An Animal Model of Neuropathic Pain with Underlying Mechanisms that Are Sympathetically Independent

Jin-Hun Sohn*, Bae Hwan Lee** ***, Sehun Park*, Imgap Yi*,
Yong Gou Park** ****

*Dept. of Psychology, Choongnam National University, **Yonsei Medical Research Center,
***Brain Research Institute, ****Dept. of Neurosurgery, Yonsei University College of Medicine

ABSTRACT: A new animal model is proposed of neuropathic pain in rats. In this model, damage to the tibial and sural nerves produces behavioral and electrophysiological signs of profound pain. In the experiment, under pentobarbital anesthesia, male Sprague-Dawley rats were subjected to surgical injuries to the tibial and sural nerves by tightly ligating and then sectioning out 10mm-long part of each nerve. The rats showed apparent signs of spontaneous pain. They also showed mechanical allodynia in responding to von Frey filament stimulation and cold allodynia to aceton treatment when these treatments were given to the paws of the injured side. The neuronal responses in the L4 or L5 spinal cord were further recorded with sympathetic manipulations, intravenous injection of phentolamine, an alpha-antagonist, or electrical stimulation of the preganglionic nerve fibers in order to determine whether the neuropathic pain in this model is a sympathetically maintained (SMP) or sympathetically independent pain (SIP). Neither manipulation resulted in any significant change in the neuronal response, which suggests that the sympathetic nervous system is not involved in the development of neuropathic pain in this model. In conclusion the new animal model has been consistant in producing neuropathic behaviors and seems useful for the investigation of sympathetically independent pain.

Key words: neuropathic pain, animal model, sciatic nerve, sympathetic nervous system, neuronal activity

INTRODUCTION

It has been shown that peripheral nerve injury or soft-tissue injury can cause severe chronic pain in humans (Bonica, 1990; Loh and Nathan, 1978; Mitchell, 1872). This pain syndrome has a rapid onset of spontaneous, constant, and burning pain that may be easily exacerbated by light mechanical stimulation, temperature change and emotional disturbances (Bonica, 1990; Loh and Nathan, 1978; Merskey, 1986), and that is frequently accompanied by increased sympathetic efferent activity (Bonica, 1990). According to clinical studies, neuropathic pain can be divided into two groups: sympathetically maintained pain (SMP) and sympathetically independent pain (SIP) (Frost, Raja, Campbell, Meyer, & Khan, 1988; Wahren, Torebjork, & Nystrom, 1991). The diagnosis of SMP can be made if the pain is removed by sympathetic interruption.

Animal models have been developed to understand the mechanisms of neuropathic pain. Most of the models involve the sciatic nerve, and the animals produce abnormal pain in the hind paw. The sciatic transection model has been developed by Wall et al. (Wall, Devor, Inbal, Scadding, Schonfeld, Seltzer, & Tomkiewicz, 1979) and used in many early studies. This model, however, does not mimic the details of neuropathic pain resulting from partial nerve injury. Three different animal models using partial nerve injury have been proposed in order to elucidate the mechanisms of human neuropathic pain syndrome. Bennett and Xie (1988) have developed one paradigm, a partial constriction of the sciatic nerve produced by making loose ligatures around it (chronic constriction injury (CCI) model). Partial sciatic nerve ligation (PSL) model was produced by a single tight transneural ligation surrounding only part of the sciatic nerve (Seltzer, Dubner, & Shir, 1990). Kim and Chung (1992) developed another experimental model for peripheral neuropathy using segmental spinal nerve ligation (spinal nerve ligation (SNL) model). Some other animal models have employed the manipulation

of the whole sciatic nerve. DeLeo et al. (DeLeo, Coombs, Willenberg, Colburn, Fromm, Wagner, & Twitchell, 1994) developed a cryoneurolysis model. Rats receiving a freeze injury to the sciatic nerve exhibit autotomy and mechano-allodynia. Markus and his colleagues (Markus, Pomeranz, & Krushelnycky, 1984) shown that the collaterally innervated territory was the source of abnormal pain sensations and this has been termed as collateral hyperalgesia model. Vallin and Kingery (1991) observed the saphenous-mediated pressure and heat hyperalgesia following the sciatic injury (adjacent neuropathic hyperalgesia).

These animal models have been regarded as successful in producing SMP. For example, Neil et al. (Neil, Attal, & Guilbaud, 1991) observed that adren-ergic blockade by repetitive systemic injections of guanethidine before or after the neuropathic nerve injury substantially decreased hyperalgesia to heat and cold using the neuropathic pain model developed by Bennett and Xie (1988). Shir and Seltzer (1991), using their own neuropathic model, demonstrated that chemical sympathetic block performed after nerve injury relieved sensory disorders, such as mechanical allodynia and thermal hyperalgesia. Kim et al. (Kim, Na, Sheen, & Chung, 1993) also reported that surgical sympathectomy relieved that signs of both mechanical allodynia and heat hyperalgesia in their neuropathic pain model rats. But neither cryoneurolysis nor adjacent neuropathic hyperalgesia was sensitive to the manipulation of the sympathetic nervous system (Vallin and Kingery, 1991; Willenbring, Beauprie, & Deleo, 1995; Willenbring, DeLeo, & Coombs, 1995). Thus these animal models have been known to represent SIP.

On the other hand, each animal model mentioned above has some limitations. For example, injured and intact nerve fibers coexist in the same nerve trunk; there is a regeneration over ligation site; it is sometimes very tricky to produce injury; and so on. In order to overcome these problems, the present study was conducted to develop a new animal model of neuropathic pain and to determmine whether this new animal model represents SMP or SIP through the behavioral and electrophysiological studies.

EXPERIMENT I
MATERIALS AND METHODS

Subjects and Surgery

Thirty-six male Sprague-Dawley rats (200-250g) were used. Under pentobarbital anesthetia (55mg/kg), neuropathic injury was produced in 4 groups of rats as follows : Group A - tight ligation and section of the tibial and sural nerves, leaving the common peroneal nerve intact; Group B - ligation and section of the common peroneal and sural nerves, leaving the tibial nerve intact; Group C - ligation and section of the common peroneal and tibial nerves, leaving the sural nerve intact; Group D - sham operation. A complete hemostasis was confirmed and the wound was closed with muscle and skin sutures.

Behavioral Tests

Behavioral signs representing three different components of neuropathic pain (mechanical allodynia, cold allodynia and spontaneous pain) were examined on all the rats for 2 weeks postoperatively and compared among four different neuropathic injured groups.

Mechanical allodynia : Rats were placed on a metal mesh floor under a transparent plastic dome which we built, and innocuous mechanical stimuli were applied with a von Frey filament (8mN of bending force) to the sensitive area of the foot. A von Frey filament was applied 10 times (once every 3-4 s) to each hind paw. The frequency of foot withdrawal expressed in percentage was used as the index of mechanical allodynia.

Cold allodynia: To quantify cold sensitivity of the foot, brisk foot withdrawal in response to acetone application was measured. The acetone was applied 5 times (once every 5 min) to each paw. The frequency of foot withdrawal expressed in percentage was used as the cold allodynia index.

Spontaneous pain: To measure spontaneous ongoing pain, each rat was placed on a acrylic plate at room temperature and covered by a transparent plastic dome (8x8x18cm). After 5 min. of adaptation, the cumulative duration of time that the rat held its foot off the floor was used as the ongoing pain index.

RESULTS
Mechanical allodynia : The frequency of foot withdrawals to repeated mechanical stimulation was
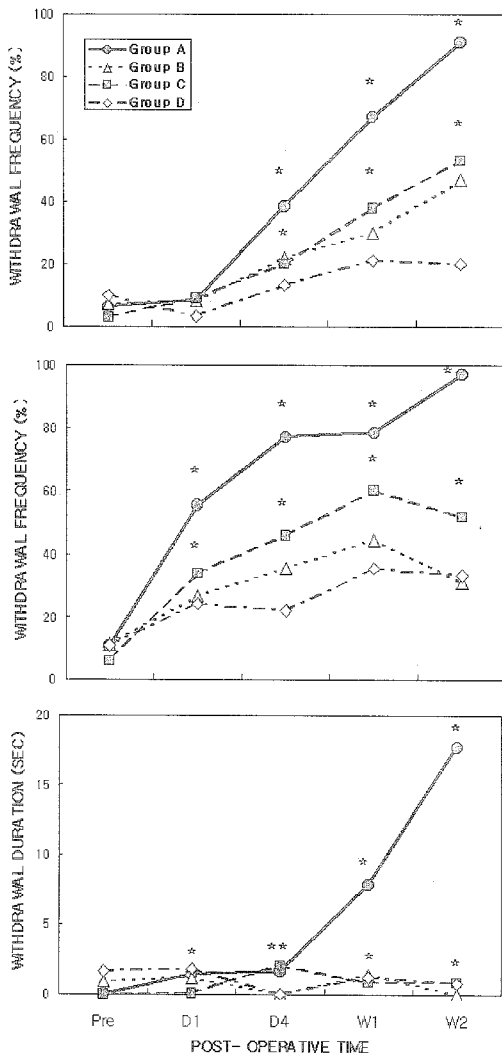
Fig. 1. Development of mechanical allodynia (a), Cold allodynia (b) and spontaneous pain (c) in rats (N=36) with different types of neuropathic injury : Group A; tibial and sural section, B; peroneal and sural section, C; peroneal and tibial section, D; sham operation. Foot-withdrawal frequency for mechanical and cold allodynia and accumulated duration of foot-lifting for spontaneous pain were measured. D; day, W; week. Asterisks indicate significant differences compared to preoperative baselines. (*p<.05)

plotted for each group against time (Fig 1a). Note that rats did not respond to stimulation of the paw before the neuropathic surgery, and that the contralateral (intact) paw remained unresponsive to mechanical stimulation in all groups (data not shown). The most vigorous mechanical allodynia was developed in Group

A. Group C showed moderate sensitivity to von Frey stimulation. In contrast, Groups B and D did not respond to von Frey filament stimulation applied to the paw of the injured side (Fig. 1a).

Cold allodynia: The frequencies of foot withdrawals to repeated cold stimulation (with acetone bubble) were plotted for each group against time (Fig 1b). Rats did not respond to stimulation of the paw before the neuropathic surgery. After the neuropathic surgery, however, rats began to withdraw the foot when acetone was applied to the paw of the injured side. The contralateral (intact) paw remained unresponsive to acetone in all groups (data not shown). Group A rats showed the most vigorous cold allodynia. Group C rats showed moderate sensitivity to acetone. Unlike the other group rats, Groups B and D rats did not respond to acetone applied to the paw of the injured side.

Spontaneous pain: As indicated by the cumulative duration of time that the rat held its foot off the floor, the spontaneous pain was very profound in Group A, but absent or rare in Groups B, C or D (Fig. 1c).

EXPERIMENT II

MATERIALS AND METHODS

Subjects and Surgery

Fifteen male Sprague-Dawley rats (250-300g) were used. Under pentobarbital anesthetia, neuropathic injury was produced by tightly ligating and sectioning the tibial and sural nerves, leaving the common peroneal nerve intact. A complete hemostasis was confirmed and the wound was closed with muscle and skin sutures.

Behavioral Tests

Behavioral signs representing two different components of neuropathic pain (mechanical allodynia and cold allodynia) were examined for 2 or 3 weeks postoperatively. The procedure was the same as described in Experiment I.

Electrophysiology

Immediately after the completion of behavioral tests, the rats were subjected to electrophysiological study.

Between 2 to 4 weeks after neuropathic surgery, single unit recordings were made from the L4 or L5 spinal cord. Anesthesia was induced initially with a bolus injection of urethane (1.25g/kg, i.p.) and maintained by intravenous infusion of pentobarbital sodium (10mg/kg/h) throughout the experiment. Polyethylene tubings of the appropriate sizes were inserted into the trachea to prevent asphyxia, into the right jugular vein for systemic drug administration and into the ventral artery of the tail to monitor the blood pressure. The end-tidal CO^2 was monitored using a capnometer and kept within the normal range (around 4 vol%). The spinal cord was exposed by laminectomy at the level of T10-T13. The animal was mounted on a spinal investigation frame. A heated mineral oil pool (36°C) was made over the exposed tissue to prevent drying.

For unit recordings, a tungsten microelectrode (12Mohm, 125um diameter, A-M System, USA) was used. Single units were identified by responsiveness to peripheral stimulation with von Frey filament or acetone. Once a single unit was idenfied, phentolamine (10ug) was injected into the jugular vein in order to determine whether adrenergic block attenuates the neuronal responses of dorsal horn cells to external stimulation. Electrical stimulation (2-100Hz, 10-40sec, monophasic pulse (0.1ms, 0.5-7mA)) of the preganglionic nerve fibers (T13) was also made in order to investigate whether sympathetic activation affects neuronal responses in the spinal dorsal horn to external stimulation.

A window discriminator and computer interface (CED 1401) was used to make post-stimulus time histograms. Because of the irregular discharge pattern, the responses to sympathetic manipulations were analyzed by comparing the total number of activities before and only 5 min and 1 hour after the sympathetic manipulation.

RESULTS

Behavioral signs of allodynia

Neuropathic pain behaviors were tested with von Frey filament for mechanical allodynia and with acetone for cold allodynia. After the tibial and sural nerves were injured, the rats showed vigorous withdrawal responses to external stimuli. Fig. 2 shows the time courses of the mechanical and cold allodynia.
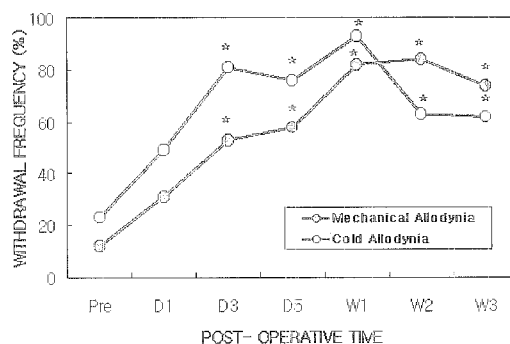
Fig. 2. Development of mechanical and cold allodynia in rats (N=15) with injury to the tibial and sural nerves. D; days, W; week. Asterisks indicate significant differences compared to preoperative baseline.(*p<.05)

Even on the first day after injury, some rats became sensitive to mechanical or cold stimulation.

Responsiveness of spinal neurons to sympathetic manipulation

After the completion of behavioral tests, the rats were subjected to the electrophysiological study. The neuronal responses to von Frey filament or acetone were recorded. Sympathetic manipulations were conducted to determine whether the present animal model represents SMP or SIP.

Phentolamine, an alpha-adrenergic antagonist, was applied intravenously to rats showing behavioral signs of neuropathic pain. The neurons were excited when von Frey filament or acetone was applied to the sensitive area of the foot. If the present animal model represents SMP, phentolamine would suppress the responses of the spinal dorsal horn neurons to the pain-provoking stimulus. Phentolamine did not show any effect on the firing rates of the spinal dorsal horn cells to mechanical stimulation (p>.05), but significantly decreased the neuronal responses to cold stimulation (p<.05) (Fig. 3).

The sympathetic nervous system can be activated by electrical stimulation of the preganglionic nerve fibers. If the present animal model represent SMP, sympathetic activation would increase the neuronal responses of spinal neurons to pain-provoking stimuli. As shown in Fig. 4, electrical stimulation of the sympathetic nervous system did not affect the neuronal
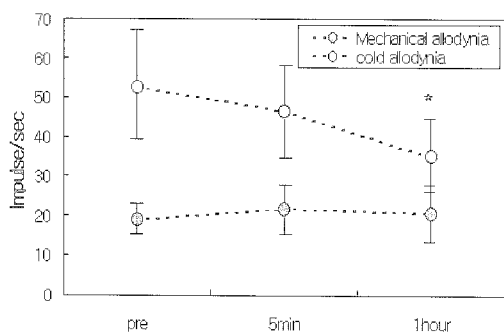
Fig. 3. Changes in responses of dorsal horn neurons to (a) mechanical (filled circles) or (b) cold stimulation (unfilled circles) following intravenous injection of phentolamine. Neuronal responses before, 5 min and 1 hour after phentol- amine treatment are expressed as impulses per second. (*p<.05)
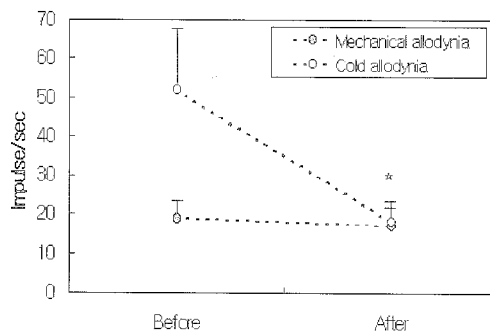

Fig. 4. Changes in responses of dorsal horn neurons to (a) mechanical (filled circles) or (b) cold stimulation (unfilled circles) following electrical stimulation of the preganglionic nerve of the sympathetic nervous system. Neuronal responses before and after electrical stimulation are expressed as impulses per second. (*p<.05)

responses in the spinal dorsal horn to mechanical stimulation (p>.05), but significantly suppress the neuronal responses to cold stimulation (p<.05) (Fig. 4).

DISCUSSION

This study was conducted to test whether the new animal model developed in our pilot studies successfully produces neuropathic pain and further whether the induced pain is sympathetically maintained or independent. Profound pain behaviors were produced in rats that were injured in the tibial and sural nerves. These rats showed severe mechanical allodynia, cold allodynia, and spontaneous pain (Fig. 1). Behavioral signs of neuropathic pain peaked at 2 weeks postoperatively and then gradually decreased (Fig. 2). The rats with the common peroneal and tibial nerves injured showed moderate neuropathic pain symptoms. Neuropathic pain symptoms were absent or very rare in rats with injuries of the common peroneal and sural nerves and were not significantly different from the sham operative control group.

Although we do not have the definitive answers as to why there were differences in magnitude of neuropathic pain symptoms among the nerve injured groups, it seems that injured sensory components of the tibial and sural nerves contribute to the development of neuropathic pain, and that motor

components of the common peroneal nerve contribute to the expression of the neuropathic pain behaviors.

In the electrophysiological test, the neuronal responses to von Frey filament or acetone were recorded. Sympathetic manipulations were conducted to decide whether the present animal model represents SMP or SIP. If the present animal model represents SMP, sympathetic activation would increase the neuronal responses of spinal neurons to pain-evoking stimuli, and sysmpathetic blockade would decrease them. If the present animal model represents SIP, sympathetic manipulations would not affect the neuronal responses.

There were puzzling results that behavioral responses, or withdrawal responses changed in a similar fashion with almost same frequencies at completion of the test in response to cold and mechanical stimulation (Fig. 1) but nuronal firing rates at pre- treatment stage were substantially different (Fig. 3 and 4). One plausible explanation is that both the small size of the subjects (15 rats for EXPERIMENT II) and different cell populations of the two groups because of occasional cell-death during single cell recording might have resulted in intergroup heterogeneity. However, more plausible explanation is that behavioral and neuronal responses would not come together. In other words, neuronal firing responses to cold and mechanical stimulations may have different stimulation-response ratios, while withdrawal

responses have similar stimulation-response ratios.

Neither phentolamine, an alpha-antagonist, nor electrical stimulation of the sympathetic nervous system changed the firing rates of the spinal dorsal horn cells to mechanical stimulation (Fig. 3 and 4), which suggests that the present animal model represents SIP rather than SMP, at least, for mechanical allodynia.

Interestingly, the firing rates of the spinal dorsal horn cells in response to cold stimulation decreased not only with phentolamine but also with electrical activation. However, these results do not support that the present model represents SMP since electrical activation resulted in a significant decrease rather than an increase in the neural firing rate. If this model is to represent SMP, electrical stimulation should have increased the firing rate. With further consideration, this seemingly paradoxical results could be interpreted differently. The dorsal horn cells may respond differently to different stimulation. The pre-operative firing responses to cold stimulation was substantially higher than those to mechanical stimulation. Over the time course of 1 hour after sympathetic treatments, the high firing rate with cold stimulation may naturally decrease to the baseline level as seen in th low firing rate with mechanical stimulation, and therefore sympathetic treatment itself did not cause any change in the neuronal responses of the dorsal horn cells.

If this is the case, change in th firing of the cells in the same direction to the baseline levels responding to cold stimulation and no changes from the baseline levels responding to mechanical stimulation may in fact indicate that the underlying mechanisms of the present model of neuropathic pain are sympathetically independent, that is represent SIP. This is consistent with results of other behavioral studies that surgical or chemical sympathectomy did not attenuate behavioral signs of neuropathic pain including mechanical allodynia, cold allodynia, and spontaneous pain (Lee et al. unpublished observations).

It has been reported that sympathetically maintained pain is prominent in some models but not in others. The CCI model's abnormal pain sensations are only partly dependent on the sympathetic nervous system, and even this partial dependency is seen only in the first few days after the injury (Bennett and Xie 1988; Perrot, Attal, Ardid, & Guilbaud, 1993). In the PSL and SNL models, however, the abnormal pain

symptoms are completely reversed by sympathectomy, even when the intervention is done many weeks or months after the nerve injury (Kim et al. 1993; Shir and Seltzer 1991). But cryoneurolysis or adjacent neuropathic hyperalgesia model has not been sensitive to sympathetic manipulation and thus known to represent SIP. Therefore, the characteristics of the present animal model are similar to those of cryoneurolysis or adjacent neuropathic model.

In SMP models, sensory and sympathetic nervous systems have been known to interact with each other in producing neuropathic pain symptoms. For example, there occurs sprouting of sympathetic postganglionic fibers in the dorsal root ganglia (DRG) after the spinal nerve ligation (Chung, Kim, Na, Park, & Chung, 1993; Chung, Lee, Yoon, & Chung, 1996) or sciatic nerve transection (McLachlan, Janig, Devor, & Michaelis, 1993). This sympathetic sprouting after peripheral nerve injury may be related to SMP. But theirs is no evidence that this sort of interaction exists in SIP. The pathogenic mechanisms of SIP are still uncertain and may involve many different components.

The present animal model has a number of advantages. For example, this model is very simple to produce injury, can discriminate between the injured and uninjured nerve fibers, has sufficient interval between the dorsal root ganglion and injury site. Most of all, this model can produce profound and reliable pain behaviors. Recently, we observed even the crossed-withdrawal response. This response is an unusual reflex which has never been reported yet and which can be produced by mechanical or thermal stimulation applied to the uninjured side. This indicates that the new model may produce exceptionally severe neuropathic pain. These advantages may enable the new animal model to be a useful tool in elucidating the mechanisms of neuropathic pain.

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