Influence of the sympathetic nervous system in the development of abnormal pain-related behaviours in a rat model of neuropathic pain

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Abstract

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INFLUENCE OF THE SYMPATHETIC NERVOUS SYSTEM IN THE DEVELOPMENT OF ABNORMAL PAIN-RELATED BEHAVIOURS IN A RAT MODEL OF NEUROPATHIC PAIN

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Abstract—This study evaluated the effect of surgical sympathectomy on pain-related behaviours in a well established model of peripheral mononeuropathy produced by loose ligatures around the common sciatic nerve in the rat. Behavioural abnormalities include spontaneous abnormal position of the hindpaw after the nerve constriction, indicative of “spontaneous pain”, and changes in responses to mechanical or thermal stimuli applied to this paw. These changes are usually maximal at week 2 after the surgery, stable until weeks 3–4, and disappear between weeks 8 and 12.

To assess the role of the sympathetic nervous system in the development and persistence of these abnormalities, four groups of rats were behaviourally tested: (i) rats receiving a complete sham surgery, (ii) rats with a sciatic nerve constriction produced by loose ligatures around the common nerve trunk plus a sham sympathectomy, (iii) rats receiving a lumbar sympathectomy with a sham nerve ligature, and (iv) rats receiving a simultaneous surgical lumbar sympathectomy and a sciatic nerve constriction.

The efficacy of the sympathectomy was assessed by the measure of the noradrenaline level in the sciatic nerve. Sympathectomy reduced selectively or even prevented the abnormal reaction to cold temperature and to heat (45°C) in rats with a peripheral mononeuropathy. In contrast, the abnormal reaction to mechanical pressure was not influenced, and the behavioural abnormalities indicating spontaneous pain were still present. Sympathectomy alone resulted in a reduction of the vocalization threshold to pressure on both hindpaws, but also a short-lasting increased tolerance to cold immersion.

This study confirms the selective role of the sympathetic nervous system in affecting the development and maintenance of some abnormal pain-related behaviours to thermal stimuli in rats with a moderate, but persistent, constriction of one sciatic nerve.

A rat model of neuropathic pain, generated by four loose ligatures around the common sciatic nerve in the Sprague–Dawley rat strain, replicates some pain-related disorders seen in human peripheral neuropathy, and is now commonly used in several laboratories with different approaches. In particular, these animals develop abnormal pain-related behaviours, which, according to the taxonomic classification of the IASP, may be named “alldynia” and “hyperalgesia” to mechanical and thermal (hot and cold) stimulation of the nerve-injured paw. They also exhibit an abnormal position of this paw, possibly reflecting the reluctance of the animal to place it on the floor. The abnormal sensitivity is maximal between two and three weeks after the beginning of the constriction, with a recovery at eight to 10 weeks. Some pain-related behaviours have also been observed for the contralateral limb, and are clearly related to the intensity of abnormal reactions observed from the nerve-injured paw.

Several aspects of neuropathic pain often seem to be related to a pathological control exerted by the sympathetic nervous system. In this respect, the abnormal thermal sensitivity, particularly marked for cold stimulus in rats with the ligated sciatic nerve, may reflect an involvement of the sympathetic system. Indeed, cold “allodynia” is sometimes used to assess the presence of sympathetic maintained pain in humans. It has been demonstrated that sciatic nerve constriction in rats induces dramatic axonal degeneration not only of myelinated, but also unmyelinated fibres. It is not known to what extent damaged unmyelinated fibres are afferent or efferent. Initial findings, however, suggest the involvement of the sympathetic system in some of the behavioural signs observed in this mononeuropathic model: (i) the mononeuropathy is associated with abnormal variations in paw temperature and (ii) sympatholytic treatment with...
guanethidine selectively prevents or suppresses the exaggerated sensitivity to thermal stimuli, mainly to cold stimuli.\textsuperscript{1,15}

To further analyse the influence of the sympathetic system in the development of the various behavioural abnormalities seen in mononeuropathic rats, the effects of surgical sympathectomy on the emergence of pain-related behaviours occurring spontaneously, or elicited by mechanical or thermal stimulation in the model of neuropathy in the rat, were evaluated in the present study. Preliminary data have been reported.\textsuperscript{18}

**EXPERIMENTAL PROCEDURES**

**Animals**

Albino male Sprague-Dawley rats (Charles River, France), all eight weeks old, weighing 175-200 g on arrival, were used: \( n = 36 \) for the behavioural study, \( n = 25 \) for the biochemical assessment of the sympathectomy. The animals were allowed to habituate to the colony room for at least one week before the beginning of the experiment.

For the behavioural study, rats were randomly assigned to one of four different surgical groups:

- Sham-operated rats in which the medial portion of the sympathetic chain and both sciatic nerves (at the mid-thigh level) were exposed but not injured (\( n = 9 \)).
- Sciatic nerve-constricted rats, in which the left sciatic nerve was ligated and a sham surgical procedure performed to expose the right sciatic nerve and the portion of the sympathetic chain (\( n = 9 \)).
- Sympathectomized rats in which a transperitoneal sympathectomy was performed and both sciatic nerves were exposed but not injured (\( n = 9 \)).
- Sympathectomized rats with a sciatic nerve constriction in which (i) a transperitoneal sympathectomy, (ii) a loose ligature on the left sciatic nerve and (iii) a sham surgical procedure exposure of the right sciatic nerve were performed (\( n = 9 \)).

For the high-performance liquid chromatography analysis of noradrenaline level (see below), rats were assigned in five groups in which rats received surgical procedures similar to those described above and one group in which the rats, considered as control rats, did not receive any surgical procedure (\( n = 5 \) in each group).

**Surgical procedures**

The neuropathy was produced according to the method described in detail by Bennett and Xie\textsuperscript{12} and Attal et al.\textsuperscript{13}

Briefly, rats were anaesthetized with sodium pentobarbital (50 mg/kg, i.p.) and four ligatures (5.0 chromic gut, about 1 mm spacing) were tied loosely around the common sciatic nerve at the level of the mid-thigh, so that circulation through the epineural vasculature was not interrupted. A sham procedure consisted of exposing the sciatic nerve without placing ligatures.

A lumbar sympathectomy was performed under deep pentobarbital anaesthesia (50-60 mg/kg, i.p.). The left sympathetic abdominal chain was exposed using a medial transperitoneal approach. A complete dissection of the sympathetic chain from L2 to L5 was performed with the aid of a dissection microscope (10-40 times magnification).

After segmental identification of the ganglia according to the criteria of Baron et al.\textsuperscript{(1} in Ref. 4), and Kotlenburg et al.,\textsuperscript{14} the left ganglia L2-L5 were cut out. It was always possible to remove the appropriate section of the sympathetic chain in one piece. Due to the fusion of lumbar ganglia, in 80% of Sprague-Dawley rats\textsuperscript{15} the sympathectomy often involved the contralateral part of the sympathetic chain. As a result, the sciatic nerve ligation was performed on the left side. The wound was washed with saline and closed in layers (chromic gut 4.0). A sham sympathectomy consisted of a similar surgical procedure in which the sympathetic chain was exposed but not removed.

Since these experimental conditions induce a certain amount of suffering, the guidelines on ethical standards for experimental pain in animals proposed by the Committee for Research and Ethical Issues of the IASP were strictly followed.\textsuperscript{16} In particular, the experimental duration was as short as possible and the number of animals used was kept to a minimum. In addition to the general housing conditions used for normal rats (in an animal room at a constant temperature of 22°C with a 12 h alternating light-dark cycle), special precautions were taken: after surgical procedure, rats were housed in individual cages, then three to four days later, a maximum of three per cage. The floors of the cages were covered with sawdust in order to minimize the possibility of painful mechanical stimulation. Food and water were available ad libitum. Operated animals were able to eat and drink unaided.

**Biochemical measurements**

In the five groups of rats used for this part of the study (with or without a sciatic nerve constriction), a portion of the sciatic nerve from 5 mm above the level of the constriction to 5 mm below this level (equivalent to 150-200 \( \mu g \) protein) was taken at two weeks following surgical procedure, the time of maximum abnormal behaviour. The sciatic nerve segments were homogenized by sonication in 100 \( \mu l \) 0.1 M HClO\textsubscript{4} containing 0.1 mM EDTA and 0.01% cysteine. After centrifugation at 35,000 \( \times G \) for 40 min, the filtered supernatant was frozen to \(-80^\circ\text{C} \) until it was used for analysis. The pellet was dissolved in 0.4 M NaOH at 4°C overnight and used for the measurement of proteins.

The level of noradrenaline was measured by using high-performance liquid chromatography with electrochemical detection, as described previously.\textsuperscript{17} The column used for separation of the compounds was an ESA HR80 (8 cm, packed with 3 \( \mu \)m spherical octadenylsilane; ESA, Inc., Bedford, MA, U.S.A.) and the buffer solution was the ESA CAT-A-Mobile-Phase. The flow rate was set at 1.5 ml/min; the analysis was performed at 25°C. The electrochemical detection was made with a Coulchem 5100 (ESA) detector equipped with an analytical cell model 5014 (serial coupled differential pulse detector) set at +0.05 V to reduce the background level compared to the mobile phase, and the second at 0.35 V for noradrenaline measurement. The retention times, peak heights, calibration curves and concentrations were obtained from a data processor (Merck D-2000). In preliminary experiments, a control of the identity of noradrenaline in the sciatic nerve extracts was made by verifying the identity of the peak height ratios observed at different potentials for the sciatic extracts and standard compounds.

**Behavioural test procedures**

**Measurement of the rat paw temperature**

The cutaneous hind paw temperature was measured on the external part of the plantar part in the territory of the sciatic nerve, using a thermistor probe (Thermocouple Digi-Sense Thermometer, Model No. 8528-10; Cole-Parmer Instrument Company, Chicago, IL, U.S.A.; 15 mm diameter). To avoid possible changes due to the different thermal stimulations measurements were done at the beginning of the test session at 09.00 in a quiet room maintained at constant temperature (23-25°C). To avoid stress induction, paw temperature was measured without straining the rats. Rats were left in their cage and the thermocouple was gently placed on the external part of the plantar surface. Stable temperature readings were obtained after 20 s with a precision of 0.1°C. For each
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**Nociceptive behavioural tests.** Four different behavioural tests, applied randomly by one experimenter with a delay of at least 4 h between each series of tests, were systematically performed in all rats before and at different time points after the surgical procedure. Post-surgery testing sessions started two days after surgery and were performed every three days until the third week, then once a week for up to two months. Since rats with sciatic nerve constriction exhibit abnormal positions of the operated hind paw, even a blind experimenter initially unaware of the conditions of the experiments would rapidly distinguish experiments from control rats: therefore, it was impossible to perform the behavioural studies blindly. However, rats were randomly assigned in groups of four for a series of tests, and before each testing session the experimenter was unaware of the previous results obtained by each of the individual animals.

To avoid sensitization and experimenter effects, the behavioural tests were performed in the same order and on two consecutive days as follows: (i) the score of "spontaneous pain"; (ii) measure of the vocalization threshold to paw pressure; (iii) measure of the struggle latency to paw immersion in a 45°C water bath, on the first day, and (iv) measure of the struggle threshold to paw immersion in a cold water bath. on the second day.

**Score of spontaneous pain.** The method described for the formalin test and extensively used was adapted as described initially (see Refs 2, 3, 36 and 54). Each rat was placed in a Plexiglas cylinder (19 x 31 cm), allowed to habituate for a few minutes before the test and observed for three consecutive 5-min periods. Six different positions of the nerve-injured paw, reflecting an increasing reluctance of the rats to place the paw normally on the floor, were rated: 0. the score of "spontaneous pain"; 1. the rat was touching the floor with the hind paw; 2. the rat was licking its paw. For each 5-min period the time spent in each position was noted, expressed in seconds, and the score was calculated according to the formula \( \frac{T_1 + T_2 + T_3 + T_4 + T_5}{300} \) (where \( T_1, T_2, \ldots \) are the times expressed in seconds, spent in positions 1, 2, … over a total of 5 min). Then, using three consecutive score values, the mean score was calculated for each particular rat.

**Vocalization threshold to paw pressure.** The vocalization threshold to paw pressure was determined by a method adapted from the Randall-Selitto description, with a Basile Analgesymeter (Apex) as currently used in our group; a constant increasing pressure is applied to the hind paw until the rat squeaks with the 1 mm tip diameter of the pusher of the Basile (Apex). The probe was positioned between the third and fourth metatarsus of the dorsal part of the paw, in the sciatic nerve territory. For each rat, two stable consecutive threshold determinations (expressed in grams) were carried out and averaged. In these conditions and according to the taxonomy proposals of the IASP, a decrease in thresholds for vocalization would reflect mechanical "allodynia": a nociceptive reaction for a non-nociceptive stimulus in normal conditions.

**Struggle latency to 45°C.** The struggle latency to cold immersion in a hot water bath was determined by immersion of one hind paw in a 45°C water bath (in the noxious range), as described previously. Each rat was carefully handled and wrapped in a towel so that only the limbs and the head were free. The hind paw, including the heel and the digits, was immersed in the 45°C water bath until the rat struggled. For each rat, two consecutive latency determinations (expressed in seconds) were carried out (with an interval of at least 30 min) and averaged. According to this procedure and the taxonomic proposals of the IASP, a decrease in latency for struggle reflects "hyperalgesia": an exacerbated reaction to an initially nociceptive stimulus.

**Struggle threshold to cold.** To evaluate the struggle threshold to cold, one hind paw was immersed in successive cold water baths (10, 8, 6, 4, 2°C) until the rat struggled (with a cut-off at 15 s to avoid stress reaction or tissue damage). An interval of at least 30 min was allowed before applying the next cold stimulation. For each rat, two stable consecutive threshold determinations (expressed in °C) were carried out and averaged. An increase in the cold temperature threshold was indicative of cold "allodynia" according to the taxonomic proposals of the IASP, a nociceptive reaction for a non-nociceptive stimulus in normal conditions.

### Statistical procedure
To evaluate the influence of surgical procedures, ANOVA was used, taking the control threshold prior to the surgical procedure as the co-variable and the values obtained at different times after surgery as the variable. For comparison of each time point, a Tukey test was used. A paired Student t-test was employed to compare the pre- and the maximally modified post-operative values, as well as for the comparison between the nerve-injured and the contralateral paw.

### RESULTS
The mean weight of all animals increased over the two month observation, reaching 400–500 g at the end of the observation period without any significant differences between normal, neuropathic and sympathectomized rats. The biochemical measurement of noradrenaline in the common sciatic nerve, performed in 25 additional rats by high-performance liquid chromatography with coulometry detection.

<table>
<thead>
<tr>
<th>Nerve constriction</th>
<th>Sympathectomy</th>
<th>Nerve constriction + sympathectomy</th>
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<tr>
<td>Control</td>
<td>Sham operated</td>
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<td>5.9</td>
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<td>6.0</td>
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<td>9.0</td>
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<td>7.5</td>
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With the unexplained exception of one rat with sciatic nerve constriction, there was a dramatic drop in noradrenaline after the three types of surgical lesion; n. d., non detected.

| Table 1. Biochemical measurement (high-performance liquid chromatography with electrometry detection) of noradrenaline in the common sciatic nerve in control rats without surgery, and in rats two weeks after sham surgery, a loose sciatic nerve constriction, a surgical lumbar sympathectomy or both lesions (n = 3 in each group); results are expressed in pmol/mg protein |
showed a dramatic drop in noradrenaline in rats with either a sympathectomy, a sciatic nerve constriction or both lesions at week 2 after surgery, as compared to normal and sham-operated rats (Table 1).

The basal skin temperature was slightly different in the various groups of rats (Fig. 1), but was comparable for both hindpaws in each rat and in each group. The vocalization threshold to paw pressure, the struggle latency to 45°C stimulus and the struggle threshold to cold were similar for both hind paws in each rat and in each group (Table 2).

**Sham animals**

As expected, the vocalization threshold to paw pressure, the struggle latency to 45°C and the struggle threshold to cold from both hind paws remained steady without significant differences from pre-operative values [ANOVA, all $F(13,112) = 1.16$, ns]. In addition, the animals exhibited no abnormal position of the paws (score of spontaneous pain = 0).

**Mononeuropathic animals**

**Paw temperature.** After an initial increase in the temperature of the nerve-injured paw at post-operative days 2–3, the temperature decreased and remained steady between days 16 and 28 (when the rats had clear abnormal pain-related behaviours; Fig. 1A, open squares). A comparable time course was seen for the contralateral paw (Fig. 1B, open squares).

**Score of spontaneous pain-related behaviour.** In contrast to the sham rats, the sciatic nerve-ligated rats never placed their paw normally on the floor, as described previously.\(^3\) The mean score of spontaneous pain related behaviour, using the abnormal hind paw position as a behavioural parameter, was maximal two weeks after sciatic nerve constriction (mean score of spontaneous pain = 1.71 ± 0.23; Tukey test, $P < 0.01$), still significant at week 3, then regularly decreased over the observation period with normalization at week 8 [ANOVA, $F(13,112) = 14.9$, $P < 0.001$; Fig. 2].

**Vocalization threshold to paw pressure.** As described previously,\(^3\) the sciatic nerve constriction produced a significant modification of the vocalization threshold to paw pressure on the nerve-injured paw compared to the pre-operative value [ANOVA,
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Many animals developed a transient hypoalgesia from the nerve-injured paw in the first five post-operative days, but the mean vocalization threshold did not differ significantly from the mean control value (398 ± 63 g) on post-operative day 3 (Fig. 3A). Subsequently, a marked decrease in this threshold, which may reflect "allodynia" to mechanical stimulation, was observed for the nerve-injured paw. This maximal change in threshold was reached between the second and third weeks (mean vocalization threshold = 180 ± 12.5 g on week 2; Tukey test, \( P < 0.01 \)); recovery to pre-operative values were observed between weeks 8 and 10.

For the contralateral paw, the mean vocalization threshold was 281 ± 10 g at week 2, but this decrease was not significant compared to the mean pre-operative threshold [ANOVA, \( F(13,112) = 1.63, P = 0.08 \)]. However, since there was an inter-individual variability of the time point at which the maximal mechanical sensitivity was reached, the mean value obtained at a specific time point (for instance at post-operative week 2) could minimize the degree of the abnormal behaviour. Thus, the peak values obtained for each paw in each rat were also analyzed irrespective of the time point of their occurrence. The minimal mean vocalization threshold to paw pressure was 170 ± 16 g on the nerve-injured paw and 243 ± 11 g on the contralateral paw (representing 53% and 74% of pre-operative values, respectively), both significant as compared to the pre-operative values (Student’s paired t-test, \( P < 0.01 \)).

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Struggle latency to 45°C. Compared to the pre-operative value, the latency for a struggle reaction to 45°C regularly decreased for both paws over the observation period, with struggle reaction peaking between weeks 2 and 3 after surgery [ANOVA, \( F(13,112) = 6.24, P < 0.01 \); Fig. 3B]. The mean struggle latency was 4.0 ± 0.8 s for the sciatic nerve-injured paw and 7.0 ± 1.0 s for the contralateral paw at week 2; recovery to the pre-operative values occurred between six and eight weeks.

Struggle threshold to cold. Compared to the pre-operative value, the threshold of the struggle reaction to cold for the nerve-injured paw increased over the observation period [ANOVA, \( F(13,112) = 3.67, P < 0.001 \)]. The maximal change in threshold temperature peaked at week 2 and remained stable until week 4; the mean struggle threshold was 9.33 ± 0.44°C at day 18 (Tukey test, \( P < 0.01 \); Fig. 3C). Recovery to the pre-operative values was observed at week 9. The mean threshold for a struggle reaction to cold for the contralateral paw was non-significantly increased \( 1.67 ± 0.37°C \) at day 18; ANOVA, \( F(13,111) = 0.87, P = 0.57 \).

Sympathectomized animals

Paw temperature. The time course of the skin temperature was comparable for the two paws, and comparable to that obtained in animals with a ligated sciatic nerve. After an initial warming following the sympathectomy, the temperature returned to the pre-surgery value: at week 2, the temperature was not
significantly different from the initial value (Fig. 1, open diamonds). With sympathectomy alone, there was no spontaneous pain-related behaviour (mean score of spontaneous pain = 0).

**Vocalization threshold to paw pressure.** Overall, sympathectomy alone produced a significant decrease in the vocalization threshold to paw pressure for both hind paws as compared to the pre-operative values [ANOVA, $F(13,112) = 5$ and 3.6 for each paw respectively; $P < 0.001$], which was more pronounced between weeks 2 and 3 after surgery (Fig. 4A). At this time point the mean vocalization threshold was $220 \pm 19$ g for the left paw (Tukey test, $P < 0.01$) and $249 \pm 24$ g for the right paw (Tukey test, $P < 0.05$). Recovery to pre-operative values occurred at week 6. Taking into account the maximal individual threshold decrease, the mean vocalization threshold to paw pressure reached $198 \pm 11$ g for the left paw (i.e. 59% of the pre-operative value) and $218 \pm 15$ g for the right paw (i.e. 66% of the pre-operative value) (differences significant for the two paws; Student’s paired $t$-test, $P < 0.01$).

**Struggle latency to 45°C.** Compared to the pre-operative value, the latency of the struggle reaction to 45°C was slightly decreased, significant for the left paw [mean value $11.5 \pm 0.9$ s at day 9; ANOVA, $F(13,112) = 2.35$, $P < 0.01$] but not significant for the right paw [ANOVA, $F(13,112) = 0.99$, $P < 0.46$; Fig. 4B].

**Struggle threshold to cold.** Compared to the pre-operative value, the threshold for struggle reaction to cold was significantly although transiently decreased for both hind paws [ANOVA, $F(13,112) = 2.73$, $P < 0.01$; Fig. 4C]. The maximum change was seen at day 12: at this time point, the mean struggle thresholds were $2.7 \pm 0.3$ and $3.1 \pm 0.3$°C for the left and right paws, respectively.

**Mononeuropathic rats with a sympathectomy**

**Paw temperature.** The time course of the paw temperature was comparable for the two paws, exhibiting a similar pattern as the other groups (Fig. 1, filled circles). In particular, between weeks 2 and 4 (time of the maximal pain-related behaviours in neuropathic rats), the paw temperature was approximately 26°C both in neuropathic rats (open squares) and in neuropathic animals with a sympathectomy (open diamonds).

**Score of spontaneous pain-related behaviour.** The score of spontaneous pain [ANOVA, $F(13,112) = 9.50$, $P < 0.001$] was not modified by sympathectomy as compared to the score observed in animals with the sciatic nerve constriction (Fig. 2, open squares). However, the peak of spontaneous pain was reached more rapidly, at day 9 vs day 15, with the mean score of spontaneous pain at day 9 being $1.8 \pm 0.2$ (Tukey test, $P < 0.01$).

**Vocalization threshold to paw pressure.** The post-operative vocalization threshold to paw pressure was significantly lower than before surgery [ANOVA, $F(13,112) = 6.25$, $P < 0.001$; Fig. 5A]; the threshold decrease was also observed for the opposite paw, although to a lesser extent [ANOVA, $F(13,112) = 3.88$, $P < 0.001$]. After sympathectomy, the mean minimal vocalization threshold to paw pressure declined to $153 \pm 13$ g (45% of the pre-surgery value) and $211 \pm 15$ g (61% of the pre-surgery value) for the
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nerve-injured and the contralateral paw, respectively (significant differences for both paws; Student's paired t-test, \(P < 0.01\)).

**Struggle latency to 45°C and to cold.** The sympathectomy partially but significantly prevented the abnormal responses to heat stimulation for the nerve-injured paw [ANOVA, \(F(13,112) = 0.99, P = 0.45\); Fig. 5B]. In contrast, the decrease of the struggle latency for the contralateral paw was still significant compared to the pre-surgery value [ANOVA, \(F(13,112) = 1.92, P < 0.05\)]. The sympathectomy completely prevented the abnormal reactions to cold stimulation for the two paws (Fig. 5C); the struggle threshold did not significantly differ from the pre-surgery values [ANOVA, \(F(13,112) = 1.07, ns\)].

**DISCUSSION**

In the present study we confirmed that using highly integrated behavioural tests,\(^2,3,36,37,53\) well quantified behavioural abnormalities indicative of painful neuropathy develop following loose constrictive ligation of the sciatic nerve in rats. These abnormal behaviours reached their peak between the second and third weeks after surgery and were spontaneously reversible eight to 10 weeks after surgery. These behaviours and their time course, repeatedly observed and highly reproducible in our group, either with 4-0 and 5-0 chromic gut or silk, sharply contrast the short latency and duration abnormal withdrawal threshold to high thermal stimulus (three to five days) described as a consequence of a toxic effect of chromic gut.\(^46\) In our study, following sciatic nerve constriction alone, there was a progressive development of a decrease in the vocalization threshold to mechanical stimulation, the presence of spontaneous pain-related behaviour implicating the nerve-injured paw, and a decrease in the struggle latency to 45°C, by several seconds. We also observed a clear increase in the struggle threshold to mild cold thermal stimulation, a measure not previously studied, and which emphasizes the importance of the abnormal reaction to cold in this model. Another important factor is that with all of the phasic tests, abnormal reactions were also detected on the contralateral paw, not only with mechanical stimuli, as repeatedly observed in this model\(^12,3\) and in inflammatory pain models,\(^3,31\) but also with thermal stimuli. Such phenomena are reminiscent of mirror pain commonly seen in human causalgias.\(^11\) The putative mechanisms responsible for a “secondary distant hyperalgesia” are likely to be due to cross-talk between neuronal populations at the spinal and/or the supraspinal level, and have been extensively discussed elsewhere.\(^31\)

In this study, surgical sympathectomy, the efficacy of which was verified by the biochemical analysis of noradrenaline in the injured sciatic nerve, prevented the abnormal reactions to cold and heat. In clear contrast, the abnormal reaction to mechanical stimuli (on both sides) and “spontaneous pain” persisted. These data are in agreement with previous studies in the same model using chemical sympathectomy induced by repeated injections of guanethidine.\(^51,54\) In these first experiments, abnormal reactions to 10 and 46°C were significantly reduced or suppressed by the chemical depletion of noradrenaline, while abnormal reactions to mechanical stimulation or scores of

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**Fig. 5.** Time course of the three phasic behavioural tests for both hind paws at different time points of the study after a sciatic constriction and a sympathectomy (filled symbols). (A) The vocalization threshold to paw pressure was significantly decreased for both hind paws. (B) In contrast, there was no decrease in the struggle latency to 45°C. (C) There was no change in the struggle threshold to cold.
spontaneous pain remained unchanged. Our results are also in full agreement with those obtained after guanethidine treatment in another rat model of neuropathic pain in which the sciatic nerve was partially ligated transversally,58 but contrast with studies of rats with ligation of the two spinal nerves L3 and L6,41 in which surgical and chemical sympathectomy relieved pain-related behaviours to heat and mechanical stimulus as well.42,43 These differences may be due to the different types of nerve injury, but also to the different tests used to assess the nociceptive behaviours. The use of paw withdrawal tests, which are obviously under supraspinal controls, but still present in spinalized animals,32 are extremely different to the tests used in our present study, which are directly integrated at the supraspinal level. Furthermore, the differential effects of several manipulations (morphine injection, spinothalamic tract or thalamic lesions) have strongly emphasized functional differences between the paw withdrawal and the vocalization thresholds to pressure.37,40,55 Moreover, the struggle latency to thermal stimulus is thought to be a more completely organized reaction than the paw withdrawal.22 The differences between our data and those of Kim et al.42 might also be related to the relatively older age and higher weight of the rats used in our study, since in younger rats pain-abnormal reactions are considered to be more pronounced.50

Our present data are in agreement with clinical observations of sympathetically maintained pain (SMP) in which cold "allodynia" has been considered as a significant clinical sign of sympathetic dysfunction.10,11,26,49 The distinctive consequences of sympathectomy on thermal and mechanical abnormal reactions in mononeuropathic rats remain to be elucidated; however, our study of the effect of sympathectomy alone on test behaviour further substantiates the understanding of the systems involved.

Why does sympathectomy relieve abnormal thermal reactions in neuropathic rats?

Role of a putative paw re-warming? A putative paw re-warming may occur after sympathectomy via an increase in cutaneous blood flow,33,34 thus being responsible for the temperature change of the struggle threshold to cold stimuli in sympathectomized rats (Fig. 5B), and consequently in mononeuropathic rats with a sympathectomy (Fig. 5C). However, several arguments can be put forward to reject the major role of this re-warming in the present study: (i) in fact this re-warming did not appear to be specific for the sympathectomy and in any case was not durable, since it was not detected between weeks 2 and 4 after surgery (the time of the maximum pain-related behaviours in rats with sciatic nerve constriction); (ii) the struggle threshold to cold or the struggle latency to 45°C were comparable in the various groups of rats, even with different basal skin temperatures (Fig. 1, Table 2); (iii) sympathectomy alone did not induce a decrease in the struggle latency to hot temperatures; and (iv) on the contrary, when associated with the sciatic nerve constriction, sympathectomy dramatically reduced the pain behaviour elicited by immersion of the paw in a 45°C water bath (struggle latency increased by several seconds).4

Furthermore, the paw temperature may be biased by the test, since we measured the skin temperature and not the deep tissue temperature, in particular, the cutaneous vasculature within the dermis, where noradrenergic sympathetic innervation is predominant.28 However, with the same measuring technique it is possible to detect a clear paw re-warming in neuropathic rats with 100 μg/kg i.v. of clonidine,38,39 but lower doses of clonidine (30–50 μg/kg, i.v.) relieve or suppress abnormal reactions to thermal stimulation (without influence on skin temperature). Thus, the re-warming of the paw by the sympathetic lesion does not appear to be the major and certainly not the only mechanism of its beneficial effect on the hyper-responsiveness to thermal stimulation exhibited by the neuropathic rats. Furthermore, in patients suffering from SMP, the positive effect of phenotolamine also appears to be unrelated to changes in skin temperature,50,62 even if opposite vasomotor effects of the sympathetic and somatic systems observed in normal human subjects have a role in the frequent temperature skin fluctuations of SMP patients.51

Role of noradrenaline depletion? That the total depletion in noradrenaline in the sciatic nerve improves the abnormal reactivity to thermal stimulation could appear paradoxical, since in the mononeuropathic rats there is already a profound alteration in the peripheral content of noradrenaline, as initially shown53 and confirmed by the biochemical controls performed in the present study. However, it has been extensively demonstrated that, under normal conditions, the influence of sympathetic fibres on afferent fibres is usually minimal in rodents, but dramatically increases after a nerve injury as demonstrated by the use of adrenergic agonists.20,34,35,56–58,66 Increased sensitivity to adrenergic substances has also been observed in patients suffering from causalgia or SMP after direct application of noradrenaline or derivatives,17,65 while the plasma concentration of noradrenaline seemed to be decreased in the affected limb.21 The increased sensitivity may be due to several mechanisms, including a recently described noradrenergic sprouting within the dorsal root ganglion itself,34,35,47 however, it should be stressed that this particular phenomenon has, so far, been observed for large afferent fibres, and it is not known whether it exists for cold and warm receptors. Thus, receptor dysfunction may underlie the pathological process, which is then suppressed by sympathectomy. However, the dissociative effects depend on the stimulus modality inducing the pain-related behaviour, i.e. the lack of effect on mechanical abnormal reactions was unexpected.
Why sympathectomy does not relieve abnormal reactions to paw pressure nor the "spontaneous" pain-related behaviour in neuropathic rats

In addition to the lack of effect on abnormal reactions to paw pressure, there was no change in scores of "spontaneous" pain-related behaviour. This similarity further substantiates the hypothesis that this behaviour reflects the reluctance of the rats to place their paw normally on the floor. As mentioned above, similar effects have been observed previously with a chemical sympathectomy in this rat model and in another model of neuropathic pain. The abnormal reactivity to paw pressure was enhanced by sympathectomy, and significant effects were observed for the paw contralateral to the injured nerve. In fact, these data fit well with those obtained in rats with a sympathectomy alone. Our results with sympathectomy in nerve-injured rats are in keeping with our results in rats with sympathectomy alone, in which there was a significant bilateral decrease in the vocalization threshold to paw pressure, likely responsible, in our opinion, for the lack of relief by sympathectomy of abnormal mechanical responses in neuropathic rats. The time course of the effect of the sympathectomy on mechanical reactions (maximum reached rapidly and recovery at the third week after surgery) fits well with the morphological degeneration and regeneration signs reported after sympathectomy in the cat.1

The abnormal mechanical reaction after sympathectomy is also reminiscent of early experimental reports and numerous clinical observations, and the pathogenesis remains obscure. However, a transitory ephaptic connection between low-threshold mechanoreceptors and polymodal nociceptors, consecutive to degeneration and regeneration of Schwann cells, expression of adrenoceptors on peripheral afferents and/or a central link between the autonomic nervous system and primary afferents, may be possible mechanisms (references in Refs 12, 13, 20, 35 and 66).

Nevertheless, the differences in the modulation exerted by the sympathetic system according to the stimulus modality cannot be explained by these various data so far; it can only be proposed that different sensory receptors possibly differentially located in the skin or dermis are differentially innervated and modulated by the sympathetic system.29

Our findings emphasize the importance of using a variety of nociceptive tests based on various stimulus modalities and intensities in analyzing the significance of tonic and phasic activity of the sympathetic system for each abnormal pain behaviour. Moreover, since sympathectomy alone produced small transient abnormal behavioural reactions to mechanical stimulation, a study of the consequences of this procedure alone could be relevant for the pathophysiological investigation of post-sympathectomy pain syndrome.

CONCLUSION

Surgical sympathectomy selectively reduced or even prevented the abnormal reaction to cold temperature and to heat (45°C) in rats with a peripheral mononeuropathy. In contrast, the abnormal reaction to mechanical pressure was not influenced, and the behavioural abnormalities indicating spontaneous pain were still present. Sympathectomy alone resulted in a reduction of the vocalization threshold to pressure on both hind paws, but also a short-lasting increased tolerance to cold immersion. This study confirms the selective role of the sympathetic nervous system in affecting the development and maintenance of some abnormal pain-related behaviours to thermal stimuli in rats with a moderate, but persistent, constriction of one sciatic nerve.

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