Mechanically-evoked itch in humans

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1. Introduction

Itch had generally been recognized as a part of pain, as in the ‘intensity’ hypothesis by Max von Frey [7], before the hypothesis of the ‘labeled line’ for itch was suggested by the finding of mechano-insensitive C-fibers and spino-thalamic tract neurons responding to histamine [2,25]. Recent findings of the involvement of gastrin-releasing peptide and its receptor in itch-specific pathways [32] as well as distinct subsets of transient receptors potential A1 (TRPA1)-positive neurons in chroloquine-induced itch [36] have strengthened the labeled-line hypothesis. On the other hand, the findings that polymodal C-fibers and polymodal spino-thalamic tract neurons are activated in association with cowhage-induced itch [13,18] as well as that nociceptive myelinated nerves are involved in cowhage-induced itch [21] are rather contradictory to the labeled-line hypothesis. However, it is still an open question whether the cowhage-induced activation of polymodal C-fibers represents itch, or maybe, only the pain-related sensory components such as pricking, stinging, and burning sensations that frequently accompany cowhage-induced itch [20]. The simplest way to solve this question would be to demonstrate mechanically-evoked itch similar to cowhage-induced itch in quality and intensity. In reality, however, mechanical stimulation to the skin by von-Frey filaments, which supposedly co-activates large myelinated fibers, can only

evoke tactile sensation, not itch, even though it activates cowhage-sensitive neurons according to electrophysiological studies [18]. Moreover, reproducible mechanically-evoked itch that is comparable to histamine- or cowhage-induced itch in intensity and quality has been shown only under specific conditions accompanied by itch sensitization, which is touch- or pinprick-evoked itch in a surrounding area of experimentally-induced or disease-caused itch [10,23], but not under normal healthy conditions.

It is not only cowhage but also histamine and electrical stimulation that very frequently evokes itch and pain at the same time [11,15,30]. Although most animal studies employ scratching behavior as the sign representing itch sensation to differentiate itch from pain, scratching does not always reflect itch but also pain [26]. One solution for this problem is a recently-reported cheek application model in mice, in which itch causes scratching but pain causes wiping [29]. However, there still remains the question whether scratching on the cheek reflects pure itch, or maybe, reflects a mixed sensation of itch and pain. Therefore, the cheek application in mice is a useful, but not a perfect model to differentiate itch from pain. The zero-availability of experimental stimuli for human subjects that can constantly evoke itch without any simultaneous pain-related sensory components accompanying is thus apparently a

major obstacle in studying differentiation of itch and pain.

The present study deals with our newly developed experimental method with mechanical stimuli to reproducibly evoke intense pure itch in healthy human subjects without any pain-related sensations mixed, which strongly supports the presence of mechano-sensitive nerves involved in itch. This method is supposed to be highly useful for future investigation on differentiation of itch and pain.

2. Materials and Method

2.1. Subjects

Ten healthy Japanese volunteers (six men and four women at the age of 32.0 ± 6.5 years, mean ± SD), who had taken no medications for the previous two weeks, participated in the present study after they gave their informed consent in a written form. The study was approved by the internal ethics committee at Kyoto University.

2.2. Overview of study design

The following six sessions of experiments were performed by the same investigator in the same subjects with at least one-week intervals between each session.

The room temperature was kept at 23°C throughout the study.

In the *first* session, mechanical stimulation using the below-mentioned special probe was applied to vellus hairs of the chin, cheek, and forehead of all subjects and male beard instead of vellus hairs in the male subjects. Light touching of chin by a cotton swab was also performed in all subjects as a control. The maximum intensity of itch during a 90-second stimulation was assessed.

In the *second* session, mechanical stimulation was applied to the chin four times with intervals that were enough for itch to diminish. The first and fourth ones were the standard stimulation with vibration of the probe and with contact to vellus hairs. In the second one, the probe vibrated without contact to vellus hairs. In the third one, the probe contacted to vellus hairs but not vibrated.

In the *third* session, histamine H_1 receptor antagonist (H_1 blocker) and its placebo were orally administered in a randomized, double-blind and crossover manner with a one-week interval between administrations of H_1 blocker and placebo. Three hours after the H_1-blocker/placebo administration, mechanical stimulation was applied for 90 seconds to the face and, after a 15-minute interval, to the arm. After another 15-minute interval, histamine iontophoresis was applied for 60 seconds to the face and, after a 15-minute interval, to the arm. Itch sensation and flare reaction were assessed during

the 90-second mechanical stimulation or for 90 seconds after the 60-second histamine iontophoresis. At the end of the session, the subjects were given questions to characterize in detail the itch sensation they felt.

In the *fourth* session, mechanical stimulation was applied to the chin for 10 minutes, and then, after a 15 minute interval, histamine-iontophoresis to the arm for 10 minutes. Itch intensities were continuously assessed during the 10-minute stimulation/iontophoresis.

In the *fifth* session, touch-alloknesis was assessed on the face at three different time points after the 90-second mechanical stimulation to the face.

In the *sixth* session, mechanical stimulation with different frequencies (1-50 Hz) and amplitudes (0.2-1.0 mm) was applied to the identical area of the face. The change in itch intensity caused by the frequency/amplitude modulation was assessed.

2.3. Mechanical stimulation (Fig. 1)

In all the sessions, the subjects lay down on a bed with the face up, arms beside the trunk, the back of hands facing up, and eyes closed. Their head position was fixed by means of a headrest that is made of durable polyurethane foam and specifically designed for experimental settings (TOYO MEDIC, Tokyo, Japan). The probe for mechanical

stimulation was a 30 cm long, 1 cm wide and 2 mm thick acryl plate with a stainless-iron wire loop (the wire diameter: 0.05 mm, the loop diameter: 1.5 cm) attached at one end. The other end of the probe was fixed to a piezo-actuator (4 x 1.5 x 1.5 cm in size, Mess-tek Co Ltd, Saitama, Japan), so that the electrically-controlled horizontal vibration of the piezo-actuator could result in a swing of the probe in the range of 0-0.2 degree and 0-50 Hz (Fig. 1A). The piezo-actuator was fixed by means of a metal stand in the position where the bottom of the wire loop could touch vellus hairs of the subjects but not the skin (Fig. 1B), except for the part of the *second* session in which the wire loop was moved without contact to vellus hairs. The horizontal movement of the wire loop caused by the swing of the probe was calculated to be in the range of 0-1.0 mm, which was confirmed with a digital magnifier (DinoLite Pro LWD®, Thanko Co Ltd, Tokyo, Japan). The position of the wire loop was always monitored during stimulation by the examiner using the digital magnifier to make sure that the wire loop would touch vellus hairs in the identical area and would not touch the skin surface. On the face, mechanical stimulation was applied to the middle of the chin, which was 1 cm beneath the lower lip. On the arm, it was applied to the middle point between the wrist and the elbow on the volar aspect of forearm. Except for the *sixth* session, the frequency/amplitude was fixed at 8 Hz/1.0 mm. In the *sixth* session, stimulation with the 1 Hz frequency and the 1.0

mm amplitude was first applied. Then, the frequency of stimulation was increased from 1 Hz to 50 Hz step by step without stopping stimulation, whereas the amplitude was fixed at 1.0 mm. At each frequency, stimulation was kept for 10 seconds and itch rating was evaluated. After the 10 second stimulation at 50 Hz, stimulation was stopped for 15 minutes to wait for itch to completely diminish. Then, stimulation was restarted with the 50 Hz frequency and the 1.0 mm amplitude. The frequency was then decreased from 50 Hz to 1 Hz step by step every 10 seconds, whereas the amplitude was fixed at 1.0 mm. In the same way, another set of experiment was performed, in which the amplitude of stimulation was changed step by step between 0.2 mm and 1.0 mm in the ascending and descending directions with the frequency fixed at 8 Hz.

2.4. Histamine-iontophoresis

In the *third* and *fourth* sessions, histamine dihydrochloride (Wako Pure Chemical Industries Ltd, Tokyo, Japan) 0.1 mg/mL in water was iontophoretically applied with a 0.1 mA constant current to the same skin area of the face and arm where mechanical stimulation was applied before. The subjects kept the same physical position as they did for mechanical stimulation. The 0.5 cm^2 cotton applicator was connected to the positive terminal of the iontophoreser (Nihon-Koden Ltd, Tokyo, Japan). The counter electrode

was placed on the skin 3 cm aside from the applicator.

2.5. Assessment of itch intensity

In all the sessions, the subjects were asked to express itch intensities on a numerical rating scale from 0 (no sensation) to 10 (worst itch imaginable) using fingers or verbally depending on the area that the stimulation/iontophoresis was applied to (face: using fingers, arm: verbally).

2.6. Characterization of itch

At the end of the *third* session, the subjects were asked whether or not they wanted to scratch the stimulation site. If they answered ‘yes’, they were further asked to describe whether or not the itch sensation had any of the following characteristics: crawling, tickling, stinging, burning, stabbing and pinching.

2.7. Assessment of flare area

In the *third* session, the superficial blood flow in the skin was measured by means of a laser Doppler imaging scanner (Moor instruments, Devon, UK) before and 5 minutes after mechanical stimulation or histamine-iontophoresis. The scanner was

positioned 30 cm above the skin, and provided flux values in the 6 x 6 cm rectangular skin area at the resolution level of 180 x 180 pixels after scanning. To determine the flare size, the mean flux value plus two times of its standard deviation obtained from the baseline imaging was set as the threshold, and the pixels with higher values than the threshold were regarded to have a significant vasodilatation.

2.8. Histamine H_1 receptor antagonist

The histamine H_1 receptor antagonist administered in the *third* session was olopatadine hydrochloride 5 mg (Allelock®, Kyowa Hakko Kogyo Co Ltd, Tokyo, Japan).

2.9. Assessment of touch-alloknesis

In the *fifth* session, the skin area in the face 1 cm aside from the spot where the 90-second mechanical stimulation had been applied was tested for touch-alloknesis by gently stroking the skin over a 1 cm length three times at a 1 Hz frequency with a commercially-available cotton swab (Johnson and Johnson, New Jersey, USA). The intensity of touch-alloknesis was assessed at three different time points: right after, 60 seconds after, and 810 seconds after the termination of mechanical stimulation. The set of a 90-second piezo-actuator stimulation with three times of gentle stroking of the skin

surface by a cotton swab was performed two times with a 15-minute interval. The first one was performed only to assess the time course of itch intensity caused by piezo-actuator movement, whereas the second one was performed only to assess the intensity of cotton swab-induced touch-alloknesis, because the simultaneous evaluation of piezo-actuator induced itch and touch-alloknesis was difficult for subjects.

2.10. Statistical analyses

Wilcoxon's matched-pairs sign test was applied for statistical analyses to compare itch intensities and flare areas between H1-blocker and placebo as well as between face and arm. For the comparison of maximal itch intensities among three different sites (chin, cheek, and forehead), Wilcoxon's matched-pairs sign test with Bonferroni correction was performed, after the presence of a significant difference among the three sites was confirmed by Kruskal-Wallis test. *P*-values less than 0.05 were regarded to be significant in all analyses except for Bonferroni correction in which *P*-values less than 0.0167 were regarded to be significant.

3. Results

3.1. Comparison of mechanically-evoked itch; vibration of vellus hairs in chin, cheek, forehead, vibration of male beard hairs in chin, and cotton-swab touching to chin (Fig.2A)

In the *first* session, vibratory stimulation of vellus hairs induced itch in chin, cheek and forehead, among which the maximum intensity of itch in the chin (10th, 25th percentiles, median, 75th, 90th percentiles; 2.9, 5, 5, 6.75, 7.1) was the highest and significantly higher (P = 0.005) than in the cheek (10th, 25th percentiles, median, 75th, 90th percentiles; 1.9, 2, 2.5, 3, 3) and forehead (10th, 25th percentiles, median, 75th, 90th percentiles; 0, 1, 2.5, 3, 3.1). No itch was induced by vibratory stimulation of male beard hairs or cotton-swab touching in the chin.

3.2. Control trial; vibration without contact to vellus hairs and contact to vellus hairs without vibration as compared to standard vellus hair vibration (Fig. 2B)

In the *second* session, itch was evoked by the standard vibration of vellus hairs, whereas no itch was evoked by vibration only or vellus hair contact only.

3.3. Itch intensity and flare area (Fig. 2C)

In the *third* session, mechanically-evoked itch on the face was as intense as

histamine-induced itch on the arm. Itch was rarely evoked on the arm by mechanical stimulation. In contrast, histamine-induced itch on the face (10th, 25th percentiles, median, 75th, 90th percentiles; 5.4, 15.25, 25, 41.5, 66.8) was significantly weaker than that on the arm (10th, 25th percentiles, median, 75th, 90th percentiles; 23.5, 36, 49.5, 69.75, 89.1) (P = 0.0051). The H1 blocker did not affect mechanically-evoked itch, whereas it almost completely suppressed histamine-induced itch on the face and arm with a significant difference as compared to the placebo (P = 0.0051 on face, P = 0.0077 on arm, comparison of AUC of itch ratings).

Mechanical stimulation did not induce any flare on the face or arm, whereas histamine induced flare on both regions. Histamine-induced flare on the face (10th, 25th percentiles, median, 75th, 90th percentiles; 3.77, 3.9, 4.07, 4.99, 5.57 cm^2) was smaller than that on the arm (10th, 25th percentiles, median, 75th, 90th percentiles; 5.17, 8.6, 12.93, 15.49, 19.73 cm^2) (P = 0.0051). Histamine-induced flare was suppressed by the H1 blocker with a significant difference as compared to the placebo (P = 0.017 on face, P = 0.0051 on arm).

3.4. Characterization of itch (Fig. 3)

In the *third* session, both of mechanical stimulation and histamine-iontophoresis

induced a desire to scratch in all the subjects. The burning and stinging characteristics were only associated with histamine-induced itch, not with mechanically-evoked itch.

3.5. Itch intensity under a prolonged stimulation/iontophoresis (Fig. 4A)

During the 10-minute mechanical stimulation on the face in the *fourth* session, itch quickly became as intense as the maximum within 10 seconds, but started to attenuate in 60-90 seconds and was rarely perceptible at the end. In contrast, histamine-induced itch on the arm gradually increased, taking 2-3 minutes to reach the maximum intensity, but stayed constant until the end.

3.6. Touch-alloknesis caused by mechanical stimulation (Fig.4B)

In the *fifth* session, it sometimes took several minutes (10th, 25th percentiles, median, 75th, 90th percentiles; 29, 45, 130, 415, 566 seconds) before mechanically-evoked itch completely diminished, although rapidly attenuated right after the 90-second stimulation. Touch-alloknesis was distinct right after the mechanical stimulation terminated. However, it became weaker at 60 seconds and was not present any more at 810 seconds when mechanically-evoked itch almost diminished, too.

3.7. Change in itch intensity caused by frequency/amplitude modulation of mechanical stimulation (Fig. 5.)

In the *sixth* session, when the frequency was changed in the ascending way from 1 to 50 Hz with the amplitude fixed at 1.0 mm, the itch intensity gradually became enhanced and reached the maximum median rating (median, 5) at 9 Hz. At higher frequencies than 9 Hz, however, the itch intensity reduced. When the frequency was changed in the descending way from 50 to 1 Hz, the itch intensity gradually reduced down to the minimum median rating (median, 0) at 20, 10 and 9 Hz. At lower frequencies than 9 Hz, however, the itch intensity became higher and reached the maximum rating (10th, 25th percentiles, median, 75th, 90th percentiles; 0, 1, 1.5, 3, 5.1) at 6 Hz with a significant difference as compared to the rating at 10 Hz (10th, 25th percentiles, median, 75th, 90th percentiles; 0, 0, 0, 0, 2.3) (P = 0.043). On the other hand, change in amplitude, whether it was ascending or descending change, with the frequency fixed at 8 Hz did not cause any significant change in itch intensity, although the tendency of higher itch intensities at higher amplitudes existed. At any frequency or amplitude, the characteristics of mechanically-evoked itch did not change, and were the same as had been described by the subjects in the *third* session.

4. Discussion

Mechanically evoked itch is a typical phenomenon of itch-sensitization associated with patients with chronic pruritus such as atopic dermatitis [12]. Even under healthy conditions, however, mechanical stimuli such as contact of wool fibers to the skin *sometimes* trigger itch, although the intensity of itch in those cases is not as intense as histamine-induced itch [35]. Other experimental reports published decades ago have also described that *short* itch sensation can *occasionally* be evoked by mechanical stimuli with a fine tungsten wire to the skin of healthy subjects when it hits a randomly-distributed 'itch point' [7,28]. However, no studies had ever successfully demonstrated that any specific mechanical stimuli could *reproducibly* evoke itch that is as intense as histamine-induced itch. The present study is the first of its kind demonstrating that application of vibratory mechanical stimuli to facial hairs of healthy human subjects can reproducibly evoke pure itch sensation that is as intense as, and sometimes more intense than, histamine-induced itch. Notably, all subjects experienced itch with this stimulus in the chin and cheek. This provides direct evidence of mechano-sensitive nerves involved in itch in human skin that had been suggested by microneurographical studies with cowhage-induced itch but not been verified by mechanical stimuli that could induce itch.

The present study has also demonstrated that, to be different from histamine-induced itch, mechanically-evoked itch is not nociceptive, i.e., never includes pain-related sensory components in it. Not only histamine but also other so-far reported experimental methods to evoke itch in human skin, such as cowhage spicules [15,20,30], serotonin [9], bradykinin [9], and electrical stimulation [11] frequently induces itch with pain-related components like pricking, burning and stinging sensations mixed. The present study is the first of its kind to demonstrate experimentally-induced itch in healthy human skin without any pain-related components.

There was a clear difference between histamine-iontophoresis and mechanical stimulation in body regions where they were more likely to induce itch. Histamine-iontophoresis induced more intense itch on the arm than on the face in the present study. This is consistent with the previous studies with histamine application to human skin, in which the majority of subjects did not feel any itch sensation, or felt only weak itch, after histamine application to the face or head, whereas they felt intense itch on the trunk or extremities [3,17,24]. Less histamine-induced itch on the face might attribute to the less innervating histamine-sensitive neurons in the face as compared to other body regions, although no much information on the expression pattern of histamine receptors in the skin nerve endings is available in literature [24]. On the other

hand, mechanical stimuli induced strong itch on the facial skin, especially in the region between the lower lip and the chin, whereas only tickling sensation without any desire to scratch was induced on the arm. This indicates difference in neuro-anatomical features between the face and other areas, although unsuccessful induction of itch by mechanical stimuli in other areas than face in the present study does not necessarily mean absence of neurons for mechanically-evoked itch in those areas. Of note, mechanical stimuli induced itch in the face only when they were applied to a specific type of facial hairs that are thin, short and barely visible with naked eyes, i.e., vellus hairs, whereas the same vibratory stimuli to terminal hairs such as eyebrow or male beard hairs did not. The density of vellus hairs is reportedly higher on the face as compared to other parts, such as 439 hairs/cm^2 on the face vs. 85 hairs/cm^2 on the back [5]. Vellus hairs causing itch on the face might be evolutionary vestiges of facial hairs in other mammals that play defensive roles as a part of sensory organs. This type of itch might represent a part of non-inflammatory itch we experience in our daily life.

The following question inevitably arises from the present study: Which neural pathway serves for mechanically-evoked itch? The pathway for histamine-induced itch does not fit this type of itch, since histamine-sensitive neurons including C-nerves and spinothalamic tract neurons have been found with microneurography to be insensitive to

mechanical stimuli. A very recent study has suggested that a part of mechano-sensitive C-nociceptors is likely in charge of itch induction [16]. However, mechano-sensitive nociceptive neurons, which majorly consist of A-delta and C fibers in the periphery [1,22], are unlikely because mechanically-evoked itch in the present study only needed a very weak innoxious mechanical stimulation to hairs, and moreover, was never accompanied with pain-related sensory components. This also indicates that the pathway for mechanically-evoked itch is different from the one for cowhage-induced itch that is frequently accompanied by pain-related sensations, although it is not fully understood whether polymodal C or A-delta nerves are in charge of cowhage-induced itch [6,14,18,21]. Myelinated tactile neurons that are responsive to movement of individual hairs, i.e., hair follicle afferents, have been reported to exist in human facial skin [33]. According to that report, they had a receptive field limited to a single hair, and discharged only after hair movement, not during maintained pressure of skin with a von-Frey filament. This feature of hair follicle afferents fits mechanically-evoked itch in the present study. However, the long-lasting itch after the termination of mechanical stimulation in the present study is not consistent with their fast-adaptation to mechanical stimuli [33]. On the other hand, low-threshold mechano-sensitive C afferents, i.e., C-tactile neurons, which have been identified in human facial skin and other hairy skin

regions but not glabrous skin [19,34], seem to be a more suitable candidate. They are characterized by 1) low mechanical thresholds, 2) intermediate adaptation and after-discharge, 3) more intense response to slowly moving stimuli than to rapidly moving stimuli [4,19,27,34], and 4) fatigue during mechanical stimulation repeated at short intervals [19], all of which are consistent with the following features of mechanically-evoked itch in the present study: 1) A very small movement of vellus hairs induces itch, 2) itch is evoked during the continuous movement of hairs, and lasts for a while after the movement terminated, 3) the itch intensity is the highest at the vibration frequency less than 10 Hz, and decreases at higher frequencies, and 4) the repeated stimuli gradually decreases itch intensity. What is against this hypothesis of tactile neurons for mechanically evoked itch is that no C-tactile neurons, but only myelinated hair follicle afferents, have been reported to exist in the area adjacent to facial hairs by a previous microneurographical study [33]. However, the facial hairs employed by that study were male beard hairs and not vellus hairs, to be different from the present study. In fact, the vibration of male beard hairs did not evoke itch but only tickling sensation in the present study (Fig. 3B). This hypothesis of C-tactile neurons for mechanically-evoked itch is also consistent with the anatomical report that all hair follicles develop dense and complex neural networks with numerous unmyelinated fine

caliber C-fibers [8]. It is also of note that stroking stimuli to the facial skin, which simultaneously stimulated both the skin surface and vellus hairs, did not induce any itch. This indicates that co-activation of myelinated tactile nerves inhibits the signal conduction of unmyelinated mechano-sensitive nerves involved in itch as explained by the gate control theory or that simultaneous activation of a large number of mechano-sensitive nerves involved in itch induces tactile sensation instead of itch. The latter possibility is rather low, though, because touching the facial skin with a cotton swab never evoked itch, even if it was very gently touched to the skin. Thus, the data in the present study are favorable to the hypothesis that C-tactile neurons are involved in mechanically-evoked itch. Needless to say, however, it remains to be confirmed by future neurographical studies whether or not this hypothesis is true. Furthermore, C-tactile neurons would be only part of neurons involved in itch, as indicated by the fact that histamine induces itch at glabrous skin [17] where C-tactile neurons are reportedly absent.

By the way, the fact that increase in the vibration amplitude did not cause statistically significant increase in the itch intensity in the present study might be explained by softness of vellus hairs that can absorb increased movement of the probe before conveying it to the hair follicle.

The 90-second mechanical stimulation on the face in the present study caused itch-sensitization in the skin area adjacent to the stimulated skin spot, where a gentle stroking of the skin with a cotton swab, which usually only evokes touching sensation, also caused itch, i.e., touch-alloknesis. Touch-alloknesis is not only associated with chronic pruritus such as atopic dermatitis [9], but also is possibly present in healthy human subjects when itch is continuously induced by experimental methods such as histamine application and transcutaneous electrical stimulation [11,31]. Alloknesis in the present study supports the hypothesis that itch sensitization can be caused by a continuous activation of peripheral neurons involved in itch whether or not they are histamine-sensitive C nerves [11].

In conclusion, mechanical stimulation to the facial vellus hairs in the present study is a novel experimental method to evoke itch in healthy human subjects. The purity of mechanically evoked itch without pain-related sensory components is unique and advantageous in terms of differentiation of itch and pain. The combination of this method with other investigative approaches such as microneurography and functional brain imaging is expected to bring more insights into the neurophysiological mechanism of itch sensation.

Figure Legends

Fig. 1 The distant (upper left picture) and close-up (upper right picture and lower illustrations) view of mechanical stimulation on the face. The electrically-controlled piezo actuator (A) horizontally vibrated in the range of 0-0.2 degrees at a frequency of 1-50 Hz, which led to a horizontal vibration of the stainless-steel wire loop (B) with an amplitude of 0-1.0 mm. The wire loop could touch and vibrate only vellus hairs, not the skin surface.

Fig. 2 (A) Maximum itch intensity during the 90s vibration of vellus hairs in the chin, cheek and forehead as compared to the 90s vibration of male beard hairs in the chin and gentle touching of the chin skin by a cotton swab. Vibration of vellus hairs induced itch in the chin, cheek and forehead, among which the most intensely in the chin, whereas no itch was evoked by male beard stimulation or skin touching by a cotton swab. Box plot shows median (line in center of box), 25th and 75th percentiles (bottom and top of box, respectively), and 10th and 90th percentiles (bottom and top error bars, respectively) (n = 10). (B) Comparison between standard vibration of vellus hairs in the chin as compared to vibration only without probe contact to vellus hairs and probe contact to vellus hairs only without vibration. The standard vellus hair vibration (first and

second stimulation) induced itch, whereas no itch was evoked when the probe vibrated without contact to vellus hairs (third stimulation) or when the probe contacted to vellus hairs without vibration (fourth stimulation). Squares and circles correspond to median (n = 10). (C) The itch intensity and flare area induced by mechanical stimulation and histamine-iontophoresis on the face and arm in the subjects who had taken H1-blocker (olopatadine hydrochloride 5 mg) or placebo 3 hours before. The time course of itch intensity was assessed during the 90-second mechanical stimulation or for 90 seconds after the 60-second histamine-iontophoresis. The flare area was measured 5 minutes after the end of stimulation/iontophoresis. Mechanical stimulation (left panel) evoked itch on the face that was as intense as histamine-induced itch on the arm, although no itch on the arm or no flare at all. Mechanically-evoked itch was not suppressed by the H1-blocker. Histamine-iontophoresis (right panel) induced itch and flare, although weaker on the face than on the arm. All histamine-induced reactions were significantly suppressed by the H1-blocker as compared to placebo. Squares correspond to median. Box plot shows median (line in center of box), 25th and 75th percentiles (bottom and top of box, respectively), and 10th and 90th percentiles (bottom and top error bars, respectively) (n = 10). AUC stands for area under the curve. Asterisks represent a statistical significance (*P< 0.05, **P< 0.01).

Fig. 3 Characteristics of itch induced by mechanical stimulation and histamine-iontophoresis. All subjects felt itch (=desire to scratch) with both mechanical/histamine stimulation. To be different from histamine-induced itch, none of the subjects characterized mechanically-evoked itch as burning or stinging.

Fig. 4 (A) The time course of itch intensity during a 10-minute mechanical stimulation on the face or histamine-iontophoresis on the arm. Mechanically-evoked itch rapidly reached the maximum rating but gradually decreased, whereas histamine-induced itch took a few minutes to reach the maximum but stayed intense after that. (B) The time course of itch intensity for 15 minutes in total during and after a 90-second mechanical stimulation on the face as well as touch-alloknesis 1cm aside right after, 60 seconds after, and 810 seconds after the mechanical stimulation ended. Touch-alloknesis was intense right after mechanical stimulation ended, but gradually decreased and was not present any more when mechanically-evoked itch diminished. Squares and circles correspond to median (n = 10).

Fig. 5 Change in itch intensity caused by modulation of frequency (left panel) or

amplitude (right panel) of mechanical stimulation on the face. When the frequency was changed in the ascending way from 1 to 50 Hz with the amplitude fixed at 1.0 mm, the itch intensity (left panel, unfilled squares) gradually became enhanced and reached the maximum median rating of 5 at 9 Hz. At higher frequencies than 9 Hz, however, the itch intensity reduced. When the frequency was changed in the descending way from 50 to 1 Hz, the itch intensity (left panel, filled squares) gradually reduced down to the minimum median rating of 0 at 20, 10 and 9 Hz. At lower frequencies than 9 Hz, however, the itch intensity became higher and reached the rating of 1.5 (median) at 6 Hz with a significant difference as compared to the rating at 10 Hz (P = 0.043) (middle magnified panel). On the other hand, change in amplitude, whether it was ascending or descending change (right panel, unfilled and filled squares), with the frequency fixed at 8 Hz did not cause any significant change in itch intensity, although the tendency of higher itch intensities at higher amplitudes existed. The characteristics of mechanically-evoked itch were same at any frequency/amplitude as in Fig. 3. Squares in the left and right panels correspond to median. Box plot in the middle panel shows median (line in center of box), 25th and 75th percentiles (bottom and top of box, respectively), and 10th and 90th percentiles (bottom and top error bars, respectively) (n = 10). The asterisk represents a statistical significance (*P< 0.05).

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Conflict of interest

No conflict of interest to declare.

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Fig. 1

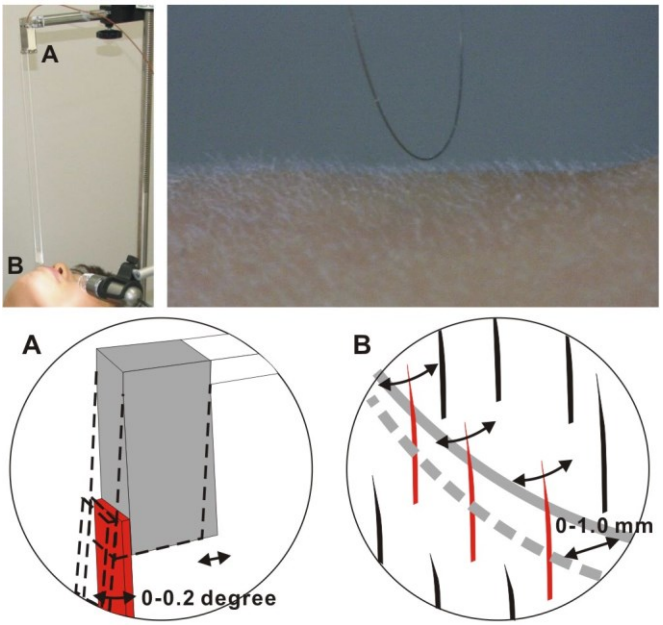


Fig. 2

	Crawling	Tickling	Stinging	Burning	Stabbing	Pinching
Mechanical stimulation	9/10	9/10	0/10	0/10	0/10	0/10
Histamine-iontophoresis	4/10	3/10	8/10	3/10	0/10	0/10

Fig. 3

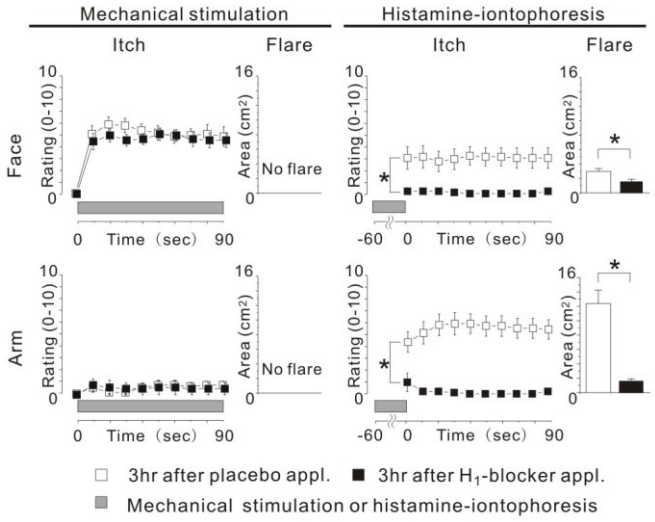

Mechanical stimulation
Histamine-iontophoresis
Itch
Flare
Face
Arm
Rating (0-10)
Area (cm²)
No flare
Time (sec)
0
90
-60
10
16
*
3hr after placebo appl.
3hr after H1-blocker appl.
Mechanical stimulation or histamine-iontophoresis

Fig. 4

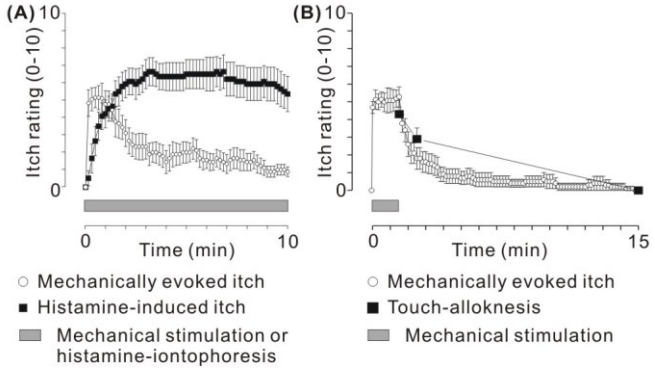
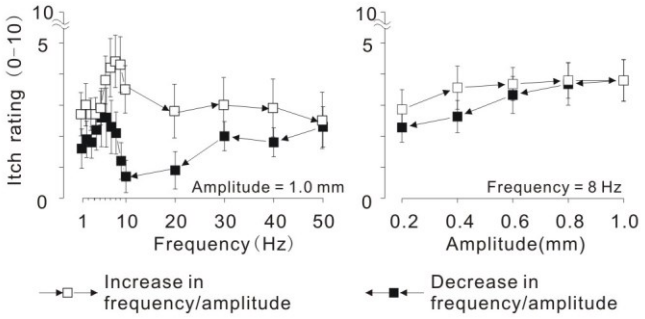

(A)
10
Itch rating (0-10)
0
0
Time (min)
10
Mechanically evoked itch
Histamine-induced itch
Mechanical stimulation or
histamine-iontophoresis
(B)
10
Itch rating (0-10)
0
0
Time (min)
15
Mechanically evoked itch
Touch-alloknesis
Mechanical stimulation

Fig. 5


Itch rating (0-10)
10
5
0
Amplitude = 1.0 mm
1 10 20 30 40 50
Frequency (Hz)
Increase in frequency/amplitude
10
5
0
Frequency = 8 Hz
0.2 0.4 0.6 0.8 1.0
Amplitude(mm)
Decrease in frequency/amplitude