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A Thesis for the Degree of Master of Science

**Chocolate as a comfort food increases working memory
by reducing high-beta EEG oscillation in adolescents**

컴포트 푸드로서의 초콜릿 섭취로 인한
청소년의 하이베타 뇌파의 감소와
작업기억능력의 증가 효과

August, 2016

By

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Abstract

Comfort food is mostly known as any food consumed by individuals, often during periods of stress, which evokes positive emotions. In many researches, comfort food has been discussed as a cause of obesity and some metabolic diseases. However, in recent years, its psychological effect of relieving stress attracts people's attention. Moreover, previous studies showed the effects of stress on memory function. In this study I frame a hypothesis that the stress relieving effect of comfort food will also lead to increase in working memory function. In this study, chocolate was used as a representative of comfort food. Digit span task and Sternberg task were used to measure working memory capacity. In forward digit span task and Sternberg task, working memory scores increased after chocolate intake. I also found distinct electroencephalography (EEG) oscillatory patterns in high beta (23-36Hz) and theta (4-7Hz) band. Decrease in high beta which is related to stress relieving and increase in theta which reflect working memory improvement were

detected. These results indicate that increases in working memory capacity is due to the stress relieving effect of comfort food, which is reflected by high beta activity.

Keywords: Comfort food, Chocolate, Stress, Working memory, High-beta band, Theta band, Digit span task, Sternberg task

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I . Introduction

1.1. Comfort food

Lifestyle behaviors are themselves influenced by stress and the way how people are coping with it (Lindquist, Beilin et al. 1997). Emotional eating is one of the stress induced behavior (Torres and Nowson 2007). When stressed, people tend to be more vulnerable to emotional eating, and under this circumstance, people are more likely to intake more comfort foods (Kandiah, Yake et al. 2006). Comfort food is mostly known as “Any food consumed by individuals, often during periods of stress, which evokes positive emotions” (Dube, LeBel et al. 2005, Locher, Yoels et al. 2005). The notion of “comfort food” has become more popular since 1997 when it was added to the Oxford English Dictionary. Generally, the term “Comfort food” is commonly used for certain foods with stress-reducing effects which are mostly calorie-dense foods containing high amounts of carbohydrates and/or fats. Under stress, people tend to choose more snack-type comfort foods to reduce stress and provoke positive emotion such as desserts and chocolate, and meal-type comfort foods such as

burgers, meat items and pizza (Kandiah, Yake et al. 2006). It has been identified that the intake of comfort foods actually buffer all major physiological and behavioral responses to stress, and the key neural circuits underlying these effects (Ulrich-Lai, Christiansen et al. 2010).

1.2. Stress and working memory

Stress profoundly affects the neural correlates of memory formation. Over-stress reactions such as increase of stress hormones most frequently affects memory particularly through the hippocampus, prefrontal cortex and the amygdala regions (Henckens, Hermans et al. 2009). Stress has been demonstrated to both improve and impair working memory. Working memory is defined as a system which is needed to store and manipulate information during a short period of time (Baddeley 1992). Working memory is affected to a greater extent by stress compared to long term memory (Foyle and Hooey 2007). It is known that working memory consists of two storage systems: the

phonological loop and the visuospatial sketch pad, and the central executive acts as a supervisory system (Baddeley 2000, Roux and Uhlhaas 2014).

1.3. My hypothesis and present study

My primary hypothesis is that comfort food will increase the working memory capacity since comfort food reduces stress. I used chocolate as a representative of comfort food because it is a highly preferred comfort food (Kandiah, Yake et al. 2006). Digit span task (Clark, Veltmeyer et al. 2004) and Sternberg task (Jensen and Tesche 2002) were used to measure working memory function. Digit span task is common tool to measure working memory span (Conway, Kane et al. 2005). Sternberg task is also commonly used to measure working memory in association with electroencephalography (EEG) measurement (Roux and Uhlhaas 2014). EEG was simultaneously measured during the whole experiment.

II . Materials and methods

2.1. Participants

Forty, right-handed high school students (25 females, 35 males, ages: 16-18) with normal visual acuity, hearing ability and motor activity were participated in the study. They were voluntarily involved in the study and got the reward of 15 dollars. All subjects provided informed consent before participating, and the study was approved by the Institutional Review Board (IRB # 1601-001-003) at Seoul National University, Korea. Participants were separated into comfort food and non-comfort food groups by K means clustering algorithm (SPSS, IBM) based on comfort food rating of chocolate which is calculated by 7-score scale (Wansink, Cheney et al. 2003). To eliminate the possibility that the effect could be due to learning effects, which means that score might be increased because participants get used to the task experiment, I conducted the same correlation analysis in the control group who ate no chocolate. The experimental process of control group was same as the chocolate-treated group except no chocolate was given between session 1 and session 2. Grouping was randomly conducted. Participants visited the laboratory on one occasion only. The Participants with BMI over 23 was excluded from the study. Any food intake was prohibited from 2 hours before the experiment.

Figure 1

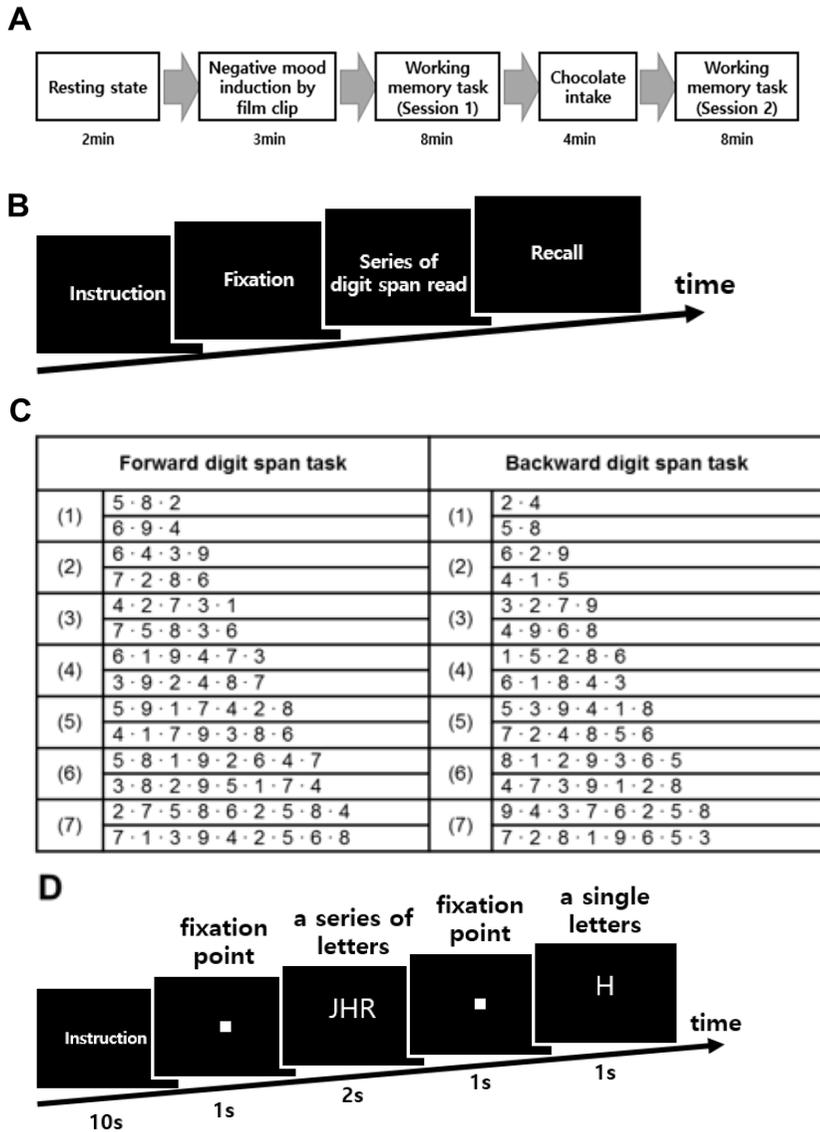


Figure 1. Experimental design. (A) Entire procedure of the experiment. (B) Procedure of the Digit span task. (C) Numbers used for digit span tasks. Series of numbers were spoken to participants and then participants repeated this numbers in forward or backward order. (D) Procedure of the Sternberg task. Series of letters were presented to participants. Thereafter the single letter was shown to the participants to judge whether it had appeared in the previous letters or not. Both Sternberg task and digit span task were used as working memory indicators.

2.2. Materials and procedures

The comfort food group and the non-comfort food group were involved in same experimental design. Participants were instructed about the fundamental principle and the procedure of the experiment. In a quiet room, each participant was instructed to sit on a comfortable armchair with neutral shoulder position and the elbow at 90° flexion in front of a 56 inch wide monitor to show the introductions and stress evoking film. Sternberg task was conducted using an 18 inch monitor which was located at a distance of 60 cm from the participants. During EEG preparation they were instructed with further procedures and answered the survey on the table. In the survey, participants answered questions about basic information such as age and BMI and comfort food rate of chocolate based on how much they consider it as comfort food which was expressed as 7-score scale (Wansink, Cheney et al. 2003). When the experiment started, participants were instructed to close eye for 2 minutes as an EEG stabilization period. Thereafter, they were instructed to watch the 3-minute film clip which could evoke negative feeling, especially stress (Schaefer,

Nils et al. 2010). Then, working memory task started. First task was digit span task including forward and backward memorizing trial. Fixation icon was on the screen during the task since digit span task is totally dependent on auditory processing. Sternberg task was conducted continuously. When the first session ended, they ate 15 g of chocolate and conducted the whole session again. Chocolate was chosen as a representative of comfort food because it is the highly preferred comfort food (Kandiah, Yake et al. 2006). The total duration of the experiment was approximately 40 minutes. EEG was measured simultaneously during the whole experiment.

2.3. Film clip

A film clip from American History X was used as stress inducer which is available in the internet for free. The story is about a neo-Nazi (who was played by Edward Norton) killing an African-American man, smashing his head on the curb. This clip was verified as the mostly negative feelings-evoking film among others by assessment study about emotion-eliciting films (Schaefer,

Nils et al. 2010). However, this can be different from individual to individual so participants who were not evoked negative feelings by this film were extracted from the experiment.

2.4. Digit Span task

Participants conducted the digit span memory task (Wechsler 1945) and Sternberg task to measure working memory capacity. Digit span task were containing both forward and backward memory task. Digits read aloud. Participants were required to give immediate ordered recall. At a particular span length, if the participants recalled digits correctly, the span length was then increased by two digits. Span was taken as the maximum length if performed without error. However, if participants answered wrong 2 times consequently, the task ended and number of correct answers were calculated as score (Klingberg 2010). In the first trial they spoke out the digits in order (forward), but in the second trial they did it in reverse order (backward).

2.5. Sternberg memory scanning task

Sternberg task (Sternber.S 1966), a standard measurement of working memory (Howard, Rizzuto et al. 2003), were presented after digit span task ended. In Sternberg task, participants were presented with a series of letters. Thereafter, a single letter was presented to participants to judge whether it had appeared or not in the previous letters. The number of letters increased as session progressed (set size: 3, 5 and 7). Each trial included the presentation of a series of letters (simultaneously) for 2 s, followed by a fixation point for 1 s, and a single letter for 1 s. Subjects pressed a right button if the single letter was presented in the previous letters and left button if it was not presented. They were instructed to press the button as quickly and accurately as possible.

2.6. Electrophysiological recordings

A 32-channel EEG system (WEEG 32a) along with a customized EEG-based real-time brain mapping software (Telescan, LAXTHA Inc., Korea) was used to acquire data on the cortical activity in the regions of interests (ROI)

during the experiment. Scalp electrodes (Ag-AgCl) on the specific locations were detected according to an extended 10/20 system. The procedure was conducted in an electrically shielded and sound attenuated experimental room. 32 scalp sites including Fz, Cz and Pz were recorded using the electrode cap (ECI. Inc., USA). All scalp electrodes were referred to linked electrodes placed on the left and right mastoid (right-reference, left-ground). Eye movements and blinks were eliminated by EOG filtering system. Impedance was maintained at 10 k Ω or less. The EEG was recorded continuously with 0.7–46 Hz analogue bandpass and a sampling rate of 256 Hz. After data collection, the EEG was segmented into 16 bit with respect to event markers. The epochs were baseline-corrected, and those contaminated with artifacts were rejected before averaging. The threshold for artifact rejection was $\pm 90 \mu\text{V}$ (EOG-filtering) in all channels. The epoch was averaged for target and standard stimulus separately.

2.7. Data analysis

Correlation between data was calculated using Pearson's correlation coefficient. Group differences were calculated by t-test. Statistical significance was considered at the 5% level. All statistical analysis were performed using the computer software SPSS (IBM Inc, US). Wavelet transformation and brain mapping was conducted using and MATLAB (Mathworks Inc, USA).

Figure 2

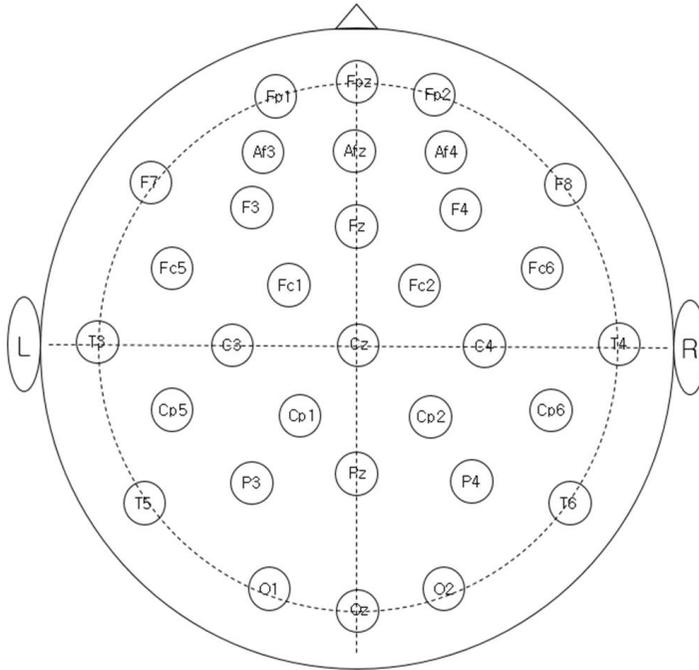


Figure 2. Location of 32 electrodes based on 10/20 system. Prefrontal regions (Fp1, Fp2) and frontal regions (F3, F4) were regions of interest. High beta EEG oscillations (23-35 Hz) in these area were analyzed to measure stress related signal, and theta EEG oscillations (4-7 Hz) were analyzed to measure working memory-related signals.

III. Results

Whole experiment which consists of 2 sessions, was conducted in 40 minutes (Fig. 1). Several working memory tasks were used with EEG measurement. 32 channels were used according to the 10/20 system (Fig. 2).

3.1. Forward digit span score was positively correlated with comfort food rating score.

Digit span task is mainly used to measure the working memory capacity of individuals. Forward digit span task is related to phonological loop system and backward digit span task is related to central executive system (Gregoire and VanderLinden 1997). I conducted these task to measure which system of working memory is affected by emotional effects of comfort food. Forward and backward trial were separately analyzed. As more people consider chocolate as comfort food, more forward digit span performance increased ($r=0.4929$, $p<0.05$) after chocolate intake (Fig. 3A). To eliminate the possibility that the effect could be due to learning effects, which means that score might

be increased because participants get used to the task experiment, I conducted the same correlation analysis in the group without chocolate. In the group without chocolate, there was no significant difference in session 2 forward digit span task compared to session 1 ($r=0.1566$, $p>0.05$) (Fig. 3B). In backward digit span there was no significant relationship between task performance and comfort food rating ($r=-0.1999$, $p>0.05$, data not shown).

Figure 3

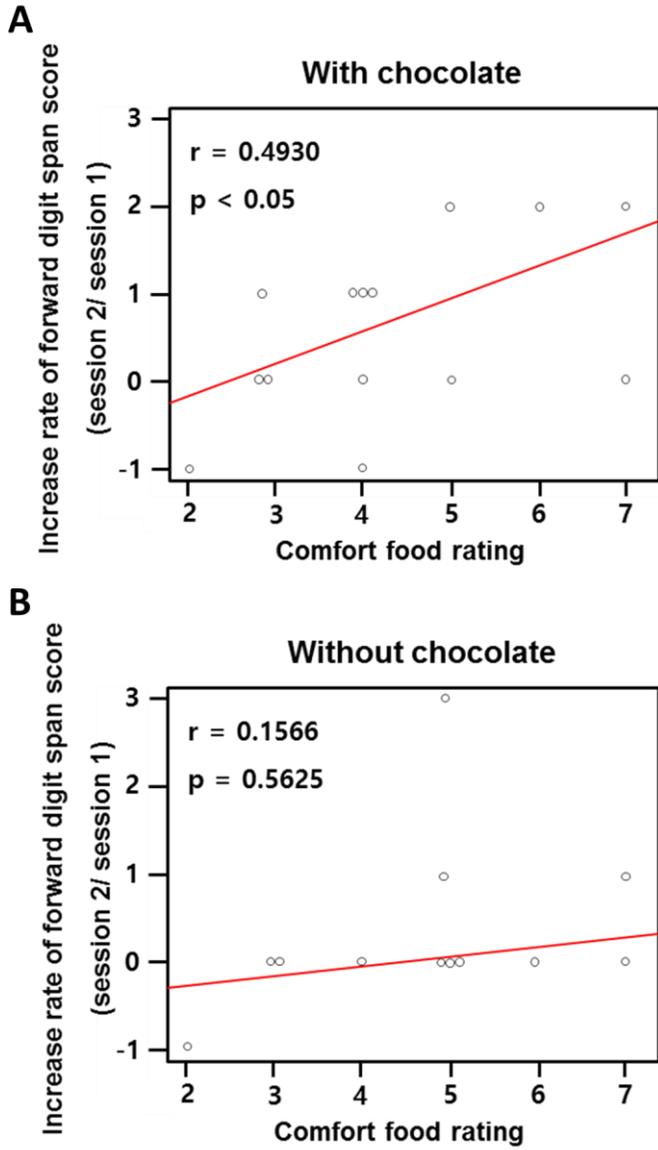
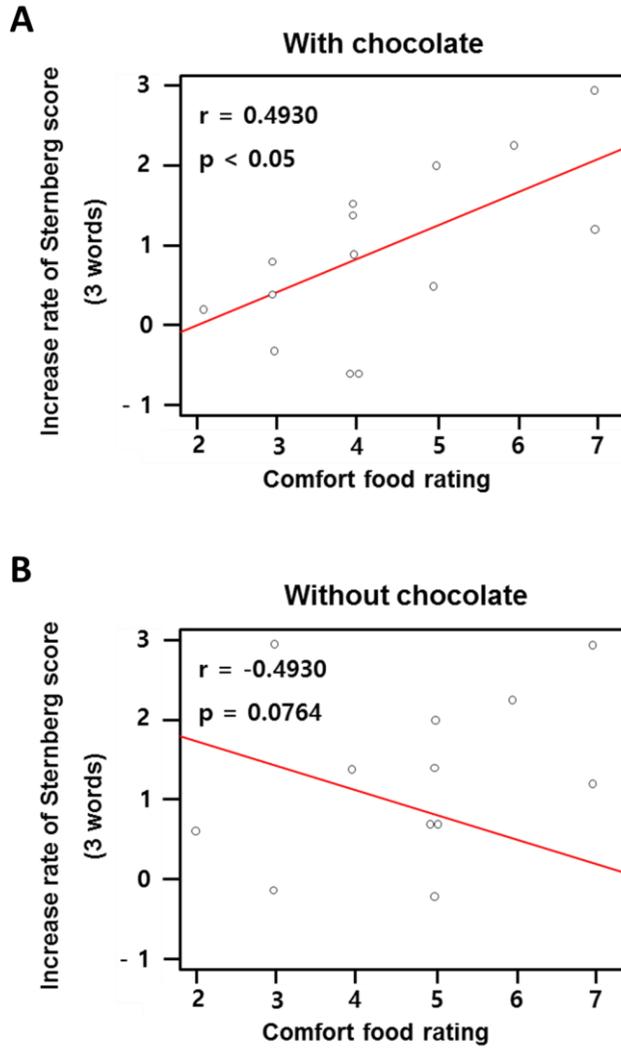


Figure 3. Correlation between digit span task score and comfort food rating score (A) Increase rate of forward digit span task score depending on comfort food rating score was significant in the chocolate-intake group (n=14, $r=0.4930$, $p<0.05$). Increase rate was calculated by dividing score after chocolate intake by score before chocolate intake. (B) A relationship between digit span task score and comfort food rating score in the group without chocolate was analyzed to see the learning effect and time effect. The group without chocolate showed no significant relationship (n=12, $r=0.1566$, $p>0.05$).

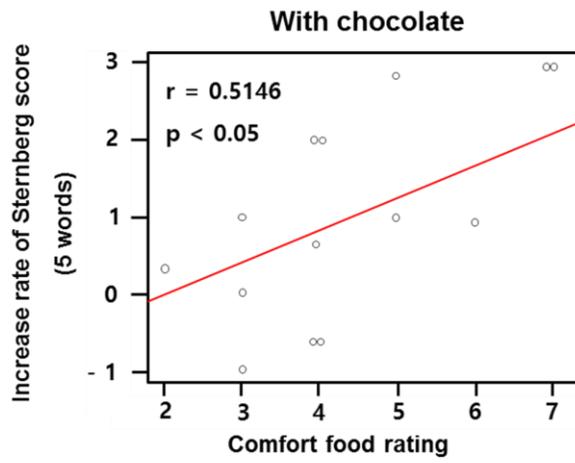
3.2. Sternberg task score was positively correlated with comfort food rating score.

In the Sternberg task, correlation between Sternberg score and comfort food rating score were calculated depending on the length of presented letters to measure the working memory capacity. The number of letters increased as session progressed (set size: 3, 5 and 7). The number of correct answers were calculated as score. Score of 3 words Sternberg score showed significant correlation with comfort food rating score ($r=0.4930$, $p<0.05$) (Fig. 4A). To see the learning effect I compared this result with the group without chocolate intake. In the group without chocolate, increase rate of the Sternberg score tended to decrease as the as the comfort food rating increased ($r= -0.4930$, $p=0.0764$) (Fig. 4B). In the 5 word Sternberg trial, score showed significant correlation with comfort food rating ($r=0.5146$, $p<0.05$) (Fig. 4C). However in the group without chocolate intake, there was no significant effect ($r=-0.3952$, $p=0.1449$) (Fig. 4D). In the 7 word Sternberg trial, there was increasing tendency but no significant correlation ($r=0.4360$, $p=0.08$) (Fig. 4E). The group without chocolate intake did not show significant effect ($r=-0.1210$, $p=0.6674$) (Fig. 4F).

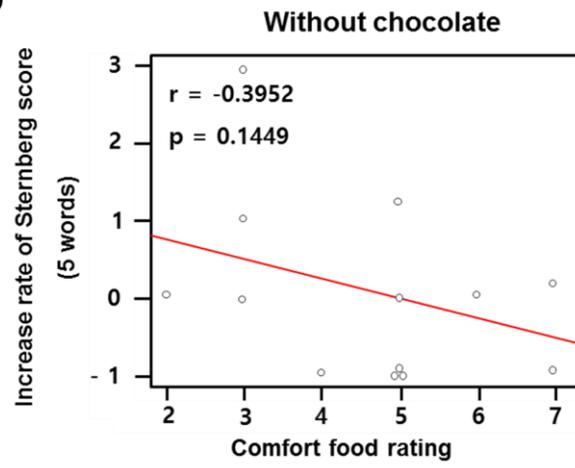
Figure 4



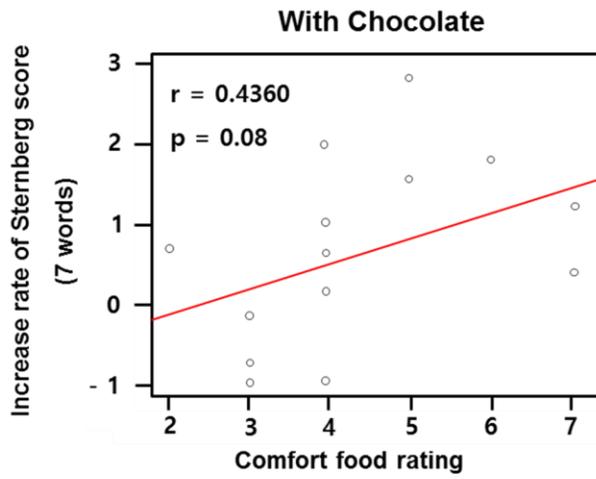
C



D



E



F

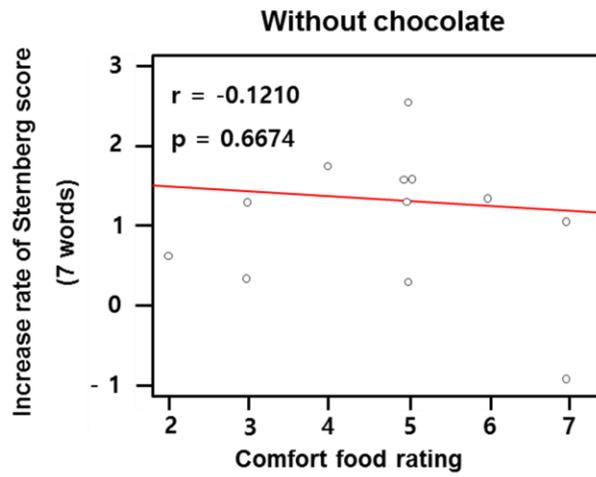


Figure 4. Correlation between Sternberg task score and comfort food rating score. Increase of Sternberg task score was calculated by dividing score of session2 by score of session1 (n= 26). Sternberg task consists of three trials depending on the length of letters. (A) 3 word digit span Sternberg score (r=0.4930, p<0.05) of the chocolate-treated group. (B) 3 word digit span Sternberg of the chocolate-non-treated group score (r= -0.4930, p=0.0764). (C) 5 word digit span Sternberg score (r=0.5146, p<0.05) of the chocolate-treated group. (D) 5 word digit span Sternberg score of the chocolate-non-treated group (r= -0.3952, p=0.1449). (E) 7 word digit span Sternberg score of the chocolate-treated group (r=0.4360, p=0.08). (F) 7 word digit span Sternberg score of the chocolate-non-treated group (r= -0.1210, p=0.6674).

3.3. EEG results

3.3.1. High beta band increased after watching stress-evoking film clip.

A film clip from American History X was used as a stress inducer. I analyzed high beta oscillation to measure the stress related signal. High beta (23-36 Hz) data in prefrontal cortex were analyzed since it is related to stress and negative emotion [16]. The high beta activity in prefrontal region was analyzed to examine whether high beta activation increases after watching the stress evoking film clip. High beta activity significantly increased in prefrontal area after watching the film clip ($p < 0.05$) (Fig. 5). These results indicate that the film clip induced stress related responses in the prefrontal area of the subjects.

Figure 5

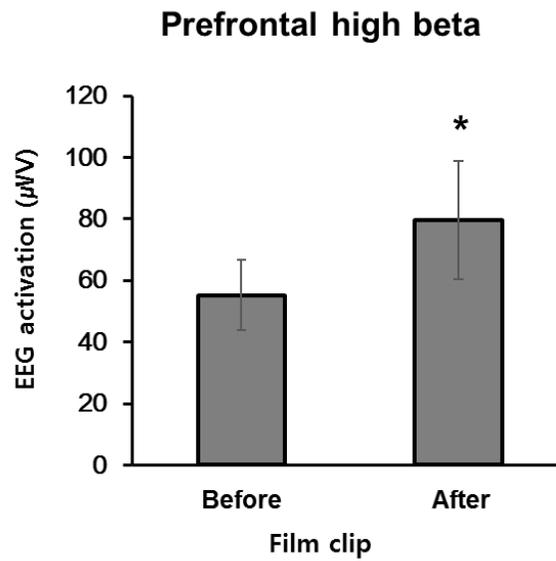


Figure 5. Increases of high beta activities after watching film clip which evokes negative feelings. Data are presented as mean \pm S.E. * $p < 0.05$ vs. Before watching film clip (n=36).

3.3.2. High beta band during forward digit span task decreased in comfort food group after chocolate intake.

Since high beta activity (23-36 Hz) reflects stress related signal (Alonso, Romero et al. 2015), I measured the high beta activity in prefrontal (Fp1, Fp2) and frontal (F3, F4) regions during working memory task to analyze the change of stress related signal after chocolate intake in both comfort and non-comfort food group. As consistent with the results of forward digit span task, prefrontal high beta activity was significantly lower in the comfort food group compared to the non-comfort food group ($p < 0.05$) (Fig. 6A). High beta activity in the frontal region also decreased significantly in comfort food group ($p < 0.05$) (Fig. 6B) during forward digit span task. However, in the backward task digit span, there was no significant change of high beta activity in either comfort or non-comfort group ($p > 0.05$) (data not shown). In Sternberg task, there was no significance in high beta change between two groups ($p > 0.05$) (data not shown). Brain mapping shows distinct significant decrease of high beta in the comfort food group compared to the non-comfort food group during forward digit span task after chocolate intake (Fig. 6C). However, there was no such significance in the group without chocolate who did not eat chocolate (Fig. 6D). These results indicate that the stress reducing effect is due to chocolate as comfort food.

Figure 6

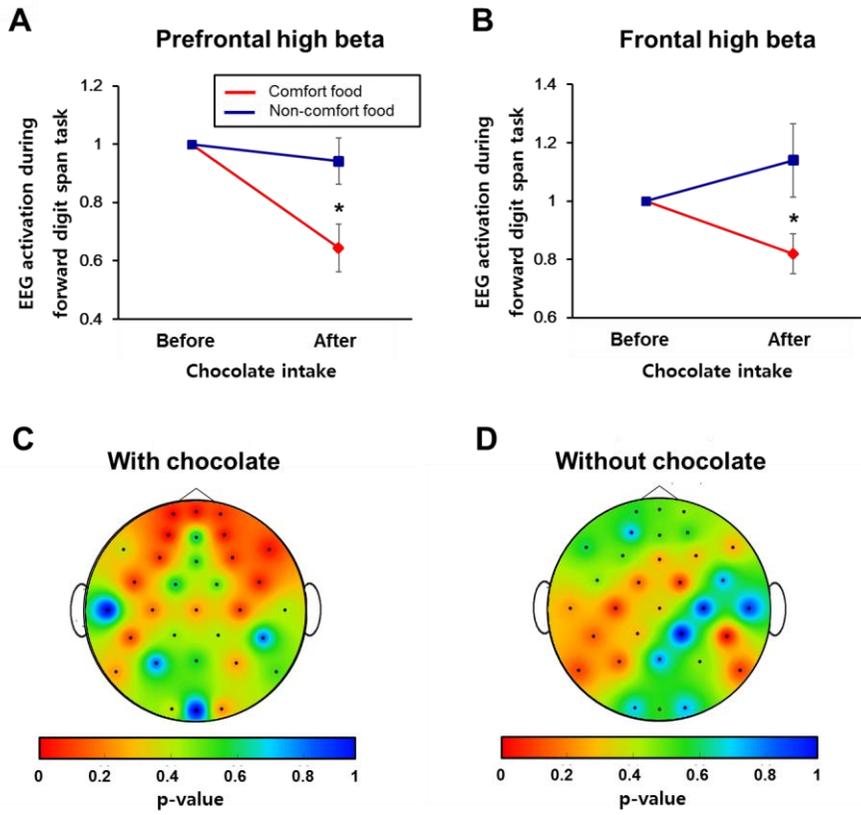


Figure 6. High beta (23-35 Hz) tendencies during forward digit span task. (A) Relative high beta values before and after chocolate intake in the prefrontal regions (A) and the frontal regions (B) of both comfort and non-comfort food groups (n= 26). Data are presented as mean \pm S.E. * $p < 0.05$ vs. Non-comfort food group. Brain mapping of p-value comparing high beta EEG between comfort and non-comfort food group with chocolate (C) or without chocolate intake (D). Image are presented using MATLAB.

3.3.3. Theta band changes during Sternberg task increased in comfort food group after chocolate intake.

Since the theta activity (4-7 Hz) during Sternberg task reflects working memory capacity (Maurer, Brem et al. 2015), I analyzed theta activity in the prefrontal cortex (Fp1, Fp2) and the frontal cortex (F3, F4) during Sternberg task. Among the whole brain areas, prefrontal area of the brain was highly activated during the Sternberg task. The comfort food group showed a significant increase in prefrontal theta activity during Sternberg task compared to the non-comfort food group ($p < 0.05$) (Fig. 7A). This result indicates that working memory capacity increased significantly in the comfort food group after chocolate intake compared to the non-comfort food group. In forward digit span task, prefrontal and frontal theta activity showed no significant change in the comfort food group compared to the non-comfort food group ($p > 0.05$) (Data not shown). In backward digit span task, there also was no significance ($p > 0.05$) (Data not shown). However, in Frontal area, there was no significant theta activity in comfort food group compared to non-comfort group ($p < 0.05$) (Fig. 7B). To eliminate the time effect which is natural phenomenon as time flows, whole brain activity was analyzed. Prefrontal theta activity increased significantly in comfort food group only after they ate chocolate ($p < 0.05$) (Fig. 7C, 7D).

Figure 7

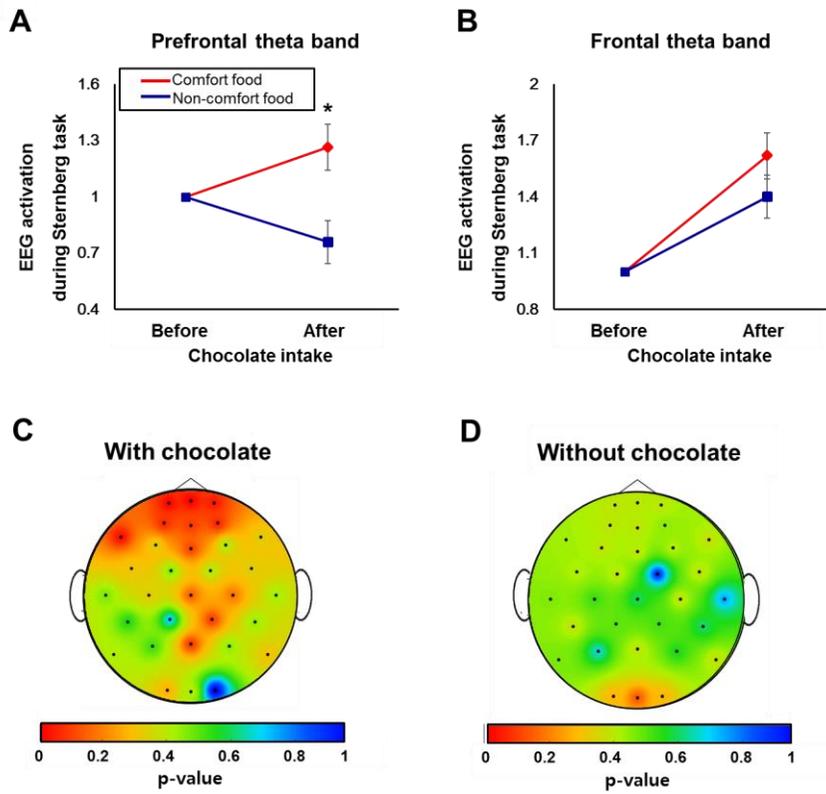


Figure 7. Theta (4-7 Hz) tendencies during Sternberg task. (A) Relative theta values before and after chocolate intake in the prefrontal regions (A) and the frontal regions (B) of both comfort and non-comfort food groups (n= 26). Data are presented as mean \pm S.E. * $p < 0.05$ vs. Non-comfort food group. Brain mapping of p-value comparing high beta EEG between comfort and non-comfort food group with chocolate (C) or without chocolate intake (D). Image are presented using MATLAB.

IV. Discussion

In this study I aimed to demonstrate relationship between working memory and comfort food. The main hypothesis that chocolate as comfort food will increase working memory capacity by reducing stress was determined by working memory task scores and related EEG signal.

4.1. Increase in working memory capacity after comfort food intake

The behavioral results demonstrated that working memory capacity significantly increased after chocolate intake in comfort food group in several working memory tasks (Fig. 3, 4). I assumed that if the working memory increased after chocolate intake in comfort food group, it might be due to stress relieving effect of individual's comfort food since decrease in stress improves working memory performance (Roosendaal 2002, Mrazek, Franklin et al. 2013). In the forward digit span task which showed increase in working memory capacity after chocolate intake, stress related high beta (23-36 Hz) EEG oscillation in prefrontal and frontal areas significantly decreased in comfort food group (Fig. 6). Backward digit span task showed no significant tendency. Backward digit span task involves more complicated processing and thus calls

for a larger involvement of the central executive resources (Gregoire and VanderLinden 1997). The central executive is an attentional control system engaged in strategy selection, memory control and coordination of the various processes related to short-term storage and general processing tasks (Desposito, Detre et al. 1995). Therefore based on data, comfort food intake only affected temporal memory storage system which is related to phonological loop, rather than central executive. This might be due to age related characteristic. Sternberg task, that sequential encoding is involved, is also related with phonological loop. It can activate phonological loop to maintain sequenced working memory items by subvocal rehearsal (Roux and Uhlhaas 2014). In comfort food group the Sternberg task score increased compared to non-comfort food group even though both group ate chocolate (Fig. 4). From these working memory tasks results, I can assume that comfort food intake increase working memory by affecting phonological loop system. However, in Sternberg related high beta EEG oscillation did not decreased after chocolate intake. Stress relieving effect is possible candidate which caused working memory improvement. However, since comfort food evokes individual's emotional craving, it could work as reward which means that working memory increase in Sternberg task might be due to reward system in brain. Recent studies have demonstrated the effects of brain areas related to reward on memory-related areas that are involved in memory encoding, processing and storage (Pochon, Levy et al. 2002). There

also is a study that reward-related brain regions are related to increase in capacity of working memory (Kawasaki and Yamaguchi 2013).

Since tasks scores which is related to phonological loop increased, I expected that the theta activity would be increased because tasks related to sequential encoding which activate the phonological loop supports the maintenance of sequenced working memory items thorough subvocal rehearsal which increases theta activity (Roux and Uhlhaas 2014). However it only increased during Sternberg task not in forward digit span task (Fig. 7). Sternberg task is well known task which is highly related to theta (4-7 Hz) band activation (Raghavachari, Kahana et al. 2001, Jensen and Tesche 2002) but there rarely is evidence on relationship between digit span task and theta band itself. I assumed that other variables exist.

4.2. The future food: Healthy comfort food

Our approaches on comfort food is different from majority of studies covering comfort food. Keyword such as obesity, metabolic syndrome, cardiovascular disease, and diabetes are frequently used words in comfort food studies since comfort food is highly related to emotional and impulsive eating and stress induced eating (Duyff and Association 2002, Kandiah, Yake et al. 2006, Tomiyama, Dallman et al. 2011, Tryon, DeCant et al. 2013). The reason

I investigated psychological effects of comfort food is not to promote the consumption of unhealthy comfort food. The reason I have to eat healthy food is apparent. However, most of healthy food (ex> functional food, food extracts and food supplements) restrict happiness of eating food and freedom to choose their favorite food. If person who has emotional eating habit restrict their eating, it could result in more serious impulsive eating (King and Gibney 1999). I expect that our future food will be “healthy comfort food”. We will eat what I want and be healthy by new technologies and researches. New food material will be used (ex> Meat made by soybeans, vegetable egg etc.) and food technologies are being developed and innovated year by year (Coutand, Cyr et al. 2008, Johanningsmeier, Harris et al. 2016). In this manner I expect that healthy comfort food also be developed and evolved continuously. In the further research, I am planning to demonstrate that comfort food which is made in healthy way could still result in positive psychological effects.

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VI. 국문 초록

컴포트 푸드는 스트레스 상황에서 개인이 긍정적 기분을 고취시키기 위해 먹는 음식으로 정의된다. 선행 연구들에서 컴포트 푸드는 비만이나 대사증후군 같은 부정적인 영향에 초점을 맞추어 연구되어왔다. 본 연구는 컴포트 푸드의 긍정적 심리적 효과에 대한 것이다. 연구의 가설은 컴포트 푸드가 스트레스를 낮추어 줌으로써 궁극적으로는 작업기억능력에도 긍정적인 영향을 미친다는 것이다. 본 연구에서는 초콜릿을 컴포트 푸드의 대표 식품으로 사용하였다. Digit span task와 Sternberg task는 작업기억능력을 측정하기 위해 실험 중 사용되었다. Forward digit span task와 Sternberg task의 점수는 초콜릿을 컴포트 푸드로 여기는 그룹에서 초콜릿 섭취 후 증가하는 경향을 보였다. 이에 대한 메커니즘을 규명하기 위해 뇌파를 측정하였다. 컴포트 푸드 그룹에서 초콜릿 섭취 후에 스트레스와 관련된 하이베타파(23-36Hz)가 감소하는 것을 확인하였으며 작업기억능력과 관련된 세타파(4-7Hz)는 증가하

는 것을 확인할 수 있었다. 본 연구는 콤포트 푸드로서의 초콜릿의 스트레스 저하 효과를 확인하고 이와 관련된 뇌과학적 메커니즘을 최초로 규명하였으며 신체적으로뿐만 아니라 정신적으로도 건강한 미래의 식품에 대한 방향성을 제시하였다.

주요어 : 콤포트 푸드, 초콜릿, 스트레스, 작업기억능력, 하이베타과, 세타과, Digit span task, Sternberg task

학번 : 2014-22466