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Thermal hyperalgesia assessment for rats after spinal cord injury: developing a valid and useful pain index

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Abstract

BACKGROUND CONTEXT: Ongoing research to understand the mechanism behind pain is heavily dependent on animal testing. However, unlike humans, animal subjects cannot directly communicate with researchers to express the degree of pain they are experiencing. Therefore, measuring the presence of pain in animal studies is based on behavioral tests. The use of arbitrary values for determining the presence of pain in animal studies is an oversimplification of a complex and cortically dependent process.

PURPOSE: The purpose of the present study was to identify a statistically supported latency time indicator that can be used as an accurate index for hyperalgesia to thermal stimuli in Sprague-Dawley rats subjected to T9 contusive spinal cord injury (SCI).

STUDY DESIGN: A statistical analysis of latency of withdrawal from stimulus-mediated spinal reflex in 979 Sprague-Dawley rats that had been subjected to a T9 contusive SCI was performed.

METHODS: This is a retrospective review of a large research database derived from a series of studies performed evaluating thermal hyperalgesia in rats after SCI. Sprague-Dawley rats underwent a T9 contusive SCI and were tested for withdrawal latency from a heat stimulus. Assessment was done preinjury and on Postinjury Days 21, 28, 35, and 42 of the chronic phase of injury via a plantar withdrawal test.

RESULTS: The baseline test results of the 979 rats showed a significant resemblance to the normal distribution. The observed change in withdrawal showed mean latency drops of 0.42 second (standard error of the mean [SEM], 0.18; p=.026), 0.57 second (SEM, 0.19; p=.004), 0.63 second (SEM, 0.19; p=.002), and 0.69 second (SEM, 0.19; p=.0003). The standard deviation from the mean at all four postsurgical assessments was between 2.8 and 2.9 seconds.

CONCLUSIONS: Interpretation of withdrawal latency times as a marker for thermal hyperalgesia must be based on an appreciation for the normal distribution of pain scores. Recognizing that withdrawal latency is normally distributed both before and after injury allows for rational assignment of animals to groups designated as hyperalgesic and nonhyperalgesic. Two point nine seconds faster than the mean latency time is a statistically reliable indicator of thermal hyperalgesia in Sprague-Dawley rats subjected to contusive SCI. Repeated testing of animals to establish the presence or absence of thermal hyperalgesia beyond 21 days is not necessary in the absence of intervention.

Keywords: Spinal cord injury; Thermal hyperalgesia; Plantar test; Spinal reflex; Withdrawal latency time; Statistical analysis; Neuropathic pain

FDA device/drug status: Not applicable.

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Introduction

Neuropathic pain is defined as secondary to a lesion or dysfunction of the nervous system [1]. Damage to either the peripheral or the central nervous system is a well-defined cause of such pain [2,3]. Neuropathic pain as a result of spinal cord injury (SCI) is a significant clinical problem, affecting up to 80% of SCI patients [4,5] (National Spinal Cord Injury Statistic Center 2006). Neuropathic pain is often a significant detriment to quality of life in patients with SCI [6,7]. Better understanding of the mechanism behind neuropathic pain through studying animal models of pain is central to the development of effective therapies. However, direct assessment of the subjective sensation of pain in animal models is not straightforward.

Thermal hyperalgesia (TH) and mechanical allodynia are commonly used indicators for the presence of pain in animal models [8]. Thermal hyperalgesia testing developed by Hargreaves et al. [9] assesses an animal's withdrawal of a hind paw to a thermal noxious stimulus. We have used this method of pain assessment after a contusive SCI in a rat model in a variety of studies since 2004. In some of our previous work, we have defined a decreased withdrawal latency of greater than 2 seconds compared with baseline values to be indicative of the development of TH. This value was selected based on previous studies investigating peripheral nerve injuries in which a reliable and consistent change in withdrawal latency was elicited through ligation of the sciatic nerve [10]. Others and we have questioned whether this value is too arbitrary, given our observed variability in withdrawal latency time and the variability on pain response in human patients with SCI. In patients with SCI, there is significant variability in the degree of neuropathic pain with nearly identical injury [11,12]. Our laboratory and others have resorted to reporting differences between treatment and control groups as statistically significant (or not), irrespective of the characteristics of individual animals within the groups or clinical importance of the magnitude of difference [13,14].

Therefore, we examined the response of animal subjects to thermal stimulation through a retrospective review of a large prospectively collected database of animals injured in a series of experimental studies.

Materials and methods

Information regarding every animal operated on in our laboratory has been entered into a common database, and information regarding all assessments performed on each animal has been recorded. All studies were approved by the local institutional animal care and control committee. All animals were treated in accordance with published National Institute of Health standards. Data derived from animals subjected to experimental treatments were included up to the point that they received the treatment. No new surgery was performed for this analysis.

Contusive SCI

To induce contusive SCI in rats, we used the MASCIS Impactor protocol as described previously [15–20]. Briefly, adult male Sprague-Dawley rats (250–300 g) were anesthetized with gaseous isoflurane in oxygen (5% for induction and 3% for maintenance) throughout the duration of surgery. A T9 laminectomy was performed under aseptic conditions without disrupting the dura mater. Stabilizing vertebral clamps were placed at T8 and T10, and the animal was positioned in the MASCIS Impactor (Model II; WM Keck Center for Collaborative Neuroscience, Rutgers University, Piscataway, NJ, USA). The spinal cord was injured by releasing a 10-g rod (2.5 mm diameter) from a height of 12.5 mm. Bupivacaine (Sensorcaine-MPF 0.25%, 0.20 mL; Fresenius Kabi USA, LLC, Lake Zurich, IL, USA) was administered subcutaneously as a local anesthetic and the wound was closed in layers. Throughout the procedure, body temperature was maintained at 37°C with a constant temperature heating pad. The animals were then returned to their cages after recovering from anesthesia. Animals underwent daily manual bladder expression until bladder control was reestablished, and each animal received cefalexin antibiotic (0.10 mL; 330 mg/mL in saline) for 7 days after injury.

Basso, Beattie, and Bresnahan field locomotion test

Animals underwent an open-field locomotor test on Postinjury Days 2, 7, 14, 21, 28, 35, and 42. Once animals showed consistent forelimb and hind limb coordination (scoring 15 in the Basso, Beattie, and Bresnahan locomotor rating scale) [21], they were tested for TH.

Plantar test

Baseline reaction time was measured for 979 male Sprague-Dawley rats (250–300 g). Three sets of left and right hind paw reaction latencies to the thermal noxious stimulus were collected by placing an animal inside the Plexiglas apparatus (Plantar Test; Ugo Basile, Comerio VA, Italy). A movable focused beam of radiant heat was applied under the sole of one hind paw (Plantar Test, Biological Research Apparatus; Ugo Basile). When the animal retracts its paw from contact with the beam, a photocell turns off the heat, and the latency time is automatically recorded with a built-in timer. The strength of stimulation is 60 IR and is adjusted to produce baseline latencies of 8 to 10 seconds (typically 45°C–47°C). Animals were first acclimatized in the apparatus for 30 minutes before any measurements were taken for consistency. Each measurement was taken with the intermission of 5 minutes in between the measures. Testers were blinded to the animal's experimental group. No measurements aside from the behavioral baseline and locomotion tests were taken before 21 days in our study, and all rats were sacrificed after 42 days.