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Measuring Pain Withdrawal Threshold using a Novel Device in "Pseudo-continuous" Mode

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Running title: Pain Withdrawal Measurements in "Pseudo-continuous" Mode

Abstract

The study of pain and analgesia is an important area of biomedical research that has led to a significant number of advances in the treatment of acute and chronic pain. This study introduces a novel approach to mechanical testing of pain withdrawal of a rat hind paw to a stimulus. This systematic method involves a modified electronic esthesiometer controlled by an IDEA drive that allows for consistency in experiments. The device gives the experimenter computer control of the step size and velocity of approach of the probe stimulus. We discuss here some of the limitations in the current techniques used and illustrate how this device will result in reduced errors during an experiment. The standard method primarily involves manually raising the probe towards the animal. The data presented herein shows how the computer controlled *pseudo-continuous* mode of operation is effective in determining the pain threshold with a lesser deviation from the mean.

Introduction

In 1864, three surgeons, S. W. Mitchell, G. R. Morehouse, and W. W. Keen, produced one of the first publications addressing neurological disorders in their book, "Gunshot wounds and other injuries of the nerves." This book is one of the first publications to address the idea that neurological disorders can be characterized by pain in the affected area (Xinning et al. 2014). Today, clinical and basic science research shows that chronic neuropathic pain is caused by lesions in the peripheral or central nervous systems present in many varied forms (Dworkin et al 2003, Kim and Chung 1992). The behavioral study of pain has led to a significant number of advances in the treatment of acute and chronic pain. These types of experiments include measuring the withdrawal threshold of limb to a thermal, mechanical, electrical, or chemical stimulus. Mechanical testing of pain response can reveal either mechanical allodynia or hyperalgesia. A limb withdrawal in response to a light touch, a pressure, or a brushing evidences allodynia, which is pain to a normally non painful stimuli (Bove 2006). Hyperalgesia is increased sensitivity pain as a result of peripheral nerve damage.

The current method used for quantifying mechanical pain is based on an early esthesiometer, developed by the German physiologist M. von Frey who utilized horse hairs of varying lengths and diameters that would buckle under a specific force. The pain threshold was determined as the bending force of the weakest filament applied that resulted in limb withdrawal in the tested animal or human. Recently the horse hair has been replaced with nylon (Semmes and Weinstein monofilaments) with increasing diameters that bend when a specific value is reached (Weinstein 1993). The advent of electronic force transducers has produced new forms of esthesiometry; these are either electronically controlled by a motor or manually moved by the experimentalist. To use an electronic esthesiometer, a motor controls a probe, to which a force transducer is connected. The probe applies pressure in a linear motion to an area of skin until the threshold is reached, at which point the subject moves the limb and the probe is removed (Moller et al. 1998). Although both the electronic and manual method of esthesiometry rely on the transference of a force, there is a difference in the outcome of the methods. For example, transfer of force in the manual system may not be constant each time (Chong and Cros 2004), while the electronic system may have a rather continuous motion, and thus monitor a true reaction to the stimulus. The purpose here is to present a novel method of measuring and testing hyperalgesia to a mechanical stimulus. This proposition is aimed to increase experimental sensitivity and reproducibility.

Materials and Methods

The device consists of a captive actuator with a 38.1 mm (1.5 in.) stroke. An anti-rotation cap allows the shaft to actuate without an external guide mechanism and is designed to lift up to 2 kg of mass. The mass of the transducer atop the motor is 100 g. This mass plus the reaction force on the animal's plantar surface are within the limits of the motor. The minimum step size is 0.006 mm. The motor is computer controlled using a programmable IDEA drive (HaydonKerk Motion Solutions). The drive is electronic with a fully programmable control unit that uses a Graphic User Interface, giving the experimenter access to control the rate and size of the steps.

The cylindrical force transducer delivers the stimulus on the same plane and axis as the linear actuator (Figure 1). The flow chart in Figure 1 shows the basic electronic schematic. The data acquisition card varied between the WINDAQ system and the CLAMPEX data acquisition systems. The design is flexible to work with any signal data acquisition systems available.

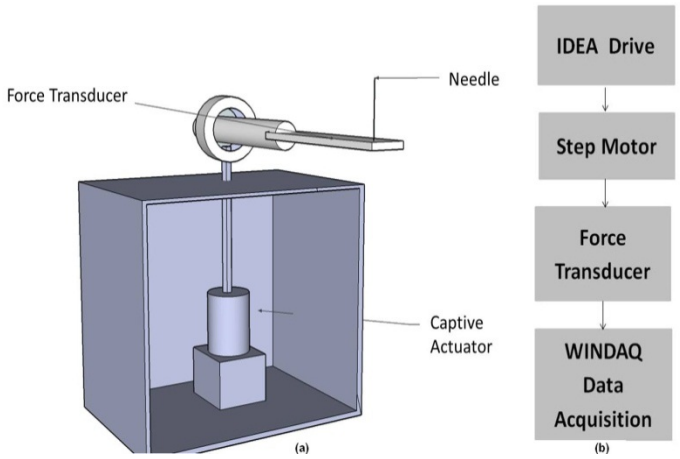

Figure 1. (a) Diagram of the automated device used the measure the PWT. (b) Flow chart of the components in the design.

The experiment was performed on Sprague-Dawley rats, under IACUC protocol #3393 (Evaluation of efficacy of novel analgesic compounds in rat model of neuropathy, University of Arkansas for Medical Sciences). The rats were kept in the test cages for at least 30 minutes prior to experiment. This allowed them to acclimatize to the test environment. They were resting during the experiments.

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The experiments were conducted on three male rats and each test was repeated five times for each rat. The device was placed under each rat and the probe approached the rat at a rate of 1mm/sec. The experiment stopped after the hind paw was withdrawn indicating the pain threshold. The data were compared using the SigmaStat statistical package.

Results and Discussion

The motor functions in a *pseudo-continuous* mode. This means that during testing, the very small increments in the step size will be an almost "continuous" motion. The exact force representing the pain threshold can be determined since the experiment is identical for each test. The results presented here show how the technique allows for the maximum threshold force to be determined as well as the reduced deviation from the mean values.

Figure 2 shows typical results from the experiments. The background noise shown is due to small vibrations that may exist in the laboratory, which is generally averaged out. . The actual force can be determined using Newton's second law, $F = mg$, where m is the mass (kg) and g = 9.8m/s^2.

Figure 2a and 2b show an increase in force, followed by a sharp drop. The peak is recorded as the pain threshold response for that experiment. From the similarities in the results, it can be seen that the programmable esthesiometer does not alter the experiment but rather the way the data is recorded: the force is increased in set increments. The manual esthesiometer has been considered to move in incremental steps as well, however, the experimenter's approach to the animal is subjective and may not always be constant.

Figure 3 shows the overall data obtained from 5 experiments on 3 different rats using the automated programmable esthesiometer and the manual esthesiometer. In each case the experiment was performed 5 times. The error bars represent the standard deviation of the values obtained from the experiment and will be used here to compare the two different methods to determining the pain withdrawal

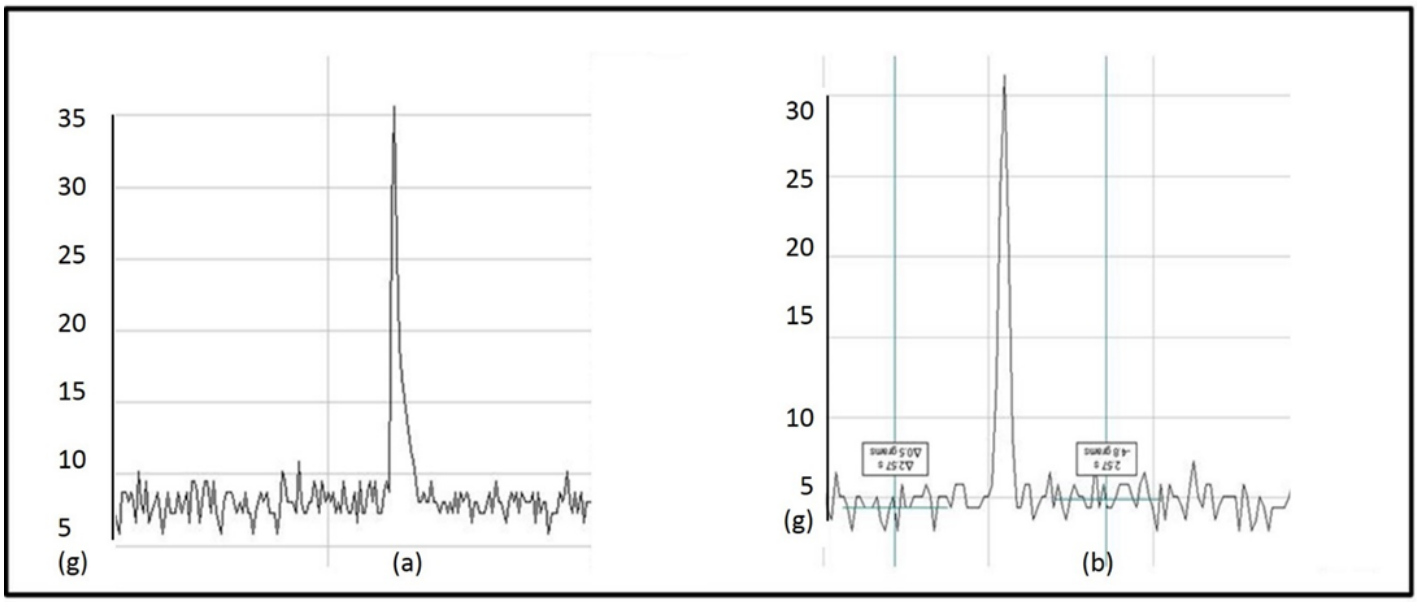

Figure 2. (a) Typical data using programmable esthesiometer. (b) Typical data using manual esthesiometer. The horizontal axis (not shown) is time. The vertical axis is grams. The vertical axis shows the raw data obtained from the experiments. The force is then calculated using F=mg, where m is the mass (kg) and g = 9.8m/s^2

threshold. The data obtained from the manual experiment shows between 50-60% standard deviation, while the data obtained from the automated device shows 10-25% deviation from the mean. This is at least two-fold decrease in error using the programmable esthesiometer.

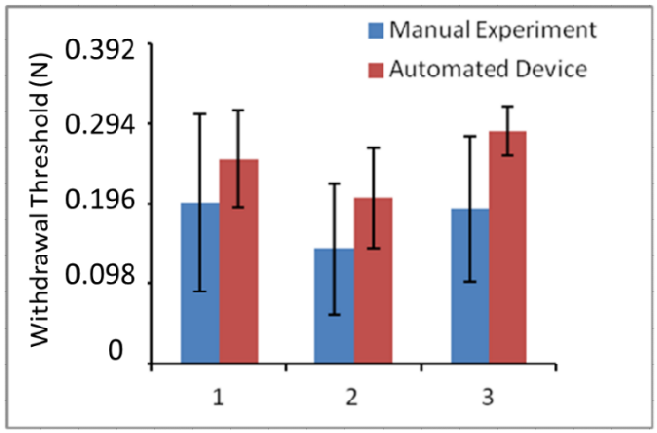

Figure 3. Comparison of withdrawal thresholds obtained from the manual experiment and the automated device ($p<0.1$).

While the actual thresholds obtained from the two experiments are not significantly different, and should not be, the automated device produced a tighter group of data when compared to the manual method. In all 3 subjects, the same trend is observed. The automated device shows a reduction in the percent variation from the mean compared to the manual experiment. The rats in the experiment were all normal control rats. There are usual variations between the rats themselves because of size. However, the purpose here is not to compare the pain withdrawal threshold but to compare the difference obtained in the threshold from the two described methods.

Conclusion

While the technique of using a force transducer is not new, the method we have described is new and permits an innovative way of collecting data. Each iteration of the experiment is guaranteed to be performed in exactly the same way each time. The rate of approach to the animal is kept constant regardless of the experimenter. The goal of this paper was not to compare the actual values obtained. Rather, the data presented illustrate that using a programmable esthesiometer operating in small increment steps (*pseudo-continuous)* results in a greater consistency in each experiment and yields data with lesser variation. Thus this new method can could ultimately lead to better models of behavioral response and a better understanding the factors contributing to these various ailments.

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