Anesthesiology 2002; 96:633–40 

# A Combination of Gabapentin and Morphine Mediates Enhanced Inhibitory Effects on Dorsal Horn Neuronal Responses in a Rat Model of Neuropathy

*Elizabeth A. Matthews, Ph.D.,* Anthony H. Dickenson, Ph.D.†*

***Background:*** **Peripheral nerve damage can result in severe, long-lasting pain accompanied by sensory deficits. This neuropathic pain remains a clinical problem, and effective morphine analgesia is often limited by intolerable side effects. The antiepileptic gabapentin has recently emerged as an alternative chronic pain treatment. Improved management of the diverse symptoms and mechanisms of neuropathic pain may arise from combination therapy, based on multiple pharmacologic targets and low drug doses.**

***Methods:*** **The authors used the Kim and Chung rodent model of neuropathy to induce mechanical and cold allodynia in the ipsilateral hind paw. *In vivo* electrophysiologic techniques were subsequently used to record evoked dorsal horn neuronal responses in which the effects of systemic morphine and gabapentin were investigated, both individually and in combination.**

***Results:*** **Morphine (1 and 4 mg/kg) inhibited neuronal responses of control rats but not after neuropathy. Gabapentin (10 and 20 mg/kg) inhibited neuronal responses in nerve injured rats and to a lesser extent in sham rats but not in naive rats. In the presence of gabapentin (ineffective low dose of 10 mg/kg), morphine (1 and 3 mg/kg) mediated significant inhibitory effects in all experimental groups, with the greatest inhibitions observed in spinal nerve–ligated and sham-operated rats. After neuropathy, inhibitions mediated by morphine were significantly increased in the presence of gabapentin compared with morphine alone.**

***Conclusions:*** **After spinal nerve ligation, the inhibitory effects of systemic morphine on evoked dorsal horn neuronal responses are reduced compared with control, whereas the effectiveness of systemic gabapentin is enhanced. In combination with low-dose gabapentin, significant improvement in the effectiveness of morphine is observed, which demonstrates a clinical potential for the use of morphine and gabapentin combinational treatment for neuropathic pain.**

DAMAGE to the peripheral and central nervous system can lead to the development of neuropathic pain in which patients often experience a combination of sensory deficits with spontaneous and stimulus-evoked pain (allodynia and hyperalgesia). Because of the multiplicity of causes of neuropathy, ranging from trauma to viral infections to diabetes, and the number of possible resultant symptoms, the underlying dysfunctional mechanisms are likely to be diverse. This may contribute to the problematic clinical management of nerve injury pain. Neuropathic pain and epilepsy share neuronal hyperexcitability as a common underlying mechanism. There are established antiepileptic drugs that target the generation of neuronal hyperexcitability, and some of these have been proven to be effective in the treatment of various forms of neuropathic pain.[1] One of the most useful methods used to assess clinical analgesic efficacy is "numbers needed to treat," which refers to the number of patients that have to be treated before one patient experiences more than 50% pain relief. Excitability blockers, antidepressants, and opioids can be useful therapies for neuropathic pain, but the number needed to treat is approximately 3, even for the most effective agents, and their use can be limited by unfavorable side effects (see Sindrup and Jensen[1]). The diverse mechanisms and symptoms of neuropathic pain lend credence to the idea that combination therapy, based on multiple pharmacologic targets and low drug doses, could improve both the pain relief and side effect profiles.

The anticonvulsant gabapentin is widely becoming accepted as an alternative treatment for various types of neuropathic pain[2] because it provides reasonable efficacy and is well-tolerated. Designed as an analog of the inhibitory neurotransmitter γ-aminobutyric acid (GABA)[3] to cross the blood–brain barrier, it does not clearly modulate GABA receptor function, and the mechanisms of its anticonvulsant–analgesic actions remain undetermined (see Taylor *et al.*[4]). Gabapentin does not bind to any known neurotransmitter receptor but binds to a unique site in the central nervous system identified as $\alpha_2\delta$, a voltage-dependent calcium channel modulatory accessory subunit.[5] Animal models have shown the antinociceptive abilities of specific voltage-dependent calcium channel blockers in line with electrophysiologically observed reductions in spinal cord hyperexcitability and highlight the differential role each subtype plays in nociception, often dependent on the nature of the pain state.[6–8]

Morphine acts *via* a number of central nervous system sites, including the spinal cord, where presynaptic and, to a lesser extent, postsynaptic μ-opioid receptors modulate nociceptive transmission. μ-Opioid receptors are G-protein coupled, and on extracellular binding of morphine, a conformational change in the receptor is elicited that subsequently opens potassium channels. The resultant neuronal hyperpolarization leads to a decrease in the opening of voltage-dependent calcium channels, and at presynaptic locations, a reduction in the release of neurotransmitter from the afferent nerve ensues. This

* Research Fellow, † Professor of Neuropharmacology.

Received from the Department of Pharmacology, University College London, London, United Kingdom. Submitted for publication July 9, 2001. Accepted for publication November 1, 2001. Supported by a Neuroscience Ph.D. grant funded by Wellcome Trust, London, United Kingdom (to Dr. Matthews under supervision of Dr. Dickenson).

Address reprint requests to Dr. Matthews: Department of Pharmacology, University College London, Gower Street, London WC1E 6BT, United Kingdom. Address electronic mail to: e.matthews@ucl.ac.uk. 

presynaptic action is common to all opioid-mediated inhibitory effects at other sites, the net result being a reduction in neuronal excitability. Because of the differing ionic mechanisms of inhibition, it could be predicted that morphine and gabapentin would interact positively, through concomitant decrease of excitation and increase of inhibition. Controversies exist regarding the effectiveness of morphine in neuropathic pain, but it seems that dose escalation may be an essential issue.[9]

Thus far, there are no *in vivo* electrophysiologic studies investigating the role of gabapentin in conjunction with morphine in the processing of neuropathic pain. This study uses the spinal nerve ligation model, confirmed by behavioral testing, to induce a neuropathic state. Subsequent to this, electrophysiologic studies of dorsal horn spinal neurons were made to investigate the effects of low does of subcutaneously delivered gabapentin and morphine on a wide range of electrical and natural-evoked neuronal activity. Because of the serious difficulties in conducting combination studies in patients with neuropathic pain, it is hoped this approach may provide a guide to potential improvements in the treatment of this type of pain.

## Materials and Methods

*Spinal Nerve Ligation*

Male Sprague-Dawley rats, initially weighing 130–150 g, were used in this study. All experimental procedures were approved by the Home Office (animal procedures section, London, United Kingdom) and follow the guidelines under the International Association for the Study of Pain. Selective tight ligation of spinal nerves L5 and L6 and a sham procedure were performed as first described by Kim and Chung (1992).[10] Briefly, during gaseous anesthesia, the rat was placed in a prone position, a midline incision was made from L4–S2, and the left paraspinal muscles were separated from the spinous processes. Part of the L6 transverse process was removed to expose the L4 and L5 spinal nerves, and L6 was identified lying just under the sacrum. Using 6-0 silk thread, the left spinal nerves L5 and L6 were tightly ligated distal to their dorsal root ganglion and proximal to their conjunction to form the sciatic nerve. Hemostasis was confirmed, the wound was sutured, and the animal recovered from anesthesia. A sham operation was performed to produce a control group, whereby the surgical procedure was identical to that of the experimental group, but spinal nerve ligation was omitted.

*Behavioral Testing*

For 2 weeks after surgery, the rats were housed in groups of four in plastic cages under a 12/12 h day/night cycle, and their general health was monitored. Successful reproduction of the neuropathic model was confirmed by behavioral testing at postoperative day 14, assessing the sensitivity of both the ipsilateral and contralateral hind paws to normally nonnoxious punctate mechanical (von Frey filaments) and cooling (acetone) stimuli. Rats were placed in transparent plastic cubicles on a mesh floor and were allowed to acclimatize before tests were initiated. Foot withdrawals to trials of ascending von Frey filaments (bending forces of 1, 5, and 9 g: 9.9, 49.5, and 89.1 mN, respectively), considered nonnoxious under normal circumstances, were quantified. In a trial, a single filament was applied 10 times to the plantar surface of the foot, through the mesh floor, for 2–3 s each time. A period of 3–4 min was left before commencing with the next filament. Foot withdrawals to the application of a drop of acetone to the plantar region of the foot were quantified. In a trial, a drop of acetone was gently squirted through the mesh floor *via* a syringe, five times, at 5-min intervals. The number of foot withdrawals were measured on both the ipsilateral and contralateral hind paws for each stimulus modality and were expressed as Difference scores = ipsilateral response − contralateral response, as in the original article[10] and as previously described in Chapman *et al.* (1998).[11]

*Spinal Cord Electrophysiology*

Subsequent to behavioral testing, the operated rats were used for electrophysiologic studies at postoperative days 14–17. Briefly, anesthesia was induced with 3% halothane in a mixture of 66% $N_2O$ and 33% $O_2$, and a cannula was inserted into the trachea. A laminectomy was performed (vertebrae L1–L3) to expose segments L4–L5 of the spinal cord, and the level of halothane was reduced to 1.8%. Extracellular recordings of single convergent neurons, located deep within the dorsal horn ($> 500$ $\mu$m), receiving input from the toe region ipsilateral to the spinal nerve ligation or sham procedure, were made using a parylene-coated tungsten electrode (2 M$\Omega$ impedance, 0.005-in uninsulated tip). Neurons selected responded to both noxious (pinch) and nonnoxious (touch) stimuli.

**Cell Characterization.** Any spontaneous activity exhibited by a neuron was recorded over 10 min. Action potentials evoked by natural stimuli applied constantly over 10 s were quantified by the application of both punctate mechanical (von Frey filaments, 9 and 75 g) and thermal (constant water jet at 45°C) stimuli applied to the center of the neuron's receptive field. On each separate application, the von Frey filaments were applied to the exact same location, and pressure was applied so as to bend the filament to the same degree. This mediated an accurate and consistent bending force as indicated by the acquisition of stable predrug control responses. The thermal response to 45°C was determined by subtracting the response to 32°C (a nonnoxious temperature so as to ascertain any mechanical re-

sponse evoked by the water jet) from the response to 45°C. All responses to natural stimuli were normalized by the subtraction of any spontaneous activity over 10 s measured before the application of each stimulus. A representative response of a neuron to punctate mechanical stimuli (von Frey filaments, 9 and 75 g) and noxious heat (constant water jet at 45°C) is shown in figure 1. Response of the neuron to transcutaneous electrical stimulation was established by insertion of two fine needles into the center of its peripheral receptive field and, for the majority of cells involved, adjacent toes. A test consisted of a train of 16 stimuli (2-ms-wide pulse at 0.5 Hz at 3 times the threshold required to evoke a C-fiber response), and a poststimulus histogram was constructed. The thresholds were determined by increasing the electrical stimulus from 0 mA until an action potential was evoked in the corresponding latency band. Electrically evoked action potentials were separated on a latency basis into Aβ fibers (0–20 ms), Aδ fibers (20–90 ms), C fibers (90–300 ms), and postdischarge (300–800 ms). Examples of a representative response of a neuron to a single electrical pulse at 3 times the C-fiber threshold (as seen on the oscilloscope) and a poststimulus histogram after a train of 16 electrical stimuli is shown in figure 1. The "input" is the number of action potentials (90–800 ms) evoked by the first stimulus of the train. "Excess spikes" is a measure of "wind-up," which is increased neuronal excitability to repeated constant stimulation. Excess spikes was calculated as the total action potentials (90–800 ms) after the 16-stimulus train minus the input times 16.

**Pharmacologic Studies.** The testing protocol, initiated every 10 min, consisted of an electrical test followed by the natural stimuli, as described. Stabilization of the neuronal responses was confirmed with at least three consistent predrug responses (< 10% variation) for all measures. These values were then averaged to generate predrug control values with which to compare the effect of drug administration on the subsequently evoked responses. Morphine sulfate and gabapentin (a gift from Parke Davis, Cambridge, United Kingdom) each were dissolved in saline and were administered subcutaneously into the scruff of the neck in volumes of 250 μl. The effect of morphine alone (1 and 4 mg/kg applied cumulatively) was monitored until exertion of maximum effect (a minimum of 50 min). The drug combination protocol consisted of a single dose of gabapentin (either 10 or 20 mg/kg), monitored for 60 min, preceded by two doses of morphine (1 and 4 mg/kg). Reversal of opiate-mediated effects was assessed by spinal application of 5 μg naloxone.

**Statistical Analysis.** The results were calculated as maximum percentage inhibition from the averaged predrug value for each neuron, and the overall results for each dose were expressed as mean ± standard error of the mean of the normalized data. Statistical analysis of maximal drug effect for each measurement at each dose compared with its averaged predrug value was determined by paired *t* test on raw data. An unpaired *t* test on the normalized data was used for the comparison of drug effects between different experimental groups and for

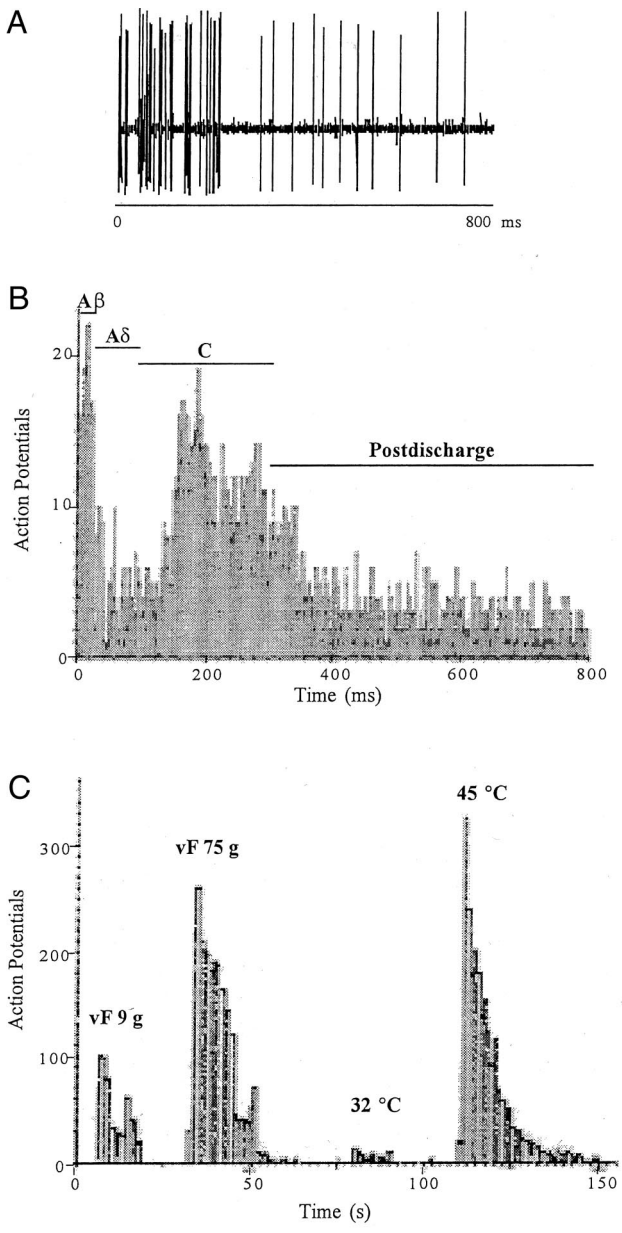


**Fig. 1. (*A*) Representation of the evoked dorsal horn neuronal response to a single electrical stimulus and 3 times C-fiber threshold, as seen on the oscilloscope. (*B*) An example of a typical poststimulus histogram produced after a train of 16 electrical stimuli at a frequency of 0.5 Hz at 3 times C-fiber threshold. The action potentials evoked in a single dorsal horn neuron by the different primary afferent fiber types and the postdischarge are displayed. (*C*) A typical rate recording, showing the evoked action potentials to natural stimuli applied for a period of 10 s each. The mechanically evoked response to innocuous von Frey (9 g) and noxious von Frey (75 g) filaments are displayed. The response to noxious heat over 10 s (water jet at 45°C) was normalized by subtraction of the response to water jet at 32°C over 10 s so as to remove any mechanically evoked activity due to the water jet itself.**

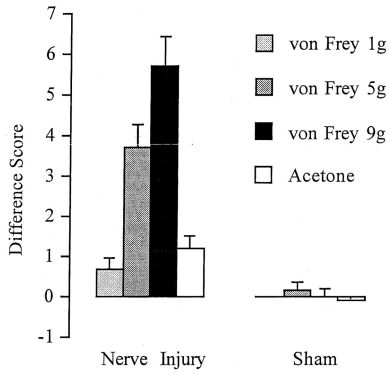


**Fig. 2. Establishment of mechanical and cooling allodynia in the ipsilateral paw 14 days after spinal nerve ligation. Data are presented as the mean difference score ± standard error of the mean (n = 24 for spinal nerve–ligated animals, n = 19 for sham-operated control group) in the withdrawal response to punctate mechanical stimuli (von Frey filament bending forces of 1, 5, and 9 g) and cooling stimulus (drop of acetone) applied to the plantar surface of the hind paws (trials of 10 for the mechanical stimuli and 5 for the cooling stimulus). Difference score = (ipsilateral response frequency) − (contralateral response frequency).**

comparison of the inhibitory effect of morphine in the presence and absence of gabapentin. The level of significance was $P \geq 0.05$.

## Results

*Behavioral Studies*

During the postoperative period, the animals showed normal weight gain and maintained good general health. Rats subjected to spinal nerve ligation showed abnormal foot posture ipsilateral to nerve injury whereby toes were held together in a "guarding" behavior. This did not occur either in the contralateral hind paw or in the sham-operated rats. Successful replication of the nerve injury model was confirmed by behavioral testing at postoperative day 14 (fig. 2), which showed the presence of mechanical and cooling allodynia in the injured hind paw of spinal nerve–ligated rats. Evoked allodynia, in response to innocuous mechanical (von Frey filaments, bending force 1–9 g) and cooling (acetone) stimuli, was displayed as a brisk withdrawal, accompanied in some cases by shaking and licking of the foot ipsilateral to spinal nerve ligation. Consistent withdrawal responses were not exhibited by the control group or by the contralateral hind paw of the experimental group, and when present, were never accompanied by the pain-like behaviors displayed by the lesioned hind paw of spinal nerve–ligated rats. The development of neuropathic behaviors has previously been shown to be evident at postoperative day 2, maximal at days 7–12, and still maintained at day 14.[7]

*Spinal Cord Electrophysiology*

**Cell Characterization.** The numbers of ipsilateral dorsal horn neurons characterized in each group were 24 in spinal nerve–ligated rats, 19 in sham-operated rats, and 19 in naive rats. All neurons had a receptive field over the left ipsilateral hind paw. No significant differences were found between experimental groups in the mean values of recorded neuron depth or responses evoked by electrical and natural stimulation. However, the mean level of ongoing spontaneous activity recorded from neurons in nerve-injured rats was 1.73 ± 2.53 Hz, which was significantly higher ($P < 0.05$) than that observed in sham and naive rats (0.39 ± .89 and 0.42 ± 0.71 Hz, respectively). It is also worth noting that 71% of neurons characterized in spinal nerve–ligated rats showed spontaneous activity at a rate greater than 0.1 Hz in comparison with only 37% of characterized neurons in sham-operated rats and 39% of naive rats.

**Pharmacologic Studies.**

***Effects of Morphine Alone.*** The effect of subcutaneously administered morphine (1 and 4 mg/kg applied cumulatively) on the electrically and naturally evoked dorsal horn neuronal responses was tested in spinal nerve–ligated, sham-operated, and naive animals. Morphine produced a dose-related inhibition of the evoked responses in neurons recorded from naive and sham-operated animals, with clear effects seen at approximately 40–50 min. The greatest effect was seen in the sham group (figs. 3A–D). In addition to the significances indicated on the measurements displayed in figure 3, in the sham group, both doses of morphine significantly inhibited the excess spike and heat response in comparison to predrug control values, as did 4 mg/kg for the von Frey 9-g evoked response (n = 6, $P < 0.05$). In addition, for the naive group, the top dose of morphine also significantly inhibited the excess spike measurement (n = 6, $P < 0.05$). Morphine was noticeably less effective at inhibiting the evoked responses in nerve-injured rats in comparison with naive and sham-operated rats (figs. 3A–D). Two out of 7 cells showed an increase in their response, resulting in an average excitation for the input (fig. 3C) and excess spike measurements at the low dose. The von Frey–evoked responses were significantly inhibited. No statistical significance was determined for the direct comparison of the effects of morphine between experimental groups. Morphine-mediated inhibitions were reversed with spinally applied naloxone, and for the majority of neuronal responses, this often exceeded control values, indicative of some activation of endogenous opioid systems.

***Effects of Gabapentin Alone.*** The effect of subcutaneously administered gabapentin (10 and 20 mg/kg), on the electrically and naturally evoked dorsal horn neuronal responses, was tested in spinal nerve–ligated, sham-operated, and naive animals. Gabapentin produced a dose-related inhibition of the evoked responses in neurons in spinal nerve–ligated and sham-operated animals, with clear effects seen at approximately 40–50 min. The greatest effect was observed in the neuropathic animals

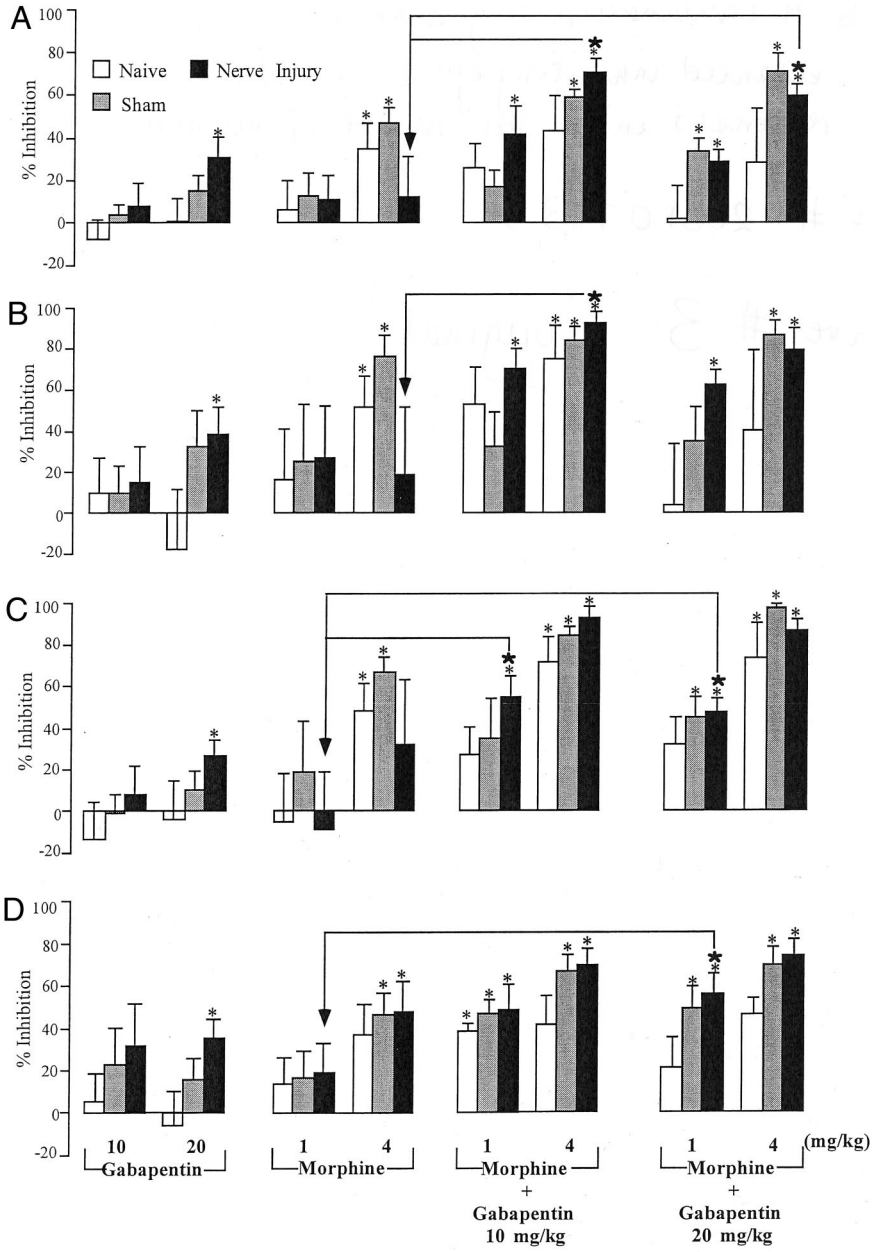


**Fig. 3. Effect of subcutaneously administered morphine and gabapentin, both individually and in combination, on evoked dorsal horn neuronal responses (see Methods) recorded from spinal nerve–ligated, sham-operated, and naive rats (n = 6–10 for each experimental group used in each drug testing protocol). (*A*) C-fiber, (*B*) postdischarge, (*C*) input, and (*D*) von Frey (75 g) measurements are shown. Data are expressed as maximal mean percent inhibition of the predrug values ± standard error of the mean. *Statistically significant inhibitory response compared with predrug control value; ★Significantly greater inhibitory effect of morphine in the presence of gabapentin compared with morphine alone at a specific dose ($P \leq 0.05$).**

for which statistically significant inhibitions (n = 10, $P < 0.05$) compared with predrug control values were achieved with 20 mg/kg for all measurements (excluding A fibers, which were relatively spared) (figs. 3A–D). In contrast, in the naive group, gabapentin did not have a constant inhibitory effect on all neuronal measurements. With the exception of the heat response, either or both doses of gabapentin produced an overall excitation of the responses recorded from four out of seven neurons. This was most marked for postdischarge (fig. 3B) and excess spikes (data not shown). No statistical significance was determined for direct comparison of the effects of gabapentin between experimental groups.

***Effect of Morphine in the Presence of Gabapentin.*** The effect of subcutaneously administered morphine (1 and 4 mg/kg applied cumulatively), 60 min after the subcutaneous administration of either 10 or 20 mg/kg gabapentin, on the electrically and naturally evoked dorsal horn neuronal responses was tested in spinal nerve–ligated, sham-operated, and naive animals. In combination with gabapentin, morphine produced a dose-related inhibition of the evoked responses in neurons in naive, sham-operated, and nerve-injured animals, with clear effects seen at approximately 40–50 min, and these were reversed by spinally applied naloxone (fig. 4). Maximal inhibitions within each experimental group were achieved with a combination of 10 mg/kg gabapentin and 4 mg/kg morphine (figs. 3A–D). In each experimental group, these inhibitions were greater than those observed with morphine alone, with the greatest effect observed in the nerve injury group. Further to the measurements displayed in figure 3, in the presence of

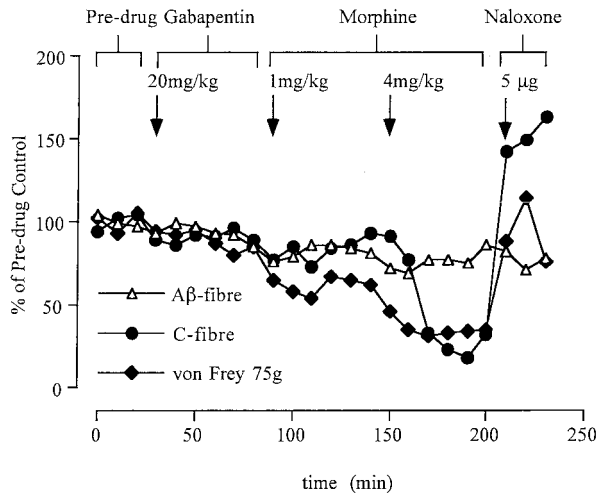


**Fig. 4. Time course of the effect of subcutaneously applied gabapentin followed by morphine, with spinal naloxone reversal, on the evoked response of a typical dorsal horn neuron (see Methods) recorded from a spinal nerve–ligated rat. Examples of the effect on the Aβ-fiber (Δ), C-fiber (●), and von Frey (75 g) (◆) measurements are shown with the cumulative dose indicated. Data are expressed as percent of predrug control value.**

10 mg/kg, both doses of morphine significantly inhibited the von Frey 9-g and heat-evoked responses in all three experimental groups. When morphine was used in combination with 20 mg/kg gabapentin, the levels of inhibition reached were no greater than those achieved in the presence of 10 mg/kg gabapentin (figs. 3A–D). The effects of the gabapentin–morphine combination were fairly comparable between sham and spinal nerve ligation groups. However, in the neuropathic group, there was a more marked increase in the inhibitions achieved with morphine in the presence of gabapentin compared with the effects of morphine alone (fig. 3). Because in the sham group the levels of inhibition mediated by morphine alone were greater than compared with that observed after nerve ligation, the increase observed in the presence of gabapentin in the sham group was not as marked. However, statistical significance was found for input (fig. 3C) using 4 mg/kg morphine in conjunction with 20 mg/kg gabapentin and for heat (data not shown) using 1 mg/kg morphine with 20 mg/kg gabapentin. The neuronal responses of the naive group were least inhibited by the drug combination (figs. 3A–D) for which no statistically significant difference was found in the comparison of the effects of morphine in the presence of gabapentin to morphine alone.

## Discussion

Unilateral tight ligation of L5 and L6 spinal nerves produced reproducible nociceptive syndromes in the lesioned hind paw. A clear withdrawal reflex with associated aversive behaviors, indicative of the development of mechanical and cooling allodynia, was produced as previously described.[10,11] Thus, all spinal nerve–ligated animals used for the electrophysiology and subsequent pharmacology exhibited neuropathic signs; sham-operated animals did not. This is the first electrophysiologic study examining the effect of a combination of gabapentin and morphine on spinal processing of sensory information after nerve injury. In naive, sham-operated, and spinal nerve ligation groups, significant inhibitions of the evoked dorsal horn neuronal responses were achieved using a combination of the two drugs at doses that, when administered alone, lacked any effect. The greatest improvement in the effectiveness of morphine after gabapentin treatment occurred after neuropathy, in which systemic morphine was almost ineffective.

### *Effect of Morphine Alone*

The current study has shown that subcutaneously administered morphine had a reduced inhibitory effect on the electrically evoked dorsal horn neuronal responses in rats subject to spinal nerve ligation in comparison with sham-operated and naive animals. In addition, morphine dose escalation did not improve the inhibition mediated in nerve-injured rats, whereas its effect was dose-related in sham and naive groups. Doses used here were identical to those shown to have effects in behavioral studies of neuropathy and inflammation.[12] A reduced sensitivity of evoked neuronal responses in spinal nerve–ligated rats to systemic morphine has previously been demonstrated in a similar electrophysiologic study.[13] However, the antiallodynic and antinociceptive abilities of morphine in behavioral studies involving neuropathy are somewhat variable and seem to be dependent on the model of neuropathy used, behavioral assessment, and nature of stimuli used, alongside the route of morphine administration. In accordance with the current study, a reduced effectiveness of intrathecally administered morphine after neuropathy has been observed[14–17] and may relate to the observed spinal reduction of opioid receptors ipsilateral to spinal nerve ligation.[18] Spinal morphine activates spinal $\mu$ receptors, the majority of which are located on the presynaptic terminals of nociceptive C-fiber primary afferent fibers to suppress neurotransmitter release and, to a lesser extent, postsynaptic $\mu$ receptors, which result in a hyperpolarization of dorsal horn neurons. A lack of opioid receptors on the terminals of large-diameter, low-threshold A$\beta$ fibers that convey nonnoxious information explains the nociception-specific actions of morphine, as seen in the current study, whereby in all three experimental groups, A$\beta$ fiber–evoked responses were relatively spared in comparison with the C fiber–mediated responses (see also Dickenson and Suzuki[19]).

### *Effect of Gabapentin Alone*

It has been demonstrated here that subcutaneously administered gabapentin, at the highest dose used (20 mg/kg,

which was intentionally low for the main focus of the study but interestingly within the range used clinically), had a greater inhibitory effect on the evoked neuronal responses in rats subject to spinal nerve ligation in comparison with sham-operated and naive animals. Effects of gabapentin were established at approximately 40–50 min. The systemic doses used here and the time course of effects observed electrophysiologically are in accordance with those seen behaviorally.[12] Behavioral studies show gabapentin has a negligible effect against physiologic sensory nociception,[20–23] but is effective in pathophysiologic situations in which central sensitization is present. In various models of nerve injury, gabapentin has been shown to reduce any resultant mechanical or thermal allodynias and hyperalgesias. Systemically, gabapentin is antinociceptive and antiallodynic in nerve injury models[12,20,24–26] and reduces evoked dorsal horn neuronal responses after spinal nerve ligation.[27] Here, gabapentin was not without inhibitory effect in the experimentally appropriate sham-operated control group, but there was a tendency for the neuronal responses of the unoperated naive group to be facilitated. This is in accordance with other similar studies and may result from the ability of the drug to act in inflammatory states[27,28] because for sham surgery, the initial invasive injury may be sufficient to induce some inflammatory response.

Effective systemic doses of gabapentin used in preclinical studies are within a similar range (10–300 mg/kg), which fits well with the doses used in this investigation. Using the spinal route of administration, therapeutic doses of gabapentin required are much lower (10–100 $\mu$g per animal), which indicates a spinal site of action, but the mechanisms of its anticonvulsant–analgesic effects remain elusive. Gabapentin is an analog of GABA designed to cross the blood–brain barrier, but it does not seem to modulate GABA receptor function, and there are no other known neurotransmitter receptor targets (see Taylor *et al.*[4]). A gabapentin binding site has been identified as the modulatory $\alpha_2\delta$ subunit of voltage-dependent calcium channels,[5] and there is an established role for these channels in nociception, the contribution of the different subtypes dependent on the nature of the pain.[6–8] Thus, interaction of gabapentin with $\alpha_2\delta$ may influence neuronal excitability because voltage-dependent calcium channels mediate the release of excitatory neurotransmitters critical for wind-up and central sensitization (see Matthews and Dickenson[7]).

### *Effect of Gabapentin and Morphine Coadministration*

The current investigation has convincingly shown that after nerve injury, the evoked dorsal horn neuronal responses, shown to be refractory to systemic morphine, become susceptible to the inhibitory actions of morphine in a dose-related manner when in the presence of systemic gabapentin (itself at a dose that alone mediated negligible neuronal inhibitions). Morphine- and gabapentin-mediated inhibitions were reversed with spinally applied naloxone, and for the majority of neuronal responses, this often exceeded control values, indicative of some activation of endogenous opioid systems. Furthermore, the ability of spinal naloxone to reverse the combined effects of the drugs provides a means to nullify the effects of an overdose but, importantly, reveals that the site of interaction of the systemically applied drugs ultimately resides within the spinal cord. In behavioral studies, systemic gabapentin has been shown to have effects that peak 1 h after administration and that are still apparent at 5 h.[20] This indicates that gabapentin would still be active throughout the entire time course of an experiment in the current study in which morphine was administered 1 h after gabapentin and then continued for a further 2 h. Thus, there is great clinical relevance for the treatment of poorly opioid-responsive neuropathic pain for which many cases morphine dose escalation becomes limited by adverse side effects. The majority of the evoked neuronal responses were significantly inhibited by a combination of gabapentin and morphine, with the greatest effects seen on the C-fiber, postdischarge, input, and von Frey–evoked responses. These observations are important because high-threshold C fibers specifically relay nociceptive information, postdischarge is a measure of spinal cord hyperexcitability, and von Frey–evoked responses may relate to behaviorally observed mechanical allodynia or hyperalgesia, all important features of neuropathy. In contrast, the A$\beta$-fiber response was relatively spared, which may indicate that physiologic touch sensations would be left intact.

There are many benefits to a multidrug regimen. Because neuropathic pain has many causes and resultant symptoms, the underlying dysfunctional mechanisms are likely to be diverse—hence its problematic clinical management. The use of gabapentin to target the excitatory system and morphine to target the inhibitory system tackles a sensitized spinal cord from two angles. It is also likely to offer a better side effect profile because as shown in this study, only low doses of both gabapentin and morphine, each alone being ineffective, were required to mediate significant effects. The use of gabapentin in conjunction with morphine may also impart important characteristics of gabapentin-mediated antinociception seen in preclinical studies, which morphine lacks—in particular, its ability to block both static and dynamic components of mechanical allodynia[12] and the lack of tolerance development with chronic use.[21]

There is an increasing amount of clinical data in support of the control of various neuropathic pain with gabapentin, such as postherpetic neuralgia, painful diabetic neuropathy, multiple sclerosis, and trigeminal neuralgia, to name a few.[2] Two separate randomized, double-blind studies in postherpetic neuralgia patients[29] and patients with diabetic peripheral neuropathy[30] reported

significant reductions in pain scores compared with placebo over a period of 8 weeks. Furthermore, improvements in sleep and mood were noted for the gabapentin-treated patients, and tolerance did not seem to develop. Reported adverse effects included somnolence and dizziness; however, occurrence of these was minimal and, when experienced, only mild to moderate. Alongside other studies and case reports, it has been proposed that gabapentin is a useful first-line treatment for chronic neuropathic pain, especially when other therapies fail.

The clinical use of opioids in neuropathic pain is more complex, and varied responsiveness is reported, which may be due to the different types of pain, with differing causal dysfunctions. There have been many randomized, double-blind controlled trials conducted in this area,[31] and morphine effectiveness ranges from opioid resistant[32] to modest pain relief[33] to good pain relief.[34] It is widely believed that neuropathic pain is less susceptible but not resistant to systemic morphine,[35,36] as observed in the current study. Documented morphine side effects include sedation, nausea, vomiting, constipation, respiratory depression, and tolerance, which may prevent dose escalation and thus provide inadequate analgesia.[37] Interestingly, the results obtained from this study are generally in accordance with the observed clinical data surrounding the use of morphine and gabapentin alone in neuropathic pain. The limitations of opioids have led to the concept of combination therapy in the hope of attaining a favorable balance of analgesia and side effects with a reduction in the dose. We have demonstrated here the potential for the use of low dose morphine and gabapentin combinational treatment after neuropathy.

## References


1. Sindrup SH, Jensen TS: Pharmacologic treatment of pain in polyneuropathy. Neurology 2000; 55:915–20
2. Nicholson B: Gabapentin use in neuropathic pain syndromes. Acta Neurol Scand 2000; 101:359–71
3. Satzinger G: Antiepileptics from gamma-aminobutyric acid. Arzneimittelforschung 1994; 44:261–6
4. Taylor CP, Gee NS, Su TZ, Kocsis JD, Welty DF, Brown JP, Dooley DJ, Boden P, Singh L: A summary of mechanistic hypotheses of gabapentin pharmacology. Epilepsy Res 1998; 29:233–49
5. Gee NS, Brown JP, Dissanayake VU, Offord J, Thurlow R, Woodruff GN: The novel anticonvulsant drug, gabapentin (Neurontin), binds to the alpha2delta subunit of a calcium channel. J Biol Chem 1996; 271:5768–76
6. Vanegas H, Schaible H: Effects of antagonists to high-threshold calcium channels upon spinal mechanisms of pain, hyperalgesia and allodynia. Pain 2000; 85:9–18
7. Matthews EA, Dickenson AH: Effects of spinally delivered N- and P-type voltage-dependent calcium channel antagonists on dorsal horn neuronal responses in a rat model of neuropathy. Pain 2001; 92:235–46
8. Matthews EA, Dickenson AH: Effects of ethosuximide, a T-type Ca(2+) channel blocker, on dorsal horn neuronal responses in rats. Eur J Pharmacol 2001; 415:141–9
9. Portenoy RK, Foley KM, Inturrisi CE: The nature of opioid responsiveness and its implications for neuropathic pain: new hypotheses derived from studies of opioid infusions. Pain 1990; 43:273–86
10. Kim SH, Chung JM: An experimental model for peripheral neuropathy produced by segmental spinal nerve ligation in the rat. Pain 1992; 50:355–63
11. Chapman V, Suzuki R, Dickenson AH: Electrophysiological characterization of spinal neuronal response properties in anaesthetized rats after ligation of spinal nerves L5-L6. J Physiol 1998; 507:881–94
12. Field MJ, McCleary S, Hughes J, Singh L: Gabapentin and pregabalin, but not morphine and amitriptyline, block both static and dynamic components of mechanical allodynia induced by streptozocin in the rat. Pain 1999; 80:391–8
13. Suzuki R, Chapman V, Dickenson AH: The effectiveness of spinal and systemic morphine on rat dorsal horn neuronal responses in the spinal nerve ligation model of neuropathic pain. Pain 1999; 80:215–28
14. Ossipov MH, Lopez Y, Nichols ML, Bian D, Porreca F: The loss of antinociceptive efficacy of spinal morphine in rats with nerve ligation injury is prevented by reducing spinal afferent drive. Neurosci Lett 1995; 199:87–90
15. Ossipov MH, Lopez Y, Nichols ML, Bian D, Porreca F: Inhibition by spinal morphine of the tail-flick response is attenuated in rats with nerve ligation injury. Neurosci Lett 1995; 199:83–6
16. Wegert S, Ossipov MH, Nichols ML, Bian D, Vanderah TW, Malan TP Jr, Porreca F: Differential activities of intrathecal MK-801 or morphine to alter responses to thermal and mechanical stimuli in normal or nerve-injured rats. Pain 1997; 71:57–64
17. Yamamoto T, Sakashita Y: Differential effects of intrathecally administered morphine and its interaction with cholecystokinin-B antagonist on thermal hyperalgesia following two models of experimental mononeuropathy in the rat. ANESTHESIOLOGY 1999; 90:1382–91
18. Porreca F, Tang QB, Bian D, Riedl M, Elde R, Lai J: Spinal opioid mu receptor expression in lumbar spinal cord of rats following nerve injury. Brain Res 1998; 795:197–203
19. Dickenson AH, Suzuki R: Function and dysfunction of opioid receptors in the spinal cord, Progress in Pain Research and Management. Edited by Kalso E, McQuay H, Wiesenfeld-Hallin Z. Seattle, IASP, 1999, pp 17–44
20. Hunter JC, Gogas KR, Hedley LR, Jacobson LO, Kassotakis L, Thompson J, Fontana DJ: The effect of novel anti-epileptic drugs in rat experimental models of acute and chronic pain. Eur J Pharmacol 1997; 324:153–60
21. Field MJ, Oles RJ, Lewis AS, McCleary S, Hughes J, Singh L: Gabapentin (neurontin) and S-(+)-3-isobutylgaba represent a novel class of selective antihyperalgesic agents. Br J Pharmacol 1997; 121:1513–22
22. Yoon MH, Yaksh TL: The effect of intrathecal gabapentin on pain behavior and hemodynamics on the formalin test in the rat. Anesth Analg 1999; 89:434–9
23. Shimoyama N, Shimoyama M, Davis AM, Inturrisi CE, Elliott KJ: Spinal gabapentin is antinociceptive in the rat formalin test. Neurosci Lett 1997; 222:65–7
24. Abdi S, Lee DH, Chung JM: The anti-allodynic effects of amitriptyline, gabapentin, and lidocaine in a rat model of neuropathic pain. Anesth Analg 1998; 87:1360–6
25. Field MJ, Hughes J, Singh L: Further evidence for the role of the alpha(2)delta subunit of voltage dependent calcium channels in models of neuropathic pain. Br J Pharmacol 2000; 131:282–6
26. Pan HL, Eisenach JC, Chen SR: Gabapentin suppresses ectopic nerve discharges and reverses allodynia in neuropathic rats. J Pharmacol Exp Ther 1999; 288:1026–30
27. Chapman V, Suzuki R, Chamarette HL, Rygh LJ, Dickenson AH: Effects of systemic carbamazepine and gabapentin on spinal neuronal responses in spinal nerve ligated rats. Pain 1998; 75:261–72
28. Stanfa LC, Singh L, Williams RG, Dickenson AH: Gabapentin, ineffective in normal rats, markedly reduces C-fibre evoked responses after inflammation. Neuroreport 1997; 8:587–90
29. Rowbotham M, Harden N, Stacey B, Bernstein P, Magnus-Miller L: Gabapentin for the treatment of postherpetic neuralgia: A randomized controlled trial. JAMA 1998; 280:1837–42
30. Backonja M, Beydoun A, Edwards KR, Schwartz SL, Fonseca V, Hes M, LaMoreaux L, Garofalo E: Gabapentin for the symptomatic treatment of painful neuropathy in patients with diabetes mellitus: a randomized controlled trial. JAMA 1998; 280:1831–6
31. Dellemijn P: Are opioids effective in relieving neuropathic pain? Pain 1999; 80:453–62
32. Arner S, Meyerson BA: Lack of analgesic effect of opioids on neuropathic and idiopathic forms of pain. Pain 1988; 33:11–23
33. Rowbotham MC, Reisner-Keller LA, Fields HL: Both intravenous lidocaine and morphine reduce the pain of postherpetic neuralgia. Neurology 1991; 41:1024–8
34. Likar R, Spendel MC, Amberger W, Kepplinger B, Supanz S, Sadjak A: Long-term intraspinal infusions of opioids with a new implantable medication pump. Arzneimittelforschung 1999; 49:489–93
35. Jadad AR, Carroll D, Glynn CJ, Moore RA, McQuay HJ: Morphine responsiveness of chronic pain: double-blind randomised crossover study with patient-controlled analgesia. Lancet 1992; 339:1367–71
36. Cherny NI, Thaler HT, Friedlander-Klar H, Lapin J, Foley KM, Houde R, Portenoy RK: Opioid responsiveness of cancer pain syndromes caused by neuropathic or nociceptive mechanisms: A combined analysis of controlled, single-dose studies. Neurology 1994; 44:857–61
37. Foley KM, Inturrisi CE: Analgesic drug therapy in cancer pain: Principles and practice. Med Clin North Am 1987; 71:207–32