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Short communication

Capsaicin-sensitive cutaneous primary afferents convey electrically induced itch in humans

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Abstract

Specially designed transcutaneous electrical stimulation paradigms can be used to provoke experimental itch. However, it is unclear which primary afferent fibers are activated and whether they represent pathophysiologically relevant, C-fiber mediated itch. Since low-threshold mechano-receptors have recently been implicated in pruriception we aimed to characterize the peripheral primary afferent subpopulation conveying electrically evoked itch in humans (50 Hz stimulation, 100 μs square pulses, stimulus-response function to graded stimulus intensity). In 10 healthy volunteers a placebo-controlled, 24-hour, 8% topical capsaicin-induced defunctionalization of capsaicin-sensitive (transient receptor potential V1-positive, 'TRPV1^{+}') cutaneous fibers was performed. Histaminergic itch (1% solution introduced by a skin prick test lancet) was provoked as a positive control condition. Profound losses of warmth and heat pain sensitivity (pain threshold and supra-threshold ratings) as assessed by quantitative sensory testing, were found as indicative for efficient TRPV1-fiber defunctionalization (all outcomes: P <0.0001). The topical capsaicin profoundly, and with equal efficaciousness, inhibited itch intensity evoked by electrical stimulation and histamine (-89 ± 4.1% and -78% ±4.9%, respectively, both: P <0.0001 compared to the placebo patch area). The predominant primary afferent substrate for electrically evoked itch in humans, using the presently applied stimulation paradigm, is concluded to be capsaicin-sensitive polymodal C-fibers.

1. Introduction

Itch in both experimental and clinical itch studies is frequently elicited by various transcutaneous electrical stimulation paradigms [1–6]. These method has been used to document increased itch sensitivity in chronic itch patients [5–7] and allow for temporal control and customization of the stimulation intensity, opposite to the more thoroughly investigated chemical itch models [8]. On the other hand electrical stimulation is non-selective, unphysiological, and it is unclear which primary afferent fibers that are involved in conveying electrically elicited itch [1,2]. Notably, electrical stimulation often produces co-sensations such as tapping, buzzing or tingling, generally associated with activity of large myelinated primary afferents. Recently, low-threshold mechano-receptors (LTMRs), i.e. C-tactile and Aβ-fibers, have been implicated in mechanically evoked itch in response to stimuli that are probably below the threshold of pruriceptive nociceptors [9,10]. This prompts reconsideration as to whether electrically evoked itch paradigms actually probes the afferent units that are spontaneously active and sensitized in patients with chronic itch or whether an entirely different pathway is activated. In humans prolonged topical application of high-concentration capsaicin can profoundly defunctionalize dermo-epidermal nociceptive fibers expressing transient receptor potential V1 channel (TRPV1) [11], and has previously been shown to almost entirely inhibit warmth/heat pain sensations as well as itch evoked by activation of both C-mechano-insensitive (histaminergic) and polymodal C-fibers (cowhage-induced) [12]. In the present double-blinded, placebo-controlled, cross-over study such an ablation of capsaicin-sensitive dermo-epidermal C- and Aδ-fibers in human skin performed, validated by psychophysical warmth/heat pain assessments. Subsequently, electrically evoked itch sensitivity was tested in the capsaicin/placebo-pretreated areas and histaminergic itch was used as a positive control.

2. Materials and methods

Ten healthy male volunteers were enrolled (23.6 years, range 21-25). The local ethics committee approved the protocol (N-20160026). Subjects were informed about the procedures involved in the study and gave their written informed consent prior to the experiment. The study consisted of three sessions and lasted approximately 2.5 hours in total. The first session was a screening where the electrical paradigm was tested and non-responders were excluded; defined as a peak itch of <20 ($VAS_{0\text{-}100}$), prompting N=3 exclusions. Then capsaicin and placebo patches were applied for 24 hours and removed (session two). In the third session, 24 hours after patch removal the sensory tests were conducted in both the placebo and capsaicin-treated area.

2.1. Capsaicin-induced fiber ablation: Two 4x4cm areas, 3 cm apart, on the medial aspect of the volar forearm were pretreated with either an 8% capsaicin patch (Qutenza®, Grüenthal, Germany) or a placebo patch for 24 hours. Distal versus proximal application sites were randomized in a balanced manner. The application of additional opaque occlusion was utilized to blind the subjects (taking advantage of the poor localizability of chemo-nociceptive stimuli) and to blind the investigators performing the psychophysiological tests. 24 hours after patch removal the test session was performed. This technique has previously been used to study the skin under capsaicin-sensitive fiber depleted conditions [11,12].

2.2. Validation of capsaicin-induced fiber ablation: A 3x3cm thermal probe was attached to the 4x4cm patch application skin areas. The probe was connected to a Pathway sensory stimulator (Medoc, Ramat Yishai, Israel), controlled by Medoc Main Station software. The baseline temperature was always 32˚C and for warmth detection and heat pain threshold ('WDT' and 'HPT', respectively) ramping stimuli of 1˚C/s were delivered until the subjects identified the associated threshold (first perception warmth and first perception heat pain) by pressing a stop button. Hereafter the temperature returned to 32˚C, at a rate of 1˚C/s. WDT and HPT were performed in triplicates and averaged. Suprathreshold heat pain sensitivity (SHPS) was assessed by two ramps-and-hold stimuli lasting 1s at

50˚C with ascending and descending ramp rates of 5˚C/s. Subjects rated each evoked stimulus on a numerical rating scale from 0='no pain' to 10='worst imaginable pain'.

2.3 Electrically evoked itch stimulus-response function: Two surface electrodes were attached 2 cm apart within the pretreated 4x4 cm. Ramp stimuli were applied at 50Hz with a pulse duration of 100μs, using an increasing current intensity (0.05 mA/s). Current intensity started at 0.4 mA and ended at 6.4 mA (2-minute duration per stimulus ramp). This stimulation paradigm is described in details elsewhere [2,4]. The stimulus-response curve was constructed by simultaneous ratings of itch intensity obtained using a 100-mm digital visual analog scale (VAS) on a tablet: 'no itch'=0 mm, and 'worst imaginable itch'=100-mm. Itch intensity ratings were conducted continuously and sampled at 0.2Hz.

2.4. Histamine evoked itch: Histamine dihydrochloride 1% solution (Allergopharma, Germany) was applied using a 1-mm shouldered skin prick test (SPT) lancet. A drop was placed in the center of the placebo or capsaicin-pretreated area and pricked with the SPT lancet using a 120g weighted device (Aalborg University, Denmark) as previously described [13]. Immediately hereafter a 10-minute VAS-recording of the itch intensity was initiated using the same approach as described for electrically evoked itch.

2.5. Statistics: Analyses were performed with SPSS 25 (IBM, Armonk, USA). The studentized residuals for all variables were normally distributed according to Shapiro-Wilks test. Thermal validation tests were assessed using paired-samples t-tests. The main outcome of itch following capsaicin/placebo pretreatment was assessed by two-way RM-ANOVAs with two factors: *treatment* (levels: capsaicin and placebo) and *provocation method* (levels: electrical and histamine-induced). Post hoc testing was adjusted with the Bonferroni procedure. $P < 0.05$ was considered significant.

3. Results

3.1. Validation of capsaicin-induced ablation: The 8% capsaicin ablation induced significant sensory desensitization to warmth, heat pain and suprathreshold heat stimuli (lowest t_9=7.1; all outcomes: $P < 0.0001$, Fig. 1A-C). As an example, pain in response to a short 50˚C heat pulse was reduced from 7.7±0.3 (NRS_{0-10}) in the placebo-treated area to only 1.7±0.3 in the capsaicin-treated area.

3.2. Itch inhibition: The capsaicin-induced fiber ablation inhibited histamine- and electrically evoked itch to the same extent (Fig. 2A-C). The RM-ANOVAs for both peak and mean showed a profound capsaicin-mediated inhibition of itch evident from the main effect of *treatment* ($F_{1,9}$ = 63.3, P<0.0001; mean itch and $F_{1,9}$ = 65.5, P<0.0001; peak itch). Both for mean and peak itch there were no main effect of *provocation method* (lowest test F-value: $F_{1,9}$ = 2.5, P=0.15; peak itch) signifying that histamine and the electrical stimulation evoked comparable itch intensities. The *treatment* x *provocation* interaction was also insignificant demonstrating that the capsaicin-ablation equally inhibited electrical and histamine-evoked itch (lowest test F-value: $F_{1,9}$ = 2.2, P=0.18; peak itch, Fig. 2C). It was noted that the histamine provocations, but never the electrical stimulation procedure, produced wheal and flare reactions in all subjects. Histamine-evoked flares were significantly smaller in capsaicin pre-treated skin (data not shown) as indicative for inhibition of neurogenic inflammation.

4. Discussion

Defunctionalization of capsaicin-sensitive fibers in human skin inhibited itch evoked by electrical itch stimulation paradigm and histamine with equal efficacy. The applied capsaicin ablation approach generally targets subsets of Aδ- and C-fibers expressing $TRPV1^+$, while leaving C-tactile and Aβ-fibers fibers intact since these are TRPV1-negative. Under these conditions histaminergic itch receptiveness is as expected almost entirely inhibited. This is

aligned with studies suggesting that this pathway relies on C-mechano-insensitive TRPV1$^+$ fibers [12,14,15]. The fact that cowhage-induced itch is also highly susceptible to inhibition by capsaicin pretreatment suggests that most polymodal C-fibers are also TRPV1$^+$, and this thus represent a commonality between the two most well-elucidated human primary afferent pathway of itch [12].

The electrically induced itch was effectively inhibited, thus suggesting that this sensation is primarily mediated by TRPV1$^+$ C- and possibly, Aδ-fibers. A hallmark of C-mechano-insensitive fiber activation is the neurogenic flare response [16], which was never observed after the electrical stimulation. Hence, these units are unlikely to be involved in transmission of itch in the present stimulation paradigm. Moreover, C-tactile and Aβ units can be excluded because these are unresponsive to the capsaicin-ablation [11] and thus would have left a much more intact itch response to the presently applied electrical stimulation. This in turn leaves polymodal C-fibers as the likeliest candidate, which corroborates and extends on an earlier finding by Mochizuki *et al.* (2008) based on conduction velocity data from EEG-recorded sensory evoked potentials also pointing towards C-fibers as a whole [17].

The purported itch-conveying Mas-related G-protein receptor D (MrgprD)-subset of non-peptidergic nociceptors is known to terminate very superficially in the epidermis and do not significantly co-express TRPV1 [18–20]. Consequently, it can be inferred that these fibers either do not transmit electrically evoked itch of the present stimulation paradigm or that they are inadvertently defunctionalized by the capsaicin-treatment. Further studies using itch provocations with β-alanine (an MrgprD-agonist) following a topical capsaicin-ablation could clarify this. In this context, general caution have to be considered with regards to the specificity, fiber subpopulation preferentially and effectiveness of the 8% capsaicin ablation technique, which remains to be fully elucidated [11]. It should be noted that while the sensory effects of the present capsaicin pretreatment are pronounced, it does not represent a complete loss as demonstrated particularly by both the conservation of some degree of heat sensitivity as well as the mild itch response to histamine in a few subjects. Moreover, the high stimulation frequency (≥50 Hz, short duration stimuli [1,2]) required is nominally against the notion of C-nociceptors as the substrate for electrically evoked itch because it is significantly above the natural instantaneous frequency of the majority of such units. Lastly, this study does not refute that the recently highlighted pruriceptive LTMRs [9,10] are present in hairy human skin but simply demonstrate that they are not the predominant substrate for the presently applied electrical itch elicitation paradigm.

5. Conclusion

In conclusion, we suggest that electrically induced itch (evoked by a unique stimulation paradigm) is conveyed predominantly by capsaicin sensitive (TRPV1$^+$) polymodal C-fibers similarly to the fibers activated by some chemical itch provocation models, e.g. cowhage. This primary afferent subpopulation is also implicated in itch and sensitization for itch in chronic pruritic clinical conditions [21]. Moreover, besides targeting a relevant fiber population the temporal controllability of electrical itch induction techniques enables neuroimaging methods and studies on rapid pruriceptive modulation processes where repeated controlled itch provocations can be applied (e.g. to screen centrally acting antipruritics or to assess counter-stimulatory mechanisms).

References

[1] A. Ikoma, H. Handwerker, Y. Miyachi, M. Schmelz, Electrically evoked itch in humans, Pain. 113 (2005) 148–154.

[2] H.H. Andersen, A.I.M. van Laarhoven, J. Elberling, L. Arendt-Nielsen, Modulation of Itch by Conditioning Itch and Pain Stimulation in Healthy Humans, J Pain. (2017).

[3] A.I.M. van Laarhoven, F.W. Kraaimaat, O.H. Wilder-Smith, P.C.M. van de Kerkhof, A.W.M. Evers, Heterotopic pruritic conditioning and itch--analogous to DNIC in pain?, Pain. 149 (2010) 332–7.

[4] D.J.P. Bartels, A.I.M. Van Laarhoven, E.A. Haverkamp, O.H. Wilder-Smith, A.R.T. Donders, H. Van Middendorp, P.C.M. Van De Kerkhof, A.W.M. Evers, Role of conditioning and verbal suggestion in placebo and nocebo effects on itch, PLoS One. 9 (2014) e91727.

[5] A.I.M. van Laarhoven, F.W. Kraaimaat, O.H. Wilder-Smith, P.L.C.M. van Riel, P.C.M. van de Kerkhof, A.W.M. Evers, Sensitivity to itch and pain in patients with psoriasis and rheumatoid arthritis, Exp Dermatol. 22 (2013) 530–534.

[6] A.I.M. van Laarhoven, D.J.O. Ulrich, O.H. Wilder-Smith, N.E.E. van Loey, M. Nieuwenhuis, N.J.A. van der Wee, A.W.M. Evers, Psychophysiological Processing of Itch in Patients with Chronic Post-burn Itch: An Exploratory Study., Acta Derm Venereol. 96 (2016) 613–8.

[7] A.I.M. van Laarhoven, F.W. Kraaimaat, O.H. Wilder-Smith, P.C.M. van de Kerkhof, H. Cats, P.L.C.M. van Riel, A.W.M. Evers, Generalized and symptom-specific sensitization of chronic itch and pain, J Eur Acad Dermatology Venereol. 21 (2007) 1187–1192.

[8] H.H. Andersen, J. Elberling, L. Arendt-Nielsen, Human Surrogate Models of Histaminergic and Non-histaminergic Itch., Acta Derm Venereol. 95 (2015) 771–777.

[9] S. Bourane, B. Duan, S.C. Koch, A. Dalet, O. Britz, L. Garcia-Campmany, E. Kim, L. Cheng, A. Ghosh, Q. Ma, M. Goulding, Gate control of mechanical itch by a subpopulation of spinal cord interneurons., Science. 350 (2015) 550–4.

[10] M. Fukuoka, Y. Miyachi, A. Ikoma, Mechanically evoked itch in humans, Pain. 154 (2013) 897–904.

[11] F. Henrich, W. Magerl, T. Klein, W. Greffrath, R.-D. Treede, Capsaicin-sensitive C- and A-fibre nociceptors control long-term potentiation-like pain amplification in humans, Brain. 138 (2015) 2505–2520.

[12] H.H. Andersen, J.B. Marker, E.A. Hoeck, J. Elberling, L. Arendt-Nielsen, Antipruritic effect of pretreatment with topical capsaicin 8% on histamine- and cowhage-evoked itch in healthy volunteers: a randomized, vehicle-controlled, proof-of-concept trial, Br J Dermatol. 177 (2017) 107–116.

[13] H.H. Andersen, J. Elberling, S. Lo Vecchio, L. Arendt-Nielsen, Topography of itch: evidence of distinct coding for pruriception in the trigeminal nerve, Itch. 1 (2016) 1–10.

[14] L.M. Johanek, R. a Meyer, T. Hartke, J.G. Hobelmann, D.N. Maine, R.H. LaMotte, M. Ringkamp, Psychophysical and Physiological Evidence for Parallel Afferent Pathways Mediating the Sensation of Itch, J Neurosci. 27 (2007) 7490–7497.

[15] W.S. Shim, M.H. Tak, M.H. Lee, M. Kim, M. Kim, J.Y. Koo, C.H. Lee, M. Kim, U. Oh, TRPV1 Mediates Histamine-Induced Itching via the Activation of Phospholipase A2 and 12-Lipoxygenase, J Neurosci. 27 (2007) 2331–2337.

[16] M. Schmelz, K. Michael, C. Weidner, H. Torebjörk, H. Handwerker, Which nerve fibers mediate the axon reflex flare in human skin?, Neuroreport. 11 (2000) 645–648.

[17] H. Mochizuki, K. Inui, K. Yamashiro, N. Ootsuru, R. Kakigi, Itching-related somatosensory evoked potentials, Pain. 138 (2008) 598–603.

[18] M.J. Zylka, F.L. Rice, D.J. Anderson, Topographically distinct epidermal nociceptive circuits revealed by axonal tracers targeted to Mrgprd., Neuron. 45 (2005) 17–25.

[19] M. Wooten, H.-J. Weng, T. V Hartke, J. Borzan, A.H. Klein, B. Turnquist, X. Dong, R.A. Meyer, M. Ringkamp, Three functionally distinct classes of C-fibre nociceptors in primates., Nat Commun. 5 (2014) 4122.

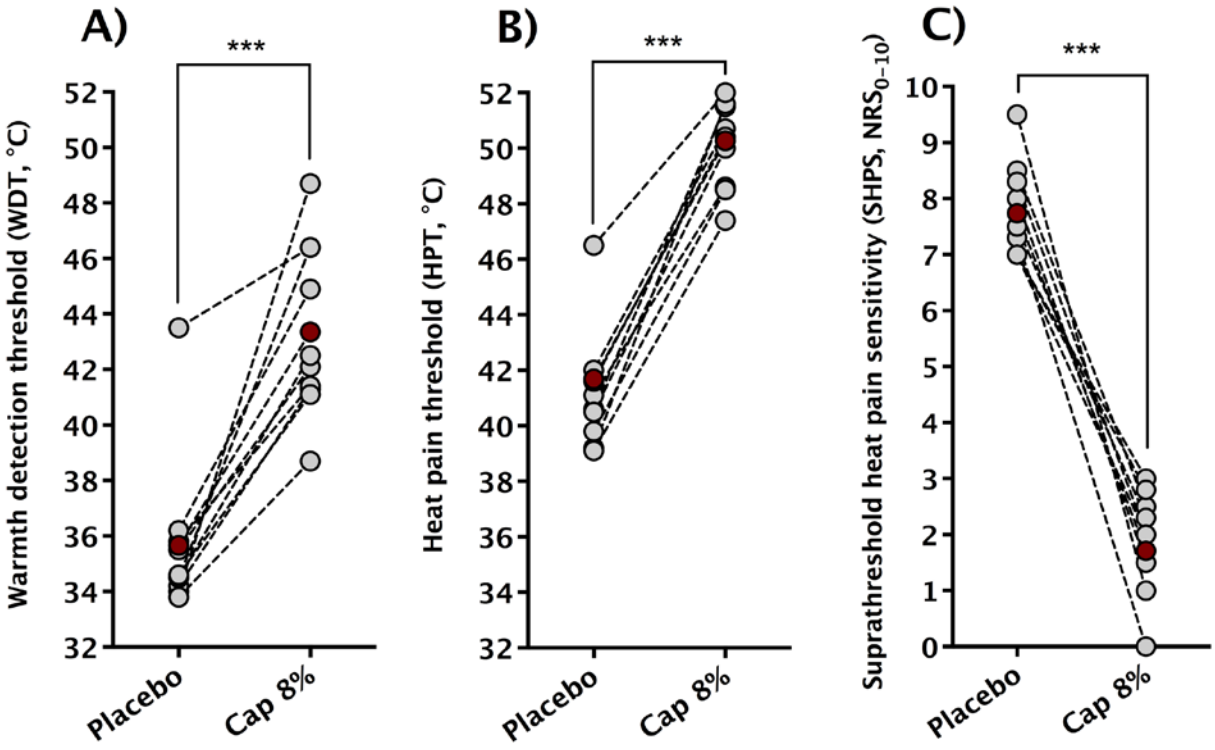
[20] D. Usoskin, A. Furlan, S. Islam, H. Abdo, P. Lönnerberg, D. Lou, J. Hjerling-Leffler, J. Haeggström, O. Kharchenko, P. V. Kharchenko, S. Linnarsson, P. Ernfors, Unbiased classification of sensory neuron types by large-scale single-cell RNA sequencing, Nat Neurosci. 18 (2015) 145–153.

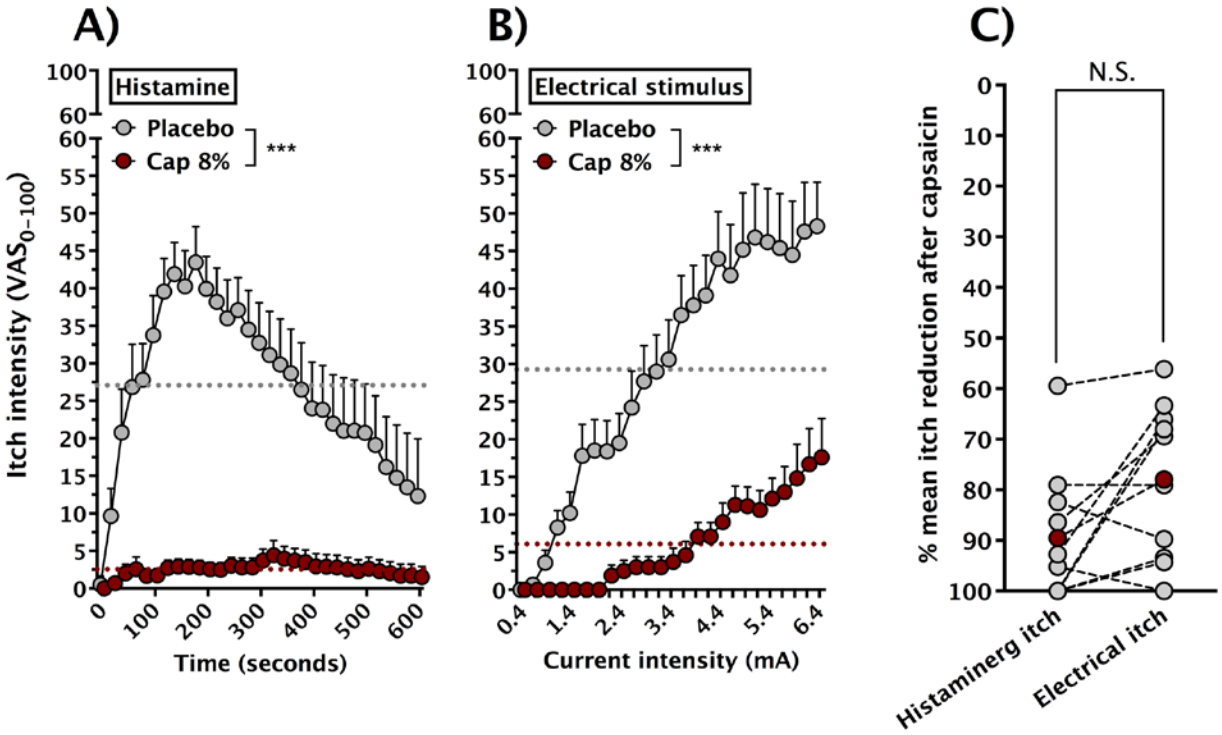
[21] H.H. Andersen, J. Elberling, H. Sølvsten, G. Yosipovitch, L. Arendt-Nielsen, Nonhistaminergic and mechanical itch sensitization in atopic dermatitis, Pain. 158 (2017) 1780–1791.

Figure legend

Figure 1: A, **B** and **C**) Validation of capsaicin-induced sensory desensitization. Individual subject (grey dots) and mean (red dots) increase in warmth detection threshold (**A**), heat pain threshold (**B**) and suprathreshold heat pain sensitivity (**C**). Cap = capsaicin. *N* = 10 for all plots. Error bars represents standard error of mean, *** = *P* < 0.0001.

Figure 2: A) Histamine evoked itch intensity in capsaicin (red dots) versus placebo (grey dots) treated skin areas. Dashed lines represent the overall mean itch intensities. **B**) Electrically evoked itch in capsaicin (red dots) versus placebo (grey dots) treated skin areas. Dashed lines represent the overall mean itch intensities. **C**) Similar percentage-wise itch reduction observed for the histamine provocation and the electrical stimulation paradigm. Grey dots represent individual subject data; red dots represent the overall mean. N.S. = not significant. Cap = capsaicin. *N* = 10 for all plots. Error bars represents standard error of mean, *** = *P* < 0.0001.




A)
Histamine
Placebo
Cap 8%
***
Itch intensity (VAS0-100)
Time (seconds)
B)
Electrical stimulus
Placebo
Cap 8%
***
Current intensity (mA)
C)
N.S.
% mean itch reduction after capsaicin
Histaminerg itch
Electrical itch