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Effect of Facial Expression of Mother on 15–21-Month-Old Infants Using Salivary Biomarker

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The objective of this study is to determine the possibility of evaluating stressful events such as facial expressions of mothers in linguistically incommunicable infants using salivary alpha-amylase (sAMY) activity. Eleven healthy Japanese infants between 15 and 21 months old were enrolled. Both a smile and grimace of their own mothers were shown to the subjects for 1 min as a stressor. sAMY activity in the infants was analyzed using a sAMY monitor before and after exposure to the stressors. Both facial expressions and behaviors of the subjects were recorded using a video camera and then the data were analyzed using a behavior coding system. Furthermore, the emotions of the subjects during the test period were subjectively evaluated by their mothers using a visual analogue scale (VAS). A significant correlation was observed between the mothers and the infants in terms of psychological state evaluated using the behavior coding system ($p < 0.01$, correlation coefficient (R) was 0.81). The sAMY activity during the grimace exposure was significantly higher than that during the smile exposure ($p < 0.05$). However, a correlation between VAS score and sAMY activity was observed ($p < 0.05$, $R = -0.26$). It was indicated that salivary biomarkers may be used for evaluating noninvasively the stressful events experienced by infants .

1. Introduction

Observations and self-reports of stressful event exposure, appraisals, and distress ratings are useful for improving the quality of life, but their reliability and validity are limited particularly in linguistically incommunicable infants.[1] Regarding the quantitative evaluation of psychosomatic effects, conventional methods are used,

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including subjective evaluations using a questionnaire and physical measurements such as electroencephalography (EEG), blood pressure and heart rate measurements. However, these physical measurements require the subjects to be physically restrained. Thus, identifying salivary biomarkers that reflect human stress-responsive peripheral systems has the advantage of being noninvasive.[2]

Communication in growing infants is very important because they develop through communication with mothers and caregivers.[3,4] We have been interested in evaluating stressful events in infants because they are unable to communicate verbally. In general, caregivers read pleasure-pain feelings of infants based on their facial expressions and behavior. However, these nonverbal signs may be overlooked. Researchers reported that infants over 3 months of age could already recognize their mothers' smile or grimace as a comfortable or an uncomfortable stressor, respectively.[5] Furthermore, there is a study that infants about 7 months of age can already recognize the standard facial expressions of surprise, fear, happiness, anger, and sadness.[6] The human face plays a fundamental role in social communication for infants. Many studies have shown that infants recognize the differences between positive and negative facial expressions at the behavioral or neural level.[7,8] This important ability to recognize facial expressions of emotions develops early in humans. Thus, we used a mother's smile as a comfortable stressor and a grimace as an uncomfortable stressor.

The human stress system is controlled by both the hypothalamus-pituitary-adrenocortical axis (HPA axis), which secretes cortisol, and the sympatho-adrenomedullary (SAM) system, which secretes catecholamines. If biomarkers related to these stress systems can be noninvasively measured, they can be useful as a stress index. In particular, salivary cortisol (sCORT) is a stress hormone whose level is associated with the level of serum cortisol.[9]

Recently, reports on the evaluation of human psychological stress using salivary alpha amylase (sAMY) activity have been reported.[10,11] In the 1970s, it was revealed that in the salivary gland, the secretion levels of water from alpha receptors and proteins such as amylase from beta-receptors increase as a reaction to peripheral adrenalin.[12,13] Because changes in sAMY are blocked by beta-blocker dosing, salivary amylase secretion can be considered as a direct mechanism of adrenergic activity.[14] Amylase is secreted from the salivary gland in response to sympathetic stimuli.[15] Chatterton *et al*. reported that there is a good association between the concentration of salivary amylase and levels of blood cathecholamines.[16] A study showed that in the presence of a stressor, sAMY activity significantly and promptly increases compared with the sCORT level.[17] Thus, sAMY can be considered as a useful indicator for estimating the activities of the SAM system.

There are some reports on the evaluation of stress in infants by measuring the sCORT level.[18–21] In studies of infants, performing measurements at rest is a difficult task; therefore, the use of sAMY, which can be analyzed instantly, is considered desirable. However, there have been no reports on the evaluation of stress in infants using sAMY.

The objective of this study is to determine the possibility of evaluating stressful events such as facial expressions of mothers in linguistically incommunicable infants using sAMY activity. The smile and grimace of mothers were used as the positive and negative stimuli, respectivity. Both the positive and negative facial expressions and behaviors of the infants and the mothers were estimated by a behavior coding method. sAMY activity and sCORT level were measured at the same time before, during, and after stressor exposure. By comparing the results, the usefulness of sAMY for evaluating stressors in infants was studied.

2. Method

2.1 *Subjects*

The subjects were eleven healthy Japanese infants of 15 to 21 months old (six boys and five girls, mean ± SD: 17.6 ± 1.9 months). Infants that regularly took naps were selected. During the test period, the subjects were prohibited from taking any food or drinks other than water. The stressors were presented in a randomized-double-blind design.

The study protocol was approved by the Ethical Committee of Iwate University. The study protocol was clearly explained to all the mothers of the subjects in both spoken and written forms, specifically focusing on the purpose of the study, the precise procedures that would be used, and any possible adverse effects. Signed, informed consent was obtained from each mother who enrolled in the study.

2.2 *Measurement of biomarker*

To conduct the experiment in a daily life environment, a handheld salivary amylase activity monitor (salivary amylase monitor, 59-103, Nipro Co., Japan) was used.(22,23) This handheld monitor consists of a disposable test strip and an optical analyzer (130 ×87×40 mm^3, 190 g), which is incorporated within an automatic saliva transcription device. Prior to the test, the saliva-collecting test strip was placed in the mouth of a subject several times in order to accustom them to the collection of saliva. The mothers collected saliva using the teststrip and then analyzed the salivary amylase using the salivary amylase monitor.

To measure the sCORT level, a small triangular-shaped absorbent sponge attached to a plastic rod was inserted in the mouth of an infant (length, 70 mm swab; 5029, Sorbette, Salimetrics LLC, USA). Saliva samples were collected and placed in a plastic sample tube. The collected saliva samples were frozen and stored at −30°C until analysis. The sCORT level was assayed using enzyme-linked immunosorbent assay kits (1-3002, Salimetrics LLC, PA).

2.3 *Stress evaluation*

The experiments were conducted at the same time of the day for each subject to avoid variation of the circadian condition. The subjects and their mothers were left on their own in an experimental room, which reproduced the environments similar to those in their daily life (Fig. 1, baseline period). Thirty-five minutes after entering the room, the mother's facial expressions were shown to the subject for 1 min. While the mother was presenting stimuli to the subject, the subject sat in a baby chair face to face with the mother with no physical contact between them (stress period). sAMY activity was measured at the baseline and poststress periods. The sCORT level was measured

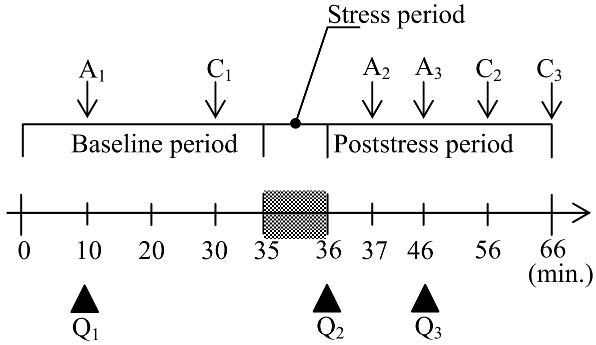

Fig. 1. Protocol for evaluating the responses of infants to psychological stress. $A_{1\text{–}3}$: collection of saliva samples and analysis of salivary amylase activity, $C_{1\text{–}3}$ collection of saliva samples and analysis of salivary cortisol concentration, $Q_{1\text{–}3}$ scoring using visual analog scale.

at the baseline period, 20 and 30 min after the stress period, because the reaction of this hormone is delayed by 20–30 min after the presentation of stimuli.

Changes in facial expressions of both the mother and infant from entering into the room until the end of the test were video-recorded. Using a behavior coding system (PTS-113 version 2292 DKH Corporation, Japan),[24,25] behavior analysis was conducted. The coding system was used to calculate the continuation time of the facial expression. The percentage of time during which a smile appeared was classified as a positive value, whereas that during which still faces and grimaces appeared was classified as a negative value. The subjects were categorized into five classes on the basis of the extracted facial expressions and behaviors (Table 1). Furthermore, the mothers filled out forms using VAS to evaluate their infants' emotions.[26]

2.4 *Statistical analyses*

Within-group comparisons were performed using the paired Wilcoxon test (SPSS 14.0J, SPSS Japan, Tokyo, Japan). Unless otherwise stated, all data are expressed as mean ± standard deviation (SD). Correlation analysis was performed and the correlation coefficient is shown using R.

3. Results

Figure 2 shows the differences between positive and negative values (psychological state) in behavior analysis. The psychological state of the mothers during the smile exposure showed a significantly higher value in the stress period than in the baseline and poststress periods ($p < 0.05$). On the other hand, the psychological state of the mothers during grimace exposure showed a significantly lower value in the stress period than in the baseline and poststress periods ($p < 0.05$). The psychological state of the infants during the smile exposure showed a significantly higher value in the baseline

Table 1
Categories of behavior and facial expressions determined by behavior analysis.

Category of infants	Classification
Smile	+
Still face	−
Trying to run away	−
Category of mothers	**Classification**
Smile	+
Grimace	−

+; positive, −; negative

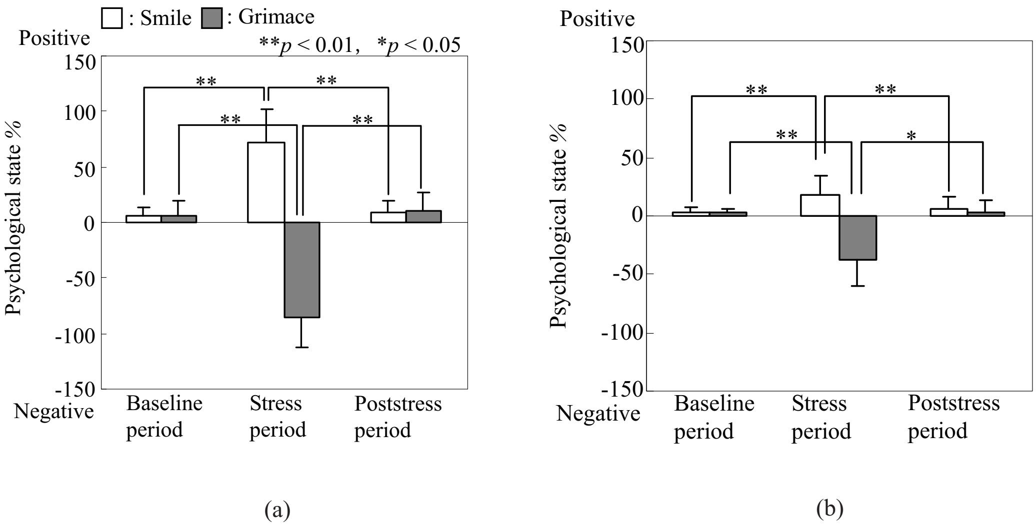

Fig. 2. Psychological state of mothers and infants estimated by behavior analysis. (a) Psychological state of mothers. (b) Psychological state of infants.

and poststress periods than in the stress period ($p < 0.05$). On the other hand, the psychological state of the infants during the grimace exposure showed a significantly lower value in the stress period than in the baseline period ($p < 0.05$). Furthermore, a significant correlation was observed in the psychological state between mothers and infants ($p < 0.01$, $R = 0.814$).

The sAMY activity during the grimace exposure was significantly higher than that during the smile exposure in the stress period ($p < 0.05$, Fig. 3). However, no significant difference was observed between them in the baseline and poststress periods. A correlation between the VAS score and sAMY activity was observed ($p < 0.05$). However, no significant correlation was observed between the psychological state in behavior analysis and sAMY activity, not only for the infants but also the mothers.

Concerning the sCORT level in the poststress period, a significant difference was

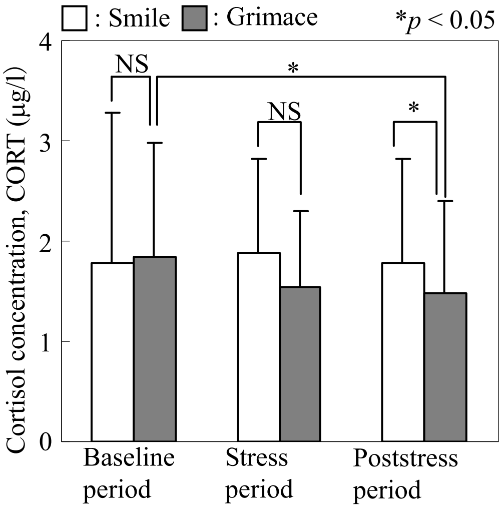

Fig. 3. sAMY activities in infants in baseline, stress, and poststress periods (NS: not significant).

observed between the smile and grimace exposures ($p < 0.05$, Fig. 4). Furthermore, a significant difference in the sCORT level was observed between the baseline and poststress periods ($p < 0.05$). No significant difference in the sCORT level was observed between the smile and grimace exposures in both the baseline and stress periods. A correlation was observed between the sCORT level and psychological state activity of infants ($p < 0.05$). On the other hand, no correlation was observed between sAMY activity and sCORT level.

4. Discussion

Psychologically, it is interesting that a relatively high correlation between the psychological states of mothers and infants was observed. It was indicated that a mother's facial expressions are stimulating to her infant.

The sAMY activity during the grimace exposure was significantly higher than that during the smile exposure and a correlation between VAS score and sAMY activity was also observed. These indicated that the exposure to acute psychologically negative stimuli could have an effect on the SAM system of infants.

The sAMY activity reflected the facial expressions of mothers in the stress period; on the other hand, the sCORT level reflected them in the poststress period. The timing of collecting saliva samples may have accounted for the fact that no correlation was observed between both salivary biomarkers. It is possible that the release from a negative grimace stimulus resulted in the decrease in the sCORT level in the poststress period owing to the latency of sCORT secretion.

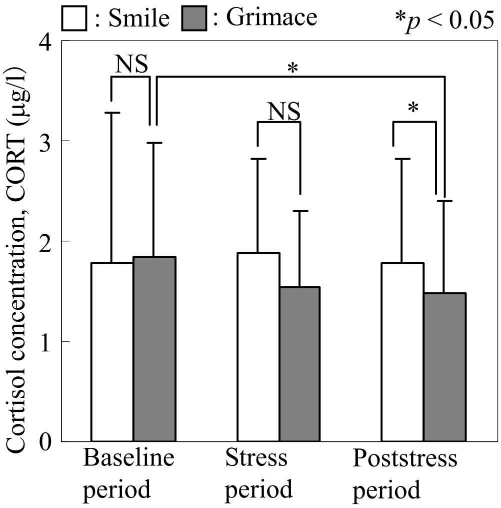

Fig. 4. Salivary cortisol concentrations in baseline, stress, and poststress periods (NS: not significant).

5. Conclusions

A correlation between the psychological states of mothers and infants was shown using the behavior coding system. The major finding of this study is that the facial expressions of mothers may affect the sympathetic nervous system of infants. In summary, salivary biomarkers can be useful tools for evaluating stressful events in psychosomatic studies of linguistically incommunicable infants.

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