Physiology & Behavior 184 (2018) 226–234

Contents lists available at ScienceDirect

Physiology & Behavior

journal homepage: www.elsevier.com/locate/physbeh

An automated method to detect and quantify fungiform papillae in the human tongue: Validation and relationship to phenotypical differences in taste perception

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ARTICLE INFO

Keywords:
Fungiform papillae
PROP
Thermal taster status
Tongue images
Automated counting
Colour segmentation

ABSTRACT

Determination of the number of fungiform papillae (FP) on the human tongue is an important measure that has frequently been associated with individual differences in oral perception, including taste sensitivity. At present, there is no standardised method consistently used to identify the number of FP, and primarily scientists manually count papillae over a small region(s) of the anterior tip of a stained tongue. In this study, a rapid automated method was developed to quantify the number of FP across the anterior 2 cm of an unstained tongue from high resolution digital images. In 60 participants, the automated method was validated against traditional manual counting, and then used to assess the relationship between the number of FP and taste phenotype (both 6-n-propylthiouracil (PROP) and Thermal Taster Status). FP count on the anterior 2 cm of the tongue was found to correlate significantly with PROP taster status. PROP supertasters (PSTs) had a significantly higher FP count compared with PROP non-tasters (PNTs). Conversely, the common approach used to determine the number of FP in a small 6 mm diameter circle on the anterior tongue tip, did not show a significant correlation irrespective of whether it was determined via automated or manual counting. The regional distribution of FP was assessed across PROP taster status groups. PSTs had a significantly higher FP count within the first centimetre of the anterior tongue compared with the PNT and PROP medium-tasters (PMT), with no significant difference in the second centimetre. No significant relationship was found with Thermal Taster Status and FP count, or interaction with PROP taster status groups, supporting previous evidence suggesting these phenomena are independent. The automated method is a valuable tool, enabling reliable quantification of FP over the anterior 2 cm surface of the tongue, and overcomes subjective discrepancies in manual counting.

1. Introduction

Human taste papillae on the dorsal surface of the tongue can be classified in three types: fungiform, circumvallate and foliate papillae [19]. Papillae are distributed in a specific pattern. Fungiform papillae (FP) are mushroomed-shaped pink structures located on the anterior two-thirds of the tongue with a higher density being present on the tongue tip compared to other areas of the tongue [26]. Taste buds and mechanoreceptors located in FP are innervated by both gustatory and trigeminal nerve fibres [46]. Therefore, these papillae are thought to be associated with oral sensitivity [24,33]. The circumvallate papillae are large structures arranged in an arc on the posterior tongue, whilst foliate papillae are clustered at the edges of the tongue. Filiform papillae are numerous threadlike elevations covering most of the tongue surface, however they have no taste function.

The number and shape of FP are highly variable across individuals [23,25], and in some cases FP number has been associated with individual differences in oral perception. FP density is frequently reported to be higher in individuals who are classified as supertasters of the bitter substance 6-n-propylthiouracil (PROP) compared with PROP medium tasters (PMTs) and PROP non-tasters (PNTs) [1,2,9,10,28]. However, a number of recent studies have failed to show an association between FP count and PROP rating [11,14,21]. Some evidence reports PROP supertasters (PSTs) to be more sensitive to many oral sensations, including prototypical tastants [8], irritants [34] and tactile stimuli [10], than PMTs and/or PNTs. However, others have identified no

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https://doi.org/10.1016/j.physbeh.2017.12.003
Received 10 October 2017; Received in revised form 29 November 2017; Accepted 3 December 2017
Available online 06 December 2017

difference across PTS groups for taste [22,45] or trigeminal [42,47] stimuli.

Recently, a taste phenotype termed Thermal Taster Status has been discovered [7], whereby 20 to 50% of individuals (known as thermal tasters (TTs)) perceive a 'phantom taste' when the tongue is thermally stimulated (warmed and/or cooled) [1,15,48]. Elicited phantom taste sensations include prototypical tastes (sweet, sour, salt, umami, bitter) or other oral sensations (mint, metallic, spicy), with reported sensations varying with the temperature regime of warming or cooling [1,7,18,48]. Previous studies have shown that the anterior tip of the tongue is most sensitive to perceiving temperature and phantom taste [7]. TTs have been reported to have higher sensitivity to pure taste stimuli at supra-threshold levels [7,15,16]. The detection threshold of sucrose [31,48] and difference threshold tartaric acid [31] have been reported to be lower in TTs than thermal non-tasters (TnTs). This heightened sensitivity to pure tastes may be linked to the number of FP located on the tongue tip. Only one previous study has assessed the relationship between FP density and Thermal Taster Status, but reported no significant correlation [1].

The counting of FP in humans is generally achieved using one of the following non-invasive methods; video microscopy [23] which requires high-quality images to be obtained over 30–60 min, and is therefore limited to the research laboratory, or digital photography [40] which provides a rapid method for obtaining high quality images. The current methods for counting FP require staining of the tongue with a dye (blue food colouring), to provide contrast between the FP (which appear pink) and the filiform papillae (coated blue), before a high quality digital image is captured [23]. FP are then manually detected over a small region of the anterior tongue, typically a 5–10 mm circular template placed onto the tongue tip close to midline. Manual counting of FP can be subjective and has been shown to be highly variable across assessors counting the same tongue image [29], however this variability has been shown to significantly decrease with training [29]. To reduce discrepancies between assessors, Nuessle and colleagues [29] proposed the 'Denver Papillae Protocol' as a standardised method to characterise FP, this method is based on manual counting papillae in a dyed 10 mm circle of the tongue. Recently, automated methods to detect FP from digital images have been proposed [32,36,37,43], however most of these methods still require staining of the tongue or specialised methods to acquire the image. Overall, the lack of a standardised approach to measuring FP density contributes to discrepancies between studies, especially when exploring variation across taste phenotypes, thus highlighting the need for a robust, accurate method that can be applied outside of the laboratory environment and across large scale studies.

The primary objective of this study was to develop an automated method to detect and quantify distribution and density of FP across the anterior 2 cm of the tongue from a high resolution digital image, and to validate the automated counting against traditional manual counting of FP. Secondary objectives were to use this automated method to locate the area(s) of the tongue with the highest FP count, and to assess the relationship between the number of FP and both PROP taster status and Thermal Taster Status phenotypes.

2. Methods

2.1. Participants and protocol

The study was approved by the University of Nottingham Medical School Research Ethics Committee. All volunteers gave informed consent before enrolling in the study. Recruitment questionnaires were used to screen out any volunteer who had a known taste dysfunction or were on medication that could affect their taste sensitivity. Sixty healthy volunteers (43 female, age 18–60 years) participated in the study. The 60 participants were chosen from 200 subjects recruited and assessed for taste phenotype, to include equal number across PROP tasters with 20 PNTs, 20 PMTs and 20 PSTs. Across the cohort this resulted in Thermal Taster Status being characterised with 29 TTs, 26 TnTs and 5 uncategorised (Uncat).

Participants attended two separate sessions, each session lasting approximately one hour. During the first session participants were trained to correctly use the general Labelled Magnitude Scale (gLMS), their PROP taster status was determined, and images of the tongue were collected for FP measures. Thermal Taster Status was determined in the second session. A gLMS [17] was used to collect all intensity ratings. The gLMS scale is a category ratio scale used to measure intensity of sensation comprising of categories of 'no sensation' (0 mm), 'barely detectable' (1.4 mm), 'weak' (6 mm), 'moderate' (17 mm), 'strong' (35 mm), 'very strong' (53 mm), and 'strongest imaginable sensation of any kind' (100 mm). Participants were familiarised and trained on how to use the gLMS prior to data collection, in order to increase validity [3].

2.2. PROP taster classification

PROP taster status was defined based on the intensity ratings of 0.32 mM of PROP (Sigma Aldrich, UK) prepared in deionised water from a reverse osmosis unit, presented and classified according to the method described by Lim et al. [20]. Participants were instructed to apply the PROP solution by rolling a cotton bud (Boots pharmacy, UK) that had been saturated in the solution across the whole tongue for approximately 3 s, and to then rate the intensity of the perceived taste of the PROP solution when it reached its maximum on the gLMS [17]. A 2 min break was given for palate recovery and palate cleansing with deionised water, before a second replicate was conducted. The mean intensity rating from the two replicates were used to classify participants as PNT (< barely detectable), PMT (range between barely detectable and < moderate), or PST (> moderate) [20].

2.3. Thermal taster (TT) classification

Thermal Taster Status was assessed using a Medoc Pathway thermode (Medoc, Israel) with intra oral ATS (Advanced Thermal Stimulator), based on the method described by Bajec and Pickering [1]. The thermode (16 × 16 mm square surface) was applied to the anterior tongue tip, the area most responsive to thermal taste [7]. A mouthpiece was engineered to ensure the thermode was in contact with the tongue in a standardised position and with standard pressure across both replicates and assessors. Two warming trials (from 15 °C to 40 °C) and two cooling trials (from 35 °C to 5 °C) were conducted, employing a temperature ramp of 1 °C/s for all trials. Prior to each warming/cooling trial, a baseline temperature of 35 °C was held for 10 s. The warming trial started at 35 °C, cooled to 15 °C and re-warmed to 40 °C at which it was held for 1 s. The cooling trial started at 35 °C, cooled the tongue to 5 °C where it was held at this temperature for 10 s before returning to baseline (35 °C). Warming trials always preceded cooling trials to avoid possible adaptation from the intense, sustained cold stimulation [15]. After the warming trial, participants were told to wait until their tongue temperature and sensation had returned to normal before proceeding onto the next trial, with a minimum of a two-minute break between the trials. If a thermally induced taste was perceived in either the warming or cooling trial, the subjects were asked to indicate the taste quality perceived from a selected list ('sweet', 'salty', 'bitter', 'sour', 'umami', 'other please specify'), and rate the perceived intensity on the gLMS. TTs were classified as those individuals who perceived a taste above weak intensity during both replicates of either the warming or cooling trial. TnTs were classified as those individuals who did not perceive a taste on any replicate of any trial. Participants who did not meet the criteria for a clear TT or TnT categorisation were labeled as uncategorised (Uncat).