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Mechanical frequency and stimulation-site-related differences in vibrotactile detection capacity along the lip vermilion in young adults

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Abstract Accurate clinical assessment of the infraorbital and mental branch of the trigeminal nerve is aided by an appreciation of the variations in sensitivity that may exist along the surface of the perioral region under examination. The purpose of this investigation was to map the mediolateral spatial and frequency variations in vibrotactile detection capacity to inputs delivered to the upper lip (UL) and lower lip (LL) vermilion. Mechanical vibrotactile inputs at frequencies of 5, 10, 50, and 150 Hz were delivered to three locations on the vermilion of the UL and LL: midsagittally and laterally (left and right) at a point halfway between the midsagittal plane and the oral angle. An adapted staircase tracking method was used to converge upon a threshold value for each test frequency at each stimulation site. The results indicated that midsagittal vermilion sites were significantly more sensitive to our range of vibrotactile inputs compared to lateral vermilion locations. In addition, no significant differences in sensitivity as a function of laterality or between the UL and LL vermilion sites were noted. Greater midline sensitivity to vibrotactile stimulation suggests that receptive fields at this location may be of greater density and/or demonstrate greater overlap compared to lateral vermilion sites.

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Introduction

The lip vermilion has been shown to be one of the most sensitive areas in the orofacial region to tactile stimuli [4, 7, 17, 18, 22, 33]. As revealed by microneurography of the infraorbital nerve in man, dense networks of low-threshold cutaneous mechanoreceptors, possessing overlapping and generally small receptive fields (RFs), encode events associated with oral behaviors such as speech or facial gesturing [19, 20, 27]. In light of this underlying neural substrate, accurate diagnosis of an orofacial sensory condition is facilitated by an appreciation of numerous stimulus-response-related factors including spatial and frequency differences in sensitivity of the test area in question [23]. Depending on the form of the stimulus used, changes in sensitivity from one area to the next within a given structure may not reflect pathology per se, but rather, may represent an expected regional variation [23].

Previous investigations using two-point discrimination and pinprick magnitude estimation tasks have suggested that differences in tactile sensitivity may exist along the mediolateral surface of the perioral area [7, 23, 33]. Additionally, a limited number of reports have provided some normative data on select features of orofacial vibrotactile detection capacity of the lip [4, 17, 18, 22, 31]. Unfortunately, the latter studies have several differences among them that limit the generalizability of their results. For example, Kesarwani et al. [22] and Posnick et al. [31] used a single test frequency (TF) at 120 Hz to assess vibratory detection at only one location on the lip vermilion. The use of a single TF is problematic given that the skin of the lower face is populated by multiple receptor channels that can be activated individually by different frequency inputs in the range from 1 to 150 Hz [4]. Only two studies could be found that have used multiple TFs within this specific range to assess vibrotactile detection capacity in the lip vermilion [4, 18]. Unfortunately, these particular investigations only evaluated perceptual capacity at single test sites on the lip vermilion, thus, not providing a complete description of any threshold variations that might have existed along the full surface of the vermilion.

Given that microneurographic and histological data suggest that variations in peripheral innervation likely exist in the orofacial system [10, 15, 17, 19, 20, 28], it is reasonable to suggest that thresholds may not be uniform along the entire surface of the vermilion. To the best of our knowledge, no study to date has used several different TFs to assess and formally compare vibrotactile detection capacity of midsagittal as well as lateral vermilion sites in the same group of subjects. Because psychophysical assessments are typically variable among human subjects [11, 23], a more consistent evaluation of vibrotactile sensitivity differences across a range of TFs in the same pool of subjects may provide a more representative picture of labial vibrotactile sensitivity changes. As such, the purpose of this study was to systematically examine stimulation site and TF variations in vibrotactile detection thresholds (VDTs) along the surface of the upper lip (UL) and lower lip (LL) vermilion in normal young adult subjects.

Materials and methods

Subjects

Eleven normal young adults, between 20 and 29 years of age, were randomly selected from the population of students at the University of Georgia. All subjects had normal or corrected-to-normal vision, normal speech, and were in good physical health with no history of neuromuscular or craniofacial disorder. All subjects provided informed consent to participate in this investigation. This study was conducted in accordance with NIH regulations for the ethical treatment of human subjects. All work was approved by the University of Georgia Institutional Review Board for the safety of human subjects.

Cutaneous stimulation

A mechanical stimulus generator system, mounted onto an articulated microscope arm, delivered sinusoidal vibrotactile inputs to the vermilion of the UL and LL at three different sites: midsagittally and on the right/left lateral vermilion at a point halfway between the midsagittal plane and the oral angle (see Fig. 3 for placements). The stimulus system consisted of a Bruel and Kjaer Model 4810 Minishaker (Norcross, GA, USA), a flat-surfaced nylon stimulus probe (surface area=0.5 cm²), and a rigid surround (17-mm OD with a probe-surround gap= ~ 1 mm). A Schaevitz (Fairfield, NJ, USA) microminiature linear variable differential transformer (LVDT) was serially coupled to the outboard end of the Minishaker to provide displacement information of the probe with 1-µm resolution (Fig. 1). The output of an arbitrary digital waveform generator (Wavetek Model-29, Everett, WA, USA) was conditioned by a power amplifier (Bruel and Kjaer Model 2706) and provided the input signal to the Minishaker. Synthesized waveforms from the waveform generator were 1 s in duration with a 150 ms linear rise–fall decay to eliminate the possibility of on/off mechanical transients. Probe displacement signals from the LVDT were digitized at 5 kHz on a 16-bit A/D (ADInstruments, Colorado Springs, CO, USA). LVDT output was digitized in calibrated units of microns. General system calibration checks were performed by the principal investigator prior to subject testing to ensure consistency and accuracy in the output of the LVDT.

Vibratory detection threshold testing

Subjects were seated in a chair equipped with a head support and fitted with a customized bite block (Extrude XP, Kerr, Orange, CA, USA) to stabilize movement of the mandible and to maintain a slight separation of the lips (approximately 1 cm) during stimulus presentation. The bite block was positioned posteriorly over the molars and trimmed to a small size to avoid any possible interference of the bite block with the lip vermilion. Subjects were instructed to let their rear molars gently rest on the bite block when inserted and to maintain a relaxed lower face posture during the entire psychophysical testing period. The rigid surround about the contactor probe was then placed on a given stimulation site with a 1,000-um contactor preload indentation. This same preload indentation level was applied during all TF and stimulation site testing combinations to ensure consistency in the magnitude of



Fig. 1 The orientation of the mechanical stimulus generator system with reference to the subject is shown. The system was configured with a flat-tipped 0.5-cm² nylon probe positioned orthogonally onto the surface of the lip vermilion at six different test sites, whereas the subject sat quietly and completed the psychophysical detection task. Headphones delivered narrowband auditory masking centered upon each stimulus frequency

contact made between the apparatus and the vermilion surface.

Following subject setup, vibrotactile stimulation was delivered to each of the six stimulation sites at each of the following TFs: 5, 10, 50, and 150 Hz. This range of sinusoidal vibrotactile inputs corresponds well to the frequency responsivity of cutaneous mechanoreceptor populations in the glabrous skin of the lower face [4]. All subjects were provided with a standard instructional set for the task and were asked to respond to the detection of a vibrotactile stimulus event by depressing a handheld button switch "as soon as they felt" the input. The Transistor Transistor Logic (TTL) signal generated by the subject's handheld response switch was digitized in synchrony with the vibratory test burst transduced by the LVDT.

An adapted staircase tracking method was used to converge upon an estimated threshold value for each TF at each stimulation site [16]. Signal amplitude changes were accomplished manually by the examiner with respect to the subject's perceptual response using a programmable logarithmic attenuator (Tucker-Davis, Model PA 4, Alachua, FL, USA) scaled in 1-dB steps. Operationally, positive subject detection trials required that the rising edge of the TTL signal fall within a restricted time range beginning at the midpoint of the vibratory burst and 2.5 s later at the end of the sampling window. All responses that fell outside of this time range were operationally defined as negative detection trials. This general procedure was used to minimize the effects of any guessing strategy by the subject. The choice of whether to increase or decrease the test signal amplitude was performed in real time by the examiner by monitoring the digitized trial and the timing of the subject's response relative to the vibratory burst on a computer monitor.

At a given stimulation site, initial amplitude levels for each TF were presented at supramaximal magnitudes followed by signal attenuation in 6 dB descending steps until the first negative detection event was recorded. After this first negative response, the next stimulus event presented to the subject was increased in amplitude by 6 dB until the subject responded positively again. From this point onward, the TF signal amplitude was continuously bracketed up and down using progressively smaller amplitude step sizes until a level was reached at which half the TF signals were detected and oscillated about a change in amplitude of 1 dB for more than five consecutive trials. Threshold for a single TF at a given stimulation site was achieved within approximately 30-50 trials. A total of 24 TF/stimulation site assessment blocks were required to complete the entire protocol (six stimulation sites×four TFs). A single assessment block consisted of the presentation and threshold determination of one of the four TFs at one of the six stimulus location sites. The testing order for stimulation site and the order of TF delivery within a given site were randomized and counterbalanced across all subjects. To mask any auditory cues associated with the Minishaker, subjects wore circumaural headphones

through which narrowband noise centered at each TF was delivered.

Subjects were provided with a 5- to 10-min period at the beginning of the session to gain familiarity with the assessment method. Total subject testing time lasted approximately 3.5 h. Frequent rest intervals of approximately 3–5 min were programmed into the session and were provided to each subject to avoid concentration fatigue. Rest periods were always taken after the complete assessment of a given TF. Subjects were consistently encouraged to focus on the task and verbally reminded during rest periods to continue their vigilance on the requirements of the task.

Data analysis

The LVDT signals were measured offline from the digitized records to determine peak-to-peak displacement values in microns. The last eight trials of a given TF series having at least four decision reversals were evaluated in order to estimate VDT. Probe displacement measures from the positive response detection trials within these last eight trials were averaged to obtain a single threshold estimate for each TF in microns. Peak-to-peak displacement measurements were performed on those periods in the middle 700 ms of the 1-s digitized LVDT waveform. A two-way repeatedmeasures ANOVA (SPSS v10.0) was performed on the main effects of TF and stimulation site (α =0.05). The interaction effect of the two main factors was also calculated. The repeated-measures ANOVA design incorporated the use of the Greenhouse–Geisser correction [14, 21]. All parametrics in this report are presented in microns.

Results

Pooled subject VDT means and standard errors for each TF vs stimulation site are shown in Fig. 2. Pooled subject VDT means collapsed across TF are shown in Fig. 3 to provide a composite depiction of site-related threshold differences. Results from the full model two-factor repeated-measures ANOVA indicated a significant main effect for stimulation site [F(5,50)=7.33, p=0.001] and for TF [F(3,30)=155.81, p<0.001]. No interaction effect between stimulation site and TF was noted [F(15,150)=2.71, p=0.08]. In general, threshold values for all test frequencies were consistent with previously reported psychophysical vibrotactile data in normal young adults obtained with either an alternative forced choice paradigm or a computerized maximum likelihood procedure, respectively [2, 4].

Stimulation site effects and planned comparisons

To isolate sources of variation of the significant main effect of site, planned comparisons were conducted between



Fig. 2 Mean vibrotactile detection thresholds (micrometers) are shown for the six vermilion stimulation sites at each of the four test frequencies (hertz). Error bars represent the standard error of the mean. Each data point represents the mean threshold pooled across N=11 subjects. Midsagittal psychometric functions for the UL and LL are shown with heavy dashed lines

the midsagittal vs left or right stimulation sites and between the left and right lateral sites for both the UL and LL. An adjusted alpha level of 0.008 (α =0.05/6 planned comparisons) was used to control for family-wise error. Paired sample *t* tests revealed significantly greater sensitivity at midsagittal stimulus sites than at lateral locations for three of the four comparisons: left LL vs midsagittal LL (*p*< 0.001), left UL vs midsagittal UL (*p*=0.008), and midsagittal UL vs right UL (*p*=0.002). The midsagittal LL vs right LL comparison, whereas not significant (*p*=



Fig. 3 Mean vibrotactile detection thresholds and standard errors (in parentheses) are shown for each stimulation site (N=11) collapsed across all test frequencies. All values in this figure are reported in units of micrometers

0.018), did approach our adjusted significance level. No significant differences between the left and right side VDT comparisons from the lower (p=0.37) or upper (p=0.73) vermilion of the lip were noted. Planned comparisons between UL and LL stimulation sites, collapsed across frequency, were also conducted. An alpha level of 0.017 (α =0.05/3 planned comparisons) was used for this analysis. Paired sample *t* tests revealed no significant differences in vibrotactile sensitivity for the following comparisons: left LL vs left UL (p=0.382), midsagittal LL vs midsagittal UL (p= 0.095), and right LL vs right UL (p=0.594).

TF effect and planned comparisons

To isolate sources of variation for the significant main effect of TF, planned comparisons were conducted between the six pairwise combinations of TFs collapsed across stimulation site using an adjusted alpha level of 0.008 (α = 0.05/6 planned comparisons). Paired sample t tests revealed significant threshold differences among all pairwise TF combinations: 5 Hz vs 10 Hz (p<0.000), 5 Hz vs 50 Hz (p<0.000), 5 Hz vs 150 Hz (p<0.000), 10 Hz vs 50 Hz (p< 0.000), 10 Hz vs 150 Hz (p<0.000), and 50 Hz vs 150 Hz (p < 0.002). As observed in Fig. 2, the lowest vibrotactile thresholds were consistently achieved with the 50-Hz TF at all stimulation sites. In comparison to the 50-Hz threshold values, the VDTs obtained at TFs of 5, 10, and 150 Hz were approximately 9.8, 4.3, and 1.1 times greater, respectively. In general, midsagittal VDT estimations were the lowest across all TFs compared to all lateral stimulation site thresholds. The midsagittal VDT psychometric functions obtained in this investigation were qualitatively similar in shape and threshold value to previously reported orofacial vibrotaction data obtained with a similar range of TFs, at a similar test site, yet by different methods of estimation [4, 18].

Discussion

Site of stimulation effects

Generally, vibrotactile sensitivity was found to be significantly greater at the midsagittal vermilion sites on the UL and LL compared to the lateral stimulation sites (Figs. 2 and 3). The only exception to this result was the borderline nonsignificant contrast between the midsagittal LL vs right LL. It is likely that this single nonsignificant contrast is an outcome of our alpha adjustment procedure and the inherent variability in the subjects' pooled perceptual responses. Excluding this comparison on statistical grounds, the bulk of our results are fully consistent with previous data suggesting that overall differences in tactile sensitivity may exist along the mediolateral surface of the vermilion [7, 23]. This can be clearly seen in Fig. 3, where the averaged midsagittal VDTs from the UL and LL, collapsed across TF, are consistently lower than averaged

VDT for lateral stimulation sites. Midsagittal sensitivity was consistently at its best across all TFs when compared to thresholds obtained at lateral stimulation sites (Fig. 2).

Data from human microneurographic recordings of the lower two divisions of the trigeminal nerve suggest that RFs are not uniformly distributed in either the LL [10] or UL [19, 20, 28]. Additionally, histological data have shown anatomical variations in peripheral innervation within the orofacial system of primates [15]. As such, greater vibrotactile sensitivity at the midsagittal vermilion sites may suggest that orofacial receptor densities and/or RF overlap differ at these locations compared to more lateral regions of the vermilion. It is generally accepted that a large degree of afferent fascicle overlap is correlated with higher tactile acuity [19, 20, 28]. Consistent with this suggestion, Nordin and Thomander [28], examining innervation zones of 66 nerve fascicles in the infraorbital nerve, reported that approximately half of all RF tested reached the midline to some extent with some RF extending 2-3 mm into the contralateral side. Extracellular afferent recordings in the cat have also demonstrated that RF overlap at the midline of the perioral region is possible up to 2 cm contralaterally [8]. Considering that the width of our probe tip was held constant and straddled the midsagittal plane of the vermilion by approximately 3–4 mm on either side, the significant decrease in midsagittal VDT compared to those obtained from lateral sites is consistent with neural integration effects associated with either increased RF density and/or an increased overlap of RFs. Confirmation of this hypothesis awaits additional fine-grain anatomical and microneurographic analyses on the distribution of cutaneous mechanoreceptors in the lip vermilion of humans.

Laterality differences in tactile sensitivity are well known for limb and hand systems [24, 36]. On the contrary, the literature regarding laterality differences in tactile sensitivity in the orofacial region is somewhat mixed. For example, James et al. [18] reported 1.33 times greater vibrotactile thresholds on the right side of the face at TF of 10 and 100 Hz, whereas Lee and Essick [23] identified greater pinprick sensitivity on the left side of the face. In contrast, other reports have found no laterality differences in the head and neck [36] or in the face during two-point discrimination tasks [7, 33], vibrotaction [22, 31], and grating orientation discrimination [37]. Our data are in agreement with those reports demonstrating no laterality differences in tactile sensitivity.

Questions of tactile sensitivity differences in the UL vs the LL have consistently shown that identical sensitivities are found for a variety of neurosensory procedures including spatial resolution [7, 31, 37], cutaneous pressure threshold, and moving two-point discrimination [31]. Our results show that vibrotactile sensitivity to a range of TF is also relatively equal along the midsagittal plane and laterally on the upper and lower vermilion. These data are in accord with the only other report that compared UL vs LL vibrotactile sensitivity (at a single paramedial testing site) using a similar range of TF [18].

VDT frequency effects

The significant variations in threshold as a function of TF are consistent with psychophysical data suggesting that as many as four channels of mechanoreceptive fibers possessing partially overlapping sensitivities can be differentially recruited to generate an operating range for the perception of vibration extending from 0.4 to greater than 500 Hz in glabrous skin [5]. In the orofacial region, however, histological and electrophysiological analyses have demonstrated that only three receptor channels may exist, those resembling the rapidly adapting type I (RA I) and the slowly adapting type I and II (SA I and II) receptor classes [19, 29, 25, 27]. Conspicuously absent in the perioral region of humans and primates are Pacinian type responses [4, 15, 18, 28]. Pacinian receptors have been shown to be most sensitive to frequency inputs in the range of 250 to 300 Hz [4, 5]. In their absence, it has been hypothesized that general vibrotactile detection capacity in the lip vermilion may be mediated instead by RA I units (correlated to the Meissner receptor) [32]. Histological data have confirmed the existence of Meissner end organs in the perioral skin of primates [15]. The nominal operating response range for the RA I receptor class has been suggested to span from 3 to 100 Hz [5] and corresponds well with the best frequency sensitivity demonstrated by our subjects to the 50-Hz TF. It is still possible that other receptor channels, such as the SA I and SA II, may also contribute to the overall capacity for vibrotactile detection in the skin given that mechanoreceptors are suggested to possess partially overlapping sensitivities to different input frequencies [5].

Functional uses

From a practical standpoint, perceptual neurosensory testing provides an efficient means to noninvasively map the integrity and health of orofacial neural pathways involved in the transmission of somatosensory inputs to central brain regions [4, 9, 11, 17]. Alterations in sensory functioning that could conceivably impact speech and other oromotor behaviors have been reported after treatment in regions innervated by the infraorbital and mental nerves [1, 3, 26, 29, 30, 35]. Several testing procedures have been employed to assess orosensory capacity in humans including twopoint discrimination [33], oral stereognosis [6], directional discrimination [13], texture discrimination [34], and vibrotactile detection [4]. Assessments such as these can be used in clinical settings to diagnose the nature and distribution of trigeminal system impairment resulting from the progression of disease processes, trauma, and/or surgical treatments [12].

An important advantage of vibrotactile testing methods is that it allows the experimenter to precisely modify a given stimulus parameter whereas maintaining other stimulus-related variables constant. Through the use of different TF, vibrotactile testing may allow the practitioner to selectively activate different afferent channels, potentially providing greater levels of diagnostic resolution and a better understanding as to the temporal characteristics of recovery of trigeminal low-threshold mechanoreceptors. These data are hypothesized to be of importance given that mechanosensory inputs generated during speech and other functional facial movements are suggested to underlie the sensory guidance of skilled motor control in this region [19, 20, 27, 28].

Conclusions

The present study reported four findings regarding the sensitivity of the lip vermilion to a range of vibrotactile stimuli: (1) midsagittal sites were significantly more sensitive to a range of vibrotactile inputs compared to lateral vermilion locations, (2) VDT varied as a function of frequency with the best thresholds achieved for the 50-Hz TF at all stimulation sites, (3) no significant differences in sensitivity as a function of laterality were noted, and (4) the UL and LL vermilion stimulation sites were found to be equally sensitive. The greater midline sensitivity to vibrotactile stimulation may suggest that RF at this location may be of greater density and/or demonstrate greater overlap compared to lateral vermilion sites.

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