Responses of spinal dorsal horn neurons to foot movements in the rat with sprained ankle

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Acute ankle injuries are common problems and often lead to persistent pain. To investigate the underlying mechanism of ankle sprain pain, the response properties of spinal dorsal horn neurons were examined after ankle sprain. Acute ankle sprain was induced manually by overextending the ankle of a rat hind limb in a direction of plantarflexion and inversion. Weight bearing ratio (WBR) of the affected foot was used as an indicator of pain. Single unit activities of dorsal horn neurons in response to plantarflexion (PF) and inversion (Iv) of the foot or ankle compression (Cp) were recorded from the medial part of the deep dorsal horn, laminae IV-VI, in normal and ankle sprained rats. One day after ankle sprain, rats showed significantly reduced WBR on the affected foot and this reduction was partially restored by systemic morphine. The majority of deep dorsal horn neurons responded to a single stimulus modality. After ankle sprain, the mean evoked response rates were increased significantly and after-discharges were developed in recorded dorsal horn neurons. The ankle sprain-induced enhanced evoked responses were significantly reduced by morphine, which was reversed by naltrexone. The data indicate that movement-specific dorsal horn neuron responses were enhanced after ankle sprain in a morphine dependent manner, thus suggesting hyperactivity of dorsal horn neurons is an underlying mechanism of pain after ankle sprain.

Keywords: acute ankle sprain, dorsal horn neuron responses, foot movements, inflammatory pain, morphine effect
INTRODUCTION

Joint injuries of various causes can result in both persistent pain and locomotion disorder (Schaible et al. 2002; Schaible and Grubb 1993; Schaible and Schmidt 1988). Among joint injuries, ankle sprain is the most common musculoskeletal injury (Kannus and Renstrom 1991) and more than 25,000 patients require medical care for ankle sprain every day in the United States (Kannus and Renstrom 1991; Lynch and Renstrom 1999). The majority of them involves disruption of the lateral ligament complex (Garrick and Requa 1988) induced by excessive plantar flexion and inversion of the foot. A significant portion of patients who once had an ankle sprain suffer persistent pain and recurrent sprains (Anandacoomarasamy and Barnsley 2005; Kern-Steiner et al. 1999; Konradsen et al. 2002; Koo et al. 2002).

Despite the prevalence of ankle sprains, the detailed mechanism of ankle sprain pain has not been studied systematically. Thus, much of the understanding of ankle sprain pain relies on clinical descriptions and evidence from other joint disorders, such as joint inflammation. It is thus assumed that ankle sprain pain would be similar to the pain mechanism of inflammation. Persistent pain induced by joint inflammation has been studied extensively. Factors that initiate inflammation as well as chemicals released due to inflammation have shown to sensitize nociceptor terminals and thus lead to peripheral sensitization (McDougall and Larson 2006; Schaible et al. 2009; Stein et al. 2009). In addition, the enhanced response activity of the spinal dorsal horn neurons has been shown after administration of inflammatory agents, kaolin/carrageenan or Freud's adjuvant, into the joint capsule (Neugebauer et al. 1996; Neugebauer et al. 1995; Schaible 2004; Schaible et al. 2002; Schaible and Grubb 1993; Schaible and Schmidt 1988). Thus both peripheral and central sensitizations may be involved in persistent pain caused by joint inflammation.
Several animal models of musculoskeletal injury exist. These include carpal tunnel syndrome (Diao et al. 2005), muscle strain injuries (Song et al. 2004), Achilles tendon injuries (Huang et al. 2004; Messner et al. 1999) and lumbar strain by cyclic lumbar flexion (Claude et al. 2003). The most relevant animal model for the present study, however, is the rat ankle sprain pain model produced by manually overextending the lateral ankle ligaments (Koo et al. 2002). This model mainly affects joint ligaments in the hind limb with minor effects on cutaneous tissue and produces spontaneous pain and impairment of movement. Using this model of ankle sprain, the present study investigates the physiological responses of deep dorsal horn neurons that are evoked by various movements of the hind foot and compression applied to the ankle joint in normal and ankle sprained rats.

MATERIALS AND METHODS

Experimental animals

Adult male Sprague-Dawley rats (weighing 200–350 g) were purchased from Harlan Sprague-Dawley Co. (Houston, TX) and used for this study. Rats in the experimental group received acute ankle sprain. Normal naïve rats were used as controls for both behavioral testing and electrophysiological recordings. Among 80 ankle sprained rats, 32 were used for behavior testing and 48 for electrophysiological recordings. Animals were housed in plastic cages with soft bedding with free access to food and water under a 12/12 h reversed light-dark cycle (dark from 8 AM to 8 PM). All animals were acclimated for 7 days before any experiment. All experimental procedures are approved by the Institutional Animal Care and Use Committee of the University of Texas Medical Branch and are in compliance with the Guide for the Care and Use of Laboratory Animals by the National Institute of Health.
Ankle sprain induction

Under gas anesthesia (halothane 3% in air for induction and 1.5 ~ 2% for maintenance), ankle sprain was induced manually as described in a previous study (Koo et al. 2002). While holding the leg, the left hind foot was overextended repeatedly in the direction of simultaneous inversion and plantar flexion for 60 times during 1 min period with gradual increase of bending force. The same procedures were repeated one more time. At the end, the ankle could be rotated to a position of 180° inversion (paw facing completely upward). Anesthesia was discontinued and the rats recovered from anesthesia within 5-10 min and were returned to their cages.

Behavioral testing

To estimate the level of pain in the sprained ankle, the amount of weight bearing force on the sprained foot was measured one day before and after ankle sprain. The experimental time of one postoperative day was chosen because our previous study suggested that the level of pain after ankle sprain was the maximum at one postoperative day as judged by the degree of reduction in weight bearing force (Koo et al. 2002). On the day of behavioral testing, each rat was weighed and then allowed to walk through a long rectangular plastic tube (10 cm width, 10 cm height, and 60 cm length) with a scale (Acculab, Pocket pro 250-B, Newton, PA, USA) located midway down the tube that only underlay the right-hand half of the tube. Thus, when the rat walked in one direction the weight on the left foot could be determine and when the rat came down the other way, the weight placed on the right foot could be measured. The weight signals from the scale were fed into an oscilloscope and the weight bearing forces were calculated using a data acquisition system (CED 1401 plus with Spike 2 program, CED, UK). To obtain a valid weight bearing force of the foot, weight bearing forces were measured 6 times and the values were averaged for each measurement. The weight bearing ratio (WBR)
was calculated based on the following formula: \( WBR = \text{(weight bearing force of the designated foot/body weight)} \times 100. \)

Surgical preparation for in-vivo electrophysiological recording

Extracellular recordings were made from the dorsal horn neurons in 48 rats, one-day after ankle sprain, as well as in 40 normal rats. Rats were anaesthetized with urethane (i.p., 1.5 g/kg) and supplemental urethane (i.p., 200 mg/kg) was administered when a sign of low anesthesia level was detected. The depth of anesthesia was monitored by observing the heart rate, the end-tidal CO\(_2\) level, and the pupil size. The rat was artificially ventilated at a rate of 80-100 breaths/min after tracheotomy and the end tidal expiration CO\(_2\) level was maintained at 3.5-4.5%. The animal was paralyzed with an initial bolus of pancuronium bromide (i.v., 1 mg/kg) and maintained by continuous infusion (0.4–0.6 mg/kg/h). A laminectomy was done from T12 to L2 vertebrae to expose the L4-L6 spinal cord segments and rats were securely fixed in a stereotaxic frame. The dura of the exposed spinal cord was then removed and the vertebral column was held rigid by clamps. The spinal cord was continually bathed in a pool of warm mineral oil held by skin flaps. Throughout the experiment the core body temperature was monitored by a rectal thermal probe and maintained at 36.5-37 °C by a heating blanket equipped with an automatic feedback control unit. At the end of the experiment, the rats were euthanized with an injection of a lethal dose of urethane (3 g/kg, i.p.) and death was confirmed by opening the chest.

Recordings of spinal dorsal horn neurons

Extracellular recordings of dorsal horn neuron activities were made from the ipsilateral lumbar spinal cord in normal rats as well as ankle sprained rats at one postoperative day (the same time point as the behavioral test) when the sprain pain is the
maximal (Koo et al., 2002). Most recordings were made using carbon fiber CARBOSTAR-1 electrodes (0.4-0.8 MΩ; Kation Scientific, MN, USA), but in some cases, 3-barrel carbon fiber CARBOSTAR-3 electrodes (0.4-0.8 MΩ; Kation Scientific, MN, USA) were used to mark recording sites by injection of methylene blue dye. A microelectrode was lowered into the cord using an electronic micromanipulator. The depth of the recording site from the dorsal surface of the cord was measured on each recording. The activity recordings were fed into an amplifier (CYBERAMP 320, Axon Ins., USA) and then displayed on an oscilloscope. Only single unit activity was isolated by using a window discriminator (WPI, USA) and a Spike 2 program (version 4, CED, UK). Responses to mechanical stimuli applied to the foot were quantified by a data acquisition system (CED140l, CED, UK)

Mechanical stimulation of the ankle

The foot movements that are critical for weight bearing and affected most by ankle sprain are plantarflexion and inversion, thus these 2 movements were chosen to activate sensory afferents originating from deep tissue. Ankle joint compression was also used to activate deep nociceptive sensory receptors. For accurate measurement of ankle joint movement, a special device equipped with a potentiometer (Bourns, Mexico) was designed and patched to the plantar surface of rat hind paw. This device measures the angle of movement accurately when the foot was passively moved. To standardize the experimental procedures, the criteria of each movement were arbitrarily defined and applied in the same way for each stimulus. The resting position is defined when the tibiotalar-tarsometatarsal (TT-TM) angle was 90°. For plantarflexion stimulus, the foot was placed at the resting position where the TT-TM angle is 90° and then slowly plantar flexed to 190°, the maximum plantarflexed position. For inversion stimulus movement, the foot was rotated medially from the resting position (0° on horizontal plane) up to 120°. For each movement stimulus, the foot
was slowly moved from the starting position to the maximally moved position during the first 2 seconds, held at the maximally moved position for 12 seconds, and then moved back to the starting position during the last one second. The range of each movement was set where the joint showed appreciable resistance felt at the maximum point but did not induce tissue injury. For compression stimulus, the pressure was applied medio-laterally to the ankle by using a pair of large blunt forceps (20 cm long; contact area, 4 mm x 4 mm) equipped with strain gauges (Yu et al. 2002). Each compression stimulus started from 0 g intensity but quickly reached to 1500 g within 2 sec and then held at the maximum intensity for 13 sec and then quickly released. The intensity of each stimulus was calibrated and fed into the data acquisition system (CED1401, CED, UK) to synchronize with neuronal responses. Thus the total compression stimulus was applied for 15 sec.

**Experimental design for evoked neuronal response measurement**

Neurons showing evoked responses to plantarflexion, inversion movement of the foot or compression of the ankle were subjected to study. Neurons responding to cutaneous stimulation (brushing or squeezing of the foot skin) were excluded from the study. To establish reliable responses of each neuron, neuronal responses were recorded initially 2-3 times by repeating the same mechanical stimulus at 10-min intervals. When the responses to each stimulus produced no more than 10% variations from the original, they were considered as stable responses. When a neuron that responded to a specific stimulus modality was identified, the spontaneous background activity was recorded for 60 sec followed by the response recordings in response to a specific movement of the foot and then compression of the ankle. The response recordings were made 3 times with the same stimulus repeated 3 times and the average of the 3 recordings was used as the average response to that stimulus. If spontaneous activity is present, the evoked responses were calculated by subtracting the
background spontaneous activity. The recorded neurons were classified based on their responses to different mechanical stimuli; (1) plantarflexion (PF) neurons were those responding only to plantarflexion movement, (2) inversion (Iv) neurons were those responding only to inversion movement, (3) compression (Cp) neurons were those responding only to compression of the ankle joint. Neurons responding to multiple stimuli were rare and not included in the data analysis. Recordings of one or 2 neurons were made from each animal.

**Drug administration**

Morphine (2 or 5 mg/kg, morphine sulfate; Sigma, St Louis, MO) or naltrexone (10 mg/kg; Sigma, St Louis, MO) was dissolved in saline and given intraperitoneally (i.p.) at a volume of 1 ml/kg. The drugs were prepared immediately before each use. Control groups received the same volume of saline. In behavioral experiments, morphine effects (2 or 5 mg/kg, i.p.) were tested 60 min after injections (Fig. 1). In electrophysiological experiments, morphine (2 mg/kg, i.p.) was given immediately after control recordings and the dorsal horn neuron responses to morphine were recorded 30 min after morphine treatment. Immediately after, naltrexone (10 mg/kg, i.p.) was injected and the responses were recorded again at 30 min after the naltrexone treatment (60 min after morphine injection).

**Histology**

To determine the location of the recording site, methylene blue was iontophoretically injected (1~3 μA for 3 min) using a 3-barrel carbon fiber microelectrode (CARBOSTAR-3, Kation Scientific, MN, USA) immediately after the recordings from single neurons of 10 randomly selected rats (one neuron in each rat). The spinal cord was removed after perfusion with fixative containing 4% paraformaldehyde and then cryosectioned at 30 μm thickness,
mounted on slides and examined under a light microscope. The location of methylene blue injection sites was plotted from 10 rats (one site per rat, total 10 sites; Fig. 5A).

Data analysis and statistics

Statistical significance was determined using one-way repeated measure ANOVA with the Duncan’s multiple comparison post-hoc test for behavioral tests and unpaired student t-test for the others, using Sigmastat (ver. 3.0, SPSS, USA). All data are presented as the mean ± S.E. Values of p < 0.05 were considered significant.

RESULTS

Weight bearing force on the sprained ankle is reduced partly due to pain

Rats with a sprained ankle limped on the injured side and frequently tended to the sprained ankle by licking or biting, and showed an occasional irritable jump one day after ankle sprain. Postmortem examination showed that the manual ankle sprain manipulation produced grade I or II type of ankle sprain, which included over stretching and minor tears of lateral ankle ligaments: the calcaneofibular and anterior and posterior talofibular ligaments. In addition, several other ligaments, which connect various tarsal bones, such as calcaneocuboid, talonavicular, and talocalcaneal ligaments were also stretched. In addition, a minor hemorrhage on the extensor retinaculum and swelling of the ankle joint were found. This lateral ankle sprain without rupturing any lateral ankle ligaments is equivalent to ankle sprain grade I or II in humans (Cotler 1984).

To examine pain after sprain, stepping forces of the limbs during locomotion were analyzed before and after ankle sprain. Since the stepping forces of the forelimb were not
significantly changed after ankle sprain of hind limb or morphine treatment (Koo et al. 2002), detailed analysis was limited to the affected hind limb. Fig. 1A is an example of stepping force recordings of the forelimb and hind limb in a normal rat. Normal rats step on the forelimb (F) first followed by the hind limb (H). The peak stepping force of the hind limb is on average 64% of the body weight. One day after ankle sprain, weight bearing forces of the injured hind limb decreased to 27% of body weight (Fig. 1B & C).

To test whether the decreased weight bearing on the sprained foot is due to pain, the effect of an analgesic drug, morphine, on weight bearing forces was examined one day after ankle sprain. Systemic injection of morphine, 2 or 5 mg/kg, significantly improved the weight bearing forces of the affected foot (the maximum effect was observed at 1 hr after morphine; Fig. 1C). This suggests that the reduction of weight bearing force after ankle sprain is at least in part due to pain.

Dorsal horn neurons respond to deep afferent stimuli of the foot in a modality dependent manner

The evoked responses of the dorsal horn neurons that respond to foot movements or an ankle compression were recorded from normal rats and compared to that of ankle sprained rats. About 70% of 37 and 59 neurons recorded from normal and ankle sprained rats, respectively, showed background spontaneous activity of 1Hz or higher. However, the rates of individual units were highly variable and no consistent and significant changes after ankle sprain were found.

The majority of dorsal horn neurons responded only to one specific foot movement, and hence, these neurons were defined by the corresponding movement type. Thus 3 types of neurons were examined: plantarflexion (PF), inversion (Iv), and ankle compression (Cp) neurons.
Examples of evoked responses of plantarflexion (PF) neurons in a normal rat and a rat one day after ankle sprain are shown in Fig. 2A and 2B, respectively. In normal rats, PF neurons (n = 16) showed an increasing evoked activity during plantar flexion (from the TT-TM angle 90° to 190°) and the activity peaked when the foot was maximally plantarflexed. The response thresholds to PF were ranged from 96° to 135° (118.3 ± 4.6°). While holding the foot at the maximum PF position, the evoked responses were gradually decreased from the peak response but still maintained significantly high levels. The response activity was quickly stopped when the foot was returned to the basal position (Fig. 2A). One day after ankle sprain, PF neurons (n = 21) showed significantly enhanced peak and mean evoked responses as compared to that of normal rats. In addition, moderate levels of responses were sustained even after the foot was returned to the basal position, thus showing significant levels of after-discharges (Fig. 2B). The group data of these changes obtained from normal and ankle sprained rats is shown in Fig. 2C. The after-discharges were developed after ankle sprain as shown in Fig. 2D. However, a significant change in the response thresholds of the plantarflexion neurons could not be determined.

The properties of 11 inversion specific neurons were recorded from normal rats and 18 additional from one day post-ankle sprained rats, as shown in Figure 3. The dorsal horn neurons that responded only to inversion movement showed the average threshold angle of 33.8 ± 8.1º in normal control rats (Fig. 3A & 3C). One day after ankle sprain, the threshold angle was decreased to 15.2 ± 3.9º and the response rates were increased (Fig. 3B & 3C). The after-discharge rates were also increased after ankle sprain (Fig. 3D).

For compression responsiveness, activity recordings were made from 10 dorsal horn neurons of normal rats and 20 more from ankle sprained rats (Fig. 4). The compression intensity ranged from 0 to 1500 g. In ankle sprained rats, the evoked responses were significantly increased as compared to that of normal rats (Fig. 4A-4C). The response
activities completely ceased when the stimulation was removed in normal rats (Fig. 4A & Fig. 4D) whereas neurons in ankle sprained rats showed prominent after-discharges (Fig. 4B & 4D).

Neurons responding to ankle stimuli are located in the medial deep dorsal horn

The locations of 10 recording sites (4 from normal and 6 from ankle sprained rats) were identified by methylene blue injection at the end of the recordings and then recovered by a histological preparation. All recorded sites (all modalities) were located in the medial part of the deep dorsal horn (laminae IV-VII) (Fig. 5A). The depths of the recording sites ranged 772 – 1193 µm from the spinal cord surface of the L5 to L6 spinal cord.

Among 40 recorded cells in the normal rats, plantarflexion, inversion, and compression neurons were 16 (40%), 11 (27.5%), and 10 (25%), respectively. The remaining 3 cells were multi-responsive neurons (Fig. 5B). In ankle sprained rats, the proportion of each type neuron does not change, suggesting that phenotype of dorsal horn neurons do not change after ankle sprain (Fig. 5C, n=64).

Enhanced dorsal horn neuronal responses are sensitive to morphine

After acute ankle sprain, weight bearing forces (WBF) of the affected foot were decreased and these reduced WBF were partially restored with systemic morphine (Fig. 1). To see if enhanced responses of dorsal horn neurons to ankle stimulation after the sprain are sensitive to systemic morphine as well, 5 dorsal horn neurons (1 neuron per rat) were examined after ankle sprain. Of these 5 neurons, 3 were plantarflexion (PF) neurons and 2 were inversion (Iv) neurons. Fig. 6 shows an example of a PF and an Iv neuron to corresponding foot movement stimuli before and after morphine (2 mg/kg, i.p.), and after naltrexone injection. The evoked responses reduced after intraperitoneal injection of
morphine (2 mg/kg) and then recovered by injection of naltrexone (10 mg/kg, i.p.), an opioid receptor antagonist. Not only the evoked responses, but also the after-discharges were changed by opioid ligands, particularly in the PF unit. The data suggest that an enhanced part of dorsal horn responses may be related to nociceptive inputs.
DISCUSSION

The relationship between painful behaviors and dorsal horn neuron responses to foot movements is investigated in the rat after ankle sprain. The weight bearing ratios (WBR) of the affected foot are greatly reduced following ankle sprain and then recovered significantly by morphine. Extracellular recordings show that the majority of dorsal horn neurons respond exclusively to a specific foot movement and neurons responding to multiple inputs are rare. After ankle sprain, the evoked responses are increased in plantarflexion, inversion and compression responsive neurons. In addition, after-discharges were developed so that discharges continue for some time after the termination of these stimuli. These changes in dorsal horn neuronal responses to ankle movements likely underlie the reduced WBR following sprain of the ankle. Neurons recorded in this study were located in the medial part of the deep dorsal horn (laminae IV-VII) (Fig. 5A). Their locations are consistent with previously recorded neurons receiving nociceptive afferent inputs from the ankle joint or those that respond to flexion/extension of the ankle joint (Schaible and Ebersberger 2009; Schaible and Grubb 1993; Willis and Coggeshall 2004).

In recent years, roughly 25% of all sports-related injuries involve ankle sprain, which damage the lateral ankle ligament complex (Hume and Gerrard 1998; Puffer 2001). The lateral ankle ligament complex is composed of 3 ligaments in humans: the anterior and posterior talofibular ligaments and the calcaneofibular ligament. In human patients, ankle sprain is categorized into 3 grades based on the severity of injuries: ligaments are stretched without any tear (grade I), one or more ligaments are partially torn (grade II) or completely torn (grade III). It usually happens during an unanticipated hyper-plantarflexion in combination with hyper-inversion of the fixed foot with external rotation of the tibia (Cotler 1984; Lynch and Renstrom 1999; Safran et al. 1999). Typically, the lateral ankle ligament
injury is likely accompanied by injuries to other joint structures, such as the joint capsule, the subtalar ligament (Lynch and Renstrom 1999), the tibiofibular ligament (Safran et al. 1999), and the muscle tissues surrounding the ankle complex (Hertel 2000). By post-mortem autopsies, the extent of ligament injuries in our rat ankle sprain model was identified. The ligaments affected by sprain maneuvers include the calcaneocuboidal, the talonavicular, and the talocalcanean ligaments in addition to the major lateral ankle ligaments. Although the involved ligaments are elongated or partially torn in some cases and thus widen the gaps between bones (Koo et al. 2002), there is no sign of complete tear. Thus our ankle sprain model mimics the grade I or II of human ankle sprain (Cotler 1984). In addition, there was a minor hemorrhage on the extensor retinaculum and swelling of the ankle joint, thus indicating inflammation. These rats showed a significant recovery in 4 days after ankle sprain, thus the recovery rate is also comparable to that of grade I and II ankle sprained patients shown in clinical studies (Lynch and Renstrom 1999; Safran et al. 1999).

In this study, we grouped the dorsal horn neurons according to responding stimulus modality, such as plantarflexion, inversion and compression of the ankle. Our results indicate that >90% of the dorsal horn neurons responding to ankle stimulation show exclusively a specific stimulus modality and relative proportions of the populations did not change after ankle sprain. This seems to be consistent with the previous data in which 50% of primary afferent nerve fibers innervating the rat knee joint respond predominantly or exclusively in one directional knee movement (Just et al. 2000).

The most prominent changes in the responses of dorsal horn neurons after ankle sprain are a marked enhancement of the evoked responses to ankle stimulation and the development of prominent after-discharges. The enhancement of evoked responses suggests that dorsal horn neurons are hyperactive after ankle sprain. The exact cause of such hyperactivity, however, is not clear. One possibility is that dorsal horn neurons are known to
develop sensitization after receiving sustained noxious peripheral input and nociceptive input after sprain. Another possibility is that ankle sprain has likely caused nociceptor sensitization and sensitized nociceptors are hyperreactive to ankle stimulation, and then, hyperresponses of dorsal horn neurons could be secondary to enhanced evoked responses of nociceptors. However, the above two possibilities are not mutually exclusive and, in fact, it is likely that both factors are contributing to hyperactivity of dorsal horn neurons in this particular case. In conclusion, ankle sprain injury induced the reduction of weight bearing force on the affected foot and the enhancement of evoked activity as well as development of after-discharges in the dorsal horn neurons receiving afferent inputs from foot movement and ankle compression. The present study showed neuronal basis for pain associated with ankle sprain and described some response characteristics of dorsal horn neurons receiving inputs from the sprained ankle. A partial recovery of weight bearing force and a reduction of dorsal horn responsiveness after morphine treatment suggests that these changes may be related to pain after ankle sprain.

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**DISCLOSURES**
No conflicts of interest, financial or otherwise, are declared by the authors.

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Figure 1. Changes in weight bearing forces of the rat hind limb after ankle sprain and the effect of morphine treatment. A and B show examples of weight bearing force traces of the forelimb (F) and hind limb (H) in a normal rat and one day after ankle sprain, respectively. As shown in C, stepping the forelimb is followed by the hind limb in normal locomotion (N), and the stepping force of the hind limb is about 64% of the total body weight. One day after ankle sprain (Sp), the weight bearing ratio decreased to 27%. Morphine treatments (M2 = 2 mg/kg and M5 = 5 mg/kg of morphine, i.p.) induced a recovery of the weight bearing forces in a dose dependent manner. Saline treatment (S) had no effect. Value of ordinate was expressed as percent ratio of the stepping force to body weight. Values are mean ± S.E. Abscissa indicate animal groups. * indicates the value is significantly different (P<0.05) from the normal; # indicates the value is significantly different (P<0.05) from the sprained condition (Sp). Groups: N, normal (n=10); Sp, sprain (n=8); Sp+S, sprain + saline (n=8); Sp+M2, sprain + morphine 2 mg/kg (n=8); Sp+M5, sprain + morphine 5 mg/kg (n=8).

Figure 2. Response characteristics of spinal dorsal horn neurons to plantarflexion of the foot in normal and ankle sprained rats. Plantarflexion was initiated from the tibiotarsal-tarsometatarsal (TT-TM) angle 90° (baseline) and ended at 190°. The neurons responding exclusively to plantarflexion were recorded in normal (A) and sprained (B) rats. Insets show raw recordings of single spikes. C shows the mean evoked responses to plantarflexion and D shows after-discharges of the dorsal horn neurons recorded from normal (n=16) and one day after ankle sprained rats (n=21). Asterisks indicate values significantly difference from the normal value (*, P<0.05; **P<0.01).
Figure 3. Response characteristics of spinal dorsal horn neurons to inversion of the foot in normal and ankle sprained rats. Inversion was initiated at 0° on the horizontal plane and medially rotated up to 120°. The neurons responding exclusively to inversion were recorded in normal (A) and sprained (B) rats. Insets show raw recordings of single spikes. C shows the mean evoked responses to inversion and D shows after-discharges of the dorsal horn neurons recorded from normal (n=11) and one day after ankle sprained rats (n=18). Asterisks indicate values significantly different from the normal value (*P<0.05; **P<0.01).

Figure 4. Response characteristics of spinal dorsal horn neurons to compression of the ankle joint in normal and ankle sprained rats. Compression was applied from medial to lateral sides of the ankle with a pair of forceps equipped with compression force output reading. The neurons responding exclusively to compression were recorded in normal (A) and ankle sprained (B) rats. Insets show raw recordings of single spikes. C shows the mean evoked responses to compression and D shows after-discharges of the dorsal horn neurons recorded from normal (n=10) and one day after ankle sprained rats (n=20). Asterisks indicate values significantly different from the normal value (*P<0.05; **P<0.01).

Figure 5. The location of recorded neurons in the spinal cord and afferent modalities of the neurons receiving inputs from the ankle. A: A schematic drawing of the spinal cord showing locations of 10 recorded neurons. Each location was marked with an injection of methylene blue after the recording session and the marked spot was recovered by histological procedure. Marked spots are clustered in the medial deep laminae of the dorsal horn and neurons having different afferent modalities were mixed within the same region. B & C: Pie charts showing the proportion of recorded dorsal horn neurons that respond to plantarflexion (PF), inversion
(Iv), or compression (Cp) stimuli in normal (B, n=40) and ankle sprained rats (C, n=64). The proportion of afferent modalities is about the same in normal and ankle sprained rats.

Figure 6. Effects of morphine and naltrexone on the responses of dorsal horn neurons recorded one day after ankle sprain. A & B: One neuron each responding to plantarflexion (A) and inversion (B) is shown. Both morphine and naltrexone effects were tested 30 min after intraperitoneal injections. Two more PF and one more Iv neurons were tested with similar results. PF: plantarflexion; Iv: inversion.
Fig. 2.

A Normal

B Sprain

C Evoked response

D After-discharge
Fig. 3.

A Normal

B Sprain

C Evoked response

D After-discharge

Rates (imp/sec)

Normal Sprain
**Fig. 4.**

Panel A: Normal response showing a sharp decline in impulse rate with higher amplitudes.

Panel B: Sprain response showing a slower decline in impulse rate with lower amplitudes.

Panel C: Evoked response with a comparison between normal and sprain conditions.

Panel D: After-discharge with a comparison between normal and sprain conditions.

Legend: * and ** indicate statistical significance.
Fig. 5.
Fig. 6.

Control

Morphine

Naltrexone

$10 \text{s}$

$50 \text{ imp/sec}$

$100^\circ$

$50 \text{ imp/sec}$

$100^\circ$