

Gabapentin Normalizes Spinal Neuronal Responses That Correlate with Behavior in a Rat Model of Cancer-induced Bone Pain

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Background: Cancer-induced bone pain is a major clinical problem for which current treatments lack full efficacy. Gabapentin is licensed for use in neuropathic pain yet is also effective against inflammatory stimuli in animals.

Methods: A rat model of cancer-induced bone pain using the MRMT-1 cell line injected into the tibia was established to investigate the efficacy of acute (10, 30, 100 mg/kg) and chronic (30 mg/kg) systemic gabapentin on electrophysiological superficial dorsal horn neuronal responses to natural and noxious electrical stimuli, as well as on pain-related behavior.

Results: In electrophysiological studies gabapentin worked both acutely (100 mg/kg) and chronically (30 mg/kg) to normalize the hyperexcitable superficial dorsal horn neuronal response, significantly reducing electrical-evoked and mechanical-evoked but not thermal-evoked responses. The behavioral study showed that chronic gabapentin (30 mg/kg) significantly attenuated pain behavior in MRMT-1 rats, restoring responses to preoperative baseline degrees, and that this attenuation was accompanied by a reversion to normal (non-MRMT-1) wide-dynamic-range: nociceptive specific superficial dorsal horn neuronal profiles.

Conclusions: Pain-related behavior in this rat model of cancer-induced bone pain is strongly linked to hyperexcitability of a population of superficial dorsal horn neurones. Gabapentin normalizes the cancer-induced bone pain induced dorsal horn neuronal changes and attenuates pain behavior. It may therefore provide a novel clinical treatment for cancer-induced bone pain.

ONE of the most common causes of pain in patients with malignant disease is cancer-induced bone pain (CIBP) affecting patients with primary bone sarcomas and those with bone metastases.¹⁻³ The skeleton is the third most common site of metastatic spread,⁴ occurring in 60–84% of cases.² The pain consists of a triad of states: tonic (background) pain, spontaneous pain, and movement-induced (incident) pain.^{3,5} With disease progression chronic pain develops, exacerbated by the severe episodes of incident pain.⁶

Pharmacological intervention (including nonsteroidal antiinflammatory drugs, opioids, and bisphosphonates) is used in conjunction with other therapies (including radiotherapy, chemotherapy, hormonal therapy, and sur-

gical intervention) to alleviate CIBP.² These interventions are often effective in controlling tonic pain but not incident pain. The degree of analgesia required to combat the flares of incident pain renders unacceptable side effects, and the presence of incident pain is predictive of reduced opioid efficacy.⁷⁻⁸

Until recently, investigation of novel drugs in the treatment of CIBP was hampered by a lack of suitable animal models.⁹ The original protocol involving intramedullary injection of a murine osteosarcoma line has been developed using different cancers, including melanoma, breast, colon, and fibrosarcoma in rats and mice.¹⁰⁻¹² These models all closely mimic the development of CIBP seen in humans and have allowed the unique dorsal horn pathophysiology found in CIBP to be investigated.^{9,13} Immunohistochemistry findings suggest that central sensitization occurs,¹³ and *in vivo* electrophysiology shows the dorsal horn is hyperexcitable, with unique features not previously reported for either inflammatory or neuropathic pain states.¹⁴⁻¹⁵

Damage within the bone from extensive osteoclast activity and cancer growth results in peripheral nerve damage coupled with a chronic inflammatory infiltrate and may contribute to central sensitization.¹⁶ Thus, drugs active in these pain states could have a role in attenuating the hyperalgesia. One such drug is the anti-convulsant gabapentin, used widely in clinical neuropathies; it has also been shown to selectively inhibit noxious-evoked responses of dorsal horn neurones in models of neuropathy and after inflammation, albeit with a lag period of a few days depending on the dose.¹⁷⁻²⁰ The aim of this investigation was to determine the efficacy of systemic gabapentin on the hyperexcitable dorsal horn neuronal responses and behavioral responses to pain in rat intratibial MRMT-1 CIBP. Furthermore, the neuronal and behavioral measures in these studies enable the relation between spinal plasticity and behavior to be gauged.

Materials and Methods

All procedures described were approved by the Home Office (animal procedures section, London, United Kingdom) and follow the IASP guidelines and the animal ethics committee at University College London, London, UK.

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Animals

Experiments were carried out on male Sprague-Dawley rats (University College animal house, University College London, UK) weighing approximately 170 g at the time of surgery and 300 g at electrophysiology. Rats were housed maximum five per cage on a 12 h day-12 h night cycle for at least 1 week before surgery. Food and water were available *ad libitum*.

Preparation of Cells

Syngeneic MRMT-1 rat mammary gland carcinoma cells were donated by the Novartis Institute (London, UK) and prepared as described previously.^{7,14} Cells were cultured in RPMI 1640 (Gibco, Invitrogen Ltd., Paisley, UK) with 10% fetal bovine serum, 1% L-glutamine and 2% penicillin/streptomycin (Gibco, Invitrogen Ltd.). Brief exposure to trypsin (0.1%w/v) was used to release the adherent cells from the flask; they were then quenched with an equal volume of 10% fetal calf serum and centrifuged for 3 min at 1200 rpm. The resulting pellet was washed twice with 10 ml of Hank's balanced salt solution (Gibco, Invitrogen Ltd.) without Ca^{2+} , Mg^{2+} , or phenol red and was finally suspended in 1 ml Hank's solution. Cells were diluted in Hank's medium to the required concentration for injection (3×10^3 cells/10 μl) and kept on ice.

Osteotomy and Injection of Cells

The surgical procedure was the same as described previously.¹⁴ Briefly, anesthesia was induced and maintained *via* a nose cone with halothane (1.5–2%) in a 66:33 mixture of nitrous oxide and oxygen. The left leg was shaved and the skin was disinfected with chlorhexidine (Animalcare Ltd, York, UK). A small incision was made over the anteromedial surface of the distal end of the tibia to expose the bone. A 0.7-mm dental drill attachment was used to bore a hole through the periosteum and a 2-cm length of polythene tubing was fed 1 cm into the intramedullary cavity of the tibia. Ten microliters of cells were injected into the tibia *via* a Hamilton syringe and the tubing withdrawn. The hole was plugged with bone restorative material (LD Division, Dentsply Inc., Milford, Delaware), the area was irrigated with 0.5 ml 0.9% saline, and the wound was closed with a metal clip. The procedure was identical for sham-operated animals except these were injected with Hank's media alone. After surgery rats were placed in a thermo-regulated recovery box until they had fully regained consciousness, at which time they were returned to their home cages.

Drug Administration

Gabapentin (a gift from Pfizer, Sandwich, UK) was dissolved in saline to give 30 mg/kg (in a volume of 1 ml/kg) for the chronic behavioral study and 10 mg/kg, 30 mg/kg, and 100 mg/kg (in the same volume of 250 μl) for the acute pharmacologic study. All injections were given subcutaneously in the scruff of the back of the neck.

Rats were divided into three treatment groups. The first group (n = 15), all having received intratibial injection of MRMT-1 cells, were given twice-daily injections (every 12 h: 8 AM and 8 PM) of gabapentin 30 mg/kg between days 11 and 14 postsurgery, with a single injection on the morning of day 15. Twelve of the animals in this group were subsequently used for electrophysiological characterization of superficial dorsal horn neurons. A second group (n = 6) acting as a control for the gabapentin-treated animals were inoculated with MRMT-1 cells and received twice-daily injection of saline according to the same schedule. The third group of animals made up of MRMT-1-injected (n = 7) and sham-operated rats (n = 6) received no postoperative treatment and were used for electrophysiological pharmacological study of the acute systemic effects of gabapentin.

Behavioral Testing

Animals were left to acclimatize to the area for 30 min before testing. Behavioral signs of ambulatory-evoked pain were assessed preoperatively and on postoperative days 1, 5, 7, 11, 12, 13, 14, 15, and 18 using the Ugo Basile model 7750 Rotorod (Linton Instruments, Diss, Norfolk, UK). The apparatus was set to accelerate from 0–20 revolutions per minute (rpm) over 60 s and the time in seconds maintained on the beam before each rat fell was recorded (with a maximum cut-off of 150 s). Ambulation was observed and scored as follows: normal = 0, slight limping = 1, marked limping = 2, avoidance of use of limb = 3. Only rats scoring between 90 and 120 s on two training sessions before surgery were used for experimentation. The use of the Rotorod test has been reported to be a reliable and consistent means of assessing ambulatory-evoked pain.^{13–15,21}

Behavioral measurement of mechanical and cold allodynia was carried out at the same time points as ambulatory-evoked pain. The testing area consisted of transparent plastic cubicles on a mesh-floored platform. Mechanical sensitivity was assessed by application of four von Frey filaments with increasing bending forces of 1, 5, 9, and 15 g (North Coast Medical Inc., Morgan Hill, California). Each filament was applied 10 times to the plantar surface of both ipsilateral and contralateral hindpaws for approximately 2–3 s, with a period of at least 5 min separating ascending von Frey filaments. The number of lifts (brisk withdrawal of the hindpaw) observed of the maximum total of 10 was expressed as percentage response. Cold sensitivity was assessed by five applications of a drop of acetone, using a 1-ml syringe, to the plantar surface of the hindpaw. As with mechanical testing, this was carried out on both ipsilateral and contralateral hindpaws, with each application separated by at least 5 min. Again, the number of lifts observed of the maximum total of five was expressed as percentage response.

Spinal Cord Electrophysiology

Briefly, anesthesia was induced with 3% halothane in a mixture of 66% nitrous oxide and 33% oxygen and a cannula was inserted into the trachea. A laminectomy was performed to expose the spinal cord insertions of L4, L5, and L6 spinal nerves, and the concentration of halothane reduced to 1.8% for maintenance of anesthesia. Extracellular recordings of superficial dorsal horn (SDH) neurones (<300 μm from surface of cord) receiving afferent C-fiber and A-fiber input from the ipsilateral hindpaw were made using a parylene-coated tungsten electrode (A-M systems, Carlsborg, Washington).

Cell Characterization

Characterization of SDH neurones was carried out as described previously,¹⁴⁻¹⁵ on day 15 postsurgery (*i.e.*, after the final injection of gabapentin) and on day 18 postsurgery (2 days after cessation of gabapentin treatment). The search stimuli were gentle tapping over the hindpaw to locate potential neurones, and then electrical and natural stimuli were used to fully characterize the neurone. Briefly, a train of 16 electrical stimuli (2 ms pulse width, 0.5 Hz) at three times the threshold current for C-fiber activation were applied transcutaneously to the center of the receptive field. The responses evoked by A-beta (0–20 ms), A-delta (20–90 ms), and C-fiber (90–350 ms) were separated according to latency. The C-fiber responses evoked by the first stimulus of the train are referred to as input and the responses recorded between 350 and 800 ms as postdischarge. Wind-up was calculated as the total number of action potentials evoked minus the input multiplied by the total number of stimuli in the train.¹⁶ Action potentials evoked by natural stimuli applied for 10 s to the center of the receptive field were quantified by the application of von Frey filaments (bending forces of 1, 5, 9, 15, 30, and 75 g) for punctate mechanical stimuli, a standard artists brush for nonnoxious dynamic mechanical stimuli, constant water jet at 32°, 35°, 38°, 40°, 42°, 45°, 48°, and 50°C for thermal stimuli, and constant water jet at 4°C for cold stimuli. Any response to 32°C was subtracted from subsequent thermal and cold responses to account for the mechanical component of the response to the water jet. SDH neurones were classified as nociceptive specific (NS) or wide-dynamic-range (WDR) based on their responses to static mechanical and thermal stimuli, with NS taken as those responding (with more than 10 action potentials per stimulus) at von Frey 9 g and above and 42°C plus for heat.

Pharmacologic Studies

WDR SDH neurones were identified for pharmacologic study, as we have shown that this spinal neuronal population is altered by the peripheral pathology.¹⁴⁻¹⁵

The protocol, carried out every 20 min, consisted of an electrical test followed by natural stimuli exactly as de-

scribed above with the exception that fewer temperatures were tested (35°, 40°, 45°, 48°, and 50°C for thermal and 4°C for cold). After three consecutive stable control trials (<10% variation for all parameters) neuronal responses were averaged to give predrug control values to which subsequent responses were compared. Gabapentin (10 mg/kg, 30 mg/kg, and 100 mg/kg) was administered subcutaneously into the scruff of the back of the neck in a volume of 250 μl , and the evoked response of the neuron was followed for 50 min (testing at 10, 30, and 50 min) before the next dose was given.

Data Analysis

Data are presented as mean \pm SEM unless otherwise stated. Statistical analyses were performed using the Mann-Whitney U test with GraphPad Prism 3.0 (GraphPad, San Diego, CA) software and significance was set at $P < 0.05$.

Results

All rats continued in good health after surgery as assessed by normal weight gain, general activity, and grooming. In agreement with our previous findings,¹⁴⁻¹⁵ the animals injected with MRMT-1 showed progressive development of evoked mechanical allodynia and hyperalgesia and cold sensitivity on the ipsilateral hindpaw and ambulatory evoked pain (Rotorod). The contralateral hindpaw responses were the same as the sham group responses on both hindpaws and remained stable throughout the postoperative period, indicating that none of the behavioral tests cause tissue damage or hypersensitivity. These findings were identical with previous published findings.¹⁴⁻¹⁵

Chronic Systemic Gabapentin

Behavior. A total of 21 MRMT-1-injected rats were used in the chronic behavioral study, 15 of which received twice-daily injection of gabapentin 30 mg/kg between postoperative days 11 to 15, and six of which received twice-daily injection of saline. The gabapentin-treated MRMT-1 animals showed an attenuation of the enhanced withdrawal responses to mechanical stimuli caused by CIBP compared to saline-injected animals, which was significant ($P < 0.05$) by postoperative day 12 for von Frey 1 g (fig. 1A) and by day 13 for higher von Frey filaments (fig. 1B). We would interpret this as indicative of inhibition of allodynia and hyperalgesia. It is notable that gabapentin did not abolish the withdrawal response to mechanical stimuli but returned it to the preoperative/sham baseline degree (fig. 1B). The results for the ambulatory-evoked pain (as assessed by the Rotorod test) mirrored the von Frey results with a significant reduction of the deficits ($P < 0.05$) in the gabapentin-injected group compared with saline-injected rats

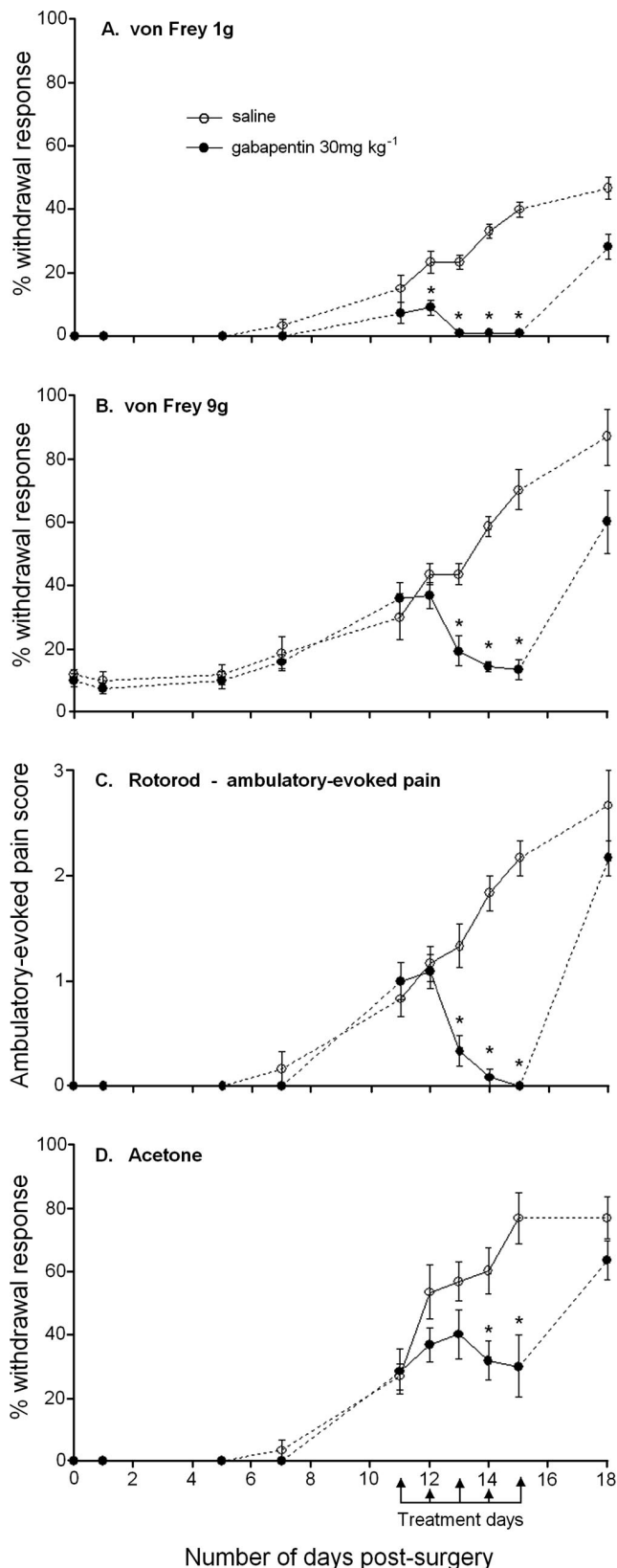


Fig. 1. The effect of chronic gabapentin treatment on mechanical allodynia (A, B), ambulatory-evoked pain (C), and cold allodynia (D) in MRMT-1 injected rats. Each data point in the saline-treated group (open circle) represents the mean \pm SEM of six rats and in the gabapentin-treated group (closed circle) the

by postoperative day 13 (fig. 1C). The withdrawal response to acetone was also significantly lower ($P < 0.05$) in the gabapentin-treated animals by day 14 (fig. 1D). Unlike with the mechanical and ambulatory-evoked pain responses, gabapentin did not normalize the response to acetone but prevented it from further increasing beyond the degree it had reached at the time treatment was initiated.

The results from day 18 (from a total of nine rats, 2 days after cessation of gabapentin treatment) revealed the reemergence of hyperalgesia and allodynia in this group that was equivalent to the saline-treated rats (*i.e.*, rats never having received gabapentin) for all of the behavioral tests (fig. 1, A-D).

Gabapentin 30 mg/kg treated normal animals, $n = 4$ (same dosing regime) showed no alteration in their motor function (limping, Rotorod) nor in their responses to von Frey filaments or acetone when compared to non-treated or gabapentin solute (normal saline) treated normal animals

Electrophysiological Studies

Characterization of Dorsal Horn in Response to Chronic Gabapentin Dosing. A total of 30 SDH neurons were characterized (from six rats) on postoperative day 15 after the final dose of gabapentin at the time point when pain behavior was reduced to the degree of sham-operated rats (*i.e.*, without cancer). A total of 32 cells (from another six rats) were characterized on postoperative day 18 (and 2 days after cessation of gabapentin) by which time pain behavior had returned to the same degree of allodynia and hyperalgesia as the saline-treated MRMT-1 rats. A total of 30 cells were characterized from six animals who received MRMT-1 cells and normal saline vehicle and a total of 31 cells from six sham-operated animals.

In sham-operated animals the ratio of WDR:NS was 26%:74%, in MRMT-1 treated animals it was 47%:53%, as we have previously reported.¹⁴ Here, almost identical results were seen in that the ratio of WDR:NS in gabapentin-treated CIBP rats on day 15 was 33%:66% and on day 18 had returned to 50%:50% (fig. 2). This suggests that chronic gabapentin treatment attenuates spinal cord plasticity and so shifts the abnormally high WDR neuro-

mean \pm SEM of 15 rats. Animals received twice-daily subcutaneous injections of saline/gabapentin 30 mg/kg on postoperative days 11 to 14 inclusive at 12-h intervals, with a final injection on the morning of day 15. Gabapentin-treated rats show a reduction in withdrawal responses to von Frey 1 g (A) compared with saline-treated rats significant ($P < 0.05$) from day 12 and to von Frey 9 g (B) significant from day 13. The ambulatory-evoked pain score (C) in gabapentin-treated rats was also significantly reduced compared with saline-treated rats from day 13 and the withdrawal response to acetone (D) from day 14. On day 18, 2 days after cessation of treatment, there was no longer a significant difference between the groups with gabapentin-treated rats returning to the same degree of mechanical and cold allodynia, and ambulatory-evoked pain, as saline-treated rats.

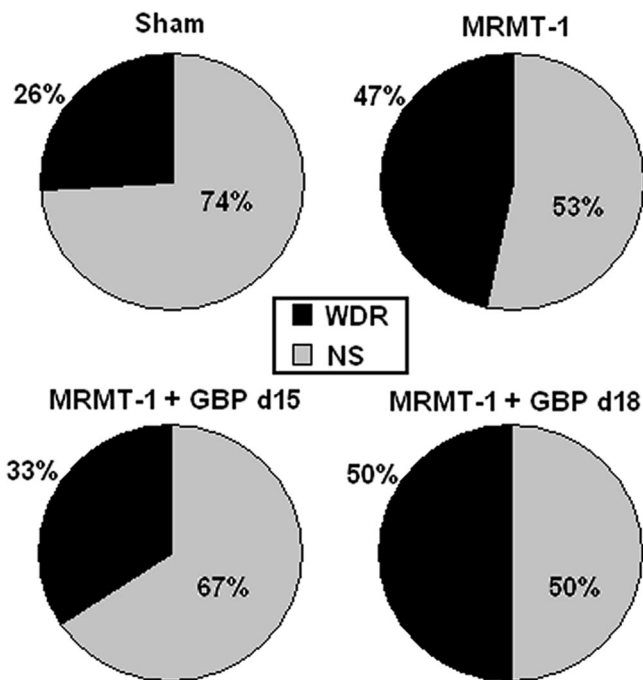


Fig. 2. The effect of chronic gabapentin treatment on proportions of wide-dynamic-range (WDR) and nociceptive specific (NS) neurones in the superficial dorsal horn (SDH) of MRMT-1 injected rats. In sham-operated animals the ratio of WDR:NS was 26%:74% ($n = 33$ cells), which changed in MRMT-1 treated animals ($n = 28$ cells) to 47%:53%.¹⁴ In MRMT-1 injected rats receiving gabapentin treatment (twice-daily subcutaneous injections of gabapentin 30 mg/kg on postoperative days 11–14 and a single injection on day 15), characterization of SDH neurones on day 15 ($n = 30$ cells), when pain-related behavior was returned to a normal (*i.e.*, sham-operated rat) degree, revealed a WDR:NS ratio of 33%:67%, approaching the normal ratio seen in sham-operated animals. Characterization on postoperative day 18 ($n = 32$ cells), 2 days after cessation of gabapentin treatment when animals had returned to the same degree of pain behavior as saline-treated rats revealed a return to the characteristic cancer-induced bone pain state with a WDR:NS ratio of 50%:50%.

nal population (characteristic of this rat model of CIBP) back towards the predominant high-threshold situation seen in sham animals. Importantly, cessation of treatment and the reemergence of pain-related behaviors result in a return to the CIBP state and increased WDR ratio.

The WDR cells characterized in gabapentin-treated rats on day 15 (number of animals = 6; total of 30 neurones, of which 10 were WDR) showed significantly reduced ($P < 0.05$) input and C-fiber evoked responses to electrical stimuli compared with cells characterized in MRMT-1-injected animals receiving no postoperative drug treatment (number of animals = 6; total number of neurones 30, of which 14 were WDR) (fig. 3). The A-beta and postdischarge evoked responses to electrical stimuli were also reduced such that there was no longer a significant difference to sham-operated rats (fig. 3). In addition, by day 18, 2 days after the cessation of gabapentin the evoked neuronal response (input, C-fiber, post-

discharge) had returned to the same degree as MRMT-1 treated rats with no gabapentin treatment (fig. 3).

On day 15 postsurgery, the mean response to mechanical stimuli (von Frey filaments) of WDR neurones in the MRMT-1 gabapentin-treated rats was significantly reduced ($P < 0.05$) compared with MRMT-1 rats receiving no treatment (fig. 4A), and this reduction was such that the degree of response was similar to that seen in sham-operated animals. Characterization of WDR cells (number of animals = 6; total number of neurones = 32, of which WDR = 16) on day 18 (2 days after cessation of gabapentin) revealed that the mechanical-evoked response was returning to the degree of response in untreated MRMT-1 rats (fig. 4A). Similar normalization of the CIBP-induced increases in brush responses was also seen suggesting that the drug can influence both the abnormal static and dynamic mechanical responses. It is of note that gabapentin normalizes both the A-beta response to noxious electrical stimulation and the brush-evoked response in CIBP. However, to investigate the effect of gabapentin on A-beta fiber evoked responses it would be necessary to carry out the test at supra-A-beta thresholds rather than at the supra-C-fiber thresholds used here. Chronic gabapentin treatment did not have a significant effect on the thermal-evoked response of WDR neurones at either day 15 or day 18 (fig. 4B).

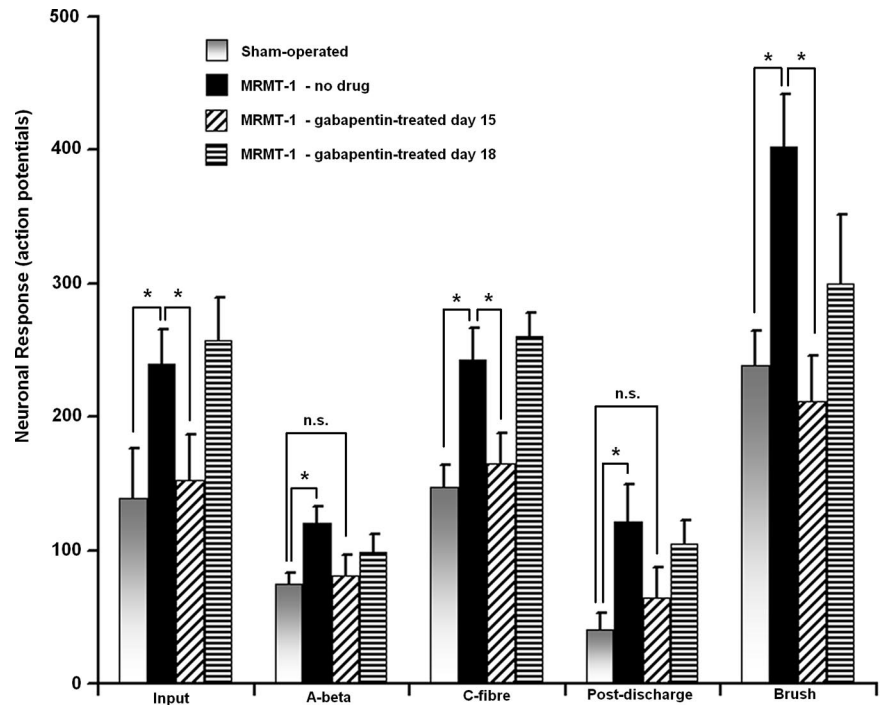
Effects of Acute Systemic Gabapentin

In each of the two groups, MRMT-1 operated animals ($n = 7$) and sham-operated animals ($n = 6$), a single WDR neurone was isolated per rat per experiment. These cells showed increased responses in the MRMT-1 group compared with shams, consistent with results reported previously¹⁴ and elsewhere in this study. This was particularly notable for input and C-fiber-evoked responses to electrical stimuli where cells in the sham group gave average predrug control action potential (AP) values of 131 ± 19 and 133 ± 9 , respectively, and those in the cancer group were 201 ± 23 and 206 ± 41 respectively. Postdischarge and wind-up responses also showed a tendency to be increased in MRMT-1-injected animals. Again, corresponding with previous findings, the nonnoxious brush-evoked and the noxious mechanical-evoked responses were greater in the cancer group compared with sham-operated animals (fig. 5).

Gabapentin had no significant effect on A-fiber-evoked responses to electrical stimuli or thermal-evoked responses (data not shown). The input, C-fiber, postdischarge, and wind-up responses to electrical stimuli in the MRMT-1 group were significantly reduced ($P < 0.05$) compared with shams with the highest dose of gabapentin (100 mg/kg). Furthermore the C-fiber-evoked response was significantly reduced ($P < 0.05$) (113 ± 11 AP in sham-operated rats and 82 ± 11 AP in MRMT-1-injected rats) with gabapentin 30 mg/kg (fig. 5).

The increased responses to noxious mechanical stim-

Fig. 3. The effect of chronic gabapentin treatment on electrical-evoked and natural-evoked responses of wide-dynamic-range (WDR) superficial dorsal horn (SDH) neurones in MRMT-1 injected rats. SDH WDR neurones in MRMT-1 injected rats ($n = 13$ cells) show significantly ($P < 0.05$) increased electrical-evoked responses (input, A-beta fiber, C-fiber, post-discharge) and natural-evoked responses (nonnoxious brush) compared with sham-operated rats ($n = 9$ cells).¹⁴ Electrophysiological characterization of WDR SDH neurones in MRMT-1 injected rats having had chronic gabapentin treatment (twice-daily subcutaneous injections of gabapentin 30 mg/kg on postoperative days 11–14 with a single injection on day 15) on the final day of treatment ($n = 10$ cells) showed a significant reduction ($P < 0.05$) in electrical-evoked (input, C-fiber) and brush-evoked responses compared with MRMT-1 injected rats receiving no drug treatment. The A-beta and postdischarge evoked responses to electrical stimuli were reduced such that there was no longer a significant difference to the degree of response of neurones in sham-operated rats. Characterization of WDR SDH neurones in MRMT-1 injected rats 2 days after cessation of gabapentin treatment (postoperative day 18, $n = 16$ cells) revealed that the electrical-evoked and natural-evoked responses had returned to the same degree as in MRMT-1 injected rats receiving no drug treatment. The input and C-fiber evoked responses were significantly increased ($P < 0.05$) compared with the responses at day 15 (final day of gabapentin treatment).



uli seen in MRMT-1-injected animals were reduced to sham degree with 30 mg/kg gabapentin (fig. 5). The brush-evoked response was also reduced in MRMT-1-injected rats from 468 ± 36 AP to 247 ± 34 AP (sham predrug control was 243 ± 74 AP) with 100 mg/kg gabapentin (fig. 5).

As found with the chronic treatment study, gabapentin did not have a significant effect on thermal-evoked responses at any dose.

Discussion

In this study we have confirmed our previous findings that injection of MRMT-1 cells into the rat tibia results in the progressive development of mechanical and cold allodynia as demonstrated by behavioral testing and a characteristic dorsal horn pathophysiology of increased proportion of WDR neurones in the superficial lamina and heightened electrical-evoked and natural-evoked responses of these cells.¹⁴

We have shown that chronic systemic administration of gabapentin reduces the aberrant behavioral responses to mechanical stimuli (von Frey filaments) to normal preoperative baseline degrees and also significantly reduces cold allodynia as assessed by the acetone test. The 2-day lag seen before gabapentin significantly reduced behavioral responses is in agreement with other published findings in a model of neuropathic pain, in which multiple doses of gabapentin were necessary to produce

behaviorally measurable antihyperalgesia.^{20,22} Gabapentin at 30 mg/kg did not affect normal motor function in a group ($n = 4$) of normal animals (data not shown). The impairment of ambulation seen on the Rotorod in the MRMT-1 group was a result of the destruction of the tibia rather than the gabapentin, as function returned to normal during gabapentin treatment. Furthermore, we show that the attenuation of pain is reversed when gabapentin treatment is stopped with the return of mechanical allodynia and grade 3 ambulatory-evoked pain on the Rotorod after 2 days.

Characterization of SDH neurones at the time of maximum antihyperalgesia (day 15 postsurgery and the fifth day of gabapentin treatment) confirmed that gabapentin reversed the dorsal horn pathophysiology, significantly reducing electrical-evoked and mechanical-evoked responses of WDR neurones in addition to normalizing the ratio of WDR to NS neurones. Two days after cessation of gabapentin treatment the dorsal horn returned to the hyperexcitable state seen in untreated MRMT-1 CIBP, with an increased proportion of hyperexcitable superficial WDR neurones. This shows that the changes occurring after gabapentin treatment, which lead to attenuation of pain and normalization of dorsal horn neuronal responses, are not the result of permanent changes such as anatomical reorganization. Indeed, the rapid return to the pain state would mitigate against gene changes being involved and would argue for changes in neuronal physiology as the determinant of the behavioral allodynia and

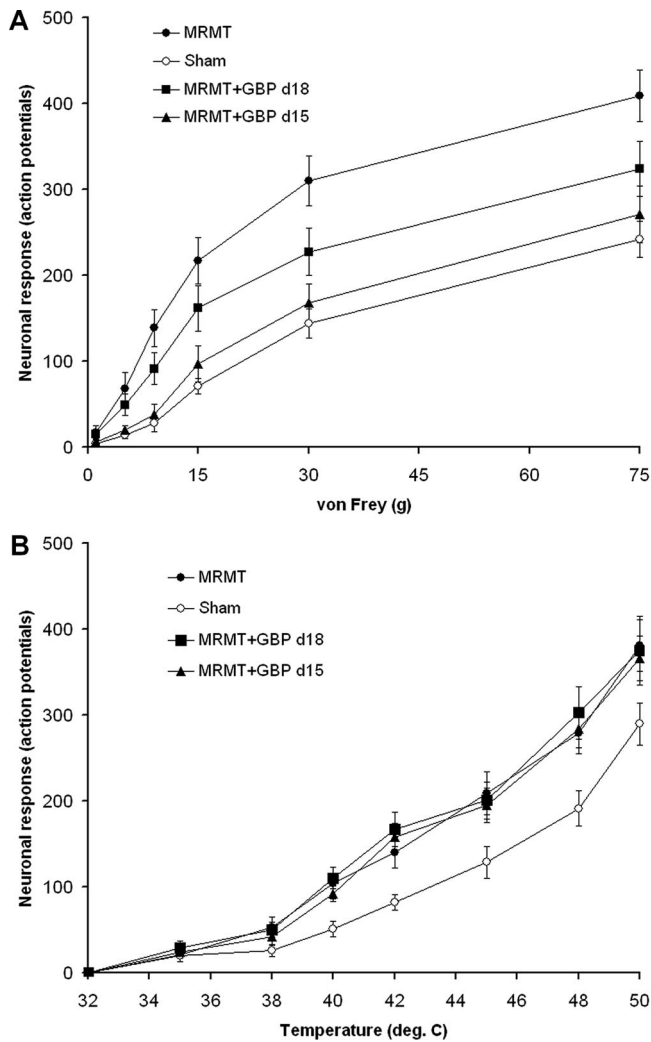


Fig. 4. The effect of chronic gabapentin treatment on mechanical-evoked (A) and thermal-evoked (B) responses of wide-dynamic-range (WDR) superficial dorsal horn (SDH) neurons in MRMT-1 injected rats. WDR SDH neurons in MRMT-1-injected rats ($n = 13$ cells) show significantly increased ($P < 0.05$) responses to mechanical-evoked (A) and thermal-evoked (B) stimuli compared with sham-operated rats ($n = 9$ cells). The mean response to mechanical stimuli (A) of WDR neurons in the MRMT-1 gabapentin-treated rats (twice-daily subcutaneous injections of gabapentin 30 mg/kg on postoperative days 11–14 with a single injection on day 15) on the day of the final injection was significantly reduced ($P < 0.05$) compared with MRMT-1 rats receiving no treatment, such that the degree of response was similar to that seen in sham-operated animals. Characterization of WDR cells ($n = 16$) on day 18 (2 days after cessation of gabapentin) revealed that the mechanical-evoked response was returning to the degree of response in untreated MRMT-1 rats. Chronic gabapentin treatment had no significant effect on thermal-evoked responses of WDR SDH neurons at any time point (B).

hyperalgesia. These findings provide further evidence for a correlation between the dorsal horn neuronal pathophysiology and pain behaviors in CIBP.¹⁵ In addition, they suggest a pivotal role for plasticity in superficial SDH neurons in CIBP pain behavior development. The shift in the ratio of NS:WDR would appear to be attributable to a *de novo* gaining in the ability of low

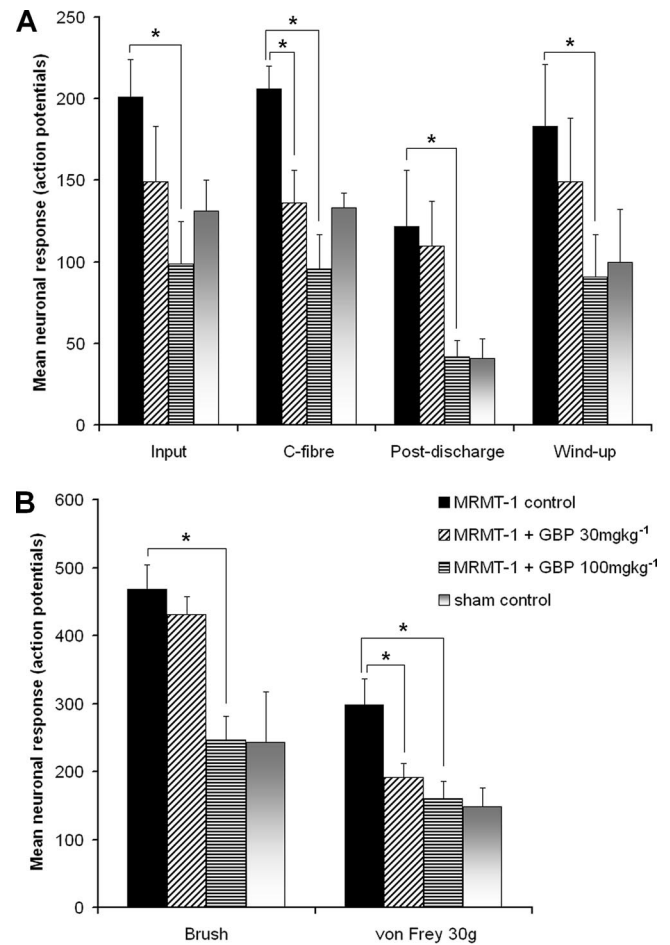


Fig. 5. The effect of acute systemic gabapentin on electrical-evoked (A) and mechanical-evoked (B) responses of wide-dynamic-range (WDR) superficial dorsal horn (SDH) neurons in MRMT-1 injected rats. Electrical-evoked responses (A) of WDR SDH neurons ($n = 7$) in MRMT-1 injected rats were reduced compared with control values, significant ($P < 0.05$) at 30 mg/kg gabapentin for C-fiber-evoked responses and 100 mg/kg for input, postdischarge, and wind-up. The reduction was such that the degree of response was similar to sham-operated rat controls. Mechanical-evoked responses (B) were also significantly reduced ($P < 0.05$) with gabapentin (at 30 mg/kg for von Frey 30 g and 100 mg/kg for brush).

threshold mechanical inputs to activate NS neurons (attributable to either an increase in excitatory inputs or a reduction in inhibition). This is paralleled by increased responsiveness of the overall WDR neurone population at the time as pain-related behavior. Gabapentin abolished the aberrant behavior and reset the neuronal responses. Thus we believe that this confirms the idea that the allodynia and hyperalgesia seen after CIBP is in part attributable to spinal hyperexcitability and that central sensitization is responsible for the pain behavior. Gabapentin removed the behavior but, rather than abolishing the neuronal responses, simply normalized the neuronal changes. Finally, the data further support the concept that gabapentin is antihyperalgesic and as the treatment ceased and the neuronal excitability was reestablished, there was a reemergence of pain behavior.

The neuronal plasticity we report is likely to result from peripheral originating mechanisms that drive central spinal changes. Thus, the hyperresponsivity of the ipsilateral hindpaw may be a reflection of several events. Peripheral mechanisms may reflect a large area of primary hyperalgesia, extending from the tibia into the adjacent paw. In addition the increased afferent drive into the spinal cord and the subsequent excitation and central sensitization could evoke a secondary hyperalgesia over the hindpaw. The mechanisms of the observed changes in neuronal excitability in CIBP have not been elucidated. One important factor must be the increased primary afferent input into the superficial (and thus indirectly to the deep) lamina. In addition, lamina I is not only the source of major ascending projections to both areas of the brain involved in affective and autonomic components of pain but is the origin of a spinal-supraspinal loop, activating descending facilitation acting on the dorsal horn.²³ The changed ratio of WDR:NS neurones in the superficial lamina appearing to drive the CIBP behavioral changes may be attributable to a combination of increased excitation (arising from primary afferents or descending facilitation) or decreases in intrinsic inhibition. Chronic treatment with gabapentin causes a decrease in the number of WDR neurones, a reduction in the hyperexcitable state of the remaining WDR neurones, and thus a normalization of the dorsal horn.

The mechanism of action of gabapentin, although not fully understood,²⁴⁻²⁵ may be an interaction with the $\alpha_2\delta$ subunit of voltage-dependent calcium²⁶ which would be expected to reduce transmitter release and neuronal activity. The significantly increased input response (representing the excitability produced by afferent activity) seen in MRMT-1 injected rats could be indicative of increased neurotransmitter release as a result of the cancer-induced osteoclast activation and subsequent bone destruction¹⁶ that was normalized by gabapentin. N-type and P-type calcium channels have been shown to be involved in neuropathic and inflammatory pain states,²⁷⁻³¹ and, furthermore, nerve injury-induced up-regulation of the $\alpha_2\delta$ -1 subunit is reported to correlate with the antiallodynic effects of gabapentin.³² The ability of gabapentin to normalize and reduce these enhanced responses could result from potential actions on altered voltage-dependent calcium channel activities reducing transmitter release and the monosynaptic activation of the spinal superficial neurones that drive the plasticity. The mechanisms behind the state-dependency of the effects of gabapentin as shown here in terms of a normalization of CIBP-related behavior and neuronal hyperexcitability yet a total lack of effect on normal measures remain unknown.

Although the dorsal horn alterations in CIBP have been shown to have unique characteristics that differentiate it from neuropathy or inflammation,^{9-10,14} the bone destruction wrought by cancer cells combines both inflam-

matory infiltrate and peripheral nerve destruction.^{16,33-36} The acute effects of systemic gabapentin can be compared directly with other animal models. Gabapentin has been shown to reduce the evoked responses of dorsal horn neurones and have antiallodynic effects in the spinal nerve ligation and chronic constriction injury models of neuropathic pain.^{18-19,37-38} In addition, it is also effective in inflammatory pain. It has been shown to block the late phase of the formalin response,³⁹ inhibit C-fiber and postdischarges,¹⁷ and reduce both thermal and mechanical hyperalgesia in the carrageenan model of inflammation.³⁹

We found the acute effect of gabapentin in CIBP was similar to that reported in both neuropathy and inflammation, although in CIBP there was no effect on the neuronal responses to thermal stimuli, which agrees with some studies in animal models of inflammatory and neuropathic pain⁴⁰⁻⁴¹ and clinical investigations⁴²⁻⁴³ but not with others.^{39,44-45} In addition, the acute doses of gabapentin we have used in this study are the same as those used in an inflammatory pain model¹⁷ and the chronic behavioral dose we have used was the minimum effective dose in our acute study. This may indicate that normal adjuvant doses of gabapentin may be helpful in controlling CIBP in humans. It is of interest that a single systemic dose of gabapentin 30 mg/kg had no effect on behavioral responses (shown by the lag period), although had some effect on reducing dorsal horn excitation (deep lamina C fiber responses). This apparent disparity may be an effect of dose; for a single dose of gabapentin to have an effect on all dorsal horn responses it was required to be given at 100 mg/kg. The acute reduction in lamina V C-fiber responses may not be sufficient to translate into behavioral responses; however, over time gabapentin at 30 mg/kg normalizes the dorsal horn response and this latter neuronal change may be a prerequisite for behavioral responses to alter.

In conclusion, we have shown that gabapentin normalizes dorsal horn neuronal responses and pain behavior in a rat model of CIBP. We suggest that increased excitation (from primary afferents or descending facilitation) may be responsible for the plasticity in superficial dorsal horn neuronal populations in that there is an increased proportion of WDR to NS neurones in the superficial spinal cord. The data presented here provide strong evidence for a correlation between SDH hyperexcitability and behavioral allodynia and hyperalgesia. Finally, the ability of gabapentin to normalize SDH neuronal responses and pain behavior in this rat model, at clinically relevant doses, suggests that it could be effective in treating humans with CIBP.

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