

Appendix S1

SUPPLEMENTARY MATERIAL AND METHODS

A total of 21 healthy Caucasian subjects (25 ± 4.9 years; 9 males, 12 females) were recruited. Exclusion criteria included ongoing pain, medication use, as well as previous or current musculoskeletal, neurological, allergic, or dermatological diseases. All subjects signed an informed consent and the regional ethics committee approved the protocol (N-20180003). Seven 2×2 cm areas were marked on the volar aspect of the forearms. The provocations were performed with β -alanine (2%, 10% and 50% w/v in isotonic saline) and histamine 1% by SPT lancets, or with 30–35 spicules of cowhage (inserted by gentle rubbing). To assess the potential importance of repeated punctures, β -alanine 10% was applied by 1, 5 or 25 punctures. A weighted 60 g SPT device was used. For the 5 or 25 SPT procedures, all punctures were conducted in rapid succession (less than 1 puncture per second) and were delivered in a circular skin area with a diameter of 0.5–1 cm. Repeated puncturing of the exact same skin spot was avoided. The intensity of any itch sensation was obtained in a continuous manner using a computerized visual analogue scale (VAS), ranging from 0="no itch" to 100="worst imaginable itch". Subjects were instructed to rate the itch intensity for 10 min after substance application.

Mechanical itch sensitivity was assessed using von Frey filaments (13.7, 19.6, 39.2 mN), by stimulating the area immediately surrounding the itch provocation before vs. after the itch rating. The subjects evaluated the evoked sensation of itch on a numerical rating scale (NRS₀₋₁₀) with the same labels as the VAS described above. The method is explained in detail elsewhere (11). Because multiple discontinuous ratings were required to assess mechanical itch sensitivity, an NRS rather than a VAS was used in accordance with several previous psychophysical studies (3, 11, 12). We currently use the term "mechanical itch sensitivity" to describe itch evoked by controlled cutaneous mechanical stimulation and use the term mechanical "hyperknesis" specifically to describe the sensory state in which exaggerated itch is perceived in response to such stimulation. For a recent review on the terminology and mechanisms of allo- and hyperknesis, see (13).

Neurogenic flare was investigated using a full-field laser perfusion imager (FLPI-2, Moor Instruments, Axminster, UK) 10 min after each itch provocation. The axon-reflex-flare area size was quantified as the area exhibiting a $>50\%$ increase in superficial perfusion relative to the background level. Statistical testing was conducted with repeated-measure analysis of variance (ANOVA) corrected for multiplicity by the Sidak procedure (performed in SPSS).