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Effects of Interstimulus Delay on the Sensory Discrimination: Application of Bi-Lateral Tasting for Assessment of Food and Personal Products

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Abstract

For sensory evaluation of food or personal products which have a considerable carry-over or fatigue, it is necessary to apply appropriate resting or mouthrinsing between the testing. However, interstimulus delay caused by such resting or mouthrinsing increases memory load as well as the session length required for the test. Therefore, as a solution to this problem, an alternative technique, bi-lateral tasting has been examined in comparison to normal full mouth testing. Three 2-AFC procedures with different memory requirements were used to investigate the most efficient method for assessing the irritating sensation of toothpaste: a full mouth brushing with 20 min rest (interstimulus interval) between toothpastes, a full mouth brushing with no rest between toothpastes, and a bi-lateral brushing. Significant carry-over effects were observed in toothpaste evaluation. The adapted bi-lateral testing resulted the highest sensitivity and appeared to be applicable for toothpaste evaluation with the advantage of taking less time with higher sensitivity.

Key words: bi-lateral tasting, difference test, toothpaste, interstimulus interval, memory

Introduction

The problem with assessing any set of stimuli that must be placed in the mouth is that the perception of any given stimulus is affected by stimuli that were assessed immediately beforehand. Placing a stimulus in the mouth affects the environment of the mouth and thus the sensory input from subsequent stimuli. If a sequence of foods is to be tasted in an experimental session, one of the first things that the experimenter must do is to figure out how to return the mouth to its original state before tasting the next stimulus. Thus, the experimenter must determine the appropriate interstimulus procedure to be used in any experiment, before starting. This may involve rest periods or mouth rinses.

Physiologically, the effects taking place in the mouth can involve a loss or even a gain in the perceived intensity of a subsequently tasted stimulus. Generally taste and smell involve adaptation, which involves a loss of sensitivity. Irritant stimuli can cause either increases or decreases in sensitivity. Some food or personal products involve significant irritation as well as taste and smell. For example, spicy food such as tomato-based salsa which contains capsaicin elicits lingering oral heat and involves sensitization and desensitization (Allison *et al.*, 1999). To allow the mouth to recover from the effects of a stimulus placed in the mouth, the usual procedure is to allow a rest period or mouthrinsing before tasting the next stimulus. The longer the rest period or the more mouthrinses taken, the better will be the recovery of the mouth to its initial state. However, longer rest periods or more rinsing allows more opportunity for the judge to forget the sensations elicited by the first stimulus (Avancini de Almeida *et al.*, 1999; Cubero *et al.*, 1995). So if the judge rinses a lot, he cleans the mouth but forgets the taste of the first stimulus. If he does not rinse much, he can remember the taste of the first stimulus but the taste of the second stimulus will be distorted by the altered condition of the mouth.

For the most sensitive tests, stimuli should be compared close in time. Yet, interstimulus rests or

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mouthrinses can prohibit this. Previous researches have demonstrated how delays of a few seconds between testing two stimuli, can reduce discrimination performance (Lau, *et al.*, 2004; Kinchla and Smyzer, 1967). One solution to this problem is a technique developed in the Sensory and Psychophysics laboratory at University of California, Davis, which has been called 'half mouth tasting' or 'bi-lateral tasting. This solves the problem of memory and any 'carry-over' effects. If, after cleaning the mouth, two stimuli are tasted immediately after each other, on opposite sides of the tongue, the first stimulus will not affect the oral environment for the second stimulus. This technique will work as long as the stimuli can be confined to opposite sides of the mouth. With liquid stimuli like beverages, this requires some skill on the part of the judge. Investigation has shown that many people have this skill and many do not. Yet the skill can be acquired. However, bi-lateral tasting of liquids is the most difficult case. Solid or semi-solid stimuli are easier to confine to one side of the mouth.

Different sides of the mouth may have different sensitivity. When this is a problem, it can be solved by counterbalancing the sides on which each stimulus is presented. However, for many measurements, it is not necessarily a problem. In fact, it can even be exploited.

Bi-lateral tasting has been successively used for taste testing where sensitive discrimination tests were required. In a study of the effects of irritant stimuli like sodium chloride, capsaicin, nicotine, piperine, or carbonation, bi-lateral tasting has been invaluable (Dessirier *et al.*, 1997, 1998, 1999, 2000, 2001a, 2001b; Simons *et al.*, 1999). In this case, the taste stimuli are put on the each side of the tongue for comparisons by using small size of filer papers. The question to be studied here is whether bi-lateral tasting technique can be applicable for assessing real food or personal products with fatiguing taste. One such fatiguing product is toothpaste. There has been little published research on toothpaste tasting techniques. Hyde *et al.* (1981) investigated how brushing the tongue with toothpaste tended to raise taste threshold for sucrose, NaCl, and citric acid but not caffeine. Yet, his interest was in taste perception and not in the development of better tasting procedures.

In this study, the technique of bi-lateral tasting was applied to brushing the teeth. It will be called 'bi-lateral brushing'. With bi-lateral brushing, two toothpastes were applied simultaneously with two separate toothbrushes, to opposite sides of the mouth. Judges were required to report which toothpaste was the more irritating. This method was compared with an alternative procedure where judges cleaned the teeth (full mouth) with two toothpastes, with a 20 min rest between the two brushings. Again, judges were required to report which toothpaste produced the more irritation. The 20 min rest between brushing would help the mouth to recover from the effects of the first toothpaste but would facilitate forgetting the sensations it elicited. With such memory problems, it would seem that judges might need training to use this method; it would probably not be suitable for untrained consumers. It is also a time consuming method and any alterative technique that took less time, would be an advantage. A third method was compared. Here, judges brushed their teeth with two toothpastes immediately after each other, with a brief mouthrinsing procedure in between. This method was advantageous as far as memory was concerned but not as far as any carry-over effects were concerned. Thus, for full mouth tasting, a method designed to inhibit forgetting (mouth-rinsing) and a method designed to allow the mouth recover (20 min rest) were compared with bi-lateral brushing.

The logic was to compare two toothpastes with different levels of irritants. The task of the judges was to report which toothpaste was more irritating. If there were carry-over effects, it would be anticipated that when the more irritating toothpaste was tasted first, the second less irritating toothpaste might feel more irritating than the first toothpaste. This would lead to errors in the discrimination task. This could be due to persistence of irritation (the sensation from the first toothpaste continuing while the second toothpaste was being assessed thus, by addition, increasing the overall intensity) or sensitisation (the first toothpaste rendering the nociceptor system more sensitive so that the second less irritating toothpaste might appear more irritating than it normally would have done). Either way, such carry-over effects would cause mistakes in judging

which toothpaste was more irritating.

Thus, with any full mouth tasting method, with a short time between the stimuli, carry over effects could lead to errors in discrimination. In the method that used a 20 min rest between brushing the teeth, errors might occur because the sensation elicited by the first toothpaste may not be remembered accurately while the second toothpaste was being judged. The hypothesis to be tested here was that bi-lateral brushing might eliminate both these effects. Thus, the design examined the numbers of errors when both the full mouth methods and bi-lateral methods were used. For both of the full mouth methods, the stronger toothpaste was tasted both first and second. For the bi-lateral (half mouth) method, the stronger toothpaste was presented both to the left and to the right sides of the mouth. Thus, with this counterbalancing required with each of the three testing procedures (hence two sessions for each procedure), each judge was tested in six experimental sessions.

Materials and Methods

Judges

Seventeen judges: students, visitors, and friends (12 females, 5 males; age range 20-32 yrs.) were recruited from the campus of the University of California, Davis.

Stimuli

Two toothpastes with the same flavor but different irritant (menthol) levels were used. The stronger one (higher irritant level) was designated as T1. The weaker one was designated as T2. The toothpastes were applied with toothbrushes obtained from a local store (Tek Professional Straight Toothbrushes: 6/12's Soft; Playtex Products Inc. Dover Delaware). The toothbrushes came in four colours: red, blue, green and purple. Each judge was assigned a pair of brushes to be used exclusively by him. For convenience of identification, the two brushes were different colours. The colours of the brushes were assigned randomly over judges. The data obtained indicated that the colour of the brushes did not bias the judgments in any way.

A smear of the appropriate toothpaste was applied to each brush. Excess toothpaste was not used so as to avoid excess frothing in the mouth; this avoided any 'crossing over' effects during the half mouth brushing. The quantity of toothpaste applied to the brushes ranged 0.45-1.15 g (mean weight 0.8 g) for the bi-lateral (half mouth) condition and 0.41-1.38 g (mean weight 0.84 g) for the full mouth condition.

Procedure

Judges came for at least seven sessions. Six were experimental sessions while the seventh was a preliminary practice session. Some judges required more than one practice session: one judge required 2 practice sessions, three required 3 sessions, one required 4. During the experimental sessions, judges were required to assess which of the two toothpastes (T1 or T2) was the most irritating. The procedure for judging the more irritating of the two is variously called the paired comparison method or 2-AFC (2 Alterative Forced Choice).

The six experimental sessions were split into three conditions. In one condition, judges performed 2-AFC tests using the bi-lateral (half mouth) brushing procedure. In a second condition, judges brushed their teeth using the whole of the mouth with a 20 min wait between toothpastes. In the third condition, judges brushed their teeth using the whole of the mouth with no waiting period between toothpastes. In this condition, judges rinsed the mouth immediately after brushing with the first toothpaste and then immediately brushed with the second toothpaste. Judges took either one or two rinses according to the residual sensation in the mouth after the first toothbrushing.

The presentation of the toothpastes T1 and T2 in each condition was counterbalanced. For the bi-lateral (half mouth) brushing, toothpaste T1 was applied to the left hand side of the mouth; T2 was applied to the right hand side. In a second session, these sides were reversed. In the other two whole mouth brushing conditions, T1 was applied before T2 in one experimental session, while the reverse was true for the second session. Thus, with two experimental sessions for each of the three conditions, judges performed a total of six experimental sessions. Judges performed the six experimental sessions in an assigned random order. When one judge performed a

given random order, a second judge always performed the reverse order. This provided counterbalancing for any learning effects that might have taken place during the experiment.

In the bi-lateral (half mouth) condition, the judges first took a single mouthrinse. They were then presented with two toothbrushes, with the toothpastes already applied and moistened with water. They then inserted a brush into each side of the mouth. The toothbrushes were positioned between the cheek and the outer surface of the teeth; the bristles facing the teeth. They then began brushing (backwards and forwards) the outside surfaces of the teeth, confining their action to the appropriate single side of the mouth. In this way, they generated two sets of foam, each confined to a separate side of the mouth. Judges parted the teeth slightly to allow the foam to stimulate the respective sides of the tongue. They continued brushing until the irritation had built up to easily perceptible levels. At this point, they had their first opportunity to judge which toothpaste was the more irritating. They then expectorated and rinse the mouth once or twice depending on how well they managed to expel the foam from the mouth. They then paused to consider the residual irritation in the mouth. At this point, they had a second opportunity to judge which side of the mouth was more irritating. This second judgment was used as a confirmation of the first judgment. All judges were internally consistent in their two judgments. However, some judges found that they were unable to decide on one of the judgments, so they used the other judgment opportunity to make their final assessment. Judges reported that they found the bi-lateral (half mouth) brushing procedure easy to perform. The time taken for brushing was recorded for each judge. These brushing times ranged 8-94 sec (mean time 41.5 sec).

The two full mouth conditions were the same as the bi-lateral (half mouth) condition, with the following modifications. The judges were presented with the toothbrushes one at a time. When they were brushing the teeth, they brushed on both sides of the mouth rather than one side. They also brushed on the inside surfaces of the teeth as well as the outside. This allowed the foam to stimulate the tongue, now on both sides of the mouth. They were timed while they were brushing their teeth with the first toothpaste and it was arranged that they brushed for an equal amount of time with the second toothpaste. They brushed until the irritation was easily perceptible and then noted the intensity of irritation. They also noted the intensity of irritation after expectoration as in the half mouth condition. After completing the brushing protocol with the second toothpaste, they reported which of the two toothpastes elicited the more irritation. The second toothpaste was presented either after a 20 min rest period or after 1-2 mouth rinses. The brushing times for the full mouth conditions ranged 11-91 sec (mean time 38.6 sec).

Session lengths for the bi-lateral (half mouth) brushing ranged 2-34 min (mean time 7 min 36 sec), depending on how much the judge wished to stay and talk. For the full mouth condition with 20 min interstimulus rest, session lengths ranged 21-35 min (mean time 26 min 36 sec). For the full mouth condition with the interstimulus rinsing, session lengths ranged 3-35 min (mean time 7 min 24 sec).

Before starting any of the experimental sessions, judges were given practice sessions. For these sessions, a variety of commercially available toothpastes of differing irritation were used. Judges learned to identify the irritation sensation elicited by the toothpastes. They also practiced the various brushing procedures. They were not allowed to begin the experiment until it was assured that they were capable of estimating the degree of irritation and were confident in the use of the various brushing procedures.

The final protocols adopted in this experiment were the result of lengthy preliminary experimentation not reported here.

Results

Each of the 17 judges performed once in each of the six experimental sessions. The number of judges who performed correctly in each experimental condition was noted. For the statistical analysis of the data, the binomial comparison of proportions (binpro) was employed by looking up the binomial table and d¢ values were calculated by using IFPrograms (Institute for Perception, Richmond, VA) and the results were

given in Table 1. While the results of binomial analysis indicates the significance of the results for each test protocol, the results of d¢ values, indicates the performance of the test protocol measuring the difference between the two stimuli and allows the comparisons between the different test protocols.

As seen in Table 1, the bi-lateral (half mouth) brushing condition elicited the highest proportion of correct responses and d¢ values. Examining the overall responses for each protocol condition (N=34), the proportion correct for bi-lateral (half mouth) brushing (30/34) is significantly greater (binpro, p=0.052) than for full mouth brushing with interstimulus mouthrinsing (22/34). For the full mouth protocol with 20 min rest, the difference in proportions did not reach significance (binpro, p=0.14).

However, an examination of the overall responses (N=34) does not indicate the true picture. The full mouth performance was comparable to the bi-lateral (half mouth) performance when the stronger stimulus was assessed after the weaker stimulus. However, when the stronger stimulus was assessed first, the number of correct responses was significantly reduced. The proportion correct for full mouth brushing with interstimulus mouthrinsing (6/17) was significantly less (binpro, p=0.0004) than the proportion for full mouth brushing (22/34). The same is true for full mouth brushing with 20 min rest (11/17), although the difference in proportions did not reach the significance (binpro, p= 0.11).

Such comparisons between protocols of each experimental session can be more clearly seen in terms of d' values. As seen in Table 1, for bi-lateral brushing and full mouth brushing with 20 min rest, d' values calculated from the two different sessions (stronger stimulus on 'left or right', or 'first or second') was not significantly different, yet, for full mouth brushing only with interstimulus rinsing, d' value calculated from the session whereas the stronger stimulus was taken second was significantly higher than d' value calculated from the session with the other condition. In fact, when the stronger stimulus was taken first, negative d' value was obtained indicating that judges tended to give reverse response.

Discussion

The procedure with 20 min interstimulus interval (delay) between toothbrushing would have appeared to create a considerable memory load on the judges, who would have had to remember the sensations elicited by the first toothpaste when tasting the second toothpaste. To reduce this memory load, the two toothpastes could be tasted soon after each other, with mouthrinsing to reduce any carry-over effects. This latter procedure clearly did not improve performance. It was seen that any gain in eliminating forgetting effects was more than offset by the loss due to carry-over effects. In fact, the performance in the full mouth conditions with 20 min rest was surprisingly good. Judges did not have to

Table 1. Number of correct responses and d' value for each protocol

Protocol	Session	No. of corrects		d′ (variance of d′)	
		For each session (Out of 17)	For each protocol (Out of 34)	For each session[1)]	For each protocol[2)]
Bi-lateral (half mouth) brushing	Stronger stimulus on left	16	30	2.21[a] (0.473)	1.68[f] (0.157)
	Stronger stimulus on right	14		1.31[a] (0.255)	
Full mouth with 20 mins interstimulus interval	Stronger stimulus taken first	11	24	0.53[b] (0.195)	0.77[ef] (0.103)
	Stronger stimulus taken second	13		1.02[b] (0.244)	
Full mouth only with interstimulus rinsing	Stronger stimulus taken first	6	22	-0.53[c] (0.167)	0.53[e] (0.097)
	Stronger stimulus taken second	16		2.21[d] (0.473)	

[1)]Within protocols, d' values with the same letters are not significantly different (chi-square test, p<0.01).
[2)]Between protocols, d' values with the same letters are not significantly different (chi-square test, p<0.01).

undergo any particularly long training regime to be able to remember the perception of the first toothpaste, while brushing with the second toothpaste.

Performance in the bi-lateral (half mouth) condition, however, was superior to both full mouth techniques. The difference reached statistical significance for the full mouth with interstimulus mouthrinsing; for the full mouth with 20 min rest, the trend was apparent, while the significance levels were close to 0.1. This would indicate that the trend was present but would need a larger sample of data to reach significance levels of 0.5.

Thus, the procedure with 20 min interstimulus interval (delay) between toothbrushing of full mouth faired quite well in this study but not as well as bi-lateral (half mouth) brushing. Even if the two procedures had been equal in sensitivity, the bi-lateral (half mouth) procedure has the advantage of taking less time. It is also important to note that the slight difference in sensitivity between the two tests might be much larger if the difference in irritation potential between the two toothpastes were smaller. The closer the sensations elicited by the two toothpastes, the more difficult it is to compare the sensations from the second toothpaste with the decaying memory of the first toothpaste.

Thus, it would appear that the bi-lateral (half mouth) brushing technique has the advantage of taking less time with a slight increase in sensitivity. This has the potential to increase the number of toothpastes that can be tested in one session. The number can be doubled by testing two toothpastes initially and then two more after a 20 min rest. Further research might be able to reduce the 20 min resting time and so increase even further the number of toothpastes that could be tested in a single session.

Standards testing procedures (including resting and mouthrinsing) are available for the sensory evaluation of ingredients eliciting oral heat, such as red peppers and low heat chilies (ASTM 1994; Scoville 1912) and finished products, such as salsa (Allison *et al.*, 1999). Yet, these reported procedures recommend a long interstimulus delay which limits the number of samples can be tested in a session. For example, for tomato salsa, it was reported that seven samples of medium-heat salsa could be tested daily with at least 16 min between samples and liberal rinsing with crackers and water (Allison *et al.*, 1999). Therefore, application of bi-lateral tasting technique to evaluate oral heat of such spicy food should be explored further to improve evaluation efficiency.

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