Mechanical and Thermal Hyperalgesia and Ectopic Neuronal Discharge After Chronic Compression of Dorsal Root Ganglia

XUE-JUN SONG, SAN-JUE HU, KENNETH W. GREENQUIST, JUN-MING ZHANG, AND ROBERT H. LAMOTTE
Department of Anesthesiology, Yale University School of Medicine, New Haven, Connecticut 06520

Song, Xue-Jun, San-Jue Hu, Kenneth W. Greenquist, Jun-Ming Zhang, and Robert H. LaMotte. Mechanical and thermal hyperalgesia and ectopic neuronal discharge after chronic compression of dorsal root ganglia. J. Neurophysiol. 82: 3347–3358, 1999. Chronic compression of the dorsal root ganglion (CCD) was produced in adult rats by implanting a stainless steel rod unilaterally into the intervertebral foramen, one rod at L₃, and another at L₄. Two additional groups of rats received either a sham surgery or an acute injury consisting of a transient compression of the ganglion. Withdrawal of the hindpaw was used as evidence of a nocifensive response to mechanical and thermal stimulation of the plantar surface. In addition, extracellular electrophysiological recordings of spontaneous discharges were obtained from dorsal root fibers of formerly compressed ganglia using an in vitro nerve-DRG-dorsal root preparation. The mean threshold force of punctate indentation and the mean threshold temperature of heating required to elicit a 50% incidence of foot withdrawal ipsilateral to the CCD were significantly lower than preoperative values throughout the 35 days of postoperative testing. The number of foot withdrawals ipsilateral to the CCD during a 20-min contact with a temperature-controlled floor was significantly increased over preoperative values throughout postoperative testing when the floor was 4°C (hyperalgesia) and, to a lesser extent, when it was 30°C (spontaneous pain). Stroking the foot with a cotton wisp never elicited a reflex withdrawal before surgery but did so in most rats tested ipsilateral to the CCD during the first 2 postoperative weeks. In contrast, the CCD produced no changes in responses to mechanical or thermal stimuli on the contralateral foot. The sham operation and acute injury produced no change in behavior other than slight, mechanical hyperalgesia for ~1 day, ipsilateral to the acute injury. Ectopic spontaneous discharges generated within the chronically compressed ganglion and, occurring in the absence of blood-borne chemicals and without an intact sympathetic nervous system, were recorded from neurons with intact, conducting, myelinated or unmyelinated peripheral nerve fibers. The incidence of spontaneously active myelinated fibers was 8.61% for CCD rats versus 0.96% for previously nonsurgical rats. We hypothesize that a chronic compression of the dorsal root ganglion after certain injuries or diseases of the spine may produce, in neurons with intact axons, abnormal ectopic discharges that originate from the ganglion and potentially contribute to low back pain, sciatica, hyperalgesia, and tactile allodynia.

INTRODUCTION

Trauma, degenerative disorders and other diseases of the lumbar spine in humans can lead to chronic low back pain, sciatica, hyperalgesia, and other painful conditions of uncertain cause. Peripheral nociceptors may be chronically activated in injured tissue, and injured primary afferent neurons that retain their central connections may have development of ectopic discharges with potential nociceptive consequences. Conversely, a complete loss of afferent input from denervated tissue may give rise to pain of central origin (for review, Devor 1994).

Animal models of painful sequela in humans after nerve injury have provided behavioral evidence for ongoing pain and cutaneous hyperalgesia (Bennett and Xie 1988; Kim and Chung 1992; Seltzer et al. 1990). Electrophysiological recordings from primary sensory neurons with transected peripheral axons indicate that the somata in the dorsal root ganglion (DRG) can become hyperexcitable. DRG cells can become a source both of ectopic spontaneous discharges, in the absence of peripheral receptor activation, and abnormal activity, evoked by sympathetic stimulation and/or endogenous chemicals such as norepinephrine (e.g., Wall and Devor 1983; Xie et al. 1995). Such abnormal activity, if occurring in the appropriate nociceptive afferent neurons, may maintain a state of central sensitization of nociceptive neurons in the dorsal horn and, as a consequence, cause chronic pain and cutaneous hyperalgesia. Determination of the role of each functional class of afferents is problematic when axotomy has removed the injured neuron from its peripheral receptors. However, it may be possible to do this in an animal model of neuropathic pain in which ectopic spontaneous discharges and other abnormal neuronal properties develop in neurons that do not undergo axotomy. Such abnormal properties may develop after spinal injuries or disorders that mechanically or chemically affect the DRG without affecting conduction in the spinal nerve or root.

Recently, it was discovered that mild hyperalgesia to radiant heat develops in rats on the plantar surface of the foot after a chronic compression injury of the ipsilateral DRG (CCD model) produced by the implantation of a metal rod in the intervertebral foramen. After removal of the rod, ectopic discharges originating in the formerly compressed ganglion were electrophysiologically recorded in vivo from myelinated dorsal root fibers (Hu and Xiang 1998). This preparation provides a model of DRG compression in humans as a consequence of an acutely herniated lumbar disc, foraminal stenosis, tumors, or other injuries or diseases of the spine.

The purpose of the present study, in which a similar procedure was used to compress the DRG, was threefold. The first was to measure the withdrawal threshold to mechanical stimulation of the skin. The reason for this is that cutaneous hyperalgesia in response to mechanical stimuli is more common than the response to heat after nerve injury (Chaplan et al. 1994). The second was to control the temperature of the stimulus on the skin during measurements of
the withdrawal thresholds to heat in the rat. Although the
temperature of a heat stimulus to the skin is closely related
to the magnitude of evoked pain in humans (Hardy et al.
1952; LaMotte and Campbell 1978), the stimulus tempera-
ture is typically not controlled in behavioral measurements
of withdrawal to heat after nerve injury in the rat (e.g.,
Bennett and Xie 1988; Hu and Xing 1998; Kim and Chung
1992). The third purpose was to use an in vitro electrophys-
ilological recording method (Zhang et al. 1997b) to deter-
mine whether ectopic discharges due to DRG compression
persist in vitro in the absence of blood-borne chemicals and
a functioning sympathetic nervous system.

Preliminary results of the present study have been published
in abstract form (Song et al. 1997).

M E T H O D S

Surgical procedure

ANIMALS. Seventy adult, male, Sprague-Dawley rats weighing 200–
250 g were housed in groups of 3–4 in 40 × 60 × 30-cm plastic cages
with soft bedding under a 12:12 h day:night cycle. The rats were kept
5–7 days under these conditions before and up to 5 wk after surgery.

CCD. Forty rats were anesthetized with pentobarbital sodium (40
mg/kg, ip, supplemented as necessary). On the left side, the paraspinous
muscles were separated from the mammillary process and the trans-
verse process and intervertebral foramina of L4 and L5 exposed. A
fine, sharp, stainless steel needle, 0.4 mm in diameter with a right
angle to limit penetration (Fig. 1), was inserted approximately 4 mm
to the intervertebral foramen at L4, and again at L5, at a rostral
direction at an angle of 30–40° below the vertebral horizontal (Hu and Xing 1998). Once the
needle was withdrawn, a stainless steel rod, L-shaped, 4 mm in length,
and 0.63 mm in diameter, was implanted into each foramen, one at L4
and the other at the L5 ganglion. The purpose of compressing two
DRGs instead of one was to increase the number of compressed
neurons innervating the plantar surface of the hindpaw. Each insertion
was guided by the mammillary process and the transverse process
and oriented as described for the needle. As the rod was moved over
the ganglion, the ipsilateral hindleg muscles typically exhibited one or
two slight twitches. After the rod was in place, the muscle and skin
layers were sutured. An oral antibiotic, Augmentin, was administered
for 7 days.

ACUTE INJURY PRODUCED BY TRANSIENT ROD INSERTION. In six
rats, the surgical procedure was identical to that described, except that
the rod was temporarily inserted into each intervertebral foramen, left
in place for 5 s, and then withdrawn.

SHAM SURGERY. In eight rats, the surgical procedure was identical
to that described but without the needle stick and rod insertion.

Behavioral testing

The rats were tested on each of 3 successive days before surgery.
After surgery, the animals were inspected every 1 or 2 days during the
first 14 postoperative days and at weekly intervals thereafter. For
general observation, the rats were placed on a table, and notes were
made on each animal’s gait, the posture of each hindpaw, the condi-
tions of the hindpaw skin, and growth of nails.

FOOT WITHDRAWAL TO PUNCTATE MECHANICAL INDENTATION.
The incidence of foot withdrawal was measured in response to
mechanical indentation of the plantar surface of each hindpaw with
a sharp, punctate cylindrical probe. Mechanical stimuli were ap-
plicated with seven modified, Von Frey–type nylon filaments, each
differing in the bending force delivered (5, 10, 20, 40, 80, 120, and

160 mN) but each fitted with the same metal cylinder with a flat tip
and a fixed diameter of 0.1 mm (LaMotte et al. 1998). The rat was
placed on a metal mesh floor and covered with a transparent plastic
dome (20 × 25 × 15 cm). The animal rested quietly in this
situation after an initial few minutes of exploration. After 15
minutes, the test was begun. Each filament was applied from
underneath the metal mesh floor to the plantar surface of the foot.
Each filament was applied to 10 different spots spaced across
nearby the entire extent of the paw (Fig. 2A). The duration of each
stimulus was 1 s, in the absence of withdrawal, and the interstimu-
lus interval was 10–15 s. The filaments were given in order of
ascending force with a given filament delivered to each spot
alternatively from one paw to the other in sequence from the 1st to
the 10th spot. The incidence of foot withdrawal was expressed as
a percentage of the 10 applications of each stimulus as a function
of force. Measurements were taken on 3 successive days before
surgery on 12 and 6 rats respectively subjected to chronic and acute
compression and on 8 rats subjected to sham surgery. Postopera-
tive tests were made 1, 4, 7, 10, and 14 days after surgery and
weekly thereafter until 5 wk. Fewer measurements of mechanical
thresholds were made in rats tested with heat and cold. For these,
thresholds were obtained 1 day before surgery and again on post-
operative day 2. Rats used in the electrophysiological experiments
were similarly tested once before and once after the surgery but
received an additional test on the day of electrophysiological
recording.

FOOT WITHDRAWAL TO INNOCUOUS MECHANICAL STIMULI. Twelve
rats were tested for foot withdrawal to innocuous tactile stimulation 3
days before rod implantation and again on postoperative days 1, 4, 7,
10, 14, 21, 28, and 35. A wisp of cotton pulled up but still attached to
a cotton swab was stroked mediolaterally across the plantar surface
of the skin through the floor of the testing apparatus. Six strokes were
delivered to each foot, alternating between feet after each stroke. The
number of withdrawals was expressed as a percentage of the six
strokes for each foot.

FOOT WITHDRAWAL TO HEAT. Twelve rats were tested for foot
withdrawal in response to heat before and after rod implantation. The
heat stimuli, each of 5-s duration, were delivered to the plantar surface
of the hindpaw through the mesh floor by means of a temperature-
controlled contact thermode with a surface area of 2 × 3 mm

FIG. 1. Intended relationships between the positions of the implanted rods
and the dorsal root ganglion (DRG) and lumbar vertebrae. L-shaped rods were
inserted 4 mm into the L4 and L5 intervertebral foramina in a rostral direction
at an angle of 30–40°.
INDENTATION AND TO HEAT. The percentages of withdrawal for base temperature within shoot (C°C). The temperature cooled passively back to in the numerical order indicated in Fig. 2. Each heat stimulus had a stimulus temperature was applied every 25 s to each of six locations, thermocouple that was attached to the resistor and located at the desired value within (LaMotte et al. 1998). The stimulus temperature was maintained at a desired value within ±0.1°C by an electronic circuit that controlled the current to the resistor in relation to a temperature signal from a thermocouple that was attached to the resistor and located at the skin-thermoregulatory interface. Stimulus temperatures of 41, 43, 45, 47, and 49°C were each applied from a base temperature of 39°C in ascending order of temperature. Starting with the lowest temperature, each stimulus temperature was applied every 25 s to each of six locations, in the numerical order indicated in Fig. 2B. Each heat stimulus had a trapezoidal waveform with a ramp rate of 19°C/s and minimal overshoot (≤0.2°C; Fig. 2C). The temperature cooled passively back to base temperature within <5 s after the 49°C stimulus and sooner for stimuli of lesser temperature. The thermocouple was mounted to a load cell (Senosetek) so that the force between the thermocouple and the skin could be maintained between 150 and 250 mN (Fig. 2C). Once an adequate contact was made, the probe was held in place at the base temperature for 5 s, after which the test stimulus was delivered. The test stimulus was maintained for 5 s, unless a withdrawal occurred, at which point the thermocouple was brought away from the cage floor. The incidence of foot withdrawal was expressed as a percentage of the six applications of each stimulus as a function of temperature.

CALCULATION OF THE THRESHOLD WITHDRAWAL TO MECHANICAL INDENTATION AND TO HEAT. The percentages of withdrawal for each rat were plotted as functions of the forces and temperatures delivered. To estimate the threshold value at a 50% withdrawal level, a logistic linear transformation, logit (P) = ln(p/q), was obtained, where p is the percentage of withdrawals for each stimulus and, q = 1 – p (LaMotte and Mountcastle 1975). Logit (P) plotted against stimulus intensity (x) was approximately linear. The best estimate of the line was a linear regression function L = Ax + B, with A as the slope and B as the intercept. The threshold was defined as the x value at the 50% level (when L = 0) and estimated by −B/A. For an observation of P = 0, or P = 100%, the corresponding logit is infinite. If the curve reached an asymptote at either of these values, all values beyond the first 100% and below the first 0% were eliminated, and a substitution made of 1/2n and (2n – 1)/2n for the last and first instances of 0 and 100%, respectively (Berkson 1953), where n is the number of trials. The accuracy of threshold estimates obtained by the logistic transformation was indicated by a covariance coefficient of R² > 0.85 with most R² values obtained >0.9, thereby representing linear relationships between L and x.

FOOT WITHDRAWAL TO NEUTRAL, COOL, AND COLD TEMPERATURE STIMULATION. Eight rats were tested for foot withdrawals after they were placed on surfaces of neutral, cool, or cold temperature. The rat was placed on a temperature-controlled aluminum plate and confined beneath an inverted, transparent, plastic cage with dimensions of 20 × 25 × 15 cm. The floor was cooled to 4°C or 21°C by a temperature-controlled water bath or warmed to 30°C by an electric blanket that maintained the stated temperature through feedback from a thermistor in contact with the floor. After a few minutes of exploration, the rats became quiescent. After 5–10 min of adaptation, the number of withdrawals and the cumulative time that the rat held its foot off the floor were recorded during a period of 20 min. Foot lifts associated with locomotion or body repositioning were not counted. Measurements were taken on days 3, 2, and 1 before surgery; 1, 4, and 7 days after surgery; and then once weekly for 5 wk. The sequence of plate temperatures on each day of testing was 30, 21, and 4°C, each separated by a 30-min interval in the home cage.

MEASUREMENT OF SKIN TEMPERATURE. The rat was anesthetized with a half dose of pentobarbital sodium (25 mg/kg, ip) and placed on its abdomen on a table. The skin temperature of the plantar surface of each hindpaw was measured for 12 rats before rod implantation and again on the 7th postoperative day. A thermocouple (model Bat-12, Physiotech) was taped on the surface of the hindpaw and stable temperature readings recorded within 20–30 s.

FIG. 2. Locations of test sites for application of Von Frey filaments and the thermode to the plantar surface of the hindpaw. The numbers on the hindpaws indicate the order in which each test site was stimulated for a given set of stimuli. A: test sites for Von Frey filaments. L, lateral; M, medial. B: test sites for the thermode. C: temperature trace obtained (top) while maintaining the contact force between the thermode and the hindpaw (bottom). Scale on abscissa is in ms.

Determination of the incidence and site of origin of spontaneous activity

Microfilament recordings were made from dissected dorsal root fiber strands in 12 rats that had received ipsilateral rod implantation and in 12 normal nonsurgical rats. The L₄ and L₅ DRG with the attached dorsal roots and sciatic nerve were removed from the rat, placed in a recording chamber, and perfused with oxygenated artificial cerebrospinal fluid (ACSF, pH 7.3) (Zhang et al. 1997b). The total length of peripheral and spinal nerve from the distal tip of the nerve to the DRG was ~3 cm. The rat was killed by intracardiac injection of an overdose of pentobarbital sodium. Fiber recordings were typically obtained from the dorsal roots of each ganglion (see Fig. 9). The method of Govrin-Lippmann and Devor (1978) was used to measure the incidence of spontaneous activity in myelinated afferent fibers whose conduction velocities could be measured by electrical stimulation of the sciatic nerve. For each fiber bundle of approximately equal diameter (30–50 μm), the sciatic nerve was stimulated with a gradually increasing intensity of current (0.1–0.5 ms square-wave pulses, 1–2 Hz) up to 10 mA. The number of different action potential waveforms was counted, and the incidence defined as the number of fibers (waveforms) that were spontaneously active divided by the total number recruited in the strand.
Statistical tests

Differences in mean threshold over time were tested with repeated measures analyses of variance followed by post hoc pairwise comparisons. Using SAS/STAT software (SAS Institute, Cary, NC). The Genmod procedure and Poisson distribution were used to test the significance of differences in the mean number of withdrawals on the temperature-controlled floor. Unless otherwise stated, statistical results described as significant in RESULTS are based on a criterion of $P < 0.01$.

RESULTS

General observations

All animals appeared in good health throughout the study. They gained weight during the test period, were well groomed, and exhibited no self-inflicted wounds. No abnormal gait or posture was seen in the sham-operation control group. However, after acute or chronic compression, all rats showed development of varying degrees of abnormality in gait and posture.

POSTURE AND GAIT. The CCD rats were often seen to raise the affected hindpaw from the floor and hold it in a protected position next to the flank while standing or sitting. When the affected hindpaw was touching the floor, it sometimes appeared that the rats were reducing the weight placed on it by leaning to the other side or by sitting on the opposite haunch. In resting position, CCD rats intermittently exhibited a sudden licking of the ipsilateral hindpaw on the surgical side, accompanied by gentle biting or pulling on the nails with the mouth. This type of behavior began on the 1st postoperative day, was expressed most often during the first 2 postoperative weeks, but continued less frequently up through the 5th wk. For the rats in the acute compression group, this kind of guarding posture occurred only during the first few days after surgery but without biting or pulling toes. In one exceptional rat, it persisted for 1 wk. None of the rats in any group exhibited any signs of autotomy or abnormal nail growth.

When a noxious mechanical or heat stimulus was applied to the hindpaw during preoperative testing, the reflex withdrawal was of small amplitude and brief, typically lasting 1–2 s. Postoperative withdrawals to the same stimuli delivered ipsilateral to a chronic compression were typically of greater amplitude and excessive duration during which the paw might be held in the air 2–15 s but sometimes 20–60 s. Such a paw lift was accompanied by exaggerated aversive behavior such as licking the stimulated paw and/or pulling on the nail with the mouth.

MOTOR BEHAVIOR. Each CCD rat exhibited some ataxia while walking within the first 15–24 h after surgery and thereafter exhibited some guarding of the ipsilateral paw. In general, the affected hindpaw was placed clumsily while walking and the toes, which before surgery were spread apart while walking or standing, were together but not ventroflexed. The hindpaw was everted, and the animal stood and walked with the medial edge of the hindpaw in contact with the floor. This was notable within the first 2 wk after surgery but was less so during subsequent weeks. In a few cases, the rat walked without allowing the hindpaw to touch the floor. All rats walked normally and with toes spread apart in normal fashion when momentarily escaping a prod by the experimenter even within a few days after the operation, suggesting that no permanent motor deficit was responsible for the abnormal gait and posture. That is, it seems that any abnormal posture and gait most likely served the purpose of preventing aversive sensory stimulation.

Withdrawal threshold to punctate mechanical indentation

Before surgery, the mean incidence of foot withdrawal increased monotonically with filament bending force (Fig. 3A, left). The distributions of force thresholds obtained the day before and the 7th day after ipsilateral DRG compression are presented in Fig. 3B. The time course of changes in threshold on each foot after each surgical treatment is shown in Fig. 3C. Threshold values were statistically analyzed separately for each foot. For a given foot, thresholds obtained in each of the three surgical groups were analyzed with a two-way ANOVA (group $\times$ days) with repeated measures on days. Post hoc contrasts determined the significance of differences between the average of the three preoperative tests and the mean obtained for each postoperative test. The same statistical analyses were applied to the slopes of the logistic functions from which the thresholds were derived. For thresholds and slopes obtained contralateral to a surgical operation there were no significant differences due to experimental treatment or days of testing. That is, postoperative thresholds and slopes were not significantly changed over preoperative values.

For the thresholds on the ipsilateral foot, the effects of group and days and the group $\times$ days interaction were each statistically significant. Thresholds on the foot ipsilateral to the chronic compression decreased significantly below baseline on the 1st postoperative day and remained so through the last day of testing. In contrast, thresholds ipsilateral to either an acute compression (transient rod insertion) or a sham operation were significantly lower than baseline only for the 1st day of postoperative testing. The mean threshold ipsilateral to the rod implantation decreased from a preoperative value of 57.7 $\pm$ 3.8 mN (averaged for the 3 days of testing) to 21 $\pm$ 5.8 mN 1 day after surgery. The mean threshold response to Von Frey filaments reached 25.6 $\pm$ 3.5 mN on the 7th day and increased to 37.8 $\pm$ 2.4 mN by the 3rd postoperative week (Fig. 4C). However, all postoperative thresholds on the ipsilateral foot remained significantly lower than the preoperative mean up to the last week of testing.

For some rats, a postoperative decrease in threshold was accompanied by a notable increase in the slope of the psychometric function relating the proportion of withdrawals to the force of indentation (Fig. 3A, right). There were also incidences of a postoperative decrease in slope, particularly on the foot contralateral to a surgical site. However, when the slopes were averaged for a given foot within a given experimental group, there were no significant changes between preoperative and postoperative means. There were also no significant differences in slope between experimental conditions.

Tactile allodynia

The rats did not exhibit reflex withdrawals to stroking with the cotton wisp before rod implantation (Fig. 4). On the 1st day of postoperative testing, most of the rats exhibited a reflex withdrawal to at least one of the strokes with cotton wisp when
the wisp was applied to the foot ipsilateral to the compressed DRG (tactile allodynia). In contrast, none responded to the same stimulus applied to the contralateral foot. The incidence of withdrawal of the ipsilateral foot to the wisp decreased during subsequent tests, so that none of the rats withdrew to this stimulus by the end of the 3rd postoperative week. A McNemars $\chi^2$ test, coupled with a prior test of significance by means of a Cochran Q test, was used to determine the significance of differences between results obtained on different test days. It was found that the percentage of withdrawals obtained on either the 1st or the 14th postoperative day for the foot ipsilateral to the CCD was significantly higher than that obtained on any preoperative day on either foot.

**Withdrawal threshold to heat**

The mean temperature thresholds for heat stimulation were obtained before and after rod implantation and analyzed separately for each foot. Before surgery, the mean incidence of withdrawal for each foot increased monotonically with stimulus temperature (Fig. 5A). The distributions of thresholds on the 3rd preoperative and 7th postoperative days are presented in Fig. 5B. The measurements of thresholds and slopes obtained from the logistic functions for a given foot were analyzed using one-way ANOVAs with repeated measures over days with post hoc contrasts for each foot between individual postoperative means and the grand mean of the three preoper-
ative means. On the 1st postoperative day, there was a significant decrease in the mean withdrawal threshold from the preoperative grand mean ipsilateral but not contralateral to the compressed ganglia (Fig. 5C). The mean postoperative threshold remained significantly lower on the ipsilateral foot on each subsequent test with the exception of day 35, the last day of testing. During the postoperative period, probability levels of significance were ≤0.01 with the exception of days 21 (P = 0.04) and 35 (P = 0.1). Postoperative means on the contralateral foot remained unchanged from the preoperative grand mean through the last day of testing. There was no significant change over preoperative values in the slopes of the logistic functions obtained after surgery for each foot.

In the presence of tactile allodynia during the 1st week or two after the operation, withdrawal responses were sometimes obtained in response to the mere contact between the thermode (held at the base temperature of 39°C on contact). In such cases, the experimenter waited until the foot returned to the floor of the testing apparatus and tried again. Sometimes, several such contacts were made before the foot remained in contact with the thermode for the required 5-s period before onset of the test temperature. Thus, during the first 2 postoperative weeks in those cases when allodynia to mechanical contact was present, it is possible that a small proportion of withdrawal responses were partially biased one way or the other. Nevertheless, the decreased postoperative temperature thresholds persisted on the ipsilateral foot well after the disappearance of tactile allodynia to light contact.

Foot withdrawal on a temperature-controlled floor

Before surgery, only the cold (4°C) plate elicited occasional lifting of either hindpaw (Fig. 6). The duration of elevation of the paw was typically 2–5 s. Foot lifting did not occur on the cool or thermally “neutral” plates. After surgery, there was a significant increase over preoperative values in the number of short- (<2 s) and long-duration withdrawals of the ipsilateral but not the contralateral foot in response to the cold plate. The longer duration withdrawals were accompanied on occasion by licking the paw or gentle biting or pulling of the nails with the mouth. There were also significant increases over preoperative values in the number of short- but not long-duration withdrawals to the cool and neutral plates. These increases were significantly less than those obtained in response to the coldest plate. Because the increases in number of withdrawals were not different for the cool and neutral plates, they were probably due to the occurrence of spontaneous pain rather than a reaction to temperature.

Skin temperature

The mean difference in skin temperature for the two hindpaws for 12 rats was measured before CCD as −0.08 ± 0.2°C and on the 7th day after CCD as −0.01 ± 0.12°C (ipsilateral foot temperature minus contralateral). These differences were not significantly different from zero (Student’s t-tests, P > 0.1).

Determination of the location of the implanted rod relative to the DRG

Rod locations were determined in 35 rats, 1–40 days after rod implantation. Each rat was anesthetized with pentobarbital sodium (40 mg/kg, ip). A laminectomy was performed at the level of L₄-L₅. Each of the two rods, the two ganglia, and their dorsal roots and spinal nerves were identified and exposed. The position of each rod with respect to the DRG was examined under a dissection microscope and classified into one of eight categories (Fig. 7). Most of the rods (64 of 70 or 91.4%) were in the positions of A₂–₄, B₂–₄, and C₂–₄, i.e., entirely or partly over the DRG. Six rods (8.6%) were in the positions of A₁, B₁, or C₁. Seven rods (10%) were in the position of A₄, B₄, or C₄, indicating the possibility that the rod may have pressed against the dorsal root. However, there was no indication that the behavioral measurements of hyperalgesia in rats with a deviant rod position were different in any way from those of the other animals.

FIG. 4. Mean percentage withdrawal in response to a cotton wisp before and during chronic compression of the DRG. For each day of testing, the number of withdrawals to six strokes of the cotton wisp is expressed as a percentage and averaged for the 12 rats for the foot ipsilateral and contralateral to the rod implantation.
Ectopic activity in dorsal root fibers

Action potentials could be evoked by electrical stimulation of the sciatic nerve in 1,034 myelinated fibers recorded in 12 rats, 1–35 days after the onset of chronic DRG compression. The mean conduction velocity of these fibers was 17.1 ± 1 m/s (range 2–58.5 m/s). All rats exhibited hyperalgesia, as evidenced by a postoperative decrease in threshold ipsilateral to the compressed ganglia. The mechanical thresholds decreased from 56.3 ± 2.3 mN (range 40–75 mN) before to 24.3 ± 2.7 mN (range 10–40 mN) after surgery on the day of recording. Of the 1,034 fibers activated by sciatic nerve stimulation, 945 were silent, and 89 exhibited ectopic spontaneous discharge generated within the formerly compressed DRG. The incidence of spontaneously active A-fibers was therefore 89/1,034 or 8.61%.

The incidence of spontaneous activity was also measured for 12 nonsurgical normal rats. There were 12 spontaneously active and 1,216 silent A-fibers activated by electrical stimulation of the sciatic nerve. The mean conduction velocity (CV) of these fibers was 18.2 ± 0.39 m/s. The incidence of spontaneously active A-fibers, 12/1,216 or 0.98%, was significantly lower than that obtained from the rats with formerly compressed DRGs ($\chi^2$ test). The patterns of spontaneously active A-fibers were expressed as regular (Fig. 8A) or irregular (e.g., Fig. 8B). The patterns were additionally classified as either nonbursting (e.g., Fig. 8A and B) or with bursting discharges that were either regular in occurrence (e.g., Fig. 8D) or irregular (Fig. 8, C and E). Seventy-five percent of the A-fibers with bursting discharges were recorded during the 1st postoperative week. This percentage was significantly higher than the remaining 25% recorded 8 to 35 days after rod implantation ($\chi^2$ test).

Twenty-seven spontaneously active C-fibers with a mean CV of 0.7 ± 0.06 m/s, obtained by electrical stimulation of the sciatic nerve, were recorded from finely dissected dorsal root
fibers in five rats that exhibited behavioral hyperalgesia to mechanical indentation. An additional 16 fibers could not be activated by stimulating the sciatic nerve but were identified as C-fibers on the basis of the shapes of their action potentials. All of the spontaneously active C-fibers recorded exhibited discharges with irregular interspike intervals and a low frequency that was typically 0.5–1 Hz.

After the incidence of spontaneous activity was determined, a subset of fiber bundles was reexamined for spontaneous activity. Then, the spinal nerve was cut a few millimeters distal to the DRG and, after 5 min, the bundles again examined for the presence of spontaneous activity (Fig. 9). Next, the same bundle was transected 0.5 mm proximal to DRG and again examined for spontaneous activity over the next few minutes. The approximate location of the origin of ectopic activity was examined for 45 A- and 12 C-fibers from rats with compressed DRGs and for 10 A-fibers from nonsurgical normal rats. In the compressed group, 44 of the 45 A-fibers and all 12 of the C-fibers continued firing after the sciatic nerve was cut just distal to the DRG. Each of these fibers became silent after the dorsal root was transected (Fig. 9), suggesting that the ectopic generation site for this fiber originated within or close to the DRG. There was only one A-fiber that stopped firing after the spinal nerve was cut, suggesting that the ectopic generation site for this fiber originated distal to the ganglion. The spontaneously active A-fibers from the nonsurgical normal rats continued firing after cutting.
off the sciatic nerve but became silent after dorsal root transection.

**DISCUSSION**

The results of this study provide further characterization of an animal model of neuropathic pain produced by a chronic compression of the DRG (CCD model of Hu and Xing 1998). It is possible that the behavioral and electrophysiological effects of DRG compression as characterized for the rat can occur in humans, consequent to such disorders as intervertebral foraminal stenosis, a herniated intervertebral disc, or a tumor that impinges on the DRG. For example, a laterally herniated disc accompanied by a rupture of the annulus fibrosus may alter the properties of DRG somata by the exertion of pressure and the release of inflammatory substances such as phospholipase A2 and cytokines from the nucleus pulposus (Cavanaugh 1995; Devor 1996; Kawakami et al. 1996; Olmarker and Myers 1998). However, dysesthesias such as hyperalgesia and tactile allodynia are not commonly reported in such cases in humans. On the other hand, it is possible that localized, mild cutaneous dysesthesia may go unnoticed, given more pressing symptoms and in the absence of careful, quantitative sensory testing.

**METHODS USED TO MEASURE WITHDRAWAL THRESHOLD.** Our behavioral testing procedures differed in some respects from those commonly used to measure withdrawal thresholds to mechanical and thermal stimulation of the rat hindpaw (Lamotte et al. 1998). First, we varied the bending force of a Von Frey-type nylon filament independently of the filament diameter. The diameter of the tip was held constant by attaching 0.1-mm diameter rods to nylon filaments of differing diameter. The advantage of this method was that preoperative thresholds were easily obtained without the need for thicker filaments with blunt tip diameters that could sometimes lift the foot without eliciting a withdrawal. A second methodological feature was our use of a small contact thermode instead of a radiant heat stimulus to measure threshold withdrawal to heat. The advantage of this method was that withdrawal could be expressed as a function of stimulus temperature as opposed to the latency of withdrawal to a stimulus of unknown temperature. Maintaining a steady contact between the skin and thermode was aided by measuring the contact force with a load cell attached to the thermode. A disadvantage of the method was the initial withdrawals elicited by mechanical contact when tactile allodynia developed. However, repeated attempts to

**FIG. 8.** Typical discharge patterns of spontaneous activity recorded in vitro from myelinated dorsal root fibers of previously compressed DRGs.

**FIG. 9.** Determination of the locus for generation of ectopic discharges after chronic compression of the DRG. At the end of some experiments, spontaneous activity in identified fibers was recorded from dorsal root filaments (R) before and immediately after transection of the spinal nerve (A) and again after transection of the filament (B). The spontaneous discharges were eliminated by the second cut but not by the first. Thus, the DRG was the most likely site for the generation of ectopic discharges.
apply the thermode were eventually successful despite this drawback. The third feature of the behavioral testing procedure that was unusual for threshold testing in the rat was the use of the method of constant stimuli in which the same stimulus intensities were delivered the same number of times during each test. This method provided a psychometric function relating intensity to response frequency from which a slope as well as a threshold were derived. The slope of this function—that is, the rate of increase in the frequency of withdrawal with stimulus intensity, may have increased more rapidly than normal after an experimental treatment, although this did not typically occur in the present study. Previous investigators have more commonly used a modified method of limits (or threshold-tracking procedure) to measure withdrawal thresholds in rats (e.g., Chaplan et al. 1994). The latter method obtains the threshold more efficiently but may be more prone to experimenter error and to response biases of the rat whose behavior influences what stimuli are to be delivered.

MECHANICAL HYPERALGESIA. The withdrawal threshold to punctate mechanical indentation was significantly decreased on the foot ipsilateral to the compressed ganglia. In addition, the magnitude and duration of the withdrawal responses were greatly exaggerated and often accompanied by aversive behavior, such as licking and/or shaking the paw. In contrast, the magnitude and duration of withdrawal reflex of the postoperative contralateral paw or of either paw before CCD were of small amplitude and brief duration. There were no significant changes on the contralateral paw that were consistent among rats. The mirror-image changes on the contralateral feet, as reported by Seltzer et al. (1990) and Kim and Chung (1992) in their models, were not commonly found with the present model.

TACTILE ALLODYNA. A marked sensitivity to innocuous tactile stimulation ipsilateral to the compression developed in CCD rats. Beginning on the 1st postoperative day and lasting in some cases through the 2nd week, a reflex withdrawal could often be obtained in response to innocuous stroking with a cotton wisp. The withdrawal was sometimes exaggerated in amplitude and duration and accompanied by licking the paw. These signs suggest that a light touch, which never evoked a reflex withdrawal in control animals, could elicit pain in the paw ipsilateral to the compressed DRG. To our knowledge, this kind of mechanical allodynia has not been reported using other animal models of neuropathic pain (e.g., Bennett and Xie 1988; Seltzer 1990; Kim and Chung 1992). The latter investigators defined mechanical allodynia in terms of lowered thresholds to punctate indentations. Alldynia in response to stroking the skin may be particularly relevant to the touch-evoked allodynia sometimes reported by human patients with neuropathic pain.

THERMAL HYPERALGESIA. The withdrawal temperature threshold to heat was significantly decreased on the hindpaw ipsilateral, but not contralateral, to the compressed DRG. This difference was not based on a difference in skin temperature between the ipsilateral and the contralateral foot, as might occur after a peripheral nerve injury that results in a significant loss of sympathetic innervation of the skin. In the present model, the skin temperature was not significantly different between the ipsilateral and the contralateral feet after CCD. Consistent with our result, Hu and Xing (1998) observed a significant reduction in the latency of foot withdrawal to radiant heat stimuli after DRG compression. There was clear evidence that the CCD produced cold hyperalgesia. Exposure to the cold metal plate, chilled by ice water, elicited a significant increase in the incidence of withdrawal of the foot ipsilateral to the DRG compression. These results were similar in some respects to those observed by Bennett and Xie (1988) and Choi et al. (1994) for their animal models of peripheral neuropathy models after nerve injury.

Taken together, the present results indicate that a CCD produced mechanical and thermal hyperalgesia similar in some respects to that reported to occur after peripheral or spinal nerve injuries (Bennett and Xie 1988; Choi et al. 1994; Kim and Chung 1992; Seltzer et al. 1990).

SPONTANEOUS PAIN. Rats with compressed DRGs exhibited behavior indicative of spontaneous pain (e.g., Attal et al. 1990). Observations of the general behavior of the rat suggest that spontaneous pain developed in the hindpaw ipsilateral to the implanted rods. While resting, the rats were often seen to raise the affected hindpaw from the floor and occasionally shake it and hold it in a protected position. Sudden licking of the hindpaw on the surgical side was often accompanied by gentle biting or pulling on the nails with the mouth. In spite of the presence of signs of spontaneous pain, none of the rats in any of our groups exhibited the autotomy that can occur after nerve transection (e.g., Wall et al. 1979).

THE ABSENCE OF MOTOR DEFICITS. Although rats in the present study may have appeared awkward in placing their affected hindpaw while resting or walking, they exhibited normal motor behavior when attempting to escape gentle prodding by the experimenter. This indicated that no obvious motor deficits accompanied the abnormal behavior resulting from CCD and suggested that motor nerves were not significantly damaged. This is in contrast to peripheral neuropathy models in which the animals exhibit permanent foot deformity after nerve injury (Bennett and Xie 1988; Kim and Chung 1992).

INCIDENCE AND SITE OF ORIGIN OF SPONTANEOUS ACTIVITY. Ectopic, abnormal neuronal activity can contribute to chronic pain of peripheral nerve origin (e.g., Devor 1994). Spontaneous activity originating from the somata is rarely observed in DRG cells with normal, uninjured axons (Wall and Devor 1983). However, it is common when peripheral axons are injured (Burchiel 1984; De Santis and Duckworth 1982; Kajander et al. 1992; Study and Kral 1996; Wall and Devor 1983; Xie et al. 1995; Zhang et al. 1997b). After a peripheral nerve injury, ectopic discharges can originate at the injury site and/or within the DRG containing the cell bodies of the injured neurons (De Santis and Duckworth 1982; Kajander et al. 1992; Kirk 1974; Wall and Devor 1983). In the present study, the DRG was both the injury site and the site of origin of ectopic discharges. Although these discharges had characteristics similar to those of peripherally axotomized neurons (e.g., Babbage et al. 1996; Zhang et al. 1997a,b) they were produced in the present study by neurons with intact axons. Therefore, axotomy is not a prerequisite for the presence, and various patterns, of ongoing ectopic discharge. Because the ectopic discharge was recorded in vitro, it was not dependent on either a blood supply or a functioning sympathetic nervous system. Possibly it reflects an increase in the intrinsic excitability of the somal membrane (Zhang et al., 1999). Approximately 9% of the myelinated afferents activated by electrical stimulation of the sciatic nerve was spontaneously active after DRG compression. This percentage is in approximate agreement with the value of

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PHYSIOLOGICAL BASIS FOR ECTOPIC DISCHARGES AFTER CCD. Most of the implanted rods in the present study were located entirely or partially over the DRG. An increase in external pressure produced directly or indirectly by the rod may produce an intraneural edema and possibly hemorrhage in the endoneural space of the DRG as suggested by Rydevik et al. (1989) in their studies of dorsal root compression. Action potentials can be evoked from uninjured DRG neurons by the local application of mechanical stimuli to the ganglion in vivo (Howe et al. 1977) and in vitro (Sugawara et al. 1996). In addition to possibly directly activating DRG cells, the chronic mechanical pressure from an implanted rod may have produced ischemia and compromised the delivery of oxygen and nutrients. Rod implantation may have elicited an inflammatory process and a release of cytokines, nerve growth factors, inflammatory mediators, and other substances that have been shown in other studies to directly activate and/or change the properties of DRG neurons and increase their excitability (e.g., Wagner and Myers 1996; Waxman et al. 1994). Release of such chemical factors may have contributed, in a recent study, to the deceased stimulus thresholds for foot withdrawal ipsilateral to a prior surgical exposure of the lumbar dorsal root and DRG (Olmarker and Myers 1998). The threshold in that study was further reduced when the same surgical procedure was combined with a mechanical displacement of the dorsal root and application of material from the herniated nucleus pulposus to the root. It is possible that a waning inflammatory process contributed to the gradual decrease in allodynia to the cotton wisp within the first 2 wk after rod implantation. The allodynia disappeared near the end of the 2nd wk. If so, more persistent factors would be responsible for contributing to the hyperalgesia to mechanical and thermal stimuli that continued up to the last day of testing.

The number of DRG cells that degenerated as a result of the chronic compression injury has not been quantified. Preliminary histological examination of a few of our compressed DRGs suggests minimal cell loss (unpublished observations). This is consistent with our finding of no difference in the number of A-fibers in the dorsal roots of compressed versus nonsurgical DRGs that could be electrically activated by stimulation of the sciatic nerve. Nevertheless, it is conceivable that the loss of even a small population of DRG neurons may have contributed to the development of hyperalgesia and tactile allodynia. Wallerian degeneration in DRG neurons has been implicated in the production of neuropathic pain and cutaneous hyperalgesia (e.g., Ramer et al. 1997). Cytokine and growth factor activation by immune cells responsive to the process of neuronal degeneration may increase the excitability of uninjured neighboring neurons with axons in the same nerve or with cell bodies in the same ganglion. Indirect evidence for such a mechanism is provided by recent behavioral and electrophysiological studies in nerve-injured animals (Ali et al. 1999; Li et al. 1999).

In a pilot study (Z. Zheng and R. H. LaMotte, unpublished observations) we found that both the incidence of ectopic discharges recorded from dorsal root A-fibers from compressed DRGs and the proportion of fibers activated by sciatic nerve stimulation were lower when the implanted rods were left in place than they were after the rods were removed. In a pilot study with two rats, a rod was implanted over a single DRG (L4) resulting in ipsilateral hyperalgesia and allodynia that were alleviated when the rod was removed 4 days after implantation. Ectopic discharges in dorsal root A-fibers were recorded in vitro from the formerly compressed DRG 4 days after rod removal. The incidence of spontaneous activity was lower than it was when recorded in the present study within 12 h of rod removal. The results of these preliminary studies suggest that there is a long-term change in the properties of a subpopulation of formerly compressed DRG neurons that persists for at least several days after the compression is removed and the hyperalgesia is alleviated. Based on the preliminary recordings obtained in vivo, it appears that the compression of the DRG produces abnormal ectopic discharge in some afferents while blocking conduction in many others.

It is possible that the activity of nociceptive dorsal horn neurons is disinhibited by the decreased activity of myelinated primaryafferent neurons and enhanced by spontaneous and evoked activity in a subpopulation of nociceptive afferents. Similar concepts have been suggested to explain the hyperalgesia and tactile allodynia surrounding an area of analgesia produced by acute nerve transection (e.g., Denny-Brown 1965; Henson 1965) and to account for the larger sized cutaneous sensory area when spinal nerves instead of dorsal roots are sectioned (possibly because of nociceptive ectopic discharges from the DRG) when the method of “remaining sensibility” is used to map dermatomes (Kirk 1974; Kirk and Denny-Brown 1970).

Abnormal spontaneous activity, if present in appropriate C-nociceptive afferents, could maintain central sensitization or enhanced excitability of dorsal horn neurons (Simone et al. 1991; Woolf 1983). A chronic state of central sensitization could form the basis for chronic hyperalgesia and tactile allodynia. It is therefore hypothesized that ongoing neuropathic ectopic discharges from chronically compressed DRG neurons contribute to the cutaneous thermal and mechanical hyperalgesia observed in the present study.

In summary, a unilateral chronic compression of the L4 and L5 DRGs resulted in rapid-onset, long-lasting, mechanical and thermal hyperalgesia accompanied by tactile allodynia on the ipsilateral hindpaw. Ectopic discharges originating from the DRG were similar in some respects to those occurring after peripheral axotomy but were exhibited by neurons with intact axons and were not dependent on an intact circulatory system or sympathetic nervous system. Unlike the effects of peripheral nerve injury, there were no motor deficits or changes in skin temperature. Experimental compression of the DRG in animals may be useful for investigations of the cellular basis for pain and hyperalgesia after spinal injuries and other disorders that affect the DRG in humans.

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Present addresses: X.-J. Song, Research Institute, Parker College of Chiropractic, Dallas, TX; S.-J. Hu, Institute of Neuroscience, Fourth Military Medical University, Xian, China; and J.-M. Zhang, Dept. of Anesthesiology, University of Arkansas Medical Science, Little Rock, AR.

Address for reprint requests: R. H. LaMotte, Dept. of Anesthesiology, Yale University School of Medicine, 333 Cedar St., New Haven, CT 06510.
REFERENCES


