Introduction

The 65-year-old or older population exceeded 30 million with rapid aging in Japan. It is predicted to peak with about 39 million in 2042 and the 75-year-old or older population will continuously increase thereafter. The Ministry of Health, Labour and Welfare promotes the construction of the Integrated Community Care System targeting 2025 [1], and how to prevent a need-for-nursing care state in the elderly is an urgent issue. Regarding the prevention of nursing care, improvement of sociality of the elderly, such as an increase in conversation employing the life review/reminiscence therapy, and subsequent improvement of nighttime sleeping hours have been reported [2-4]. In addition, prevention of nursing through amusement devices has recently been attracting attention, and improvement of the motor function by using amusement devices has been reported [5]. Matsuguma et al. performed research and development of a game machine for standing up-
sitting down training, and investigated the influence of the entertainment value of the game on the positiveness of patients/users and improvement of persistence. Specifically, fatigue strength was significantly decreased by voluntary training with the game on subjective evaluation, and the subjects tended to consider that ‘I want to do it again’ and ‘it was fun’, showing a positive attitude [6]. It was suggested that ‘pleasant stimulations’, such as ‘enjoying’ and ‘having fun’, which are entertainment values of amusement, increase motivation, positiveness, and activeness, influencing improvement of the motor function. Regarding communication through the game, they reported that new communication between patients and rehabilitation staff and among patients were constructed [6, 7]. It was suggested as described above that amusement increases motivation and positiveness, promotes activeness, and produces new communication [6, 7]. However, no study on the physiological function demonstrating ‘pleasant stimulation’ by interaction through amusement has been performed, or the effect of this ‘pleasant stimulation’ on sleep.

In preceding studies on mental stress, analysis of the endocrine system or heart rate variability was used as a physiological index of stress analysis [8, 9]. Yamaguchi et al. focused on salivary amylase as a new index of sympathetic nerve activity. They clarified that salivary amylase may serve as a stress index responding more rapidly than cortisol, and unpleasant stimulation elevates salivary amylase activity and inversely, pleasant stimulation decreases it, showing a possibility to distinguish comfort and discomfort based on salivary amylase [10, 11]. Shimizu et al. investigated ‘laughing stimulus’-induced changes in physiological indices, and suggested that stress was reduced by laughing based on the salivary amylase level, and sympathetic nerve activity was enhanced while being stimulated with laughing, followed by enhancement of parasympathetic nerve activity, and returning to the resting state [12]. Takada et al. performed intervention of the elderly with ‘pleasant conversation’, and observed that it was appropriate for intervention of the elderly because its physical and mental burdens were small, and fulfillment of the mind and the effect of relaxation were observed after the conversation [13]. However, all these were transient effects of short-time intervention, and the effect of long-term intervention was not investigated. A long-term effect is necessary from the viewpoint of preventing needs for nursing. In addition, these studies used the low frequency (LF) component of spectral analysis of heartbeat R-R interval, which is an index of parasympathetic nerve enhancement, and LF/high frequency (HF) component, which is an index of sympathetic nerve enhancement, as indices of autonomic nerve activity [12, 13]. However, to estimate autonomic nerve activity by spectral analysis, it is essential to have uniform respiration to a specific depth during measurement of cardiac cycle data [14], and Sawada pointed out that spectral analysis of the R-R interval of heartbeats is not appropriate to trace reflections of sympathetic nerve activity of the heart [15]. In such a background, Toichi et al. found a method to evaluate cardiac autonomic nerve activity based on heartbeat R-R interval data using the Lorenz plot. They stated that this method is capable of evaluating sympathetic and parasympathetic nerve activities individually, which has been difficult using spectral analysis, and its usefulness has been reported [16].

Thus, we intervene in interactions among the elderly through one-month amusement, and investigated time-course changes in the autonomic nerve activity calculated using the Lorenz plot and salivary amylase level and the influence on sleep.

Materials and Methods

Subjects

The subjects were elderly females (age: 89.0 ± 4.7 years old) who periodically visited a day care service center for the elderly in Japan. The level of care needed was between Support Need 2 and Long-Term Care 2. Regarding ADL, the Barthel Index (BI) [17] was 87.2 ± 11.2, requiring support/assistance to some extent, but the grade of independence was high (Table 1). The cognitive function was evaluated using the Mini-Mental State Examination (MMSE) [18]. The score was 26.8 ± 2.6, and it was lower than 23 in 2 subjects, but their scores correspond to mild on the severity judgment [19].

<table>
<thead>
<tr>
<th>Table 1. Basic attributes of the subjects</th>
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<tr>
<td>Gender</td>
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<td>Age (years)</td>
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<td>Level of care needed</td>
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<td>BI (point)</td>
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<td>K6 Scales (point)</td>
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For evaluation of mental stress, the Japanese version of the Kessler Psychological Distress Scale (K6) [20] was used. Reliability and validity of social survey using K6 have been evaluated in Japan. The score range of K6 is 0–24, and mental stress increases as the score increases. A recent study proposed a cut-off value of 10 or higher for mood/anxiety disorder in the Japanese version [21], in which the score of the subjects was 4.3 ± 3.8 including 2 subjects with a score of 10, but no marked change in the cognitive function, words, or behavior suggesting serious depression was noted throughout the survey period (Tables 1 and 2). Of 15 subjects to whom the study content was explained beforehand and consent was obtained, 9 subjects in whom saliva could be collected and the sleep condition could be measured were included in analysis. This study was conducted with the approval of the Research Ethics of Hiroshima University (approval number: E-318), conforming to the Declaration of Helsinki.

**Survey items**

The survey was performed from October 2015 to December 2015. The survey period was comprised of 3 amusement introduction periods: before amusement introduction (pre-amusement), and one week and one month after amusement introduction. In each period, the heart rate (HR) and autonomic nerve activity were measured, saliva was collected, and the sleep condition was measured.

**Amusement**

Blackjack was introduced. Staff of the day care service center for the elderly served as a dealer, and the subjects were players. In Blackjack, the dealer and players match games. The basic rule is that players collect cards so as to make the total score close to but smaller than 21 and greater than that of the dealer. Regarding the card score, ‘A’ was scored 1 or 11 (whichever convenient), and ‘2–10’ cards were scored as their numbers, respectively. Since numbers printed in the designs of trump cards are small, reading and calculating the numbers are difficult. Thus, the card designs were removed and only numbers were printed (Fig. 1). To increase activeness, cards were spread and the players picked up cards for the game (Fig. 2b), the dealer did not distribute cards (Fig. 2a).

**Measurement of heart rate and autonomic nerve activity**

HR and changes in the autonomic nervous system on heart rate variability analysis were measured from before amusement to after amusement using a wearable heartbeat sensor, WHS-1 (UNION TOOL Co.) directly applied to the chest with a Blue Sensor (METS Inc.) electrode pad. The amusement time was 13:30–14:30, and data were collected during this period in all amusement introduction periods. Based on the R-R interval data measured in 60-minute electrocardiography, HR (number of beats/min) and the indices of sympathetic (CSI: cardiac sympathetic index) and parasympathetic (CVI: cardiac vagal index) nerve activities were calculated using the Lorenz plot analysis program [22]. In this Lorentz plot, the measured values of continuous heartbeat R-R intervals were presented as \( I_1, I_2, \ldots, I_n \).

<table>
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<th>Table 2. Association between the amusement introduction period and heart rate, autonomic nervous system, sleep condition, Kessler Psychological Distress Scale (K6 Scales) (n=9)</th>
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<tr>
<td><strong>Pre-amusement</strong></td>
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<td>HR (/min)</td>
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<tr>
<td>CSI</td>
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<td>CVI</td>
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<td>Sleep efficiency (%)</td>
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<td>Sleeping hours (min)</td>
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<td>MMSE (point)</td>
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<td>K6 Scales (point)</td>
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\( p \text{ value: repeated measures one-way ANOVA} \)

\( *a: \) pre vs 1month, \( p = 0.004 \) by Bonferroni

Fig. 1. Cards labeled with an only a letter or number.
Heartbeat R-R intervals of $I_k$ were plotted on the horizontal axis and those of $I_k + 1$ were plotted on the vertical axis, and the plots are distributed in an oval pattern with straight line $I_k = I_k + 1$ as a longitudinal axis. The major axis component $L$ (horizontal to the line $I_k = I_k + 1$) and minor axis component (vertical to the line $I_k = I_k + 1$) were calculated, and the $L/T$ ratio was regarded as the CSI value and the area value of $L$ and $T$, $\log(L \times T)$, was regarded as the CVI value [16]. An increase and decrease in the CSI value indicate promotion and suppression of sympathetic nerve activity, respectively. An increase and decrease in the CVI value indicate promotion and suppression of parasympathetic nerve activity, respectively.

Measurement of change in the salivary amylase level
Saliva was collected between 11:30 and 12:00 before lunch and between 14:45 and 15:00 after amusement. To collect saliva samples for measurement, the subjects held a swab in their mouth for 3 minutes (a maximum of 5 minutes) using Salimetrics Oral Swab (Salimetrics LLC). Saliva was separated from the swabs by centrifugation at 3,500 rpm for 15 minutes and frozen. For the measurement, the BG5 PNP method (corresponding to JCCLS) was used. The salivary amylase level before amusement was subtracted from that after amusement in all periods, and changes were determined. There are several amylase measurement methods, such as enzyme and blue starch methods. The standard value is different among the measurement methods and individual variation is large [23]. Since changes in individuals were investigated in this study, the change was determined by subtracting the amylase level after the amusement from that before the amusement.

Measurement of sleep condition
The sleep condition was measured at night on the day the subject performed amusement. They wore Activewatch Spectrum Plus (Philips Respironics GK) on their wrist from 15:00 to pick-up time on the following morning. Body movement and ambient light were subjected to quantitative analysis using Actiware, and the sleep efficiency (%) and sleeping hours (min) were calculated.

DATA ANALYSIS
The means and standard deviations of HR, CSI, CVI, sleep efficiency, sleeping hours, and change in the amylase level in the amusement introduction period (pre-amusement and one week and one month after amusement introduction) were determined. Repeated measures one-way ANOVA was performed regarding the period as one factor and HR, CSI, CVI, sleep efficiency, sleeping hours, and difference in the amylase level as dependent variables. For multiple comparison, the Bonferroni method was used. The normality of HR, CSI, CVI, sleep efficiency, sleeping hours, and the change in the amylase level were confirmed using histograms and the Kolmogolov-Smirnov test ($p = 0.200$). Statistical analysis was performed using EZR Ver 1.32 [24] setting the significance level at $p < 0.05$.

Results
Significant differences among the amusement introduction periods were observed in CVI (Table 2, repeated measures one-way ANOVA): The value at pre-amusement was 6.0 and that at one month after amusement introduction was 5.4, showing a significant decrease. The heart rate, CSI, sleep efficiency, and sleeping hours were not significantly different. The change in the salivary amylase level was significantly different among the amusement introduction periods (Fig. 3, repeated mea-
sures one-way ANOVA). The values at pre-amusement and one month after amusement introduction were 130,634 and −111,627 U/L, respectively, showing a significant decrease at one month.

Discussion

The amusement introduction period and CVI showed a significant association, and CVI was significantly lower at one month after amusement introduction than that at pre-amusement (Table 2). The results of this study were consistent with those of previous reports [12, 13, 25], but these studies investigated the effect of short-time intervention, whereas our study introduced amusement and followed the subjects for a long period, which initially clarified the association between amusement and parasympathetic nerve activity. Regarding sympathetic nerve activity, no association with the amusement introduction period was noted. In the amusement, Blackjack, the cards were shuffled by the subjects before the game, the cards were distributed, and the number of chips was determined from the distributed card. The subjects calculated the sum of the 1st and 2nd cards in their head to decide on pulling the 3rd card. These processes may include many stress stimulations required for active processing. Cardiovascular response to stress stimulation includes active and passive copingings [26]. Positive coping means actively confronting stimulation, such as competition in mental arithmetic and reaction time, i.e., active challenge and competition in response to stimulation that one can deal with. In passive coping, one cannot move, remaining in a passive attitude against stress stimulation and continue to pay attention and surveillance [15]. Sympathetic nerve activity is promoted and parasympathetic nerve activity is suppressed in a situation requiring active coping whereas sympathetic nerve activity is suppressed and parasympathetic nerve activity is promoted in a situation requiring passive coping [26]. Therefore, active coping during the amusement may have suppressed parasympathetic nerve activity, resulting in a low CVI value. On the other hand, it has been reported that ‘laughing stimuli’ of comic dialogues stimulate and enhance both sympathetic and parasympathetic nerve activities, and the mean parasympathetic nerve activity level was inhibited while being stimulated [25], in which the subjects did not constantly receive ‘laughing stimuli’ and changes also appeared in movements, such as instantaneous ‘laughing’, ‘laughing aloud’, ‘laughing with body movement back and forth’, and ‘laughing holding one’s sides’ [25]. It was also reported that sympathetic nerve activity was significantly enhanced and parasympathetic nerve activity was significantly suppressed in subjects who felt a funny video as ‘funny’, compared with those in subjects who did not perceive the video as funny [27]. In addition, it has been suggested that ‘laughing stimulus’-induced enhancement of sympathetic nerve activity occurred while being stimulated with laughing, followed by enhancement of parasympathetic nerve activity and returning to a resting state [12]. In our study, instantaneous stimuli, such as laughing and regret, and resting, such as waiting for one’s turn and conversation,
were repeated in the interactions during the amusement, suggesting that enhancement and suppression of sympathetic and parasympathetic nerve activities repeated. No significant association was observed between the amusement introduction period and sympathetic nerve activity. Averaging the total time performing the amusement may have influenced this. Considering that analysis averaging the entire range with stimulation investigates only a part of the characteristics in studies on ‘laughter stimulation’, Kobayashi tried to investigate time-series data to qualitatively analyze the characteristics of response to ‘laughter stimulation’ in individual data [25]. We may also have been able to closely analyze changes in sympathetic and parasympathetic nerve activities during the amusement by time-series analysis, such as ‘immediately after initiation of the amusement’ and ‘10 minutes after initiation of the amusement’.

Our study did not aim at evaluation of the amusement-induced autonomous nervous activity during a short period, but one-month intervention with the amusement may have suppressed parasympathetic nerve activity by promoting active coping behavior, and interaction through the amusement may have suppressed excess sympathetic nerve activity.

Significant differences were noted in the change in the salivary amylase level among the amusement introduction periods, and it was significantly lower at one month after amusement introduction than that at pre-amusement (Fig. 3). The interaction during the amusement may have given comfortable stimuli [10, 11, 28], and reduced the salivary amylase level. There are 2 control mechanisms reducing the salivary amylase level: The sympathetic nervous-adrenal medullary system (SAM system) inhibits norepinephrine release from the adrenal medulla through suppressing sympathetic nerve activity, and another system suppresses sympathetic nerve activity and directly acts on the nerve to inhibit enzyme secretion from the salivary glands, suggesting that one of these mechanisms induced the low amylase level [28]. Shimizu et al. reported that the salivary amylase level was significantly decreased by intervention with ‘laughing stimuli’, showing stress reduction by laughing: Laughing induced mental changes, such as reduction of negative emotion and feeling better, and these psychological changes were involved in the reduction of the salivary amylase activity level [12]. Based on these reports, the significant decrease in the change in the salivary amylase level during the amusement introduction period suggests that the subjects felt amusement-induced laughing and regret and interaction through the amusement comfortable.

Regarding the sleep efficiency and sleeping hours, no significant association with the amusement introduction period was noted, but the mean sleeping hours increased by about 50 minutes at one month after amusement introduction compared with that at pre-amusement (Table 2). The awake time in the bed becomes longer in the elderly [29, 30], and the sleep efficiency decreases [30]. Regarding treatment of insomnia in the elderly, it has been reported that dozing of elderly subjects at a nursing home can be prevented by setting a specific social activity [31], and an individual social activity program for residents with dementia at a nursing home improved the sleep efficiency and reduced nocturnal awakening [32]. Since the subjects of our study were users of day care services for the elderly, their sleep efficiency was originally high and the sleeping hours were long based on the sleep data, which may have resulted in the absence of significant association of sleep efficiency and sleeping hours with the amusement introduction (Table 2). However, the sleeping hours extended while maintaining sleep efficiency, suggesting that the interactions through the amusement positively influenced their sleep conditions at night.

Generalization of these study results may be problematic because the frequency of using a day care service center for the elderly varied among the elderly female subjects, but the internal validity of the results may have been high because they mostly stayed at home when they did not use the services. This study followed the subjects for one month and clarified the associations between the amusement introduction and parasympathetic nerve activity and salivary amylase.

Conclusion

Based on the physiological indices: heart rate, heart rate variability analysis, and salivary amylase, it was suggested that amusement-induced laughing and regret or interaction through the amusement influenced the autonomic nerve system and they felt comfortable.

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References


