Modulation of Noxious and Non-Noxious Spinal Mechanical Transmission From the Rostral Medial Medulla in the Rat

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Zhuo, M., and G. F. Gebhart. Modulation of noxious and non-noxious spinal mechanical transmission from the rostral medial medulla in the rat. J Neurophysiol 88: 2928–2941, 2002; 10.1152/jn.00005.2002. Modulatory influences on spinal mechanical transmission from the rostral medial medulla (RMM) were studied. Noxious stimulation, produced by von Frey-like monofilaments, and non-noxious stimulation, produced by a soft brush, was applied to the glabrous skin of the hind foot. At 28 sites in RMM, electrical stimulation facilitated responses to noxious mechanical stimulation at low intensities (5–25 μA) and inhibited responses of the same neurons at greater intensities (50–100 μA) of stimulation. At 24 and 9 other sites in RMM, stimulation at all intensities only inhibited or only facilitated, respectively, responses to noxious mechanical stimulation of the hind foot. Stimulus-response functions to mechanical stimulation were shifted leftward by low intensities and decreased by high intensities of stimulation. Inhibitory influences were found to descend in the dorsolateral funiculi; facilitatory effects were contained in the ventral spinal cord. Descending modulation of non-noxious brush stimulation revealed biphasic facilitatory-inhibitory effects (9 sites in RMM), only inhibitory effects (14 sites) and only facilitatory effects (8 sites). The effects of electrical stimulation were replicated by intra-RMM administration of glutamate; a low concentration (0.25 nmol) facilitated and a greater concentration (2.5 nmol) inhibited spinal mechanical transmission, providing evidence that cells in RMM are sufficient to engage descending influences. Descending modulatory effects were specific for the site of stimulation, not for the spinal neuron, because modulation of the same neuron was different from different sites in RMM. These results show that spinal mechanical transmission, both noxious and non-noxious, is subject to descending influences, including facilitatory influences that may contribute to exaggerated responses to peripheral stimuli in some chronic pain states.

Introduction

Spinal nociceptive transmission is subject to descending modulatory influences from supraspinal sites (see Fields and Basbaum 1999; Mason 2001; Sandkühler 1996 for recent overviews). Inhibitory modulation is most commonly studied and is generally represented as selective for spinal nociceptive transmission (as opposed to non-nociceptive transmission). For example, inhibitory modulation from the periaqueductal gray (PAG) or rostral medial medulla (RMM) has been reported to be relatively selective for nociceptive responses of spinal dorsal horn neurons, including spinothalamic tract cells (e.g., Bennett and Mayer 1979; Duggan and Giersmith 1979; Fields et al. 1977; Oliveras et al. 1974a), although some have reported inhibitory effects on non-nociceptive spinal input (e.g., Carstens et al. 1981; Du et al. 1984; Gray and Dostrovsky 1983; McCreaery et al. 1979).

It is also appreciated that descending modulatory influences can enhance or facilitate spinal nociceptive transmission (e.g., Haber et al. 1980; McCreaery et al. 1979; Zhuo and Gebhart 1992, 1997). The RMM has been well documented to contribute both inhibitory and facilitatory influences on spinal nociceptive transmission. Importantly, it has been shown that chemical activation of cell bodies in the RMM, and not only fibers of passage that also would be affected by electrical stimulation, produce inhibitory and/or facilitatory effects on spinal nociceptive transmission (e.g., Drower and Hammond 1988; Helmchen et al. 1995; Thomas et al. 1995; Urban and Gebhart 1997; Urban and Smith 1994; Zhuo and Gebhart 1990, 1992, 1997). Most studies have employed thermal stimulation as the peripheral noxious stimulus. Fewer studies (e.g., Gerhart et al. 1981; Gray and Dostrovsky 1983; Haber et al. 1980; Kajander et al. 1984; McCreaery et al. 1979) have studied the effects of stimulation in the RMM on spinal transmission of cutaneous mechanical input. McCreaery et al. (1979) and Haber et al. (1980) noted excitatory effects of electrical stimulation in RMM on spinal mechanical transmission but did not investigate biphasic or bidirectional influences from RMM and were uncertain whether effects were produced by excitation of cells in RMM. The present study was thus undertaken to evaluate parametrically the effects of electrical and chemical stimulation in the RMM on noxious and non-noxious spinal mechanical transmission.

Methods

Animals

Adult, male Sprague-Dawley albino rats (Biolab., St. Paul, MN) weighing 270–450 g were used in experiments. Rats were anesthetized with an intraperitoneal injection of pentobarbital sodium (45 mg/kg; Nembutal, Abbott Laboratories, Abbott Park, IL), and catheters were inserted into the trachea for mechanical ventilation, into a femoral vein for intravenous administration of drugs, and into a femoral artery for the measurement of arterial blood pressure and heart rate. All wound margins were covered with a local anesthetic ointment. The Animal Care and Use Committee, The University of Iowa approved the research protocol.

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The lumbar spinal cord was exposed by laminectomy between T13 and L1. Rats were suspended by vertebral clamps rostral and caudal to the laminectomy, and a pool for agar (1.75% in saline) was made to minimize respiratory movements of the spinal cord. The head of the rat was fixed in a stereotaxic apparatus, and a hind foot was placed in a paraffin wax model with the plantar surface upward.

During the recording session, rats were paralyzed with pancuronium bromide (0.4 mg initially and 0.2 mg/h intravenously thereafter) and mechanically ventilated. Anesthesia was maintained by inhalation of a gaseous mixture of nitrous oxide and oxygen (2:1) and a continuous intravenous infusion of pentobarbital sodium (5–10 mg · kg^−1 · h^−1). Arterial blood pressure and heart rate were recorded continuously throughout the experiments, and body temperature was maintained at 37 ± 0.5°C with a water-circulating heating pad and overhead lamps. Tungsten microelectrodes (Micro Probe, Clarksburg, MD; 0.8–0.95 MΩ) were used for extracellular recording of single neurons in the L4–L5 spinal segments.

Peripheral stimulation
Mechanical stimulation (touch, pressure, and pinch) applied to the glabrous skin of the plantar surface of the ipsilateral hind foot was used to search for spinal units. Isolated units, continuously monitored by analog delay, were subsequently tested for responses to non-noxious brush, noxious pressure stimuli, and noxious heat. BRUSH. A reproducible non-noxious mechanical stimulus was produced using an instrument (B413 Tactile Stimulator, Bioengineering, The University of Iowa) that moved a soft camera lens brush (5 × 10 mm) across the skin at rate of 1 cm/2.2 s. Each stimulus consisted of one continuous forward and reverse excursion of the brush across the glabrous skin of the hind foot within the receptive field of the unit. Baseline responses of spinal units to brush of the skin were defined as the mean of three consecutive measurements at 3-min intervals.

PRESSURE. Von-Frey-like stimulation with nylon monofilaments (Semmes-Weinstein Anesthesiometer; Stoelting, Chicago, IL) was applied within the receptive field of the unit. Filaments of different thickness, requiring different pressures to bow the filament (28.8–125.9 g), were applied for 10 s at 3-min intervals. These intensities of stimulation were considered in the noxious range based on stimulus duration and hindpaw withdrawal in rats. Baseline responses of spinal units to pressure was the mean of three consecutive measurements at 3-min intervals.

HEAT. Radiant heat from a 50-W projector lamp (50°C, 15 s) was focused on the glabrous skin within the receptive field. A copper-constantan thermocouple (ANSI type T, 0.13 mm diam, Omega Engineering, Stamford, CT) placed in the center of the field of heat stimulation allowed for feedback control of temperature at the air-skin interface. Heat stimuli were given at 3-min intervals; this resulted in stable spinal unit responses over the course of an experiment (e.g., Zhuo and Gebhart 1992, 1997). Baseline responses of spinal units to noxious heating was the mean of three consecutive measurements taken at 3-min intervals.

Focal electrical brain stimulation
Focal electrical brain stimulation, 5–100 μA, consisted of continuous 100-Hz constant current cathodal pulses of 100-μs duration. Brain stimulation was started 5 s before and continued during peripheral stimulation of the skin of the hind foot. Monopolar stimulating electrodes (34-gauge; 0.15 mm diam), guided stereotaxically in the vertical plane (incisor bar at +3.3 mm) (Paxinos and Watson 1986), were inserted into the brain through a 26-gauge (0.45 mm OD) guide cannula. The electrodes were cut to extend 2 mm beyond the tip of the guide cannula. Typically, an electrode was lowered to a site in the RMM, and the effect of stimulation on spinal sensory transmission was tested as described in the preceding text before advancing the electrode 0.5 mm and repeating the procedure; typically, two sites in one electrode track were tested in each experiment.

Glutamate microinjection
Three-barrel glass micropipette electrodes were constructed with tip diameter of 15–40 μm. Barrels contained 10 or 100 mM L-glutamate in saline, or saline (control), or a tungsten microelectrode (Micro Probe; 0.8–0.95 MΩ). A pneumatic picupump was used to inject small volumes of L-glutamate. One or more short-duration (10–50 ms) pressure (10–25 psi) pulses to a pipette barrel was employed to administer glutamate or saline into the RMM. Injection volumes were measured directly by monitoring the movement of the fluid meniscus in a pipette barrel with aid of a ×120 compound microscope equipped with a fine reticle. The volume of glutamate was determined by the distance the fluid meniscus was moved by pressure and the inside diameter of the pipette barrel (i.e., volume = distance × inside area). Before glutamate microinjection, electrical stimulation at the same site on responses of spinal units to brush, pressure, or noxious heating of the skin was always tested.

Spinal dorsolateral funiculus (DLF) transection
In some experiments, the cervical spinal cord was also exposed. To transect the DLF, a small pledget of Gelfoam soaked in 1% lidocaine was applied briefly to the cervical spinal cord. The ipsilateral and/or contralateral DLF was then cut using a pair of fine scissors. A reversible fall in arterial blood pressure was produced by DLF transection: all measurements were made only when arterial blood pressure recovered to near the pretreatment baseline (30–60 min later).

Ventrolateral funiculus (VLF) lidocaine microinjections
In some experiments, two 26-gauge guide cannulas, 2 mm apart, were advanced into the cervical spinal cord (C1–C3) in the coronal plane to penetrate the pia matter. Microinjection of lidocaine (4%, 0.5 μl) was made into the ipsilateral and/or contralateral VLF(s) through a 33-gauge (0.20 mm, OD) injection cannula inserted through and extending 2 mm beyond the end of the 26-gauge guide cannula. Injection of lidocaine was done by an electrically driven syringe pump at a speed of 0.5 μl/1.5 min. Progress of the microinjection was continuously monitored by following the movement of an air bubble in a length of calibrated tubing between the syringe and the cannula. This procedure produced a reversible functional blockade in the ventral part of spinal cord (Jones and Gebhart 1987; Sandkühler et al. 1987; Zhuo and Gebhart 1997).

Histology
At the end of experiments, rats were killed with an intravenous overdose of pentobarbital sodium. Anodal electrolytic lesions were made in the brain stem and spinal cord to mark the sites of stimulation and spinal cord recording as well as lidocaine microinjection. The brain, lumbar, and cervical regions of the spinal cord were removed and fixed in 10% Formalin, frozen and cut in 40 μm coronal sections, mounted on glass slides and stained with cresyl-violet. The extent of transection of the DLF(s) was reconstructed.

Data and statistics
Spontaneous activity of spinal dorsal horn neurons was counted during the first 5 s of the period of analysis (before brain stimulation or stimulation of the skin was started). Responses to mechanical pressure, brush, or noxious heating of the skin were counted during stimulus application and are presented as total number of impulses (or as a percentage of that number) minus baseline activity. Data are presented as means ± SE. Statistical comparisons were made using
either one- or two-way ANOVAs (Newman-Keuls test for post hoc comparison). Student’s t-test was applied for comparisons between paired groups. In all cases, $P < 0.05$ was considered significant.

RESULTS

Unit sample

A total of 42 units recorded in the lumbar spinal cord of 29 rats were studied. All units responded to noxious and non-noxious mechanical stimulation. Thirty-two units responded only to mechanical stimuli (non-noxious brush and noxious pressure), and 10 units responded to non-noxious brush, noxious mechanical pressure, and noxious heating ($50^\circ$C) of the plantar surface of the glabrous skin of the hind foot. Accordingly, all units studied were wide dynamic range type, class 2 spinal units that respond to noxious and non-noxious stimuli. Microelectrode penetrations were made only to 1.2 mm below the dorsum of the spinal cord, and all units studied were histologically confined to dorsal laminae I–VI. The effects of electrical stimulation or glutamate microinjection into the RMM on spontaneous unit activity as well as unit responses to hind foot stimulation of the skin were studied.

Spinal nociceptive mechanical transmission

GENERAL. Both descending facilitatory and inhibitory effects of electrical stimulation on responses of spinal units to noxious pressure (28.8–125.9 g) applied to the skin of the hind foot were observed. At 24 of a total 61 sites in the RMM, electrical stimulation produced intensity-dependent inhibition of spinal unit response to noxious pressure of the skin. At 28 of the remaining 37 sites in the RMM, electrical stimulation produced biphasic effects, facilitating responses to noxious pressure at lesser intensities (5–25 $\mu$A) and inhibiting responses of the same spinal units at greater intensities (50–100 $\mu$A) of stimulation. At the nine remaining sites in the RMM, electrical stimulation at all intensities tested (5–100 $\mu$A) only facilitated responses of spinal units to noxious pressure applied to the skin. The effects of stimulation at 57/61 sites are described below; four sites were located outside the areas of principal interest.

BIPHASIC EFFECTS. Most sites of stimulation were located in n. gigantocellularis (NGC; $n = 19$), five sites of stimulation were located in n. gigantocellularis pars alpha (NGCa), and three were located in n. raphe magnus (NRM). An example of stimulation-produced biphasic modulation is given in Fig. 1A. In this example, the response to 28.8 g pressure during 25 $\mu$A electrical stimulation in the NGC is 162.4% of the control response (440 total imp/20 s) while stimulation at 50 $\mu$A attenuated the response to 78.1% of control (239 total imp/20 s). Figure 2A summarizes effects of stimulation at 27 sites in RMM; electrical stimulation at 10 $\mu$A produced a significant mean 130.5 ± 4.6% facilitation of responses to noxious pressure, whereas 50 $\mu$A stimulation significantly reduced responses of the same units to a mean 70.8 ± 7.9% of control. The mean recruitment index for inhibition between 10 and 50 $\mu$A stimulation (% inhibition/20 $\mu$A increase in stimulation intensity) was 31.3 ± 3.7.

FACILITATORY EFFECTS. At nine sites in the RMM (5 in NGC, 1 in NGCa, and 3 in NRM), stimulation at all intensities tested (10–100 $\mu$A) only enhanced responses to noxious pressure to a mean 131.9 ± 11.6–186.6 ± 34.8% of control, respectively. Data are summarized in Fig. 2B.

Because low intensity stimulation facilitates responses from both biphasic and facilitatory sites of stimulation in RMM, we examined whether there were differences in effects produced by stimulation at sites in NGC ($n = 24$), NGCa ($n = 6$), and NRM ($n = 6$). At a mean stimulation intensity of 16.3 ± 3.8 $\mu$A, unit responses were facilitated to 146.5 ± 11.6, 127.8 ± 6.0, and 125.0 ± 9.6% of control from NGC, NGCa, and NRM, respectively [$F(2,68) = 0.94$]. Neither the magnitude of
facilitation produced nor intensity of stimulation (range: 15–25 μA) differed among these three areas.

INHIBITORY EFFECTS. An example of stimulation-produced inhibition is given in Fig. 1B; data from 21 experiments (11 in NGC, 6 in NGCα, 4 in NRM) are summarized in Fig. 2. Electrical stimulation (10–100 μA) only inhibited responses of spinal units to noxious pressure applied to the skin. As in the preceding text, we examined whether there were differences in effects produced at sites in NGC, NGCα, and NRM and noted none either in the magnitude of inhibition (to a mean 33.8 ± 6.9, 49.1 ± 10.4, and 52.8 ± 9.2% of control, respectively; F(2,36) = 0.03) or intensity of stimulation that produced significant inhibition (6.3 ± 9.1, 43.8 ± 4.2, and 40.0 ± 10.0 μA, respectively). We also compared inhibition produced at these 21 sites of stimulation with inhibitory effects produced at biphasic sites of stimulation. The estimated (extrapolated) mean threshold for stimulation-produced inhibition was 8.5 ± 2.5 μA, which is significantly less than the estimated threshold intensity of stimulation that produced inhibition from biphasic sites in the RMM (33.1 ± 3.1 μA). The mean recruitment index for inhibition (% inhibition/20 μA) from these 21 inhibitory sites was 26.9 ± 4.0, which did not significantly differ from the recruitment of inhibition from the biphasic sites of stimulation (31.3 ± 3.7% inhibition/20 μA). The inhibition produced by electrical stimulation at 50 μA, however, produced a significantly greater inhibition of responses (to a mean 39.2 ± 5.1%) than produced from biphasic sites of stimulation at the same intensity (mean: 70.8 ± 7.9%).

SPONTANEOUS ACTIVITY. At sites in the RMM where electrical stimulation biphasically modulated responses to noxious mechanical transmission, spontaneous activity of units was not significantly affected by electrical stimulation at the intensities tested (10–100 μA). Similarly, at sites in the RMM where electrical stimulation only inhibited or only facilitated responses to noxious pressure, spontaneous activity of units was not significantly affected.

INTENSITY CODING. Responses of spinal units to noxious mechanical pressure applied to the skin were positively accelerating functions in the intensity range tested (see Fig. 3, A and C). Stimulus-response functions (SRF) for seven spinal units

![Figure 2](image-url)  
**Fig. 2.** Summary of descending modulation of spinal nociceptive mechanical transmission from the RMM. A: responses to noxious pressure of the skin are represented as a percentage of the control response (total number of impulses in 10 s) against the intensity of stimulation for biphasic (n = 27), only inhibitory (n = 21), or only facilitatory (n = 9) effects. B: stimulation sites drawn on representative coronal brain sections; abbreviations as in Fig. 1. Some symbols are obscured.

![Figure 3](image-url)  
**Fig. 3.** Effects of stimulation in the RMM on stimulus-response functions (SRF) to graded pressure applied to the skin. A and C: SRFs of individual spinal neurons in the absence of stimulation in the RMM. Data are plotted as the response to pressure applied to the skin (total number of impulses in 10 s) against intensities of pressure. B and D: summary of effects of stimulation on SRFs of the units illustrated in A and C, respectively. Data are presented as mean response in the absence (●) and presence (○) of stimulation. In B, an inhibitory intensity of stimulation at sites illustrated in E (●) significantly reduced the slope of the SRF of the 7 units studied. In D, a facilitatory intensity of stimulation at sites illustrated in E (● and ○) shifted the SRF leftward.
and their modulation by stimulation at inhibitory intensities of stimulation are presented in Fig. 3, A and B, respectively. Electrical stimulation at a mean intensity of $47.9 \pm 11.9 \mu A$ significantly inhibited responses of these seven units to 75.9 g pressure applied to the skin to a mean 63.8 $\pm 7.4\%$ of control and significantly decreased the slope of the SRF without affecting the extrapolated threshold for response (Fig. 3B).

SRFs for seven spinal units and their modulation by stimulation at facilitatory intensities are presented in Fig. 3, C and D, respectively. Electrical stimulation at a mean intensity of $17.1 \pm 5.5 \mu A$ significantly facilitated responses of these seven spinal units to 75.9 g pressure of the skin to a mean $123.9 \pm 7.7\%$ of control and produced a parallel leftward shift of the SRF without changing its slope (Fig. 3D).

LATENCY TO EFFECT. The apparent latency to stimulation-produced facilitation and inhibition of spinal mechanical nociceptive transmission was determined by employing a cumulative sum technique (Ellaway 1978) and bin-by-bin analysis of unit responses (Gerhart et al. 1983). Electrical stimulation was given during a relatively stable rate of unit response (10-s duration) to noxious pressure of the skin. Unit activity for 500 ms before the onset of electrical stimulation was averaged to generate a reference baseline and the cumulative sum of unit activity 500 ms before and 1,500 ms after the onset of stimulation was plotted. The apparent latency to facilitatory or inhibitory effects was defined as the time from the onset of stimulation to the point when the cumulative sum of the histogram significantly deviated from the baseline (Fig. 4A). In eight experiments, the mean latency to inhibition of unit response to noxious pressure applied to the skin was determined to be $112.2 \pm 39.7$ ms (range, 19.7–339.5 ms; mean intensity of stimulation, 56.3 $\pm 3.8 \mu A$). In another five experiments, the apparent latency for facilitation of unit response to noxious pressure applied to the skin was determined to be $290.5 \pm 76.9$ ms (range, 125.0–511.0 ms; mean intensity of stimulation, 19.0 $\pm 7.8 \mu A$). The latency to facilitation was significantly longer than for inhibition.

SITe SPECIFICITY. To determine whether descending facilitatory and inhibitory influences from different sites in RMM similarly affect the same spinal unit, the effects of electrical stimulation at the same intensity (10 $\mu A$), but different sites in the RMM, were studied on the same spinal units. An electrode was lowered into RMM and the effect of stimulation at 10 $\mu A$ was characterized. The electrode was subsequently lowered 0.5 or 1.0 mm and the same intensity of stimulation tested on response of the same spinal unit to noxious pressure. Summary data are shown in Fig. 5, A and B. The scatter-diagram clearly shows that for each unit studied, the same intensity of stimulation at two different sites (connected by a line in 5B; mean distance between stimulation sites = $0.9 \pm 0.2$ mm) produced significantly different effects. At 10 stimulation sites (Fig. 5B, ○), electrical stimulation at 10 $\mu A$ produced an inhibitory effect or no effect on responses of spinal units to noxious pressure applied to the skin; the mean effect was inhibition to 71.8 $\pm 9.2\%$ of control (Fig. 5A, inset). At the second 10 sites of stimulation in the same experiments, electrical stimulation at the same intensity facilitated responses of the same spinal units to the same intensity of noxious pressure to a mean $126.8 \pm 4.8\%$ of control. The effects produced significantly differ.

GLUTAMATE MICROINJECTION. At sites where glutamate was microinjected, the effect of electrical stimulation on response of spinal units to noxious pressure applied to the skin was characterized first. At five sites in the RMM (4 in NGC, 1 in NGCa), electrical stimulation at 17.5 $\pm 4.3 \mu A$ increased responses of spinal units to noxious pressure to a mean $123.3 \pm 6.3\%$ of control and inhibited responses of the same units to 40.4 $\pm 17.0\%$ of control at greater intensities of stimulation (75.0 $\pm 14.4 \mu A$). Microinjection of glutamate (10 mM, 25 nl) produced a rapid onset (mean: $2.6 \pm 1.0$ min), short-lasting (5–10 min) facilitation of responses of spinal units.
to noxious pressure of the skin (mean 127.0 ± 6.3% of control; Fig. 6A). This glutamate-produced facilitation was reproducible. Responses to noxious pressure of the skin were increased to a mean 125.8 ± 5.8% of control by a second microinjection of glutamate into the same sites 15 min after the first microinjection of glutamate, a time by which responses of units returned to baseline. There was no significant difference between the magnitude of facilitation produced by the first and second microinjection of glutamate, indicating an effect by reversible activation of cell bodies. Microinjection of glutamate at a greater dose (100 mM, 25 nl) into the same five sites after responses to noxious pressure returned to baseline produced significant inhibitory effects on responses of the same units to noxious pressure of the skin (to a mean 63.6 ± 8.8% of control, n = 5; Fig. 6A).

**Spinal DLF transection.** To investigate the spinal pathway(s) mediating descending facilitation and/or inhibition from the RMM, ipsilateral first (n = 6), contralateral first (n = 3), and ultimately bilateral (n = 9) transections of the DLF were performed at the cervical level of the spinal cord. The spontaneous activity of units (n = 9) was not significantly affected by either a unilateral (ipsi- or contralateral) transection of the DLF or ultimately bilateral transection of the DLFs. Similarly, ipsilateral DLF transection (n = 6) did not significantly affect baseline responses of spinal units to noxious pressure of the skin; responses to noxious pressure were slightly increased from baseline (568 ± 92 total imp/20 s) to 716 ± 169 total imp/20 s (P > 0.05). Similar results were found after contralateral transection of the DLF (from 989 ± 149 total imp/20 s to 1,193 ± 117 total imp/20 s; P > 0.05) and ultimately bilateral transection of the DLFs (from 715 ± 117 to 869 ± 121 imp/20 s; P > 0.05).

In nine experiments (Fig. 7D, ○ and •), electrical stimulation at a mean 57.5 ± 9.2 μA produced inhibition of spinal mechanical nociceptive transmission (to 48.5 ± 7.0% of control; Fig. 7A). The inhibitory effect of electrical stimulation was attenuated by either an ipsilateral (n = 6) or contralateral (n = 3) DLF transection (Fig. 7A). Bilateral transections of the DLF completely abolished the inhibitory effect (to a mean 105.2 ± 9.4% of control; P < 0.01). In four of these nine experiments, stimulation-produced inhibition (to 40.6 ± 12.3% of control) was completely abolished by bilateral transection of
the DLFs and a facilitation of responses to noxious pressure became apparent (to 129.0 ± 12.0% of control) at the same
intensities of stimulation (56.3 ± 18.8 μA).

At nine sites in RMM (Fig. 7D, ● and ○), electrical stimulation at 24.4 ± 3.9 μA facilitated responses of units to noxious pressure (to a mean 119.2 ± 5.1% of control). An ipsilateral (n = 6) or contralateral (n = 3) DLF transection did not significantly affect stimulation-produced facilitation of spinal mechanical nociceptive transmission (Fig. 7B). Bilateral transections of the DLF (n = 9) also did not affect stimulation-produced facilitation of responses (120.5 ± 5.6% before vs. 149.3 ± 16.2% of control after DLF transections). Although not statistically significant, responses to noxious pressure tended to be greater after bilateral transection of the DLFs.

VENTRAL SPINAL CORD LIDOCAINE MICROINJECTIONS. In nine experiments, ipsilateral first (n = 6), contralateral first (n = 3) and ultimately bilateral, microinjections of lidocaine were made into the VLF in the cervical spinal cord. The spontaneous activity of spinal units was not significantly affected by either unilateral or bilateral microinjections of lidocaine. Baseline responses of spinal units to noxious pressure were increased (n = 2), decreased (n = 2), or not changed (n = 2) by ipsilateral lidocaine microinjection. Subsequent bilateral blockage of the VLF by lidocaine also did not significantly affect responses of spinal units to noxious pressure (283 ± 65 total imp/20 s vs. 453 ± 167 total imp/20 s; P > 0.0 5).

At six sites in the RMM, electrical stimulation at 10.8 ± 3.0 μA significantly facilitated spinal unit responses to noxious pressure (to a mean 145.5 ± 12.5% of control). Lidocaine microinjection into the ventral part of the spinal cord ipsilateral to the brain stem stimulation site completely abolished the facilitatory effect produced by electrical stimulation (mean: 145.5 ± 12.5% before vs. 88.5 ± 5.1% of control after lidocaine injection; P ≤ 0.01; Fig. 8A). In three experiments, lidocaine microinjection into the VLF contralateral to the brain stem stimulation site did not affect stimulation-produced facilitation in two experiments and abolished effects in one experiment. Further lidocaine microinjections into the ipsilateral VLF abolished the facilitatory effects of electrical stimulation in those two experiments. Electrical stimulation at greater intensities (mean: 75.0 ± 14.4 μA) at the same brain stem sites significantly inhibited responses of spinal units to noxious
Spinal non-nociceptive mechanical transmission was completely abolished. Descending inhibitory effects were not affected while descending facilitatory effects were completely abolished.

Spinal non-noceceptive mechanical transmission

GENERAL. Electrical stimulation in RMM produced both facilitatory and inhibitory effects on responses of spinal units to non-noxious brush of the skin of the hind foot. At 14 of 31 sites in the brain stem, electrical stimulation produced intensity-dependent inhibition of responses. At 9 of the remaining 17 sites in the brain stem, electrical stimulation produced biphasic effects, facilitating responses to brush at lesser intensities (<50 μA) and inhibiting responses of the same units at greater intensities (50–200 μA) of stimulation. At the remaining eight sites, electrical stimulation at all intensities tested (10–100 μA) only facilitated responses of spinal units to brush of the skin.

BIPHASIC EFFECTS. As summarized in Fig. 9A, 10 μA stimulation in NGC (n = 7) or NGCα (n = 2) produced a significant mean 136.9 ± 11.5% facilitation of responses to brush of the skin; 100 μA stimulation significantly reduced responses of the same units to a 62.6 ± 11.2% of control. The mean recruitment index for inhibition at these nine biphasic sites of stimulation (% inhibition/20 μA increase in stimulation intensity) was 29.7 ± 7.5.

FACILITATORY EFFECTS. An example of stimulation-produced facilitation of responses of a spinal unit to brush of the skin is given in Fig. 10. In this example, the response to brush of the skin during 10 or 50 μA stimulation was facilitated to 120 and 130% of control (125 total impulses), respectively. Stimulation at eight sites in the RMM (3 in NGC, 2 in NGCα, and 3 in NRM) produced only facilitation of responses to brush of the skin at all intensities of stimulation tested (10–100 μA). The data are summarized in Fig. 9A.

Because low-intensity stimulation facilitates responses from both biphasic and facilitatory sites in RMM, we examined whether there were differences in effects produced by stimulation at sites in NGC (n = 10) and NGCα (n = 4). Because no biphasic sites of stimulation were located in NRM and only three facilitatory sites were studied, data from NRM were incomplete. At mean stimulation intensities of 10.0 ± 4.9 and 15.0 ± 5.0 μA in NGC and NGCα, responses to brush of the skin were increased to 138.1 ± 10.9 and 119.2 ± 1.5% of control, respectively. There were no differences in either the magnitude of facilitation produced or the intensity of stimulation required.

INHIBITORY EFFECTS. At 14 sites in RMM (9 in NGC, 3 in NGCα, and 2 in NRM), responses of spinal units to brush of the skin were only inhibited by electrical stimulation at all intensities tested (10–100 μA). Data are summarized in Fig. 9A. The estimated mean threshold of stimulation for inhibition was 4.5 ± 2.5 μA, which was significantly less than the estimated threshold for inhibition of response from biphasic sites of stimulation (31.9 ± 5.9 μA). The inhibition of responses to brush produced by 50 μA electrical stimulation at inhibitory sites was significantly greater (to 67 ± 4.6% of control) than the inhibition produced from biphasic sites (mean: 96.3 ± 14.7%) at the same intensity of stimulation.
Meaningful comparisons between effects produced by stimulation in NGC, NGCα, and NRM are compromised by the limited number of sites (5) studied in NGCα and NRM. Stimulation in NGC (n = 9) at a mean intensity of 66.7 ± 8.3 μA inhibited responses to brush to 58.2 ± 8.7% of control.

SPONTANEOUS ACTIVITY. There were no effects on spontaneous activity produced by stimulation at biphasic, inhibitory, or facilitatory sites in the RMM.

LATENCY TO EFFECT. The apparent latencies to stimulation-produced facilitation and inhibition were determined as described in the preceding text. RMM stimulation was given during a relatively stable rate of unit response to non-noxious brush of the skin. The first 500-ms period of recording was used to generate a reference base, and the cumulative sum of unit activity 500 ms before and 1,500 ms during stimulation was plotted. The apparent mean latency for descending facilitation by electrical stimulation was determined to be 169.7 ± 26.2 ms (range, 22.0–306.7 ms; n = 11). There was no significant difference between the latencies to facilitation and inhibition of unit responses to non-noxious brush of the skin (Fig. 4, C and D).

SITE SPECIFICITY. To examine whether a spinal unit received both descending facilitatory and inhibitory influences from different sites in RMM, the effects of electrical stimulation at the same intensity, but different sites in the RMM, were studied on the same spinal units. The scatter diagram in Fig. 5C shows that for each unit studied, the same intensity of stimulation at different sites (connected by a line) in the RMM (mean distance between sites; 0.6 ± 0.1 mm) produced significantly different effects. At six stimulation sites (Fig. 5D, ▽), electrical stimulation at a mean 16.7 ± 6.7 μA produced inhibition or no effect on responses of spinal units to brush (to a mean 77.6 ± 7.5% of control). However, at the second, more ventral of the six sites, electrical stimulation at the same intensities facilitated responses of the same spinal units to brush to a mean 116.0 ± 3.7% of control (P < 0.001).

GLUTAMATE MICROINJECTION. The example in Fig. 11 shows that L-glutamate microinjection into a site in the RMM facilitated the response of a spinal unit to brush to 194% of control 1 min after glutamate administration. Data from five experiments are summarized in Fig. 6B. Electrical stimulation (10 μA) at five sites facilitated responses to brush to a mean 114.4 ± 4.8% of control and inhibited responses of the same units to 72.4 ± 6.8% of control at greater intensities of stimulation (66.3 ± 11.8 μA). Microinjection of glutamate (10 mM, 25 nl) into these biphasic sites significantly increased responses to brush of the skin to a mean 122.9 ± 7.6% of control. Glutamate-produced facilitatory effects were rapid in onset (mean 3.8 ± 1.0 min), short-lasting (≤10 min) and reproducible. Responses of spinal units to brush of the skin returned to 95.4 ± 3.4% of control when tested 10 min after administration of glutamate. The facilitatory effect of glutamate microinjection was blocked by lidocaine blockage of the ventral spinal cord (Fig. 8).
mate was tested again by a second glutamate microinjection into the same five sites. The facilitation produced by the second administration of glutamate was to $130.3 \pm 10.7\%$ of control, which was not significantly different from the facilitation produced by the first microinjection of glutamate. A third, greater dose of glutamate (100 mM; 25 nl) microinjected after responses to brush returned to preinjection baseline produced a significant attenuation of responses of the same units to brush of the skin (to a mean $71.4 \pm 11.4\%$ of control, $n = 5$; Fig. 5B).

**RMM MODULATION OF NOCICEPTIVE AND NON-NOCICEPTIVE TRANSMISSION.** We also compared descending influences on responses of the same nine spinal units to noxious pressure and non-noxious brush. Electrical stimulation in the NGC ($n = 7$), NGCα ($n = 1$), or NRM ($n = 1$) produced biphasic ($n = 7$) or only inhibitory ($n = 2$) effects on responses to noxious pressure applied to the skin. Responses of the same spinal units to non-noxious brush of the skin were biphasically modulated ($n = 4$), only facilitated ($n = 2$), only inhibited ($n = 2$), or not affected ($n = 1$) by the same intensities of stimulation (10–100
μA) in the same sites. At three of these nine sites, electrical stimulation biphasically modulated responses of the same three spinal units to both noxious pressure and non-noxious brush of the skin. However, at the other six sites, stimulation modulated responses of the same spinal units to noxious pressure or non-noxious brush differently.

In seven different experiments, stimulation-produced modulation of responses of the same spinal units to noxious heating (50°C) and noxious pressure of the skin were compared. Stimulation at four (NGC, 3; NGC,1) sites produced the same modulation (biphasic, n = 2; inhibitory, n = 2) of responses of the same units to 50°C heating and noxious pressure applied to the skin. At two other sites (1 each in NRM and NGC), responses of spinal units to 50°C skin heating were biphasically modulated (n = 1) or only inhibited (n = 1), whereas responses of the same spinal units to noxious pressure were only inhibited (n = 1) or only facilitated (n = 1), respectively. Stimulation at one site in the NGC inhibited responses of a spinal unit to 50°C skin heating and facilitated responses to noxious pressure (to 139% of control at 50 μA). For three of the same seven spinal units, responses to non-noxious brush were also studied. Responses of these three spinal units to noxious pressure were biphasically modulated (n = 2) or inhibited (n = 1) by stimulation, whereas responses of the same units to brush were only facilitated (n = 2) or not affected (n = 1).

**Discussion**

The present study demonstrates that electrical stimulation and glutamate microinjection in the RMM produce intensity-dependent biphasic (facilitatory and inhibitory) modulation of spinal mechanical transmission, both noxious and non-noxious. Stimulation-produced inhibition but not stimulation-produced facilitation was also intensity dependent for both noxious and non-noxious mechanical transmission. Both stimulation- and glutamate-produced effects were rapid in onset, short-lasting and reproducible. Descending modulatory effects were specific for the site of stimulation in the brain stem, not for the unit recorded in the spinal cord, because modulation of the same spinal units was shown to be different from different sites in the brain stem. The estimated latencies to effect significantly differed for facilitatory and inhibitory modulation of noxious mechanical spinal transmission and are conveyed in different spinal pathways. Descending inhibitory effects are contained in the DLFs while descending facilitatory effects are contained in the ventral part of the spinal cord.

**Descending inhibition of spinal nociceptive mechanical transmission**

Spinal nociceptive transmission is known to be subject to descending inhibitory influences from supraspinal structures, including the PAG, NRM, and NGC. Electrical stimulation in these supraspinal structures attenuates or completely inhibits animal behavioral responses to noxious mechanical stimulation of the skin (e.g.,Fields et al. 1977; Gray and Dostrovsky 1983; Haber et al. 1980; McCreery et al. 1979; Oliveras et al. 1974a; Willis et al. 1977; Yezierski 1990) or stimulation of afferent Aδ and C fibers (Gerhart et al. 1981, 1983; Haber et al. 1980; Willis et al. 1977). In these earlier investigations, only electrical stimulation, which nonselectively activates cell bodies and fibers of passage, was used. We found in the present study that glutamate microinjection replicated the effects of electrical stimulation, producing rapid onset, short-lasting inhibition of responses of spinal units to noxious pressure of the skin, revealing that activation of cell bodies in the RMM is sufficient to inhibit spinal mechanical nociceptive transmission. Stimulation-produced inhibition in the present study was selective for stimulus-evoked responses because spontaneous activity of units was not significantly affected by stimulation at the same intensities.

**Descending facilitation of spinal nociceptive transmission**

Descending facilitatory influences from supraspinal structures on spinal nociceptive mechanical transmission also have been noted. McCreery et al. (1979) reported that single pulse electrical stimulation in the NGC or NRM of the cat increased the excitability of STT neurons activated by sustained mechanical pressure applied to the skin (followed by prolonged suppression). Haber et al. (1980) reported that electrical stimulation in or near the NGC in the monkey produced excitatory effects on 4 of 41 (9.7%) wide dynamic range STT neurons. When all spinal neuron types are incorporated in the analysis, including five low-threshold STT cells, 9 of 57 (19%) were excited by NGC stimulation. The excitatory effect included increases in spontaneous activity and responses of spinal units to noxious mechanical stimulation of the skin. In another report, electrical stimulation in PAG or NRM (1 site each) facilitated responses of only 2 of 138 spinal dorsal horn neurons to noxious pinch of the skin in cats, but facilitation may have been obscured by the brain stem stimulation artifact (Gray and Dostrovsky 1983). In a study of spinomesencephalic tract cells in the cat, Yezierski (1990) reported that electrical stimulation at 25 of 32 sites (78%) in the brain stem, including the NGC, NRM, and n. reticularis magnocellularis, produced excitation followed by inhibition (n = 16) or only excitation (n = 9) of responses, including to noxious mechanical stimulation of the skin.

In the present study, electrical stimulation at 36 of 57 sites in the RMM (63%) produced biphasic (facilitatory and inhibitory, n = 27) or only facilitatory (n = 9) modulation of spinal nociceptive mechanical transmission. The low percentage of stimulus sites that produced facilitatory effects in earlier work can be explained by the predominant focus in those studies on inhibition and stimulating electrically at threshold or suprathreshold intensities to produce inhibition. Earlier studies either did not parametrically vary stimulation intensity or, if they did, did not test low intensities of stimulation. We have repeatedly noted that facilitatory effects on spinal nociceptive transmission are produced at lesser intensities of electrical stimulation (Zhuo and Gebhart 1992, 1997; Zhuo et al. 2002).

Similarly, low concentrations of glutamate have been demonstrated by us to reliably and reproducibly facilitate spinal nociceptive transmission (Zhuo and Gebhart 1992, 1997; Zhuo et al. 2002). In the present report, glutamate was shown to...
reproductively facilitate spinal mechanical nociceptive transmission. The effect of glutamate, like that of stimulation, appeared to be selective for stimulus-evoked responses because spontaneous activity of units was not affected by intra-RMM glutamate injection.

**Spinal pathways**

Descending influences traveling in the DLFs are generally considered responsible for inhibitory modulation of spinal nociceptive transmission from supraspinal structures, including the PAG and RMM (see Basbaum and Fields 1984; Gebhart and Randich 1990 for reviews). In the present study, descending inhibition of spinal nociceptive mechanical transmission was blocked by transection of the DLFs, consistent with previous results (Zhuo and Gebhart 1992, 1997). Further, removal of this descending inhibitory pathway uncovered descending facilitatory effects. Electrical stimulation at intensities that inhibited responses to noxious mechanical stimulation before bilateral DLF transections produced a modest enhancement of responses to the same stimulus after DLF transections. These results are in good agreement with our previous studies of modulation of noxious thermal stimulation (Zhuo and Gebhart 1990, 1992, 1997) and with the work of others (e.g., Jones and Gebhart 1987; McCleery et al. 1979; Mokha et al. 1986; Sandkühler et al. 1987; Willis et al. 1977). One interpretation of these outcomes is that descending inhibitory and facilitatory influences are simultaneously active/engaged in the RMM. We have previously suggested that this outcome likely reflects the presence of prepotent, tonic descending inhibition, which when removed by transection of the DLFs, permits expression of normally present, but overridden facilitatory influences.

Spinal pathways for descending facilitatory or excitatory modulation have been less well studied. We found here that stimulation-produced facilitation but not inhibition of spinal mechanical nociceptive transmission was blocked by reversible lidocaine-produced local anesthesia of the ventral part of spinal cord, suggesting that descending facilitation is primarily conveyed in the ventral spinal cord. This is consistent with previous results (Zhuo and Gebhart 1990, 1992, 1997) and related work in which we found that descending facilitatory influences on spinal neurons, whether produced directly in RMM (Urban and Gebhart 1997) or indirectly by activation of vagal afferent fibers (see Randich and Gebhart 1992), were confined to the ventral part of the spinal cord in the rat.

**Modulation of spinal nociceptive and non-nociceptive transmission**

**Inhibitory modulation.** Previous studies have addressed the selectivity of descending inhibitory influences on spinal nociceptive and non-nociceptive transmission. In studies of spinal wide dynamic range or class 2 dorsal horn neurons (including ascending tract neurons), it has been reported that responses of spinal units to noxious stimulation (e.g., pinch, squeeze, heating of the skin or stimulation of afferent Aδ and C fibers) are more effectively inhibited by electrical stimulation in the RMM or PAG in terms of percentage of units inhibited and/or the magnitude of inhibition produced (Beall et al. 1976; Carstens et al. 1981; Gebhart et al. 1981, 1983; Haber et al. 1980; Lovick and Wolkencroft 1979; Willis et al. 1977; Zhang et al. 1991). Preferential effects of supraspinal modulation for spinal nociceptive transmission are not always noted (e.g., Duggan and Grierson 1979; Oliveras et al. 1974a), and animals are reported sometimes to be hyperreceptive during stimulation-produced analgesia to non-noxious stimuli (e.g., Mayer et al. 1971; Oliveras et al. 1974a).

In comparing descending effects on different classes of spinal dorsal horn neurons, electrical or chemical (morphine) stimulation in the PAG or NRM has been reported to selectively inhibit responses of class 2 or class 3 spinal units to noxious stimulation whereas responses of class 1 spinal units to non-noxious stimulation was not affected (Bennett and Mayer 1979; Fields et al. 1977). Others report that both class 2 and class 1 spinal units are similarly modulated from both the midbrain and medulla, although the stimulation threshold required to inhibit responses of nociceptive neurons (including class 2 and 3) is generally lower than required to inhibit non-nociceptive neurons (class 1) (Dostrovsky et al. 1983; Gray and Dostrovsky 1983). In the present study, electrical and chemical stimulation in the RMM inhibited responses of spinal class 2 neurons to noxious pressure and non-noxious brush of the skin of the hind foot. The magnitude of inhibition produced by electrical stimulation was greater for noxious than non-noxious mechanical transmission. For example, 50 μA stimulation in RMM attenuated responses to noxious pressure to ≤40% of control whereas responses to non-noxious brush were attenuated to 67% of control. At comparable intensities of stimulation in NGC (63.6 and 66.7 μA), responses to noxious pressure were reduced to 34% of control and responses to brush were reduced to 58% of control, respectively. This is consistent with our previous experience studying inhibition of responses to noxious heating of the skin (Zhuo and Gebhart 1992, 1997) and is consistent with the reports cited in the preceding text. We did not study class 1 spinal neurons, but speculate that descending inhibitory influences on non-nociceptive spinal transmission are qualitatively the same but quantitatively different from descending inhibition of spinal nociceptive transmission.

**Facilitatory modulation.** Facilitation of responses of spinal dorsal horn neurons (including STT neurons) to non-noxious stimuli by electrical stimulation in supraspinal structures has been previously reported. Haber et al. (1980) reported that electrical stimulation in the NGC produced excitatory or mixed (excitatory and inhibitory) effects on three of five STT class 1 neurons. Stimulation in the NRM also facilitates responses of class 1 neurons to non-noxious stimuli (Fields et al. 1977). Recording intracellularly, Light et al. (1986) demonstrated that stimulation in the NRM (n = 22) or PAG (n = 4) produced an excitatory postsynaptic potential (EPSP) following an inhibitory postsynaptic potential (IPSP) in 31 of 46 (67%) dorsal horn neurons (class 1) recorded in spinal laminae I and II. Dubuisson and Wall (1979) reported that medullary raphe stimulation produced descending excitatory effects on responses of spinal dorsal horn laminae I and 2 neurons in the cat, including those only responding to non-noxious stimuli (brush and touch). It was demonstrated in the present study that spinal class 2 neurons are subject to descending facilitatory effects from the RMM. In 17 of 31 sites in RMM (55%), electrical stimulation produced biphasic modulation (n = 9) or only facilitatory (n = 8) effects on responses of spinal units to non-noxious brush of the skin. Such facilitatory effects were
reproduced by glutamate microinjection in the RMM, suggesting that facilitatory influences on spinal nociceptive and non-nociceptive transmission can arise from cells located in RMM.

**Significance**

Accumulating evidence suggests an important role for the RMM in the development and maintenance of exaggerated responses to peripheral stimuli after tissue injury (i.e., hyperalgesia and allodynia). Supraspinal contributions to hyperalgesia have been established in inflammatory, neurogenic, neuropathic, and illness-induced models of hyperalgesia (see Urban and Gebhart 1999 for review). In experiments where hyperalgesia has been established, spinal cord transection, intra-RMM lidocaine or ibotenic acid, and electrolytic lesions of the RMM all have been reported to reverse or block the hyperalgesia. In review of these studies, we (Urban and Gebhart 1999) concluded that the RMM plays a prominent role in mediating the development and maintenance of secondary hyperalgesia and hypothesized that facilitatory influences from the RMM were central to these findings. More recently, Porreca and colleagues have investigated the role of the RMM in the allodynia that characterizes a model of neuropathic pain produced by ligation of the L5 and L6 spinal nerves. Sun et al. (2001) established that the tactile allodynia, which develops after nerve ligation, is dependent on inputs to supraspinal sites. Porreca et al. (2001) subsequently documented that selective ablation of RMM cells that express the µ-opioid receptor can prevent the development or reverse established allodynia in this model of neuropathic pain. These data contribute to the growing appreciation that descending facilitatory influences likely underlie some chronic pain states. The present report documents that both nociceptive and non-nociceptive spinal mechanical transmission is subject to tonic facilitatory modulation from cells located in RMM, reinforcing their potential role in the exaggerated responses to peripheral stimuli that characterize some chronic pain states.

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