Supplemental Digital Content

Methods

Experimental design

Nineteen healthy subjects (10 females, mean age 25.3±0.7 years) were recruited for this study. Subjects were excluded if they were presenting one of the following: known anaesthetic allergy, pregnancy, lactation, previous or current musculoskeletal, neurologic, or dermatologic diseases, tattoos in the forearm areas, as well as ongoing pain and itch or medication intake. The experiment consisted of a single session lasting less than 2 hours. Every participant was adequately informed about all the procedures they would undergo. All the stimulations were randomized between the left and right forearm of the participants (Fig. S1A-B). The study was approved by the regional ethical committee (N-20180035). All participants gave signed informed consent before the participation and could withdraw from the experiment at any time.

Skin anesthetization

Local intradermal anaesthesia was performed by lidocaine (2%) in conjunction with a vasoconstrictor (adrenalin; Skanderborg Apotek, Denmark). A total of five 20 μL blebs using a 30G short intra-dermal needle were done. The small blebs ensured an even distribution in a skin area having a diameter of approximately 3 cm, and minimal discomfort due to the volume. This procedure was applied on one of the forearms of the subjects, whereas on the opposite randomized forearm saline (5 x 20 μL) was used as a placebo/control. The method has been extensively applied to anaesthetize a local region of the human skin in various settings1–3.

Heat stimulation

Reproducible thermal stimulations were performed by a CHEPS thermal probe (3 cm diameter) attached to a Medoc PATHWAY thermal stimulator (Medoc Ltd, Ramat Yishay, Israel). The thermode was kept in place by a strap. A heat stimulation ramp (38-50; ≈0.2°C/s increasing) was applied on the marked areas on the forearms. The subjects were instructed that they could stop the stimulation as soon as the pain tolerance was reached, otherwise the stimulation would automatically stop at 50°C. The stimulus was applied every 5 minutes per forearm and up to 90 minutes (resulting in a 2.5 minutes period between a stimulation on one forearm and the other).

Itch and pain intensity assessment

Itch and pain intensities were assessed using two digital analogue scales (VAS; eVAS Software; Aalborg University, Aalborg, Denmark) and sampled (0.2 Hz) during heat stimulation. The two 10 cm scales both ranged from 0 to 100 and were set to indicate: 0 “No pain” and 100 “Worst imaginable pain” and 0 “No itch” and 100 “Worst imaginable itch”. The participants can only see the outer labels (0-100), and after the recording, the VAS data are transformed to values from 0 to 100 by the application. Ratings were stopped when the stimulation ended (exactly 1 minute after the start of the stimulation) or if the participants stopped the stimulation. If the stimulation was stopped the VAS score attributed was 100 for the pain sensation and 0 for itch sensation.

Statistical analysis

The area under the curve (AUC) was calculated using the trapezoidal method explained by Bailer 4 for every eVAS recordings. This allows to normalize the pain and itch intensity throughout the thermal stimulation. To better understand and analyse the effect of different temperatures on anaesthetized skin, during the analysis we subdivided the itch and pain intensity coming from the heating ramp stimulation into 3 different temperature ranges: innocuous warm temperature range (38-42°C), set of heat pain perception (42.2-46°C) and finally noxious temperature range (46.2-50°C). Subsequently, the mean, and the standard error of the mean (SEM) of the AUCs values of every time point after the anaesthetization were calculated. Multiple paired T-tests were used for statistical analysis, comparing every time point of saline with the respective time point of lidocaine. Normality was ensured using visual inspection of Q–Q plots. The graphs were realized in GraphPad Prism 9 (GraphPad Software Inc., CA, USA).

The graphs showed in the figures (Fig. 1A-C and Fig. 2A-C) do not show a continuous rating, rather the AUCs sampled every 5 minutes for the temperature ranges indicated.



**Figure S1:** A) Schematic representation of the localization of the intradermal injection and stimulation sites. B) Schematic representation of the experiment. The two treatments were randomized for placement and order.

Bibliography

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