The Effect of Applied Force on Two-Point Discrimination Threshold in the Trigeminal Region

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Aims: To determine the effect of alterations in applied force on the two-point discrimination (TPD) threshold and to evaluate whether these effects were gender- or site-dependent. Methods: A total of 62 healthy adults were enrolled in the study and divided into two groups based on gender (men and women, n = 31 each). The TPD test was performed using a modified compass on the forehead, upper labium, and lower labium, and each site was tested with Semmes-Weinstein monofilaments (SWMs) of sizes 4.56, 5.07, and 5.46 that exerted bending forces of 6, 10, and 26 g, respectively. The differences in thresholds according to alterations in the applied forces were evaluated for different genders and sites. Results: Both gender groups showed a significant decrease in TPD thresholds with the 5.46-size monofilament than with the 4.56- or 5.07-size monofilaments. This decrease was more apparent on the forehead regardless of gender. Conclusion: These findings suggest that increased intensity of mechanical stimuli could increase tactile acuity as measured by the TPD test. J Oral Facial Pain Headache 2019;33:371–376. doi: 10.11607/ofph.2362

Keywords: face, force, gender, spatial acuity, two-point discrimination

Since being introduced by Weber in the early nineteenth century, the two-point discrimination (TPD) test has been frequently used to assess tactile spatial resolution in the skin. The TPD threshold is defined as the minimum distance between two mechanical stimuli simultaneously applied to the skin that can be perceived as two separate points.1 The TPD test has been extensively used for clinical diagnosis as well as for scientific studies due to its simple application and easy interpretation.2 TPD is usually measured using simple handheld instruments, including an unfolded paper clip, compass, disk-criminator, andesthesiometers. Copious studies have been performed to establish the test’s reliability and validity in clinical and experimental assessments; however, several studies showed considerable variabilities in TPD measurements within subjects, between subjects, and between studies.2–5 While various factors, including within-patient and clinician factors, could have contributed to these variabilities, the lack of repeatability of the applied force has long been indicated as an important methodologic weakness of using handheld instruments in the TPD test.6,7 A previous study also revealed that the manually exerted forces could vary greatly with each application of a handheld instrument.6 Accordingly, the uncontrolled application of force might affect TPD values because the increased force leads to more skin deformation, possibly leading to the altered involvement of sensory receptors.4,6

This inherent weakness of handheld instruments led to the introduction of computerized equipment with standardized pressure.8 However, this equipment is not yet popular in clinical practice because of its limited applicability and accuracy. Standardized procedures still require the use of an improved instrument. Prior to establishing the standardization of applied force in the TPD test, it is necessary to assess the effect of the applied force on TPD. Furthermore, recent studies found that there were gender or site differences in tactile sensitivity in the orofacial region, which might influence the effect of the applied force on TPD.1,9,10
The effect of applied force on TPD needs to be evaluated in different orofacial areas in different genders. Therefore, the primary purpose of the present study was to verify the hypothesis that there is a difference in TPD threshold value when different intensities of force are applied. The additional purpose was also to evaluate the gender-dependent effects of the altered forces on different trigeminal areas. Therefore, the present study was designed to examine the differences in TPD threshold in three trigeminal regions by altering the applied force.

Materials and Methods

Participants

An advertisement for the experiment was posted in the dental hospital and school of Kyungpook National University. A total of 62 healthy adult subjects between 20 and 40 years of age were selected from applicants for even gender and age distributions after a detailed explanation of the protocol. The subjects were all students and staff from the Kyungpook National University or dental hospital. This study excluded participants with a history of cutaneous damage on the experimental regions, consumption of neurologic and analgesic medications, neurologic disorders, and/or uncontrolled diabetes. Ethical approval was obtained from the Kyungpook National University Dental Hospital Institutional Review Board (KNUDH-2018-03-002).

Experimental Protocol

The TPD test was performed using a modified compass with a pair of Semmes-Weinstein monofilaments (SWMs) instead of metal prongs. The size numbers of the monofilaments (Touch Test Sensory Evaluator, North Coast Medical) used were 4.56, 5.07, and 5.46, with the given thickness and length and estimated bending forces of 4, 10, and 26 g, respectively, as given by the manufacturer (www.ncmedical.comitem_1278.html). All filament tips were uniformly blunted into a hemisphere-like shape using dental resin (CharmFil Flow Blue, Denkist) and a template in order to limit possible morphologic variation with different tip sizes (Fig 1a).

Experimental sites were selected to represent each territory of the three trigeminal nerve branches as follows: forehead (lower third of forehead), upper labium (upper third of upper labial skin), and lower labium (upper third of lower chin skin) (Fig 1b). On the forehead, the experimental site was determined to be a point on the lower third of the midline connecting the mid-lowest point of the forehead hairline to the midpoint between both eyebrows. The upper labial experimental site was the upper third point on the midline drawn from the midpoint of the nasolabial junction to that of the vermilion border of the upper lip. The lower labial experimental site was the upper third point on the midline from the midpoint of the vermilion border of the lower lip to the lowest midpoint of the chin. These sites were marked with an eyebrow pencil, and then the modified compass was applied with its legs horizontally straddled over the upper or lower third points described above.

These sites were marked with an eyebrow pencil. During the test procedure, the subjects were asked to sit upright in a comfortable chair with their eyes closed in a quiet room with controlled temperature and humidity (temperature: approximately 25°C, relative humidity: 30% to 40%). The test was performed starting at the forehead and then proceeding to the upper and lower labia with three different filament sizes in a randomized order, with an interval of approximately 30 seconds between each of the three sessions. The two-point stimuli were simultaneously and perpendicularly applied to the cutaneous surface until both filaments bowed. Each stimulus was designed to remain for approximately 1.5 seconds with an interstimulus time gap of approximately 5 seconds using an electronic metronome (MT-301, Intelli) with a rhythmic set-up of 40 beats per minute.

The compass was applied to the skin and removed according to the beat rhythm. TPD value was measured by a single examiner using the staircase method, as described in a previous study. Subjects were first instructed to specify whether they detected one point or two points; when they answered two points, it was marked as positive. The next application was performed with a narrower distance between the two tips. This procedure was repeated until subjects answered one point, which was marked as negative. Subsequently, a series with increased distances was applied. Two blank (placebo) trials were additionally...
applied after peaks 5 and 11, as described in a previous study. This procedure continued until eight positive and eight negative peaks were measured, and the threshold was finally calculated as the average of these values (in millimeters).

Statistical Analyses

The estimated sample size was determined based on a previous TPD study using the compass and staircase method with application of G*power 3.1 program. Experimental group sizes were calculated prospectively to detect a difference in TPD mean values with α-type error, set at .05, power (1 − β) of 0.80, effect size of 0.3828365, and N2/N1 ratio of 1.23 All of the data were expressed as mean ± standard deviation (95% confidence interval). Descriptive data of two-point discrimination (TPD) threshold

<table>
<thead>
<tr>
<th>No. of monofilament size/subjects</th>
<th>TPD threshold (mm)</th>
<th>P value (among sites)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Forehead</td>
<td>Upper labium</td>
</tr>
<tr>
<td>4.56</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>12.64 ± 3.77 (11.69–13.60)</td>
<td>4.66 ± 1.44*(4.29–5.02)</td>
</tr>
<tr>
<td>Male group</td>
<td>12.32 ± 3.78 (10.93–13.71)</td>
<td>5.09 ± 1.74*(4.45–5.73)</td>
</tr>
<tr>
<td>Female group</td>
<td>12.96 ± 3.79 (11.57–14.36)</td>
<td>4.22 ± 0.89*(3.90–4.55)</td>
</tr>
<tr>
<td>P value (between genders)</td>
<td>.507</td>
<td>.017</td>
</tr>
<tr>
<td>5.07</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>11.32 ± 3.70 (10.43–12.20)</td>
<td>4.49 ± 1.36*(4.14–4.83)</td>
</tr>
<tr>
<td>Female group</td>
<td>11.25 ± 3.70 (9.89–12.61)</td>
<td>4.25 ± 1.06*(3.87–4.64)</td>
</tr>
<tr>
<td>P value (between genders)</td>
<td>.885</td>
<td>.179</td>
</tr>
<tr>
<td>5.46</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male group</td>
<td>10.03 ± 2.82 (9.00–11.07)</td>
<td>4.43 ± 1.43*(3.90–4.96)</td>
</tr>
<tr>
<td>Female group</td>
<td>9.26 ± 4.22 (7.72–10.81)</td>
<td>3.90 ± 1.14*(3.48–4.32)</td>
</tr>
<tr>
<td>P value (between genders)</td>
<td>.402</td>
<td>.113</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± standard deviation (95% confidence interval). For assessment of differences among groups, Welch one-way analysis of variance with post hoc Dunnett T3 test was used unless otherwise specified. Significant values are in bold.

Results

The male and female groups had a similar age distribution with no significant difference (P = .068); the mean ages of the male and female groups were 29.45 ± 4.20 and 27.42 ± 4.42 years, respectively. Statistical analysis revealed that there was no significant difference between the gender groups in the TPD mean threshold regardless of the test site or applied force (P > .05), except for on the upper labium using the 4.56-size monofilament (Table 1). With respect to the experimental sites, both gender groups showed that the forehead had significantly higher TPD mean threshold than the upper and lower labia (P < .01) (Table 1); however, there was no significant difference in TPD mean threshold between the upper and lower labia. Two-way repeated measures ANOVA showed that there was no significant interaction between gender and monofilament size on any of the three facial sites (P > .05) (Table 2). Further statistical analyses revealed there was a significant effect of monofilament size on TPD value (P < .01 for forehead and upper labium, P < .05 for lower labium) (Table 2); however, there was no significant effect of gender on the TPD threshold (all facial sites P > .05) (Table 2). Post hoc analysis found that TPD threshold on the forehead significantly decreased with increasing monofilament size in both the male and female groups (Table 2 and Fig 2a). On the upper labium, TPD threshold significantly decreased with increasing monofilament size in the male group, while the female group showed a significant decrease in TPD value with the 5.46-size monofilament compared to the 5.07-size monofilament (Fig 2b). On the lower
labium, there was no significant difference in TPD threshold among the different monofilament sizes in the male group, and the female group showed a significant decrease in TPD value with the 5.46-size monofilament compared to the 4.56- and 5.07-size monofilaments (Fig 2c).

**Discussion**

Tactile stimuli are composed of various mechanical variables—such as velocity, duration, frequency, and intensity—that are sensed by different mechanoreceptors. The mechanical sensory system is designed to distinctively sense and then perceive the alterations in each variable in an integrated manner. Despite the simplicity and availability of the TPD test, many researchers and clinicians were concerned that the uncontrolled force in the TPD test would affect its outcome; however, the effect of the applied force on TPD values has not yet been sufficiently studied and remains questionable.

While the stimulus waveform produced by these monofilaments is impulsive, a previous study showed that their force outcomes could yield relatively stable and reliable forces for the evaluation of pressure sensitivity. The TPD test was originally designed to selectively evaluate the slowly adapting, sensory fiber–detecting tactile stimuli and nonnoxious stimuli. Accordingly, many authors recommended very light forces of 10 to 15 g to avoid an excessive stimulus to noxious sensation. A few others also suggested a force just sufficient for the subject to assess the mechanical stimulus. To determine the effect of force during the TPD test, this study was designed to include filaments with lower, higher, or equal bending force to that of the force recommended above (10 to 15 g). However, during the preliminary study, it was found that some patients showed some avoidance responses—such as withdrawal and frowning with a painful or annoying sensation—for the TPD test using 5.88-size monofilament with a bending force of 60 g (data not shown). Previous studies showed that the increase in stimulus pressure caused the increasing involvement of noxious sensation and the neural activation of the brain area associated with pain response. Therefore, it was decided that the upper limit of the monofilament should be 5.46 with a bending force of 26 g to protect subjects from feeling annoyed and to minimize the involvement of noxious stimuli.

The application of the increased monofilament size was found to decrease the TPD mean threshold in the present study. Both groups showed that TPD values decreased with the application of the 5.46-size monofilament when compared to the application

**Table 2** Summary Data of Two-Way Repeated-Measures Analysis of Variance for Differences in Two-Point Discrimination (TPD) Mean Threshold According to Altered Monofilament Size

<table>
<thead>
<tr>
<th>Source</th>
<th>Forehead</th>
<th>Upper labium</th>
<th>Lower labium</th>
<th>Forehead</th>
<th>Upper labium</th>
<th>Lower labium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group (gender)</td>
<td>0.010</td>
<td>3.759</td>
<td>1.975</td>
<td>.919</td>
<td>.057</td>
<td>.165</td>
</tr>
<tr>
<td>Size (monofilament)</td>
<td>29.216</td>
<td>10.625</td>
<td>3.504</td>
<td>&lt; .001</td>
<td>&lt; .001</td>
<td>.036</td>
</tr>
<tr>
<td>Group*size</td>
<td>1.731</td>
<td>2.052</td>
<td>1.479</td>
<td>.186</td>
<td>.138</td>
<td>.236</td>
</tr>
</tbody>
</table>

Bonferroni post hoc test. Significant values are in bold.

![Fig 2](image-url) Mean differences in the two-point discrimination (TPD) test values for the (a) forehead (FH), (b) upper labium (UL), and (c) lower labium (LL) in both gender groups. Brackets indicate standard deviation. *P < .05 and **P < .01 for comparison of 4.56 between groups. *P < .05 and **P < .01 for comparison of 5.07 between groups. SWM = Semmes-Weinstein monofilament.
of 4.56- or 5.07-size monofilaments (Table 2). These findings suggest that tactile acuity could have a tendency to increase with an increase in applied force, without noxious evocation and regardless of gender. These force-dependent alterations in tactile acuity might be related to peripheral sensing patterns due to increased stimuli intensity. A previous study found that tactile sensory intensity was more closely related to the depth of skin indentation.4 Many studies have described that all types of cutaneous mechanoreceptors are situated at a certain depth and location6,16,17; each tactile stimulus with a different intensity could be encoded as a distinctive sensory signal pattern, as various types and numbers of mechanoreceptors were activated underneath the indented skin spot. Additionally, another study using functional magnetic resonance imaging showed that the pressure alteration with different monofilaments caused central changes in the activated area within the brain.15 Contrary to the present findings, a previous study concluded that TPD value was invariant to the force level in healthy subjects during the application of two different forces (approximately 500 and 1,000 mN).18 However, their conclusion was based on experimental measurements of only four subjects under two forces of supra-threshold level, and their study did not include a statistical analysis.18 The application of supra-threshold–level force could imply a higher involvement of nociceptive receptors. Conversely, a recent study using nonpainful electrocutaneous stimuli found that increased intensity improved the consistency, as well as the accuracy, of stimulus localization.19

The increase in tactile acuity with increasing force was more obvious on the forehead than the upper and lower labia. These findings might be related to the differences in tissue properties and tactile sensitivity. Cutaneous tissue overlying the forehead area was composed of firmly attached, relatively thin soft tissue as compared to that on the upper and lower labia. Therefore, the applied force might be less distributed and have less of a buffering effect in the thinner soft tissue. Neurohistologic studies have shown possible variations in peripheral innervation density within the trigeminal region.20,21 Additionally, previous literature established that the upper and lower labia had much higher tactile sensitivity than the forehead when measured with the TPD test, which implies that it is less likely for the alteration extent to reach any significance over normal variation in TPD values.22 However, this study did not show a significant effect of gender on the TPD threshold except for a few variables, while several studies reported that women were more sensitive than men in many sensory parameters.1,19 The present results are in accordance with previous findings that the differences in the regions of the body had more effect on sensory threshold than gender or age differences, as reviewed by Rolke et al.23

Methodologically, previous studies showed that the mechanical properties of monofilaments might vary depending on temperature and moisture.24,25 Other studies indicated that mechanical sensation using probes could vary depending on the size and shape of the probe tip.4,26 Therefore, this study was performed using SWMs with uniform tips under well-controlled circumstances (temperature: approximately 25°C, relative moisture: 30% to 40%). A previous study revealed that the presence of sharp margins in flat tips decreased the TPD value,26 and another study also revealed that the sharply pointed tip induced lower TPD values than the blunted tip.1 These findings were considered to be related to the involvement of the nociceptors and to the application of the increased pressure.1 Subsequently, the blunted tip was used in the present study to minimize the unwanted involvement of noxious stimuli for better clarification of the effect of force on the TPD value. In addition, previous studies reported that elderly persons were less sensitive and more variable to mechanical stimuli than young individuals.10,27,28 Recent literature recommends the standardization of sensory tests by controlling age, gender, and site.10,23 Accordingly, for the present study, subjects between 20 and 40 years of age were recruited to minimize the possible effects of aging. Lastly, existing literature has also described the distinct functional properties of mechanoreceptors in hairy and glabrous skin.17 To minimize the effects of make-up and hair in the study, the experimental sites of the subjects were prepared with 70% ethanol, and hair was assessed to see whether it would be too thick to properly perform the TPD test.

However, despite the authors’ best efforts, the functional mechanism of tactile sensation is not simple enough to be able to control all related variables. This study had several limitations. First, the neurophysiologic mechanism underlying the altered tactile acuity could not be provided due to the limitations of the study design. Furthermore, the intake of beverages such as coffee or energy drinks was not checked, though the effect of these beverages (due to their possible stimulating actions) on the perception of tactile acuity remains to be determined. Another limitation of the present study was that various force ranges and nontrigeminal sites were not included due to the restricted availability of subjects, who were students and staff. Therefore, further studies will be required to establish a more detailed relationship between force and tactile acuity using a wider range of force. Nevertheless, the present study provided some valid and useful findings regarding the effect of applied force on TPD outcome.
Conclusions

The application of different monofilament sizes with their corresponding bending forces showed significant differences in the TPD mean threshold, which suggests that the increased intensity of mechanical stimuli without noxious evocation could increase tactile acuity.

Acknowledgments

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References