Journal of Neuroscience Methods 256 (2015) 74–81

Contents lists available at ScienceDirect

Journal of Neuroscience Methods

journal homepage: www.elsevier.com/locate/jneumeth

Clinical neuroscience

Demarcation of secondary hyperalgesia zones: Punctate stimulation pressure matters

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HIGHLIGHTS

- Secondary hyperalgesia area is a primary outcome in studies of analgesic efficacy.
- The literature indicates a lack of standardization in demarcation paradigms.
- Standardized demarcation of areas were made with different punctate stimulators.
- A highly significant correlation between applied pressure and area was demonstrated.
- Standardized assessment methods are recommended in future research.

ARTICLE INFO

Article history:
Received 21 May 2015
Received in revised form 16 August 2015
Accepted 18 August 2015
Available online 24 August 2015

Keywords:
Burn injury
Mechanical hyperalgesia
Monofilaments
Punctate hyperalgesia
Secondary hyperalgesia
Sensitization

ABSTRACT

Background: Secondary hyperalgesia is increased sensitivity in normal tissue near an injury, and it is a measure of central sensitization reflecting injury-related effects on the CNS. Secondary hyperalgesia areas (SHAs), usually assessed by polyamide monofilaments, are important outcomes in studies of analgesic drug effects in humans. However, since the methods applied in demarcating the secondary hyperalgesia zone seem inconsistent across studies, we examined the effect of a standardized approach upon the measurement of SHA following a first degree burn injury (BI).
New method: The study was a two-observer, test–retest study with the two sessions separated by 6 wk. An observer-blinded design adjusted to examine day-to-day and observer-to-observer variability in SHA was used. In 23 healthy volunteers (12 females/11 males) a BI was induced by a contact thermode (47.0 °C, 420 s, 2.5 × 5.0 cm^2). The SHA, demarcated by polyamide monofilaments (bending force: 0.2, 69 and 2569 mN) and a "weighted-pin" stimulator (512 mN), were assessed 45 to 75 min after each BI.
Results: A random effect, linear mixed model demonstrated a logarithmic correlation between elicited skin pressures (mN/mm^2) and the SHAs ($P < 0.0001$). No day-to-day or observer-to-observer differences in SHAs were observed. Intraclass correlation coefficients, in the range of 0.51 to 0.84, indicated a moderate to almost perfect reliability between observers.
Comparison with existing methods: No standardized approach in SHA-assessment has hitherto been presented.
Conclusions: This is the first study to demonstrate that demarcation of secondary hyperalgesia zones depends on the developed pressure of the punctate stimulator used.

1. Introduction

The secondary hyperalgesia area (SHA) is a circumscribed zone in normal skin near an injury with enhanced mechanical sensitivity. Secondary hyperalgesia is inducible in the majority of healthy subjects and represents evidence of central sensitization: a stimulus-response enhancing mode that may contribute to the development and maintenance of chronic pain states (Woolf, 2011).

SHA has been a primary outcome measure in a number of experimental studies testing anti-hyperalgesic drugs or investigating physiological pain paradigms (Asghar et al., 2015; Dirks et al., 2002; Letzen et al., 2014; Werner et al., 2004). Induction of secondary hyperalgesia is made by dermal capsaicin administration, repeated electrical stimulation, surgical incision, thermal injury, or

Abbreviations: BI, first degree burn injury; PS, punctate stimulator; SHA, secondary hyperalgesia.

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http://dx.doi.org/10.1016/j.jneumeth.2015.08.018
0165-0270/

by a combination of these methods. Secondary hyperalgesia is a robust and reproducible phenomenon with a duration of several hours (Pedersen and Kehlet, 1998). The borders of the secondary hyperalgesia zone are marked by stimulating from normal skin into the area of secondary hyperalgesia, a transition often described as a normal sensation changing into a burning, pricking or stinging sensation. Mechanical stimulation is either dynamic, by a brush or a cotton bud, or static, by punctate stimuli, using bendable polyamide filaments. Although, allodynic stimuli that do not cause pain in normal skin, but pain in the secondary hyperalgesia zone, are used in SHA-assessments, secondary hyperalgesia is the preferred term (Magerl et al., 1998).

In a recent experimental opioid study we used polyamide monofilaments to demarcate the secondary hyperalgesia zone, induced by a first degree burn injury (Ravn et al., 2013). Interestingly, we were unable to demonstrate any *decrease* in SHAs following morphine-infusions compared to controls, findings contrasting with previous experimental studies employing identical testing paradigms and drug-dosing schemes (Warncke et al., 1997, 2000). In these studies the authors observed a significant reduction in SHAs of 83% compared to placebo. The only important difference between the studies were the characteristics of monofilaments used in demarcation of the secondary hyperalgesia zones: in our negative study the bending force was 890 mN (91 g) (Ravn et al., 2013) while in the positive studies it was 51 mN (5 g) (Warncke et al., 1997, 2000). After induction of the burn injury the SHAs in the placebo-group in our negative study were 23 cm^2 and in the positive study 70 cm^2. The use of a rigid monofilament with a greater bending force (890 mN) therefore hypothetically could lead to smaller hyperalgesia areas than assessment with a monofilament with lower bending force (51 mN). Therefore, the *primary* objective of the present study was during *standardized* conditions, to compare the size of the secondary hyperalgesia zone, demarcated by three different polyamide filaments (0.2, 69 and 2569 mN) and a "weighted-pin" stimulator (512 mN), following a burn injury. The *secondary* objective was to examine day-to-day and observer-to-observer differences in measurements of SHAs. In addition, allowing a comparison of our results with the literature, we performed a systematic review of randomized, placebo-controlled studies, examining secondary hyperalgesia induced by a burn injury.

2. Material and methods

2.1. Study

2.1.1. Approval

The study protocol was approved by the Regional Committee on Health Research Ethics (H-1-2013-045) and the Danish Data Protection Agency (30-1097). The study was registered in ClinicalTrials.gov (NCT02286037). The original study-protocol is attached as Supplementary data (**S1**).

2.1.2. Design and randomization procedure

The study was a randomized, two-observer, test–retest study with the retest session made in mean (SD) 42 ± 3 days after first study day (Day 1; Fig. 1). The study was single-blinded, i.e., the results were blinded to the volunteers, in regard to the punctate stimulator used and the size of the secondary hyperalgesia zone. The randomization procedure was performed by a registered nurse, not participating in the study, using the randomisation software at www.randomization.org. The volunteers, numbered 1 to 24, were randomly allocated into two groups and each group was then randomly allocated to one of the observers. The observers were allocated to two examination sequences: either early Day 1 and late Day 2, or, late Day 1 and early Day 2 (cf. 2.1.5; Fig. 1). The test-order of the three monofilaments was randomized, while the examinations always ended with the "weighted-pin" stimulator.

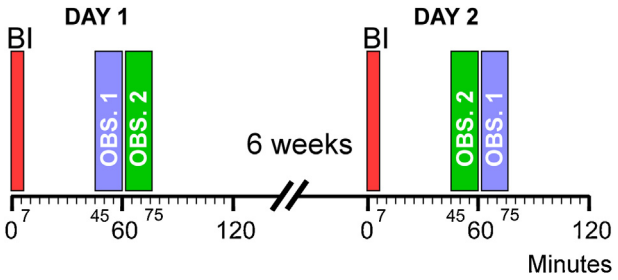

Fig. 1. *The study algorithm*. The two sessions at Day 1 and Day 2 were identical in testing sequences. The observer-order was randomized between the sessions. Each observer at post-burn times, 45 to 60 min or 60 to 75 min, demarcated secondary hyperalgesia areas by four different punctate stimulators (**PS1**–**PS4**). BI = burn injury; Obs. 1 = observer 1.

2.1.3. Volunteers

Twenty-four volunteers (12 females/12 males) were enrolled in the study. On Day 1 the volunteers were screened according to eligibility (Supplementary data **S2**) and an instant urine drug-test, testing for opioids, was performed.

2.1.4. Observers

Observer 1 (TKR) was a highly proficient investigator with more than four years of experience with quantitative sensory testing, while observer 2 (CE) had only three months of experience.

2.1.5. Study algorithm

SHAs were assessed 45 to 75 min after induction of the burn injury. Thus, each observer had 15 min to examine SHA: either as an early (45–60 min), or late (60–75 min) examination, determined by randomization between observers on Day 1. On Day 2 the procedure was repeated according to the randomization procedure, however the observer-sequence was changed, balancing time- and sequence-effects. The burn injury was induced on the exact same site as on Day 1.

2.2. Laboratory environment

The experimental procedures were performed in a quiet, bright room with a temperature range of 23–25 °C and a relative humidity (RH) of 31–33%, measured with an indoor thermo-hygrometer with external thermo-probe (THW301, Irox ETG, Bern, Switzerland). The testing sessions were made between 1st of October 2013 and 11th of November 2013, and were carried out Mondays to Fridays between 07.30 AM and 08.00 PM. The volunteers adopted a comfortable supine position during the assessments.

2.3. Burn injury

The volunteers were instructed to use a hair trimmer in the assessment area, two days before the study days, in order to avoid interference with the QST-assessments. A rectangular area, 2.5 × 5.0 cm^2, was delineated with the upper anterior corner 11 cm below the medial meniscus margin of the knee and 6 cm from the anterior margin of the tibia. The first degree burn injury (BI) was induced in the delineated area with a contact thermode (Thermotest, Somedic AB, Hörby, Sweden [active area: 2.5 × 5.0 cm^2, 47.0 °C, 420 s]). The pain intensity during the BI was rated on a visual analogue scale (VAS [0 = no pain, 100 = maximum imaginable pain]) at 0, 30, 60, 120, 180, 240, 300, 360 and 420 s after the thermode had reached 47.0 °C. The mean pain during the induction