Szechuan pepper, a widely used ingredient in the cuisine of many Asian countries, is known for the tingling sensation it induces on the tongue and lips. While the molecular mechanism by which Szechuan pepper activates tactile afferent fibres has been clarified, the tingling sensation itself has been less studied, and it remains unclear which fibres are responsible. We investigated the somatosensory perception of tingling in humans to identify the characteristic temporal frequency and compare this to the established selectivity of tactile afferents. Szechuan pepper was applied to the lower lip of participants. Participants judged the frequency of the tingling sensation on the lips by comparing this with the frequencies of mechanical vibrations applied to their right index finger. The perceived frequency of the tingling was consistently at around 50 Hz, corresponding to the range of tactile RA1 afferent fibres. Furthermore, adaptation of the RA1 channel by prolonged mechanical vibration reliably reduced the tingling frequency induced by Szechuan pepper, confirming that the frequency-specific tactile channel is shared between Szechuan pepper and mechanical vibration. Combining information about molecular reactions at peripheral receptors with quantitative psychophysical measurement may provide a unique method for characterizing unusual experiences by decomposing them into identifiable minimal units of sensation.

1. Introduction

Psychophysical studies can show how neural afferent pathways shape the relationship between physical inputs and sensory perceptions. For example, cutaneous receptors that transduce specific mechanical events can account for the relationships between stimulus energy and different tactile experiences. This relationship was previously investigated by invasive microneurographic stimulation of single neurons [1].

Natural products, such as capsaicin, mustard oil and menthol, have been a powerful tool to investigate the neuronal mechanisms of somatosensory afferent processing [2]. Their specificity for chemically activating specific types of ion channels and fibres has greatly contributed to the understanding of physiological and molecular mechanism of pain and thermal afferent pathways [3,4]. Based on these detailed understanding of the neuronal mechanisms, making use of natural products may provide an alternative tool to investigate the relationship between population of specific afferent signals and the resultant perception.

Szechuan pepper is a natural product widely used in the cuisine of many Asian countries. It is known from the unique somatosensory sensations of tingling and numbing it induces [5,6]. Previous neurophysiological studies using animal models (mice and rats) have demonstrated that the bioactive component of Szechuan pepper, hydroxyl-a-sanshool (hereafter sanshool), induces bursts of neuronal firing in specific cutaneous afferents through chemical events within the receptor membrane [7–10]. However, these studies focused on molecular receptor mechanisms and did not directly assess how chemical receptor events could relate to the tingling somatosensory experience. Here, we use psychophysical experiments on tactile frequency perception to achieve this goal. Frequency decomposition of inputs by specific classes of mechanoreceptor is a fundamental principle of tactile sensory processing [11] and accounts for the qualities of vibrotactile sensation [12]. We therefore hypothesized that Szechuan pepper activates the afferent fibres of a specific tactile frequency channel [13] to produce the percept of tingling. In this study, we investigate whether such tingling induced by...
Szechuan pepper is indeed experienced as a somatosensory event with a characteristic temporal frequency.

Psychophysical and microneurographic studies show that specific classes of skin mechanoreceptors, and their associated afferent fibres, mediate experience of specific tactile vibration frequencies \[14,15\]. Molecular studies suggested that low-threshold rapidly adapting mechanoreceptor fibres (RA1/Meissner), responding to light touch and mechanical vibration, could underlie sanshool tingling \[8,9\]. Therefore, if the RA1/Meissner receptor fibres are the main contributor for the sanshool tingling, the tingling should involve a distinct perception of frequency, corresponding to the range preferred by RA1 fibres \[16\].

2. Material and methods

(a) Participants
Twenty-eight naive participants, aged 20–37 (20 male), participated in the experiments (experiment 1, 12; experiments 2 and 3, 8; experiment 4, 8). Procedures were approved by the University College London Ethics Committee.

(b) General apparatus
Two mechanical vibrators (Ling Dynamics Systems Limited, Type 101, Royston, UK) were used to deliver vibrotactile stimuli to the finger or lips (experiments 2–4). The amplitude of the vibration was 2 mm. The contact surface touching the finger or lips was an 8 mm diameter circle (experiments 2 and 3). In experiment 4, the contact with the lips was a 15 × 30 mm² rectangle plate, covering most of the lower lip vermilion where Szechuan pepper was applied. This ensured that the stimulus worked on all the areas where Szechuan pepper induced the tingling sensation. Throughout the trials, noise-cancelling headphones suppressed the sound of the vibrator.

(c) Experiment 1
We first confirmed that the application of Szechuan pepper on the skin induces a reliable tingling sensation. Ground Szechuan pepper was mixed in a solution of 40% ethanol at a concentration of 0.05 g ml⁻¹. The suspension was applied to the vermilion of the lower lip of 12 participants (figure 1; see the drawing of the lip). As reported previously, there is an initial delay before the participants start to feel the tingling sensation \[7\]. Therefore, after the application of the pepper, a delay was imposed for at least 120 s. Then, the participants were asked whether the sensory experience on their lips matched with the following five adjectives; tingle, burn, cool, warm and numb. The selection of these adjectives was based on the previous literature \[7,17\]. ‘Tingle’ was the attribute of most interest for our study. Each adjective was presented to the participants verbally, and the participants responded to each of them by saying ‘yes’ or ‘no’. To control for the non-specific perceptual effects of the experimental situation and stimulation, two control conditions were also studied. In one control condition, 40% ethanol was applied on the lips instead of Szechuan pepper, and the participants reported their experience as before. We also included a second control condition, in which water was applied on the lips, because this should not produce any sensations comparable to those of interest here. The order of the conditions was randomized across participants. The presentation order of the adjectives was randomized across participants and conditions.

For each adjective in each condition, the number of participants responding ‘yes’ was calculated. Then, the frequency of ‘yes’ and ‘no’ responses for each adjective was compared across the three conditions using a 2 (yes or no) × 3 (Szechuan pepper, ethanol or water) \(\chi^2\) interaction test. We applied a Bonferroni correction procedure for the number of adjectives tested. Significant results for any adjective were followed up by 2 × 2 tests between pairs of conditions to identify the source of the interaction.

(d) Experiment 2
In experiment 1, we confirmed that the tingling sensation is only reliably induced by the application of Szechuan pepper on the lips but not by the application of other control liquids (see Results and figure 1). In experiment 2, we investigated the temporal characteristics of this tingling sensation induced by Szechuan pepper. Specifically, we tested whether the Szechuan pepper tingling experience has consistent and measurable frequency. Ground Szechuan pepper was applied to the lower lip of the participants as in experiment 1. When the participants claimed that they felt the tingling vividly, the experiment started.

While the participants perceived the tingle on the lips, in each trial, a mechanical vibration was applied on the participant’s right index finger for 1000 ms (figure 2(i)). After the vibration, participants were asked to judge whether the tingling sensation on the lips or the vibration of the finger was higher in frequency. The range of the mechanical vibration varied from trial to trial, according to an adaptive staircase method (QUEST; \[18\]). In this method, stimulus frequency is adaptively changed according to the participant’s response, thus allowing effective reconstruction of the psychometric function without the need to decide on a particular stimulus range \emph{a priori}. Thus, the range of mechanical vibration frequencies that the participant could select to match the tingling sensation is effectively unlimited. The experiment consisted of three sessions of 40 trials each.

A cumulative Gaussian function was used to relate the percentage of ‘higher frequency on finger’ responses to the actual vibration frequency on the finger. The point of subjective equality (PSE; judgement of ‘higher frequency’ for 50% or the trials) and the slope of the function were calculated from each regression.

(e) Experiment 3
Experiment 3 aimed to confirm that tactile frequencies of mechanical stimuli can be compared between the finger and the lips. This step was required to validate the method of comparing tingling frequency of the lips with tactile frequencies on the finger in experiment 1. Instead of judging the tingling frequency induced by Szechuan pepper on the lips, the same eight participants compared the frequency of a constant mechanical vibration applied on the lips relative to the vibration on their finger (figure 2(ii)). The constant vibration frequency on the lips was defined individually to match each of the participants’ perceived tingling frequency of the Szechuan pepper in experiment 1.

Vibration was applied first on the lips for 1000 ms, and after a 1500 ms delay, another vibration was applied to the finger. As in experiment 1, the vibration frequency on the finger varied trial-to-trial. Participants judged which of the two vibrations had higher frequency. The experiment consisted of three blocks of 40 trials.

(f) Experiment 4
In experiments 2 and 3, we found that the Szechuan pepper elicits a vibratory sensation with a subjective frequency corresponding to the range of the tactile RA1 afferent. To confirm that the Szechuan pepper indeed mimics the perceptual mechanisms of physical vibrotactile input activating the same frequency channel, in experiment 4, we tested the effect of frequency adaptation on the Szechuan pepper tingling. Selective adaptation of tuned channels is a well-established method of identifying the neural signals responsible for specific sensations, notably in studies of visual illusions \[19\]. Prolonged vibrotactile input reduces the perceived frequency/speed of a subsequent vibrotactile stimulus applied to the same skin site, possibly by selectively adapting RA channels
If the tingling induced by Szechuan pepper shares the same frequency channel as vibrotactile mechanical information, the perceived frequency of Szechuan pepper tingling should be reduced by previous mechanical vibration on the lips.

Eight new participants judged the tingling/vibration frequency on their lips as in experiments 2 and 3. The experiment spanned 2 days. On the first day, participants judged the mechanical vibration frequency on the lips (vibration condition), and on the second day, they judged the Szechuan pepper-induced tingling on the lips (Szechuan pepper condition). Forty per cent ethanol without Szechuan pepper was applied on the lips in the vibration condition to prevent non-specific effects unrelated to Szechuan pepper. On each day, two conditions were prepared. In one condition, judgement of tingling frequency was preceded by a prolonged mechanical vibration (adaptation condition). In the other condition, it was preceded by a contact to the static vibrator (control condition).

In the adaptation condition, 80 Hz mechanical vibration was applied on the lips (adapting stimulus). We chose this high-frequency because it has been shown in a previous study that the effect of tactile frequency adaptation is more robust when the adapting stimulus has higher frequency than the standard stimulus [20]. The adapting stimulus was given before each trial; 60 s for the initial trial, and 20 s for the remaining trials, as in tactile frequency adaptation paradigms [20]. In the control condition, instead of the dynamic adaptor stimulus, the vibrator remained in static contact with participants’ lips but did not move.

In the vibration condition, 500 ms after the adaptor stimulus, 50 Hz vibration was applied to the same skin site for 1000 ms. Then, a 1000 ms vibration was applied on the finger, at a frequency varying from trial-to-trial. In the Szechuan pepper condition, after the adaptor vibration, a 4000 ms rest period was given during which participants estimated the frequency of the tingling. Then the finger vibration was applied. Participants judged which area, lip or finger, experienced the higher frequency.

On each day, the adaptation and the control conditions were separated by a minimum of 20 min. This is sufficient to allow the RA1 fibres to recover from adaptation [22]. Each condition consisted of three sessions of 30 trials.

3. Results

(a) Experiment 1
All 12 participants agreed that Szechuan pepper induced a sensation described as ‘tingle’ (figure 1). Some participants also reported the sensation of tingling after the application of ethanol or water (33% and 25% of the participants, respectively). However, at the end of the experiment, some of these participants anecdotaly reported ethanol-induced tingling sensations as qualitatively different to those induced by Szechuan pepper. They described the tingling during the pepper application as ‘much more vivid and stronger in intensity’ and the ‘repetitive touch sensation was more regular in time’. The ratio of agreeing to the tingling sensation was significantly different among the conditions ($\chi^2 = 16.3$,
p < 0.005, Bonferroni corrected). Direct comparisons between the conditions revealed significantly stronger agreement for the Szechuan pepper condition compared with either ethanol control ($\chi^2 = 12.0, p < 0.005$, Bonferroni corrected) or water control conditions ($\chi^2 = 14.4, p < 0.001$, Bonferroni corrected). By contrast, agreement did not differ between ethanol and water conditions ($\chi^2 = 0.26, p > 0.6$). These results confirm that the Szechuan pepper induces a unique and reliable tingling sensation, which cannot be explained by the effect of ethanol or water.

Among the other adjectives, only ‘burn’ revealed differences in agreement between the conditions ($\chi^2 = 14.7, p < 0.005$, Bonferroni corrected). The ratio for this ‘burn’ sensation significantly differed between the Szechuan pepper and the water control condition ($\chi^2 = 14.4, p < 0.001$, Bonferroni corrected), but neither differed between the Szechuan pepper and the ethanol control condition ($\chi^2 = 4.2, p > 0.2$, Bonferroni corrected) nor between the ethanol and the water control conditions ($\chi^2 = 4.8, p > 0.1$, Bonferroni corrected). This fits with the previous notion that the Szechuan pepper also induces a burning sensation [17]. Furthermore, the milder specificity of this sensation for the Szechuan pepper to the ethanol also fits with the previous literature showing the burning experience by application of ethanol [7].

(b) Experiments 2 and 3
The temporal characteristic of the tingling sensation elicited by Szechuan pepper, the perceived frequency of the tingling, was examined. The frequency of the tingling sensations was strikingly consistent. On average, the tingle induced by Szechuan pepper on the lips was matched by a mechanical vibration frequency of 50.0 Hz on the finger (s.e. 2.4 Hz) (figure 2b,c; blue). This corresponds to the most sensitive response frequency range of RA1 fibres (10–80 Hz) [16]. In experiment 3, the Szechuan pepper was replaced with the actual mechanical vibration of the same participants’ lips. The frequency of lip vibration was held at the level that matched participants’ individual tingling experiences in experiment 2. This mechanical vibration on the lips was perceived to match finger vibrations at 46.4 Hz (s.e. 2.3 Hz) (figure 2b,c red), which was slightly underestimated compared with the actual given vibration frequency ($t = 2.0, p = 0.082$). However, even given this slight bias to underestimate vibration frequency on the lip, we confirmed that Szechuan pepper elicits a clear percept of tactile vibration in the RA1 range.

(c) Experiment 4
To test whether the tactile frequency channel activated by Szechuan pepper shares the same pathway as vibrotactile mechanical stimuli, we examined the effect of prolonged mechanical vibration on perceived pepper tingling frequency (frequency adaptation).

A two-factorial (stimulus (2: vibration or Szechuan pepper) × adaptation (2: adaptation or control)) ANOVA (repeated measure) on the perceived frequency revealed the main effect of adaptation ($F_{1,2} = 23.0, p < 0.002$) and the interaction between the two factors ($F_{1,2} = 6.4, p = 0.04$). From the pairwise comparisons, we confirmed that following prolonged 80 Hz vibratory stimulation, the perceived frequency of a 50 Hz lip vibration was reduced compared with adaptation by a preceding static force (vibration condition; $t = 4.9, p = 0.002$) (figure 3a,b; red). This ensured the successful induction of frequency adaptation on the lips, as previously reported for other skin regions [19]. More importantly, though slightly weaker, the same reduction of perceived frequency was also observed for the Szechuan pepper condition; the perceived frequency of pepper-induced tingle was significantly reduced when preceded by an adapting mechanical vibration on the lips ($t = 3.1, p = 0.017$) (figure 3a,b; blue). Transfer of adaptation from mechanically to chemically induced sensation of frequency implies that the Szechuan pepper activates the same tactile frequency channels as mechanical vibration, possibly mediated by RA1 fibres.

Finally, the same ANOVA was applied for the slope of the fitted psychometric function, which represents the sensitivity to the frequency discrimination. None of the effects reached significance (all the effects $p > 0.12$), showing that the prolonged vibration reduced the perceived stimulus frequency but without altering the discriminability of the frequency stimuli.

4. Discussion
We showed that the tingling sensation induced by Szechuan pepper has a consistent and measurable frequency within the range of the RA1 tactile frequency channel. Furthermore, by using a frequency adaptation paradigm, we demonstrated that this tingling is probably mediated by the same pathway/RA1 frequency channel that processes frequency of the mechanical vibrotactile information.

In experiment 1, we confirmed that the application of Szechuan pepper on the lips induces a tingling sensation, as has been shown previously [7,17]. However, some participants also agreed to the description ‘tingle’ when ethanol (four out of 12 participants) or even water was applied (three out of 12 participants). Interestingly, all seven of these reports involved control conditions experienced before the Szechuan pepper condition. This suggests that these responses may have reflected general tendencies to agree, rather than a discriminative response to the stimuli. By contrast, for the Szechuan pepper condition, 12 out of 12 participants agreed to the description ‘tingle’, regardless of the condition order. Furthermore, participants anecdotally described a qualitative difference between the tingling induced by the Szechuan pepper and the other control conditions. Therefore, only the Szechuan pepper reliably induced the sensation having a specific tingling quality, consistent with involvement of a specific class of afferents.

Previous studies have detected many varieties of compounds—sanshool—in the Szechuan pepper ($\alpha$, $\beta$, $\gamma$, $\delta$, hydroxy-$\alpha$, hydroxy-$\beta$) [5]. Previous psychophysical studies using derivatives of these have identified hydroxy-$\alpha$-sanshool as the compound most responsible for this unique buzzing and tingling [5,7]. From these previous reports, we believe that also in this study, the hydroxy-$\alpha$-sanshool in the raw pepper activated somatosensory fibres [9] and induced the tingling sensation. However, our interest focused on the tingling sensation itself, not the molecular biochemistry that initiates it. Previous reports did not systematically investigate the perceptual parameters of this tingling sensation, and how these may relate with the activated somatosensory fibres.

The results from the experiments 2–4 suggests that the information which elicits the tingling is conveyed by the light-touch RA1 fibres, which corroborates with the neurophysiological findings showing that the sanshool strongly activates those fibres in rats [9]. Other than the RA1 fibres, sanshool has been shown to also activate other
sets of afferent fibres, including RA2, SA1, SA2 fibres and unmyelinated C-fibres [9]. Thus, there is a possibility that the overall sensation of sanshool tingling is a result of ‘blending’ of all of these afferent fibre activations [23]. However, RA2 fibres responding to higher frequency vibration are reportedly absent from the human orofacial area [15]. Mechanical frequency detection threshold on the lower lips was indeed reported to lack pacinian-type (RA2) like frequency sensitivity [24,25]. Further, slow adapting fibres are less responsive to sanshool than rapid adapting fibres [9]. Direct stimulation of orofacial SA1 and SA2 fibres by microneurography induces only sensations of sustained pressure, and not of tingling or vibration. By contrast, microneurographic stimulation could elicit sensations of flutter-vibration on the superficial skin site only when stimulating RA1 fibres [15], in agreement with our findings. Finally, although low threshold C-fibres have been shown to respond to mechanical stimulations [26,27], they are unlikely to contribute to perceived fast repetitive mechanical stimulation, owing to their slow conduction velocity and marked preference for slowly moving stimuli. Taken together, though Szechuan pepper may activate several fibre classes, we believe that the major contributor for the temporal component (tingling) of the tingling experience is owing to the activation of the RA1 frequency channel. Szechuan pepper creates the experience of vibratory sensation on the lips despite the absence of any mechanical vibration. Sanshool effects on other sensory channels may explain the accompanying feelings of cooling and numbing [7].

In experiment 4, we showed that the preceding prolonged mechanical vibration can reduce the perceived tingling frequency by Szechuan pepper compared with when followed by a static force. This indicates that the mechanical vibration and Szechuan pepper activates the same frequency channel, thus sharing the same tactile processing pathway. Importantly, the adapting vibration did not lead to any significant change in the sensitivity for discriminating frequencies. This suggests that the adaptor vibration did not lead to the strong reduction of the intensity of the signal, which should alter the discrimination ability, but rather worked to adapt the particular (RA1) temporal processing [20,21].

Adaptation to prolonged vibrotactile input has been reported at the receptor level [28], as well as in several central sites, including the cuneate nucleus [29], thalamus [30] and cortex [31]. RA1 type neurons are reported to exist in S1 [32] and are known to underlie frequency discrimination in the RA mechanical frequency range [33]. Therefore, adaptation of tactile processing at any of several stages of the afferent pathway may have affected the firing rate of the S1 neurons. Further studies are required to clarify this point. Nevertheless, the mechanical-to-chemical transfer of adaptation reveals the shared processing pathway between Szechuan pepper and mechanical vibration, and thus indicates that the Szechuan pepper-induced tingling experience is indeed a tactile frequency experience.

Decomposing complex somatosensations into component units of neuronal activity is a critical step towards understanding how the brain constructs sensory experiences. Previous studies were able to investigate the minimal unit of somatosensory experiences using microneurographic stimulation of single afferent fibres [1]. However, natural stimuli generally activate large populations of neurons, each containing several afferents. By using an unusual chemical stimulus to activate a class of tactile receptor, we have been able to identify a discrete population of ‘labelled lines’ in the somatosensory system, based on their psychophysical properties. This offers a possibility of testing the minimal unit of somatosensation at a population level.

Spontaneous firing of the population of RA afferent fibres and/or Meissner’s corpuscle mechanoreceptor fibres are also thought to underlie the ‘pins and needles’ tingling sensation experienced after ischemia [34,35] or focal nerve compression [36]. As Szechuan pepper can activate populations of these same tactile fibres and induce similar sensations to paraesthesia, our result could provide insights into abnormal afferent fibre discharges in clinical cases of paraesthesia [37].

Finally, food is both an intense sensory experience and a primary carrier of human culture. Modern gastronomy recognizes the multisensory aspects of food, such as temperature [38], colour [39] and sound [40] on perception of flavour and taste. The specific somatosensory effects of Szechuan pepper shown here may provide a unique opportunity to investigate somatosensory contributions to taste perception. Szechuan pepper might boost taste by mimicking touch.

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Data accessibility. Data involved in this study is deposited in the Dryad repository: http://dx.doi.org/10.5061/dryad.cn667.
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