A comparison of two sciatic nerve lesions producing neuropathic pain in the rat: The chronic constriction and crush injury

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Abstract

This study provides an extensive comparison of the motor and sensory disturbances following two types of lesion of the sciatic nerve: the chronic constriction injury (CCI), which serves as a model for neuropathic pain, and the crush injury, a model widely used to study nerve regeneration.

Initially, there was a severe loss of motor function with both lesions, as measured by the sciatic function index, toe spreading and open field locomotor behaviour. In the CCI group, motor function remained impaired for over 70-80 days, whereas in the crush group, motor locomotion gradually recovered within the first 28-35 days post-injury.

CCI rats developed signs of hyperalgesia and allodynia to cold, mechanical and chemical stimulation already within a few days after lesioning, whereas in the crush group there were no signs of sensory abnormalities at this time. However, after 29-35 days, they developed sensory abnormalities similar to those in the CCI group, this onset coinciding with the return of sciatic motor function. This suggests that, analogous to the CCI lesion, a neuropathic pain syndrome develops after sciatic nerve crush, which can only be detected after regeneration and re-establishment of a functional contact between the periphery and the central nervous system, allowing the animal to perceive stimuli applied to the hindpaw.

The above data suggest that the sciatic nerve crush might provide an additional useful tool in the study of neuropathic pain.
Introduction

Following a peripheral nerve injury, in humans sometimes a painful syndrome develops. This neuropathic pain is characterised by spontaneous pain in combination with allodynia and hyperalgesia. A widely used animal model to study this condition is the chronic constriction injury (CCI), in which 4 ligatures are loosely tied around the sciatic nerve. The model induces severe sensory abnormalities, closely mimicking the clinical symptoms of neuropathic pain, as well as motor deficits. The pathogenic mechanisms underlying neuropathic pain in the CCI model have been studied extensively, and comprise dysfunctions both in the peripheral as well as in the central nervous system. Histologically, associated with this lesion is an extensive axonal loss, predominantly of the Aβ-type and, to a lesser extent, the Aδ type. Thus the periphery is innervated by C fibers and a largely reduced number of Aδ fibers.

Another experimental nerve lesion producing extensive fibre loss is the sciatic nerve crush. As with the CCI lesion, the surrounding nerve sheath is not disrupted, thus allowing guidance of the outgrowing axons during regeneration and reconnection of distal targets. The sciatic nerve crush is a much used animal model to study nerve regeneration, of which the extent and speed of regeneration is usually defined by the return of sensory and motor function. However, only few studies using the crush model specify the different sensory modalities or focus on possible pain-related behaviours associated with the lesion.

The similarities between the CCI and crush model raised the question to what extent the crush lesion produces symptoms of neuropathic pain, as seen in the CCI model. Here we systematically compare the two models with regard to the temporal correspondence between motor function recovery and changes in thresholds to different painful and non-painful stimuli.

Materials and methods

All procedures in this study were performed according to the Ethical Guidelines of the International Association for the Study of Pain and approved of by the Ethics Committee on Animal Experiments of Utrecht University.
**Animals**

Fifty male Wistar rats weighing 250-300 g at the start of the study were used. Animals were housed in groups of 2-3 in plastic cages on sawdust bedding. They were kept at a 12/12hr light/dark cycle, with food and water available ad libitum. Animals were randomly divided into 4 groups (N=12-13).

**Surgery**

Animals were anaesthetised with a single subcutaneous injection of Hypnorm (Janssen Pharmaceutical Ltd., Beerse, Belgium), containing 0.315 mg/mL fentanyl citrate and 10 mg/mL fluanisone, diluted in saline (1:2, 0.3 ml/100 g bodyweight).

In 38 animals the right sciatic nerve was exposed at mid-thigh level by blunt dissection. Hereafter, in one group of animals (N=13) the nerve was chronically constricted by placing 4 loose ligatures of 4-0 chromic catgut around the nerve, as previously described by Bennet and Xie1 (CCI-group). In the second group (N=13) the nerve was crushed by tightly squeezing it with a forceps for 30 seconds (the distance between the lesion site and the mid-plantar skin was 7.5 cm), as described by de Koning et al10 (crush-group). In the third group (N=12) the nerve was exposed for 5 minutes, without ligating or crushing it (sham-group).

Subsequently the incision was closed with silk sutures and the animals were allowed to recover for a 2-3 day period. A group of animals (N=12) which underwent no surgery was also included (control group).

**General observations**

As a measure of general health, bodyweight was monitored twice weekly for the first 5 weeks, and weekly thereafter. Autotomy was scored as follows: 0; no autotomy, 1; autotomy of one or more toenails, >1; autotomy of one or more toes (adapted from Wall et al.16). Animals showing signs of autotomy were closely monitored and excluded from testing in case of severe damage to the paw.

**Test procedures**

Testing started at post-operative day (p.o.d.) 3 and continued until p.o.d. 84. The following tests were performed:
1 Locomotor testing

1.1 Sciatic Function Index
In order to quantify disturbances in walking patterns we used the Sciatic Function Index (SFI). In short, after dipping their hind paws in developing fluid we let the animals walk over a strip of photographic paper, thus creating black footprints on the paper. Four measurements were taken from both the normal (N) and the experimental (E) paw: IT (distance between the 2nd and 4th toe), TS (distance between the outer toes), PL (foot print length) and TOF (distance to opposite foot). The SFI was calculated as follows:

\[
\text{SFI} = \frac{(EIT - NIT)}{NIT} + \frac{(ETS - NTS)}{NTS} + \frac{(NPL - EPL)}{EPL} + \frac{(ETOF - NTOF)}{NTOF} \times 55
\]

A score of $0 \pm 10$ indicates a normal function of the sciatic nerve, whereas a score of $-100$ or less indicates a complete loss of function (for further details, see de Medinacelli et al.\textsuperscript{17}).

1.2 Open field locomotor performance
To analyse locomotor recovery animals were observed in an open field. We used a scoring system in which individual symptoms were scored together with their frequency of occurrence. Sets of these symptoms were ranked according to severity of disability, analogous to the Basso, Beattie, Bresnahan (BBB) locomotor rating scale\textsuperscript{18}. Possible scores ranged from 0 (paralytic dragging of the operated hind limb) to 14 (normal gait) (see Table 1).

1.3 Toe spreading
To quantify the extent of spontaneous toe spreading the animals were held in a vertical position that causes them to flex the hind paw and spread their toes. The difference in distance between the outer toes from the right (experimental) foot was measured in mm, as described by Bijlsma et al.\textsuperscript{19}.

2 Sensory testing

2.1 Cold stimulation test
Withdrawal latency to a cold stimulus was measured by immersing the hind paws into a 4.5°C or a 10°C water bath, as previously described\textsuperscript{20}. Upon immersion of the paw an electronic circuit including a clock was closed. Withdrawal of the paw resulted in a discontinuation of the circuit, which
stopped the clock, thus allowing a careful registration of the withdrawal latency. Cut-off time was set at 10 sec. Interval time between consecutive tests was at least 10 min. to allow restoration of original foot temperature.

2.2 Mechanical stimulation test
Foot withdrawal threshold in response to a mechanical stimulus was determined using a series of von Frey filaments (Stoelting, Wood Dale, IL), ranging from 1.12 to 52.15 g. Animals were placed in a plastic cage with a metal mesh floor, allowing them to move freely. They were allowed to acclimatise

Table 1. Scoring of open field locomotor performance

<table>
<thead>
<tr>
<th>Status of operated hind paw</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paralytic dragging</td>
<td>1</td>
</tr>
<tr>
<td>Plantar placement without weight support</td>
<td>2</td>
</tr>
<tr>
<td>Plantar placement with weight support</td>
<td>3</td>
</tr>
<tr>
<td>Occasional plantar stepping and frequent or continuous heel placement</td>
<td>4</td>
</tr>
<tr>
<td>Occasional plantar stepping and occasional heel placement</td>
<td>5</td>
</tr>
<tr>
<td>Frequent plantar stepping and frequent or continuous heel placement</td>
<td>6</td>
</tr>
<tr>
<td>Frequent plantar stepping and occasional heel placement</td>
<td>7</td>
</tr>
<tr>
<td>Continuous plantar stepping and frequent or continuous heel placement</td>
<td>8</td>
</tr>
<tr>
<td>Continuous plantar stepping and occasional heel placement</td>
<td>9</td>
</tr>
<tr>
<td>Toe clearance and continuous plantar stepping without heel placement</td>
<td>10</td>
</tr>
<tr>
<td>Parallel placement or toe spreading and toe clearance and continuous plantar stepping without heel placement</td>
<td>11</td>
</tr>
<tr>
<td>Parallel placement and toe spreading and toe clearance and continuous plantar stepping without heel placement</td>
<td>12</td>
</tr>
<tr>
<td>Heel of the ground in stance and parallel placement and toe spreading and toe clearance and continuous plantar stepping without heel placement</td>
<td>13</td>
</tr>
</tbody>
</table>

* occasional: < 50%, frequent: 51-94%, continuous: ≥95% of the observation time
to this environment prior to the experiment. Von Frey filaments were applied to the mid-plantar surface of the both feet through the mesh floor. Each probe was applied to the foot until it just bent, and kept in this position for 6-8 s. The smallest filament that elicited a foot withdrawal response was considered the threshold stimulus.

2.3 Chemical stimulation test
A 5 µl drop of 50% mustard oil (n-allyl-isothiocyanate (Sigma) in ethanol) with 0.1% methylene blue added to visualise spreading was applied to the lateral dorsum of the right hind paw, as described by Bennet and Xie. The animal was then placed in an open field and observed for two minutes. During this time the total number of the following responses to the mustardoil was recorded: shaking the paw, tapping it on the floor and licking the paw.

Statistical analysis
All data are plotted as mean ± standard error of the mean (S.E.M.), for visualisation purposes.
Differences in bodyweight, SFI, toe spreading and number of responses to mustard oil application were analysed using a repeated measures analysis of variance. For post-hoc analysis of group differences a Students t-test (SFI) or Student-Newman-Keuls test (other) was performed.
All other data were analysed using non-parametric tests. For the von Frey stimulation absolute applied forces in g are plotted. In order to obtain a linear scale of applied force the logarithm of the applied force was calculated and statistical analysis was performed on the transformed data. Overall group differences in von Frey and temperature stimulation tests were analysed by a Kruskall Wallis test. For analysis of differences in open field locomotor performance scores and mechanical or cold withdrawal responses, a Mann-Whitney U test was performed, with Bonferroni-correction when necessary. Results were considered significant when p<0.05.
For open field locomotor performance scores 95% confidence intervals (CI) were calculated and scores were considered significantly different from maximum possible score if 14 was not within the 95% CI.
Results

Exclusion of animals
One sham animal died during anesthesia, thus rendering a sham-group of N=11. On p.o.d. 17 one animal in the crush-group was excluded due to severe autotomy and inflammation of the right hindpaw. On p.o.d. 51, five animals of each group were sacrificed for purpose of histology (manuscript in preparation).

General observations
Bodyweight gradually increased throughout the study. There were no significant differences in bodyweight between groups at any time point. Autotomy of the affected hindpaw was observed in both the crush and CCI group. The time of onset as well as the total number of animals showing autotomy differed between the two groups, with the crush group showing earlier and more autotomy. In this group the first signs were seen already at p.o.d. 7 and the number of animals displaying autotomy reached a maximum at p.o.d. 14 (8 out of 13 animals). In the CCI group onset was at p.o.d. 14 and the maximum (5 out of 13 animals) was reached only at p.o.d. 31 (fig. 1). Overall, autotomy in both groups was mild. Only a few animals reached a score of >1, two in the CCI group and two in the crush group, of which one was sacrificed because of the severity of autotomy.

1 Locomotor testing
There were no significant differences between control and sham animals at any time point in any of the locomotor tests.

1.1 Sciatic Function Index
Already from the first testing day after surgery (p.o.d. 3) SFI in the CCI group was significantly lower than in the crush group (-101.0 ± 2.3 vs. -93.4 ± 1.4, respectively) and remained lower until p.o.d. 73. In contrast to the CCI group, recovery in the crush group was much faster and already from p.o.d. 30 SFI in the crush-group was within normal limits (fig. 2A).
1.2 Open field locomotor performance
P.o.d. 7 was the first day the animals’ locomotor performance was observed in an open field. Locomotor performance scores in the CCI and crush groups were decreased to 4.6 ± 0.6 and 7.2 ± 0.6, respectively. Starting at p.o.d. 35 crush scores did no longer significantly differ from maximum score (14). CCI values remained significantly lower until p.o.d. 70 (fig. 2B).

1.3 Toe spreading
Toe spreading in the control and sham animals displayed a gradual increase during the first 10 days, due to growth of the animals. At p.o.d. 3 toe spreading in CCI and crush group was decreased to 13.2 ± 0.3 and 14.2 ± 0.3 mm, respectively. After p.o.d. 17 toe spreading in the crush group rapidly increased and from p.o.d. 28 no longer differed from control values. In contrast, in the CCI group, toe spreading remained significantly smaller until p.o.d. 63 (fig. 2C).
Figure 2.
Locomotor testing
(A) Sciatic Function Index. The grey area indicates the range of SFI in normal rats (0 ± 10). (for details, see de Medinacelli et al., 17). (B) Open field locomotor performance scores (see Table 1). Maximum possible score was 14, indicated by the dotted line.
(C) Toe spreading. The distance between the outer toes of the experimental hindpaw. Measurements were taken in rats held in a vertical position and actively spreading their toes.
Locomotor recovery in the crush-group took place between post-operative days 28 (toe spreading) and 35 (open field). In the CCI group functional recovery occurred later and scores remained below control level until p.o.d. 63 (toe spreading) through 73 (SFI).
Data represent mean ± S.E.M. of 12 (control), 11 (sham) or 13 (crush and CCI) rats each. (º p < 0.05 crush vs. sham; * p < 0.05 CCI vs. sham, # p < 0.05 CCI vs. crush).
2 Sensory testing
There were no significant differences between control and sham animals at any time point in any of the sensory tests. Also there were no differences in withdrawal latencies of the left, unoperated hind paw from a mechanical or cold stimulus between groups at any time point.

2.1 Temperature stimulation test
Already from the first testing day after surgery (p.o.d. 4) CCI animals developed a cold allodynia, as indicated by a significant decrease in withdrawal latency to a 4.5 °C stimulus (3.2 ± 1.0 s, cut-off value was 10 sec). Withdrawal latencies in this group remained lower when compared to sham values until p.o.d. 77. Starting at p.o.d. 29 crush animals also developed a significant cold-allodynia. Withdrawal latencies decreased to a minimum of 2.4 ± 1.1 s and gradually returned to normal level towards the end of the testing period (fig. 3A). Similar results were obtained when temperature was increased to 10 °C (data not shown).

2.2 Mechanical stimulation test
Mechanical allodynia developed in the CCI group, as demonstrated by a large decrease in withdrawal threshold to von Frey stimulation starting at p.o.d. 8 and reaching a minimum of 10.9 ± 1.8 g. Also in the crush group a mechanical allodynia developed. Thresholds were significantly lower than sham values starting at p.o.d. 29 and reached a minimum of 12.8 ± 1.6 g. In both CCI and crush animals the mechanical allodynia gradually resolved over the 12 week postoperative period (fig. 3B).

2.3 Chemical stimulation test
Both sham and control groups showed a small, gradual increase in the number of responses during the first 2 weeks. No mustardoil was applied between p.o.d. 49 and 77 and hereafter the number of responses was again reduced to initial levels in these groups. Already at p.o.d. 3, the first testing day after surgery, CCI animals exhibited significantly more behavioural responses upon application of mustardoil as compared to the sham group. Also in the crush group a hyperalgesia developed. The total number of
responses in this group was significantly increased at a single time point (p.o.d. 17) and thereafter from p.o.d. 35 until p.o.d. 49. From p.o.d. 77 on, the number of responses had normalised in both CCI and crush animals (fig. 3C).

**Figure 3. Sensory testing**

(A) Cold stimulation. Withdrawal latencies of the experimental hindpaw upon immersion in a 4.5 °C waterbath. Cut-off latency was 10 s. (B) Mechanical stimulation. Withdrawal thresholds of the experimental hindpaw to von Frey filaments (ranging from 1.12 to 52.15 g). (C) Chemical stimulation. The total number of behavioural responses upon application of 5 ml of 50% mustard oil to the dorsum of the experimental hindpaw was determined. Responses consisted of shaking, licking and tapping the paw and were counted during two minutes.

CCI rats showed cold and mechanical allodynia and chemical hyperalgesia already from the first testing day after lesioning. Animals in the crush group developed similar sensory abnormalities between p.o.d. 29 (cold and mechanical stimulation) and 35 (mustard oil). Data represent mean ± S.E.M. of 12 (control), 11 (sham) or 13 (crush and CCI) rats each (* p < 0.05 crush vs. sham; * p < 0.05 CCI vs. sham; # p < 0.05 CCI vs. crush).
Discussion

In the study presented here we describe the time course of different sensory and locomotor functions, as well as the development of a neuropathic pain syndrome, after a crush injury to the sciatic nerve.

At the first experimental day after surgery, the SFI in crush rats had decreased to approximately –95%, indicating that an effective nerve injury had been accomplished. During regeneration, return of function is reflected in an increase in SFI towards 0%. Toe spreading and the open field locomotor performance test (which contains an evaluation of toe spreading with the higher scores) both include an assessment of the function of the interosseus muscles, innervated by distal branches of the sciatic nerve. Normalization of these functional tests thus indicates a successful regeneration of the most distal parts of the sciatic nerve. In our rats, normalization of these locomotor function tests occurred between postoperative days 28 and 35, which is in good agreement with previous studies. Since in the present study autotomy was limited and usually confined to biting off one or more toenails without damage to the skin or flesh (except in one animal which was sacrificed for this reason), it had no influence on the outcome of the functional tests we used.

Upon a sciatic crush injury a profound sensory loss occurs, as has been demonstrated by using electrical stimulation of the foot sole, or mechanical or thermal stimulation. In the present study there were no differences in responses to cold and mechanical stimulation between control, sham and crush rats, early after surgery. Most animals in these groups did not respond to the highest filament tested (52.15 g) or demonstrate a positive withdrawal upon cold stimulation within 10 s (cut-off value). Thus, we were unable to detect a decreased sensitivity to these stimuli in crush animals compared to controls. Since a positive response consists of a flexion of the limb, away from the stimulus, an absence of withdrawal in crush animals cannot be subscribed to the severe motor damage associated with the lesion, as the femoral nerve which is needed for hip flexion is still intact. There were also no significant differences in the number of responses to mustard oil application between crush and control rats early after surgery. The fact that the number of responses was not lower in the crush group compared to control values,
as would be expected with a complete lesion of the sciatic nerve, might be explained by a spread of the oil to skin areas innervated by the adjacent saphenous nerve. Also, mustard oil has a very strong smell which could cause the animal to shake and lick its paw in an attempt to get rid of the oil. In contrast to early after surgery, the crush animals demonstrated increased responses to all sensory tests after postoperative day 29, temporally corresponding with the restoration of locomotor function. Through analysis of the return of positive reactions to electrical stimulation of the sole of the foot after a sciatic nerve crush, de Koning et al. estimated axonal growth to be 2.8–2.9 mm/day. By using immunohistochemical methods, Verdu and Navarro have estimated similar regeneration rates, for both sensory and alphamotor fibers. They reported an interval of about 2–3 days between histological signs of reinnervation of target sites and the onset of functional responses, due to the formation of functional end-organs such as Meissner corpuscles and neuromuscular junctions, this interval also being similar for different nerve fiber types. Our data correspond well with these findings, since the distance that the regenerating nerve had to span was 7.5 cm, which would take about 27 days. This indicates that the sensory abnormalities we observed in animals with a crush lesion occur after regeneration of the sciatic nerve and re-establishment of a functional contact between the periphery and the central nervous system. Although few studies have demonstrated nociceptive behavior after a crush injury, the disorders they described are moderate and short-lasting, and occurred within the first week after lesioning of the nerve. A possible explanation for this might be an incomplete lesion of the sciatic nerve, thus still allowing stimuli applied to the nerves peripheral territory being perceived by the animal. In contrast, we show that only late after crush, animals develop severe and long-lasting allodynia and hyperalgesia to cold, mechanical and chemical stimuli. A recent study by Bester et al. reports similar pain-related behaviors late after crush. Here we compare the time course and magnitude of these sensory abnormalities to those observed in a generally accepted model for neuropathic pain, the chronic constriction injury of the sciatic nerve. Immediately after surgery, the magnitude of motor function loss was comparable in crush and CCI animals as demonstrated by similar SFI and toe spreading scores (testing for open field locomotor behaviour was started only
at postoperative day 7). In contrast to the crush animals, in CCI animals signs of alldynia and hyperalgesia were already present at this time, consistent with previous reports. These responses are mediated through surviving fibers, that are predominantly of the unmyelinated and small myelinated class.

To date, many neurobiological mechanisms which may contribute to the pathogenesis of neuropathic pain have been identified, including changes in both the peripheral and central nervous system. One of the pathological changes following nerve injury is an alteration in the level of several neuropeptide genes and their products involved in pain processing. These changes include increases in the level of galanin (GAL), vasoactive intestinal polypeptide (VIP) and neuropeptide Y (NPY), and decreases in substance P (sP) and calcitonin gene-related peptide (CGRP) levels in DRG neurons.

Many of these plastic changes, which can contribute to the altered sensory processing present in neuropathic pain, also occur following nerve crush, such as increased NPY, VIP and GAL and decreased sP levels. There is also a considerable degree of reorganization of synaptic connectivity in response to nerve injury. Sprouting of large myelinated fibers into lamina II of the dorsal horn, an area that normally receives only small fiber input, has been described both in sciatic nerve crush and chronic constriction injury, and has been suggested to play a role in the mechanical alldynia that is present in neuropathic pain. Also, both in complete and partial nerve injuries, sympathetic postganglionic fibers start to sprout into the DRG. These sprouted neurones form synaptic varicosities within the DRG and could form an anatomical basis for a sympathetic dependency of neuropathic pain. Thus similar phenomena, contributing to the pathogenesis of neuropathic pain, occur with both lesion types.

Moreover, as has been suggested for the CCI model, the development of increased nociception is related to the preferential loss of large-diameter myelinated fibers, thus resulting in a loss of inhibitory control. After a crush lesion, the myelin sheath of regenerating nerve fibers is thinner than in an unlesioned nerve up to 12 months after lesioning. Also, both sensory and motor nerve conduction velocities remain decreased for over 6 months after crush. Functionally, as in the CCI model, these phenomena would result in a diminished large-diameter fiber mediated inhibition. On
the other hand, the increased sensitivity after a crush injury might also be in part explained by hyper-responsiveness of regenerated afferent fibers, as suggested by Andrew and Greenspan. In the experiments described here, mechanical allodynia was assessed by von Frey probing of the mid-plantar region and chemical hyperalgesia was measured by applying mustard oil to the lateral dorsum of the hindpaw, both areas normally innervated by the sciatic nerve. In contrast, cold allodynia was quantified by measuring the withdrawal latencies to thermal stimulation upon immersion of the whole hind-paw in a 4.5 °C waterbath. In this way the exact nociceptive territory could not be determined, since the sensory innervation from the hind paw is provided by both the sciatic and the saphenous nerve. Thus we cannot rule out the possibility that the saphenous nerve is involved in mediating this cold allodynia, consistent with a previously suggested role for the saphenous nerve in pain-related disorders following sciatic nerve injury. This is however not likely, since Kaupilla and Xu found no saphenous nerve mediated cold hypersensitivity following sciatic nerve section. Kingery et al. reported a temporal correspondence between recovery of motor function after a crush lesion and resolution of saphenous nerve-mediated heat and pressure hyperalgesia. However, in this present study the sensory abnormalities only occurred when the different locomotor function tests returned to normal, suggesting that these altered responses are mediated by sciatic reinnervation rather than through the adjacent saphenous nerve. Since Kingery and colleagues used larger rats and a different method to determine sciatic motor function it is difficult to compare their time course for sciatic nerve regeneration with our present data. Moreover, they focussed on saphenous nerve mediated hypersensitivity in the period preceding sciatic motor function recovery, thus making it possible that later sciatic nerve mediated sensory abnormalities remained undisclosed.

At present, the sciatic nerve crush is an animal model often used to study nerve regeneration. A consequence of the development of hyperalgesia and allodynia with this type of lesion might be a disturbance of the outcome of functional and sensory recovery tests. Moreover, we have recently demonstrated that melanocortins, often used in regeneration studies because of their positive effects on functional recovery, can worsen neuropathic pain.
Therefore, in interpreting results of these studies one should bear in mind the presence of possible confounding factors in sensory recovery, such as an increased response to a normally non-noxious stimulus. However, this does not hold true for the beneficial effects of melanocortins on locomotor recovery after sciatic nerve crush.

Next to the CCI model we used here, several other experimental animal models are available to study the mechanisms underlying the symptomatology and possible therapeutic strategies of neuropathic pain. They include partial tight ligation of the sciatic nerve\textsuperscript{54} and segmental spinal nerve ligation\textsuperscript{55}. Although the main symptoms produced by these three models (alldynia and hyperalgesia to heat, cold, and mechanical stimuli) share a high degree of similarity, there are also substantial differences, such as the extent and time course of the abnormalities and the response to sympathectomy\textsuperscript{41}.

In the present study the sensory abnormalities in CCI and crush rats are of comparable magnitude but greatly differ in their time course. In the clinical situation there is a complex relationship between the aetiology, mechanisms and symptoms of the neuropathic pain syndrome\textsuperscript{31}, and possibly the different rodent models demonstrating contrasting features involve different pathophysiological mechanisms\textsuperscript{56} and might represent different populations of human neuropathic pain\textsuperscript{41}.

Since it is not likely that a single animal model will cover the complete range of possible mechanisms, it is valuable to study additional animal models producing symptoms of neuropathic pain, such as the sciatic nerve crush model we present here, since this might provide further insight into human neuropathic pain.

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