

# Variability of Argon Laser-Induced Sensory and Pain Thresholds on Human Oral Mucosa and Skin

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The variability of laser-induced pain perception on human oral mucosa and hairy skin was investigated in order to establish a new method for evaluation of pain in the orofacial region. A high-energy argon laser was used for experimental pain stimulation, and sensory and pain thresholds were determined. The intra-individual coefficients of variation for oral thresholds were comparable to cutaneous thresholds. However, inter-individual variation was smaller for oral thresholds, which could be due to larger variation in cutaneous optical properties. The short-term and 24-hr changes in thresholds on both surfaces were less than 9%. The results indicate that habituation to laser thresholds may account for part of the intra-individual variation observed. However, the subjective ratings of the intensity of the laser stimuli were constant. Thus, oral thresholds may, like cutaneous thresholds, be used for assessment and quantification of analgesic efficacies and to investigate various pain conditions.

efficacies<sup>6</sup> and for investigation of pain syndromes.<sup>7</sup> An evaluation of mucosal thresholds in orofacial pain conditions could be of great clinical interest, but so far the laser stimulation technique has not been applied to oral mucosa or facial skin. Before pharmacological and clinical studies can be performed in this region, the variability of oral thresholds should be determined. The aim of the present study was to investigate the reproducibility and hour-to-hour and day-to-day variation of sensory and pain thresholds determined on oral mucosa compared with thresholds determined on hairy skin.

## METHODS

### Subjects

A total of 21 volunteers participated in three different experiments: nine men with a mean age of 24 years (range 23–28 years) and 12 women with a mean age of 24 years (range 20–31 years). Informed consent according to the II Declaration of Helsinki was obtained. The study was approved by the Local Ethics Committee.

### Laser Stimulation

An argon laser (Spectra Physics 168, USA) was used as the experimental pain stimulator<sup>5</sup> on the hand and oral mucosa. The output was transmitted via a quartz fiber to a specially designed handpiece, which consisted of two metal tubes assembled at a 30° angle in order to obtain an orthogonal application of the laser beam to the mucosal surface. Standardized beam diameters were secured by a fixed distance appliance. A continuous low-energy beam (0.05 W) from the argon laser marked the stimulation site. Laser output power could be adjusted from 0.05 W to 2.50 W. A 2.15 W upper limit of stimulus intensity was chosen to avoid superficial burns. The argon laser wavelengths were 488 nm (blue) and 515 nm (green), and the laser beam had a Gaussian profile. An external laser power meter (Ophir, Israel) measured the dissipated output power from the fiber. In the experiments, the stimulus

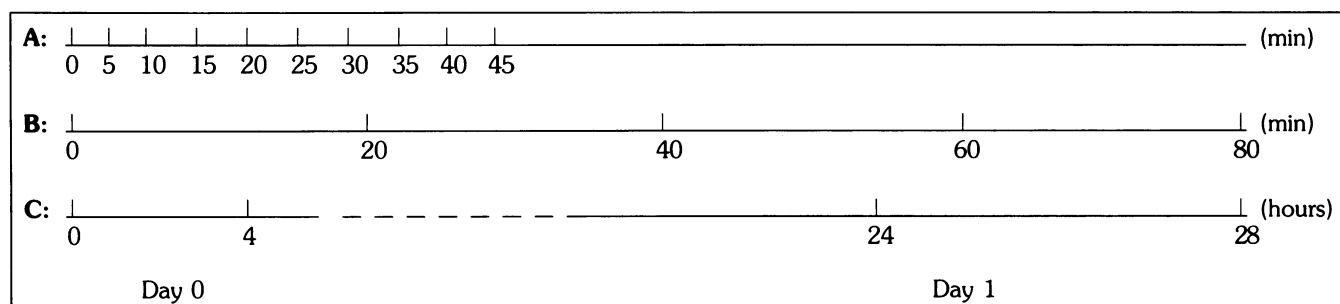
**H**igh-energy lasers are suitable for induction of experimental pain in humans, as they deliver reproducible and quantitative stimuli. Thus, cutaneous pain elicited by laser stimulation has been quantified by determination of psychophysical response thresholds.<sup>1–5</sup> In a few studies, small intra-individual and large inter-individual variations in laser-evoked thresholds have been described.<sup>3,5</sup> Cutaneous thresholds have successfully been used for the assessment of central and local analgesic

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**Figure 1.** Protocols for experiments A, B, and C. Each vertical mark indicates a test session.

duration was fixed at 200 ms, and the beam diameter was 3 mm, except on the hard palate where the beam diameter was 2 mm, to obtain a sufficient energy density to elicit pain perception.

### Threshold Determination

Prior to the experiments the subjects were carefully instructed and trained in discrimination of the laser stimuli. The sensory threshold was defined as the slightest perception of either warmth, touch, or faint pin-prick that could be realized. The pain threshold was defined as a sharp, distinct pin-prick pain perception. Both thresholds were calculated as a mean of five ascending and five descending series of stimulations, where the thresholds were reached from below and above, respectively, in a modified staircase assessment regimen.<sup>8</sup> The subjects were requested to give a verbal description of the stimuli after each threshold determination. Repeated stimulations of identical spots within the test area were avoided to exclude receptor fatigue or receptor sensitization.<sup>9</sup> The intervals between stimuli were random, with a mean of 10 sec to reduce the effect of habituation. During the experiments the subjects rested comfortably on a couch in a quiet room.

### Experimental Protocol

The reproducibility and hour-to-hour and day-to-day variations of oral and cutaneous thresholds were determined by the following three experiments (Figure 1).

**Experiment A: Variation In One Series Consisting of Ten Determinations.** Sensory and pain thresholds to argon laser stimulation were measured 10 consecutive times, each time separated by 5 min. The laser stimuli were applied on the dorsum of the left hand (C7 dermatome), the tip of the tongue, and the mucous part of the lower lip in the midline in 11 subjects.

**Experiment B: Variation Between Five Series In One Day.** On the tip of the tongue, sensory and pain thresholds were measured in five series, each consisting of three determinations. The mean of these three determinations was used for further calculations. A period of 20 min elapsed between each series. A total of 10 subjects participated.

**Experiment C: Variation Between Four Series In Two Days.** Series of three determinations were measured on the dorsum of the left hand (C7 dermatome), the tip of the tongue, the mucous part of the lower lip, and the anterolateral part of the hard palate in 10 subjects (hour 0). The mean of these three determinations was used for further calculations. Four hr later, threshold determinations were repeated (hour 4). The following day exactly the same regimen was used (hour 24) and (hour 28). The 4-hr variation was determined for each day, and the day-to-day (24-hr) variation was calculated between the days (hour 0 vs. hour 24).

### Statistics

Friedman's analysis of variance and Wilcoxon's signed rank test for paired samples were used for statistical analysis. Significance was accepted at a 5% level (two-tailed). The coefficient of variation (C.V.) was calculated intra-individually and inter-individually.

## RESULTS

### Experiment A: Variation In One Series Consisting of Ten Determinations

The perception of the argon laser stimuli at the sensory threshold level on the tip of the tongue and on the hand was consistently described as warmth and was most often described as a faint pin-prick on the mucosa of the lower lip. A distinct pin-prick pain perception was always easily recognized on the three stimulated surfaces. The subjective description of the laser stimuli remained constant

**Table 1.** Intra-individual Variation in Ten Consecutive Sensory and Pain Threshold Determinations on Hand, Tip of Tongue, and Lip Mucosa (Experiment A)<sup>a</sup>

Subject	Hand		Tongue		Lip	
	Sensory	Pain	Sensory	Pain	Sensory	Pain
1	1.2	5.1	7.3	6.6	19.3	12.2
2	1.2	11.2	0.0	6.1	2.4	5.4
3	0.0	11.3	17.8	8.2	3.9	2.2
4	0.0	12.7	6.2	9.0	6.0	5.8
5	0.0	9.8	0.9	12.2	5.2	5.4
6	1.1	6.7	0.0	5.2	10.5	3.4
7	0.0	4.3	8.1	10.4	9.4	13.6
8	10.8	4.1	18.5	8.5	13.9	7.0
9	2.9	3.5	1.9	8.7	6.7	5.2
10	5.6	3.8	1.2	6.1	5.4	6.2
11	3.9	7.8	1.8	10.2	4.5	11.0
Median	1.2	6.7	1.9	8.5	6.0	5.8

<sup>a</sup> Sensory and pain threshold numbers indicate the coefficients of variation (%).

during the 10 consecutive threshold determinations. The intra-individual C.V. for sensory thresholds in the 11 subjects ranged from 0% to 10.8% on the hand, 0% to 18.5% on the tip of the tongue, and 2.4% to 19.3% on the lower lip. The sensory C.V. on the lower lip was significantly larger than on the hand ( $P < 0.005$ ). For pain threshold determinations, the C.V. ranged from 2.2% to 13.6%, with no significant difference between the stimulated surfaces (Table 1). The inter-individual C.V. for sensory thresholds on the hand, tip of the tongue, and lower lip were 39.3%, 18.5%, and 30.3%, respectively, and for pain thresholds were 26.6%, 23.0%, and 21.4%.

On all stimulated surfaces, the median pain thresholds based on 10 determinations were significantly larger (up to 9.1%) compared with thresholds based on only three determinations ( $P < 0.032$ ).

### Experiment B: Variation Between Five Series In One Day

On the tip of the tongue the first series of sensory threshold determinations (group median 0.24 W) was significantly lower than the following four series (0.29, 0.28, 0.29, and 0.30 W, respectively; Figure 2). The same observation was made for the first series of pain threshold determinations (group median 0.65 W) and the following four series (0.73, 0.74, 0.76, and 0.76 W). For both sensory and pain thresholds, there were no significant differences between series 2, 3, 4, and 5, and the group medians varied less than 5%. The intra-individual C.V. for the five sensory and pain threshold determinations were 10.9% (range 2.9% to 19.1%) and 4.9% (range 2.5% to 23.7%), respectively.

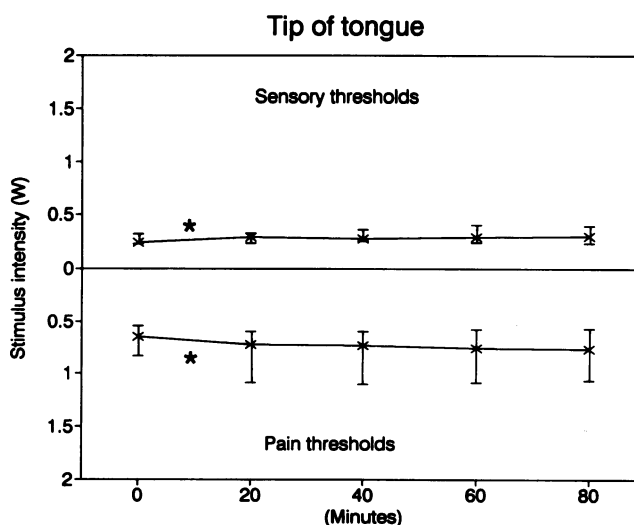
### Experiment C: Variation Between Four Series In Two Days

The sensory and pain thresholds did not differ significantly between the four series when conducted on the hand, tip of the tongue, the lower lip, or the hard palate. The subjects described the perception at both sensory and pain threshold levels consistently during the four sessions. The hour-to-hour variation for the group median thresholds on the stimulated surfaces never exceeded 9%, and day-to-day variation never rose above 8% (Table 2). The median intra-individual C.V. for sensory and pain thresholds was less than 6% (range 0% to 16.6%), with no significant difference between the stimulated surfaces.

## DISCUSSION

The variability of laser-induced thresholds on the oral mucosa has been investigated in the present study. Ten consecutive determinations, separated by 5 min, of sensory and pain thresholds on the oral mucosa and hand indicated little variation, with small ranges between the lowest and highest threshold determinations and small intra-individual C.V. (Table 1). In a previous study using the argon laser stimulation technique the intra-individual C.V. was 9.3% for sensory thresholds and 4.3% for pain thresholds on the hand.<sup>5</sup> Rohdewald and Keuth<sup>10</sup> found C.V. values ranging from 0.0% to 12.5% for electrical tooth pulp sensation thresholds with bipolar and monopolar stimulation, which was considerably less than the varia-

**Figure 2.** Median (X) sensory and pain thresholds on tip of tongue in 10 subjects. Five series separated by 20 min in 1 day (Experiment B). Vertical bars indicate range of observations. Asterisks indicate significant changes in threshold between adjacent series ( $P < 0.05$ ).



**Table 2.** Median Sensory and Pain Thresholds (Watts) on Hand, Tip of Tongue, Lip Mucosa, and Hard Palate in Ten Subjects (Experiment C)<sup>a</sup>

	Hours	Hand	Tongue	Lip	Palate
Sensory	0	0.26 (0.21–0.33)	0.24 (0.23–0.34)	0.58 (0.42–0.84)	0.94 (0.75–1.22)
	4	0.25 (0.23–0.30)	0.24 (0.23–0.36)	0.61 <sup>b</sup> (0.42–0.77)	0.92 (0.71–1.22)
	24	0.23 (0.23–0.31)	0.25 (0.23–0.40)	0.63 (0.43–0.79)	0.93 (0.78–1.22)
	28	0.24 (0.18–0.31)	0.25 (0.23–0.34)	0.63 (0.45–0.68)	0.93 (0.71–1.25)
	Pain	0	0.91 (0.45–1.23)	0.93 (0.45–1.11)	0.99 (0.67–1.26)
4		0.83 (0.42–1.23)	0.96 (0.59–1.15)	0.93 (0.59–1.16)	1.40 (0.98–2.15)
24		0.87 (0.46–1.23)	0.98 (0.60–1.15)	0.91 (0.61–1.21)	1.38 (1.06–2.15)
28		0.86 (0.38–1.24)	0.97 (0.59–1.16)	0.94 (0.62–1.20)	1.31 (1.06–2.15)

<sup>a</sup> Four series during a period of 2 days. Ranges of observations in parentheses.

<sup>b</sup> Significantly different from 0 hr ( $P < 0.05$ ).

tion of 40% to 66% in a similar study reported by Virtanen.<sup>11</sup> Electrical stimulation of the skin is characterized by a high intra-individual reproducibility, as indicated by small standard deviations on repeated measurements, and large inter-individual variation, with perception and pain thresholds ranging from 0.4 to 3.0 mA and from 1.2 to 6.0 mA, respectively.<sup>9</sup> With use of a cutaneous heat-radiation apparatus, Chapman and Jones<sup>12</sup> found an intra-individual variation of  $-2\%$  to  $6\%$  in heat pain perception compared with the values of the inter-individual variation of  $-40\%$  to  $50\%$ . In experiment A in the present study, the inter-individual variation for laser-induced thresholds was considerably larger than the intra-individual variation. Sensory and pain thresholds also showed a higher inter-individual variation on the hand compared with the oral mucosa. This observation can partly be explained by a larger inter-individual variation in skin pigmentation and hence differing light reflection and absorption properties of the skin compared with the oral mucosa.

Different variables—such as instruction to subjects, threshold determination regimen, stimulation technique, diurnal variation, and anxiety—may influence the reproducibility of psychophysically determined thresholds.<sup>13</sup> However, the present results indicate that short-term reproducibility of argon laser-induced thresholds on the oral mucosa is equivalent to values obtainable on the hand. Furthermore, the small intra-individual C.V. for laser thresholds is comparable to those found by dental electrical stimulation.<sup>10</sup> Repeated threshold determinations may increase the pain threshold level significantly, presumably due to both peripheral response plasticity<sup>14</sup> and central habituation to laser-induced pain perception.<sup>3</sup>

In experiment B the intra-individual variations for sen-

sory and pain thresholds on the tip of the tongue were very close to the findings on the hand.<sup>5</sup> However, a significant increase in both median group thresholds between the first and the following series was noted. A similar trend has also been found by Biehl et al,<sup>3</sup> indicating it may represent unavoidable habituation despite randomization of the interstimulation interval. Exclusion of the first trial score considerably reduces the variability, and this procedure has previously been recommended.<sup>13</sup> As a natural consequence of this variation, a series of training (habituation) stimuli should be included in the protocol and applied prior to the experiment.

The hour-to-hour variation for mean pain thresholds to cutaneous electrical stimulation can reach 10% depending on the time of day.<sup>15</sup> Diurnal variation in experimental pain thresholds may partly explain the hour-to-hour variation.<sup>16,17</sup> Thus, pain perception is affected by many different factors (eg, vegetative, hormonal, and psychological state factors), which themselves exhibit diurnal variation.<sup>18</sup> The low variability observed in experiment C (Table 2) could be due to effectiveness of instructions to subjects and careful determination of thresholds in a staircase assessment regimen. Finally, the argon laser evokes a distinct painful pin-prick perception, which is hard to attenuate by psychological factors and therefore easy to recognize.

## CONCLUSION

Sensory and pain thresholds determined on human oral mucosa have similar intra-individual variations as thresholds on the hand, but lower inter-individual variations. The short-term and daily variations in thresholds on both

surfaces are less than 9%. A standardized argon laser-stimulation technique can be used for evaluation of mucosal analgesia and as a tool for investigations of chronic orofacial pain conditions, such as burning mouth syndrome.

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