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Quantitative test of responses to thermal stimulation in spinally injured rats using a Peltier thermode: A new approach to study cold allodynia

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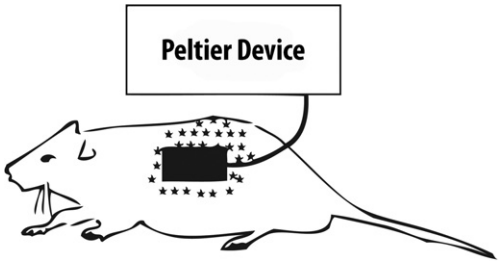
HIGHLIGHTS

- We developed a method to assess the response of female Sprague–Dawley rats to thermal stimulation (heating and cooling) to the flanks using a Peltier thermode.
- We were able to demonstrate that spinally injured rats exhibited allodynia to cooling, which was correlated with mechanical allodynia.
- No hypersensitivity to heat stimulation was observed following spinal cord injury.
- This method is useful in further studies on the mechanism and treatment of cold evoked pain.

GRAPHICAL ABSTRACT



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ABSTRACT

In this work, we described a method of testing of responses of spinally injured rats to thermal stimulation (heating and cooling) to the flank area using a Peltier thermode. With a baseline holding temperature at 32 °C and the temperature change rate of 0.5 °C/s, we measured vocalization thresholds of rats to thermal stimulation in the flank area. While normal rats did not vocalize to temperatures changes ranging from 6 °C to 50 °C, the spinally injured rats exhibited significantly increased response to cooling with average response temperature above 15 °C through the 70 day observation period after spinal cord injury. The response temperature to cooling in spinally injured rats is correlated with the magnitude of responses to cold stimulation scored after ethyl chloride spray and with the response threshold to mechanical stimulation. In contrast, we did not observe an increase in response to warm/heat stimuli. The results showed that ischemic spinal cord injury produced cold, but not heat, allodynia in rats. Furthermore, we showed that it is possible to quantitatively measure response of rats to thermal stimulation on the body using temperature as end points which may aid further studies on mechanisms and treatments of thermal stimulation, particularly cold, evoked pain.

1. Introduction

Abnormal sensitivity to thermal stimulation (cold, warm or heat) is often associated with acute or chronic pain. While heat hyperalgesia is prevalent during acute tissue injury or inflammation (Meyer and Campbell, 1981; Pogatzki-Zahn et al., 2005; Huang et al., 2006), increased sensitivity to cold (cold allodynia and hyperalgesia) is commonly seen in chronic neuropathic pain (Woolf and Mannion, 1999; Jorum et al., 2003; Jääskeläinen et al., 2005;

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Horowitz, 2007). In particular, a high percentage of patients suffering from central neuropathic pain after, for example stroke or spinal cord injury, exhibit cold allodynia (Finnerup et al., 2003; Greenspan et al., 2004).

A variety of rodent models of neuropathic pain have been developed and in some of these models thermal sensitivity has been assessed as response latency or intensity to temperatures that are often nociceptive (Hargreaves et al., 1988; Perrot et al., 1993; Choi et al., 1994; Jasmin et al., 1998; Yoon et al., 2004). Thus, the responses reflect thermal hyperalgesia rather than allodynia, despite the clinical prevalence of allodynia in neuropathic pain. Quantitative sensory testing of sensitivity to thermal stimulation is routinely used in the clinic to measure sensory abnormalities under pathological conditions (Verdugo and Ochoa, 1992; Hansson et al., 2007). In contrast, quantitative sensory testing to thermal stimulation has been rarely performed in rodents. Recently several groups have addressed this question, particular in studies of cold allodynia (Allchorne et al., 2005; Yalcin et al., 2009).

We have previously reported that photochemically induced spinal cord injury in rats produced a chronic bilateral pain-like behavioral syndrome consisting mainly of profound mechanical and cold hypersensitivity on body areas at or rostral to the dermatome of the injured spinal segments (Xu et al., 1992; Hao et al., 1996, 1998). In our previous studies, cold response was induced by ethyl chloride spray, which reduces the skin temperature to about 0 °C and the response pattern of the rats was scored (Hao et al., 1996, 1998). We have not studied responses to warm and heat stimulation in this model previously. In the current work, we assessed the temperature (both heating and cooling) required to elicit pain-like responses in spinally injured rats using a Peltier thermode, with which the surface temperature of a metal plate is controlled. We also studied the correlation between response temperature to cold stimulation and response magnitude after ethyl chloride spray and between mechanical and cold allodynia in spinally injured rats.

2. Materials and methods

2.1. Animals

All experiments were approved by the regional research ethics committee. Female Sprague–Dawley rats (Möllegård, Denmark) weighing 250–300 g at the start of the experiments were used. Animals were housed 4 per cage, irrespective of experimental groups, at constant room temperature of 22 °C and in a 12:12 h light–dark cycle with food and water available ad libitum.

2.2. Photochemically induced spinal cord ischemic injury

The rats were anaesthetized with Domitor (75 mg/kg ketamine + 1 mg/kg medetomidine in 1 ml/kg) and one jugular vein was cannulated. A midline incision was made in the skin overlying vertebral segments T12-L1. The animals were positioned beneath an argon laser beam and irradiated for 10 min with the beam directed toward vertebral segment T12 or T13 (spinal segments L3-5). Immediately prior to and 5 min after the start of the irradiation, erythrosin B (Red No. 3, Aldrich-Chemie, Steinheim, Germany) dissolved in 0.9% saline was injected i.v. at a dose of 32.5 mg/kg. A tunable argon ion laser (Innova model 70, Coherent Laser Product Division, Palo Alto, CA) operating at 514 nm was used. The average beam output power was 160 mW. During irradiation, the temperature of the rats was maintained at 37–38 °C.

2.3. Assessment of mechanical allodynia in spinally injured rats

Sensitivity to mechanical stimulation was tested by examining the vocalization thresholds to graded mechanical touch/pressure applied with calibrated von Frey hairs (Stoelting, USA). During testing the rats were gently restrained in a standing position and the von Frey hair was pushed onto the skin until the filament became bent. The frequency of the stimulation was about 1/s and ateach intensity 5–10 stimuli were applied. The intensity of stimulation which induced consistent vocalization (>75% response rate) was considered as pain threshold.

2.4. Thermal stimulation using a Peltier thermode

Peltier effect is an end-to-end transfer of heat when electric current is passed in a circuit consisting of two dissimilar semiconductors to result in cooling off one junction while heating up the other. The surface temperature of a Peltier thermode can be maintained or adjusted by varying current. The development and application of a Peltier thermal stimulator in rats has been described previously (Wilcox and Giesler, 1984). In this study, a fluid cooled, hand held Peltier thermode (active surface: 25 mm × 50 mm, control resolution: >0.02 °C, calibration uncertainty: ±0.2 °C) connected to a MSA (Modular Sensory Analyzer) Thermal Stimulator (Somedic, Sweden) was used for thermal stimulation. The baseline temperature was 32 °C and the rate of temperature change was 0.5 °C/s. Rats were held gently in a standing position and the thermode was pressed against the shaved flank area (shaded area in Fig. 1). Three heating stimuli were applied at 1 min intervals and the average temperature at which the rats vocalized was taken as heat response threshold with 50 °C as cut-off temperature. Similarly, three cooling stimuli were then applied at 1 min intervals and the average temperature at which the rats vocalized was taken as cold response threshold with 6 °C as cut-off temperature.

2.5. Measurement of cold hypersensitivity using ethyl chloride spray

During testing the rats were gently restrained in a standing position and ethyl chloride spray (Rönnings Europa AB, Sweden) was applied to the shaved allodynic flank area. The response was graded with a score of 0 = no observable response; 1 = localized response (skin twitch and contraction), no vocalization; 2 = transient vocalization, moderate avoidance and 3 = sustained vocalization and avoidance

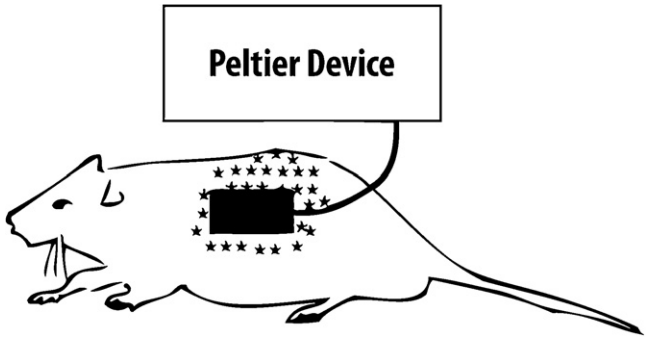

Fig. 1. Schematic illustration of a spinal cord injured rat subjected to thermal stimulation with a Peltier thermode on an allodynic area (shaded region). When a vocalization response was observed, the examiner pushed a switch which was connected to a computer for data collection and to reset the temperature of the thermode.