Research article

ASSESSMENT OF MECHANICAL ALLODYNIA BY USING DYNAMIC PLANTAR AESTHESIOMETER

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ABSTRACT

The most commonly used Von Frey filaments are productive in evaluating behavioral responses of neuropathic pain in preclinical and clinical research. To reduce the potential experimenter bias, automated instruments are being developed for behavioral assessment. In preclinical research, neuropathic pain models of nerve injury with varied etiology like chronic constricted injury (CCI) and Spared Nerve Injury (SNI) are employed to screen the analgesic drugs to treat symptoms like allodynia and hyperalgesia. The current study was aimed to validate and compare conventionally used Von Frey monofilaments and dynamic plantar aesthesiometer using different pain models. CCI and SNI rats were used to compare and validate the assessment of neuropathic pain using Von Frey monofilaments and automated dynamic plantar aesthesiometer.

Key wards: Mechanical Allodynia, Von Frey monofilaments, dynamic plantar Aesthesiometer

INTRODUCTION

The Dynamic Plantar Aesthesiometer has been designed to automate the assessment of "touch sensitivity" on the plantar surface of rats. It is known by some as the Electronic Von Frey or Plantar Von Frey instrument, but differently from Von Frey devices, Latency time, and Actual Force at the time of paw withdrawal reflex are automatically detected and recorded. In the present study, experiments were conducted with the aim to evaluate the usefulness of the Dynamic Plantar Aesthesiometer in drug-screenings assays, which are conducted on a regular basis at Neuro Search. The hopes were to find a method that could eliminate some of the unevenness in hair application techniques when testing with von Frey Monofilaments. Also the possibility of generating continues data that could be statistically analyzed with t-test were important, since testing with von Frey Monofilaments does not leave this option.

METHODS

Adult male Wistar rats, weighing 200-250g on the day of surgery were used in this study. The animals were housed on soft bedding, 2 per cage, with food and water ad libitum and the light-dark cycle was 12:12h. The experiments were performed according to the Ethical Guidelines of the International Association for the Study of Pain (Zimmerman 1993) Study protocol was approved by Institutional Ethics committee.

2.1 Surgical procedures

2.1.1 Surgery of CCI

The method of Chronic Constriction Injury (CCI) has been described in detail previously (Bennet and Xie 1988). The rats were anaesthetized with chloral hydrate (Merck Pharm Ltd 80mg/kg i.p.) and the skin of the medial left thigh was incised and the nerve was exposed by blunt dissection through the biceps femoris. Proximal to the common sciatic nerve's trifurcation the nerve was freed from surrounding connective tissue. Using a small pincette slid under the nerve, the nerve was gently lifted up and fixed. Using 40x magnification 4 ligatures (4.0 cat gut chromic suture, Ethicon) were tied loosely around the nerve with about 1 mm of spacing. The muscle and the skin were closed in two layers and the skin was closed with hidden stitches to avoid any opening of the wound by biting.

2.1.2 Surgery of SNI

The method of Spared Nerve Injury (SNI) has been described in detail previously (Decosterd and Woolf 2000). The rats were anaesthetized with chloral hydrate (Merck, Germany, 80mg/kg i.p.) and the skin of the lateral left thigh was incised. The cranial and caudal parts of the biceps femoris muscle were separated and held apart by a retractor to expose the sciatic nerve and its three terminal branches: the sural, common peroneal and tibial nerves. The tibial and common peroneal nerves were tightly ligated with 4/0 silk suture and 2-3 mm of the nerve distal to the ligation was removed. Any stretching or contact with the intact sural nerve was avoided. The muscle and the skin were closed in two layers and the skin was closed with hidden stitches to avoid any opening of the wound by biting.

2.2 Behavioral testing

All behavioral tests were conducted on animals at least one week after surgery and performed in a quiet room. Prior to each study the animals were allowed to accommodate to the testing environment for 15 min. The inclusion criteria for these studies was clearly developed mechanical allodynia, with included animals displaying withdrawal threshold of less than 2g, when tested with manually von Frey monofilaments (Stoelting, USA).

2.2.1 Mechanical Allodynia tested with Dynamic Plantar Aesthesiometer

Mechanical allodynia was measured with a Dynamic Plantar Aesthesiometer (DPA) (UgoBasil, Italy). The filament was applied as perpendicular as possible to the mid-plantar surface of the left hind paw. The filament was applied with a preset force between 0-5g or 0-50g and with a preset velocity between 0 and 20 seconds until the preset force was reached. When testing animals operated according to the SNI method, only the outer mid-plantar surface of the left hind paw was tested, according to the innervations of the hind paw by the sural nerve. Measurements were repeated from 3-9 times and the threshold determined as the average of these measurements.

2.2.2 Mechanical Allodynia tested with von Frey monofilaments

Mechanical allodynia was measured with a set of von Frey monofilaments (0,0018g – 51.1g, Stoelting, USA) and the withdrawal threshold to mechanical stimulation was determined using a modification of the Dixon up-down method (Dixon 1980). The monofilaments were applied perpendicular to the mid-plantar surface of the left hind paw with sufficient force to cause a slight buckling of the hair. Starting of with a monofilament just below 2g and continuing in either a- or descending order according to the response, the monofilaments were applied until a withdrawal threshold had been reached.

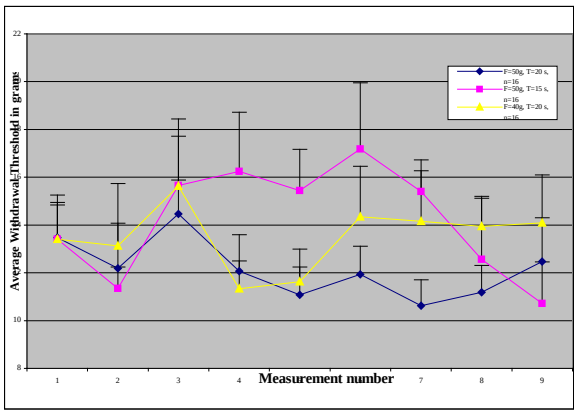

Fig.1. Parameter Testing: Withdrawal thresholds during repeated measurements with Dynamic Plantar Aesthesiometer in CCI animals. F= Force, T= Time, n= animals. Variations calculated as SEM.

2.2.3 Parameter testing with Dynamic Plantar Aesthesiometer in CCI Animals

In order to assess the most valid parameters for testing of CCI animals' different maximum forces and time frames for application of the filament were studied. Since CCI animals normally have an average respond threshold around 1 g, but can reach thresholds up to 20g if given analgesic drugs, the 0-5g range of applied force was not seen as a valid range, since a good analgesic effect of a tested drug might evoke higher thresholds than 5 grams. Therefore the broader ranging interval from 0-20 grams was chosen for the parameter testing. These tests were conducted to evaluate the influence of different time intervals or maximum forces applied, on average thresholds and on variation of repeated measurements. The three different combinations of time and force tested were: 1) F=50g, T=20s; 2) F=50g, T=15s; 3) F=40g, T=20s (Fig. 1).

2.2.4 Control Animals tested with Dynamic Plantar Aesthesiometer

In order to evaluate normal response thresholds for non-allodynic rats, control rats were tested with the parameters chosen in section 2.2.3. Both average thresholds and the effect on thresholds from repeated measurements were evaluated.

2.2.5 Comparison of Dynamic Plantar Aesthesiometer and von Frey Monofilaments

Baselines were obtained on the day of testing. Rats were given either Morphine (2mg/kg) or vehicle (0.9% NaCl) and then tested in parallel time frame at: 30, 60, 120 and 180 min with the Dynamic Plantar Aesthesiometer (F=50, T=20) (see section 2.2.1), and at: 45, 75, 140 or 200 min with von Frey Monofilaments (see section 2.2.2).

2.2.5 Parameter Testing of Dynamic Plantar Aesthesiometer in SNI Animals

In order to assess the most valid parameters for testing of SNI animals, both the range 0-5g and the range 0-20g were applied with the time set to 20 seconds in both tests (see section 2.2.1). SNI animals are extremely sensitive to mechanical allodynia and in average reach thresholds less than 0,1g when tested with von Frey Monofilaments. The two different combinations tested were 1) F=50g, T=20s and 2) F=5g, T=20s.

2.2.6 Drug testing with Dynamic Plantar Aesthesiometer in SNI Animals

Baselines were obtained on the day of testing. Rats were given either Morphine (2mg/kg) or vehicle (0,9%NaCl) and then tested at 30,60,90,120 and 180 min. with the Dynamic Plantar Aesthesiometer (F=50g, T=20s)(see section 2.2.1).

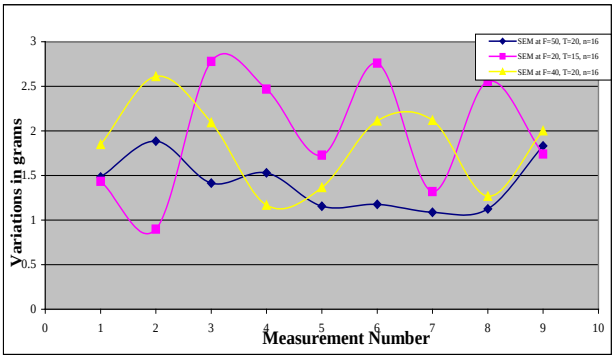

Fig. 2 Parameter Testing: SEM variations during repeated measures with Plantar Dynamic Aesthesiometer on CCI Animals. F=Force, T= Time and n= animals.

RESULTS AND DISCUSSION

3.1 Parameter testing with Dynamic Plantar Aesthesiometer in CCI Animals

As can be seen from fig. 1, the variation during repeated measurements were least pronounced at the parameter combination of F=50g and T=20s, where also the lowest variation in group averages (Fig. 2) were found. This setting was therefore chosen as standard for further studies on CCI animals.

3.2 Control Animals tested with Dynamic Plantar Aesthesiometer

As can be seen from fig.3 there were a quite high degree of variations between the four Control Animals tested in this experiment. The responses between animals were varying from 35 to 15 after nine repeated measurements, and there was a tendency to see declining threshold values as repetitions increased. However, when looking at the overall average for each Control Animal at Fig. 4 the average thresholds calculated after the repeated measurements appears basically equal and the SEM variations on the repetitions are comparable between Control Animals.

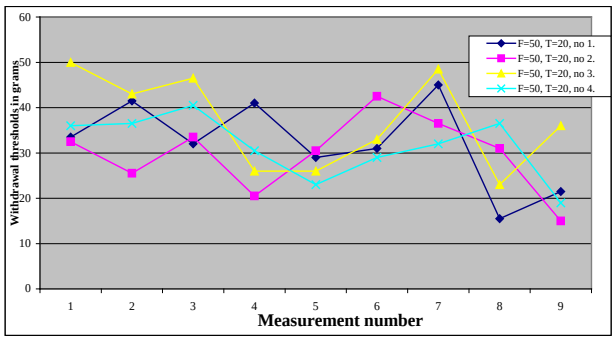

Fig. 3 Control Animals tested with Dynamic Plantar Aesthesiometer. Responses shown after repeated measurements. F= Force, T= Time, no= animal number.

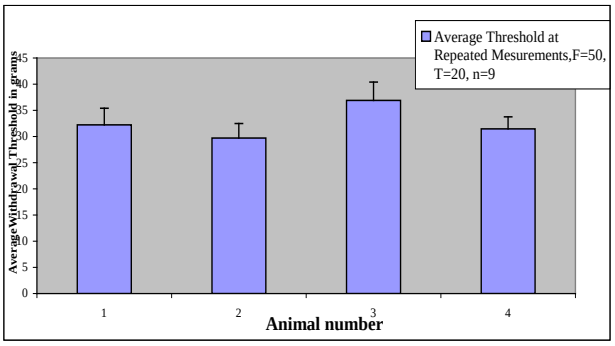

Fig. 4 Average Thresholds after Repeated Measurements in Control Animals. F= Force, T= Time, n = number of repeated measurements. Variations calculated as SEM.

3.3 Comparison of Dynamic Plantar Aesthesiometer and von Frey Monofilaments

The results from testing Morphine and Vehicle treated CCI animals with the two methods are presented in Fig. 5 and Fig. 6. The most striking difference, when looking at the Vehicle treated CCI animals, is the lack of sensitization of response patterns when testing with von Frey monofilaments. Comparing the separation in Average thresholds between Morphine and Vehicle treated CCI animals; both methods obtain good separations, even though the separation from baseline in the von Frey Monofilament test is more pronounced than with the Dynamic Plantar Aesthesiometer. It is difficult not to wonder whether or not the decline in response patterns from the Vehicle treated animals, is also pronounced in the Morphine treated CCI animals when tested with the Dynamic Plantar Aesthesiometer, thereby composing a masking of Morphine's beneficial effects on the CCI animals. The variations in the Dynamic Plantar Aesthesiometer test is however much lower than in the testing with von Frey Monofilaments.

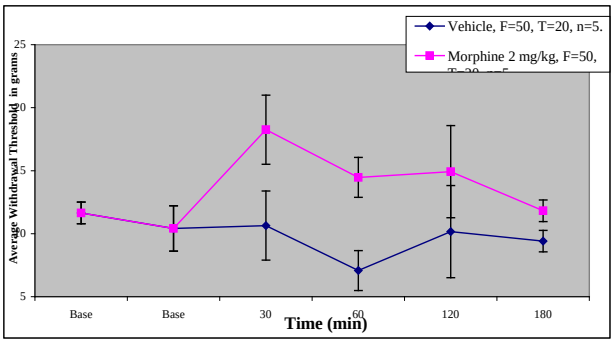

Fig. 5. Average thresholds in Morphine and Vehicle treated CCI animals tested with Dynamic Plantar Aesthesiometer. Each animal was tested three consecutive times at each time interval, and the average of these measurements were used as the animals value

at that time interval. F= Force, T= Time and n = animals. Variations calculated as SEM.

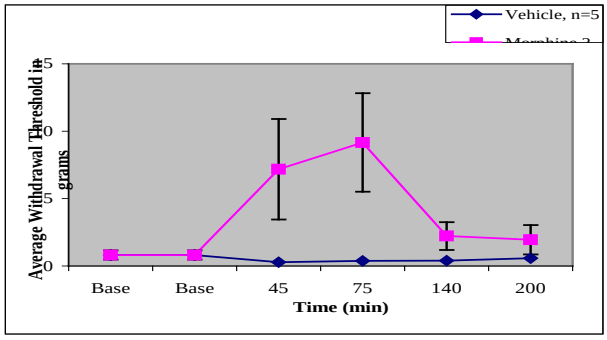

Fig. 6. Average threshold in Morphine and Vehicle treated CCI animals tested with von Frey Monofilaments. n= animals. Variations are calculated as SEM.

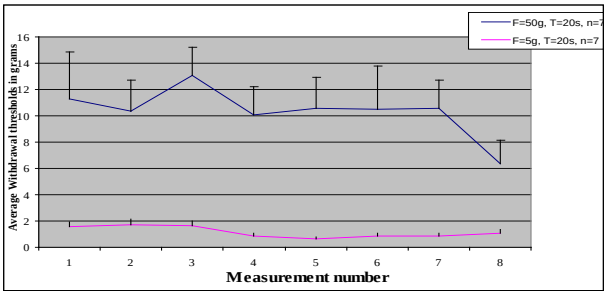

Fig. 7 Parameter testing in SNI animals. Average with drawal threshold following repeated measurements. F= Force, T= Time, n= animals. Variations are calculated as SEM.

3.4 Parameter Testing of Dynamic Plantar Aesthesiometer in SNI animals

SNI animals are often much more sensitive to von Frey Monofilaments than CCI animals, and may therefore respond differently to testing with the Dynamic Plantar Aesthesiometer. Therefore both ranges of the Dynamic Plantar Aesthesiometer (0-5 and 0-50) were tested to estimate the best test-range for these animals (Fig. 7). The results are an excellent example of the arbitrary outcome in withdrawal thresholds, when using the Dynamic Plantar Aesthesiometer. Why are the SNI animals responding around 10-12 grams in the highest interval and only around 1 to 1.5 in the lowest interval? The answer is of course the time factor for pressure application, which in these experiments was set at 20 seconds. However, it does seem strange that these ten-fold differences in withdrawal thresholds are influenced at such a high degree by the timeframes for application. The lowest variations were seen in the 0-5 range, but since the range only went up to 5 grams, it was decided to use the 0-50 range in further experiments, since there would be a risk of moving out of range when testing drugs.

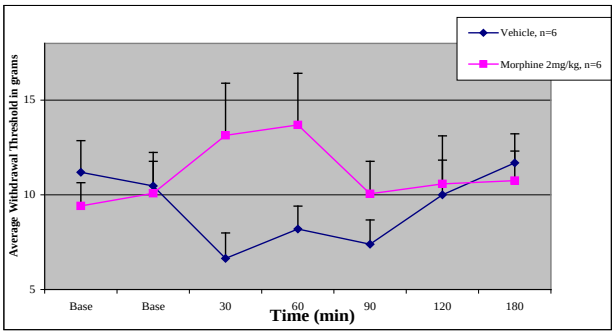

Fig. 8 Average thresholds in Morphine and Vehicle treated SNI animals tested with Dynamic Plantar Aesthesiometer. Each animal was tested three consecutive times at each time interval, and the average of these measurements was used as the animal's value at that time interval. (F=50, T=20). F= Force, T= Time and n = animals. Variations calculated as SEM.

3.5 Drug testing with dynamic Plantar Aesthesiometer

When comparing Morphine and Vehicle treated SNI animals (Fig.8) we saw a nice separation between the two groups. However the same pattern of sensitization in the Vehicle group as seen for the CCI Vehicle animals was observed. Again it must be questioned whether there is a masking effect on the Morphine treated group because of the sensitization.

3.6 General Behavioural Aspects

When using the Dynamic Plantar Aesthesiometer, there are several behavioural factors that may strongly influence the testing outcome. First, it is very important that the animal is standing on all 4 paws when tested. Second, the animals weight must be evenly distributed between the hind-legs. Third, since rats fall quite quickly asleep once accommodated to the testing environment, it becomes increasingly difficult to test the animals in the type of pain studies where drug effects are evaluated over time, as it has been done in the above studies with Morphine. Fourth, if the animals do fall asleep, the relaxation of the hind-leg seems to be giving the same response values as the baseline measurements (around 10-12 grams in CCI animals when F=50 and T=20, data not shown). This may lead to serious misinterpretations of the results from drug screenings if the experimenter is not aware of the animals' awakeness level.

CONCLUSION

The Dynamic Plantar Aesthesiometer offers a reproducible way of testing neuropathic pain animals relatively fast, and provides continuer data that can be analyzed by regular statistical techniques such as the t-test. When that is said it must also be strived that the Dynamic Plantar Aesthesiometer does not, according to the above experiments fit as a method used for drug-screenings tests where drug effects are evaluated over time. This is said for two reasons. First, the testing with approximately 30 min intervals creates a decline in withdrawal thresholds in vehicle groups, and may also be masking a positive effect in the drug groups, thereby causing a lowered separation between groups. Second, the animals falls asleep when testing this way, and therefore makes it extremely difficult to obtain reliable data. It is therefore the belief of this experimenter that the Dynamic Plantar Aesthesiometer is not suited for drug- screenings tests.

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