction of 10 WDR neurons to 60 mmHg CRD stimulation. An example of the activating reactions induced by CRD is shown in Figure 2A. After CRD, the average discharge frequency of WDR neurons increased from 2.83 ± 0.32 spikes/s of background to 9.96 ± 1.20 spikes/s ($P < 0.05$), with an increasing rate of $259.94\% \pm 26.59\%$ (Figure 2B). These data indicate

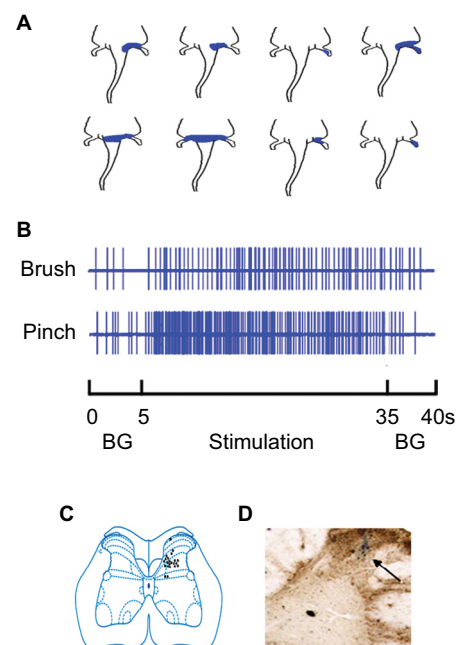


Figure 1 General features of WDR neurons.

Notes: (A) The peripheral receptive field of WDR neurons located in the L1–L3 spinal cord. (B) The response of a WDR neuron to non-noxious (brush) and noxious (pinch) stimulus. (C) The location of identified WDR neurons in the spinal cord. (D) A representative example showing the location of the WDR neuron marked by Pontamine Sky Blue.

Abbreviations: BG, background; WDR, wide dynamic range.

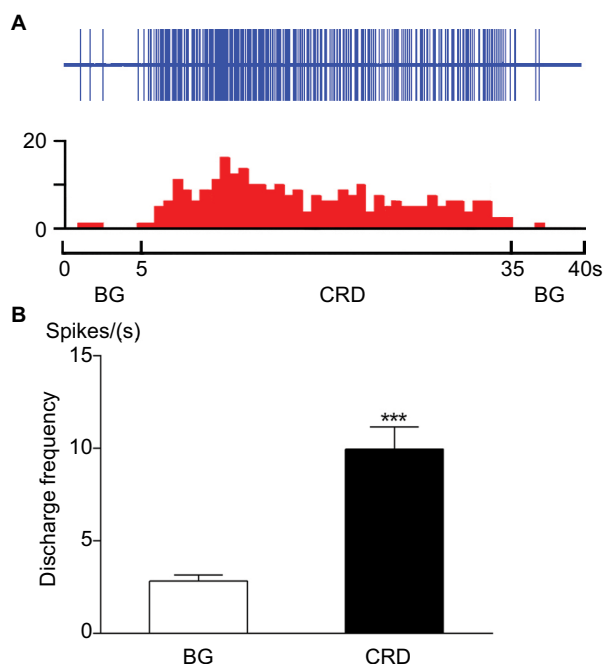


Figure 2 Responses of WDR neurons to 60 mmHg CRD stimulation.

Notes: (A) Representative example showing the activity of a WDR neuron evoked by 60 mmHg CRD stimulation. Top row shows the original unit discharges and bottom row shows the histograms. (B) The cumulative result shows that the discharge of WDR neurons increased significantly after CRD compared to BG. *** $P < 0.05$ compared with BG.

Abbreviations: BG, background; CRD, colorectal distension; WDR, wide dynamic range.

that 60 mmHg CRD stimulation could significantly activate WDR neurons and could be recognized as visceral nociception.

The response of WDR neurons to graded intensities of EA stimulation without CRD

In this section of the experiment, we examined the response of WDR neurons to graded intensities of EA stimulation without CRD. As shown in Figure 3, the activity of WDR neurons was significantly enhanced by EA stimulation applied at ipsilateral ST 36, which was located in the receptive field of these neurons. Examples of neuronal discharges evoked by 0.5–8 mA EA stimulation are shown in Figure 3A–F. The cumulative results ($n=8$) showed that the discharges of WDR neurons evoked by EA increased as the intensity of EA increased within the range 0.5–4 mA. Further increases in current intensity beyond 4 mA resulted in a saturation effect of this neuron (Figure 3G).

However, when EA stimulation was applied at contralateral ST 36 and PC 6, which were located in the nonreceptive field of WDR neurons, EA had no effect on the activity of WDR neurons (Figures 4 and 5). This is consistent with our

previous study that WDR neurons only receive the inputs of EA from acupoints located in their skin receptive fields in the normal state.¹⁸

The response of WDR neurons to different intensities of EA at contralateral ST 36 during CRD

According to our previous studies,¹⁸ during CRD-induced acute visceral pain conditions, the nociceptive responses of WDR neurons could be inhibited by EA applied at acupoints located in their nonreceptive fields. However, EA at acupoints located in the receptive fields of WDR neurons could affect the nociceptive responses of these neurons. To explore the dose–effect relationship of EA on the nociceptive response of WDR neurons, EA stimulation was applied at contralateral ST 36.

As illustrated in Figures 6 and 7, EA at 0.5 and 1 mA produced a slight but significant inhibition. After 0.5 mA of EA, the average discharge frequency of WDR neurons decreased from 8.59 ± 0.41 spikes/s of CRD to 7.30 ± 0.43 spikes/s ($P < 0.05$, $n=8$), with an inhibiting percentage of $15.36\% \pm 1.86\%$. After 1 mA of EA, the neuronal discharges decreased from 8.47 ± 0.63 spikes/s of CRD to 6.30 ± 0.63 spikes/s ($P < 0.05$, $n=8$), with an inhibiting percentage of $26.39\% \pm 3.04\%$.

EA at 2 mA produced a moderate inhibition, with the neuronal discharges decreasing from 8.87 ± 0.98 spikes/s of CRD to 4.45 ± 0.70 spikes/s, with an inhibiting percentage of $50.10\% \pm 5.53\%$ ($P < 0.01$, $n=8$). EA at 4 mA produced a strong inhibition, with neuronal discharges decreasing from 8.26 ± 0.84 spikes/s of CRD to 3.01 ± 0.48 spikes/s, with an inhibiting percentage of $64.64\% \pm 2.72\%$ ($P < 0.001$, $n=8$).

When the intensity of EA was further increased to 6 and 8 mA, the inhibiting effect of EA on nociceptive response of WDR neurons reached a plateau. After 6 mA of EA, the neuronal discharges decreased from 8.14 ± 0.75 spikes/s of CRD to 2.67 ± 0.18 spikes/s, with an inhibiting percentage of $66.37\% \pm 2.13\%$ ($P < 0.001$, $n=8$). After 8 mA of EA, the neuronal discharges decreased from 8.06 ± 0.40 spikes/s of CRD to 2.77 ± 0.35 spikes/s, with an inhibiting percentage of $65.91\% \pm 3.09\%$ ($P < 0.001$, $n=8$).

Response of WDR neurons to different intensities of EA at contralateral PC 6 during CRD

It is well known that the antinociceptive effects of EA treatment are different when EA is applied to acupoints with the same or different segmental innervation with the pain organs.^{7–9} For these experiments, EA stimulation was applied at contralateral PC 6, which innervates different segments of

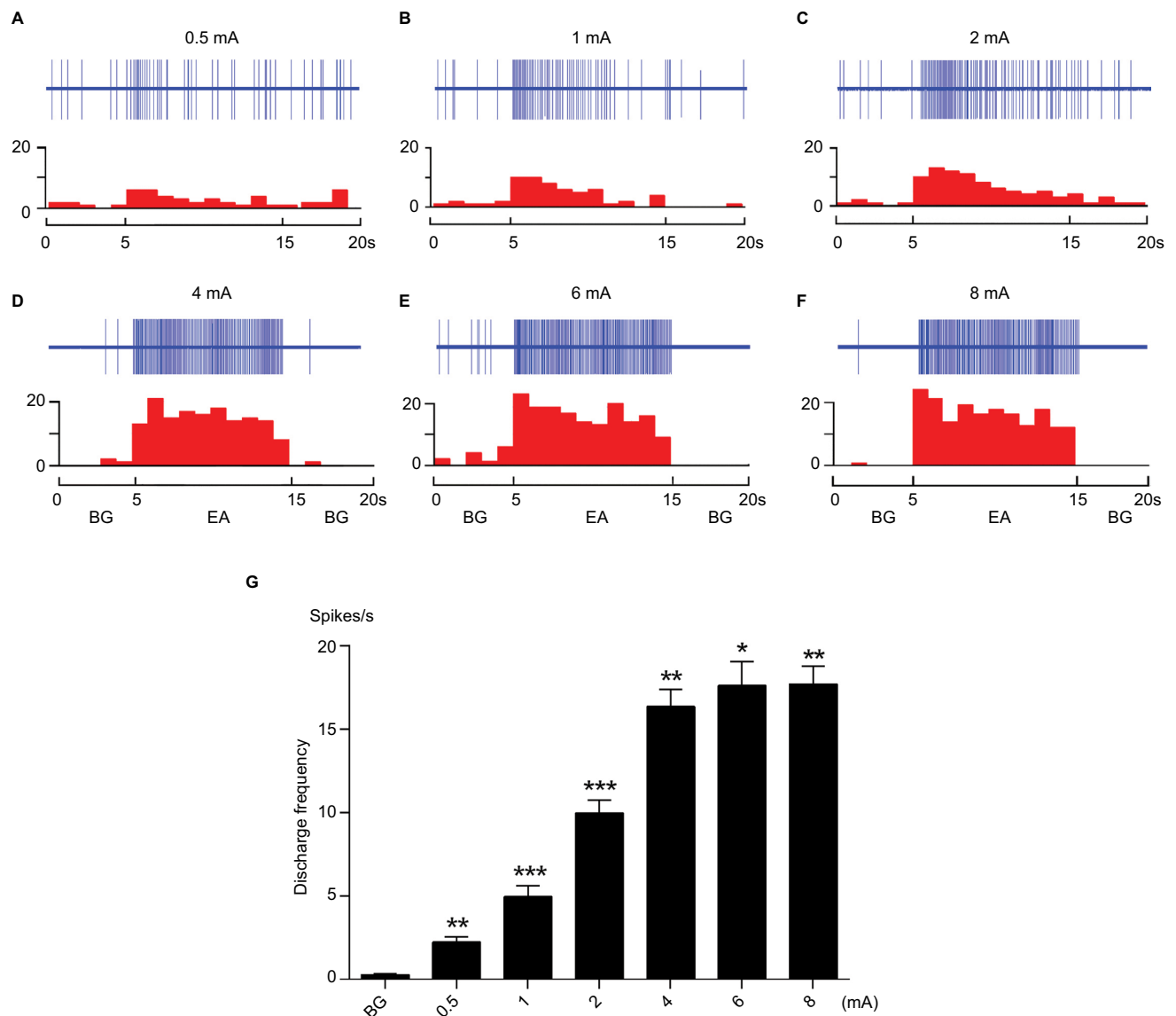


Figure 3 The responses of WDR neurons to graded intensity of EA stimulation at ipsilateral ST 36.

Notes: (A–F) Example showing the activity of a WDR neuron evoked by 0.5–8 mA EA stimulation. The top row shows original unit discharges and the bottom row represents this information in a histogram. (G) The cumulative result shows that the discharge of WDR neurons was significantly increased after EA. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared with BG.

Abbreviations: BG, background; EA, electroacupuncture; WDR, wide dynamic range.

rectum and colon, to study the dose–effect relationship of EA at heterotopic acupoints on visceral nociception. As illustrated in Figures 8 and 9, the discharges of WDR neurons evoked by CRD were inhibited by 2–8 mA EA stimulation of PC 6.

The cumulative results showed that EA with an intensity of 0.5 and 1 mA had no significant suppressive effect on CRD-induced noxious discharges of WRD neurons. Furthermore, 2 mA of EA produced a slight but significant inhibition, with the average discharge frequency of WDR neurons decreasing from 9.18 ± 0.27 spikes/s of CRD to 7.30 ± 0.45 spikes/s, and an inhibiting percentage of $19.45\% \pm 4.14\%$ ($P < 0.001$, $n = 8$).

Additionally, 4 mA of EA produced a moderate inhibition, with neuronal discharges decreasing from 8.78 ± 0.47 spikes/s of CRD to 5.51 ± 0.28 spikes/s, and an inhibiting percentage of $36.39\% \pm 3.70\%$ ($P < 0.001$, $n = 8$). Application of 6 mA of EA produced a strong inhibition, with neuronal discharges decreasing from 8.52 ± 0.50 spikes/s of CRD to 3.77 ± 0.38 spikes/s, with an inhibiting percentage of $56.12\% \pm 4.01\%$ ($P < 0.05$, $n = 8$).

Interestingly, when the intensity of EA was further increased to 8 mA, the inhibiting effect of EA on nociceptive response of WDR neurons also reached a plateau. After 8 mA

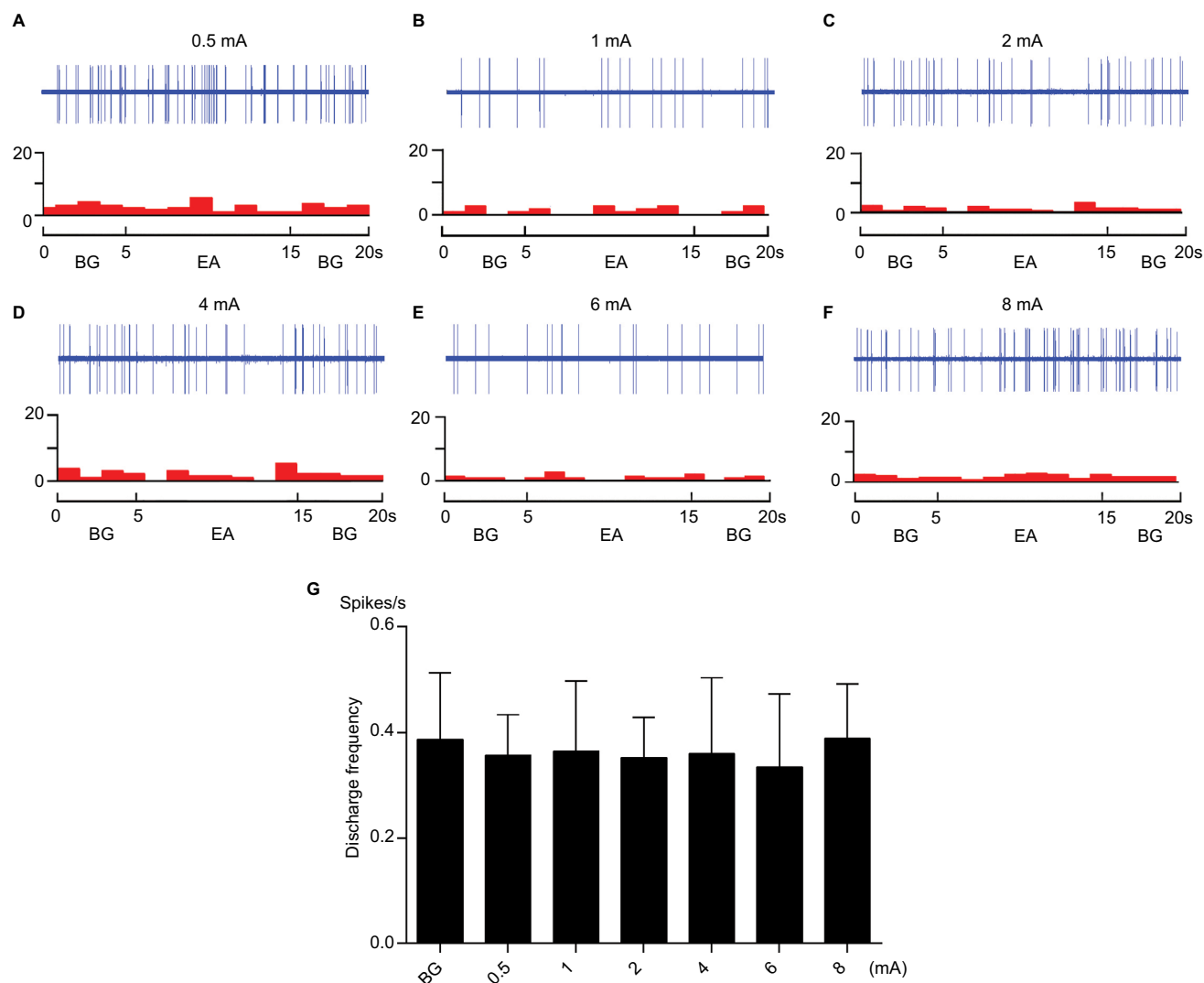


Figure 4 The responses of WDR neurons to graded intensity of EA stimulation at contralateral ST 36.

Notes: (A–F) Example of neuronal response to EA stimulation at contralateral ST 36. The top row shows original unit discharges and the bottom row shows this information in a histogram. (G) The cumulative result shows that the discharge frequency of WDR neurons was not changed after EA.

Abbreviations: BG, background; EA, electroacupuncture; WDR, wide dynamic range.

of EA, the neuronal discharges decreased from 8.20 ± 0.49 spikes/s of CRD to 3.74 ± 0.39 spikes/s and had an inhibiting percentage of $54.70\% \pm 3.69\%$ ($P < 0.001$, $n = 8$).

Discussion

Since the early 1950s, treatment with EA has been widely used for analgesia in clinical practice. According to the traditional Chinese medicine theory and modern acupuncture researches, the analgesic effect of EA treatment can be specific and extensive. EA stimulation of acupoints located in the same segmental innervation of the pain area (local acupoints) can elicit segmental analgesia, whereas stimulation of acupoints away from the pain area (heterotopic acupoints) can elicit extrasegmental analgesia.^{8,9} We, therefore, sought to

elucidate the relationship between current intensity and EA-induced local and extensive analgesia on visceral pain. This study compares the effects of different intensities of EA at local acupoints and heterotopic acupoints on the nociceptive responses of spinal WDR neurons induced by noxious CRD stimulation. The results of the present study demonstrated that the increased discharges of spinal WDR neurons evoked by CRD were inhibited by EA at both local and heterotopic acupoints, but the intensity of EA necessary to trigger segmental inhibition and extrasegmental inhibition is different. We also saw an increase in the inhibiting rate to graded intensity of EA. However, when EA exceeded a certain range, both the segmental inhibition and extrasegmental inhibition induced by EA reached a plateau. These results indicate that, within a

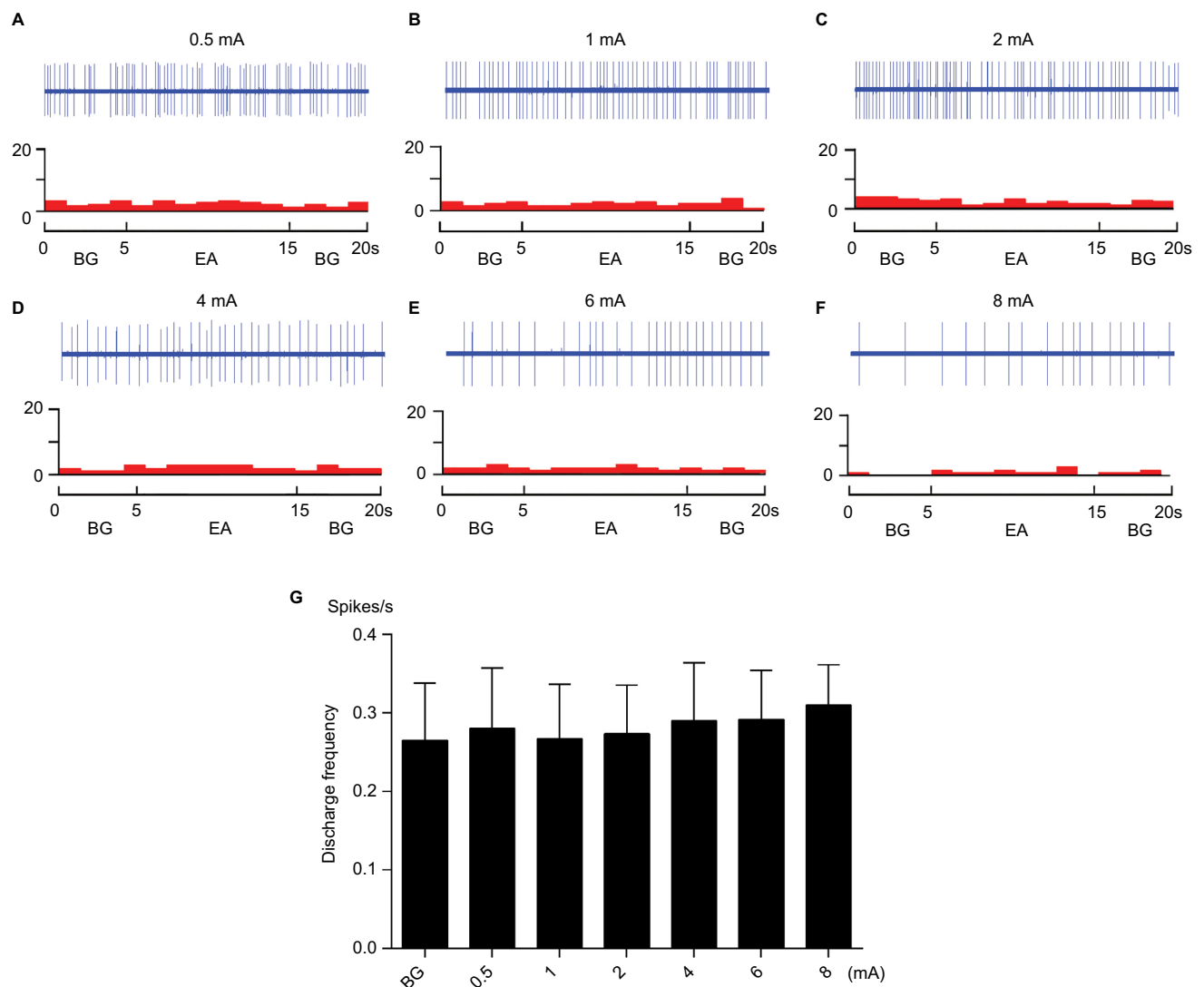


Figure 5 The responses of WDR neurons to graded intensity of EA stimulation at contralateral PC 6.

Notes: (A–F) Example of neuronal response to EA stimulation at contralateral PC 6. The top row shows original unit discharges and the bottom row shows this information in a histogram. (G) The cumulative result shows that the discharge frequency of WDR neurons was not changed after EA.

Abbreviations: BG, background; EA, electroacupuncture; WDR, wide dynamic range.

certain range, the transmission of visceral nociception could be inhibited by EA in an intensity-dependent manner.

Intensity of EA to trigger segmental inhibition and extrasegmental inhibition on visceral nociception is different

After different intensities of EA were applied to rats, increased discharges of WDR neurons induced by CRD were significantly decreased, indicating afferent impulses from visceral nociception could be inhibited by EA. However, the intensity of EA for segmental and extrasegmental analgesia is different. We found that the threshold of EA at ST 36 to trigger inhibition of the noxious response of WDR neurons was lower than that of EA at PC 6. In ST

36, EA at 0.5 and 1 mA was effective for segmental inhibition, whereas the inhibition induced by EA at PC6 occurred only when the intensity of EA increased to 2 mA. Based on earlier neurophysiological studies, electrical current in the range 0.25–0.5 mA was found to be effective for A δ -fiber activation and 1–2 mA for C-fibers activation.¹⁹ As a result, it is evident that, on visceral pain, EA-induced segmental analgesia is mediated by both A- and C-fibers, and extrasegmental analgesia is mediated only by C-fibers. Our results are consistent with previous studies on somatic pain that show that EA with a high enough intensity to excite A β -fibers is capable of producing local analgesia, but excitation of A δ - and some C-fibers induces a more potent and extensive analgesia.^{7,20,21}

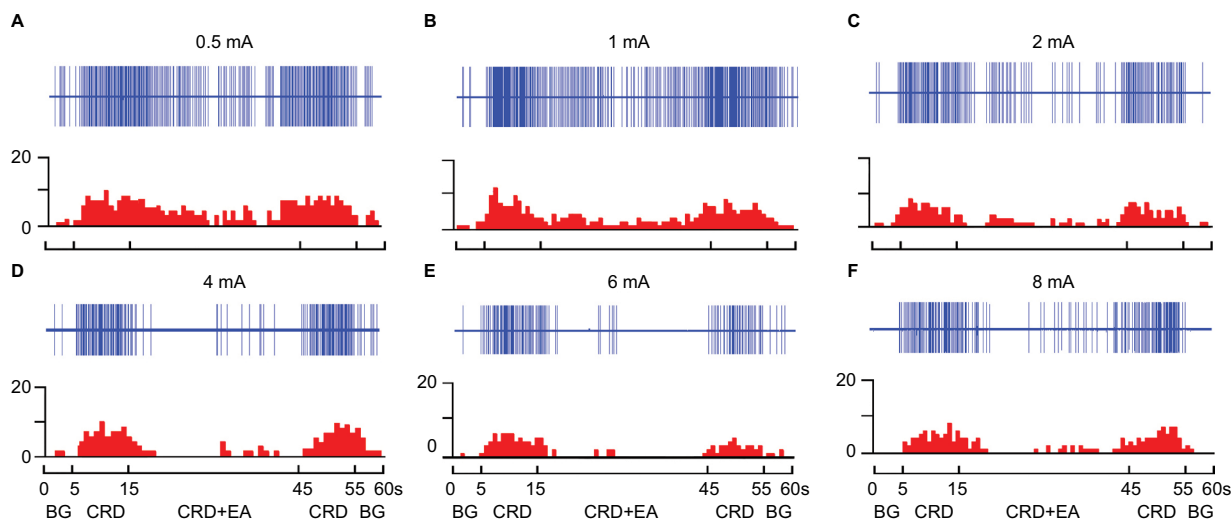


Figure 6 Examples of the response of WDR neurons to different intensities of EA at ST 36 during CRD. **Notes:** EA at 0.5 mA (A), 1 mA (B), 2 mA (C), 4 mA (D), 6 mA (E), and 8 mA (F) was applied to ST 36 during CRD. A decrease in the discharges of WDR neurons during the CRD+EA sequence was observed. Top row shows the original unit discharges and bottom row shows the histograms. **Abbreviations:** BG, background; CRD, colorectal distention; EA, electroacupuncture; WDR, wide dynamic range.

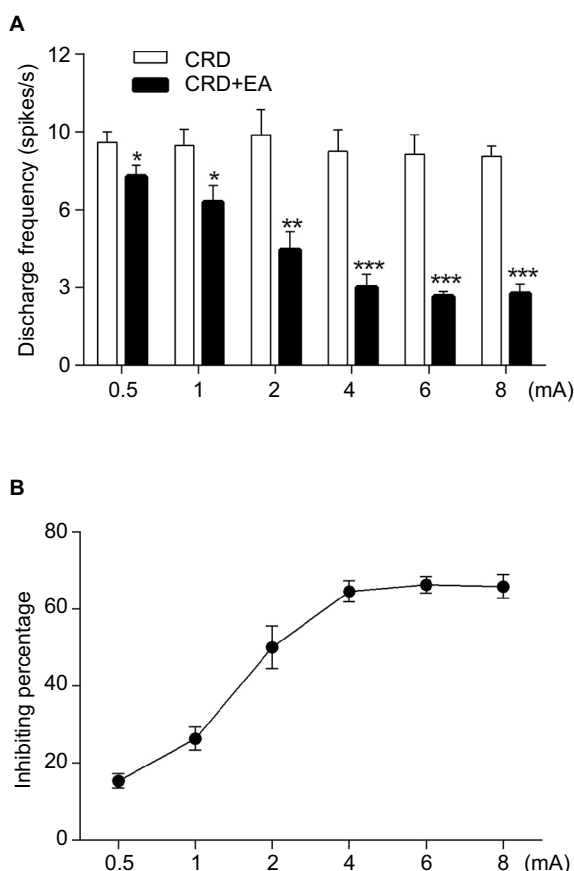


Figure 7 The effects of different intensities of EA at ST 36 on CRD-induced discharges of WDR neurons. **Notes:** (A) A histogram shows that the noxious discharges of WDR neurons induced by CRD can be inhibited by EA in the range 0.5–8 mA. Data consist of the average spikes per second (mean ± SEM). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, compared with CRD. (B) A stimulus–response curve shows that the inhibiting percentage increased as the intensity of EA increased with 0.5–4 mA. **Abbreviations:** CRD, colorectal distention; EA, electroacupuncture; SEM, standard error of the mean; WDR, wide dynamic range.

ST 36 has a unique antinociceptive effect on visceral nociception induced by EA

In this study, the inhibiting percentage of ST 36 and PC 6 on the nociceptive response of WDR neurons induced by EA was calculated. We found that EA at ST 36 produced a higher inhibiting percentage compared with EA at PC 6 with the same intensity. ST 36 is a lower confluent acupoint of the meridians of the stomach. It is innervated by the same segment of the rectum and colon and involved in the treatment of gastrointestinal diseases.^{14,18,22} The lumbar spinal WDR neurons receive ipsilateral ST36 afferent input.²³ In addition, ST36 is the most commonly used acupoint for analgesia in the clinic. It has been confirmed by many behavioral experiments that EA and acupuncture stimulation applied at acupoints ST 36 exert positive effects on rats with visceral hyperalgesia.^{24–29} These findings give evidence of the unique antinociceptive effect of ST 36 on visceral nociception.

Segmental inhibition of visceral nociception induced by low-intensity EA involved in gate control theory

Low-intensity EA applied at ST 36 produced a slight but significant inhibition on the nociceptive responses of WDR neurons. The segmental analgesia induced by low-intensity EA can be explained by the gate control theory, as proposed by Ronald Melzack and Patrick Wall in 1965.³⁰ These authors opined that activation of myelinated low-threshold afferent fibers can decrease the activity in ascending nociceptive pathways that is generated by small-diameter afferents. As such, the transmission of visceral nociceptive signals can be

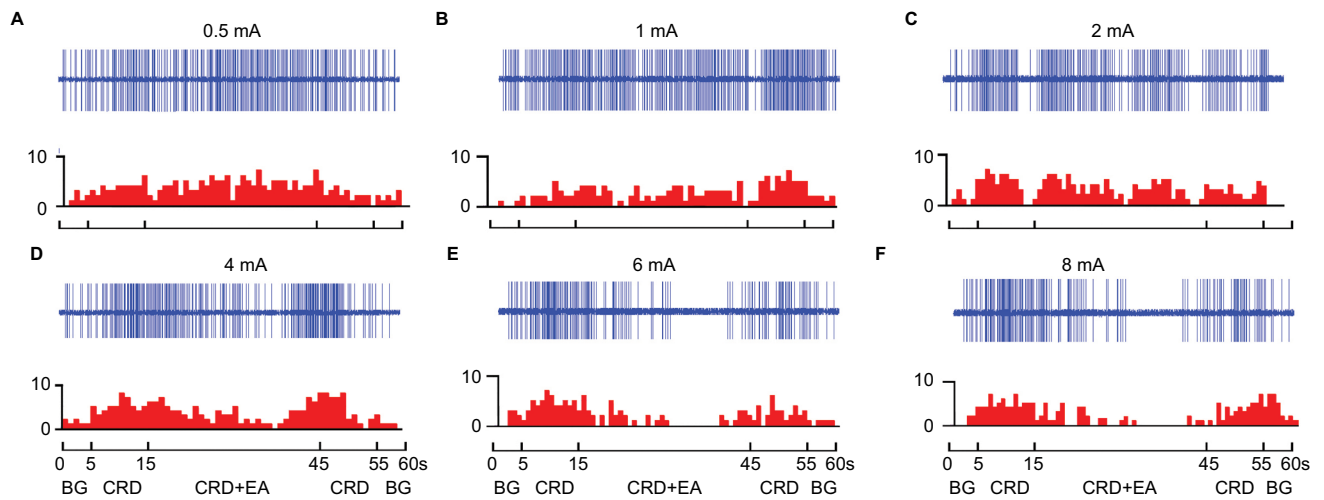


Figure 8 Examples of the response of WDR neurons to different intensities of EA at PC 6 during CRD.

Notes: EA at 0.5 mA (A), 1 mA (B), 2 mA (C), 4 mA (D), 6 mA (E), and 8 mA (F) was applied to PC 6 during CRD. A decrease in the discharges of WDR neurons during the CRD+EA sequence was observed within 2–8 mA. Top rows show the original unit discharges and bottom rows show the histograms.

Abbreviations: BG, background; CRD, colorectal distention; EA, electroacupuncture.

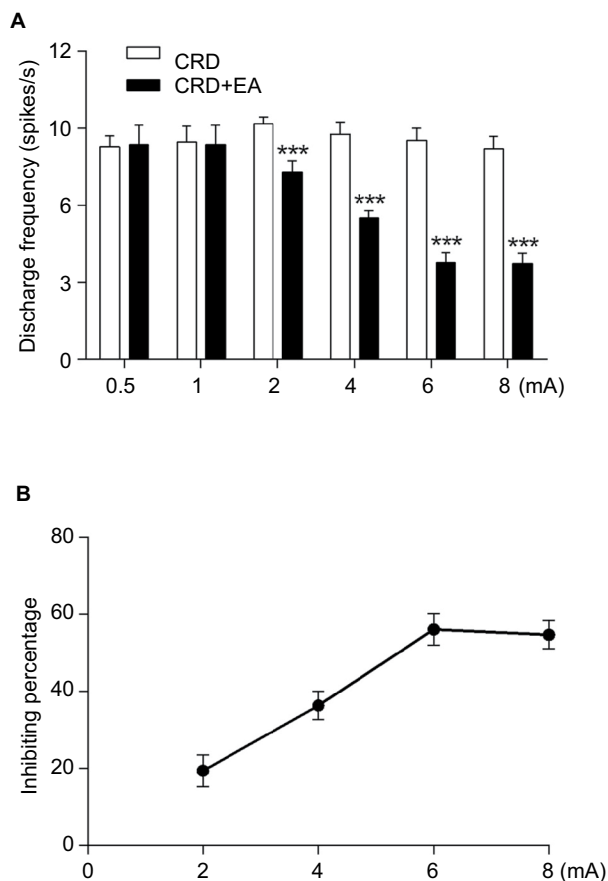


Figure 9 The effects of different intensities of EA at PC 6 on CRD-induced discharges of WDR neurons.

Notes: (A) A histogram shows that the noxious discharges of WDR neurons induced by CRD can be inhibited by EA in the range 2–8 mA. Data consist of the average spikes per second (mean \pm SEM). *** P <0.001, compared with CRD. (B) A stimulus–response curve shows that the inhibiting percentage increased as the intensity of EA increased with 2–6 mA.

Abbreviations: CRD, colorectal distention; EA, electroacupuncture; SEM, standard error of the mean; WDR, wide dynamic range.

depressed by local low-intensity stimulation. Moreover, other non-noxious stimulation, such as brushing sensitized skin, can also depress pain via gate control fibers.^{31,32}

The core of gate control theory is the segmental modulation of nociception. Our results indicate that the inhibiting effects of low-intensity EA for visceral nociception are localized to the segment of the stimulated acupoints. Similarly, previous studies have shown that the nociceptive reflex evoked by stimulating the sural nerve can be depressed by EA below the threshold of A δ -fiber activation applied at the ipsilateral local acupoints. However, heterotopic EA below the threshold of A δ -fiber activation was totally ineffective in inducing analgesia.^{8,9} In addition, the analgesic effects of local acupoints disappeared when A β -fiber function was blocked by snake venom.⁸ Consequently, low-intensity EA stimulation applied at local acupoints can inhibit nociceptive inputs at the spinal level, in which gate control may be involved and low-threshold A β -fibers are found to be crucial.

Extrasegmental inhibition on visceral nociception induced by high intensity of EA involved in diffuse noxious inhibitory control

When the intensity of EA increased to the noxious range (2 mA), the nociceptive responses of WDR neurons could be inhibited by EA at not only ST 36 but also PC 6. In other words, the extensive antinociceptive effects of EA on visceral nociception were observed on heterotopic acupoint PC 6. PC 6 is located within the medial forelimb and 3 mm above the

wrist and with different segmental innervation of the colon. It has been reported that acupuncture transcutaneous electrical stimulation of PC 6 is effective in rectal discomfort during barostat-induced rectal distension in human.³³ In addition, EA of PC 6 can suppress CRD-induced increases in mean arterial pressure, heart rate, and heart rate variability, thereby suggesting the beneficial effects of EA in relieving visceral pain and mediating autonomic nervous system function.³⁴ Furthermore, EA at PC 6 can improve the symptoms of colonic motility in irritable bowel syndrome rats.³⁵

Our results indicate that high-intensity EA could produce a more potent and extensive analgesia. This is in agreement with observations obtained on humans and animals, which show that EA stimulation within the noxious range can produce extrasegmental analgesia.^{9,36} In addition to the use of heterotopic EA stimulation for analgesia, a notable experiment revealed that heterotopic nociceptive conditioning stimulation applied to a given body location is also effective in reducing the perception of pain and response elicited by noxious test stimuli delivered at a remote body location.^{37–39}

Mechanisms underlying extrasegmental analgesia induced by heterotopic noxious stimulation involve diffuse noxious inhibitory controls (DNICs). DNIC refers to the observation that some neurons in the dorsal horn of the spinal cord and the trigeminal system are strongly inhibited when a nociceptive stimulus is applied to any part of the body. This is distinct from their excitatory receptive fields.^{40,41} Recently, researchers have hypothesized that DNIC is involved in the analgesic mechanism of acupuncture and moxibustion, as DNIC shares many properties with heterotopic EA-induced analgesia.⁴² First, DNIC was triggered only by the activation of A δ - and/or C-fibers, whereas non-noxious stimuli were completely ineffective.^{43,44} Similarly, extrasegmental analgesia can only be induced by EA exceeding the threshold of A δ - and C-fiber activation. In contrast, the analgesic effects of heterotopic acupoints decreased when the function of C-fibers was blocked by capsaicin pretreatment.⁸ Second, DNIC is a form of supraspinal descending endogenous analgesia, and the neuronal substrate for DNIC must involve supraspinal structures.^{45,46} Interestingly, we have previously observed that manual acupuncture could inhibit the nociceptive response of WDR neurons, but this effect was abolished by a blockade of the central descending pathway.¹⁴ This indicates that the effects of acupuncture on visceral nociception may not only be modulated by WDR neurons, but by many others too in the supraspinal center. Taken together, these data provide direct evidence for the involvement of DNIC in the antinociception of heterotopic EA stimulation, suggesting that EA at the

intensity of A δ - or/and C-fiber activation can trigger DNIC effects and produce extrasegmental analgesia.

Correlation between stimuli intensity and DNIC effects

Within the noxious range, different intensities of stimulation can trigger graded DNIC effects. Our results indicate that the nociceptive responses of WDR neurons could be inhibited by EA at both local acupoints and heterotopic acupoints when the intensity of EA increased to 2 mA. This demonstrates an obvious DNIC effect. Moreover, there was a positive correlation between current intensity and the degree of inhibition. The reaction characteristics of WDR neurons to EA closely match that of convergent neurons in the nucleus caudalis, recorded under similar experimental conditions.⁴⁷

The wind-up phenomenon can illustrate the reaction characteristics of WDR neurons to EA. It has previously been reported that many convergent neurons can encode the intensity of mechanical stimuli, especially within the noxious range.^{15,48,49} Wind-up is defined as a frequency-dependent facilitation to the response of convergent neurons evoked by repetitive stimulation of afferent C-fibers.^{50–52} After receiving repetitive stimulation of C-fibers, the plasticity of the neurons changes and the excitability increases. However, when the noxious inputs exceed a certain range, increased excitation within the brain cannot be elicited by noxious inputs.⁵³ The effective frequencies to elicit wind-up are in the range 0.5–2.0 Hz.^{54,55} The frequency of EA used in the present study is 2 Hz. In this condition, the excitability of the WDR neurons can be elicited by strong intensity EA stimulation. As such, within a certain range, the nociceptive response of WDR neurons was inhibited in an intensity-dependent fashion by EA stimulation.

However, it must be noted that if the electrical current is strong enough to excite C-fibers, EA treatment will inevitably cause unbearable pain in clinical practice. The present results show that when the intensity of EA exceeded 4–6 mA, the inhibiting effects of EA on visceral nociception reached a plateau. In fact, plateau saturation to the response of convergent neurons to the graded intensity of mechanical stimulation has already been reported.⁴⁸ The plateau effect on visceral nociception may be due to the saturation of the neuronal response to EA stimulation.

Limitations

One limitation of this study is that only 2.0 Hz EA was administered to rats, knowing that EA with lower or higher frequency may influence its analgesic effect. Thus, further studies are needed to explore the intensity-related effect of EA on visceral

pain under different frequencies in order to provide an optimal combination of intensity and frequency for visceral pain.

Conclusion

This study defined the relationship between current intensity and the effects of EA on visceral nociception. We demonstrated that visceral nociceptive afferents could be inhibited by EA at both local acupoints and heterotopic acupoints. At local acupoints, a positive relationship between current intensity and the extent of EA-induced analgesia was observed within 0.5–4 mA. In heterotopic acupoints, a positive relationship between current intensity and the extent of EA-induced analgesia was observed within 2–6 mA. However, further increases in the current beyond 4–6 mA resulted in both the segmental and extrasegmental analgesia induced by EA reaching a plateau. These data indicate that, within a certain range, the transmission of visceral nociception could be inhibited by EA in an intensity-dependent manner. Therefore, regulation of current intensity to optimal range may promote the antinociceptive effects of EA.

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Author contributions

LY, LL, and QQ performed the experiments; BZ and PR designed the experiments; LY and YZ analyzed the data; LY, WW, YY, and KL co-wrote the manuscript; All authors discussed the results and reviewed the final manuscript. All authors contributed to data analysis, drafting and revising the article, gave final approval of the version to be published and agree to be accountable for all aspects of the work.

Disclosure

The authors report no conflicts of interest in this work.

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