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Sciatic nerve cuffing in mice: A model of sustained neuropathic pain

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Abstract

Because of its severity, chronicity, resistance to usual therapy and its consequences on quality of life, neuropathic pain represents a real clinical challenge. Fundamental research on this pathology uses metabolic, pharmacological or traumatic models in rodents that reproduce the characteristic human pain symptoms. In 1996, Mosconi and Kruger morphologically described a model of peripheral neuropathy in which a cuff of polyethylene tubing was placed around the sciatic nerve in rats. In the present study, we evaluated the behavioral consequences of this neuropathic pain model in C57Bl/6J mice which is the main genetic background used for studies in transgenic mice. A short cuff of polyethylene tubing was unilaterally placed around the main branch of the sciatic nerve. It induced an ipsilateral heat thermal hyperalgesia lasting around 3 weeks, and a sustained ipsilateral mechanical allodynia lasting at least 2 months. We showed that this neuropathic pain model is insensitive to ketoprofen, a non-steroidal anti-inflammatory drug. Morphine treatment acutely suppressed the mechanical allodynia, but tolerance to this effect rapidly developed. The analysis of video recordings revealed that most aspects of spontaneous behavior remained unaffected on the long term, excepted for a decrease in the time spent at social interaction for the neuropathic mice. Using the elevated plus-maze and the marble-burying test, we also showed that neuropathic mice develop an anxiety phenotype. Our data indicate that sciatic nerve cuffing in mice is a pertinent model for the study of nociceptive and emotional consequences of sustained neuropathic pain.

Keywords: Neuropathic pain; Anxiety; Allodynia; Sciatic nerve; Morphine

1. Introduction

Pain is a fundamental alarm system and is crucial for survival (Le Bars et al., 2001; Scholz and Woolf, 2002). It alerts the organism to physical damages and it induces behavioral responses that protect the organism from further damage. In contrast, neuropathic pain is a severe pathology of the nervous system that offers no adaptive advantage. Neuropathic pain is defined as a form of chronic pain that results from damage or abnormal function of the central or peripheral nervous system (Abdi et al., 2004; Woolf, 2004). Patients with neuropathic pain frequently report sensory abnormalities including burning sensations, exaggerated responses to noxious stimuli (hyperalgesia), pain sensations resulting from innocuous stimuli (allodynia) and spontaneous pain episodes (dysesthesia) (Gilron et al., 2006). Neuropathic pain can also alter the patient's quality of life by interfering with emotional well-being (Galer et al., 2000). Because of its severity, chronicity

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and resistance to some classical analgesics (Gilron et al., 2006) neuropathic pain is a challenge in clinical practice.

The study of neuropathic pain requires appropriate animal models that reproduce most of the chronic pain states observed in humans. Neuropathic pain can be modelled in animals using metabolic models (such as diabetic neuropathic pain) (Courteix et al., 1993), pharmacological models (such as specific anti-cancerous treatments) (Higuera and Luo, 2004; Ling et al., 2007), or traumatic models (Bennet and Xie, 1988; Seltzer et al., 1990; Kim and Chung, 1992). In 1996, Mosconi and Kruger described a model of peripheral neuropathic pain in which short cuffs of polyethylene tubing were placed around the main branch of sciatic nerve in rats. With an ultrastructural morphometric analysis of axonal alterations, they showed that cuff-implantation minimizes the variability in the degree of nerve constriction. This model was behaviorally characterized in rats (Pitcher et al., 1999), and led to a better understanding of the pathogenesis and mechanism of peripheral neuropathic pain (Coull et al., 2003, 2005; Pitcher and Henry, 2004; Price et al., 2005). Although this cuff model was used twice in mice (Cheng et al., 2002; Benbouzid et al., 2007), it has however not yet been characterized in these species.

In the present work, we studied the behavioral consequences of sciatic nerve cuffing in C57Bl/6J mice which is the main genetic background used for studies in transgenic mice. We evaluated nociceptive parameters by using the von Frey hairs and the Plantar test to respectively assess the mechanical and the thermal nociceptive sensitivities. We completed the characterization of this model by studying its response to an opiate (morphine) and to a non-steroidal anti-inflammatory drug (ketoprofen). We also evaluated the alteration of some aspects of spontaneous behavior, and we assessed the emotional consequences of the model, more particularly with regards to anxiety. Our data indicate that sciatic nerve cuffing is a pertinent model for the study of sustained neuropathic pain symptoms in mice.

2. Methods

2.1. Animals

Experiments were performed on adult male C57Bl/6J mice (6 weeks old upon arrival, Charles River, L'Arbresle, France) group-housed 3–4 per cage and maintained under a 12 h light/dark cycle (lights on at 06:00 a.m.), with food and water available *ad libitum*. All experiments were conducted after a 2 weeks habituation period of the mice to the animal facilities. The experimental procedures described in the present manuscript have been approved by the Comité Régional d'Ethique en Matière d'Expérimentation Animale de Strasbourg (CREMEAS).

2.2. Surgical procedures

All surgeries were done under aseptic conditions and ketamine/xylazine anesthesia (ketamine: 17 mg/mL, i.p., xylazine: 2.5 mg/mL, i.p., 4 mL/kg; Centravet, Taden, France). The common branch of the right sciatic nerve was exposed and a 2 mm long splitted section of polyethylene tubing (ID = 0.38 mm, ED = 1.09 mm; PE-20, Harvard Apparatus, Les Ulis, France) was placed around it (Cuff group) (Fig. 1). The shaved skin layer was closed using suture. Sham-operated mice underwent the same surgical procedure as described above but without implantation of the cuff (Sham group). Mice of the Naïve group were only anesthetized.

2.3. Drug treatment

The 0.9% NaCl solution was used for control injections and to dissolve all drugs. The injections were done subcutaneously (s.c., 5 mL/kg). Ketoprofen (10 mg/kg; Centravet, Taden, France), a non-steroidal anti-inflammatory drug (NSAID), was acutely injected on Days 2, 7, 15 and 30 following surgery. A chronic treatment with morphine (10 mg/kg; Sigma–Aldrich, France) was administered starting 15 days after surgery. For this

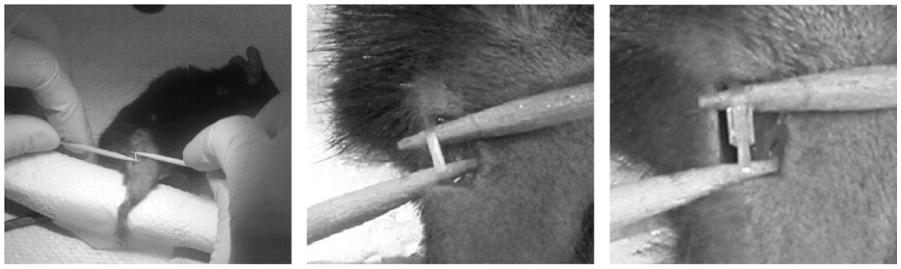

Fig. 1. Cuff-implantation surgical procedure in C57Bl/6J mice. Surgeries (left picture) were done under aseptic conditions and ketamine/xylazine anesthesia. The common branch of the right sciatic nerve was isolated (center picture) and a 2 mm section of split PE-20 polyethylene tubing was placed around it (right picture).

treatment, the mice received two injections of morphine every day (morning and evening) for a week. On an independent set of animals, morphine was also acutely injected on Day 36 post-surgery.

2.4. Response to thermal and mechanical stimuli

The latency for hindpaw withdrawal in response to thermal stimulation was determined using the Hargreaves method (Hargreaves et al., 1988). Mice were placed in clear Plexiglas® boxes (7 cm × 9 cm × 7 cm) on a glass surface and testing began after exploration and grooming behaviors ended (15 min). The infrared beam of the radiant heat source (7370 Plantar Test, Ugo Basile, Comerio, Italy) was applied to the plantar surface of each hindpaw. The experimental cut-off to prevent damage to the skin was set at 15 s. Three measures of the paw withdrawal latency were taken and averaged for each hindpaw.

The mechanical threshold for hindpaw withdrawal was determined using von Frey hairs (Chaplan et al., 1994). Mice were placed in clear Plexiglas® boxes (7 cm × 9 cm × 7 cm) on an elevated mesh screen. Calibrated von Frey filaments (Bioseb, Chaville, France) were applied to the plantar surface of each hindpaw in a series of ascending forces (ranging between 0.4 g and 15 g). Each filament was tested 5 times per paw, and the mechanical threshold was defined as 3 or more withdrawals observed out of the five trials. The effect of drug injections was evaluated before (pre-test) and 30 min after (post-test) the considered drug injection.

2.5. Video analysis

Determination of the mice behavior was performed at the beginning of the night period. It was made possible by using a video monitoring of each cage with infrared sensitive cameras (sensor CCC 320 × 240 pixels, video parameters: 30 images/min; wavelength illumination at 830 nm). Mice behavior scoring started 4 days before surgery. Between 07:00 pm and 01:00 am (6 h), which corresponds to the main activity period of the animals, an observation of the six cages containing either Sham ($n = 9$, group-housed by 3) or Cuff ($n = 9$, group-housed by 3) mice was done once every minute, for a time period of 10 min every hour. This gave us a total of 30 observations per cage per hour, and a total of 180 observations per cage per night. For each observation, the mice were considered as being engaged in one of the five following activities: locomotor activity, rest/sleep period, food/water intake, self-grooming, or contact interaction between animals. The overnight total number of observations corresponding to each behavioral activity was calculated, and the data were then expressed as percentage of time dedicated to each of these activities.

2.6. Anxiety-related parameters

Anxiety-related parameters were investigated using two paradigms: the elevated plus-maze and the marble burying.

The elevated plus-maze (polyvinyl, each arm 27 cm long and 5 cm wide with two opposite arms closed by 10 cm walls) was set 40 cm above the floor (Pellow et al., 1985). A small grey edge (0.5 cm high) around the perimeter of the open arms prevented the mouse from falling off. Mice were placed in the center of the maze and allowed to explore for 5 min. The number of arm entries and the time spent in each arm was measured. The time spent in the open-arms is a measure of animal anxiety, and the total number of arm entries is a control for locomotor activity (Barrot et al., 2005; Monteggia et al., 2007).

The marble burying test (Njung'e and Handley, 1991) was done in Plexiglas cages (37 × 23 × 18 cm) containing 5 cm of fine sawdust. Twenty five glass marbles (1 cm diameter) were placed 2.5 cm from the cage walls, so that they rested on top of the sawdust and were evenly spaced. Mice were placed individually into the cages and left undisturbed for 30 min, following which they were removed and an observer blind to the drug condition of the animals counted the unburied marbles. Marbles were considered buried if two-thirds, or more, of the surface area was covered by sawdust. The number of buried marbles is a measure of animal anxiety (Nicolas et al., 2006; Jacobson et al., 2007).

2.7. Tail suspension test

Each mouse was suspended by the proximal end of the tail with adhesive tape on a bracket (1 m above floor level) (Steru et al., 1985; Thierry et al., 1986). The subjects were observed either making escape-oriented movements, or remaining immobile. The duration of immobility was measured during a period of 6 min. This test is highly sensitive to antidepressant drugs and the duration of immobility is thought to represent a state of despair (Nestler et al., 2002; Cryan et al., 2005).

2.8. Statistical analysis

The data are expressed as mean ± SEM. Statistical analysis were performed with STATISTICA 7.1 (Statsoft, Tulsa, OK, USA), using Student T-test for 2-group comparisons or multi-factor analysis of variance (ANOVA). For the ANOVAs, the surgery procedure (Sham or Cuff) and the treatments (saline vs. drug injections) were taken as between-group factors. When needed, the paw laterality (left vs. right) and/or the time of measurement (either time-course or pre-injection vs. post-injection data) were taken as within-subject factors (with repeated measures on the same animal). Interaction

between factors was also tested. When appropriate, the Duncan test was used for post-hoc comparisons. The significance level was set at $P < 0.05$.

3. Results

3.1. Sciatic nerve cuffing and thermal response

The thermal sensitivity of the animal hindpaws was evaluated with the Hargreaves' method. Unilateral cuff-implantation ($n = 7$) caused a significant ipsilateral decrease in the latency for paw withdrawal as compared to Sham ($n = 6$) and to Naïve ($n = 5$) animals (Fig. 2A) (surgery group × paw laterality × time interaction: $F_{26,195} = 2.93$, $P < 0.0001$). This thermal hyperalgesia to a hot stimulus disappeared by 3 weeks post-surgery.

3.2. Sciatic nerve cuffing and mechanical response

The mechanical sensitivity of the animal hindpaws was evaluated using von Frey hairs. Unilateral cuff-implantation ($n = 7$) induced an ipsilateral mechanical allodynia which was not seen in Sham ($n = 6$) and Naïve ($n = 5$) animals (Fig. 2B) (surgery group × paw laterality × time interaction: $F_{26,195} = 4.76$, $P < 0.0001$). This mechanical allodynia persisted for at least 60 days. As the mechanical allodynia is both the longer lasting symptom and the most relevant nociceptive parameter for the evaluation of neuropathic pain, we then focused the pharmacological characterization of our model on this parameter.

3.3. Ketoprofen effect on cuff-induced mechanical allodynia

Acute injection of ketoprofen (10 mg/kg) or of the vehicle (saline solution) had no effect on the hindpaw withdrawal threshold in Sham animals ($n = 4$ per group) (Fig. 3A). The acute injection of ketoprofen at Day 2 post-surgery induced a moderate but significant attenuation of mechanical allodynia in Cuff mice (Fig. 3B) ($F_{1,8} = 14.45$, $P < 0.01$). This mild attenuation did not however suppress the mechanical allodynia; indeed the mechanical nociceptive threshold of the treated Cuff mice remained significantly lower than the threshold of Sham mice ($P < 0.001$). At Days 7, 15, or 30 after cuff-implantation ($n = 5$ per group), the acute injection of ketoprofen was ineffective against the cuff-induced allodynia (Fig. 3B).

3.4. Morphine effect on cuff-induced mechanical allodynia

The morphine injections started 2 weeks post-surgery. Compared to saline administration, the first morphine injection induced an analgesia in Sham mice ($n = 4$/group) ($F_{1,6} = 11.05$, $P < 0.01$) but tolerance to morphine-induced analgesia was already present by Day 3 of treatment ($P > 0.3$) (Fig. 4A). The same morphine treatment induced an analgesia which suppressed the cuff-induced mechanical allodynia ($n = 5$/group) during the first 3 days of chronic injections (Fig. 4B) ($F_{1,8} = 5.17$ on Day 1 and $F_{1,8} = 12{,}48$ on Day 3, $P < 0.05$ in both cases). At Days 5 and 6, tolerance developed and chronic morphine treatment was ineffective to alleviate the cuff-induced mechanical allodynia ($P > 0.7$) (Fig. 4B). In a separate set of animals, we also tested whether acute morphine was effective on a latter time-point after cuff-implantation. At Day 36 post-surgery, the acute injection of morphine suppressed the cuff-induced mechanical allodynia (Fig. 4C) (Morphine effect: $F_{1,7} = 1.92$, $P < 0.002$).

3.5. Cuff-implantation effect on the spontaneous behavior of mice

The weight gain of Sham and Cuff mice did not differ during the post-operative period (Fig. 5A; Table 1). On

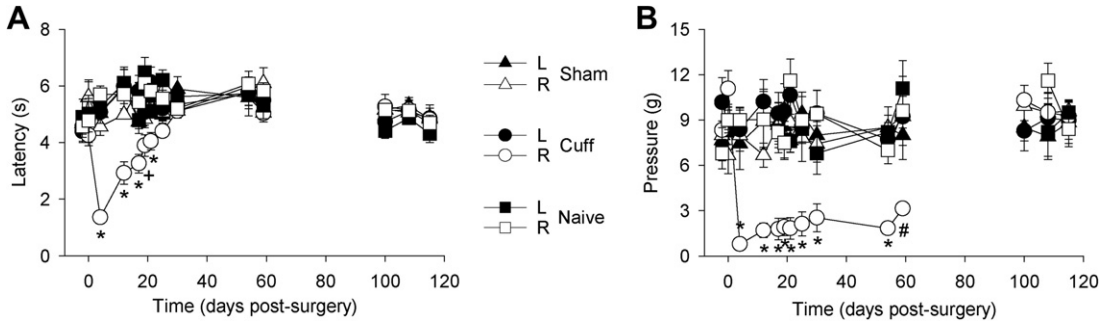

Fig. 2. Cuff-implantation and nociceptive parameters. Adult male mice underwent surgery for unilateral cuff-implantation ($n = 7$) around the main branch of the right sciatic nerve. Sham animals ($n = 6$) underwent the same surgical procedure without cuff-implantation. Naïve animals ($n = 5$) were only anesthetized and had no surgical procedure. The thermal hyperalgesia was evaluated using the Plantar test, and the mechanical allodynia was tested using the von Frey hairs: (A) the cuff-implantation induced an ipsilateral thermal hyperalgesia that disappeared around 3 weeks post-surgery and (B) the cuff-implantation induced an ipsilateral mechanical allodynia that persisted at least 2 months. Data are expressed as mean ± SEM. L = left (contralateral) hindpaw; R = right (ipsilateral) hindpaw. Duncan post-hoc: $^{*}P < 0.05$ against Sham and Naïve animals; $^{+}P < 0.05$ against Naïve animals; $^{\#}P < 0.05$ against Sham animals.

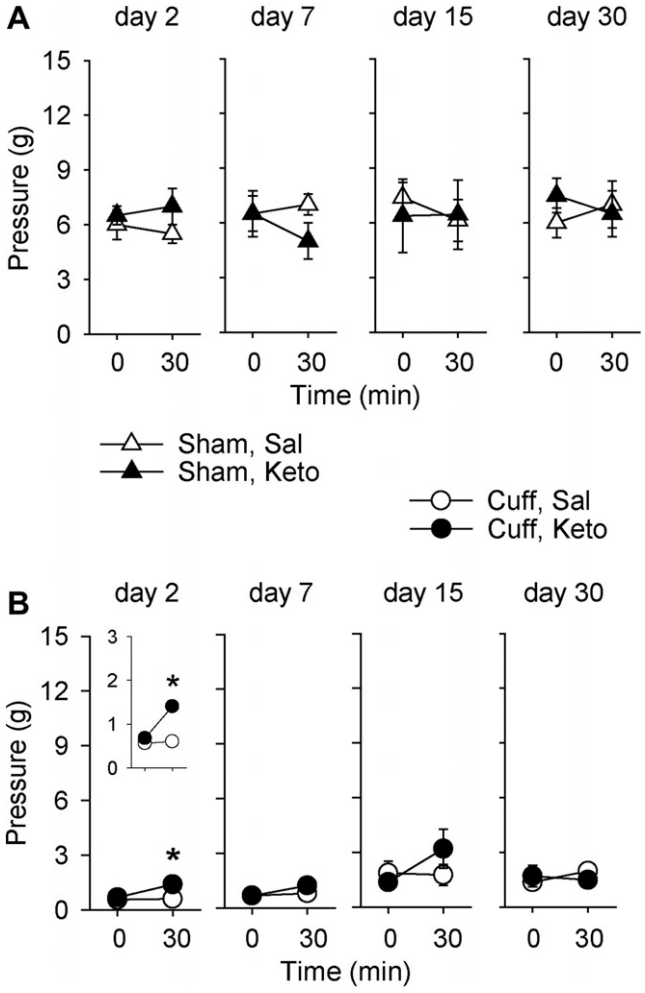

Fig. 3. Effect of an anti-inflammatory drug on cuff-induced mechanical allodynia in mice. The non-steroidal anti-inflammatory drug ketoprofen (Keto) was injected on Days 2, 7, 15 or 30 post-surgery: (A) the acute injection of ketoprofen (10 mg/kg, s.c.) did not affect the nociceptive mechanical threshold in Sham animals ($n = 4$/group) and (B) the acute injection of ketoprofen on Day 2 post-surgery induced a mild attenuation of hindpaw mechanical allodynia in Cuff mice (A close-up on this mild effect is presented in the figure insert; Duncan post-hoc: $^{*}P < 0.01$). On Days 7, 15 or 30 post-surgery, the injection of ketoprofen did not influence the mechanical allodynia in Cuff mice ($n = 5$/group). Data are expressed as means ± SEM.

the surgery day, a decrease in the time spent in locomotor activity (Time effect: $F_{9,36} = 7.23$, $P < 0.0001$) and in food and water intake (Time effect: $F_{9,36} = 2.18$, $P < 0.05$) was observed in both Sham and Cuff animals (Fig. 5B and D). These transitory decreases were associated with an increase in the time spent in rest and sleep on this surgery day (Fig. 5C) (time effect: $F_{9,36} = 6.85$, $P < 0.0001$). A slight increase in the time spent in social interactions also appeared post-surgery for both groups (Fig. 5F) (time effect: $F_{9,36} = 4.34$, $P < 0.001$).

At later time points (2 and 4 weeks post-surgery), the proportion of time spent in each activity was back to normal (Table 1), with the exception of the time spent in social interaction that decreased for Cuff animals at 4 weeks post-surgery ($P < 0.05$).

3.6. Cuff-implantation effect on mood-related parameters

The Cuff-implanted mice developed an anxiety-like phenotype as shown by the reduction of time spent in the open-arms of an elevated plus-maze (Fig. 6A) ($P < 0.002$) or by the increased number of buried marbles in the marble burying test (Fig. 6B) ($P < 0.05$). The number of arm entries in the elevated plus-maze, which was taken as an index of general activity, was not affected by cuff-implantation (Fig. 6A).

In the tail suspension test, we found no difference in the duration of immobility between Cuff and Sham animals (Fig. 6C).

4. Discussion

In the present work, we studied the consequences of unilateral sciatic nerve constriction in C57Bl/6J mice. Our data demonstrate that unilateral constriction of the sciatic nerve by cuff-implantation in mice produces an ipsilateral thermal hyperalgesia and an ipsilateral mechanical allodynia. We show that this mechanical allodynia is insensitive to the NSAID ketoprofen, while morphine suppresses it acutely. A tolerance to the morphine analgesia, however, rapidly develops. Finally, we show that the cuff-implantation has minor consequences on the spontaneous behavior of the animals, but induces an anxiogenic state 4–6 weeks post-surgery. Our data indicate that sciatic nerve constriction by cuff-implantation in mice may be a good and ethically acceptable animal model for the study of sustained neuropathic pain.

Initially developed and characterized in rats (Mosconi and Kruger, 1996; Pitcher et al., 1999), the cuff-implantation model was recently used to induce mechanical allodynia in mice (Cheng et al., 2002) but has not yet been characterized in these species. We show here that the changes of nociceptive response in mice are comparable to the observations seen in rats (Fisher et al., 1998; Pitcher et al., 1999; Kabli and Cahill, 2007). The hyperalgesia in response to hot thermal stimulation and the allodynia in response to a non-noxious mechanical stimulus are present as soon as Day 1 post-surgery and persist respectively for around 3 weeks or for at least 2 months. It is to note that we did not observe the contralateral allodynia that was reported in earlier studies conducted in rats (Pitcher et al., 1999; Pitcher and Henry, 2004). In our experiments on C57Bl/6J mice, the thermal hyperalgesia and the mechanical allodynia remained strictly ipsilateral to the cuff implantation side. These results are in good agreement with more recent studies in rats (Kabli and Cahill, 2007; Holdridge and Cahill, 2007).

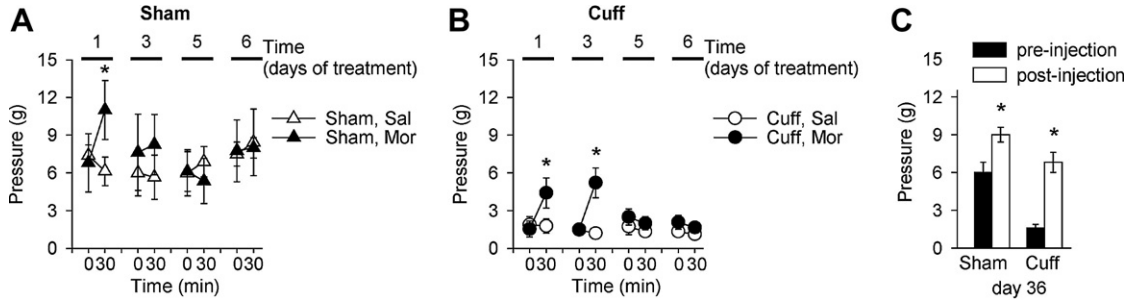

Fig. 4. Effect of morphine injection on cuff-induced mechanical allodynia in mice: (A) two weeks post-surgery, morphine injection (Mor) induced an analgesia in Sham mice ($n = 4$) on Day 1 of treatment. A tolerance to morphine was then observed with repeated injections, as the opiate had no more effect on Days 3, 5 or 6 of the treatment. (Duncan post-hoc: $^{*}P < 0.05$ against Saline treatment), (B) two weeks post-surgery, morphine injection (Mor) induced an analgesia in Cuff mice ($n = 5$) from Day 1 to Day 3 of treatment. Tolerance to morphine effect was present on Days 5 and 6 of treatment. (Duncan post-hoc: $^{*}P < 0.05$ against Saline treatment) and (C) acute injection of morphine at Day 36 post-surgery suppressed the cuff-induced mechanical allodynia. (Duncan post-hoc: $^{*}P < 0.05$ against pre-injection). Data are expressed as means ± SEM.

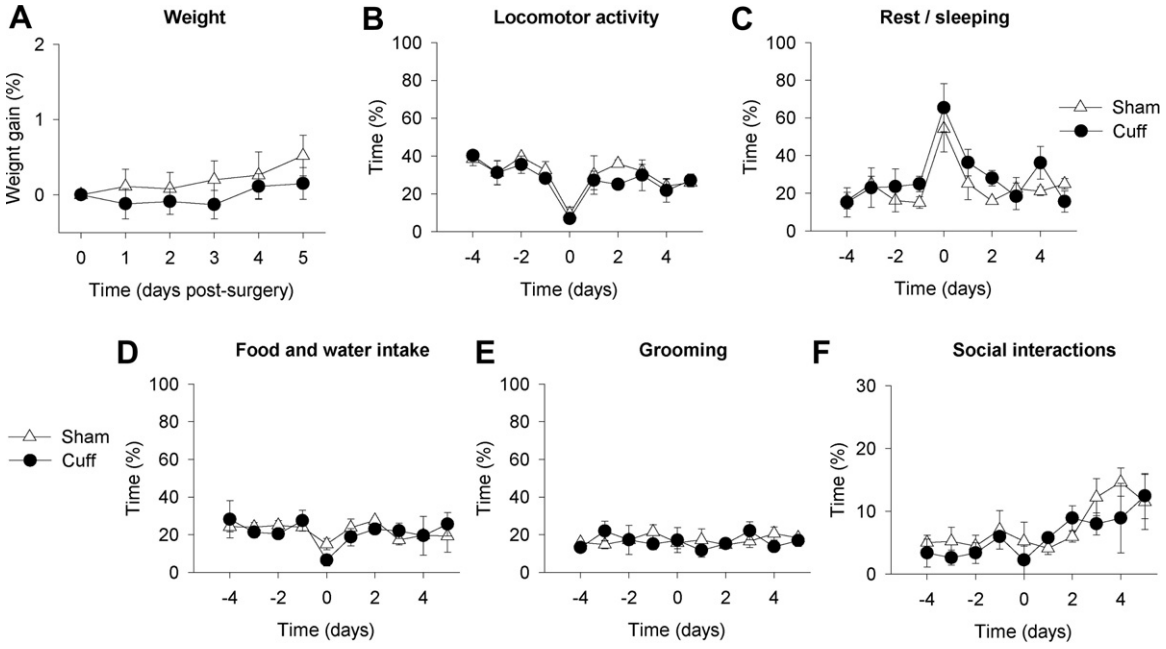

Fig. 5. Spontaneous behaviour of mice on days following cuff implantation surgery. The surgeries were done between 8:00 and 10:00 a.m. Both Sham and Cuff animals: (A) showed no difference in weight gain in the days following the surgery; (B) showed a decrease in the time spent in locomotor activity on the night following the surgery (Day 0); (C) showed an increase in the time spent at rest and sleep on the night following the surgery (Day 0); (D) showed a mild decrease in the time spent at food and water intake on the night following the surgery (Day 0); (E) showed no change in the time spent at self-grooming activities and (F) showed an increase in the time spent at social interactions (measured by the time spent in direct contact between animals) during the post-operative period. Data are expressed as means ± SEM.

Hyperalgesia and allodynia are observed in murine animal models of neuropathic pain, such as loose or tight ligations of the sciatic nerve (Sommer and Schäfers, 1998; Malmberg and Basbaum, 1998) or such as the present cuff-induced constriction model. While these parameters are always present and lasting over 2 weeks, it must be noted that their time-course can vary depending on the model and species (Malmberg and Basbaum, 1998; Pitcher et al., 1999; Lindenlaub and Sommer, 2000; Crisp et al., 2003). A striking feature observed in our present characterization is that hyperalgesia to hot thermal stimuli recovered much faster than the mechanical allodynia. Similar observations have also been documented in different models and species (Malmberg and Basbaum, 1998; Lindenlaub and Sommer, 2000; Crisp et al., 2003), which might be related to the fact that noxious heat or mechanical information involves partially independent neural pathways (Bian et al., 1998; Ossipov et al., 1999; Poisbeau et al., 2005). The mechanical allodynia that we observed in the cuff-implanted mice lasted at least 2 months. It is thus a good murine model for studies on chronic neuropathic pain and its treatment.

Table 1
Long-term effect of cuff implantation

Parameters	Second week	Fourth week
Weight (g)	$S = 23.91 \pm 0.71$	$S = 25.50 \pm 0.63$
	$C = 23.90 \pm 0.47$	$C = 25.49 \pm 0.58$
Weight gain (%)	$S = 1.59 \pm 0.42$	$S = 3.57 \pm 0.40$
	$C = 1.27 \pm 0.30$	$C = 3.86 \pm 0.41$
Locomotor activity	$S = 36.30 \pm 5.40$	$S = 33.22 \pm 4.32$
(% of time)	$C = 35.93 \pm 4.42$	$C = 35.00 \pm 4.12$
Rest or sleeping	$S = 22.96 \pm 7.44$	$S = 13.1 \pm 5.55$
(% of time)	$C = 16.67 \pm 5.90$	$C = 17.41 \pm 6.25$
Food and water intake	$S = 20.56 \pm 3.91$	$S = 26.11 \pm 3.46$
(% of time)	$C = 22.59 \pm 3.71$	$C = 29.26 \pm 3.45$
Grooming (% of time)	$S = 14.26 \pm 2.84$	$S = 21.3 \pm 2.95$
	$C = 17.78 \pm 3.15$	$C = 15.37 \pm 2.80$
Social interaction	$S = 5.74 \pm 1.02$	$S = 7.22 \pm 2.00$
(% of time)	$C = 7.04 \pm 2.48$	$C = 2.96 \pm 1.45^{*}$

Mice behavior was analyzed using video-recording at weeks 2 and 4 post-surgery. S = Sham animals; C = Cuff animals. Data are expressed as mean ± SEM.
* $P < 0.05$ against Sham mice.

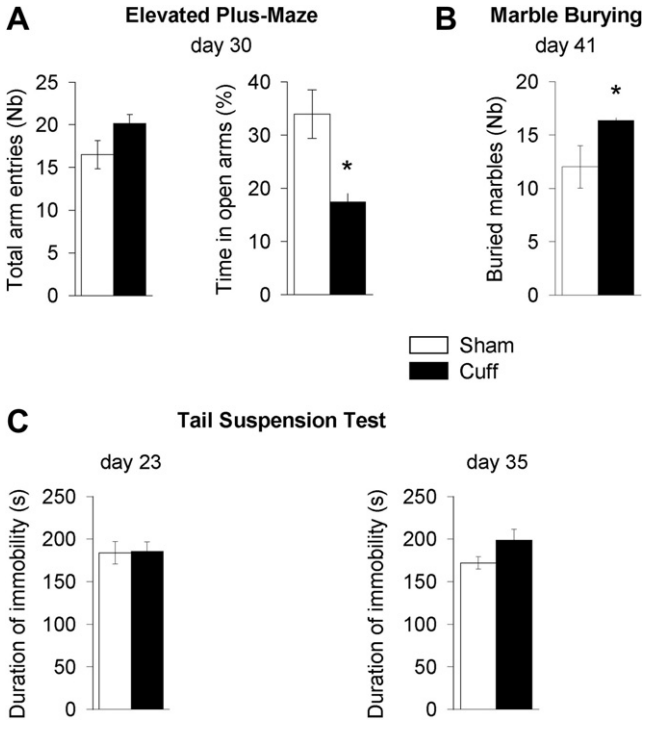

Fig. 6. Cuff-implantation and mood-related parameters: (A) In the elevated plus-maze, the number of arm entries was not different between Cuff and Sham animals, but cuff-implantation induced a decrease in the time spent in the open arms. The test was done on Day 30 post-surgery (Sham, $n = 6$; Cuff, $n = 8$), (B) at Day 41 post-surgery, Cuff animals ($n = 8$) buried more marbles than Sham mice ($n = 6$) in the marble burying test, (C) the cuff-implantation did not affect the mice immobility in the tail-suspension test. Independent set of animals were tested at either Day 23 ($n = 12$/group) or Day 35 ($n = 6$–8/group) post-surgery. Data are expressed as mean ± SEM. $^{*}P < 0.05$.

We show that the non-steroidal anti-inflammatory drug ketoprofen was mainly ineffective against the cuff-induced mechanical allodynia. This finding comforts the notion that the inflammatory component is negligible in the present model, and that the observed mechanical allodynia is a neuropathy-related parameter. On the second day post-surgery, a mild effect of ketoprofen was observed in cuff-implanted mice. This effect was however not sufficient to block the mechanical allodynia, and it probably reflects the post-surgery recovery of cuff-implantation consequences. On all the other time-points tested, the ketoprofen had no effect on the mechanical allodynia.

In our conditions and as observed in other neuropathic pain models (Bian et al., 1995; Martin and Eisenach, 2001; Ozaki et al., 2003), we show that morphine reversed the cuff-induced mechanical allodynia. This therapeutic effect is however transient and disappeared with the development of a tolerance to this drug. It should be noted that the delay for the onset of the tolerance to morphine was slightly delayed in the neuropathic conditions. Our pre-test/post-test procedure allows us to check the mechanical threshold before morphine injection on each test day. As this pre-injection threshold remained stable, this shows that the loss of morphine effect in our model reflects a real tolerance to the opiate, and not the consequence of a delayed opiate-induced hyperalgesia as can be observed in other experimental conditions (Simonnet and Rivat, 2003; Simonin et al., 2006).

Our study shows that cuff implantation had no major consequences on the spontaneous behavior and on the weight gain of the animals. We observed some changes in the spontaneous behavior during the post-operative period, with an increase in the rest and sleeping time to the detriment of locomotor activity and food and water intake. But these modifications were strictly transitory and observed in both Sham and Cuff mice and are thus to be attributed to the surgical procedure. The only long-term consequence of cuff implantation was a decrease in the time spent in social interactions at 4 weeks post-surgery. This is interesting as a decrease in social interactions has been associated with mood disorder models (Nestler et al., 2002; Berton and Nestler, 2006) and we also observed the development of an anxiety-like phenotype in the Cuff mice.

Clinical studies have revealed a relationship between chronic pain and mood disorders (Huyser and Parker, 1999; Dersh et al., 2002), each pathology making the patients potentially more vulnerable to develop the other. A first study trying to address the influence of neuropathic pain on mood-related parameters in animal models reported no effects (Kontinen et al., 1999). In this work, the authors used spinal nerve ligation in rats and studied the mood-related parameters 2 weeks post-surgery. More recent work on a longer time-course, and using tight nerve ligation in mice or rats, revealed that long-term (but not short term) neuropathic pain induces an anxiety phenotype (Narita et al., 2006; Suzuki et al.,

2007). We observed similar results in the present model: the Cuff mice showed an anxiety-like phenotype in both the elevated plus-maze and the marble burying test. This phenotype could not be attributed to locomotor deficits. Indeed, the number of arm entries in the elevated plus-maze remained unaffected and the number of buried marbles was increased in the Cuff mice. However, in the tail suspension test which is used in depression (but not anxiety) – related researches, we observed no changes in the immobility behavior which is thought to measure the state of despair of the mice (Nestler et al., 2002; Cryan et al., 2005).

In conclusion, our results show that this neuropathic pain model is valid in mice. It induces a sustained thermal hyperalgesia and mechanical allodynia, it is mainly insensitive to anti-inflammatory drugs, and it allows studying some long-term affective consequences of neuropathic pain. The use of this model in rats previously helped understanding the mechanism of peripheral neuropathic pain (Coull et al., 2003, 2005; Pitcher and Henry, 2004; Price et al., 2005), and the present study extends the validity of this model to mice, opening the possibility to work with transgenic animals.

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