Electrogustometry Thresholds, Tongue Tip Vascularization, Density, and Form of the Fungiform Papillae Following Smoking Cessation

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Abstract

The objective of this study was to evaluate differences in gustatory function and in shape, density, and vascularization of the fungiform papillae (fPap) of smokers’ tongue before and after smoking cessation. In 24 smokers (19 males, 5 females; median age: 54.6 ± 2.9 years) electrogustometry (EGM) thresholds at the chorda tympani area, at the soft palate area and at the area of the vallate papillae were recorded bilaterally. Morphology and density of the fungiform papillae (fPap) and blood vessels’ density and morphology at the tip of the tongue were examined using contact endoscopy (CE). Follow-up exams (EGM and CE) were performed on average 3.2 months after smoking cessation. Findings were compared between the 2 conditions as well as to those of a group of 24 non-smokers (median age: 55.2 ± 3.4 years; 19 males, 5 females). After smoking cessation, EGM thresholds decreased significantly (P = 0.02 or P = 0.03 depending on the tested area) but nonetheless still were quite different from those of non-smokers (P = 0.05 or 0.04 depending on the site of EGM measurement). Under CE the fPap density was higher after quitting smoking (P = 0.05) and the shape and vascularization of fPap also exhibited a trend to improvement (P = 0.05) after smoking cessation. Chronic exposure to cigarette smoke infers long lasting, although to a large extent reversible, alterations in morphology of taste buds in fungiform papillae, but rather irreversible EGM-related functional gustatory compromise, suggesting a profound physiologic effect on human peripheral taste organs.

Keywords: contact endoscopy, electrogustometry, fungiform papillae, smoking, vascularization

Introduction

The effects of smoking on taste (Fischer et al. 1983; Nin et al. 2006) and olfaction have been studied extensively. However, few studies have provided morphological data about the effects of cigarette smoking on the size and shape of the tongue papillae. Nicotine is a component of tobacco products and a gustatory stimulus. It elicits responses in both glossopharyngeal and chorda tympani (CT) taste nerves (Dahl et al. 1997) and in neurons of the nucleus of the solitary tract (Lemon and Smith 2005). Nicotine also elicited responses in single gustatory neurons from the insular cortex in monkeys (Scott et al. 1999). Nicotine is perceived as predominantly bitter by humans but its effects on other taste qualities are presently unknown. Data suggest that it could be a potential modulator of both salty and sour taste (Lemon and Smith 2005). In summary, the mechanisms underlying the detection, discrimination, and neural coding of the taste of nicotine remain unclear.

The aim of this study was to investigate if there is a difference in electrogustometry (EGM) thresholds recorded at the anterior and
posterior tongue and soft palate after smoking cessation. An additional aim was to test, if any eventually observed difference in EGM thresholds at the anterior tongue between smokers and non-smokers may be associated with any difference in density and/or morphology of fungiform papillae at that anatomical site.

**Patients and methods**

Twenty-four smokers (19 males and 5 females; age range 20–80 years; median age: 54.6 ± 2.9 years) participated in the study. Criteria for exclusion from the study were a positive history for disorders affecting gustatory function, a history of diabetes mellitus, use of angiotensin-converting enzyme (ACE) inhibitors such as captopril or ramipril, prior otological operations and neurological disorders (including facial palsy). The EGM thresholds of the smokers were compared to those of 24 randomized chosen non-smoking participants (19 males, 5 females; median age: 55.2 ± 3.4 years). The individuals of the 2 groups had therefore a very similar age and gender distribution. Volunteers participated in the study after they had been informed of its background and purpose and after they had provided written consent. The study protocol was reviewed and approved by the Institutional Review Board of The Aristotle University of Thessaloniki, Greece and was in accordance with the principles of the Helsinki Declaration. To minimize variability resulting from the examination technique, all examinations were carried out by the same examiner (PP). Four male smokers reported experiencing bad taste sensation without any respective gustatory stimulus (phantogeusia) or reported reduced taste sensation (hypogeusia) before testing. All smokers reported that they kept their cigarette usually in the midline.

**EGM testing**

Taste acuity was evaluated with EGM. Electrical stimuli were delivered with an electrogustometer (TR-06, Rion Co, Tokyo, Japan) with a single, flat, circular stainless steel stimulus probe (5 mm in diameter). The device produces low-amplitude stimuli of pre-determined duration (0.5, 1, 1.5 and 2 s). A feedback circuit controls the output current with an error quote of less than 1% (Pavlidis et al. 2013). All subjects were instructed not to smoke, drink, or eat for 1 h before beginning the testing session. First, a 30-dB stimulus was administered to test whether the subject was in a position to recognize electrogustometric stimuli. Stimulation started at the lowest stimulus amplitude (−6 dB) and increasingly stronger stimuli were presented until the subject recognized the stimulus. If the threshold for stimulus perception was not clearly determined, the next higher and lower strength stimuli were presented to the individual. The electric threshold scores were measured at 6 locations, namely paramedially on both sides of the tongue apex (each 2 cm away from the tip) at an area innervated by the CT, at the area of the vallate papillae on both sides of the tongue (innervated by the glossopharyngeal nerve) and at the soft palate (area innervated by the major petrosal nerve) bilaterally. In healthy subjects, electric gustatory thresholds for the tongue apex, vallate papillae and soft palate are generally set at levels up to 8, 14, and 22 dB, respectively. A 500-ms electric stimulus was applied (blinded test) as done in other studies (Loucks and Doty 2004). A 2-alternative forced-choice initially ascending single-staircase detection protocol was applied using a 2-down, one-up rule (Loucks and Doty 2004). The trial sequence was begun at the 8-mA current level, as in previous studies (Loucks and Doty 2004). If subjects missed a trial before reaching this criterion, the subsequent trial was presented at the next higher stimulus level. The latter process was continued until 5 consecutive correct trials occurred at a given current level. At this point, the subsequent trial was presented at the next lower stimulus level. If the first or the second 2 successive correct trials was missed at this stimulus level, the subsequent trial was presented at the next higher level, representing a reversal in the staircase. If 2 successive correct trials occurred at this level, the following trial was given at the next lower stimulus level.

**Contact endoscopy**

Contact endoscopic (CE) imaging was performed using a 30° contact endoscope (magnification × 60 and × 150; Karl Storz, Tuttingingen, Germany). Identification of fPap was first performed using a non-contact technique. Subjects were instructed to rinse their mouth with water before contact endoscopy. A CE technique was first used without staining for imaging of subepithelial vessels. After careful suctioning of the saliva, methylene blue 1% solution was used to stain epithelia and taste pores. A filter paper strip delineating an area of 1 cm² was placed in a paramedian position on the tongue tip as proposed by Negoro et al. (2004). To address the problem of continuous movement of the tongue during CE and to avoid venous congestion and hyperemia which could eventually confound CE findings, volunteers were asked to keep the tongue in a fixed position as much as possible and were advised to bite gently the tip of their tongue between their upper and lower teeth. Moreover, subjects were asked to seat in the examination chair with their head and neck supported by a pillow. Examination time by means of CE was about 30 s. No local anesthesia was necessary. A cold light source was used to minimize any heat produced at the tip of the endoscope. No changes (increase or decrease) in vascularity due to application of the endoscope on the mucosa have been observed during examination by CE.

The form of the fungiform papillae was classified to 1 of 4 types according to the following classification paradigm introduced by Negoro et al. (2004): Type 1 (egg-shaped or long ellipse type—without surface thickness), Type 2 (slight thicker surface compared to type 1), Type 3 (thick and irregular surface), and Type 4 (remarkably flat and atrophic surface). Type 1 corresponds to the healthier state and type 4 to the most pathological state. It should be noted that mushroom-shaped papillae with honey tips were counted as filiform (and not as fungiform) papillae. Due to their very light staining, fungiform papillae could be readily distinguished from filiform papillae, which stained dark (Just et al. 2006).

For evaluation of the density of fPap (number of papillae per cm²), the CE image with the highest fPap density taken from each individual was used.

The classification of the blood vessels’ morphology at the tongue apex was also performed according to Negoro et al. (2004): Type A (clear loop and wooden branch shape), Type B (unclear loop and wooden branch shape), Type C (elongated blood vessels), Type D (granular shape or dotted shape), and Type E (unclear blood vessels).
Type A represents the “healthiest” morphology and Type E corresponds to the “most pathological” morphology.

All volunteers completed the study. The findings, and particularly the CE images and the classification of the fPap, were double-checked by 2 investigators (F.P. and G.K.) to achieve consensus and minimize any possible mistakes.

Statistical analysis
The null hypothesis was that there was no statistical difference in EGM thresholds before and after quitting smoking. For statistical analysis’ computational purposes, if an EGM-threshold could not be measured at all, then it was assigned a numerical value of 36 dB. In order to examine if the numerical values of the measurement results were normally distributed, a quantile-quantile plot (QQ plot) was applied for experimental and control-subjects. This QQ plot analysis resulted in that the distribution of the numerical values was not normal for both experiments and controls. Therefore, non-parametric tests were applied. The level of statistical significance was set at $P < 0.05$. On each occasion, EGM-thresholds between 2 groups were compared using Kruskal–Wallis and Mann–Whitney tests. The following comparisons of EGM thresholds were made: between smokers before quitting smoking and controls, between smokers before and after quitting smoking, and between smokers after quitting smoking and controls. This provides useful data about the improvement or not of the EGM threshold before and after quitting smoking.

The Bonferroni correction was used as necessary. Tukey’s multiple comparison test was used to detect differences significant at the 0.05 level in mean thresholds for the various age categories. For analysis of the regression between age, form and vascularisation of fPap, the Kendall rank correlation coefficient was applied. The null hypothesis was that the 2 variables examined on each occasion were independent and that there was no difference in the findings before and after quitting smoking. The results were analyzed using SPSS software (Version 12 for Windows, SPSS Inc.).

Results
EGM thresholds
After quitting smoking, EGM thresholds decreased significantly. The measurements took place 2–35 weeks (mean: 3.2 months) after cessation. Statistical analysis showed significantly higher EGM thresholds in smokers before quitting smoking compared to non-smokers (thresholds GP-R: $P = 0.02$, threshold GL-R: $P = 0.01$, threshold CT-R: $P = 0.02$, threshold CT-L: $P = 0.02$, threshold GL-L: $P = 0.03$, threshold GP-L: $P = 0.04$) and significantly higher EGM thresholds in smokers before and after quitting smoking, and between smokers after quitting smoking and controls. This provides useful data about the improvement or not of the EGM threshold before and after quitting smoking.

The Bonferroni correction was used as necessary. Tukey’s multiple comparison test was used to detect differences significant at the 0.05 level in mean thresholds for the various age categories. For analysis of the regression between age, form and vascularisation of fPap, the Kendall rank correlation coefficient was applied. The null hypothesis was that the 2 variables examined on each occasion were independent and that there was no difference in the findings before and after quitting smoking. The results were analyzed using SPSS software (Version 12 for Windows, SPSS Inc.).

Correlation between EGM thresholds and fungiform papillae structure
Calculation of the Kendall rank correlation coefficient disclosed a strong relationship between EGM thresholds and vascularisation of the tip of the tongue in both females and males, EGM thresholds and vascular shape at the tip of the tongue and EGM thresholds and density of fPap. A definite stronger correlation with increasing age has been observed.

There was a rather strong correlation by application of Kendall’s tau ($\tau$) between EGM thresholds and vascularization ($\tau = 0.67$), EGM thresholds and shape ($\tau = 0.77$) and EGM thresholds and density of fPap ($\tau = 0.73$) in smokers before quitting smoking. The correlation was clearly lower after smoking cessation by application of Kendall’s tau ($\tau$) between EGM thresholds and vascularization ($\tau = 0.22$), EGM thresholds and shape ($\tau = 0.36$) and EGM thresholds and density of fPap ($\tau = 0.24$) in smokers after smoking cessation. All results are depicted in Table 3. In the same table, the reader can find statistical differences between the EGM thresholds of smokers before and after quitting smoking, as well between EGM thresholds of smokers before quitting smoking and non-smokers.

Discussion
Techniques used in this study may be easily implemented in both an inpatient and outpatient clinical setting. EGM can be used in the clinical routine for a variety of clinical purposes, including the improvement of taste acuity in patients suffering from various associated diseases. EGM has the advantage that the range of measurement can remain constant and always valid, that the control of stimuli (results measured in dB) is quantitative and that the period required for testing is short. Examination by means of CE has the advantage of the rapid assessment of the shape and vascularization of fPap. Moreover, from a patient’s point of view, the CE procedure is simple and the gag-reflex or other complaints which could lead to the interruption of the evaluation are avoided. Electrogustometric

Table 1. The table presents the EGM threshold (in dB) before and after quitting smoking. The sites have been chosen to evaluate the function of the chorda tympani (thresholds CT-R and CT-L), glossopharyngeal (thresholds GL-R and GL-L) and great petrosal nerves (thresholds GP-R and GP-L).

<table>
<thead>
<tr>
<th>EGM threshold</th>
<th>GP-R</th>
<th>GL-R</th>
<th>CT-R</th>
<th>CT-L</th>
<th>GL-L</th>
<th>GP-L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before</td>
<td>30.75</td>
<td>26.1</td>
<td>15.8</td>
<td>16.6</td>
<td>25.5</td>
<td>30.8</td>
</tr>
<tr>
<td>After</td>
<td>25.3</td>
<td>22.8</td>
<td>13.3</td>
<td>14.8</td>
<td>22.5</td>
<td>28.3</td>
</tr>
</tbody>
</table>

The direction is from the right (GP-R) to the left side (GP-L).
threshold assessment has good test–retest reliability. This reliability may be enhanced by the combined use of other methods such as CE. Indeed, changes in the morphology of papillary structures among smokers and non-smokers were observed with CE.

Nicotine is the main addictive component of tobacco products, mainly because of its effects on the central nervous system (CNS) (Hatsukami et al. 2008). However, the peripheral sensory impact of inhaled nicotine is also relevant in the regulation of cigarette smoking (Pritchard et al. 1996; Rose et al. 1993). Although oral somatosensory responses to nicotine occur only at relatively high concentrations (Thuerauf et al. 2006), taste responses (Iwasaki and Sato 1981) have been described at nicotine concentrations close to those found in the saliva of smokers (Rose et al. 1993). Several studies suggest that the sense of taste is relevant in the context of cigarette smoking. For instance, the ability to sense the bitter taste of phenylthiocarbamide, a trait that is genetically determined by polymorphisms of a taste receptor gene (T2R38) (Rose et al. 1993), protects from the development of addiction to cigarette smoking (Carstens et al. 1998; Vennemann et al. 2008) and reduces the positive reinforcement from smoking (Naqvi et al. 2007).

Nicotine has been associated in the past with alteration of gustatory function under conditions of chronic exposure. It has been found that EGM thresholds increase with advancing age, starting at the age of 60 years, in the areas innervated by the CT and glossohypoglossal nerves and starting at about the age of 70 years, in the areas innervated by the greater petrosal nerve (Nin et al. 2006). In a previous study, the differences in EGM thresholds, in shape and vascularization of fPap between smokers and non-smokers were illustrated (Pavlidis et al. 2009, 2013). The previous study shows the differences in EGM thresholds in smokers before and after quitting smoking. Though there is a decrease in the EGM thresholds, the shape and vascularization of fPap seem to be long-term affected by nicotine.

Nicotine and other bitter tastants have been shown to activate transducin in vitro but the participation of this taste-related G protein for bitter taste recognition in vivo has not been demonstrated (Iwasaki and Sato 1981). Additionally, a variety of results have been obtained from central and peripheral gustatory neurons regarding whether nicotine can be discriminated from other bitter tastants (Dahl et al. 1997). Thus, it is unclear whether taste responses to nicotine depend on pathways common to other bitter tastants. In particular, it is unclear if gustatory responses to nicotine depend on TRPM5, a member of the transient receptor potential (TRP) superfamily of channels (Venkatachalam and Montell 2007). TRPM5 is expressed in taste receptor cells (TRCs) and is required for peripheral transduction of bitter, sweet, and umami tastants (Zhang et al. 2003). Furthermore, this channel has been shown to participate in chemosensory detection of nicotine in the nasal cavity. Previous work has also suggested the participation of nicotinic acetylcholine receptors (nAChR) (Schiffman 2000; Simons et al. 2006) in the detection of nicotine by the peripheral taste system. The role of nAChRs in taste responses to nicotine is particularly intriguing because mcamylamine, a broad spectrum nAChR antagonist that has been used as a smoking cessation aid (Shytle et al. 2002) reduces the sensitivity to peripheral sensory stimulation by cigarette smoke. However, hexamethonium (Schiffman 2000), a different nAChR antagonist, inhibits taste responses to nicotine as well as to other tastants, suggesting that the inhibition resulting from nAChR antagonism may be unspecific.

The exact mechanisms that regulate this continuous cell renewal are still not clear, although it has been established that the brain-derived neurotrophic factor (BDNF), neurotrophin-4 and (NT-4) and a functional innervation are crucial for taste bud development and renewal (Patel and Krimm 2010). Recent studies in humans (Patel and Krimm 2010; Umene-Nakano et al. 2010) showed low BDNF serum levels in smokers compared to nonsmokers.
The use of CE provided the advantage to study the vascularization of the tongue tip and the shape of the fPap. Results of this study suggest that these 2 parameters are significantly associated with taste acuity as assessed by EGM. Nonetheless, it should be stressed again that not all fPap contain taste buds (and therefore not all fPap produce taste sensation) and that there are variations in sensitivity of fPap to chemical stimuli (Just et al. 2006).

Despite the significant differences in the shape and the vascularisation of the fPap, there were no differences found concerning the density of papillae between the 2 measurements in the smokers, before and after cessation of smoking. It has been suggested that long-term exposure of taste buds to nicotine leads to significant reduction in their size (Tomassini et al. 2007). However, changes in shape and size of the papillae are not necessarily accompanied by any simultaneous significant change in their number (Tomassini et al. 2007). An interesting finding which agrees with the above studies is the correlation between EGM thresholds and the shape and density of fPap which is strong in all age groups of both sexes. Although there is a correlation between EGM-thresholds and vascularisation, this tends to be weaker.

The lack of histological data in this study does not allow the attribution of the findings to any possible degeneration of the nerve fibers due to nicotine. Gustatory sensitivity correlates positively with the density of fPap (Pavlidis et al. 2013). Results from the analysis of the CE images suggest that even a small decrease in the density of the smokers’ papillae, compared to the non-smokers, can lead to increased EGM thresholds.

Therefore, it is important to identify specific factors that account for the variability in initial nicotine sensitivity in humans. Such factors could increase our understanding of the etiology of dependence, as well as supporting the efforts of prevention of tobacco use, especially in teenagers. One important individual difference may be due to gender. On measures of reward and reinforcement, women may be less responsive than men to manipulations of nicotine exposure, while women may be more responsive than men to manipulations of non-nicotine components of cigarette smoking, such as cues (Perkins and Scott 2008). However, because virtually all of this research has been conducted with dependent addicted smokers, it is not clear whether the sex difference in sensitivity to nicotine results from chronic exposure over years of regular smoking or may be present from the beginning of experimentation with smoking in teens.

This study supports the use of the combination of EGM and CE methods for the study of taste disorders in smokers. Both techniques provide useful clinical data in a short time frame and exhibit good test–retest reliability.

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Conflict of interest statement

We confirm that there are no financial contributions to the work and no potential conflicts of interest.

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