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Effect of 528 Hz Music on the Endocrine System and Autonomic Nervous System

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Abstract

This study examined the stress-reducing effect on the endocrine system and the autonomic nervous system of music with a frequency of 528 Hz, which has recently attracted attention as a "healing" type of music. Nine healthy participants (one man and eight women, aged 26 - 37 years) listened to 528 Hz and standard 440 Hz music on separate days. We measured salivary biomarkers of stress (cortisol, chromogranin A, and oxytocin) before and after exposure to music, and continuously recorded the activity of the autonomic nervous system. The Profile of Mood State, 2nd edition, was also administered as a subjective indicator of stress. In the 528 Hz condition, mean levels of cortisol significantly decreased, chromogranin A tended to decrease, and oxytocin significantly increased after music exposure. However, no significant change was observed in any salivary biomarkers in the 440 Hz condition. The ratio of low frequency to high frequency autonomic nervous system activity significantly decreased after exposure to both types of music, and the coefficient of variation of R-R intervals also significantly decreased, but only after exposure to 528 Hz music. Tension-anxiety and Total Mood Disturbance scores were significantly reduced after exposure to 528 Hz music, while there was no significant difference following 440 Hz music. These results suggest that the influence of music on the autonomic nervous system and endocrine system varies depending on the frequency of the music, and furthermore, that 528 Hz music has an especially strong stress-reducing effect, even following only five minutes of exposure.

Keywords

Music, Stress, Salivary Assay, Autonomic Nervous System

1. Introduction

It is well known that listening to music reduces stress. Research has investigated

many aspects of this phenomenon, and it is recognized that several constituent elements of music are involved in this stress reduction effect. Previous studies that have examined emotional arousal in response to listening to music have suggested that music arouses different emotions depending on its characteristics, such as melody, rhythm, and dynamism [1].

However, few studies have investigated how differences in the frequency of music affect the human body. In a study in which rats were exposed to musical stimuli of different frequencies, the rats' blood pressure decreased depending on the frequency; notably, this change was observed to a greater extent for music in the frequency range of 16 kHz compared to 4 kHz frequency [2]. This result suggests that music including high-frequency sound stimulates dopamine synthesis and suppresses sympathetic nervous system activity [2]. Furthermore, others report that listening to high-frequency music increases the activity of the parasympathetic nervous system and reduces stress, in comparison to listening to low-frequency music [3]. Therefore, in the present study, we examined the stress reduction effect of music with a frequency of 528 Hz by measuring its influence on the endocrine system and autonomic nervous system.

Music of the aforementioned frequency (528 Hz) has lately attracted attention as "healing" music. Usually, the reference tone of tuning is 440 Hz, and this is the international standard frequency (we refer to this as 440 *Hz music*). In this musical scale, there is no 528 Hz note. However, setting the reference tone to 444 Hz means that 528 Hz is included in the musical scale. We refer to music that is tuned and composed in this way as *528 Hz music*. In general, this specific type of music using a scale including 528 Hz is called *solfeggio frequency* music. Various effects have been ascribed to the solfeggio frequency, but none of these have any scientific basis. Accordingly, we examined the effect of such music compared to 440 Hz music.

In this study, we measured salivary biomarkers of stress (cortisol, chromogranin A, and oxytocin), which can be collected noninvasively, as indices of stress relief. It is known that listening to music affects the endocrine system and autonomic nervous system.

In the endocrine system, cortisol and chromogranin A have been used as indicators of stress. In a previous study, conducted in patients who had just undergone the stressful experience of learning in detail about the procedures involved in surgery that they would undergo the next day, salivary cortisol was significantly reduced among a group who listened to music for one hour, compared to a group who did not [4]. Additionally, in another study, which administered the Trier Social Stress Test (in which stress is caused to participants and their salivary cortisol levels thereby raised), the increase of salivary cortisol in a group who took the test while listening to relaxing music was significantly suppressed compared to that of a control group [5].

Chromogranin A is present in the submaxillary duct and is released into saliva by autonomic nerve stimulation [6]. It has also been reported that, when psychological stress is experienced, chromogranin A rises ahead of cortisol and de-

creases early after stress is relieved [7]. Furthermore, others have examined the effect on both cortisol and chromogranin A of listening to music [8] [9]. Specifically, they report that salivary cortisol and chromogranin A decrease significantly before and after listening to music [8]. In contrast, other researchers observe that, although salivary chromogranin A decreases significantly, there is no significant difference in cortisol levels [9].

In addition to the above indicators, we also focused on oxytocin. The effect of listening to music on oxytocin has recently attracted attention, with a previous study indicating that salivary oxytocin increases when participants listen to slow-tempo music compared to fast-tempo music [10].

As an additional objective index, we measured the activity of the autonomic nervous system. Finally, we also administered the Japanese short version of the Profile of Mood State, 2nd edition (POMS 2) to participants as a subjective index of stress. Both of these measures have also been used in many previous studies [11] [12]. We consider these measures to support the data provided by the above-mentioned salivary biomarkers of stress marker.

The 528 Hz music that we used in this study was soothing piano music. Some researchers report that, depending on the mental state of the listener, music preferences vary [13]. However, they also find that piano music is appreciated regardless of the listener's circumstances. Thus, we examined the stress-mitigating effect of 528 Hz music from various perspectives. This study offers a new piece of evidence for use in music therapy.

2. Methods

2.1. Participants

Participants were nine healthy adults, one man and eight women, aged 26 - 37 years (mean age: 31.1; *SD* = 1.35). We recruited participants as volunteers regardless their previous musical education. Everyone participated with full understanding of the study and gave their informed consent. They were asked to abstain from caffeine and smoking for one hour before starting the experiment. They did not apprise of which music they were listening to. This study was approved by the Ethics Committee of Shiba Palace Clinic, Tokyo, Japan (Protocol # 2018040001).

2.2. Procedure

Figure 1 shows an outline of the experimental procedure. The study was carried out in a conference room, where the room temperature was 25.0˚C - 25.5˚C and humidity was 67% - 76%. In consideration of circadian variation in cortisol and chromogranin A [14] [15], we started the experiment at 2 PM for each condition. Participants listened to the music from a seated position beside a speaker. The music was soothing piano music, and the only difference between the two musical conditions was in frequency, which was either 528 Hz or 440 Hz. Each participant took part in each of the conditions on separate days.

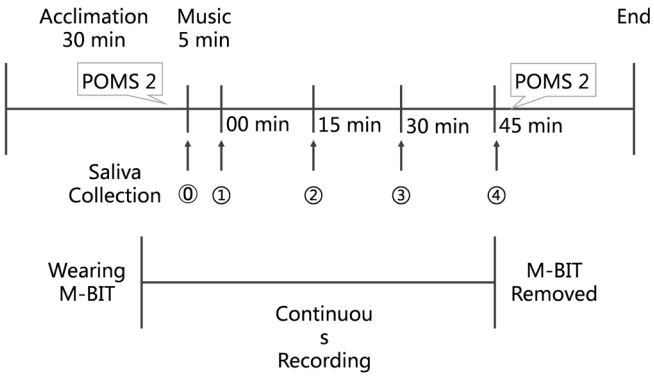

Figure 1. The experimental procedure. Participants wore a Bio Information Tracer (M-BIT) device and their electrocardiogram was recorded continuously from the acclimation phase until 45 minutes after music exposure. Participants listened to music for five minutes. Salivary samples were collected at five time points: immediately before music exposure, immediately after music exposure, 15 minutes later, 30 minutes later, and 45 minutes later. The Profile of Mood State, 2nd edition (POMS 2) was administered before and after music exposure.

2.3. Measures

2.3.1. Salivary Assay

We collected salivary samples at five time points (immediately before listening to music, immediately after listening to music, 15 minutes later, 30 minutes later, and 45 minutes later) using the Saliva Collection Aid (Salimetrics LLC, USA). We measured cortisol, chromogranin A, and oxytocin as salivary biomarkers of stress. These were measured using the Salivary Cortisol Enzyme-Linked Immunosorbent Assay (ELISA) Kit (Salimetrics LLC, USA), Human Chromogranin A ELISA Kit (Yanaihara Institute Inc., Japan), and Oxytocin ELISA Kit (Arbor Assays, USA), respectively.

2.3.2. Autonomic Nervous System

We recorded an electrocardiogram using the Bio Information Tracer (M-BIT) (Institute of Man and Science Inc., Japan). Participants wore this small wearable sensor (49 × 39 × 8 mm, 14 g) on the left side of the chest during an acclimation phase, and data were recorded continuously until 45 minutes after music exposure. The BIT analysis center analyzed these data in terms of low frequency (LF), high frequency (HF), and the coefficient of variation of R-R intervals (CVRR), and we received the averaged values of these measures over the five minutes preceding each saliva collection time point.

2.3.3. Questionnaire on Mood States

Self-reported mood states were collected using the Japanese short version of the POMS 2 scale before listening to music and 45 minutes after doing so. The POMS 2 is a questionnaire developed in the United States as a questionnaire method to evaluate mood. It is possible to quickly evaluate not only the state of emotion that lasts for a relatively long time but also the state of temporary feelings and emotions that change depending on the situation that subject is in. It is

used in a variety of fields such as clinical, workplace, and school. For example, it has been applied to the course of treatment of mental disorders, mental changes of people with physical disorders, screening in the workplace, and exercise or relaxation effect.

The POMS 2 evaluates seven mood scales of anger-hostility (AH), confusion-bewilderment (CB), depression-dejection (DD), fatigue-inertia (FI), tension-anxiety (TA), vigor-activity (VA), friendliness (F) at the same time. TMD is a general indicator such as mood disorders, psychological distress, and subjective well-being. The TMD score is calculated by the sum of the elementary scores of AH, CB, DD, FI, TA, and VA. (Since VA is weighted of negative, it is subtracted from the sum of the other five). Therefore, the score is a comprehensive evaluation of expression of negative mood.

The POMS 2 consists of 35 items questions and a respondent answer each of question with the evaluation scale of POMS 2 (0 = not at all, 1 = slightly, 2 = modestly, 3 = considerably, 4 = very). Although this evaluation scale is constant, since the number of items differs depending on the scale, the degree indicated by each scale score is not constant. In short, even though the absolute value of the score is the same, the meaning of the score differs between scales. Since it is impossible to properly compare the scales at this score, the score is converted to a standardized score, that is, a T score. The T score is a normalized standard of assessment (average value is 50 and the standard deviation is 10) so that the same value has equivalent meaning. The measurer interprets the score by converting the graded evaluation scale into the T score.

Regarding the TMD score and negative mood state (AH, CB, DD, FI, and TA), the higher the T score, the stronger the emotion concerning negative emotion or mood disorder. Regarding the positive emotional state (VA, F), the higher the T score, the more positive emotion means [16].

2.4. Data Analysis

We used the Wilcoxon signed-rank test to detect any differences between time points in each condition in this study. The threshold for statistical significance was set at $p < 0.05$.

3. Results

3.1. Salivary Assay

3.1.1. Cortisol

In consideration of the response time lag of cortisol [17] [18], we took the mean level of salivary cortisol measured immediately after music exposure as representing the level immediately before music exposure, and so on; therefore, data were only available until 30 minutes after music exposure, rather than 45 (**Figure 2**). After exposure to 528 Hz music, mean levels of salivary cortisol decreased with the passage of time and were significantly reduced 30 minutes later ($0.43 \pm 0.04 \rightarrow 0.25 \pm 0.02$, $p < 0.011$). In contrast, after listening to 440 Hz mu-

sic, mean levels of cortisol slightly decreased after 30 minutes, but there was no significant difference between the time points.

3.1.2. Chromogranin A

There was no significant difference in chromogranin A levels following exposure to either 528 Hz music or 440 Hz music (Figure 3). However, in the 528 Hz music condition, mean levels of chromogranin A showed a tendency to fall until 30 minutes after music exposure. In contrast, in the 440 Hz music condition, mean levels of chromogranin A showed a tendency to increase until 30 minutes later.

3.1.3. Oxytocin

Figure 4 illustrates the effects of music on oxytocin. Mean levels of oxytocin increased significantly immediately after listening to 528 Hz music ($37.57 \pm 1.53 \rightarrow 73.58 \pm 5.04$, $p < 0.038$). After listening to 440 Hz music, mean levels of oxytocin also increased, but the difference was not significant.

3.2. Autonomic Nervous System

An electrocardiogram could not be recorded correctly for one of the participants, so we analyzed the data from eight participants for this measure (Figure 5). In both conditions, the ratio of LF to HF decreased significantly immediately after listening to music (528 Hz music: $77.89 \pm 0.0025 \rightarrow 62.34 \pm 0.0032$, $p < 0.012$, 440 Hz music: $72.52 \pm 0.0028 \rightarrow 54.72 \pm 0.0037$, $p < 0.012$), whereas CVRR decreased significantly only immediately after listening to 528 Hz music ($6.67 \pm 0.00040 \rightarrow 5.36 \pm 0.00043$, $p < 0.025$).

3.3. POMS 2

After exposure to 528 Hz music, all negative mood scores decreased. In particular, tension-anxiety ($48.44 \pm 2.28 \rightarrow 43.67 \pm 2.45$, $p < 0.0091$) and Total Mood Disturbance ($45.56 \pm 1.99 \rightarrow 42.00 \pm 2.00$, $p < 0.0487$) decreased significantly. Additionally, all positive mood scores tended to increase. In contrast, after exposure to 440 Hz music, almost all scores showed a similar tendency to change in the same direction as observed in the 528 Hz music condition, but there was no significant difference for any score. Furthermore, anger-hostility scores (indicating negative mood) slightly increased, and friendliness scores (indicating positive mood) decreased (Table 1).

4. Discussion

The purpose of this study was to examine the effect of 528 Hz music on the endocrine system and the autonomic nervous system using objective and subjective measures. Based on salivary biomarkers, electrocardiogram, and a mood state questionnaire, we found that stress levels were reduced following five minutes' exposure to 528 Hz music, whereas this was not the case for 440 Hz music.

We found that mean levels of cortisol decreased and mean levels of oxytocin increased after listening to 528 Hz music. In general, cortisol increases via the

Table 1. Effects of music on mood as assessed by subscales of the Profile of Mood State, Second Edition.

Measures	528 Hz of Music		440 Hz of Music	
	Pre-Exposure Mean (SD)	Post-Exposure Mean (SD)	Pre-Exposure Mean (SD)	Post-Exposure Mean (SD)
Anger-Hostility	43.6 (1.75)	41.6 (1.53)	42.6 (2.00)	42.6 (2.40)
Confusion-Bewilderment	50.6 (3.10)	47.9 (2.43)	49.1 (2.63)	45.9 (2.77)
Depression-Dejection	47.2 (2.03)	44.4 (1.45)	46.4 (2.34)	45.9 (2.37)
Fatigue-Inertia	46.2 (3.04)	44.0 (2.56)	42.9 (2.42)	41.0 (3.06)
Tension-Anxiety	48.4 (2.28)	43.7 (2.45)**	44.3 (2.94)	42.4 (2.92)
Vigor-Activity	56.3 (2.11)	57.8 (4.11)	55.6 (3.53)	55.7 (4.48)
Friendliness	60.3 (3.40)	61.6 (4.22)	57.2 (4.22)	56.1 (5.20)
Total Mood Disturbance	45.6 (1.99)	42.0 (2.00)*	43.6 (1.99)	41.9 (2.48)

*$p < 0.05$; **$p < 0.01$ in pre-exposure vs post-exposure comparison using the Wilcoxon signed-rank test.

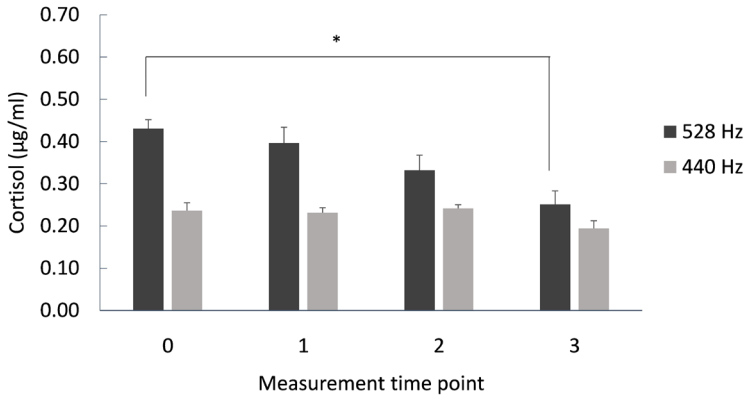

Figure 2. Effects of music on mean levels of salivary cortisol. Error bars represent standard errors. Time points are as indicated in **Figure 1** (Mean ± SE) *$p < 0.05$.

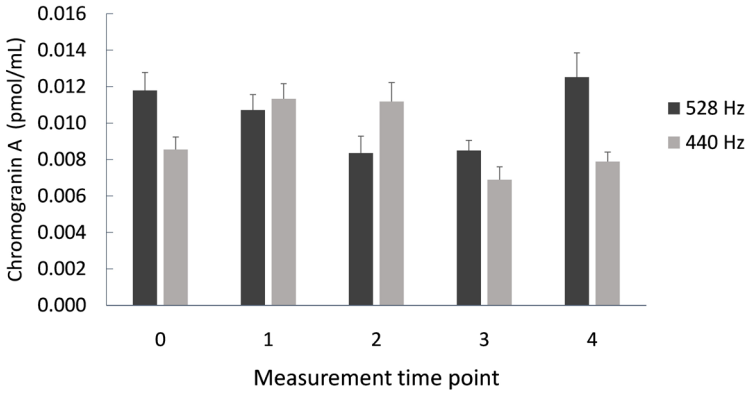

Figure 3. Effects of music on mean levels of salivary chromogranin A. Error bars represent standard errors. Time points are as indicated in **Figure 1** (Mean ± SE).

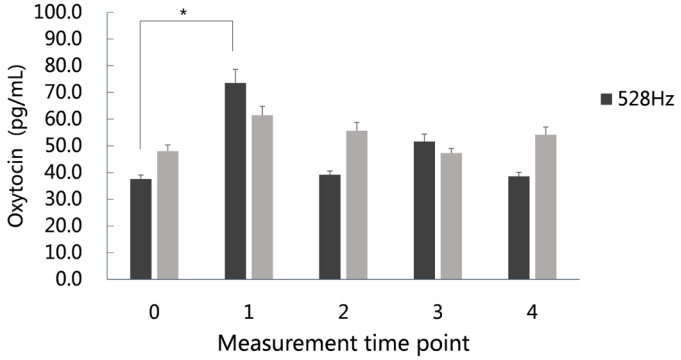

Figure 4. Effects of music on salivary oxytocin. Error bars represent standard errors. Time points are as indicated in **Figure 1** (Mean ± SE) $*p < 0.05$.

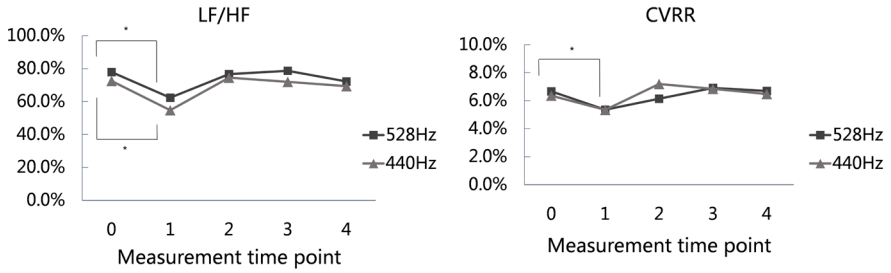

Figure 5. Effects of music on the autonomic nervous system, as measured by the ratio of low frequency to high frequency (LF/HF) and coefficient of variation of R-R intervals (CVRR). Time points are as indicated in **Figure 1** (Mean ± SE) $*p < 0.05$.

hypothalamic-pituitary-adrenal (HPA) axis in response to stress. Oxytocin is produced by the hypothalamus and released by the posterior pituitary gland. It is well known that production of oxytocin increases in the process of breastfeeding. In addition, some studies suggest that production of oxytocin increases in response to pleasant sensations or positive social interactions [19] [20]. When an individual hears music, responses to these stimuli are eventually transferred to the amygdaloid body via the medial geniculate body of the thalamus. Sensory information that causes emotions, such as music, is processed in the amygdaloid body circuit. Subsequently, signals are output to the hypothalamus, causing an oxytocin response [21] [22]. Furthermore, it is reported that oxytocin controls activation of the HPA axis [19]. Taking this information together with the present findings, we infer that mean levels of oxytocin increased in our study because the 528 Hz music was transmitted to the amygdaloid body as a pleasing sensation. Additionally, the decrease in mean level of cortisol occurred as a result of the action of oxytocin. This suggests that participants felt comfortable listening to 528 Hz music and this stimulus reduced their stress level.

Mean levels of oxytocin also increased after listening to 440 Hz music, but no significant difference was identified. In addition, mean levels of cortisol slightly decreased in this condition after 45 minutes, but this difference also failed to reach significance. In other words, we could not confirm that a stress mitigation effect occurred in the 440 Hz music condition.

Mean levels of chromogranin A showed a tendency to decrease after listening to 528 Hz music. Chromogranin A is released along with catecholamine via the sympathetic-adrenal-medullary axis in response stress. Chromogranin A is found in the duct of the submandibular gland. It is released into the saliva as a result of stimulation of the autonomic nervous system [7] [15] [23].

Concerning the autonomic nervous system, in both musical conditions, a significant decrease in LF/HF was observed immediately after listening to music. LF is considered to reflect the autonomic activity of both the sympathetic and the parasympathetic nervous systems, whereas HF reflects parasympathetic activity, and LF/HF reflects sympathetic activity [24] [25]. Additionally, CVRR significantly decreased immediately after listening to 528 Hz music. This measure represents the coefficient of variance of the R-R interval (RRI) and is calculated by dividing the standard deviation of the RRI by the mean of RRI. CVRR mainly reflects regulation of parasympathetic nervous system activity [26]. In other words, a low value of this measure indicates concentration and a heightened sense of tension [27]. Taken together, the results of the Chromogranin A, LF/HF, and CVRR analyses suggest that when the participants listened to 528 Hz music, they concentrated on listening to the music while also relaxing.

Interestingly, after listening to 440 Hz music, mean levels of chromogranin A showed a tendency to increase, while LF/HF significantly decreased in both conditions. At the same time, the cortisol, oxytocin, and POMS 2 measures did not show stress reduction. This might suggest that people were relaxed by listening to music but did not feel comfortable with 440 Hz music.

The results of the POMS 2 showed that after listening to 528 Hz music, tension-anxiety and Total Mood Disturbance scores decreased significantly. After listening to 440 Hz music, almost all scores showed a tendency to change in a similar way as in the condition with 528 Hz music; however, not only was there no significant difference in any of the scores, but anger-hostility slightly increased and friendliness decreased. On the basis of these results, it can be concluded that participants also felt subjectively relaxed after listening to 528 Hz music.

Overall, we found that participants experienced objective and subjective stress reduction after listening to 528 Hz music, while this effect could not be confirmed for 440 Hz music. In this study, participants listened to music only for five minutes in each condition. In most previous studies, participants have listened to music for 10 minutes or longer. Therefore, it might be the case that listening to 440 Hz music for longer would evoke the stress-reducing effect, especially as indexed by chromogranin A and the POMS 2, as in previous studies. However, the point remains that even if the exposure to music lasts only five minutes, listening to 528 Hz music reduces stress to the endocrine system and the autonomic nervous system. Although the sample size of this study was limited, these are interesting data and could provide support for a new approach in music therapy.

5. Conclusion

We found that music of different frequencies had different effects on the endocrine system, especially oxytocin and cortisol. Our findings suggest that 528 Hz music reduces stress even if participants listen to the music for only a short time.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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