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Behavioral and pharmacological characterization of a distal peripheral nerve injury in the rat

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Abstract

Previous rat neuropathic pain models have utilized peripheral nerve injuries that damage a significant proportion of large nerves such as the sciatic nerve or its divisions. Injuries that lead to neuropathic pain in humans may involve the peripheral extremities. The current study evaluated the behavioral effects of injury to the plantar nerves in the rat (distal nerve injury—DNI). A delayed onset of hypersensitivity to an innocuous mechanical stimulus was observed following cutting of the left plantar nerves, whereas mechanical hypersensitivity developed more rapidly in rats with either an injury near the sciatic nerve trunk (chronic constriction injury (CCI), spared nerve injury (SNI)) or a spinal nerve root (spinal nerve ligation (SNL). Similar to other nerve injury pain models, rats with injured plantar nerves also developed an early onset and persistent sensitivity to a cooling stimulus. The effects of morphine, gabapentin and imipramine on mechanical and cold hypersensitivity were evaluated in rats with a DNI, CCI and SNI. In all three models, morphine dose-dependently suppressed mechanical and cold hypersensitivity, whereas gabapentin only suppressed mechanical hypersensitivity. Imipramine had no effect on either cold or mechanical hypersensitivity in any of the nerve-injured rats. The pharmacological data suggest that the underlying basis of neuropathic pain may be similar irrespective of the site of nerve injury.

Keywords: Antinociception; Gabapentin; Imipramine; Morphine; Neuropathic pain; Plantar nerve

1. Introduction

Peripheral tissue injury leads to spontaneous pain and increased sensitivity to cutaneous stimulation. The underlying mechanism is due in part to changes in gene expression, neurochemistry and neural circuitry in regions that process sensory stimuli, including the primary afferent, spinal cord and brain (Dubner and Ruda, 1992; Mayer et al., 1999). These changes also underlie the persistence of abnormal injury-induced pain perception long after tissue healing and the presence of pain in areas in regions that are far removed from the injury site.

Pain following peripheral nerve injury is particularly debilitating, characterized by allodynia, hyperalgesia and spontaneous pain (Dworkin et al., 2003). A wide range of drugs have been used to treat neuropathic pain. Drugs that were not originally developed for pain treatment, such as anticonvulsants and tricyclic antidepressants (TCA), have been used with varying degrees of success in human (Collins et al., 2000; McQuay et al., 1995, 1996; Rowbotham et al., 1998). Opioids have also been used in neuropathic pain (Raja et al., 2002; Rowbotham et al., 2003; Watson and Babul, 1998). Due to the diverse etiology of clinical neuropathic pain, it is difficult to clarify the mechanism in humans although mechanism-based clinical approaches to diagnosis and treatment have been proposed (Dworkin et al., 1992; Max, 2000). Also, evaluation of potentially novel therapeutics in humans on a wide scale is

Abbreviations: CCI, chronic constriction injury; DNI, distal nerve injury; DRG, dorsal root ganglion; SNI, spared nerve injury; SNL, spinal nerve ligation; TCA, tricyclic antidepressant.

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time-consuming and costly. To fill these needs, animal models of neuropathic pain have been developed.

There are several trauma-evoked pain models in rodents that involve cutting or ligating a nerve at the level of mid-thigh or close to the spinal cord (Bennett and Xie, 1988; Kim and Chung, 1992; Seltzer et al., 1990). In the chronic constriction injury (CCI) model, the sciatic nerve is loosely ligated proximal to the point at which it trifurcates. In the spinal nerve ligation (SNL) model, spinal nerve roots, distal to the dorsal root ganglia, are tightly ligated. Finally, in the partial nerve injury model, 1/3–1/2 of the sciatic (proximal to the trifurcation) is tightly ligated. These models have been useful in clarifying the mechanism of neuropathic pain and have been used to validate various pain-related targets (e.g., Fox et al., 2003; Hunter et al., 1997).

Persistent pain and paresthesia may arise following trauma to distal nerve branches as well as to nerve trunks, such as injury to distal nerve branches of the lower leg (Kim and Dellon, 2001; Kumar and Jacob, 2004; Turan et al., 1997). Thus, the currently available models of trauma-induced neuropathic pain may not reflect this clinical condition. A few investigators have developed pain models with somewhat more distal nerve injuries involving cutting several branches of the sciatic nerve (tibial, peroneal and sural) and the effect of clinically relevant drugs have been evaluated in these models (Decosterd and Woolf, 2000; Erichsen and Blackburn-Munro, 2002; Lee et al., 2000).

Nerve injury in the distal limb, near the hind paw rather than at mid-thigh or near the vertebral column, could lead to a robust and long lasting hypersensitivity as observed in other rat neuropathic pain models. Ectopic activity originating from primary afferents may in part underlie the abnormal sensory perception observed following injury to nerve roots or the nerve trunk (Kajander and Bennett, 1992; Liu et al., 1999). Injury to more distal nerves may also lead to afferent spontaneous activity and subsequent neuropathic pain (Pogatzki et al., 2002), though possibly to a lesser extent or delayed onset. The current study compared the behavioral effect of cutting the medial and lateral plantar nerves near the ankle with other established rat neuropathic pain models. Also, gabapentin, imipramine and morphine were evaluated in these rats to compare the behavioral responses to these antinociceptive drugs.

2. Methods

Male Sprague–Dawley rats were obtained from Harlan (Indianapolis, IN) and weighed 100–150 g at the time of surgery. Rats had free access to food and water and were group-housed in a room on a 12-h light/dark cycle. Rats were allowed to acclimate at least 3 days prior to use in experiments. Procedures were approved by the internal institutional animal care and use committee and followed guidelines of the National Institutes of Health "Guide for the Care and Use of Laboratory Animals." Following the termination of the study, rats were euthanized by CO_2 overdose.

2.1. Peripheral nerve injury surgery

Following baseline behavioral evaluation, either a peripheral nerve injury or sham surgery was performed using aseptic technique. Anesthesia was induced with 3–4% isoflurane/O_2 and maintained with 2–3% isoflurane/O_2 (Abbott Laboratories, IL). The plantar nerves were exposed (DNI; $n=18$) by making a 1-cm incision of the skin of the left, lower medial hind limb. The gastrocnemius muscle was retracted, exposing the medial and lateral plantar nerves. The nerves were gently isolated and cut. The skin was closed with Nexaband (Veterinary Products Laboratories, Phoenix, AZ). In sham-operated rats, the plantar nerves were exposed but not cut.

In a separate group of rats, a chronic constriction injury (CCI; $n=24$) was performed on the left sciatic nerve at the level of the mid-thigh. Four 4–0 chromic gut ligatures were loosely ligated around the sciatic nerve (Bennett and Xie, 1988). A spared nerve injury (SNI; $n=17$) was performed by exposing the left common peroneal and tibial nerve branches of the sciatic nerve. Following tight ligation with a 6–0 silk suture, the nerve branches were cut distal to the ligation (Decosterd and Woolf, 2000). In both the CCI and SNI, the muscles overlying the sciatic nerve or its branches were sutured together and the skin closed with Nexaband. To produce a spinal nerve ligation (SNL; $n=16$), an incision was made on the back to expose the left L5 and L6 spinal nerve roots (Kim and Chung, 1992). The nerve roots were tightly ligated with 6–0 silk ligatures and the wound sutured shut. The skin was closed with Nexaband. In sham-operated rats, the left nerves or nerve roots were exposed but not ligated.

2.2. Sensory testing

Injured and sham-operated rats were tested 1, 2, 3, 4 and 8 weeks after surgery. Sensory testing with mechanical, cooling and heat stimuli was conducted in that order. Rats were placed on an elevated wire mesh floor and enclosed in plexi-glass containers and were not removed until the completion of both mechanical and cold sensory testing. Following cold testing, rats were then placed in the thermal testing apparatus. The numbers of stimuli presented to the rats were determined to be the minimum numbers needed to evoke reproducible and robust behavioral responses.

2.2.1. Mechanical

Rats were acclimated to the apparatus for at least 15 min prior to testing. The responsiveness to innocuous mechanical stimulation was determined by probing the lateral edge of the plantar hind paw surface with an ascending series of von Frey filaments (4.08 (1.2 g), 4.93 (9 g), 5.07 (12 g), 5.18 (15 g); Stoelting Co., Wood Dale, IL). Starting with the

lowest force, the filament was placed on the skin until it bowed slightly, with each filament presented five times at a rate of about 1/s. A different region within the testing area was stimulated with each presentation. A response was recorded if the rat withdrew its hind paw from the filament. Responses were converted into percent frequencies (%=number of responses/5 × 100).

2.2.2. Cooling

Five to ten minutes elapsed between testing of the last von Frey filament and the first application of acetone. Acetone (~100 μl) was ejected onto the lateral plantar hind paw surface via a 22-g blunted needle attached to a 1-ml[3] syringe. A response was recorded if the rat withdrew its paw in response to acetone application. The number of responses to three presentations was converted into a percent frequency.

2.2.3. Thermal

Following cold sensory testing, rats were placed in a modified apparatus described by Hargreaves et al. (1988; Stoelting Co.). Rats were placed in plexi-glass chambers which rested on an elevated glass surface. Below the glass surface was an infrared beam emitter. Following 30 min of acclamation in the chamber, an infrared beam was shined on the plantar hind paw, which simultaneously started a timer. The beam (and the timer) shut off when the rat withdrew its hind paw from the glass surface. The withdrawal latency (measured in seconds) was measured three times for the left and right hind paws. The last two latencies were used to calculate the mean withdrawal latency. A period of 3 to 5 min elapsed between testing of the same paw of a given rat. A cut-off of 20 s was used to prevent tissue damage.

2.3. Effect of drugs in neuropathic rats

In a separate group of nerve-injured rats, the effects of drugs on mechanical and cold hypersensitivity were evaluated. Drugs were tested at times when peak mechanical and cold hypersensitivity was observed, 2–3 weeks after injury in the CCI and SNI rats and 4–5 weeks after injury in the DNI rats. Because the most consistent and robust response in neuropathic rats was obtained with the 15-g filament, this filament was used to evaluate drug effects (the 15-g filament did not evoke significant hind paw lifts in rats prior to nerve injury; see Results and Fig. 1). To be included in the drug study, nerve-injured rats needed to respond three or more times to the 15-g von Frey filament out of five total presentations or two or more times out of three applications of acetone to the ipsilateral (nerve-injured) hind paw. After baseline determination in CCI (n=124), SNI (n=110) and DNI (n=118) rats, either vehicle (saline) or one of the following doses of drugs was subcutaneously (s.c.) injected in a volume of 1 ml/kg: 10, 30, 100 mg/kg gabapentin

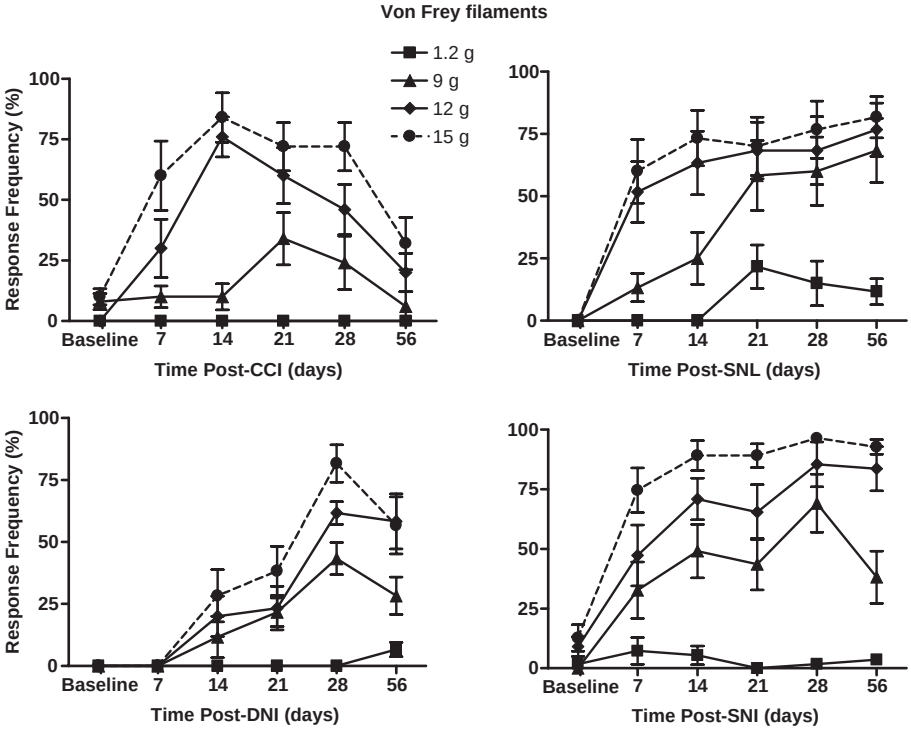

Fig. 1. Hypersensitivity to tactile stimuli over time following peripheral nerve injury. Von Frey filaments (1.2, 9, 12, 15 g) were applied five times to the lateral–plantar hind paw and the number of positive responses recorded. Rats with either a chronic constriction injury (CCI), spared nerve injury (SNI), spinal nerve ligation (SNL) or distal (plantar) nerve injury (DNI) were tested 1, 2, 3, 4 and 8 weeks following nerve injury. Data are expressed as mean±S.E.M. n=10–12 per group.

Table 1
Baseline hind paw response frequencies to mechanical stimuli in rats

	SNI (%)	CCI (%)	SNL (%)	DNI (%)
Pre-sham	3.3±1.6	5.0±1.8	0±0	1.3±0.9
Pre-injury	5.9±1.9	4.0±1.8	0±0	0±0

Total mean response frequencies (percent) to all four filaments (1.2, 9, 12, 15 g) measured prior to either a sham or nerve injury surgery of the left hind paw. Data are expressed as mean percent±S.E.M. $N=10–12$ per group.

(Toronto Research Chemicals, Canada), 20 mg/kg imipramine HCl (Sigma-Aldrich, St. Louis, MO) or 1, 3, 10 mg/kg morphine sulfate (Sigma-Aldrich). Following s.c. injection, rats were tested once every 30 min, up to 180 min post-injection.

2.4. Statistics

A two-way repeated measures analysis of variance (ANOVA) was used to compare the effect of sham surgery or the contralateral hind paw vs. the ipsilateral hind paw over time and the effect of drug treatment over time. A one-way repeated measures ANOVA was used to evaluate the effect of injury over time. Where significant treatment and time interactions were observed, the data were analyzed with Student–Neuman–Keuls as the post-hoc test. Statistical significance was taken at p value less than 0.05. To determine the dose at which drug efficacy was 50% of maximal (ED_{50}), a modified computer program utilized by Tallarida and Murray (1983) was used to calculate this value.

3. Results

3.1. Behavioral characterization

Prior to peripheral nerve surgery, the left and right hind paws were minimally responsiveness to all von Frey filaments (an overall mean response frequency to all four filaments of about 2%; Table 1, also "Baseline" in Fig. 1). Also, the rats were not responsive to acetone (0% response frequency; "Baseline" in Fig. 2). The mean withdrawal latencies to a noxious heat stimulus of the left and right hind paws before surgery are shown in Table 2. Prior to surgery, there were no significant differences between the left and right hind paw latencies between the groups and there were no differences between left and right hind paws within each group.

Following sham surgery, no significant changes in response to either von Frey filaments or acetone were observed over time, compared to pre-operative responses. Mean withdrawal latencies to heat over time for the sham-operated hind paw and were not significantly changed from baseline (Fig. 3). The contralateral withdrawal latencies of nerve-injured rats did not significantly alter from baseline pre-injury latencies (Fig. 3).

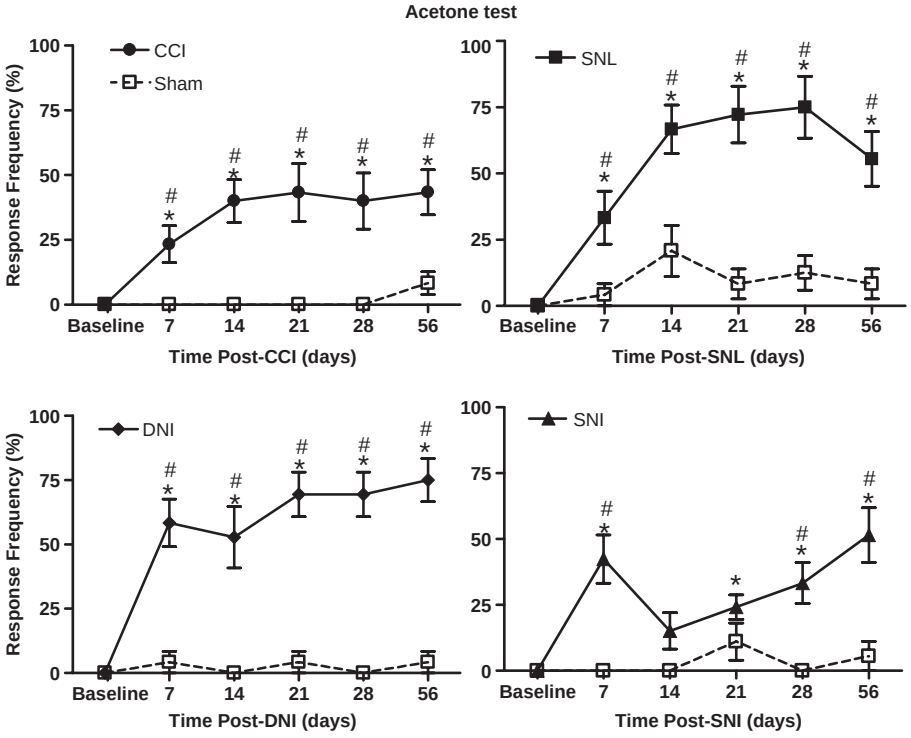

Fig. 2. Hypersensitivity to a cooling stimulus over time following peripheral nerve injury. A 100-μl drop of acetone was applied three times to the lateral hind paw and the number of responses recorded. Rats were tested 1, 2, 3, 4 and 8 weeks following nerve injury. Data are expressed as mean±S.E.M. $n=10–12$ per group. *$p<0.05$ vs. pre-operative "Baseline"; #$p<0.05$ vs. sham-operated.

Table 2
Baseline hind paw withdrawal latencies to a noxious heat stimulus in rats

	SNI (s)		CCI (s)		SNL (s)		DNI (s)	
	Left	Right	Left	Right	Left	Right	Left	Right
Pre-sham	10.6±0.7	9.2±1.0	10.7±0.4	10.0±0.5	8.4±0.3	9.0±0.4	10.7±0.6	11.4±0.6
Pre-injury	10.1±0.5	9.4±0.4	10.7±0.5	10.5±0.6	10.1±0.6	9.7±0.7	10.0±0.5	9.4±0.4

Withdrawal latencies (seconds) measured prior to either a sham or nerve injury surgery of the left hind paw. Data are expressed as mean±S.E.M. $N=10-12$ per group.

In the behavioral comparison of the neuropathic rat models, responders as well as non-responders were analyzed over time and the data are presented as mean±S.E.M. (Figs. 1–3).

The ipsilateral hind paw of rats with a CCI, DNI or SNL was everted and in some the toes were flexed. In contrast, no gross postural abnormalities or motor un-coordination was observed in rats with a DNI. The toes were extended as normal and there was no obvious gait impairment in these rats. Robust pain-related behavior was observed following either cold or mechanical stimulation in DNI rats, similar to that observed in the other nerve-injured rats (e.g., biting or licking of the toes, hind paw shaking).

3.1.1. Mechanical hypersensitivity

Beginning 1 week after nerve injury, the ipsilateral (nerve-injured) hind paw of rats with a CCI, SNL and SNI displayed increased responsiveness to von Frey filaments (Fig. 1). In these rats, responses to mechanical stimulation peaked at 2 weeks after surgery. The persistence of the increased responses varied between the groups. In CCI rats, the response frequency to the 12- and 15-g filaments decreased to less than 50% 8 weeks after surgery but in SNL and SNI rats the response frequencies remained at greater than 70% 8 weeks after surgery. By contrast, mechanical hypersensitivity in rats with a DNI increased gradually over time. Comparing the onset of hypersensitivity to the 15-g filament between the models, the responses of the DNI rats were significantly lower than those of the other models 7–21 days after nerve injury ($F(3,41)=8.7$, $p<0.001$). By 8 weeks after surgery, the response of the DNI rats to the 15-g filament was greater than that of the CCI rats but less than that of the SNI and SNL rats.

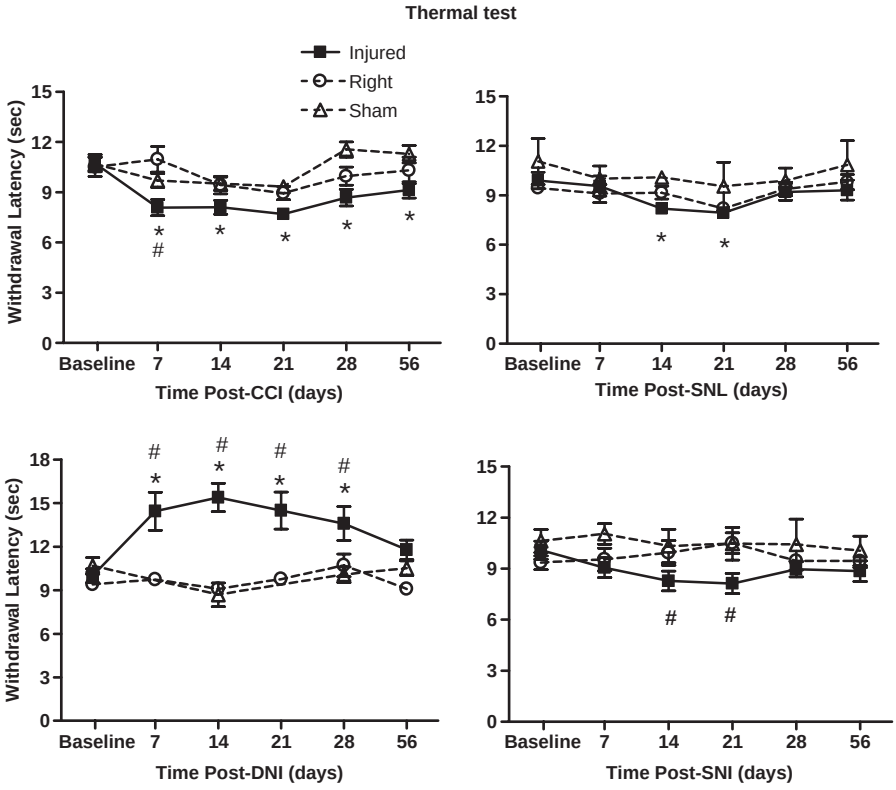

Fig. 3. Hypersensitivity to a noxious heat stimulus over time following peripheral nerve injury. A noxious heat stimulus was applied to the plantar hind paw and the latency (seconds) to respond with a withdrawal was recorded. Rats were tested 1, 2, 3, 4 and 8 weeks following nerve injury. Data are expressed as mean±S.E.M. $n=10-12$ per group. *$p<0.05$ vs. pre-operative "Baseline"; #$p<0.05$ vs. contralateral hind paw.

3.1.2. Cold hypersensitivity

One week following peripheral nerve injury, CCI, SNL, SNI and DNI rats displayed significant responses to the placement of acetone on the ipsilateral hind paw, whereas sham-operated rats did not respond to acetone (CCI: $F(1,20)=32.3$, $p<0.001$; SNL: $F(1,14)=7.9$, $p<0.014$; SNI: $F(1,15)=23.8$, $p<0.001$; DNI: $F(1,22)=90.7$, $p<0.001$; Fig. 2). Response frequencies in the CCI, SNL and DNI rats remained elevated, greater than that at baseline and of sham-operated rats, for up to 8 weeks following injury. In rats with an SNI, the response frequency was less than 25% 2 weeks after injury, but increased to about 50% at 8 weeks.

In preliminary experiments, a drop (~100 μl) of distilled water was placed on the rat's paw twice, alternating with placement of acetone. Neither the pre-operative uninjured nor the post-operative injured hind paw responded to water. Thus the response in neuropathic rats to acetone was to acetone's cooling effect rather than to the tactile sensation of the liquid.

3.1.3. Thermal hypersensitivity

The CCI, SNL and SNI rats developed thermal hyperalgesia, that is, significantly decreased withdrawal latencies of the ipsilateral hind paw compared to either the pre-operative or the contralateral withdrawal latencies (Fig. 3). In rats with a CCI, withdrawal latencies for the ipsilateral hind paw were significantly lower than the contralateral hind paw ($F(1,18)=14.1$, $p<0.001$) and baseline latencies ($F(df=5)=7.3$, $p<0.001$). There was a small decrease in withdrawal latencies of the contralateral hind paw, but the change was not statistically significant. In rats with a SNL, there were decreases in the latencies of the ipsilateral hind paw 2 and 3 weeks after surgery ($F(df=5)=3.4$, $p=0.01$). However, the latencies of the ipsilateral hind paw were not different from those of the contralateral hind paw, due to the latencies being slightly decreased from baseline ($F(1, 22)=0.5$, $p=0.5$). In rats with an SNI, a significant difference between the ipsilateral and contralateral hind paws was observed at 2 and 3 weeks post-surgery ($F(1, 20)=7.7$, $p=0.012$). The difference is due to a slight increase of the latency of the contralateral hind paw as well as a decreased latency of the ipsilateral hind paw. The ipsilateral hind paw withdrawal latency at 2 and 3 weeks were not statistically significant from baseline ($F(df=6)=1.6$, $p=0.18$).

In contrast to the other nerve injuries, DNI caused significantly increased withdrawal latencies, compared to the contralateral ($F(1, 22)=24.1$, $p<0.001$) and baseline latencies ($F(df=5)=5.5$, $p<0.001$), beginning 1 week following injury and persisting up to 4 weeks after surgery (baseline vs. time after DNI, contralateral vs. ipsilateral). The latency of the ipsilateral hind paw was no longer significantly different from baseline by 8 weeks post-surgery.

3.2. Drug effects on mechanical hypersensitivity

Prior to injection, the mean percent response frequency to the 15-g filament of all nerve-injured rats was 79.7±1.2% (Figs. 4–6). Baseline pre-injection response frequencies were not significantly different between the three neuropathic models. Gabapentin dose-dependently decreased mechanical hypersensitivity in the SNI and DNI rats, but only the highest dose of gabapentin decreased hyper-

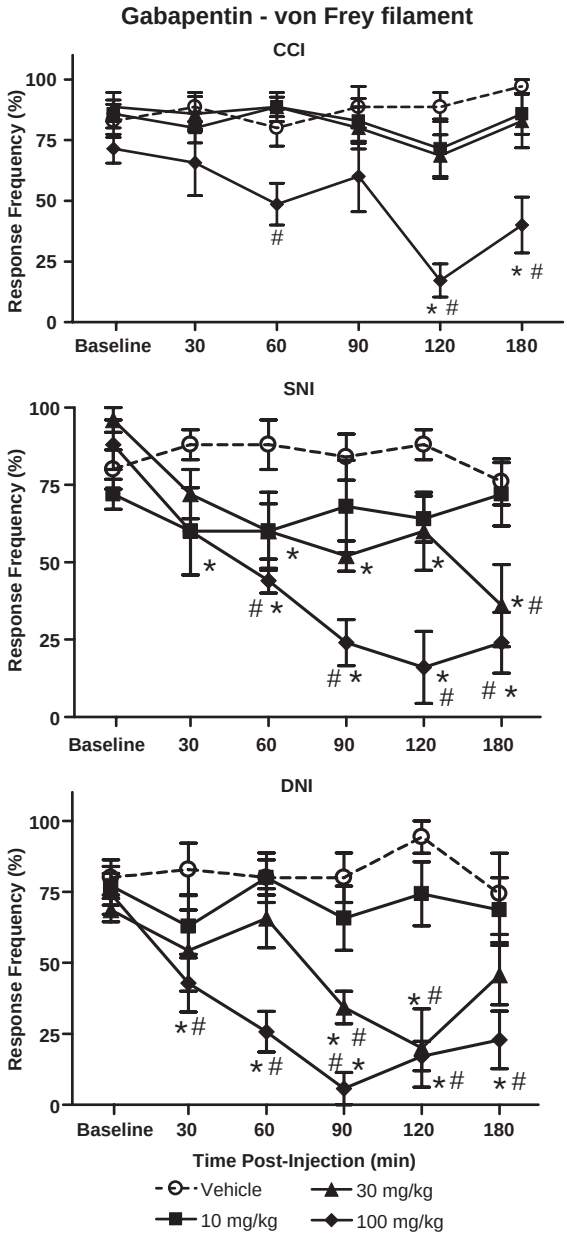

Fig. 4. Effect of gabapentin on tactile hypersensitivity in nerve-injured rats. At "baseline," 15-g von Frey filament was applied five times to the lateral–plantar hind paw and the number of positive responses recorded. Following baseline testing, chronic constriction injury (CCI), spared nerve injury (SNI) and distal (plantar) nerve injury (DNI) rats were injected subcutaneously with either vehicle, 10, 30 or 100 mg/kg of gabapentin. Data are expressed as mean±S.E.M. $n=5–7$ per group. *$p<0.05$ vs. "Baseline"; #$p<0.05$ vs. vehicle.

Table 3
Antinociceptive effect of drugs on nerve injury-evoked mechanical and cold hypersensitivity

Drug	SNI		CCI		DNI	
	Tactile	Cold	Tactile	Cold	Tactile	Cold
Gabapentin	31.9 (16.3–62.4)	NE	40.8 (24.3–68.6)	NE	19.3 (11.0–34.1)	NE
Morphine	2.2 (1.4–3.4)	1.9 (0.5–6.9)	2.9 (1.8–4.7)	1.8 (0.9–3.5)	2.2 (1.4–3.5)	2.0 (1.1–3.8)
Imipramine	NE	NE	NE	NE	NE	NE

ED_{50} (in mg/kg (95% C.L.)) of morphine calculated 60 min after injection and ED_{50} of gabapentin calculated 120 min after injection. NE—no effect. $N=5-7$ per group.

sensitivity in the CCI rats (SNI: $F(15,20)=3.3$, $p<0.001$; DNI: $F(15,24)=2.7$, $p=0.002$; CCI: $F(15,24)=1.9$, $p=0.03$; Fig. 4). In contrast, rats that were injected with vehicle continued to show hypersensitivity to von Frey stimulation throughout the observation period. The peak efficacy of gabapentin was about 120 min post-injection in all three neuropathic models, thus the ED_{50} was calculated at that time point. The ED_{50} of gabapentin in rats with a SNI and CCI was 32 and 41 mg/kg, respectively. Gabapentin in rats with a DNI was more potent compared to the other nerve injury models (21 mg/kg) but was this was not statistically significant compared with the ED_{50}s of the other models (t-test, $p>0.05$; Table 3).

Morphine dose-dependently reduced mechanical hypersensitivity in nerve-injured rats, whereas vehicle-injected rats continued to show hypersensitivity throughout the time course (SNI: $F(15,20)=4.4$, $p<0.001$; DNI: $F(15,24)=2.3$, $p=0.006$; CCI: $F(15,20)=7.0$, $p<0.001$; Fig. 5). The ED_{50} of morphine 60 min after injection was similar across the nerve injury models, about 2 mg/kg.

Since the response frequencies of the neuropathic rats to vehicle were not significantly different from each other, the response frequencies of all vehicle-injected rats were combined ($n=15$; Fig. 6). Imipramine did not alter mechanical hypersensitivity in nerve-injured rats.

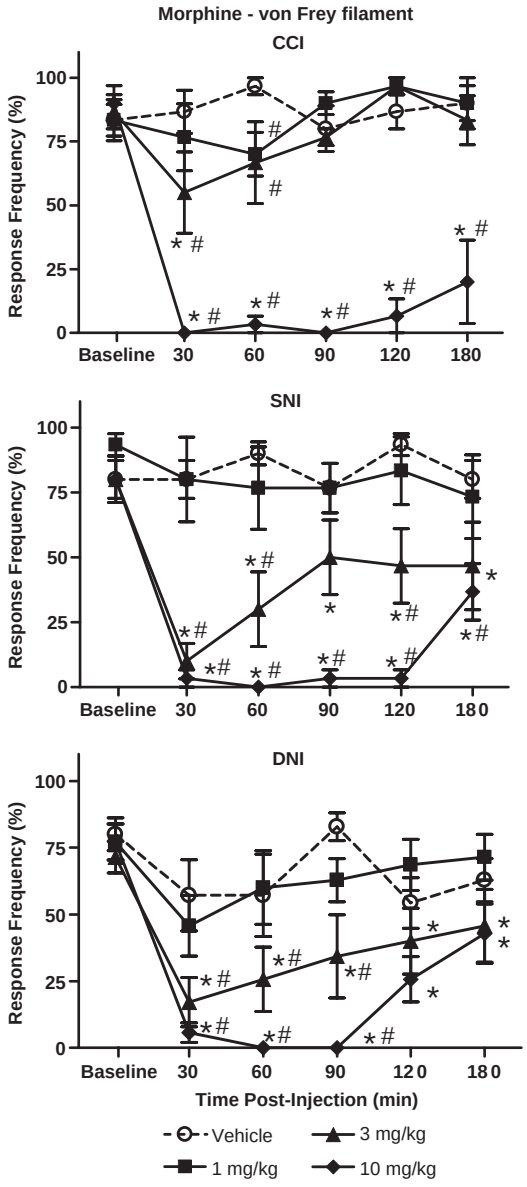

Fig. 5. Effect of morphine on tactile hypersensitivity in nerve-injured rats. At "baseline," a 15-g von Frey filament was applied five times to the lateral–plantar hind paw and the number of positive responses recorded. Following baseline testing, rats were injected subcutaneously with either vehicle, 1, 3 or 10 mg/kg of morphine. Data are expressed as mean±S.E.M. $n=6-7$ per group. *$p<0.05$ vs. "Baseline"; #$p<0.05$ vs. vehicle.

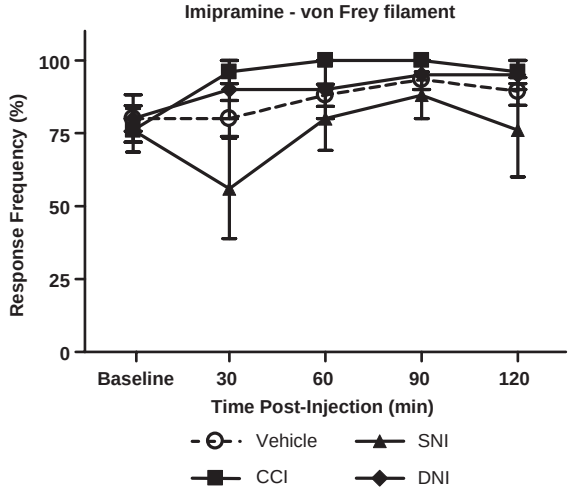

Fig. 6. Effect of imipramine on tactile hypersensitivity in nerve-injured rats. At "baseline," a 15-g von Frey filament was applied five times to the lateral–plantar hind paw and the number of positive responses recorded. Following baseline testing, rats were injected subcutaneously with either vehicle or 20 mg/kg of imipramine. Data are expressed as mean±S.E.M. $n=5-15$ per group.

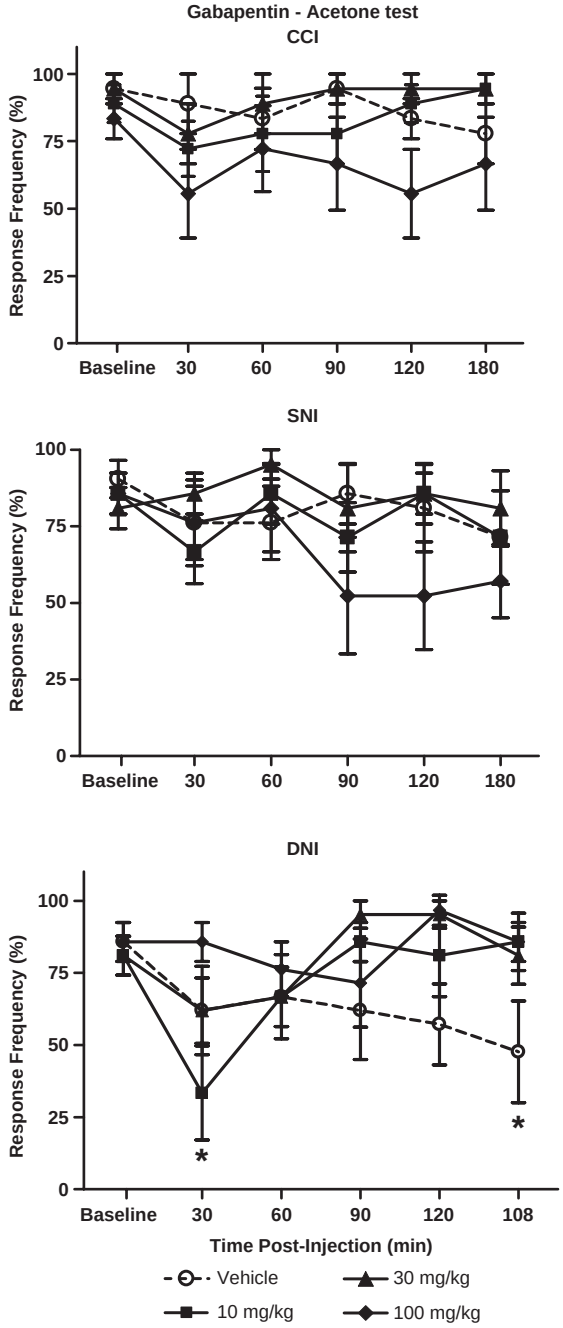

Fig. 7. Effect of gabapentin on cold hypersensitivity in nerve-injured rats. At "baseline," a 100-μl drop of acetone was applied three times to the lateral hind paw and the number of responses recorded. Following baseline testing, chronic constriction injury (CCI), spared nerve injury (SNI) and distal (plantar) nerve injury (DNI) rats were injected subcutaneously with either vehicle, 10, 30 or 100 mg/kg of gabapentin. Data are expressed as mean±S.E.M. $n=6–7$ per group. *$p<0.05$ vs. "Baseline"; #$p<0.05$ vs. vehicle.

3.2.1. Drug effects on cold hypersensitivity

Prior to injection, the mean percent response frequency of all nerve-injured rats to acetone was 85.6±1.2% (Figs. 7–9). There was no significant difference in the baseline responsiveness to acetone between the nerve injury models. Gabapentin decreased responses 30 min after injection in the DNI model ($F(15,24)=2.2$, $p=0.0011$; Fig. 7). Responses to acetone in these rats, however, decreased over time, such that responses at 180 min after vehicle injection were less than baseline. Gabapentin had no statistically significant effect in either the CCI or SNI models.

Morphine dose-dependently reduced cold hypersensitivity across all nerve injury models (CCI: $F(15,24)=3.6$, $p<0.001$; SNI: $F(15,24)=2.1$, $p=0.016$; DNI: $F(15,20)=2.6$, $p=0.003$; Fig. 8). The ED_{50} of morphine at 60 min after

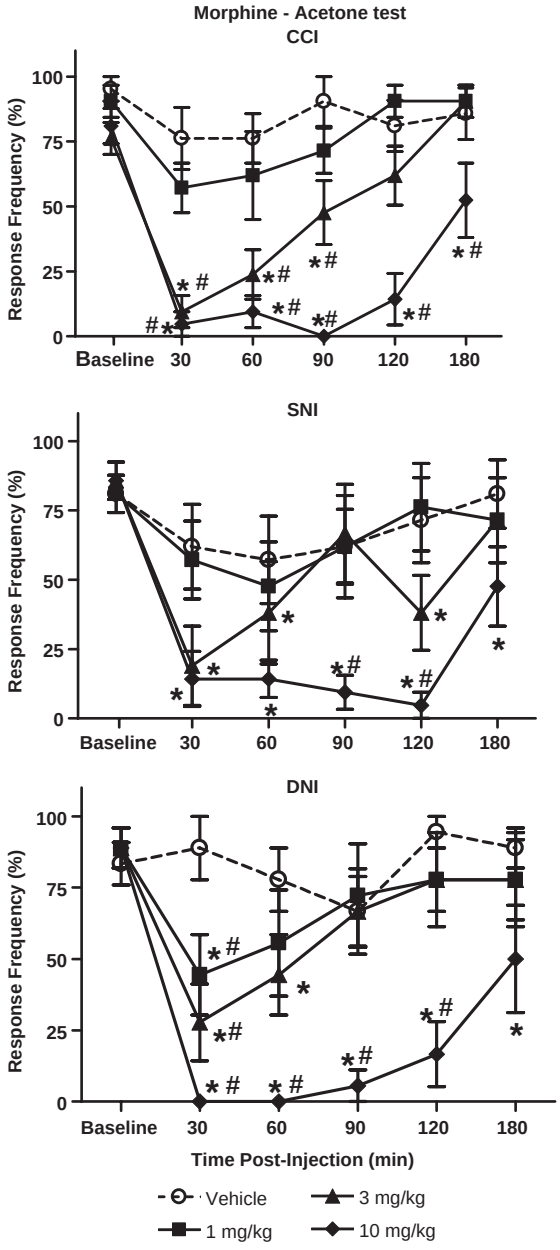

Fig. 8. Effect of morphine on cold hypersensitivity in nerve-injured rats. At "baseline," a 100-μl drop of acetone was applied three times to the lateral hind paw and the number of responses recorded. Following baseline testing, rats were injected subcutaneously with either vehicle, 1, 3 or 10 mg/kg of morphine. Data are expressed as mean±S.E.M. $n=6–7$ per group. *$p<0.05$ vs. baseline; #$p<0.05$ vs. vehicle.

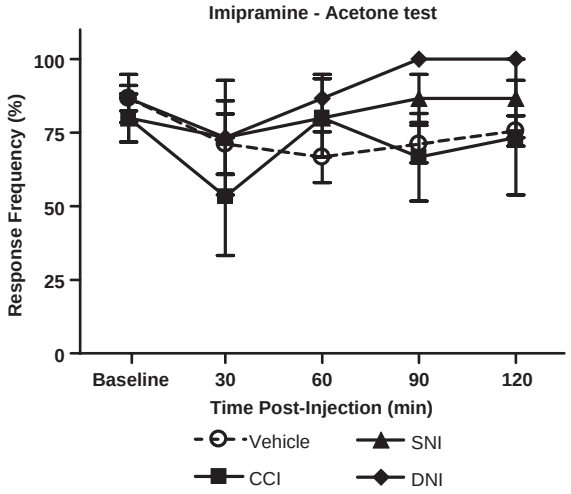

Fig. 9. Effect of imipramine on cold hypersensitivity in nerve-injured rats. At "Baseline," a 100-μl drop of acetone was applied three times to the lateral hind paw and the number of responses recorded. Following baseline testing, rats were injected subcutaneously with either vehicle or 20 mg/kg of imipramine. Data are expressed as mean ± S.E.M. $n = 5–15$ per group.

injection was similar across the nerve injury models, about 2 mg/kg (Table 3).

Imipramine did not significantly affect cold hypersensitivity (Fig. 9). Similarly, rats injected with vehicle continued to show significant cold hypersensitivity throughout the testing period (response frequencies of all nerve injured rats treated with vehicle were combined).

4. Discussion

The transection of the plantar nerves in rats leads to exaggerated pain-related behavior, demonstrated by the increased responsiveness to innocuous stimuli acetone and von Frey filaments, which is similar to that observed in other rat neuropathic pain models. However, the onset of tactile hypersensitivity in rats with a DNI was delayed relative to the other nerve-injured rats. There was a decreased sensitivity to a noxious heat stimulus in rats with a DNI, opposite of the increased sensitivity reported in other models. Morphine and gabapentin, which have been previously reported to have efficacy in rat neuropathic pain models, were efficacious in the DNI model. Imipramine, a TCA that has limited efficacy in human neuropathic pain, had no effect on the hypersensitivities evaluated in three different neuropathic pain models. The current data support the contention that nerve injury far removed from a nerve trunk or root leads to robust and persistent pain-related behaviors and that the comparable pharmacological profile suggest shared mechanisms between these nerve injury models.

Previous rat neuropathic pain models injure either a nerve trunk (CCI), sensory nerve roots (SNL) or proximal nerve branches (SNI). Although these kinds of injuries do occur in humans and may underlie neuropathic pain, neuropathies that occur at distal extremities may also lead to persistent pain. In cases of Complex Regional Pain Syndrome (CRPS or "Reflex Sympathetic Dystrophy"), the initial injury is non-invasive, such as a sprain, or follows a period of limb immobilization, such in a cast (Anandacoomarasamy et al., 2005; Guo et al., 2004). Minor surgery of a nerve of the extremities may also cause persistent pain and hypersensitivity (Kim and Dellon, 2001; Kumar and Jacob, 2004; Turan et al., 1997). In the DNI rats, long lasting hypersensitivity resulted from a relatively minor surgery, cutting of the plantar nerves proximal to the ankle. Hypersensitivity to cutaneous stimulation was detected at least 1 week following injury and lasted up to 8 weeks after injury. Abnormal hind paw positions were not observed since muscle innervation was only interrupted to the toes. The DNI model parallels the clinical observation that injury to small nerve branches leads to persistent abnormal pain perception.

One focus of the current study was to compare and contrast the degree of hypersensitivity induced by distal nerve injury with other peripheral neuropathy pain models. All neuropathic pain models, including the DNI model, displayed hypersensitivity to tactile and cooling stimulation, but as others have demonstrated, the degree and duration of tactile hypersensitivity varies from model to model (Dowdall et al., 2005; Kim et al., 1997). In the current study, the SNL and SNI rats were observed to have robust tactile hypersensitivity that persisted up to 8 weeks after injury. The CCI rats initially displayed robust tactile hypersensitivity but this subsided by 8 weeks. The sensitivity to a cooling stimulus was not uniform across models. Although both the SNL and CCI rats were hypersensitive to acetone up to 8 weeks after injury, the magnitude of the response was greater in the SNL rats. In rats with an SNI, hypersensitivity peaked 1 week after injury, dropped and then gradually increasing up to 8 weeks. It is likely that the different response profiles are due to the nature of the injury, since the age of the rats, housing conditions and testing methods were standardized throughout the experiment.

The novel finding of a slow onset of mechanical hypersensitivity in DNI rats, which took at least 2 weeks to be expressed, contrasts with the other nerve injury models, which have measurable tactile hypersensitivity days or even hours after the injury (Bennett and Xie, 1988; Decosterd and Woolf, 2000; Kim and Chung, 1992). The slow onset also contrasts with the rapid emergence of cooling hypersensitivity in these rats. The clinical finding of a delayed onset of abnormal pain perception following nerve trauma, weeks or months thereafter, has been previously reported, but the source of this phenomenon is unclear (Kim and Dellon, 2001; Kumar and Jacob, 2004; Maleki et al., 2000; Turan et al., 1997). The distinct properties of mechano-sensitive afferents and cold-sensitive afferents may underlie the separate time courses or a mild, as opposed to severe, injury may lead to a delayed onset of

some of the symptoms (Lancelotta et al., 2003; Na et al., 1993). The differential onset of hypersensitivity may also be due to injury-related reorganization of the areas of the brain that process sensory information (Kew et al., 1994; Paulson et al., 2002). The slow onset of tactile hypersensitivity in the DNI model suggests that the factor which initiates tactile hypersensitivity differs between the models.

Thermal hyperalgesia was present in the CCI and SNL models evaluated in the current study, as reported elsewhere (Bennett and Xie, 1988; Kim and Chung, 1992). However, decreased withdrawal latencies of the nerve-injured hind paw, the typical indicator of thermal hyperalgesia, is not unanimously observed (Decosterd and Woolf, 2000; Hogan et al., 2004; Tabo et al., 1999). The presence of thermal hyperalgesia in areas which are hypersensitive to tactile stimulation is inconsistently observed in neuropathic pain patients as well (Price et al., 1989, 1992; Sieweke et al., 1999). In fact, normal sensitivity or diminished responsiveness to noxious heat (hypoalgesia) has been reported in these patients. The diminished responsiveness to noxious heat coexisting with tactile and cooling hypersensitivity in the DNI rats certainly suggests a parallel to the clinical findings. Without neurophysiological evaluation, however, the interpretation of the increased withdrawal latency in the DNI rats should be cautiously interpreted, since the behavior may be due to either a lack nerve function (hypoesthesia) or a dysfunction of the intact nerves in the plantar paw that sense noxious heat.

The second focus of the current study was to uncover a potential differential pharmacology among the neuropathic pain models. If it is the case that a distinct mechanism maintains the hypersensitivity in each neuropathic pain model, then these models should have varying responses to the same drug. Despite the distal site of injury and the unique behavioral profile of the DNI model compared with the other models, the effect of reference antinociceptive drugs in this model was not notably dissimilar from the other models.

In rats with a DNI, systemic morphine attenuated both cold and tactile hypersensitivity in a dose-dependent manner. The potency of morphine on mechanical hypersensitivity in the DNI model was similar to the ED_{50}'s obtained in the CCI and SNI models (Zhao et al., 2004) but several-fold lower than that reported in the SNL model (Bian et al., 1995). The similarity of efficacies and potencies between the SNI, CCI and DNI models do not suggest a divergence of the mechanism by which morphine exerts its antinociceptive effect. Interestingly, the equi-efficacy of morphine on both cold and tactile hypersensitivity suggests a convergence of the mechanism that underlies morphine's effect, even though cold and tactile sensations are served by separate peripheral neural pathways. The current data support the contention that acute opiate treatment is efficacious in ameliorating symptoms associated with clinical neuropathic pain (Portenoy et al., 1990; Rowbotham et al., 2003).

The anticonvulsant drug gabapentin has demonstrated efficacy in rat neuropathic pain models and is currently being utilized in the treatment of clinical neuropathic pain, albeit with varying degrees of success (Backonja and Glanzman, 2003; De Vry et al., 2004; Erichsen and Blackburn-Munro, 2002; Wiffen et al., 2000). Gabapentin ameliorated mechanical hypersensitivity in the SNI, CCI and DNI rats. Gabapentin also suppressed tactile hypersensitivity in the SNL model, with an ED_{50} of 34 mg/kg, similar to the ED_{50}s obtained in the current study (Hunter et al., 1997). Others, however, have found that gabapentin did not affect tactile hypersensitivity (Decosterd et al., 2004). One possible source of the discrepancy may a difference in the testing protocol for tactile hypersensitivity. Nevertheless, the current data suggest a common mechanism or site of gabapentin's effect on tactile hypersensitivity, regardless of the injury type.

In contrast to the efficacy observed with tactile hypersensitivity, gabapentin had absolutely no efficacy on cooling hypersensitivity in any of the models. The lack of efficacy or weak efficacy of gabapentin in response to cooling (acetone, ethyl chloride) suggests that a gabapentin-sensitive mechanism that is present in tactile hypersensitivity is definitely lacking in cooling hypersensitivity (Decosterd et al., 2004; Erichsen and Blackburn-Munro, 2002). One group reported efficacy to acetone-evoked hypersensitivity, but it is not know if this was a dose-dependent effect (Rodrigues-Filho et al., 2004). Although studies have shown that gabapentin will reduce responses to cold (0–10 °C), it is not clear if the same neural mechanism is activated with both acetone and cold (Hama, 2002; Hunter et al., 1997; Kayser and Christensen, 2000; Simone and Kajander, 1996). Furthermore, Choi et al. (1994) showed that the behavioral responses of rats with an SNL on the cold plate test reflected on-going pain rather than a direct response to the cold surface. The differential gabapentin pharmacology implies that the cold water/plate test and the acetone test have distinct mechanisms. If this is so, perhaps the acetone-evoked mechanism may reflect the gabapentin-insensitive component observed in clinical neuropathic pain.

The mechanism by which imipramine and other TCAs work against neuropathic pain may be through presynaptic monoamine reuptake inhibition (McQuay et al., 1996; Sindrup and Jensen, 1999). The pre-clinical efficacy of this class of drug appears to be limited, in contrast to, for example, opiates. In the current study, imipramine had no effect on either cooling or tactile hypersensitivity. In rats, the efficacy of an acute treatment of amitryptiline in neuropathic rats appears to be selective for (either heat or pressure) hyperalgesia (Ardid and Guilbaud, 1992; De Vry et al., 2004; Esser and Sawynok, 1999). One study reported a small effect of amitryptiline on tactile hypersensitivity in SNL rats (Abdi et al., 1998). Others have found no efficacy of imipramine on hypersensitivity (Decosterd et al., 2004; Rodrigues-Filho et al., 2004). The dose of imipramine used

in the current study was similar to that which was efficacious in rat depression and non-neuropathic pain models (Korzeniewska-Rybicka and Plaznik, 1998). Although clinical reports support efficacy with TCAs, none have specifically demonstrated efficacy to evoked pain, such as hyperalgesia or cooling hypersensitivity. The conflicting pre-clinical efficacy demonstrated elsewhere and the lack of efficacy in the current study coupled with the clinical data (e.g., McQuay et al., 1996) suggests that the mechanism of action of TCAs in human neuropathic pain and rat neuropathic hypersensitivity is very divergent. The effect of TCAs on evoked pain in neuropathic pain patients should be evaluated to determine whether this is true or not.

The current study supports the notion that a distal peripheral nerve injury will lead to a persistent state of tactile and cooling hypersensitivity, comparable in severity to that seen in rats with either a nerve root or sciatic nerve injury. The data also confirm the clinical observation that neuropathic pain may occur following injury to distant nerve branches. The limited drug evaluations performed in the current study demonstrated that the pharmacology of distal nerve injury is similar to that of other models, which, combined with the behavioral data, suggests a similar mechanism, irrespective of injury type, in each of the neuropathic pain models. By contrast, Decosterd et al. (2004) claimed that each of the neuropathic pain models is mechanistically distinct, but the same behavioral tests were not performed on each of the models they evaluated. What is significant, however, is that they pointed out that drug effects, hence mechanism evaluation, are dependent on the behavioral assay ("stimulus-evoked" vs. "stimulus-induced"). Perhaps all of the peripheral nerve injury pain models, as well as clinical neuropathic pain, are mechanistically similar. To confirm this, it will be critical to identify the appropriate behavioral test and other assays (e.g., physiological) that will uncover any distinctions, which may lead to the development of either multiple treatments for a number of neuropathies or a single drug to cover most symptoms regardless of the initiating neuropathy.

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