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ORIGINAL ARTICLE

Assessment of lingual nerve functions after smoking cessation

Anne Barbara Rittich[a], Jens Ellrich[b,c] and Sareh Said Yekta-Michael[d,e]

[a]Department of Prosthodontics and Biomaterials, Centre of Implantology, University Hospital Aachen, Aachen, Germany, [b]Department of Health Science and Technology, Aalborg University, Aalborg, Denmark; [c]Institute of Physiology and Pathophysiology, Friedrich-Alexander-University Erlangen-Nuremberg, Erlangen, Germany; [d]Department of Conservative Dentistry, Periodontology and Preventive Dentistry, University Hospital Aachen, Aachen, Germany; [e]Interdisciplinary Centre for Clinical Research, University Hospital Aachen, Aachen, Germany

ABSTRACT

Objective: Cigarette smoking is associated with a variety of oral diseases. A previous study showed a reduction of thermal sensitivity in the innervation area of the lingual nerve in smokers possibly caused by a degeneration of thermosensitive receptors as a consequence of smoking. The current study investigates somatosensory changes in ex-smokers.

Materials and methods: Sensory functions in innervation areas of lingual nerve were investigated in 40 ex-smokers by psychophysical means. Functions of lingual nerve in 40 ex-smokers were compared to those in 40 smokers and 40 non-smokers. Subjects were investigated using quantitative sensory testing (QST, cold and warm detection, thermal sensory limen, heat and cold pain, and mechanical detection).

Results: Significant differences were found in both groups, ex-smokers and smokers compared to non-smokers. Cold ($p < .001$), warm (ex-smokers: $p < .01$; smokers: $p < .001$) detection thresholds and thermal sensory limen ($p < .001$) showed significantly lower sensitivity in ex-smokers and smokers in comparison to non-smokers.

Conclusions: The lower temperature sensitivity of ex-smokers compared to that in non-smokers indicates a reduction of somatosensory function of the tongue, possibly caused by irreversible nerve degeneration associated with smoking. Influencing factors leading to sensory changes could be modulation of thermo-receptors, demyelination as well as a change of the epithelial structure.

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Smoking cessation; quantitative sensory testing; lingual nerve; somatosensation

Introduction

Smoking cigarettes is directly related to several medical risk factors including cancer, low birth weight and pulmonary or cardiovascular disease [1–7]. As an example, it provokes airway irritation and cough by activating nicotinic acetylcholine receptors [8].

It is also well known that smoking is an important risk factor for oral diseases such as nicotine stomatitis, leukoplakia, candida infection, black hairy tongue, caries or periodontal disease [2,9–13]. It is as well associated with lip and the oral cavity cancers [9].

Smoking also affects the senses of taste and smell. As a result, non-smokers are able to detect salt concentrations 12–14 times lower than the smallest concentration detectable by heavy smokers [14]. While acute inhaling of cigarette smoke reversibly reduces olfactory function, chronic smoking behaviour can even lead to a permanently reduced sense of smell [15]. Additionally, the cessation of cigarette smoking leads to improvement of olfactory function [16]. Overall, smokers have a six times higher risk of olfactory deficiency compared to non-smokers, which itself depends on the duration and the frequency of cigarette smoking [17].

The lingual nerve supplies sensory innervation to the tongue. The disruption of thermal sensation and sensitisation of the lingual nerve can be measured using quantitative sensory testing (QST). This technique registers the clinical assessment of Aδ- and C-fibre function. QST has been developed as a comprehensive test battery for somatosensory function across the spectrum of primary afferents [18,19]. It measures sensory detection and pain thresholds of accurately calibrated stimuli.

A recent study showed that smoking cigarettes has an impact on the somatosensory function of the lingual nerve in tongue mucosa, which probably indicates degeneration of Aδ- and C-fibres of the lingual nerve [18]. Testing was conducted using QST on tongue mucosa. As control for systemic, extra oral effects of smoking, tests were additionally performed on the skin of the chin, which is innervated by the mental branch of the trigeminal nerve. The study showed striking effects of smoking on patients' detection of non-noxious thermal stimuli, but the ability to perceive mechanical or painful thermal stimuli was not affected. The smoking related effects were confined to the tongue. Systemic effects of smoking could be excluded [18]. The fact that no desensitisa-

CONTACT Sareh Said Yekta-Michael samichael@ukaachen.de Department of Conservative Dentistry, Periodontology and Preventive Dentistry, Interdisciplinary Centre for Clinical Research, Aachen University, Pauwelsstr. 30, 52074 Aachen, Germany

tion was observed in mental area, which is also innervated by trigeminal nerve, points at local somatosensory irritation of lingual nerve probably due to continual exposure to high doses of tobacco ingredients.

It is not known whether or not smoking can lead to irreversible reduced somatosensation of the tongue, as there are no previous studies investigating subjects who ceased smoking.

The current study addresses the hypotheses of significant differences in somatosensory function of the lingual region in ex-smokers compared to non-smokers and smokers, using QST.

Materials and methods

Data of smokers and non-smokers were reanalysed and compared to the new data set of 40 ex-smokers [18]. Overall, the results of 120 healthy participants [40 ex-smokers (20 female, 20 male; 20–64 years), 40 non-smokers (20 female, 20 male; 19–62 years and 40 smokers (20 female, 20 male; 20–56 years)] were analysed.

All participants were examined by the same trained examiner, using QST on tongue mucosa [18,20].

Volunteers participating in this study as ex-smokers fumed 20 cigarettes a day for a minimum of three years and ceased to smoke for more than six months prior to this study (mean 72.8 months). The non-smokers had never smoked. The smokers had smoked for at least three years (more than three years and above 20 cigarettes a day) [18]. Due to age and gender differences of QST parameters, 20 male and 20 female subjects were chosen. In each group half of the subjects were aged $<$40 years and the other half were aged $>$40 years [18,19].

All subjects were examined intraorally to ensure having healthy mucosa without swellings or any kind of dermal lesions. Exclusion criteria consisted of abnormalities of the mucosa identified by visual inspection, use of a removable prosthesis, previous lingual or orofacial injuries, neurological or psychiatric history, diabetes mellitus and chronic medication of any kind for at least 48h prior to examination [18]. Subjects were instructed not to eat, drink or chew gum for at least 0.5 h before examination. All participants gave their informed consent prior to their inclusion in the study according to the 1964 Declaration of Helsinki. The protocol was approved by the local ethics committee (EK 313/15).

Standardised QST protocol [18,20] was used to investigate three different somatosensory issues. Thermal detection was evaluated by recording cold detection threshold (CDT), warm detection threshold (WDT), thermal sensory limen (TSL) and paradoxical heat sensation (PHS) parameters. Cold pain threshold (CPT) and heat pain threshold (HPT) were used to assess pain processing. Finally, mechanical detection threshold (MDT) was tested. Using a computer-controlled thermode (TSA-II, Medoc Ltd., Yishai, Israel) – which is based on the Peltier thermo battery – with a stimulation area of $16 \times 16\,mm^2$, thermal stimuli were applied to the innervation area of the lingual nerve on the anterior two thirds of the tongue. Subjects were bilaterally tested in a supine position, keeping their eyes shut. For the first 20 volunteers, measuring started on the right side, whereas the other 20 participants were first tested on the left. Examination of all participants was performed at Uniklinik RWTH Aachen in Germany.

To evaluate thermal detection, the thermode started in the same way as in the preliminary study [18] from a baseline temperature of 32 °C. The range of stimulating temperatures was from 0 to 50 °C. Temperature was rising (WDT/HPT) or decreasing (CDT/CPT) at the rate of 1 °C/s. Volunteers were instructed to stop this procedure by pressing a computer mouse button to show their recognition of thermal change (WDT/CDT) or thermal pain (CPT/HPT). Thus, CDT and WDT were recorded as a difference of temperature (dT) relative to the baseline of 32 °C. In contrast, CPT and HPT were recorded as absolute temperatures [18–20]. Subjects pressed the computer mouse button once recognising heat (HPT) or cold (CPT) pain. Therefore, CDT and CPT resulted in negative values. For each value, the measurement was performed three times on the left and three times on the right part of the tongue mucosa. On both sides arithmetic means were calculated for every parameter. For the analysis of TSL, alternating warm and cold stimuli, relative to the baseline of 32 °C, were triggered. Temperature increased or decreased until the volunteer indicated a change of temperature by clicking a mouse button and telling what kind of change in temperature was recognised. Alternating stimuli were repeated twice. During this testing procedure, a subjective feeling of heat upon cooling was listed as PHS. The mean difference between values causing warm and cold detection was defined as TSL. For the measurement of MDT a standardised set of von Frey filaments (Touch-Test Sensory Evaluators, North Coast Medical, Morgan Hill, CA, USA) with forces of 0.08, 0.2, 0.4, 0.7, 1.6, 4, 6, 10, 14, 20, 40, 60, 80, 100, 150, 260, 600, 1000, 1800 and 3000 mN was applied. Method of limits was used to determine MDT. Starting with a clearly noticeable filament of 14 mN, five threshold determinations consisting of ascending and descending stimulus intensities were performed on each side of the anterior third of the tongue [19,20]. The conduct of the examination took 45 min for each side, including a demonstration of every testing method on a sample area.

Using the software IBM-SPSS statistics 23 (SPSS Inc., Armonk, NY) statistical analysis was performed to compare the data of non-smokers and smokers collected in 2012 with the new data of 40 ex-smokers. To evaluate intraindividual side-to-side comparison of all measured QST parameters, paired *t*-test was used. Due to data distribution, log-transformation was executed in the QST parameters WDT, TSL and MDT. Differences between ex-smokers, non-smokers and smokers according to gender and age for all QST parameters were investigated by multivariate testing procedure (three-way ANOVA) with the factors group (ex-smoker/non-smoker/smoker), age ($\leq$40 years/$>$40 years) and gender (male/female) using Bonferroni corrections and subsequent Student–Newman–Keuls test, when applicable (*q*, *p* value). Significance was accepted at $p < .05$. Pearson correlation was established for analysing the relationship between the time since termination of smoking and QST parameters.

Sample size calculation was based on results of a former study, which measured sensory disturbances after dental procedures [20]. Significance level was set at .05 and the desired power at 0.8. Using means and standard deviations of CDT parameter of control group and test group of the former study, minimum sample size was calculated as 40 subjects per group (p0: 3.08, p1: 3.90, α: 0.05, σ: 1.30, power: 0.8).

Results

Analysis showed no significant difference in response of the left and right sides of the innervation area of the lingual nerve in all 120 volunteers.

Three-way ANOVA with the factors group (ex-smokers/non-smokers/smokers) age ($\leq$40 years/$>$40 years) and gender (male/female) was significant for smoking behaviour ($p < .001$; power >0.8) and age ($p < .001$; power >0.8). There was an interaction between group and age ($p < .001$; power >0.8). Smoking behaviour and age interaction had a significant effect on CDT ($F = 7.2$, $p < .05$), WDT ($F = 6.4$, $p < .05$) and MDT ($F = 18.1$, $p < .001$) values.

Differences between ex-smokers, non-smokers and smokers

Results of tests of between-subjects effects for the factor group were significant for CDT ($p < .001$/power >0.8), WDT ($p < .001$/power >0.8), TSL ($p < .001$/power >0.8), and CPT ($p < .05$/power <0.8). Post hoc tests showed significant differences between smokers and non-smokers in CDT ($p < .001$), WDT ($p < .001$) and TSL ($p < .001$). Significant differences between ex-smokers and non-smokers were also found regarding CDT ($p < .001$), WDT ($p < .01$) and TSL ($p < .001$). These results show that thermal sensitivity is reduced in smokers as well as in ex-smokers in comparison to non-smokers. There were no significant differences between ex-smokers and smokers with regards to CDT ($p = .95$) and TSL ($p = .96$). There was a significant difference in WDT parameter between ex-smokers and smokers ($p < .01$; Figure 1).

CPT showed a significant difference between ex-smokers ($10.1 \pm 8.4\,^{\circ}C$) and smokers ($13.4 \pm 8.2\,^{\circ}C$; $p < .05$).

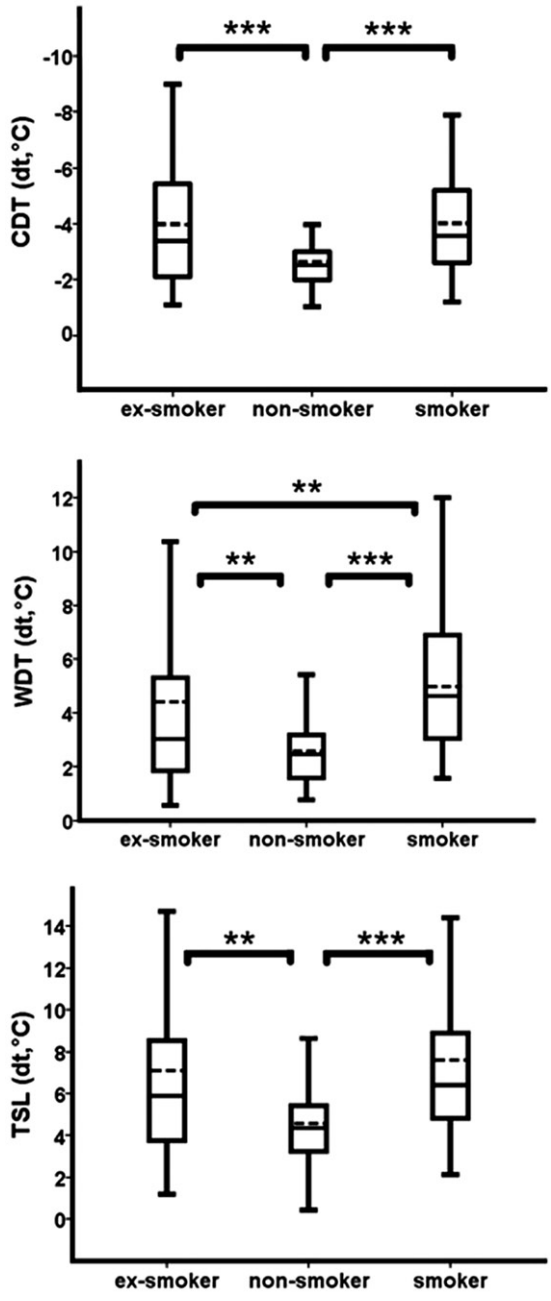

Figure 1. Differences between ex-smokers, non-smokers and smokers in the innervation area of the lingual nerve (multiple comparison, Bonferroni). Cold detection threshold (CDT), Warm detection threshold (WDT) and thermal sensory limen (TSL) were determined from 120 QST experiments in healthy volunteers (60 women, 60 men; 20 ex-smokers, 20 non-smokers, 20 smokers each). CDT and WDT were calculated as differences from baseline (32 °C; dT). Data on ex-smokers, non-smokers and smokers are presented as box plots (solid line: median, dashed line: mean, $^{***}p < .001$, $^{**}p < .01$, $^{*}p < .05$).

Differences in gender

With regards to gender, there were significant differences in WDT ($F = 4.5$, $p < .05$) and HPT ($F = 6.1$, $p < .05$) values. Regarding WDT and HPT parameters, female subjects were more sensitive than male (Table 1). In the smoker-group, female were more sensitive than male regarding CDT ($p < .05$), WDT ($p < .05$) and TSL ($p < .05$). There were no significant differences between male and female in non-smokers and ex-smokers regarding all QST parameters.

Differences in age

CDT ($F = 22.8$, $p < .001$), WDT ($F = 22.2$, $p < .001$), TSL ($F = 31.0$, $p < .001$) and MDT ($F = 9.1$, $p < .05$) showed significant differences between subjects $\leq$40 years and $>$40 years (Table 1). Subjects $\leq$40 years were more sensitive than subjects $>$40 years.

In smoker-group, there were age-dependent significant differences regarding WDT ($p < .001$), TSL ($p < .001$) and MDT ($p < .01$). In non-smokers, there were significant differences regarding CDT ($p < .01$), TSL ($p < .01$) and MDT ($p < .01$) and in ex-smokers, there were significant differences regarding CDT ($p < .001$), WDT ($p < .001$) and TSL ($p < .001$). In all groups (ex-smoker, non-smoker and smoker), younger subjects were more sensitive than older subjects.

Table 1. Gender- and age-related differences in ex-smokers, non-smokers and smokers ($n = 120$) with regard to cold detection threshold (CDT), warm detection threshold (WDT), thermal sensory limen (TSL), paradoxical heat sensation (PHS), cold pain threshold (CPT), heat pain threshold (HPT) and mechanical detection threshold (MDT).

	Age		Gender	
≤40 years	>40 years	Female	Male	
CDT (°C)	**−2.9 ± 1.6**	**−4.3 ± 2.5**	−3.4 ± 2.0	−3.9 ± 2.4
WDT (°C)	**3.2 ± 2.0**	**4.9 ± 3.5**	**3.7 ± 2.9**	**4.4 ± 3.1**
TSL (°C)	**5.1 ± 2.7**	**7.7 ± 4.9**	6.1 ± 4.1	6.8 ± 4.2
PHS (x/3)	0	0	0	0
CPT (°C)	12.1 ± 8.6	10.7 ± 8.3	12.1 ± 8.4	10.7 ± 8.6
HPT (°C)	47.7 ± 3.3	48.0 ± 2.7	**47.4 ± 3.4**	**48.3 ± 2.5**
MDT (mN)	**0.14 ± 0.1**	**0.16 ± 0.1**	0.2 ± 0.1	0.2 ± 0.1

Data is presented as mean ± SD. Significantly different results are marked in bold.

In the young age group, there were significant differences between groups (smokers, ex-smokers and non-smokers) regarding CDT, WDT, TSL and MDT. Smokers were less sensitive than both groups, non-smokers (CDT $p < .001$, WDT $p < .01$, TSL $p < .001$ and MDT $p < .001$) and ex-smokers (CDT $p < .05$, WDT $p < .01$, TSL $p < .05$ and MDT $p < .001$). No significant differences were found between ex-smokers and non-smokers.

In the old age group, there were significant differences between groups regarding to CDT, WDT and TSL. Non-smokers were more sensitive than both groups, smokers (CDT $p < .05$, WDT $p < .001$ and TSL $p < .001$) and ex-smokers (CDT $p < .001$, WDT $p < .001$ and TSL $p < .001$).

Correlation

CDT ($p = .03$) correlated negatively and TSL ($p = .04$) and MDT ($p = .01$) correlated positively to non-smoking duration (Figure 2).

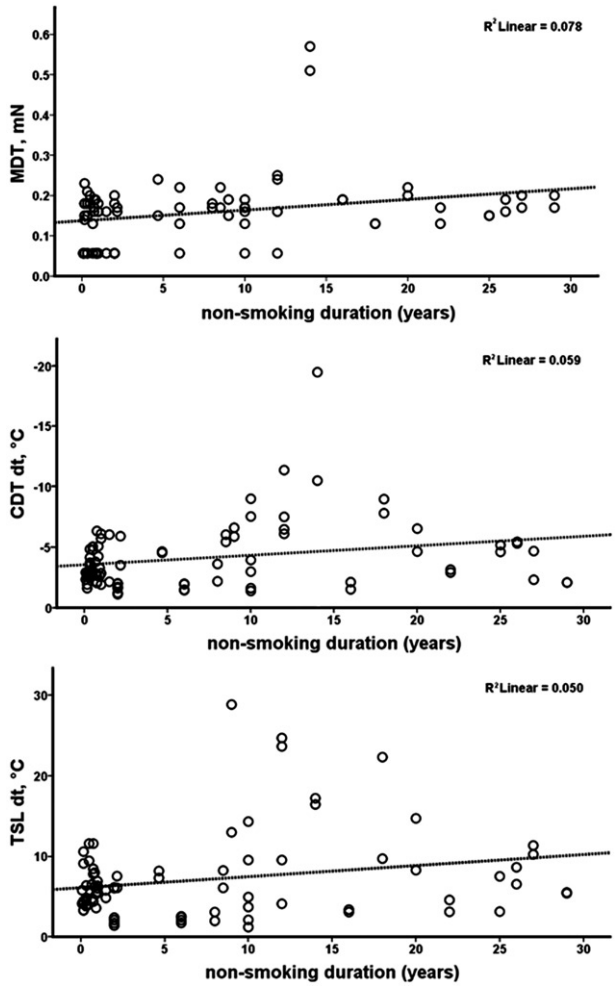

Figure 2. Correlation between duration of non-smoking (years) and the QST parameters CDT, TSL and MDT in the innervation area of the lingual nerve in ex-smokers. Data was analysed using the Pearson correlation. Each point represents the data of one subject ($n = 40$). Lines are showing linear regression curves.

Discussion

This study uses QST parameters to examine the function of Aβ-, Aδ-, and C-fibres of the tongue, with respect to differences related to smoking, age and gender.

Differences between ex-smokers, smokers and non-smokers

Compared to non-smokers, ex-smokers similar to smokers, show a reduction of thermal sensitivity in the innervation area of the lingual nerve with regards to CDT, WDT and TSL.

One possible reason for thermal sensitivity reduction in the innervation area of the lingual nerve in smokers just as in ex-smokers is a degeneration of thermosensitive receptors as a consequence of smoking.

Transient receptor potential (TRP) channels play an important role in sensation of warmth, cold, taste, irritation and chemical stimuli [21,22]. Thermo-TRP channels are activated by changes in the environmental temperature [22], which are perceived by nerve fibers, taste buds and keratinocytes in the oronasal cavity.

The most important classical thermo-TRP channels are transient receptor potential vanilloid 1-4 (TRPV1, TRPV2, TRPV3, TRPV4), transient receptor potential melastatin 8 (TRPM8) and transient receptor potential ankyrin 1 (TRPA1).

TRPV3 is stimulated by moderate heat ($T > 33\,°C$) as well as by monoterpenoids including camphor and menthol [23]. TRPV4 is activated by moderate heat between 24 and 27 °C as well as physical stimuli and chemical activators [24,25]. By contrast, TRPM8 is a cold sensitive ion channel activated between 17 and 25 °C [22,26–28]. It is also stimulated by menthol and its derivatives, eucalyptol, icilin and some other chemical activators.

The receptor activated by cooling relative to CDT is TRPV4 [22].

Gating of the channel is modulated by phosphatidylinositolbisphosphate (PIP_2) interaction and is regulated by protein kinase C (PKC) independent and dependent pathways [22,29–33].

The activation of any receptor triggering phospholipase C (PLC) causes interruption of channel activation of TRPV4 (e.g. nerve growth factor [NGF]) hydrolysing PIP_2 [30,34–36]. Studies have shown that NGF levels were elevated in the

bronchoalveolar lavage fluid of nicotine-exposed mice and current smokers because of lung fibroblasts being stimulated to release NGF [37]. The nicotine induced NGF level could be one possible reason for smoking induced TRPV4 desensitisation.

The results demonstrate that ex-smokers, just as smokers, show significant differences in CDT compared to non-smokers. The possible nerve degradation and a possible receptor desensitisation associated with smoking might not be reversible.

TRPV3 is activated by moderate heat (>33 °C) [23]. TRPV3 is strongly activated and temporarily desensitised by monoterpenoids [23].

Citral is a bioactive component of lemongrass and one of the additives added to cigarettes.

Citral activates TRP channels found in sensory neurons. It produces long-lasting inhibition of TRPV1-3 and TRPM8, while transiently blocking TRPV4 and TRPA1. Persistent citral inhibition is independent of internal calcium concentration, but it is channel state-dependent, initiating only after TRP channel opening [38]. The influence of monoterpenoids could be one possible factor for the transient desensitisation of TRPV3.

Cigarette smoking is also known as an important risk factor for multiple sclerosis (MS). In a review [39] published in 2012, the following effects of smoking on the nervous system, among others, have been identified with regards to MS. Direct evidence linking smoking to MS is limited, but possible mechanisms are immunomodulation and direct toxicity to neurons and oligodendrocytes. Cigarette smoke contains high concentrations of free radicals and two gases, among others, which have been implicated in oxidative injury to neural tissues: hydrogen cyanide and nitric oxide (NO). Neurons and oligodendrocytes are more vulnerable to oxidative stress. They have higher oxygen consumption than astrocytes, microglia or capillary endothelial cells. In animal experiments, demyelinating lesions in the central nervous system (CNS) after exposure to cyanide were detected. Cyanide absorption in humans in case of cassava consumption is associated with central and peripheral nervous system demyelinating disease [39].

NO plays an important role in regulation of blood vessel tone, synaptic function, platelet aggregation, smooth muscle regulation, and innate immune system cytotoxicity. Cigarette smoke contains free radical derivatives of NO with concentrations up to 500 ppm [39].

This review leads to the conclusion that, direct exposure to NO especially in cases of physiologically active or demyelinated axons can cause axonal degeneration and block nerve conduction [39].

This is in agreement with our results, as the smokers and ex-smokers show a reduced thermal sensitivity (CDT, WDT and TSL), but not a reduction in mechanical sensitivity (MDT). WDT reflects the function of unmyelinated C-fibres and CDT represents the function of small myelinated Aδ-fibres. These fibres might be more sensitive than the large myelinated Aβ-fibres (MDT) [40].

A histological modification of the epithelium could also be a supplementary factor for permanent somatosensory changes. In a histological analysis of 90% of rat tongues exposed to cigarette smoke (40 cigarettes a day) epithelial hyperplasia was found [41]. Morphometric analysis revealed that keratin layer thickness was greater in the tobacco group [41]. The altered epithelium strength may also have an insulation effect that causes a diminished temperature response.

In this study, CPT and WDT parameter showed significant difference between ex-smokers and smokers. CPT values were greater in smokers than in ex-smokers, which could lead to the conclusion that smokers are more sensitive to the cold-induced pain than ex-smokers. Nevertheless, the standard deviation of CPT is greater than the other QST parameters and the statistical power in CPT testing procedure was less than 0.8. Therefore, it cannot be concluded that smokers are more sensitive than ex-smokers with regard to the cold-induced pain.

WDT values of ex-smokers show lower values than those of smokers, indicating greater sensitivity of ex-smokers.

Differences regarding to gender

Regarding to gender, there was a significant difference in WDT ($p < .05$) and HPT ($p < .05$). As expected from literature, men were less sensitive than women with regards to warm stimuli in the region innervated by lingual nerve. The higher threshold for thermal stimuli gives evidence to a lower neuronal sensitivity in males 'tongue, compared to females' [18,20].

Differences regarding to age

Age related differences found in this study are also in line with published literature. There were significant differences found in CDT ($p < .001$), WDT ($p < .001$), TSL ($p < .001$) and MDT ($p < .05$). In all groups, volunteers under the age of 40 were more sensitive than subjects of older age [18–20]. Age-related dependency of thermal and mechanical parameters indicates that older subjects are less sensitive than younger volunteers.

In the old age group, non-smokers were more sensitive than both groups, smokers and ex-smokers. In the young age group, smokers were less sensitive than ex-smokers and non-smokers, but no significant differences were found between ex-smokers and non-smokers.

Young ex-smokers were more sensitive than young smokers but there were no significant differences between old ex-smokers and old smokers. These results raise the question whether there is an improvement of lingual nerve functions in young ex-smokers in contrast to the old ex-smokers. Because of the small case number of the subgroups ($n = 20$), these results must be critically interpreted. An explanation for a possible improvement of somatosensation in young ex-smokers in contrast to the old age group might be an age-dependent decrease in axon remyelination [42]. Another reason could be an age-dependent epithelial regeneration rate, but there are contradicting results in literature about increase and decrease of oral epithelium [43]. For example, one study investigated structural aspects of aging and reported about decreased mean thickness of the oral

epithelium [44]. Another study showed no age-dependent changes in the oral epithelial thickness in mice [45].

To assess these effects further studies with a higher case number should be conducted.

Correlation

The calculated correlation for the parameters CDT, MDT and TSL must be critically analysed with values of $R^2<0.1$, which indicates very weak linear correlation.

The correlation between the non-smoking duration and CDT, MDT and TSL could be interpreted as a decrease in values associated with the non-smoking duration, but long non-smoking periods are also associated with older subjects who have age-related impaired values as proven in present and former studies [18–20]. Additionally, few subjects under 40 years had ceased smoking for a period as long as volunteers older than 40 years did. Certainly, it is difficult to acquire subjects under 40 years who ceased smoking for long periods.

Limitations

In this study, WDT values of ex-smokers show lower values than those of smokers, indicating greater sensitivity of ex-smokers. Based on the study design, it cannot be concluded whether or not there is an improvement in sensitivity after smoking cessation due to the WDT factor, because the participant ex-smokers were new subjects who did not take part in the former study as smokers. In order to prove an improvement in subjects' sensitivity, volunteers should have been investigated both during smoking period and as non-smokers after smoking cessation in several year intervals.

In our study, the group of smokers analysed in the study of 2012 [18] were contacted and asked if they stopped smoking and if they would like to participate in the future study as an ex-smoker. Although many expressed the desire to quit smoking, none of the subjects had completely stopped smoking for more than 6 months since the last measurement.

Another limitation of this study was the thermal testing methodology. The baseline temperature for intraoral testing should be 37 and not 32 °C [46]. This is especially a problem for the CDT measure, as a probe $16 \times 16\,mm^2$ of 32 °C would feel cold to most people already at baseline [47]. In this study, a baseline temperature of 32 °C was selected based on a previous study, in which collected normative QST data in lingual region was measured [20]. Because of this limitation, CDT values cannot be considered valid and the results must be interpreted with caution. We recommend further examinations of the tongue with a baseline temperature of 37 °C [46,47].

Perhaps an additional limitation of the results was caused by the time elapsed since subjects ceased smoking. The minimum number of months accepted in our study was six months. The average value was 72.83 months. Nevertheless, rehabilitation of somatosensory function of lingual nerve fibres might need more than six years. A follow-up study with a longer abstinence period should be achieved with older subjects participating in each group to exclude the interaction of QST values and age.

Every year one million people across the world try to stop smoking, but only a few per cent of them succeed. It is difficult to completely give up smoking. A recent study [48] revealed that, 81% of participating smokers had experienced oral health problems during the last six months and high motivation to quit smoking was reported in only about 20% [48]. The results of this study also showed that improved general health was a major reason to quit smoking for 89% of the subjects [48]. In our study, it remains to be critically analysed whether the thermal significant differences regarding CDT, WDT and TSL (for the subjects) are also of clinical relevance. The values differ on the average by about two to three degrees. A somatosensory disorder of this extent would probably not lead to a disturbance of patients' quality of life. Although the reduction of thermal sensitivity in the innervation area of the lingual nerve has no major clinical relevance and therefore can hardly be considered a huge reason to motivate smokers to quit, patients should still get enlightened about these side effects caused by smoking.

Conclusions

This study showed that the somatosensory function of the lingual nerve of ex-smokers and smokers is significantly different from that of non-smokers. The reasons for the malfunction in ex-smokers and smokers compared to non-smokers can only be conjectured. Studies at the molecular level should be performed to investigate the reason for degradation of somatosensory function of the lingual region, which is probably caused by adaption, inactivation, desensitisation or absence of the thermo-TRP channels, or as a result of demyelination and axonal degeneration of nerve fibers or changes of the epithelial structure.

Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

References

[1] Angadi PV, Patil PV, Kale AD, et al. Myofibroblast presence in apparently normal mucosa adjacent to oral squamous cell carcinoma associated with chronic tobacco/areca nut use: evidence for field cancerization. Acta Odontol Scand. 2014;72:502–508.

[2] César Neto JB, Rosa EF, Pannuti CM, et al. Smoking and periodontal tissues: a review. Braz Oral Res. 2012;1:25–31.

[3] Tobacco smoking. IARC Monogr Eval Carcinog Risk Chem Hum. 1986;38:35–394.

[4] ARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Tobacco smoke and involuntary smoking. IARC Monogr Eval Carcinog Risks Hum. 2004;83:1–1438.

[5] Pleasance ED, Stephens PJ, O'Meara S, et al. A small-cell lung cancer genome with complex signatures of tobacco exposure. Nature. 2010;463:184–190.

[6] Maasland DH, van den Brandt PA, Kremer B, et al. Alcohol consumption, cigarette smoking and the risk of subtypes of head-

neck cancer: results from the Netherlands Cohort Study. BMC Cancer. 2014;14:187.
[7] Feng Z, Xu QS, Niu QF, et al. Risk factors for patients with multiple synchronous primary cancers involving oral and oropharyngeal subsites. Oral Surg Oral Med Oral Pathol Oral Radiol. 2016;121:360–366.
[8] Lee LY, Gu Q, Lin YS. Effect of smoking on cough reflex sensitivity: basic and preclinical studies. Lung. 2010;1:S23–S27.
[9] Just-Sarobe M. Smoking and the skin. Actas Dermosifiliogr. 2008;99:173–174.
[10] Seymour RA. Is oral health a risk for malignant disease? Dent Update. 2010;37:279–280.
[11] Warnakulasuriya S, Dietrich T, Bornstein MM, et al. Oral health risks of tobacco use and effects of cessation. Int Dent J. 2010;60:7–30.
[12] Dilhari A, Weerasekera MM, Siriwardhana A, et al. Candida infection in oral leukoplakia: an unperceived public health problem. Acta Odontol Scand. 2016;74:565–569.
[13] Wickholm S, Söder PO, Galanti MR, et al. Periodontal disease in a group of Swedish adult snuff and cigarette users. Acta Odontol Scand. 2004;62:333–338.
[14] Jackson JA. Heavy smoking and sodium chloride hypogeusia. J Dent Res. 1967;46:742–744.
[15] Gudziol H, Graul J, Bitter T, et al. Ability of smelling is reduced reversibly by acute smoking and permanently by chronic smoking. Laryngorhinootologie. 2013;92:663–666.
[16] Frye RE, Schwartz BS, Doty RL. Dose-related effects of cigarette smoking on olfactory function. JAMA. 1990;263:1233–1236.
[17] Katotomichelakis M, Balatsouras D, Tripsianis G, et al. The effect of smoking on the olfactory function. Rhinology. 2007;45:273–280.
[18] Yekta SS, Lückhoff A, Ristíc D, et al. Impaired somatosensation in tongue mucosa of smokers. Clin Oral Investig. 2012;16:39–44.
[19] Rolke R, Bron R, Maier C, et al. Quantitative sensory testing in the German Research Network on neuropathic pain (DFNS): standardized protocol and reference values. Pain. 2016;123:231–243.
[20] Yekta SS, Smeets R, Stein JM, et al. Assessment of trigeminal nerve functions by quantitative sensory testing in patients and healthy volunteers. J Oral Maxillofac Surg. 2010;68:2437–2451.
[21] Roper SD. TRPs in taste and chemesthesis. Handb Exp Pharmacol. 2014;223:827–871.
[22] Ferrandiz-Huertas C, Mathivanan S, Wolf CJ, et al. Trafficking of ThermoTRP channels. Membranes (Basel). 2014;4:525–564.
[23] Azhar Sherkheli M, Benecke H, Doerner JF, et al. Monoterpenoids induce agonist-specific desensitization of transient receptor potential vanilloid-3 ion channels. JPPS. 2009;12:116–128.
[24] Nilius B, Watanabe H, Vriens J. The TRPV4 channel: structure–function relationship and promiscuous gating behaviour. Pflugers Arch. 2003;446:298–303.
[25] Voets T, Prenen J, Vriens J, et al. Molecular determinants of permeation through the cation channel TRPV4. J Biol Chem. 2002;277:33704–33710.
[26] Erler I, Al-Ansary DM, Wissenbach U, et al. Trafficking and assembly of the cold-sensitive TRPM8 channel. J Biol Chem. 2006;281:38396–38404.
[27] Madrid R, Donovan-Rodriguez T, Meseguer V, et al. Contribution of TRPM8 channels to cold transduction in primary sensory neurons and peripheral nerve terminals. J Neurosci. 2006;26:12512–12525.
[28] Behrendt HJ, Germann T, Gillen C, et al. Chracterization of the mouse cold-menthol receptor TRPM8 and vanilloid receptor type-1 VR1 using a fluorometric imaging plate reader (FLIPR) assay. Br J Pharmacol. 2004;141:737–745.
[29] Watanabe H, Vriens J, Janssens A, et al. Modulation of TRPV4 gating by intra- and extracellular Ca^{2+}. Cell Calcium. 2003;33:489–495.
[30] Garcia-Elias A, Mrkonjic S, Pardo-Pastor C, et al. Phosphatidylinositol-4,5-bisphosphate-dependent rearrangement of TRPV4 cytosolic tails enables channel activation by physiological stimuli. Proc Natl Acad Sci USA. 2013;110:9553–9558.
[31] Mamenko M, Zaika OL, Boukelmoune N, et al. Discrete control of TRPV4 channel function in the distal nephron by protein kinases A and C. J Biol Chem. 2013;288:20306–20314.
[32] Peng H, Lewandrowski U, Muller B, et al. Identification of a protein kinase C-dependent phosphorylation site involved in sensitization of TRPV4 channel. Biochem Biophys Res Commun. 2010;391:1721–1725.
[33] Gao X, Wu L, O'Neil RG. Temperature-modulated diversity of TRPV4 channel gating: Activation by physical stresses and phorbol ester derivatives through protein kinase C-dependent and –independent pathways. J Biol Chem. 2003;278:21729–27137.
[34] Abe J, Hosokawa H, Sawada Y, et al. CA^{2+}-dependent PKC activation mediates menthol-induced desensitization of transient receptor potential M8. Neurosci Lett. 2006;397:140–144.
[35] Linte RM, Ciobanu C, Reid G, et al. Desensitization of cold- and menthol-sensitive rat dorsal root ganglion neurons by inflammatory mediators. Exp Brain Res. 2007;178:89–98.
[36] Premkumar LS, Raisinghani M, Pingle SC, et al. Downregulation of transient receptor potential melastatin 8 by protein kinase C-mediated dephosphorylation. J Neurosci. 2005;25:11322–11329.
[37] Wongtrakool C, Grooms K, Bijli KM, et al. Nicotine stimulates nerve growth factor in lung fibroblasts through an NFκB-dependent mechanism. PLoS One. 2014;9:e109602.
[38] Stotz SC, Vriens J, Martyn D, et al. Citral sensing by transient receptor potential channels in dorsal root ganglion neurons. PLoS One. 2008;3:e2082.
[39] O'Gorman C, Lucas R, Taylor B. Environmental risk factors for multiple sclerosis: a review with a focus on molecular mechanisms. Int J Mol Sci. 2012;13:11718–11752.
[40] Yekta SS, Koch F, Grosjean MB, et al. Analysis of trigeminal nerve disorders after oral and maxillofacial intervention. Head Face Med. 2010;6:24
[41] de Oliveira Semenzati G, de Souza Salgado B, et al. Histological and immunohistochemical study of the expression of p53 and ki-67 proteins in the mucosa of the tongue, pharynx and larynx of rats exposed to cigarette smoke. Inhal Toxicol. 2012;24:723–731.
[42] Adamo AM. Nutritional factors and aging in demyelinating diseases. Genes Nutr. 2014;9:360
[43] Rasha AE, Faleh S, Gabriel L, et al. Age and the architecture of oral mucosa. Age (Dordr). 2012;34:651–658.
[44] Williams DM, Cruchley AT. Structural aspects of aging in the oral mucosa. In: Squier CA, Hill MW, editors. The effect of aging in oral mucosa and skin. Boca Raton: CRC Press; 1994. p. 65–74.
[45] Cameron IL. Cell proliferation and renewal in aging mice. J Gerontol. 1972;27:162–172.
[46] Svensson P, Baad-Hansen L, et al. Guidelines and recommendations for assessment of somatosensory function in oro-facial pain conditions–a taskforce report. J Oral Rehabil. 2011;38:366–394.
[47] Pigg M, Baad-Hansen L, Svensson P, et al. Reliability of intraoral quantitative sensory testing (QST). Pain. 2010;148:220–226.
[48] Andersson P, Johannsen A. Dental patients' perceptions and motivation in smoking cessation activities. Acta Odontol Scand. 2016;74:285–290.