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'CatWalk' automated quantitative gait analysis as a novel method to assess mechanical allodynia in the rat; a comparison with von Frey testing

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Abstract

A characteristic symptom of neuropathic pain is mechanical allodynia. In animal models of neuropathic pain, mechanical allodynia is often assessed using von Frey filaments. Although the forces applied with these filaments are highly reproducible, there are various disadvantages of using this method. Testing paradigms and definitions of withdrawal threshold are not standardised. Moreover, measurements may be influenced by various conditions, such as ambient temperature, humidity, weight bearing of the limb and stress. We have therefore investigated another technique to assess mechanical allodynia, the 'CatWalk' automated quantitative gait analysis. With this computer-assisted method of locomotor analysis, it is possible to objectively and rapidly quantify several gait parameters, including duration of different phases of the step cycle and pressure applied during locomotion. We tested rats with a chronic constriction injury of the sciatic nerve, a model of neuropathic pain, both with von Frey filaments and the CatWalk method. We demonstrate that these rats minimise contact with the affected paw during locomotion, as demonstrated by a reduction in stance phase and pressure applied during stance. Moreover, these parameters show a high degree of correlation with mechanical withdrawal thresholds as determined by von Frey filaments. We therefore suggest that the CatWalk method might serve as an additional tool in the investigation of mechanical allodynia.
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1. Introduction

Neuropathic pain is a condition caused by a lesion in the peripheral or central nervous system. Clinically, it is characterised by the presence of spontaneous as well as different types of evoked pain. The latter include exaggerated responses to both noxious and non-noxious stimuli, referred to as hyperalgesia and allodynia, respectively. Allodynia to tactile stimuli is a common symptom (Price et al., 1992; Chaplan et al., 1994), and amongst the most problematic clinical phenomenon, since physical contact with the environment is difficult to avoid. To study the pathophysiological mechanisms underlying neuropathic pain symptoms such as allodynia, several animal models of neuropathic pain have been developed in the past decades. The most widely used models are the chronic constriction injury (CCI) (Bennett and Xie, 1988), partial ligation of the sciatic nerve (Seltzer et al., 1990) and tight ligation of the L5 and L6 spinal nerves (Kim and Chung, 1992). Although there are differences between these models, they all produce behavioural signs of neuropathic pain, including mechanical allodynia (Kim et al., 1997).

To date, the most commonly used method to assess mechanical allodynia in such animal models is application of a series of von Frey filaments (also known as Semmes–Weinstein filaments) to the paw. The animal's response to these filaments is used to determine mechanical withdrawal thresholds. Usually, a paw withdrawal upon probing or immediately upon release of the filament is considered a positive response (Chaplan et al., 1994). However, responses are not always clear-cut and their interpretation may vary between investigators. Most commonly, the filaments are applied to the plantar surface of the hindpaw while the animal is standing on a metal grid floor, but probing of the lateral or dorsal surface of the paw also has been

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performed. Also, various paradigms to determine withdrawal thresholds have been employed. In a frequently used testing paradigm, filaments are applied according to the up-and-down method described by Dixon (1980). Here, probing is initiated with a filament in the middle of the series, and depending on the presence or absence of a positive response, the next smaller or larger filament is tested, respectively. From the resulting pattern of positive and negative responses, a 50% withdrawal threshold can be calculated (see Chaplan et al., 1994). Alternatively, filaments can be used in order of increasing stiffness, starting with the smallest filament. With this testing in ascending order, each filament can be applied a different number of times, at various intervals, and held in position for different periods. Also, different definitions of withdrawal threshold have been employed; for example the smallest filament eliciting at least one positive response (Sato et al., 1999), or three out of three (Kauppila et al., 1998), three out of five (Ren, 1999), or four to six out of ten positive responses (Quartaroli et al., 2001). Yet another way to quantify mechanical allodynia is by testing either a complete series of filaments, or a selection of two, and calculating the total number of responses as a percentage of total number of applications (Kim et al., 1997). Thus, by using von Frey probes, assessment of mechanical allodynia can be performed in numerous ways, and may be influenced by subjectivity, thus making it difficult to compare results obtained from different studies.

In the present study, we investigated whether mechanical allodynia can also be measured in a more objective way. We produced a mechanical allodynia by subjecting rats to a CCI of the sciatic nerve. It is to be expected that an increased sensitivity to mechanical stimuli will cause the animal to exert less pressure on the affected limb during walking, and will minimise contact of this paw with the floor. We tested CCI rats on the 'CatWalk', an automated quantitative gait analysis method recently developed in our laboratory (Hamers et al., 2001). Here, we compare von Frey withdrawal thresholds in these animals with relevant data obtained from the CatWalk analysis.

2. Materials and methods

2.1. Animals and surgery

All procedures in this study were performed according to the Ethical Guidelines of the International Association for the Study of Pain (Zimmermann, 1983) and approved by the Ethics Committee on Animal Experiments of Utrecht University. Thirteen male Wistar rats weighing 250–300 g at the start of the study were used. Animals were housed in groups of two to three in plastic cages on sawdust bedding. They were exposed to a 12/12 h light/dark cycle, with food and water available ad libitum. Animals were anaesthetised with a single subcutaneous injection of hypnorm (Duphar, the Netherlands) diluted in saline (1:2, 0.3 ml/100 g bodyweight). Four loose ligatures were placed around the right sciatic nerve as described previously (Bennett and Xie, 1988). Subsequently, the incision was closed with silk sutures and the animals were allowed to recover for a 2–3-day period.

Since we previously observed that sham-surgery does not induce any changes in withdrawal thresholds to von Frey stimulation or locomotor function (unpublished results, see also Kupers et al., 1992; Vrinten et al., 2001), in this experiment we used only CCI animals.

2.2. Test procedures

Before surgery and at 2, 5, 8 and 10 weeks after surgery, the following tests were performed.

2.2.1. Mechanical withdrawal thresholds (von Frey)

Paw withdrawal threshold in response to a mechanical stimulus was determined using a series of von Frey filaments (Stoelting, Wood Dale, IL), ranging from 1.08 to 21.09 g. Animals were placed in a plastic cage with a metal mesh floor, allowing them to move freely. They were acclimatised to this environment for approximately 10 min prior to testing, to allow for behavioural accommodation. Von Frey filaments were applied to the mid-plantar surface of the operated hindpaw through the mesh floor. Probing was only performed when the animal's paw was in contact with the floor. Each probe was applied to the foot until it just bent, and was kept in this position for 6–8 s (Chaplan et al., 1994). Interval between consecutive filaments was at least 5 s. Filaments were applied in ascending order, and the smallest filament that elicited a foot withdrawal response was considered the threshold stimulus.

2.2.2. 'CatWalk' automated gait analysis

The animals traverse a walkway (plexiglass walls, spaced 8 cm apart) with a glass floor ($100 \times 15 \times 0.6$ cm^3) (length × width × thickness) located in a darkened room. Light from an otherwise completely encased white fluorescent tube (length 110 cm, 30 W) enters the distal (from the observer) long edge of this glass floor. Sufficiently far from the edge, it strikes the surface below the critical angle and is entirely internally reflected. Only at those points where a paw touches the glass, light exits the floor and scatters at the paw, illuminating the points of contact only (see Fig. 1A). Via a mirror, the corridor's floor is monitored by a Pulnix TM-765E CCD camera (Pulnix Inc., UK) equipped with a wide angle objective (Cosimar 8.5 mm) (see Fig. lB).

The intensity of the signal depends on the degree of paw floor contact and increases with pressure applied (Betts and Duckworth, 1978). The camera detects the average intensity within a rectangular area (pixel) in which total skin–floor contact may differ.

The more pressure is exerted, the larger the total area of skin–floor contact and thus the brighter the pixel (see Fig.

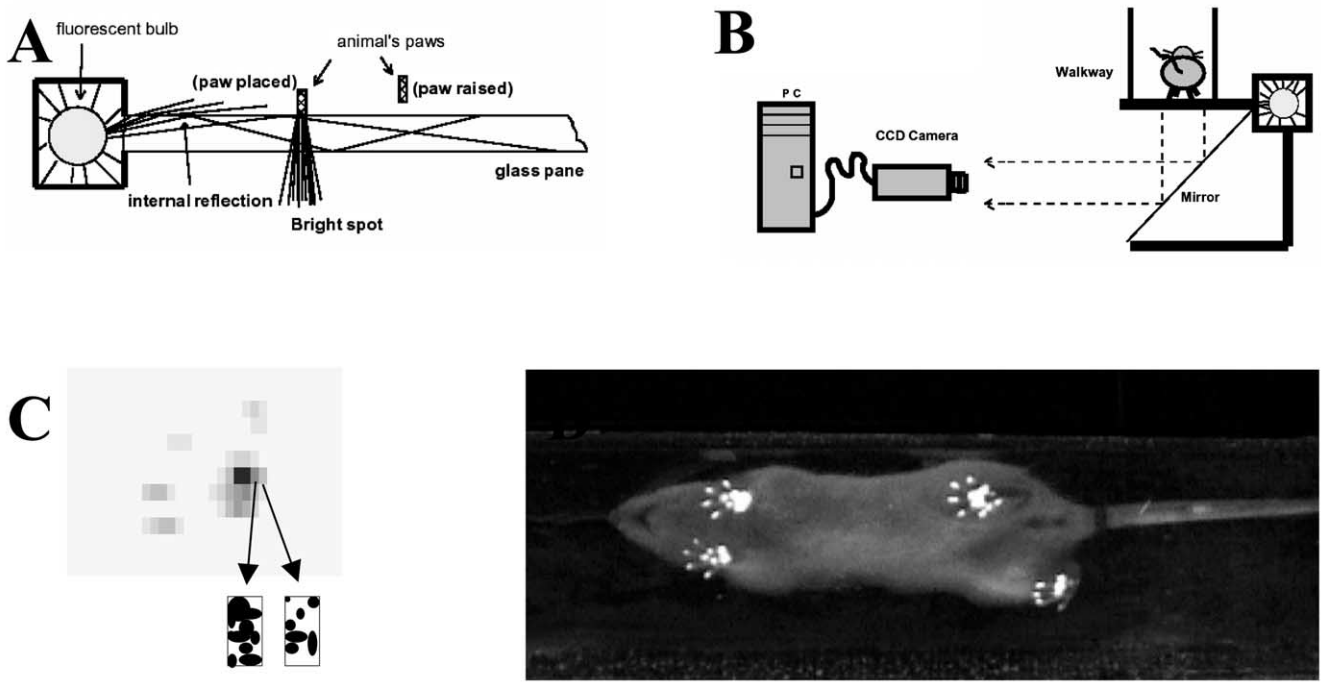

Fig. 1. Principle of the CatWalk setup. (A) Light from a fluorescent bulb is sent through a glass plane. Light rays are completely reflected internally, except where a paw is placed on the glass surface. This results in a sharp image of the paw. (B) Images are reflected by a mirror placed at a 45° angle, and recorded by a CCD video camera connected to a computer. (C) Example of a paw print during walkway crossing. The foodpad and four of the toes are clearly visible. For visualisation purposes, intensity is inverted, i.e. the darker the print in this figure, the brighter it appeared on the walkway. Influence of skin–floor contact on pixel intensity in two neighbouring pixels is shown (insets). For details see text. (D) A faint image of the rats body is visible over the paw prints, thus making it possible to identify the different paws (adapted from Hamers et al., 2001).

1C). The signal is digitised by a PClmage-SG frame grabber board (Matrix Vision GmbH, Oppenheimer, Germany) The CatWalk program acquires, compresses, stores and eventually analyses the 'videotapes' of animals crossing the walkway. All areas containing pixels brighter than a preset threshold value are also stored uncompressed, in order to circumvent compression related artefacts in the eventual analysis. Note that a very faint image of the animal crossing the walkway is present, unless all background illumination is eliminated completely (Fig. 1D). However, this image is so faint in comparison with paw prints that it does not interfere with the measurement. In effect, without the shape of the animal moving through the corridor interactive labelling of prints is far more difficult.

Data analysis is performed by first (automatically) labelling all areas containing one or more pixels above a certain analysis threshold. In a second interactive pass, these areas are assigned to one of the paws; user intervention is in most cases only required at initial contact of each paw, enabling analysis of a few seconds of walkway crossing within less than a minute. Ordered data are eventually output in ASCII-format which is readable by humans and which can be used as input of almost every spreadsheet program.

Animals are not pre-trained to cross the walkway as the Wistar rats used in our laboratory have no hesitation in crossing the walkway spontaneously with sufficient speed. A typical crossing contains six step cycles and averaged data from all step cycles in a crossing are used in the analysis.

Analysis of these recordings yields many parameters (Hamers et al., 2001), of which the following are of most interest in the CCI model:

1. step sequences (see Table 1) with their respective frequencies;
2. regularity index (RI), a measure of interlimb coordination. Interlimb coordination is complete if only normal step sequences are used during uninterrupted locomotion. The RI grades the degree of interlimb coordination as follows: $RI = (NSSP \times 4/PP) \times 100(\%)$ wherein NSSP represents the number of normal step sequence patterns and PP the total number of paw placement. Both extra PP and loss of certain PP (irregular walking on three paws) will decrease RI;

Table 1
Limb sequences in regular stap patterns[a]

Category	Sequence
Cruciate	RF-LF-RH-LH or LF-RF-LH-RH
Alternate	RF-RH-LF-LH or LF-RH-RF-LH
Rotary	RF-LF-LH-RH or LF-RF-RH-LH

[a] RF, right forelimb; RH, right hindlimb; LF, left forlimb; LH, left hindlimb; (adapted from Cheng et al., 1997).

3. intensity, a measure for the mean pressure exerted by the paw during floor contact;
4. duration of stance phase; and
5. duration of swing phase.

Since the absolute duration of stance or swing phase depends on the animal's walking speed, these parameters are transformed to a fraction of total step duration according to the following formula:fraction stance or swing phase = stance or swing phase/(stance phase + swing phase)

2.3. Data analysis and statistics

For each animal, stance or swing phase, mean intensity during stance and von Frey withdrawal thresholds are calculated as a percentage of respective pre-operative values. As such each animal serves as its own control. Von Frey withdrawal thresholds are transformed logarithmically, in order to obtain a linear scale of increasing intensities with increasing filament size. Data from stance or swing phase and mean intensity are represented as mean ± standard error of the mean (s.e.m.).

Because of the discrete nature of the von Frey data, these are represented as median and 25th–75th percentiles.

To compare differences in stance phase and mean intensity at different time points, analysis of variances (ANOVAs) were performed, followed by paired samples *T*-tests (comparisons with pre-operative values). Von Frey data were analysed using a Kruskal–Wallis test, followed by Mann–Whitney *U*-tests. Bonferroni corrections were performed.

To analyse correlations between von Frey withdrawal thresholds and mean intensities, stance or swing phase, Pearsons correlation coefficients were calculated. Data are presented as *X*–*Y* scatter plots with either % change in mean intensity, stance or swing phase on the *Y*-axis and corresponding von Frey thresholds on the *X*-axis. Regression lines were also calculated and plotted. A probability level of 0.05 was considered significant.

3. Results

3.1. Mechanical withdrawal thresholds (von Frey)

With pre-operative testing, none of the animals responded to the largest von Frey filament tested (21.1 g), on either of the hindpaws. After chronic constriction of the sciatic nerve, mechanical allodynia developed, as demonstrated by a large decrease in withdrawal threshold of the operated hindpaw at 2 weeks post-operation, to 31.0 (31.0–46.0) % of pre-operative thresholds (median and 25th–75th percentiles).

Thereafter, thresholds gradually increased to 100 (84.8–100) % of pre-operative thresholds at 10 weeks post-operatively, which is not significant from baseline (see Fig. 2).

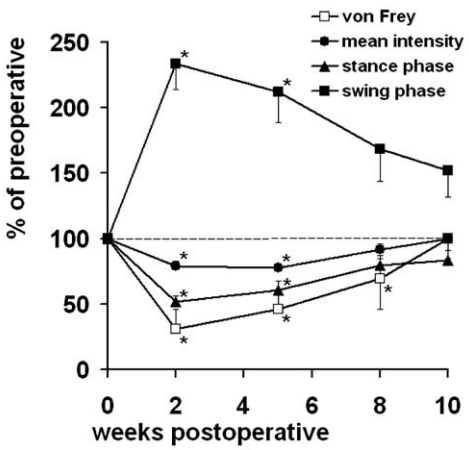

Fig. 2. Time course of different parameters obtained in rats with a CCI. Measurements were taken pre-operatively ($t = 0$), and at 2, 5, 8 and 10 weeks after CCI. Mechanical withdrawal thresholds as determined by application of von Frey filaments, mean intensity during stance, as a measure of paw pressure, and duration of stance and swing phase are plotted as percentages of respective pre-operative values. Data on intensity, stance and swing phase are obtained by the CatWalk method. Data are presented as mean ± s.e.m. (mean intensity and stance phase) or median and $_{25}tL_{75}$th percentile (von Frey) of 12 rats. (* $P < 0.05$ compared to baseline).

Withdrawal thresholds at the contralateral (non-lesioned) side remained constant throughout the experiment.

3.2. CatWalk analysis

3.2.1. General walking pattern

Before surgery, the pre-dominant step pattern is alternate (forelimb and contralateral hindlimb in sequence). After placement of the ligatures, this remains the main step pattern, although other patterns also occur (cruciate or rotary; for description of step patterns, see Table 1). Regardless of these changes in step patterns, the RI remains constant, indicating that there is no significant loss of interlimb coordination in CCI animals. Thus placement of the lesioned paw, no matter how short, is present in almost all step cycles at all time points used.

3.2.2. Intensity of the right hindlimb

Data analysis was performed with a threshold value of 40 (arbitrary units, a.u., possible range 0–255), i.e. all pixels brighter than 40 are used. The mean intensity with which the paw is placed is computed over the whole stance period. Mean baseline intensity of the right hindlimb was 86.6 ± 3.2 (mean ± s.e.m.) (a.u.). Two weeks after placement of the ligatures, intensity was significantly reduced to 79.6 ± 3.6 % of pre-operative value ($P < 0.05$) and thereafter gradually normalised to 100.3 ± 3.4% (not significant) at 10 weeks post-operatively (Fig. 2).

When these percent changes in mean intensities were plotted against percent changes in von Frey thresholds, there was a very high degree of correlation between these

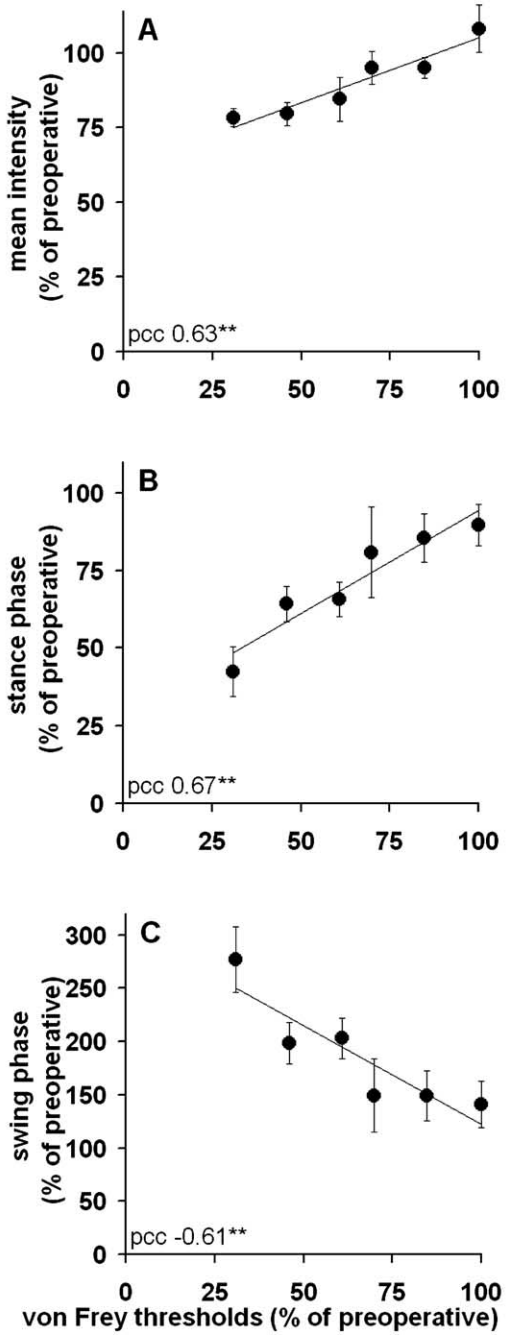

Fig. 3. Correlation between mean intensity during stance (A), stance phase duration (B), swing phase duration (C) and von Frey withdrawal thresholds in CCI rats. Data are presented as mean ± s.e.m. Linear regression lines are plotted. Pearson correlation coefficients (pcc) are calculated over 62 individual datapoints (** $P < 0.001$).

two parameters (Pearsons = 0.63, $P < 0.00\ 1$) (see Fig. 3A).

There was no consistent change in mean intensities at the contralateral side, indicating that pressure of the contralateral hindpaw does not change. Since von Frey thresholds remained constant in this paw, correlations could not be calculated.

3.2.3. Duration of stance and swing phase of the right hindlimb

Preoperatively, duration of the stance phase was 0.71 ± 0.02 (fraction of total step duration). Two weeks after surgery, the time that the lesioned paw was in contact with the floor was significantly reduced to 51.9 ± 4.4% of pre-operative values ($P < 0.05$). In the following weeks, this gradually increased to 83.6 ± 7.5 % (not significant from pre-operative values) (see Fig. 2). There was also a very high degree of correlation between this parameter and von Frey withdrawal thresholds, as demonstrated in Fig. 3B (Pearsons = 0.67, $P < 0.001$).

Initial swing phase duration was a fraction of 0.29 ± 0.02 of total step duration. At 2 weeks post-operation, this increased to 233.7 ± 19.9 % of the pre-operative value ($P < 0.05$). At 10 weeks post-operation, swing phase was again decreased to 151.8 ± 20.1 %, (not significant, see Fig. 2). Correlation between swing phase and von Frey thresholds was again very high (Pearsons = −0.61, $P < 0.001$, see Fig. 3C). There was no significant change in duration of stance or swing phase in the contralateral paw. As with the mean intensities, correlations with von Frey thresholds could not be calculated.

4. Discussion

In this paper, we describe a possible novel approach to quantify mechanical allodynia in a rat model for neuropathic pain. We found a strong correlation between von Frey mechanical withdrawal thresholds and parameters obtained from CatWalk gait analysis.

Currently, von Frey probing is one of the most frequently used methods to measure mechanical allodynia in various animal models. Although the application forces of the von Frey filaments have been shown to be objective and reproducible (Bell-Krotoski and Tomancik, 1987), there are various setbacks. One of the problems is that there are many different ways to assess and define mechanical withdrawal thresholds using von Frey probing. Moreover, bending forces of the filaments are influenced by ambient humidity and, to a lesser extent, temperature. Finally, filaments may wear off with extensive use (Andrews, 1993; Moller et al., 1998). Since testing is usually done in non-restrained animals, the experimenter has to wait for the animal to hold its paw in the right position, which then should remain the same, as weight bearing of the limb might be a confounding factor in determining von Frey withdrawal thresholds (Kauppila et al., 1998). In order to determine thresholds, multiple filaments have to be applied, which also takes time. Moreover, repetitive testing with short intervals may bias mechanical threshold determinations (Chaplan et al., 1994) and possibly disclose wind-up like pains (Jensen et al., 2001).

The CatWalk method for automated gait analysis that we used here allows for investigation of various walking para-

meters within a single run. Measurements are performed in freely moving, non-restrained animals, with minimal intervention, thus reducing the potentially confounding effects of stress (Ren, 1999). Since the time needed for an animal to cross the walkway is in the order of several seconds, it is a very rapid method, allowing relatively large groups of animals to be tested in a short time span. Moreover, data acquisition is performed by computer which allows analysis of several parameters simultaneously. In the present study, we looked at changes in mean signal intensity and the duration of different phases of a complete step cycle in rats with a CCI. We hypothesised that these parameters would be affected as a result of the increased sensitivity to mechanical stimulation in these rats.

The mean signal intensity during placement of the paw when crossing the CatWalk walkway is an estimate of paw pressure, since signal intensity decreases when less pressure is applied with the paw (Betts and Duckworth, 1978). There was a high degree of correlation between intensity measurements and von Frey withdrawal thresholds of the experimental hindpaw. This suggests that lowered mechanical withdrawal thresholds, indicative of mechanical allodynia, are paralleled by decreased pressure applied with the paw during walking. In mice subjected to sciatic nerve crush, an injury also associated with neuropathic pain (Bester et al., 2000), we also observed this decrease in applied pressure during locomotion by using CatWalk analysis (unpublished results). These observations are in agreement with earlier studies reporting a decrease in standing weight bearing of the affected limb associated with mechanical allodynia in different pain models, such as bone cancer pain (Medhurst et al., 2002), carrageenan-induced inflammation (Tabo et al., 1998) and urate arthritis (Coderre and Wall, 1988). Also, in arthritic rats, a decrease in weight load during locomotion was observed, presumably reflecting pain (Coderre and Wall, 1988; Min et al., 2001).

Duration of stance and swing phase in these CCI rats were decreased and increased, respectively, and these parameters also demonstrate a high degree of correlation with von Frey thresholds. The observation that in a rat model of inflammatory pain, i.e. carrageenan injections in the ankle joint, stance phase duration was also decreased (Angeby-Möller, personal communication) supports our findings. The use of other neuropathic or inflammatory pain models displaying mechanical allodynia (e.g. Seltzer et al., 1990; Kim and Chung, 1992; Min and Woolf, 1996) might further confirm this.

Together, our data imply that the CCI rats minimise contact with floor during walking, as demonstrated by an increased swing phase and shorter stance phase of the neuropathic hindpaw and a decrease in pressure applied during this stance phase.

Moreover, the time courses of these changes in intensity and step phases are similar to that of the von Frey withdrawal thresholds, indicating that these CatWalk parameters parallel variations in mechanical allodynia in these rats.

Since increased mechanosensitivity might be paralleled by an increase in touch-evoked responses of spinal α-motorneurones (Woolf et al., 1994), it is possible that the observed changes in gait reflect changes in motorneuron activity, rather than direct responses induced by physical contact with the floor. In this way, alterations in intensities and stance phase might represent an indirect measure of allodynia. However, since these parameters correlate very well with the response evoked by direct application of von Frey filaments, we suggest that the CatWalk method might serve as an alternative tool to assess mechanical allodynia in CCI rats. Although we have not performed any pharmacological interventions in the present study, it is very likely that future studies will reveal pharmacological sensitivity of the CatWalk method, considering the high degree of correlation between von Frey probing and this method.

A possible drawback of the CatWalk method is that it appears to be less sensitive than von Frey probing. At 8 weeks post-operation, von Frey values are significantly different from pre-operative, wherease CatWalk parameters do not reach significance (see Fig. 2). The magnitude of changes between pre- and post-operative intensities and stance phase durations was smaller than those of von Frey thresholds. However, the increase in swing phase duration is much larger. Moreover, at 10 weeks post-operation, this parameter is still increased (over 150% of pre-operative, although not significant), whereas von Frey thresholds at this time point have completely normalised. This suggests that the CatWalk method might detect small changes in mechanical sensitivity that are not detected by von Frey probing. Furthermore, the CatWalk method has several advantages over the von Frey method, such as the convenience and objectivity of data collection, and the possibility to store and review the images of rats crossing the walkway.

In summary, the CatWalk method allows for rapid and objective analysis of many locomotor parameters. In CCI rats, displaying mechanical allodynia, we demonstrated that measurements of paw pressure and duration of stance and swing phase obtained with the CatWalk method show a high degree of correlation with mechanical withdrawal thresholds as determined by application of von Frey filaments. This suggests that the CatWalk method might provide an alternative means to quantify mechanical allodynia. Moreover, since the CatWalk method is not limited to rats, it might become a practical tool to study mechanical allodynia in other animal models, in which von Frey probing can be more difficult.

References

Andrews K. The effect of changes in temperature and humidity on the accuracy of von Frey hairs. J Neurosci Methods 1993;50:91–93.

Bell-Krotoski J, Tomancik E. The repeatability of testing with Semmes–Weinstein monofilaments. J Hand Surg 1987;12:155–161.

Bennett GJ, Xie YK. A peripheral mononeuropathy in rat that produces

disorders of pain sensation like those seen in man. Pain 1988;33:87–107.

Bester H, Beggs S, Woolf CJ. Changes in tactile stimuli-induced behavior and c-Fos expression in the superficial dorsal horn and in parabrachial nuclei after sciatic nerve crush. J Comp Neurol 2000;428:45–61.

Betts RP, Duckworth T. A device for measuring plantar pressures under the sole of the foot. Eng Med 1978;7:223–228.

Chaplan SR, Bach FW, Pogrel JW, Chung JM, Yaksh TL. Quantitative assessment of tactile allodynia in the rat paw. J Neurosci Methods 1994;53:55–63.

Cheng H, Almstrom S, Gimenez-Llort L, Chang R, Ove OS, Hoffer B, Olson L. Gait analysis of adult paraplegic rats after spinal cord repair. Exp Neurol 1997;148:544–557.

Coderre TJ, Wall PD. Ankle joint urate arthritis in rats provides a useful tool for the evaluation of analgesic and anti-arthritic agents. Pharmacol Biochem Behav 1988;29:461–466.

Dixon WJ. Efficient analysis of experimental observations. Annu Rev Pharmacol Toxicol 1980;20:441–462.

Hamers FPT, Lankhorst AJ, van Laar TJ, Veldhuis WB, Gispen WH. Automated quantitative gait analysis during overground locomotion in the rat: its application to spinal cord contusion and transection injuries. J Neurotrauma 2001;18:187–201.

Jensen TS, Gottrup H, Sindrup SH, Bach FW. The clinical picture of neuropathic pain. Eur J Pharmacol 2001;429:1–11.

Kauppila T, Kontinen VK, Pertovaara A. Weight bearing of the limb as a confounding factor in assessment of mechanical allodynia in the rat. Pain 1998;74:55–59.

Kim KJ, Yoon YW, Chung JM. Comparison of three rodent neuropathic pain models. Exp Brain Res 1997;113:200–206.

Kim SH, Chung JM. An experimental model for peripheral neuropathy produced by segmental spinal nerve ligation in the rat. Pain 1992;50:355–363.

Kupers RC, Nuytten D, De-Castro-Costa M, Gybels JM. A time course analysis of the changes in spontaneous and evoked behaviour in a rat model of neuropathic pain. Pain 1992;50:101–111.

Ma QP, Woolf CJ. Progressive tactile hypersensitivity: an inflammation-induced incremental increase in the excitability of the spinal cord. Pain 1996;67:97–106.

Medhurst SJ, Walker K, Bowes M, Kidd BL, Glatt M, Muller M, Hattenberger M, Vaxelaire J, O'Reilly T, Wotherspoon G, Winter J, Green J, Urban L. A rat model of bone cancer pain. Pain 2002;96:129–140.

Min SS, Han JS, Kim YI, Na HS, Yoon YW, Hong SK, Han HC. A novel method for convenient assessment of arthritic pain in voluntarily walking rats. Neurosci Lett 2001;308:95–98.

Moller KA, Johansson B, Berge OG. Assessing mechanical allodynia in the rat paw with a new electronic algometer. J Neurosci Methods 1998;84:41–47.

Price DD, Long S, Huitt C. Sensory testing of pathophysiological mechanisms of pain in patients with reflex sympathetic dystrophy. Pain 1992;49:163–173.

Quartaroli M, Fasdelli N, Bettelini L, Maraia G, Corsi M. GV19677 1A, an NMDA receptor/glycine site antagonist, attenuates mechanical allodynia in neuropathic rats and reduces tolerance induced by morphine in mice. Eur J Pharmacol 2001;430:219–227.

Ren K. An improved method for assessing mechanical allodynia in the rat. Physiol Behav 1999;67:711–716.

Sato J, Morimae H, Seino Y, Kobayashi T, Suzuki N, Kizumura K. Lowering barometric pressure aggravates mechanical allodynia and hyperalgesia in a rat model of neuropathic pain. Neurosci Lett 1999;266:21–24.

Seltzer Z, Dubner R, Shir Y. A novel behavioral model of neuropathic pain disorders produced in rats by partial sciatic nerve injury. Pain 1990;43:205–218.

Tabo E, Eisele JH, Carstens E. Force of limb withdrawals elicited by graded noxious heat compared with other behavioral measures of carrageenan-induced hyperalgesia and allodynia. J Neurosci Methods 1998;81:139–149.

Vrinten DH, Adan RA, Groen GJ, Gispen WH. Chronic blockade of melanoeortin receptors alleviates allodynia in rats with neuropathic pain. Anesth Analg 2001;93:1572–1577.

Woolf CJ, Shortland P, Sivilotti LG. Sensitization of high mechanothreshold superficial dorsal horn and flexor motor neurones following chemosensitive primary afferent activation. Pain 1994;58:141–155.

Zimmermann M. Ethical guidelines for investigations of experimental pain in conscious animals. Pain 1983;16:109–110.