The Suppressive Effect of Electrical Stimulation on Nociceptive Responses in the Rat

Background and Purpose. The aim of this investigation was to study the effect of electrical stimulation on nociceptive responses within the lumbar levels of the rat spinal cord. Methods. A single high-energy thermal pulse produced by a surgical laser stimulator (5 W, 30 milliseconds) was applied on the plantar surface of the hind paws of male Sprague-Dawley rats. The spinal cord field potential evoked by the laser pulse was used as an indicator of thermosensitive nociceptive responses. Low-intensity single stimulation, high-intensity single stimulation, low-intensity train stimulation, and high-intensity train stimulation were applied on the common peroneal nerve with protected cuff electrodes in different trials. Results. Neither low-intensity nor high-intensity single stimulation suppressed field potentials. In contrast, low-intensity train stimulation elicited partial inhibition of field potentials. Furthermore, high-intensity train stimulation elicited biphasic inhibition at a wider range of intervals lasting for 20 seconds. Conclusion and Discussion. The results demonstrate that two modes of train electrical stimulation can produce two patterns of fast-onset (within milliseconds), short-duration (within 20 seconds) inhibition of field potentials in the spinal cord. These results provide evidence that noxious heat-related impulses are modulated by the presence of specific electrical stimulation. The clinical application of transcutaneous electrical nerve stimulation to block pain is supported. [Wang S-F, Chen Y-W, Shyu B-C. The suppressive effect of electrical stimulation on nociceptive responses in the rat. Phys Ther. 1997;97:839–847.]

Key Words: C fibers, Electrical nerve stimulation, Evoked potentials, Laser stimulation, Pain control, Transcutaneous electrical nerve stimulation.

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Electrical stimulation has been used to treat various types of painful conditions, including sciatica, headache, kidney stones, gout, hysteria, rheumatism, migraine, alcoholism, and neuralgia. Not until 1965, when Melzack and Wall proposed their gate control theory of pain, was a theoretical basis for the effect of transcutaneous stimulation in pain control provided. The gate control theory proposes that stimulation of the large caliber of myelinated fibers can inhibit the transmission of pain. Conventional (high-frequency, low-intensity) transcutaneous electrical nerve stimulation (TENS) was subsequently developed on the basis of this theory. The analgesic effect of high-intensity electrical stimulation may involve the release of endogenous morphine-like substances through the descending pain inhibitory pathway. Although the clinical effect of TENS in pain control remains controversial, a recent study indicates that interactions exist between physiological and psychological factors in patients treated with TENS. The question of whether TENS yields merely a placebo effect arises, and therefore the underlying neurophysiological mechanism of electrical stimulation-induced analgesia warrants further characterization.

Studies of cats with spinal cords transected at the C2–3 level have demonstrated that electrical stimulation can produce an inhibitory effect on the flexion reflex lasting for 10 to 100 seconds. This spinal inhibitory effect is resistant to naloxone. In anesthetized animals with intact spinal cords, electrical stimulation has been shown to produce a sustained inhibitory effect on the flexion reflex and neuronal activities of spinothalamic tract cells lasting up to 20 minutes, which can be reversed by naloxone. Spinal and supraspinal pathways can be activated by electrical stimulation. We believe, therefore, that spinal and supraspinal mechanisms should be examined separately to assess the mechanisms of electrical stimulation. The focus of this study was on spinal mechanisms.

High-intensity electrical stimulation, which activates both A-delta and C fibers, has been used as the source of pain in previous studies on analgesia induced by electrical stimulation. The intensity of the electrical current used in activating C fibers, however, simultaneously activates other sensory fibers and interacts with the activity of C fibers. To overcome this technical problem, high-power laser stimulation has been used to selectively stimulate A-delta and C fibers and produce fast and slow pain in humans with minimal activation of other large myelinated fibers. In clinical situations, high-power laser stimulation has been used in subjects in experiments and in patients for quantitative measurements of pain. Furthermore, in studies using animals, high-power laser stimulation can selectively activate non-myelinated C fibers and induce the firing of wide-dynamic-range (WDR) nociceptive neurons. The frequency of the defensive response, such as the withdrawal reflex, in anesthetized rats correlates with the energy output of applied high-power laser stimulation. Therefore, high-power laser stimulation appears to be an effective method for pain assessment.

Our study was designed to evaluate the suppressive effect of electrical stimulation acting on C-fiber activities selectively induced by laser stimulation in a spinal animal model. Single high-power laser pulses were used as test stimuli, and laser-evoked field potentials (LEFPs) were used for nociceptive measurements. Because patients with chronic pain who were treated with TENS preferred modulated stimulation modes such as frequency modulation and burst mode rather than conventional continuous stimulation, four basic units of electrical stimulation—two modes of single stimulation and two modes of train stimulation with varying intensity and frequency—were tested for their immediate effects at the spinal cord level. The effectiveness of electrical stimulation in reducing the LEFPs would provide support for the concept of using TENS to block pain.

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Spinal Cord LEFP Recording
Laser Stimulation
Electrical Stimulation
Common Peroneal Nerve
Saphenous Nerve
Sciotic Nerve
Plantar Nerve
Figure 1.
Experimental setup for stimulation and recording. Inset: Typical recording of laser-evoked field potential (LEFP).

Method

Animal Preparation
A total of 25 rats (Sprague-Dawley, male, 250–300 g) were anesthetized initially with ketamine (38 mg/kg) and Rompun* (23 mg/kg) administered intraperitoneally and supplemented as needed to maintain anesthesia. A tube (PE240) was inserted into the trachea for artificial respiration. Jugular vein catheterization was subsequently used for anesthesia and fluid infusion. Body temperature was maintained at approximately 37°C by a homeothermic blanket system. The end-tidal carbon dioxide concentration was monitored and maintained at about 3.5% to 4%. The rats were decerebrated at the intercollicular level, and their spinal cord was transected at the T8–9 level. The injection of anesthetics was discontinued following the decerebration procedure. Laminctomy was performed between T-13 and L-2, and the flap of the back skin was fixed in the frame of a spinal unit device to form a paraffin pool. The dura mater was dissected under the operating microscope so that the dorsal column and dorsal root were exposed. Both the dorsal column and the dorsal root were subsequently covered by paraffin oil. The common peroneal nerve was exposed, and a protective cuff electrode was inserted to wrap around the exposed nerve trunk. The experimental design is shown in Figure 1. The electrodes were extended by two fine wires, which were connected to the stimulator. This type of electrode can provide better contact with the nerve over an extended period.

Electrical Stimulation
Four different modes of electrical stimulation were used to test their effects on LEFPs (Fig. 2). These four modes of electrical stimulation were (1) low-intensity single stimulation (L-S), (2) high-intensity single stimulation (H-S), (3) low-intensity train stimulation (L-T), and (4) high-intensity train stimulation (H-T). The intensity was set at 5 times the threshold of the stimulated nerve for low intensity. For high intensity, 100 to 200 times the threshold of the stimulated nerve was used. The threshold was defined as the minimal intensity of electrical stimulation needed to evoke a detectable potential recorded from the surface of the spinal cord at the corresponding lumbar segment. For the L-T mode, the train duration was 1 second. For the H-T mode, the train duration was 5 seconds. A constant-stimulation isolation unit was used to provide square wave pulses, and the pulse duration was set at 0.5 milliseconds. Each of the four types of electrical stimulation preceded the laser stimulation at varied intervals (0, 100, 250, 500, and 1,000 milliseconds; Fig. 2). In the control condition, no electrical stimulation was delivered preceding the LEFP.

Laser Stimulation
A model 20 CH Carbon Dioxide Surgical Laser System† was used to stimulate the pad of a hindfoot of each rat. This carbon dioxide laser generates a laser radiation beam in the infrared spectrum at a wavelength of 10.6 μm. Laser stimulation was set at an intensity of 5 W for a duration of 30 milliseconds. At this intensity, no tissue damage was visible following exposure of the laser beam with a distance of 1 cm between the footpad and the

* Bayer AG, D-5090 Leverkusen, Bayerwerk, Germany.
† Direct Energy Inc, 3 Morgan, Irvine, CA 92718.
Drug Test
To test the involvement of the endogenous opioid in mediation of the effects, naloxone (0.2 mg), a morphine-specific antagonist, was injected intravenously 2 minutes before the condition-test procedure in one animal. Both field potentials and neuronal activities evoked by the laser pulse were evaluated.

Data Analysis
The analog signals of the LEFPs were transmitted to personal computer-based data acquisition system for online analog-to-digital conversion (sampling rate of 500/s with a Metabyte DAS-16F AD/DA interface card) and digital analysis (with Quick Basic Language). Signals were stored on hard disk for off-line data analysis in a personal computer-based data analysis system. The peak-to-peak amplitudes of the LEFPs were measured during offline data analysis. The control recording was averaged from two events of laser stimulation. Six to 12 single electrical stimulation-laser stimulation trials with varying intervals (0–200 milliseconds for L-S and H-S modes, 0–500 milliseconds for L-T mode, and 0–30 seconds for H-T mode) were recorded for each animal. A repeated-measures one-way analysis of variance was used for statistical analysis, using SYSTAT software. The maximal peak amplitude of LEFP was the dependent variable. The significance level was set at $P<.05$. Signals of the laser-evoked neuronal unit activities were sent to the X-Y plotter.

Results
Laser-evoked Field Potentials
Single laser pulses (duration = 30 milliseconds, intensity = 5 W) applied to the footpad can evoke a prominent field potential recorded on the surface of the dorsal column at the L4–5 level. The LEFPs contained one negative wave and one positive wave. The mean peak latency of the negative wave was 214.30 milliseconds (SD = 51.75). The mean peak latency of the positive wave was 343.33 milliseconds (SD = 59.76). The mean peak amplitude of the positive wave was 0.33 mV (SD = 0.13). The negative wave was small in amplitude and varied. Therefore, it was not included in the data analysis. A typical recording is shown in Figure 1 (inset).

Figure 3.
Temporal effect of low-intensity single stimulation on laser-evoked field potentials. X-axis represents the intervals of electrical stimulation-laser stimulation (in milliseconds). Y-axis represents the response percentage of change of the laser-evoked field potentials after electrical stimulation compared with the control value (100%).
**Effect of L-S Mode of Electrical Stimulation**

Low-intensity single stimulation demonstrated a minimal suppressive effect on LEFPs. The peak-to-peak amplitude of the LEFP at the 0-millisecond interval decreased to about 80% compared with the amplitude in the control condition. The control condition consisted of the LEFP recorded during laser stimulation without prior electrical stimulation. This mild suppressive effect gradually disappeared as the intervals increased. At all of the intervals tested with this paradigm, the preceding single electrical stimulation showed no effects on the LEFP (Fig. 3; n=5, F=0.15, P=.99).

**Effect of H-S Mode of Electrical Stimulation**

High-intensity single stimulation slightly suppressed the LEFP at an interval of 0 to 500 milliseconds. This suppressive effect was mild and about 70% of the amplitude in the control condition (Fig. 4), and it was not statistically significant (n=5, F=1.79, P=.131).

**Effect of L-T Mode of Electrical Stimulation**

After electrical stimulation with a train of low intensity (five times the threshold, frequency=100 Hz) for 1 second, the LEFPs were partially suppressed (Fig. 5). At the interval of 0 milliseconds, the amplitude of the evoked potentials was suppressed to about 60% of the amplitude of the control condition. The suppression gradually decreased as the electrical stimulation-laser stimulation intervals increased. When the interval was as long as 2,000 milliseconds, the amplitude of the evoked potentials became almost the same as the amplitude in the control condition.

**Effect of H-T Mode of Electrical Stimulation**

High-intensity train stimulation produced dramatically suppressive effects on the LEFPs. The amplitude of the evoked potentials was suppressed to approximately 30% to 50% of the amplitude in the control condition. These suppressive effects lasted for a few seconds (Fig. 6). When the intervals were within 20,000 milliseconds, there was a suppressive effect. The amplitude was approximately 50% of the amplitude in the control condition. At an interval of 1,000 milliseconds, a further suppression of the amplitude was noted. The amplitude was only about 30% of the amplitude in the control condition (Fig. 6).

**Discussion and Conclusions**

**Laser-evoked Neuronal Activity**

The laser-evoked neuronal activity of nociceptive neurons recorded in one rat had the same latency as that of the negative wave of the LEFPs (Fig. 7). Furthermore, the laser-evoked neuronal activity discharged for several hundred milliseconds. The higher discharge rate of neuronal activity corresponded to the negative evoked potential and the rising phase of the positive evoked potential. These highly correlated latencies of neuronal activities and field potentials support the idea that the field potentials are mostly, if not all, contributed from the activities of nociceptive neurons. While simultaneously recording the laser-evoked dorsal column potentials and laser-evoked neuronal activity, both responses were found to be partially inhibited in the L-T mode and dramatically inhibited in the H-T mode (Fig. 7). Similar results were attained with two additional animals.

**Effect of Naloxone on LEFP and Neuronal Activities**

To test for the possible involvement of endogenous opioids in mediating the suppression, naloxone (0.2 mg), an antagonist of morphine, was administered intravenously to one animal before the trials. The inhibitory effects of electrical stimulation on LEFP and neuronal activity were not reversed by naloxone (Fig. 8).

In our study, electrical stimulation suppressed LEFPs and nociceptive neural activity; stronger stimulation...
Intervals of Electrical Stimulation-Laser Stimulation

Applying laser stimulation, however, we were able to demonstrate in our study different patterns of suppression in the spinal cord. The L-T mode of electrical stimulation immediately produced a mild and partial suppression. In contrast, the H-T mode of electrical stimulation produced a biphasic suppression, an immediate and dramatic suppression at the interval of 1,000 milliseconds. The L-T mode with a stimulus intensity of 5 times that of the threshold may activate only larger myelinated fibers, whereas the H-T mode with 100 to 200 times the threshold may activate both large myelinated and nonmyelinated fibers. These results suggest that stimulating larger or small fibers can activate different pathways to suppress the neuronal activity in the spinal cord. This new finding may also be attributed to our measurement of only heat-sensitive nociceptive responses, which are specifically activated by laser pulses.

In contrast to electrical stimulation, which was used exclusively in previous studies,17-21 laser stimulation selectively activates the heat-sensitive nociceptors.43 Therefore, the use of laser stimulation is more physiological and specific in producing noxious responses. Because field potentials reflect a large population of neural activity, they cannot represent specifically the nociceptive activity. The activation of C fibers may also activate interneurons and motoneurons. In comparison with LEFPs, recording of laser-induced single neurons is a direct measurement of the neural activity during noxious stimulation. Simultaneous recordings of field potentials and single neuron activity yield the same results and, therefore, reinforce the importance of this suppressive effect.

Train stimulation appears to be important in activating different inhibitory pathways. Some evidence indicates that activation of intracellular pathways, such as Ca2+ channels and exocytotic mechanisms, is frequency dependent.46 Different frequencies of TENS applied to human subjects for 20 minutes have been shown to produced greater inhibition. Even with quite strong stimulation (200 times the threshold), the suppression was short-lived (from several milliseconds to 20 seconds) and could not be reversed by naloxone. This result is consistent with the results of a study by Shin et al.17 Our study and the study by Shin et al demonstrated that a short period of stimulation induced a fast-onset, naloxone-reversible inhibition in the spinal cord. In
produce differential release of neurochemical substances.17

The intensity of the stimulation appears to play a role in suppressing LEFPs. High-intensity electrical stimulation activates all fibers, whereas low-intensity electrical stimulation selectively activates only larger myelinated fibers. The activation of large fibers can modulate the activity induced by C fibers.2 The suppressive effects of the L-T mode of electrical stimulation correlates with the phenomenon of counterirritation, whereas the suppressive effects of the H-T mode of electrical stimulation support the concept of controlling pain by producing pain. This pain-induced analgesia is assumed to be produced by the release of endogenous morphine-like substances through the activation of the descending inhibitory pathways.3-5 In our experiment with the rat transected spinal cord, the recording from the spinal segments was still suppressed by strong stimulation. These findings have clinical implications in applying peripheral stimulation to modify pain segmentally.

Our results partially support the first mechanism of electrical stimulation described by Low and Reed.48 High-frequency, low-intensity electrical pulses (ie, traditional TENS) have a pain gate effect on C (slow) fibers in the dorsal horn due to stimulation of mechanoreceptors (A-beta fibers). Furthermore, our results demonstrate that single electrical stimulation itself is able to produce suppression. In our study, the H-S mode of electrical stimulation induced a suppressive effect. The H-T mode of electrical stimulation can produce further suppression. This phenomenon can be explained by the second mechanism of electrical stimulation described by Low and Reed,48 which states that low-frequency, high-intensity electrical pulses (ie, acupuncture TENS) stimulate A-delta fibers. These same authors hypothesized that the stimulation of A-delta fibers produces a morphine-like effect due to enkephalin release by interneurons in the dorsal horn. In our study, the suppressive effect of H-T electrical stimulation could not be reversed by naloxone, an antagonist of morphine. This finding is not consistent with the hypothesis that the H-T mode of electrical stimulation produces a morphine-like suppressive effect.

Four other possible spinal mechanisms may account for this suppressive effect in spinal cord. Decreases in axonal excitability,49,50 presynaptic and postsynaptic inhibition, and the release of adenosine and monoaminergic sub-
stances may also be involved in the mechanisms of spinal inhibition.

Electrical stimulation applied to the peroneal nerve of rats with transected spinal cords in our study demonstrated partial and strong inhibition of the nociceptive neuronal response. These results and the findings from previous animal studies provide neurophysiological evidence that noxious heat-related impulses are modulated by the presence of specific electrical stimulation. Furthermore, the existence of two separate pathways in the spinal cord for pain relief may explain why patients treated with TENS prefer modulated stimulation modes, such as frequency modulation and burst mode, rather than conventional continuous stimulation. The modulated modes may activate different pathways of analgesia by modulating the stimulation intensity and frequency. We believe that our experimental design eliminates effects arising from a supraspinal center. The observed inhibitory effect mainly resulted from intrinsic spinal mechanisms. Removal of the supraspinal descending inhibition may enhance spinal activity. In place of transcutaneous delivery of electrical stimulation (e.g., TENS), however, we used electrodes placed directly on the nerve. Clinical devices such as those that are used to deliver interventional current, with its higher frequency (4,000 Hz), can produce pulses that penetrate and stimulate deep structures or nerves. These devices may act by similar inhibitory mechanisms to yield the nerve-stimulation suppressive effect observed in our study. Further clinical trials to compare the effects of the cutaneous stimulation (e.g., TENS) and the effects of stimulation of deep tissues (e.g., interventional current) are warranted.

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