**Topological electrogustometry and chemogustometry surrogate markers of age-related gustatory decline in humans**

**Alterations of taste acquity in relation with sex and age, detected with electro- and chemogustometry.**

Names of authors removed for blind peer review

**Abstract**

**Objectives:** The primary goal was to evaluate the effect of stimulus-duration on Electrogustometry (EGM) Thrasholds, to evaluate any gender-related influences and compare the above results to those after application of Taste-Strips.

**Design:** Electrogustometry (EGM) thresholds of various stimulus duration (0.5, 1.0, 1.5, and 2.0 s) were measured in 212 non-smokers (age range: 10 – 80 years, divided into 8 age groups) without self-reported gustatory impairment. Furthermore, taste strips chemogustometry measurements in 132 participants were performed.

**Setting:** Tertiary referral medical centre.

**Participants:** 212 non-smokers, divided in 8 age-groups participated in the study.

**Main outcome measures:** EGM-Thresholds and taste-strips, duration of stimuli

**Results:** EGM-thresholds increased progressively with age and with increase in stimulus duration from 0.5 sec up to 2 sec. This pattern was consistent at all 6 anatomic areas, irrespective from gender. In contrast, in chemogustometry no differences related either to age or to gender were found.

**Conclusions:** Age-related electrophysiological and functional gustatory decline can be better documented by EGM than using chemogustometry. This superiority of EGM was not influenced by stimulus duration; nonetheless, stimulus duration should be clearly documented in future quantitative EGM-threshold recordings, given that it may significantly influence EGM amplitude threshold measurements.

**Keywords:** stimulus, electrogustometry, threshold, chemogustometry, taste strips, age, sex

1 **INTRODUCTION**

Electrogustometry (EGM), in its usual clinical applications, consists of the presentation of microampere level anodal currents to taste bud fields, typically producing sour, metallic, or composite sour/metallic sensations likely attributable to proton shift 1. Two hypotheses dominate the views regarding the mechanisms of electrical taste sensation. According to the electrolyte–chemical hypothesis, the anode creates an acid solution by electrolysis, and the proton surplus then stimulates the sour-specific taste cells. Based on the direct-effect hypothesis, electrical taste emerges by action of the current on either nerve fibers or taste receptor cells2.

The influences of several stimulus parameters, including duration, area of stimulation, and intermittency of stimulation on EGM thresholds, are poorly studied3. In particular, there is a need to further study the effect of stimulus duration on the recorded EGM-thresholds.

In most patients, the self-reported chemosensory function does not correlate reliably with psychophysical test results1,3. Moreover, many patients confuse taste and flavor perception, making it necessary to perform both smell and taste examinations. Quantitative assessment of gustatory function with taste strips is an established method for the evaluation of taste function in routine clinical evaluation and research3,4.

**The main purpose of our study was to further investigate the impact of EGM stimulus duration on the well-known phenomenon of age-related EGM-threshold deterioration in humans. Additionally, another major aim of this study was to compare the ability of EGM and chemogustometry (CGM, taste strips) methods to capture age-related gustatory functional changes.** The influence of stimulus duration (varying between values of 0.5, 1, 1.5, and 2 s) on EGM-thresholds has been measured at 6 different anatomic regions. The null hypothesis was that there is no effect of stimulus duration on the recorded EGM-thresholds or any relationship between stimulus duration and age, gender or position of the tongue.

**2 MATERIALS AND METHODS**

# Two hundred and twelve non-smoking individuals participated in the study (n=212, males=120, females n=92). For study purposes, the subjects were divided in age-groups, as proposed in previous studies4 (males: age-group 10-14 yrs 3, 15-19 yrs 4, 20-29 yrs 7, 30-39 yrs 15, 40-49 yrs 13, 50-59 yrs 15, 60-69 yrs 16, >70 yrs 3/females: age-group 10-14 yrs 3, 15-19 yrs 4, 20-29 yrs 10, 30-39 yrs 6, 40-49 yrs 9, 50-59 yrs 12, 60-69 yrs 6, >70 yrs 2). Exclusion criteria were a positive history for disorders affecting gustatory function, a history of diabetes mellitus, use of antihypertensive drugs (such as captopril and ramipril), prior otological operations and neurological disorders including facial palsy4,5,6. Volunteers participated in the study after their written consent was obtained. The protocol was reviewed and approved by the Institutional Review Board of [Blinded for review] and was in accordance with the principles of the Helsinki Declaration. To minimize variability in the examination technique, all examinations were carried out by the same examiner (PP).

**2.1 Electrogustometry**

Taste acuity was evaluated with EGM. Electrical stimuli were delivered with an electrogustometer (TR-06, Rion Co, Tokio, 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 seconds). A feedback circuit controls the output current with an error quote of less than 1% [16,17]. All subjects were instructed not to smoke, drink, or eat for one hour before beginning the testing session. First, a 30 dB-stimulus was administered to test whether the subject was capable of recognizing electrogustometric stimuli. Stimulation started at the lowest stimulus amplitude (- 6 dB), and increasingly stronger stimuli were presented until the subject identified 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 six locations, namely at the area of the vallate papillae on both sides of the tongue,defined as area A (right side) and F (left side), innervated by the glossopharyngeal nerve, para-medially on both sides of the tongue apex, defined as area C (right side) and D (left side), each 2 cm away from the tip, at an area innervated by the chorda tympani and at the soft palate, defined as area B (right side) and E (left side), 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 dB, 14 dB, and 22 dB respectively [11]. A 500-ms electric stimulus was applied, beginning at -6 dB and increasing up to +34 dB (3-400 μΑ) in 2 dB-steps. Thresholds were measured in a randomized fashion (the choice of measurement sites, one out of six, did not follow a standardized, predefined pattern) on both sides of the tongue to avoid any possible bias.

All six areas were tested applying the same stimulus duration before proceeding using different stimulus duration. This procedure resulted in a 3 – 4 minutes’ stimulus interval between the applications of stimuli of varying duration, thereby decreasing the possibility of the emergence of stimulus adaptation. The participants had been instructed to discriminate between a wide range of sensations experienced by electrical stimulation and the perception of an electrical feeling (suggesting trigeminal stimulation). The subjects answered “yes” or “no”, if they perceived any taste sensation. Subjects were kept unaware of whether or not any electrical stimulus was applied (blinded test) as done in other studies2. A two-alternative forced-choice initially ascending single-staircase detection protocol was applied using a two-down, one-up rule6. The trial sequence was begun at the 8-mA current level, as in previous studies. 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 five consecutive correct tests 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 two 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 two successive correct tests occurred at this level, the following examination was given at the next lower stimulus level. For statistical analysis, if an EGM-threshold could not be measured at all, then it was assigned a numerical value of 36 dB. In each patient, we first conducted the electrogustometry, and 15 minutes later followed the examination with taste strips.

**2.2 Taste strips**

The participants (n= 132) did not drink or eat anything except water for at least one hour before testing. The extended test consisted of 16 taste strips (and two blanks without taste) that were presented to the participants in a pseudo-randomized sequence. Testing started with the lowest concentrations to minimize adaptation and habituation processes. The strips were placed in the middle of the anterior part of the tongue and the mouth was closed (whole-mouth testing). The strips were kept for 1 minute in the mouth and they were also moved around. The subjects were asked to suck on the strips and respond according to a list of five possible answers (sweet, sour, salty, bitter, no taste). After the presentation of each taste strip, the mouth was rinsed with tap water7,8,9.

**2.3 Statistical analysis**

The null hypothesis was that there was no statistical difference in EGM-thresholds between the EGM-Thresholds after the application of stimuli of different duration (0.5, 1, 1.5, and 2 s). For statistical analysis, if an EGM-threshold could not be measured at all, then it was assigned a numerical value of +36 dB. To examine if our results were normally distributed, we applied a quantile-quantile plot (QQ plot) and non-parametric tests were applied where necessary. The level of statistical significance was set at p < 0.05. On each occasion, the EGM-thresholds between two groups were compared using Kruskal-Wallis and Mann-Whitney tests. 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.

To describe the effect size measure, we applied Cohen's d-Test (effect size measures for two independent groups) for the results of taste strips and EGM-thresholds. To examine correlations between CGM taste strip concentration and EGM stimulation threshold data with spearman's rho rank correlations have been analyzed. We defined correlations of near ± 1 as “perfect”, as “strong” if the correlation’s coefficient value (r) lied between ± 0.50 and ± 1. Correlations have been defined as “moderate” if the r lied between ± 0.30 and ± 0.49 and as “weak” if r was below 0.29.

**3 RESULTS**

**3.1 Age-related decline in EGM and CGM surrogate parameters**

EGM-thresholds increased with increasing age. As example, figures 1 and 2 depict changes of EGM-thresholds in the group of 20-29-year olds for area C (right side of tongue-tip) in males and females respectively. Comparisons among the EGM-thresholds in dB after the application of the 4 different stimulus durations have shown variable differences. The figures 3-5 show the average EGM-thresholds in both sex groups after 0.5 sec, 1, 1.5 and 2 sec-stimulation at the respective locations A, B and C. A significant deterioration (i.e. higher EGM-thresholds) with increasing age has been noticed.

EGM-thresholds (recorded in decibels, dB) at the tongue area innervated by the chorda tympani (Rapex and Lapex, areas C and D) were significantly higher in both males and females, of the age-group >70 years (p=0.036 for males and p=0.038 for females) years of age than in any of the younger age groups. In the soft-palatine area in males, the thresholds were significantly higher in subjects aged 20-29 years and ≥60 years than in the other age-groups.

Using non-parametric tests, a statistically significant difference between the EGM-thresholds recorded after 0.5 sec- and 1.0 sec-stimulation (p=0.03 and 0.04) has been found. The results were the same for both sexes. Similar results have been recorded between the EGM-thresholds after 1.0 sec and 1.5 sec-stimulation (p=0.02) as well as 1.5 sec- and 2 sec-stimulation (p=0.01). The comparison between the EGM-thresholds after 0.5 sec– and 2 sec-stimulation showed a difference of p<0.05.

**3.2 CGM age-related changes**

CGM testing with taste strips did not show any differences among the different age – and sex-groups. For all 4 concentrations of sweet-stimuli (0.4 g/ml, 0.2 g/ml, 0.1 g/ml and 0.05 g/ml) we did not find statistical differences between the sex groups of the same age (p ≥ 0.4 in every comparison). We have also found no correlation between parameters such as gender and age, gender and concentration or concentration and age (r between 0.0 and 0.15). Same findings have been recorded for the sour-stimuli in all concentrations (0,3g/ml, 0,165 g/ml, 0,09 g/ml and 0,05g/ml), for the bitter ones (1g/ml, 0.0024 g/ml, 0.0009g/ml, 0.0004 g/ml) and the four salt taste-strips (0.25 g/ml, 0.1g/ml, 0.04g/ml and 0.016g/ml). Furthermore, we have found moderate correlations between the EGM-τhresholds and CGM-τhresholds only for the highest concentrations of each stimulus for sweet, bitter and salty taste strips and EGM-thresholds for all 6 areas (r≈0.400, p=<0.001). For the sour taste a weak, not age-related, correlation could be found only with the highest stimulus concentration and stimulation threshold for all 6 areas (r≈0.250, p=<0.001).

Gender-specific alteration of surrogate parameters of EGM and CGM with increasing age are depicted on figures 3-5. Figures **3, 4 ,5 and 6** depict the distribution of thresholds at Locus C and the proportionate parts of participants’ positive sweet taste strip sensation with rising age for male and female participants respectively. The correlation coefficientsfor EGM-thresholds and for positive “sweet” taste strip with age are 0.290 and 0,001 for males and 0,220 and 0,002 for females respectively.

**3.3 Correlations between EGM- and CGM-thresholds**

Furthermore, the performed correlations between the EGM-thresholds and CGM-Thresholds did show moderate, not age-related, correlations for the highest concentrations of each stimulus for sweet, bitter and salty taste strips and EGM-thresholds for all 6 areas (r≈0.400, p=<0.001) (**Table 1**)., For the sour taste a weak, not age-related, correlation could be found with the highest stimulus concentration and stimulation threshold for all 6 areas (r≈0.250, p=<0.001).

**4 DISCUSSION**

The present study supports the hypothesis that **gustatory function, as evaluated by electrogustometry, declines with increasing age in humans. Additionally,** the relationship between lingual threshold sensitivity to low levels of electric current with stimulus duration ranging from 0.5 to 2 sec is non–monotonic. Age-related electrophysiological and functional gustatory decline can be better documented by EGM than using chemogustometry.

Only taste fibers are stimulated at currents less than approximately 300 μA. Higher current levels may stimulate pain or temperature fibers directly, causing a tingling or burning sensation which is distinct from the sour taste resulting from anodal stimulation or the bitter/soapy taste resulting from cathodal stimulation 10,11.

Electrogustometry-thresholds have been shown to increase with age12,13, although not in a uniform linear way. This finding is in agreement with previous reports6,8 according to which in the 11-15 years-of-age group (regardless of sex), the highest electric taste sensation is recorded at the areas innervated by the chorda tympani at the anterior part of the tongue. Up to 40 years of age, the threshold value curve takes a mild slope14,15. As a rule, no difference between sexes in threshold detection exists. The finding that EGM-thresholds increase non-uniformly (and certainly non-linearly) with age16,17, agrees, at least in part, with results reported by Coates (1974) who measured EGM-thresholds at the tip of the tongue in subjects with apparently normal gustatory function and found that EGM-thresholds tend to increase with age15, although differences in thresholds between young and older subjects had not been statistically significant. Krarup (1958) found that EGM-thresholds increase with age in a linear way16. We should mention a difference between our results and those of a previous study9. In our study we have found that the EGM-thresholds form a sigmoid curve in relation with EGM-thresholds recorded after 0.5 and 1.5 sec stimuli do not show any statistic significant differences between them. In addition, the EGM-thresholds recorded after 2 sec-stimuli show a significant difference compared to 0.5 sec-, 1.0 and 1.5 sec-stimuli. Loucs and Doty3 reported a non-monotonic function for the threshold values observed for both tongue regions across the stimulus durations, with the 1.0-s duration stimulus resulting in a lower threshold value (i.e., higher sensitivity) than either the 0.5- or 1.5-s durations, which did not differ in magnitude from one another.

Different tests of the gustatory function using a variety of techniques were introduced over the past few decades18,19,20. Assessment of gustatory function with impregnated taste strips is an easy, cost-effective, and reliable method2,19,20. Moreover, the process allows testing of specific areas on the tongue (e.g. for detection of chorda tympani nerve injury in middle-ear surgery or glossopharyngeal nerve injury after tonsillectomy) and has a long shelf life. While some authors prefer taste tests based on the forced-choice paradigm, others prefer non-forced choice procedures. A significant advantage of the forced-choice test is the avoidance of the subject’s response bias1. The advantage of non-forced choice models is the possibility to distinguish between the perception of tastes and correct identification of tastes. **Notably, we did not observe any similar changes in sensitivity by the application of CGM (in form of taste-strips) in the present study.**

Consequently, the taste strips-test was validated as a non-forced-choice paradigm21. Fungiform papillae density can be used as an indicator of taste function22,23 . Of interest, the results of taste-strips (chemogustometry) did not correlate with age in our study. Based on our findings, we suggest that although chemogustometry is highly reproducible, it may provide only qualitative results.

The role of physiologic adaptation to the applied EGM stimulus deserves further study. As reported above, EGM-thresholds after 2 sec-stimulation are the highest. It has been suggested that the frequency of the afferent neural discharge shows an initial decrement followed by a constant level when a sense organ is subjected to a stable continuous stimulus6,23,24 . The above could be a reasonable explanation for these results.

The observed differences in EGM-thresholds between the two sides of the tongue22,25may also need to be further investigated. In the present study, a tendency for lower EGM-thresholds at the right side of the tongue compared to the left one has been recorded, with only few exceptions in various age-groups.

**5 CONCLUSIONS**

In conclusion, we suggest that electrogustometry (EGM) has several advantages and a few limitations in clinical use. The advantages of EGM are: (i) the values of measurements can be kept constant, there is no changes in different evaluations (ii) quantitative control of the intensity of the stimulation is possible (iii) only a short period of time is required for testing; (iv) it is possible to detect even fine, subclinical taste disorders for which the patient has no subjective symptoms; (v) it is useful for topological diagnosis of lesions of taste pathways and track the evolution of these lesion over time; and (vi) it is a relative accurate quantitative method for diagnosing disorders of the glossopharyngeal nerve. In contrast, chemogustometry in the form of taste-strips, is a qualitative method. Interestingly, we did not find a change in chemogustometric measurements correlated with parameters such as age or sex. The contrasts findings of previous studies10. We suggest that age has an impact on taste acuity, but this impact may be detected only with the use of more quantitative test methods, such as electrogustometry.

**FIGURES AND TABLES**

**TABLE 1.** Correlations between EGM-Thresholds and CGM-Thresholds showing moderate correlations for sweet, bitter and salty taste strips as well as weak for sour taste strips for the highest concentrations of each stimulus and stimulation thresholds for all 6 areas.

**FIGURE1.** EGM-thresholds for area C (right side of tongue-tip) in males in age-groups 20-29, 30-39 and 40-49.

**FIGURE** **2**: EGM-thresholds for area C (right side of tongue-tip) in males in age-groups 20-29, 30-39 and 40-49.

**FIGURE 3.** EGM-thresholds after0.5 sec, 1, 1.5 and 2 sec-stimulation at the right vallate papillae region. Please notice the age-related deterioration; older age-groups show greater thresholds.

**FIGURE 4.** EGM-thresholds after0.5 sec, 1, 1.5 and 2 sec-stimulation at the right soft palate. Please notice the age-related deterioration; older age-groups show greater thresholds.

**FIGURE 5.** EGM-thresholds after0.5 sec, 1, 1.5 and 2 sec-stimulation at the right side of tongue-tip. Please notice the age-related deterioration; older age-groups show greater thresholds.

**FIGURE 6.** Distribution of thresholds at the right side of tongue-tip for proportionate part of participants’ positive sweet taste strip sensation in regard to rising age for males. R² for thresholds and for positive sweet taste strip with age are 0.290 and 0,001 for males and 0,220 and 0,002 for females respectively.

**References**

1. Bartoshuk L. Clinical evaluation of the sense of taste. [Ear Nose Throat J.](https://www.ncbi.nlm.nih.gov/pubmed/?term=Clinical+evaluation+of+the+sense+of+taste.+Ear+Nose+Throat+J+68%3A331-317.) 1989; 68:331-337.
2. Boucher Y, Berteretche MV, Fahrang F, et al. Taste deficits related to dental deafferentation: an electrogustometric study in humans. 2006; 114:456-464.3
3. Loucks CA, Doty RL. Effects of stimulation duration on electrogustometric thresholds. 2004; 81(1):1-4.
4. Negoro A, Umemoto M, Fukazawa K, et al. Observation of tongue papillae by video microscopy and contact endoscopy to investigate their correlation with taste function. Auris Nasus Larynx. 2004; 31(3):255-259.
5. Pavlidis P, Cámara RJA, Kekes G, Gouveris H. Bilateral taste disorders in patients with Ramsay Hunt syndrome and Bell palsy. Ann Neurol 2018; 83(4):807-815.
6. Kikuchi T, Kusakari J, Kawase T, et al.Electrogustometry of the soft palate as a topographic diagnostic method for facial paralysis. Acta Otolaryngol Suppl. 1988; 24(12); 458:134-138.
7. Nakazato M, Endo S, Yoshimura I,et al. Influence of Aging on Electrogustometry Thresholds. Acta Otolaryngol Suppl. 2002; 122(4): 16-26.
8. Ohla K, Hudry J, le Coutre J. The cortical chronometry of electrogustatory event-related potentials. Brain Topogr 2009; 22(2):73-82.
9. Pavlidis P., Gouveris H., Anogeianaki A., et al. Age-Related Changes in Electrogustometry Thresholds, Tongue Tip Vascularization, Density, and Form of the Fungiform Papipllae in Humans. Chem Senses 2013; 38(1):35-43.
10. Miller SL, Mirza N, Doty R. Electrogustometric thresholds: Relationship to anterior tongue locus, area of stimulation and number of fungiform papillae. Physiol Behav 2002(5); 75:753-757.
11. Tomita H., Ikeda M. Clinical use of electrogustometry: strengths and limitations. Acta Otolaryngol Suppl 2002(1); 546: 27-38.
12. Nakazato M, Endo S, Yoshimura I, Tomita H. Influence of Aging on Electrogustometry Thresholds. Acta Otolaryngol Suppl. 2002;(546):16-26.
13. Boucher Y, Berteretche MV, Fahrang F, Arvy MP, Azerad J, Faurion A. Taste deficits related to dental deafferentation: an electrogustometric study in humans. Eur J Oral Sci. 2006;114(6):456-64.
14. Doty RL, Heidt JM, Mac Gillivray MR, et al. (2016). Influences of age, tongue region, and chorda tympani nerve sectioning on signal detection measures of lingual taste sensitivity. 2016(6);155:202-207.
15. Coats AC. Effects of age, sex and smoking on electrical taste threshold. Ann Otol Rhinol Laryngol 1974 ;83(3):365-369.
16. Krarup B. Electrogustometry: a method for clinical taste examinations. Acta Otolaryngol (Stockh) 1958 (4); 49:294-305.
17. Pavlidis P, Gouveris H, Kekes G, et al. Electrogustometry thresholds, tongue tip vascularization, and density and morphology of the fungiform papillae in diabetes. B-ENT.2014; 10 (4):271-278.
18. Fǿns M. Electrogustometry III: Adaptation in electrical taste. J Laryngol Otol. 1969; 83(10):973-980.
19. Braun T, Mack B, Kramer MFR.. Solitary chemosensory cells in the respiratory and vomeronasal epithelium of the human nose: a pilot study. Rhinology 2011; 49(5):507-512.
20. Reed DR. Birth of a New Breed of Supertaster. Chemical Senses2008;33(6):489-491.
21. Landis BN, Welge-Luessen A, Brämerson A, et al. “ Taste Strips”- a rapid, lateralized, gustatory bedside identification test based on impregnated filter papers. J Neurol 2009, 256 (2):242-248.
22. Zhang Y, Hoon MA, Chandrashekar J, et al. Coding of sweet, bitter and umami tastes: different receptor cells sharing signaling pathways. Cell 2003 (3); 112: 293-301.
23. Wolf A., Illini O., Uy D., et al. A new extension to the Taste Strips test. Rhinology 2016 (1); 54: 45-50.
24. Mueller C1, Kallert S, Renner B, Stiassny K ,Temmel AF, Hummel T. Quantitative assessment of gustatory function in a clinical context using impregnated “taste strips”. Rhinology 2003; 41(1):2-6.
25. Yaginuma Y., Kobayashi T., Sai Y., et al. Predictive value of electrogustometry in the preoperative diagnosis of severity of middle ear pathology. Nihon Jibiinkoka Gakkai Kaiho 1996; 99(11): 1635-1640.