***Supplementary materials***

***Methods***

*Induction Parameters*

EMLA cream 5%: In two 4x4 cm squared areas, 2 grams of cream (1g contains 25 mg of lidocaine and 25 mg of prilocaine, AstraZeneca) were applied under topical occlusion (TegaDerm). At the end of the 3 hours application, residual ointment was removed. After sensory tests, the cream was applied in the same areas for another 3 hours. The same procedure was repeated for three consecutive days.

Histamine: Standard allergy skin prick test (SPT) lancets (with a 1 mm shouldered tip) were used to deliver a small amount of histamine approximately at the dermo-epidermal junction 37,38. A weight-controlled SPT (120 grams) was used to decrease the variability of the method. A small drop of histamine dihydrochloride (1%, in saline) was applied to previously determined areas (one pretreated with EMLA and one with placebo) on the volar forearm followed by a prick through the drop.

Cowhage: The counting was conducted by forceps using a stereo-microscope, and 25-30 spicules were gently rubbed into two 1 cm diameter skin areas (one pretreated with EMLA and one with placebo). Application of spicules breaches the keratinous layer of the skin (0.05-0.15 mm) without reaching the circulation 39,40. The active substance delivered (mucunain) has been calculated to be in the nanogram range 41. By using tape, the spicules were rapidly removed after measurements.

*Microvascular Reactivity*

Full-field laser perfusion imaging (FLPI, Moor Instruments, Axminster, Devon, UK) was used to assess cutaneous neurogenic inflammation (quantified by superficial blood perfusion) after the application of EMLA, and pruritogens. The images were obtained by placing the device 25 cm above the skin area (5 Hz display rate, 8.3 ms exposure time, 160 units of gain). The FLPI data were analyzed using a region of interest (ROI) approach. For each ROI (equivalent to the predefined cream application area) mean and peak perfusion values were extracted. The axon-reflex-flare area size was quantified as the area exhibiting a >50% superficial perfusion increase relative to the baseline perfusion level.

*Measurement of Mechanically Evoked Itch (MEI)*

For mechanically evoked itch, three von Frey filaments were used: 1.0, 1.4, 2.0 g (North Coast Medical, Gilroy, CA). The stimulation was composed by 3 pricks, and it was repeated 3 times in short succession with each filament. After each stimulation, participants were instructed to report the itch elicited on a numerical rating scale (NRS) from 0 to 10 (0 = “no itch”; 10 = “worst imaginable itch”) and a total average was calculated. Participants were blinded during stimulations 42.

*Measurement of Mechanical Pain Thresholds (MPT) and Mechanical Pain Sensitivity (MPS)*

For MPT and MPS, a pin-prick set (MRC Systems GmbH, Germany) was used. The set is composed by 8 needles with a diameter of 0.6 mm and different force applications: 8, 16, 32, 64, 128, 256 and 512 mN. For both measurements, participants were blinded during the entire procedure.

MPT: starting with the lightest, each stimulator was applied at a rate of 2 s on, 2 s off in an ascending order until the subject reported a perception of sharpness or pain. If the pain perception was not perceived by the participant with any of the seven pin stimulators, a value of 1024 mN was assigned (this value corresponds to the pin prick immediately above the highest pin of 512 mN). Using the “method of limits”, a total of five threshold determinations were obtained by series of ascending or descending stimuli. The final threshold is the geometric mean of the five thresholds obtained.

MPS: starting with the lightest, each stimulator was applied in an ascending order, and the subject was asked to give a pain rating for each stimulus on a NRS from 0–10 (0 = “no pain”; 10 = “worst imaginable pain”). This procedure was performed twice. This procedure is adapted from 42.

*Thermal Sensitivity*

Warm detection threshold (WDT) and heat pain threshold (HPT) were measured using a thermal stimulator Medoc Pathway (Medoc Ltd, Ramat Yishay, Israel). A probe (3x3 cm) with a baseline temperature of 32 ˚C was placed on each area. An ascending ramp stimulus (1 °C/s) was delivered until the subject identified the relevant threshold and clicked a button on a mouse. For WDT the threshold was the perception of a temperature change (warm sensation); for HPT was the detection of a painful sensation. After the click, the temperature returned to the baseline at a rate of 5°C/s. A cut-off temperature of 52°C was used. The results were calculated as the arithmetic mean of the thresholds obtained from three repeated ramps.

The same devise was used to measure Supra-threshold heat sensitivity (STHS). The subjects rated the pain (from 0 to 10) to two supra-threshold heat pain stimuli (starting and ending at 32°C with an increasing ramp of 5°C/s, 3 s plateau at 50°C and decreasing at 5 °C/s, 2 minutes apart between stimulations) applied on the treated/placebo areas. The result was the average of the two values obtained.

*Assessment of Itch and Pain*

After pruritogens, a visual analogue scale (VAS) with two bars was used to assess the intensity and duration of itch and pain. Participants rated itch and pain intensity continuously for 9 minutes (output sampled at 0.2 Hz) on two computerized 100 mm VASs from 0 to 100 (eVAS Software, Denmark), with 0 indicating “no itch/pain” and 100 “worst imaginable itch/pain”.

***Supplementary table 1***



*Table 1:* means and SDs of mechanical and thermal sensitivity tests, FLPI (mean, peak, and flare area), and peak and AUC of itch and pain induced by pruritogens. MEI mechanically evoked itch, MPT mechanical pain threshold, MPS mechanical pain sensitivity, WDT warmth detection threshold, HPT heat pain threshold, STHS supra-threshold heat sensitivity, FLPI full-field laser perfusion imaging.