THE RECOVERY PERIOD OF THE TASTE RESPONSES OF RAT CHORDA TYMPANI AFTER APPLICATION OF TOOTHPASTE

Yasuyuki TOYONO and Junzo NABESHIMA
Biological Science Laboratories, Lion Corporation
202 Tajima, Odawara, Kanagawa Prefecture, 256, Japan
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Abstract......The recovery period of the taste responses of rat chorda tympani after application of toothpaste was examined.

The response observations of four kinds of essential taste stimuli, 0.1 M sodium chloride, 0.005 M quinine hydrochloride, 1.0 M sucrose, and 0.05 M tartaric acid, were repeated every 2 or 5 minutes. After five observations of summed responses of the chorda tympani with each taste stimulus, a toothpaste solution diluted 3 to 1 was applied to rat tongues for 3 minutes and then they were washed with distilled water for 1.5 minutes. Thereafter, observations of the responses to each taste stimulus were repeated at 2 or 5 minute intervals for 30 or 60 minutes.

The responses caused by four kinds of essential taste stimuli became smaller immediately after application of toothpaste, and then gradually returned to normal. The same experimental procedure was also applied using an application of distilled water instead of an application of toothpaste to rat tongues. A t-test was performed between the response ratios after application of toothpaste and those after application of distilled water. Significant differences disappeared at 18 minutes after application on the 0.1 M sodium chloride stimulus, at 23 minutes on the 0.005 M quinine hydrochloride stimulus, at 15 minutes on the 1.0 M sucrose stimulus, and at 15 minutes on the 0.05 M tartaric acid stimulus.

The authors estimated that all kinds of taste responses recovered within at least 50 minutes.
Key words: taste, toothpaste, chorda tympani, recovery period

INTRODUCTION

It is well known that some foods and drinks become unsavory after cleaning of the teeth with toothpaste. Nitta et al. (1973) observed the effects of several surfactants,
ingredients of toothpaste, upon the taste responses of rat chorda tympani, and found that ionic surfactants suppress taste responses more markedly than nonionic surfactants. Sugihara (1979) obtained almost the same results as Nitta et al. (1973) from a comparative examination of a nonionic surfactant, sucrose fatty acid ester, with sodium dodecylsulfate, an anionic surfactant. Furthermore, Skoubys and Zilstorff-Pedersen (1955) reported that menthol, which is also an ingredient of toothpaste, increased the taste threshold in man for 5-10 minutes, though it decreased it later. These studies did not however, describe the effects of toothpaste itself on taste responses.

It is important to examine how long it takes to recover from the change in taste after cleaning the teeth with toothpaste, since a long recovery period is uncomfortable in our daily lives. Due to psychological factors, it is very difficult to examine accurately the recovery period in man. Therefore, it is necessary to establish an experimental method in animals which is applicable for estimating the recovery period of changed taste in man.

The present research was undertaken to estimate the recovery period of the taste responses of rat chorda tympani after application of toothpaste and to determine whether it provides satisfactory information for this aim.

MATERIALS AND METHODS

Animals: Forty-eight male Sprague Dawley rats (Charles River Japan, Inc., Atsugi city, Japan), ranging in weight from 250–350 g, were used.

Recording of responses: After each rat was anesthetized with an intraperitoneal injection of sodium pentobarbital (85 mg per kg body weight), the trachea was cannulated to prevent disturbance of respiration by the applied solutions. Then, the head was immobilized by applying a metal clamp to it, and the animal was grounded via this head clamp. Next, the chorda tympani nerve was exposed, freed from surrounding tissues, and cut at the point where it enters the skull as described by Nitta et al. (1973). Then, it was put on a 100 μm diameter platinum electrode, and a silver wire was placed on the surrounding tissue as an indifferent electrode. Impulses from the nerve were recorded with an ink-writing recorder (Ohkura, Desk Top Recorder) via a preamplifier (San-Ei Instrument, 6R06), an integrator (San-Ei Instrument, Type 1317) (the time constant being 0.5 seconds), and a main amplifier (San-Ei Instrument, 6L5). The tip of the tongue was clipped and pulled out of the mouth for application of solutions. Gustatory stimulation and application of toothpaste were performed to the anterior two-thirds of the tongue, which contains the fungiform papillae innervated by afferent axons of the chorda tympani.

Gustatory stimulation: Four kinds of solutions, 0.1 M sodium chloride, 0.005 M quinine hydrochloride, 1.0 M sucrose, and 0.05 M tartaric acid, were used as representatives of four essential tastes. Each solution was kept at 25°C to avoid any effect of temperature (Yamashita and Sato, 1965), and was applied to the tongue gravitationally at a flow rate of about 16 ml per minute. The sucrose solution was applied to the tongue for 1 minute, while the others were applied for 5 to 10 seconds. Their application was
The recovery period of taste response carried out every 2 or 5 minutes.

*Application of toothpaste:* The composition of the toothpaste used in this experiment is shown in Table 1. The toothpaste was diluted 3 to 1 by distilled water and mixed sufficiently by a magnetic stirrer. Application was also performed gravitationally at a flow rate of about 11 ml per minute.

<table>
<thead>
<tr>
<th>components</th>
<th>concentration (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>secondary calcium phosphate</td>
<td>50.0</td>
</tr>
<tr>
<td>sorbitol</td>
<td>10.0</td>
</tr>
<tr>
<td>glycerin</td>
<td>10.0</td>
</tr>
<tr>
<td>sodium carboxymethyl cellulose</td>
<td>0.50</td>
</tr>
<tr>
<td>carrageenan</td>
<td>0.50</td>
</tr>
<tr>
<td>sodium saccharin</td>
<td>0.15</td>
</tr>
<tr>
<td>spearmint</td>
<td>0.35</td>
</tr>
<tr>
<td>peppermint</td>
<td>0.35</td>
</tr>
<tr>
<td>menthol</td>
<td>0.20</td>
</tr>
<tr>
<td>anethole</td>
<td>0.10</td>
</tr>
<tr>
<td>ethyl parahydroxybenzoate</td>
<td>0.05</td>
</tr>
<tr>
<td>butyl parahydroxybenzoate</td>
<td>0.05</td>
</tr>
<tr>
<td>sodium dodecylsulfate</td>
<td>2.00</td>
</tr>
<tr>
<td>deionized water</td>
<td>25.75</td>
</tr>
</tbody>
</table>

*Observation:* After five observations of summated responses of the chorda tympani with each taste stimulus, the tongue was treated with the toothpaste solution for 3 minutes and washed with distilled water for 1.5 minutes. Then, the observations of the responses to each taste stimulus were repeated at 2 or 5 minute intervals for 30 or 60 minutes.

*Calculation:* The response value was determined by the height from the base line to the peak of the summated response curve. The mean of 5 response values obtained before application of toothpaste was used as the control value and the response ratio was calculated as follows:

\[
\text{response ratio} = \left( \frac{\text{response value}}{\text{control value}} \right) \times 100
\]

Six experiments using 6 rats were carried out on one of the four kinds of gustatory stimuli, and the means and standard errors were calculated on the response ratios obtained after application of toothpaste.

The same experimental procedure was also applied to animals treated with distilled water instead of toothpaste.

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RESULTS

Figure 1A shows the summated response of the chorda tympani to the 0.1 M sodium chloride stimulus. The response became smaller at 2 minutes after application of toothpaste (B), and recovered gradually (C, D). Washing of the rat tongue with distilled water removed each taste stimulus and the toothpaste stimulus quickly, and the summated response curve returned to the base line.

The response ratio of the summated response of the chorda tympani to 0.1 M sodium chloride fell to 44.5% (mean, n=6) at 2 minutes after application of toothpaste and recovered gradually as shown in Figure 2, while, application of distilled water instead of toothpaste resulted in a slight increase in the response ratio (108%, n=6) at 2 minutes after application and no remarkable change in the response ratio with elapsing time (Figure 2). A t-test was performed between the response ratios after application of toothpaste and those after application of distilled water. As a result, any significant difference (p<0.05) was observed to disappear at 18 minutes after application.

The response ratios at 2 minutes or at 5 minutes after application of toothpaste were 16.9% (mean, n=6) to 0.005 M quinine hydrochloride, 56.6% (mean, n=6) to 1.0 M sucrose, and 55.0% (mean, n=6) to 0.05 M tartaric acid, respectively. All response ratios recovered gradually as shown in Figure 3, Figure 4, and Figure 5.

On the other hand, those after application of distilled water were 114.0% (mean, n=6) to 0.005 M quinine hydrochloride, 95.5% (mean, n=6) to 1.0 M sucrose, and 103.3% (mean, n=6) to 0.05 M tartaric acid. However, they declined gradually as shown in Figure 3, Figure 4, and Figure 5. Significant differences (p<0.05) after application of 0.005 M quinine hydrochloride, 1.0 M sucrose, and 0.05 M tartaric acid disappeared at 23 minutes, 15 minutes, and 15 minutes, respectively.

![Figure 1](image)

**Fig. 1** The summated response of the chorda tympani to the 0.1 M sodium chloride stimulus was observed (A). The response became smaller after application of toothpaste (B), and recovered gradually (C, D).

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Fig. 2 Effect of toothpaste on the summated responses of the chorda tympani to 0.1 M sodium chloride (filled circles). The responses after application of toothpaste are expressed in percentages of the control responses (response ratios). Each point and bar indicates the means and S. E. from 6 experiments. The open circles and bars indicate the means and S. E. of response ratios in rats treated on the tongue with distilled water instead of the toothpaste solution. Any significant difference (p<0.05) disappeared at 18 minutes after application.

Fig. 3 Effect of toothpaste on the summated responses of the chorda tympani to 0.005 M quinine hydrochloride (filled circles). The responses after application of toothpaste are expressed in percentages of the control responses (response ratios). Each point and bar indicates the means and S. E. from 6 experiments. The open circles and bars indicate the means and S. E. of response ratios in rats treated on the tongue with distilled water instead of the toothpaste solution. Any significant difference (p<0.05) disappeared at 23 minutes after application.
Fig. 4  Effect of toothpaste on the summated responses of the chorda tympani to 1.0 M sucrose (filled circles). The responses after application of toothpaste are expressed in percentages of the control responses (response ratios). Each point and bar indicates the means and S.E. from 6 experiments. The open circles and bars indicate the means and S.E. of response ratios in rats treated on the tongue with distilled water instead of the toothpaste solution. Any significant difference (p<0.05) disappeared at 15 minutes after application.

Fig. 5  Effect of toothpaste on the summated responses of the chorda tympani to 0.05 M tartaric acid (filled circles). The responses after application of toothpaste are expressed in percentages of the control responses (response ratios). Each point and bar indicates the means and S.E. from 6 experiments. The open circles and bars indicate the means and S.E. of response ratios in rats treated on the tongue with distilled water instead of the toothpaste solution. Any significant difference (p<0.05) disappeared at 15 minutes after application.
The recovery period of taste response

DISCUSSION

It is very difficult to examine accurately the recovery period from changes in taste responses in man due to psychological factors. Therefore, it is necessary to establish an experimental method in animals which is applicable for estimating the recovery period of changed taste in man.

Diamant et al. (1965) clarified that neural responses are closely associated with psychophysical responses with regard to the intensity of taste, therefore suggesting the possibility that the taste responses of rat chorda tympani may be used in estimating the recovery period of changed taste in man.

Each taste response is composed of two states as shown in Figure 1. The former state involves a phasic strong response which is completed in 1-2 seconds, while the other is a steady state following the phasic state. Bealer (1978) found that the intensities in the phasic state of rat chorda tympani responses reflect well the results obtained from behavioral observations. Therefore, in this study, the intensities of the responses were obtained from the phasic state.

Hellekant et al. (1979) indicated that the intensity of rat chorda tympani responses changes with elapsing time. Their results signify that the time which lapses until the response ratio reaches 100% is not necessarily the same amount of time required for the changed taste to recover. In fact, the results of the present research showed that the taste responses to 0.005 M quinine hydrochloride, 1.0 M sucrose, and 0.05 M tartaric acid declined gradually even if distilled water was applied to the rat tongue instead of toothpaste. Consequently, the recovery period was estimated by comparison with control response ratios which resulted from application of distilled water to the rat tongue instead of toothpaste.

The results of the t-test showed that any significant differences disappeared at 18 minutes after application of the 0.1 M sodium chloride stimulus, at 23 minutes after application of the 0.005 M quinine hydrochloride, at 15 minutes after application of the 1.0 M sucrose, and at 15 minutes after application of the 0.05 M tartaric acid. Though significant differences disappeared at the each time, the extra period should be considered for estimating the recovery period. The authors estimated from Figures 2-5 that all kinds of taste responses recovered within at least 50 minutes.

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REFERENCES