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Original Article

Effects of acute, sub-chronic and chronic chocolate consumption with different percent of cocoa/sugar on memory and EEG waves in rats

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ABSTRACT

Introduction: People mainly have a major interest in eating some palatable foods such as chocolate and sweet foods that influence brain functions. This study investigated the effects of acute, sub-chronic and chronic chocolate consumption with different percent of cocoa/sugar on learning, memory, memory consolidation and electroencephalogram (EEG) waves in rats.

Methods: Thirty-five male Wistar rats were allocated to five main groups containing control and sucrose as well as dark, milk and white chocolate groups. All groups were freely fed with chow, different kinds of chocolate and sucrose separately for 1,7 and 14 days as acute, sub-chronic and chronic food consumption. Also, memory and memory consolidation were evaluated using a passive avoidance test on days 1,7 and 14. In addition, brain electrical activity was evaluated by EEG.

Results: Acute and sub-chronic dark and milk chocolate consumption significantly improved latency after day 1 and particularly day 7. In addition, only the chronic dark chocolate diet showed a significant enhancement in latency after 14 days. White chocolate and sucrose diets did not have significant effects on three latencies. The milk and dark chocolate diets changed nearly all brain waves of EEG, while the sucrose diet did not affect any of them.

Conclusion: Unlike sucrose and white chocolate, dark chocolate (acute, sub-chronic and chronic consumption) and milk chocolates (acute and sub-chronic consumption) had beneficial effects on memory and nearly all electrical brain activity probably due to high levels of cocoa and perhaps its antioxidant effect. Hence, these types of diets modified brain homeostasis and increased conscious state and relaxation reduction.

Keywords:
Chocolate
Memory
EEG
Sucrose
Rats

Introduction

Effect of food habits was indicated on some brain functions (Knezevic et al., 2016), as to intake palatable and addictive foods (consumption raising of foods) affect different aspects of behavioral, cellular and molecular brain functions (Avena et al., 2008; Songsamoe et al., 2019). Some foods such as different types of chocolate and sweet foods are known as the major palatable dietary. Also, those are very available and full usage during the past decade (Freeman et al., 2018; Samerphob et al., 2020). These foods affect different aspects of brain functions such as hyperactivity, anxiety, cognition, memory and mood (Crichton et al., 2016; Lange and Lange 2021; Meier et al., 2017; Messereli 2012;

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Sokolov et al., 2013) which are assessed by different methods such as behavioral and electrophysiological tests. Generally, each waveform of EEG signal can be analyzed in different brain parts, and the resulting data explain the brain functions and behavior; as the electrical activities were reported at multiple sites of the brain cortex by EEG, and are useful in studying recognition, learning and memory, as well as mood impairments (Hanslmayr et al., 2011; Kirk et al., 2021). However, beta waves indicate a normal alert and conscious situation; alpha waves indicate a mentally relaxed and physically situation; theta waves indicate deep meditation, reduced consciousness, awake but not consciousness; and delta waves represent the loss of bodily awareness and deep sleep (Campbell, 2009; Takahashi et al., 2005). In food research, the EEG techniques were also classified into several objectives, containing investigation of food consumption effects on brain functions, motivational tendency, behavioral and emotional responses of consumers and also the appearance of taste, flavor and food texture (Songsamoe et al., 2019). Indeed, the changes of the EEG spectrum of different waveforms (such as alpha, beta, theta, etc.) interpreted the brain functions of the consumer after consuming food (Labbe et al., 2011).

Moreover, white sugar is one of the most important sweet foods that is commonly used as palatable food in different forms such as granulated sugar, sugar cubes and sweetened products (Clarke, 2000; Kendig, 2014). In addition, the commercially available chocolate is derivated from various percentages of cocoa powder, milk, butter and sugar (Betsy, 2012). There are different types of chocolate (with increasing amounts of cocoa/sugar) including white chocolate (just contain cocoa butter and sugar), milk chocolate (up to 60% cocoa powder) and dark chocolate (at least 70% cocoa powder) (Torrico et al., 2018). Cocoa and its products affect cognition, mood and behavior (Martin et al., 2020; Sokolov et al., 2013). Furthermore, brain function may be impaired by prolonged elevations of blood glucose (Jacob et al., 2002), whereas another study reported that glucose treatment reduces memory deficits in rats fed high-fat diets (Greenwood and Winocur, 2001). Also, brain electrical activity depends on blood glucose in the brain (Serres et al., 2004).

Despite the extensive reported research on the different aspects of portable foods, no published report is yet available on the comparing effects of acute, sub-chronic and chronic chocolate on changes of memory and EEG waves. Hence, this study designed to investigate the effects of acute, sub-chronic and chronic of some palatable foods from white sugar cubes (as high-sugar food, about 100% sucrose) to different types of chocolate with various chocolate sugar content (different percent of cocoa/sugar) containing white, milk and dark (high-cocoa food, about 97% cocoa) chocolate as spectra from sucrose to dark chocolate (cocoa, sugar and the combination of them) on memory, memory consolidation and brain electrical activity (EEG waves) in rats.

Materials and methods

Animals

In this study, thirty-five male Wistar Rats (initial weight 200–250 g) were obtained from Pasteur Institute, Tehran, Iran. The animals were maintained under controlled light conditions (12 h light/dark; lights at 07 a.m.–7:00 p.m.) and humidity (50% ± 5%), in the cages. The room temperature was set to 23 ± 2° C, and water was made available ad *libitum.*

The Ethics Committee of Animal Use at the Isfahan University of Medical Sciences approved the study (IR.MUI.REC.1397.359) as all experiments were conducted in compliance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23, revised 2011). After a period of one week for habituation, the animals were assigned to the following five groups (n =7): Control (Co) group, in which animals were freely fed with only rodent's food (Chow); Sucrose (Suc) group, in which the rats were freely fed only with sugar cubes; and three chocolate groups containing the white chocolate (White Choc), milk chocolate (Milk Choc) and dark chocolate (Dark Choc) groups, in which the rats were freely fed with only white chocolate; milk and dark chocolate; respectively. The experiment duration was 14 consecutive days for all groups. Finally, all the rats were performed a passive avoidance test as a behavioral experiment at 13:00-14:00 on days 1, 7 and 14. Also, the EEG waves were recorded on day 15 (Figure 1).

Experimental procedures

Food diets

The mono-diet or single-food diet is a simple eating pattern that involves eating just one food item or food group for all meals throughout the day. Propo-

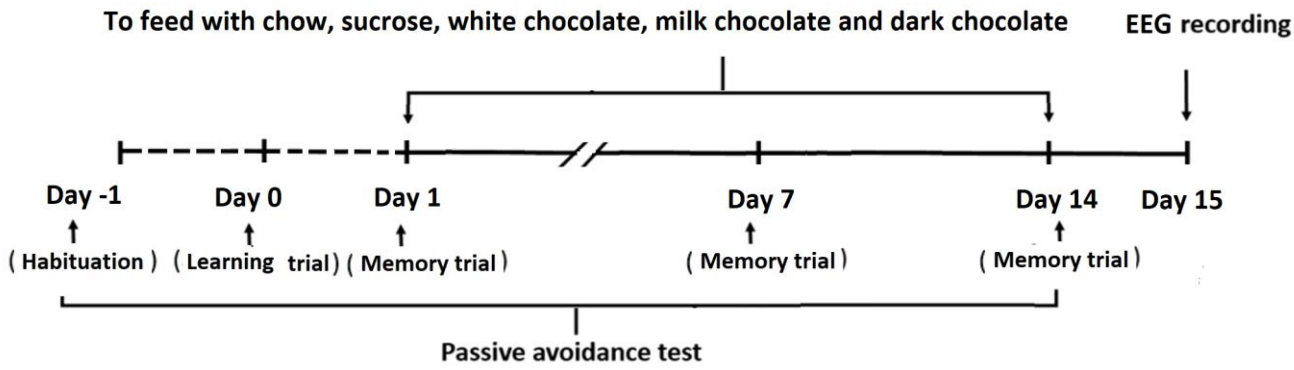

FIGURE 1. The study experimental design.

nents claim that the mono-diet can quickly affect some physiologic systems such as the brain. In other words, diet patterns influence mental health such as cognition, emotion and behavioral performance (Bidi et al., 2016). In this study, different experimental rats consumed respectively the solid chow, sugar cubes (solid sucrose), commercially white, milk and dark chocolate (solid chocolate; Farmand Co., Alborz, Iran) that have slightly different nutrients like vitamins, minerals (such as sodium, potassium, calcium, magnesium, zinc, and iron) and so on (Kruszewski and Obiedziński, 2018). Therefore, some addictive palatable foods from various types of chocolate with different ratio of cocoa/sugar containing dark (high-cocoa food, about 97% cocoa; almost sugarless), milk (60% cocoa powder) and white (0% cocoa powder) chocolate diets to white sugar cubes (as high-sugar food, about 100% sucrose) diets were used in the present study. Therefore, the spectra of the quantity of both cocoa powder and sugar (cocoa/sugar ratio) were investigated as different types of chocolate with respect to routine food (chow) on brain functions.

Experimental Paradigms

Passive avoidance test

In this study, learning, memory and memory consolidation were measured by the passive avoidance (PA) test in three intervals. The protocol of the passive avoidance test was similar to a previous study (Dastgerdi et al., 2018). According to that, the passive avoidance apparatus had identical light and dark rooms with sliding guillotine doors and grid floors. This test was conducted with three phases (300 s) containing habituation, learning and memory trials. A single electric shock (0.5 mA, 50 v and 2 s; once) was delivered to the animal's foot through the grid floor in the learning phase. The initial latency time to the dark room entrance was recorded before inducing the electric shock. In this experiment, the latencies differences between the initial and after 1 day were translated as the occurrence of learning (Dastgerdi et al., 2018). Also, the latency time of entry to the dark room was measured after 1, 7 and 14 days (up to a maximum delay of 300 s) as memory assessment in a different time. In addition, the total dark stay (DS) time was assigned as either memory consolidation and/or storage of new information (Dastgerdi et al., 2018).

Stereotaxic surgery

In this research, one day after exposure to the final experimental session (day-15), the animals were anesthetized by intraperitoneal (i.p.) injections of urethane (1.5 g/kg; Sigma, USA) (Hosseini et al., 2017). Then, the rats were being placed in a stereotaxic frame (Stoelting Co., USA). The rats' skull was exposed and drilled over dura two small holes using a bergma reference point and a standard miniature drill equipped with a 0.5 mm diameter. The holes were on the left and right sides of the skull (AP=2 mm; ML=±1.5 mm). Then, electrodes were placed sub-cranially (at the level of the cortex) for EEG recording. The relevant interfaces to the recording device are connected to the rat and the device.

EEG electrophysiological study

In order to assess brain electrical activity, the brain waves are recorded by electroencephalography (EEG) recording. Indeed, alteration of memory efficiency and cognitive state will be revealed by changes in EEG waves (Ndaro and Wang, 2018). In addition, some previous reports have applied the EEG method to investigate the role of food consumption on brain functions (Songsamoe et al., 2019).

In the recording of EEG, rats were placed on a suitable and good pad. Then, they covered during the experiment to record better signals. In each anesthetized rat, the EEG waves were recorded for nearly 20 minutes. Signals of low-pass were filtered at 0.5–3 kHz and sampled at 1 kHz. Also, these signals were additionally passed through an analog to digital interface to a computer. Next, the obtained data were analyzed by using the eTrace analysis software for transferring data to a data acquisition system (Data Acquisition, Science Beam-D3111; eProbe- eTrace Experiment software; Parto Danesh Co., Tehran, Iran).

In EEG, the power of beta (13–30 Hz), alpha (8–13 Hz), theta (4–8 Hz) and delta (1–4 Hz) waves were confirmed (Jurkowlaniec et al., 2003; Radahmadi et al., 2017). However, different types of brain waves recorded from the brain at all times (conscious or unconscious situations) (Jalilifar et al., 2016). These waves were recorded spontaneously, and those are different from exciting waves. It is noticeable that their ratio is very important in different conditions. Sine, these waves are measured relatively, therefore, determining the percentage for the ratio of each type of wave to the total power is very important in among awake, asleep, anesthesia condition and so on in experimental investigation (Hagihira, 2015). Moreover, the total power of these four frequency bands was taken as the full percentage of 100%. Finally, the percentage for the ratio of each frequency band to the total power was calculated for all groups (Radahmadi et al., 2017; Rahimi et al., 2019).

Statistical analysis

All behavioral and electrophysiological data were reported as means ± standard error of mean. The between-group comparisons were performed using one-way Analysis of variance (ANOVA) followed by LSD post-hoc test for multiple comparisons. Also, comparisons of initial latency with latency after 1 day, as well as within-group comparisons of latencies in 1, 7, and 14 days were analyzed using the paired Student's t-test. A P-value of less than 0.05 was considered as statistically significant. All calculations were performed using SPSS 24 (SPSS Inc., Chicago, IL, USA).

Results

Behavioral results

Figure 2 A-D, respectively, shown the initial latency and the latency in different time intervals (after 1, 7 and 14 days) in the PA test for all experimental groups. The one-way ANOVA did not show significant differences in initial latency in all experimental groups (figure 2. A).

Data showed no significant differences in latencies 1, 7 and 14 days in both sucrose and white chocolate groups when compared with the control group (Figure 2 B-D), indicating that acute, sub-chronic and chronic sucrose and white chocolate consumption did not improve memory.

The latencies of days 1 and 7 were significantly higher in the milk chocolate group than the control group (respectively P=0.019 and P=0.008) (Figure 2.B and C). Whereas, the latencies of the dark chocolate group showed significant (P=0.009, P=0.000 and P=0.019) enhancements compared with the control group on days 1, 7 and 14, respectively (Figure 2 B-D). It indicated that indicating that the acute, sub-chronic and chronic dark chocolate consumption improved memory. Whereas, the acute and sub-chronic milk chocolate consumption enhanced memory.

In comparison with the sucrose group, only the latency of day 7 was significantly (P=0.020) higher in the milk chocolate group (Figure 2 C). In addition, the latencies after 1, 7 and 14 days significantly increased in the dark chocolate group (P=0.048, P=0.000 and P=0.023; respectively) in comparison with the sucrose group (Figure 2 B-D).

As shown in Figure 2.B, compared to the white chocolate group, the latency of 1 day significantly (P=0.044) increased in the milk chocolate group. Whereas, the latencies of days 1, 7 and 14 in the dark chocolate group had significant (P=0.021, P=0.019 and P=0.009; respectively) enhancements in comparison with the white chocolate group (Figure 2 B-D).

Comparisons between latencies of initial and three trials were analyzed using the paired sample t test to evaluate within group latency changes. In the current study, the initial latency vs. latency of day1; latency of day1 vs. day7; latency of day7 vs. day14 and latency of day1 vs. 14 compared (Figure 3). The comparison between latency initial and latency after 1 day shows learning and comparisons of other trial sessions indicated the changes of memory trend.

Significant differences were observed between initial latency and latency after 1 day in all experimental groups (P=0.000 in the milk and dark chocolate groups,

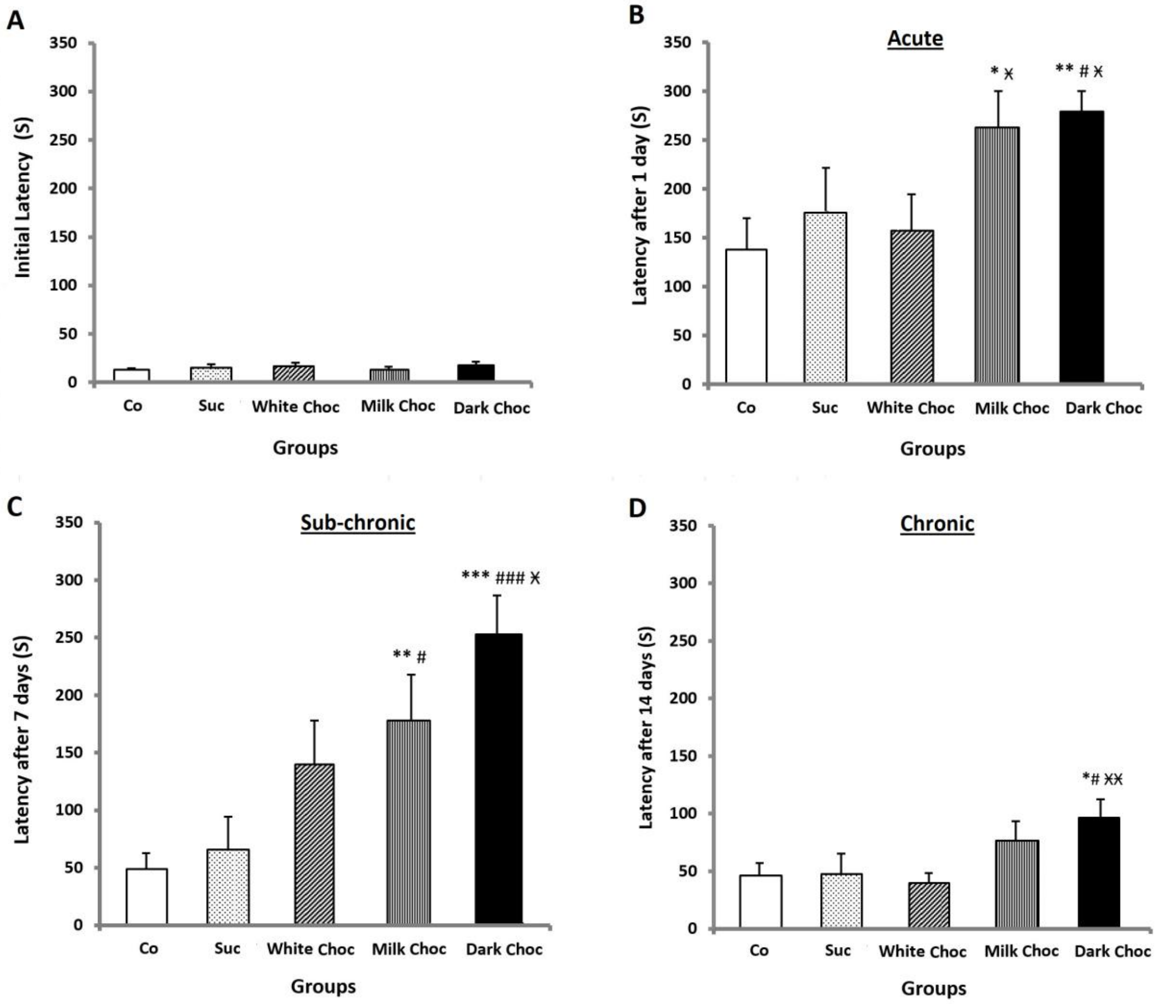

FIGURE 2. A) Initial latency before foot shock. B-D) Latency to enter the dark room (of the passive avoidance test) for acute, sub-chronic and chronic food consumption in all experimental groups (1, 7 and 14 days after foot shock; n=7). Data represented as mean±SEM (One-way analysis of variance followed by LSD post hoc test). $^{*}P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$ with respect to control group; $^{\#}P<0.05$ and $^{\#\#\#}P<0.001$ with respect to sucrose group; $^{X}P<0.05$ and $^{XX}P<0.01$ with respect to white chocolate group.

P=0.008 and P=0.007 respectively in the control and white chocolate groups, P=0.012 in the sucrose group) (Figure 3). It is indicated that there was learning in all experimental groups with different levels.

As shown in Figure 3, significant differences were observed in latencies of day 1 vs. day 7 of the control and sucrose groups (P=0.005 and P=0.007, respectively), whereas, similar comparisons had no significant differences in all three chocolate groups.

On the other hand, there were significant differences in latencies of day 7 vs. day 14 of the white, milk and dark chocolate groups (P=0.031 and P=0.043 respectively in white and milk chocolate groups, P=0.003 in dark chocolate group) after receiving the electrical foot shock (Figure 3). However, totally, the latencies of day 1 vs. day 14 had a significant difference in all experimental group (P=0.019, P=0.018 and P=0.031 respectively in the control, sucrose and white chocolate group; P=0.002 in the milk chocolate and P=0.000 in the dark chocolate groups) (Figure 3), indicating an effective role of the milk and particularly dark chocolate diets on memory trend.

As shown in Figure 3, the latencies of days 1, 7, and 14 showed a decreased trend in all experimental groups that were more serious in the normal and sucrose diets. These were probably due to time passage; because there were no repeated shocks. As shown in Figure 3, this descent trend.

The total DS times in the sucrose and white chocolate groups had no significant differences compared with the control group on days 1, 7 and 14. However, the total DS times in the dark and milk chocolate groups were

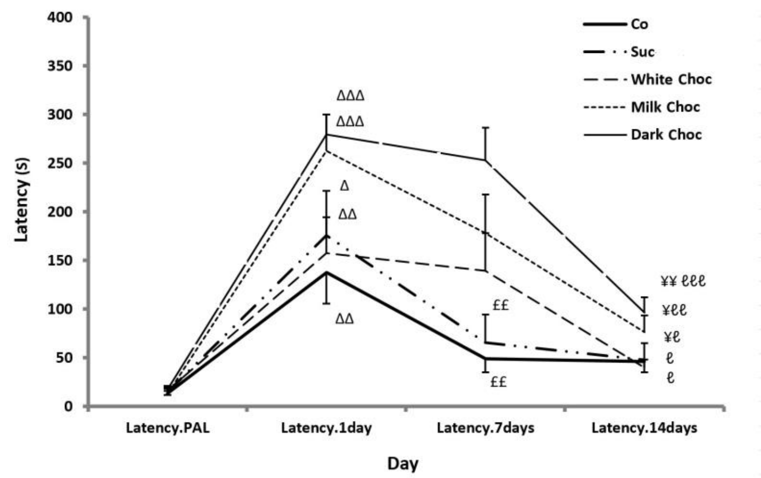

FIGURE 3. Trend line of latency before and after foot shock (within groups) in passive avoidance test during learning (initial latency before foot shock and 1 day after foot shock), and memory test (among 1, 7, and 14 days after foot shock) in all experimental groups (n=7). Data represent as mean±SEM (One-way analysis of variance followed by LSD post hoc test). $^{\Delta}P<0.05$, $^{\Delta\Delta}P<0.01$ and $^{\Delta\Delta\Delta}P<0.001$ initial latency vs. latency after 1 day; $^{££}P<0.01$ for the latency values in day 7 vs. day 1; $^{¥}P<0.05$ and $^{¥¥}P<0.01$ for the latency values in day 14 vs. day 7; $^{ℓ}P<0.05$, $^{ℓℓ}P<0.01$, $^{ℓℓℓ}P<0.001$ for the latency 7 days vs. day 14.

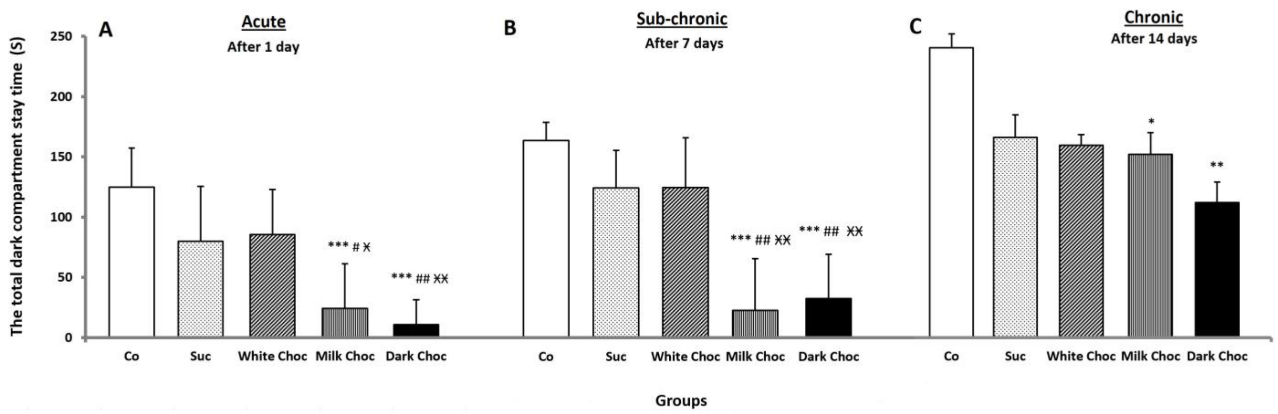

FIGURE 4. Total stay time in the dark room (of the passive avoidance test) for acute, sub-chronic and chronic food consumption in all experimental groups (1, 7 and 14 days after foot shock, n=7). Data represent as mean±SEM (One-way analysis of variance followed by LSD post hoc test). $^{*}P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$ with respect to control group; $^{\#}P<0.05$ and $^{\#\#}P<0.01$ with respect to sucrose group; $^{X}P<0.05$ and $^{XX}P<0.01$ with respect to white chocolate group.

significantly (in both P=0.000) lower than the control group on days 1 and 7. Whereas, the total DS times of day14 significantly (in the milk chocolate: P=0.036 and dark chocolate: P=0.003) differed from the control group, indicating that the milk and dark chocolate diets improved the memory consolidation for 14 days (Figure 4). Whereas, the milk and dark chocolate groups were significantly different compared to the sucrose group (in milk chocolate group: P=0.020 and P=0.004, respectively on day1 and 7; in dark chocolate diet: P=0.005 and P=0.010 on day1 and 7) (Figure 4).

The milk and dark chocolate groups were significantly different compared to the white chocolate group (in the milk chocolate group: P=0.011 and P=0.004, respectively on days 1 and 7; in the dark chocolate diet: P=0.004 and P=0.009 on days 1 and 7) (Figure 4).

Electroencephalography results

The EEG traces were represented for all experimental groups (Figure 5). Also, Figure 6. A-D indicated the responses of the different EEG brain waves in the cortex of experimental groups.

The percentage of beta waves of total power showed significant differences in the white, milk and dark chocolate groups as compared with the control group (P=0.047, P=0.011 and P=0.035, respectively) (Figure 6.A). There was a significant (P=0.001) decrease in the percentage of beta waves in the white chocolate group

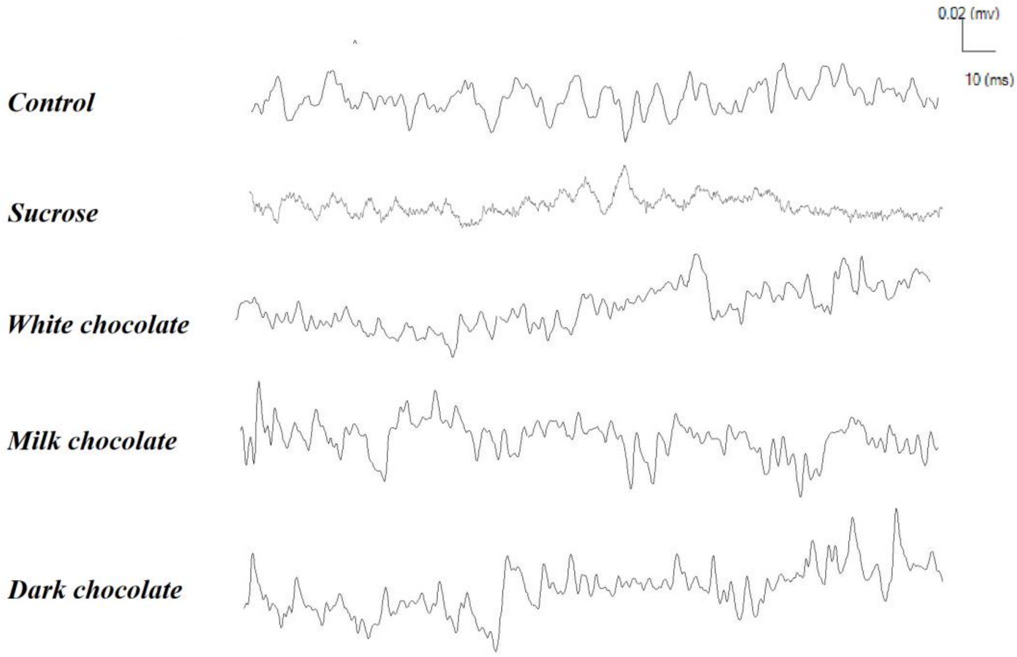

FIGURE 5. Representative EEG trace for all experimental groups.

compared to the sucrose group (Figure 6A). Also, the percentage of beta waves was significantly higher in the milk and dark chocolate groups than the white chocolate group (P=0.000 in both of them) (Figure 6A).

The percentage of alpha waves of total power showed significant enhancement in the dark chocolate group in comparison with control, white and milk chocolate groups (P=0.010, P=0.037 and P=0.044; respectively) (Figure 6B).

There were significant decreases in the percentage of theta waves of total power in milk and dark chocolate groups compared with the control group (in both P=0.009). In addition, theta waves were significantly lower in milk and dark chocolate groups than the sucrose group (P=0.023 and P=0.025, respectively). Also, in the milk and dark chocolate groups, the theta waves significantly (in both P=0.002) decreased in comparison with the white chocolate group (Figure 6C).

As shown in Figure 6.D, in the milk and dark chocolate groups, the percentage of delta waves of total power had significant decreases as compared to the control group (P=0.29 and P=0.019, respectively). In addition, there was a significant (P=0.003) increase in the white chocolate group compared to the sucrose group in delta waves. Finally, in the milk and dark chocolate groups, the percentage of delta waves significantly (P=0.000 in both) decreased in comparison with the white chocolate group.

Discussion

This study investigated the effects of acute, sub-chronic and chronic additive palatable foods such as chocolate consumption with different percent of cocoa/sugar on learning, memory, memory consolidation and electroencephalogram (EEG) waves in rats.

The present finding showed learning happened in the dark chocolate diet better than other addictive palatable diets, probability due to enhancement of cocoa content (Figure 3). Epicatechin, as one of the components of dark chocolate could improve learning in snail (Fernell et al., 2016). Also, some reports indicated that the flavanols of dark chocolate and cocoa may be beneficial for human brain functions (Martin et al., 2020; Rajaram et al., 2019).

According to other data in the present study, the acute, sub-chronic and chronic consumption of both sugar cubes and white chocolate diets did not change memory and memory consolidation when compared to the normal diet (Figures 2 and 4). There were a few reports about long-term consumption of sugar on brain functions (as distinct from the documented short-term effects of glucose on it) (Kendig, 2014). Giles et al.

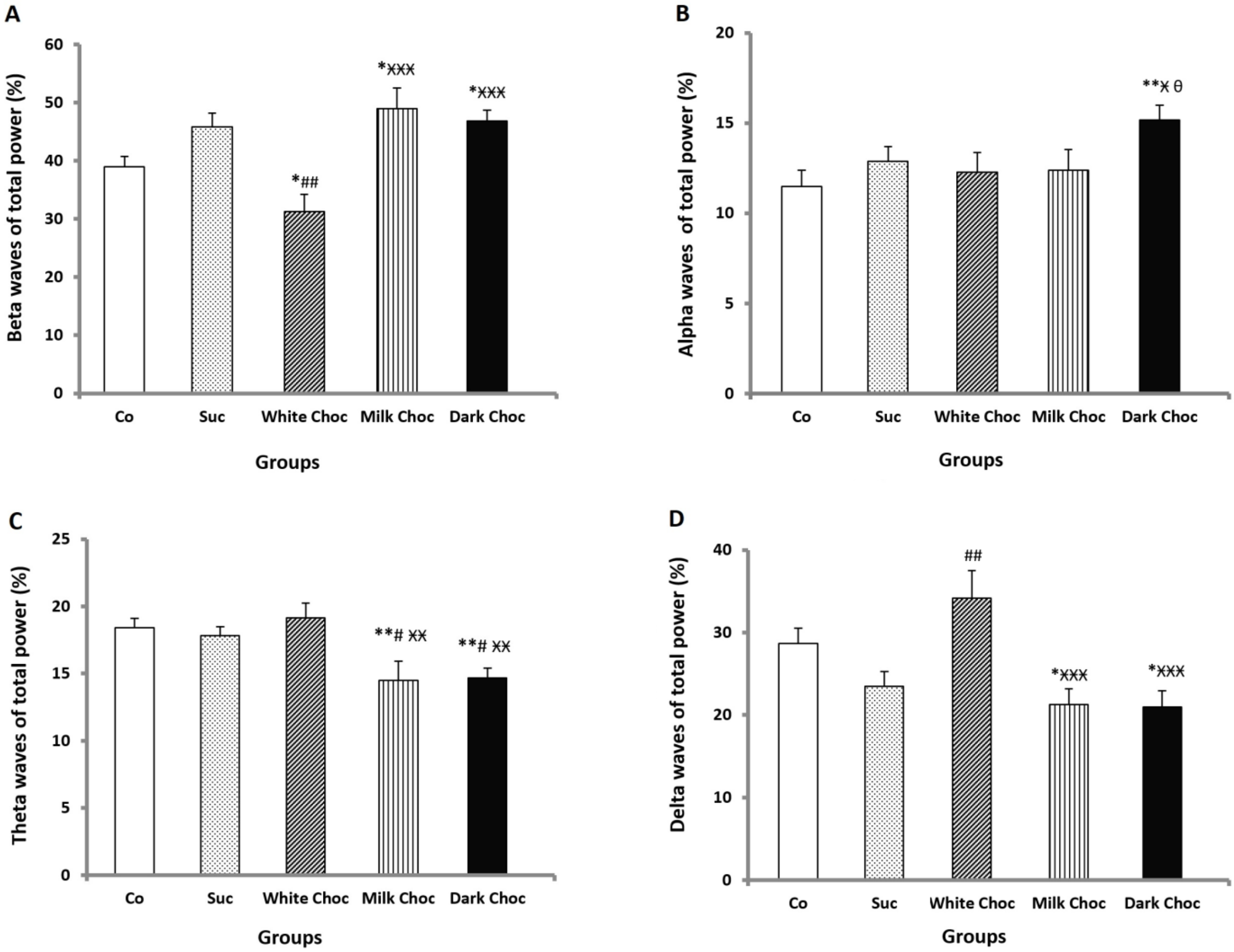

FIGURE 6. Comparison of percentages of different waves of total power (%) in all groups. A) Beta waves of total power (%), B) Alpha waves of total power (%), C) Theta waves of total power (%) and D) Delta waves of total power (%). Data represent as mean±SEM (One-way Analysis of variance followed by LSD post hoc test). $^{*}P<0.05$ and $^{**}P<0.01$ with respect to the control group; $^{\#}P<0.05$ and $^{\#\#}P<0.01$ with respect to sucrose group; $^{X}P<0.05$, $^{XX}P<0.01$ and $^{XXX}P<0.001$ with respect to white chocolate group; $^{\theta}P<0.05$ with respect to milk chocolate group.

(2018) failed to find significant differences between sugar and normal consumption on learning and memory (Giles et al., 2018). Other previous studies reported that cognitive dysfunctions can be induced by the feeding of chow saturated with sugar, sucrose solution and high caloric diet in healthy rats (Chepulis et al., 2009; Kendig, 2014; Molteni et al., 2002). Different mechanisms were suggested for these cognitive dysfunctions such as elevated neurotransmitters, free radicals, central and peripheral inflammation markers (Beilharz et al., 2016; Berridge, 2009; Westover and Marangell, 2002; Wise, 2006). Also, impairment of learning and memory was shown following consumption of high simple carbohydrates diets (e.g., mono and disaccharides) (Altermann Torre et al., 2020), and/or a combination of fat and sucrose (Abbott et al., 2019). In contrast, Miser et al. also interpreted a positive effect of fructose (as one kind of carbohydrates) on learning and memory (Messier et al., 2007). It seems that the lack of memory improvement in the white chocolate and sucrose diets probably happened due to flavonoid-free (Lamport et al., 2020).

In this study, acute and sub-chronic milk and particularly dark chocolate consumption improved memory (on days 1 and 7) compared to normal diet (Figure 2), whereas, chronic dark chocolate diet improved memory until long duration (for 14 days). In addition, memory consolidation happened in all three trials in milk and dark chocolate diets (figs. 4). Moreover, it seems the beneficial effect of dark chocolate on cognition probably presents the role of high flavanol and epicatechin content with antioxidant properties on the improvement of brain functions (Fernell et al., 2016; Rendeiro et al., 2012; Whyte and Williams, 2015). In this way, Lamport et al. demonstrated that verbal memory improved after two-hour consumption of dark chocolate (short-term consumption) and it was better than the white chocolate diet (with low flavanol content) (Lamport et al., 2020).

Only acute milk chocolate diet improved memory (on day 1) compared to white chocolate diets, whereas, all three manners (acute, sub-chronic and chronic) dark chocolate consumption improved memory with respect to the white chocolate diet (Figures 2). Also, the differences between milk and dark chocolate diets were not considered on memory in different intervals (Figures 2). Therefore, current findings supported an important role of milk and dark chocolate on brain health, although the effect of dark chocolate was more permanent on memory improvement. In this way, a previous study showed chocolate (with cocoa concentration 30-70%) improved memory (Lamport et al., 2020), and this cognitive improvement related to flavonoid-enriched cocoa (Lamport et al., 2020; Scholey and Owen, 2013) and/or long-term consumption of it (Socci et al., 2017). However, it was found the memory trend curve had a mildly decreased slope in the dark chocolate diet with respect to other diets (Figure 3). It seems that the beneficial effect of the dark chocolate diet can be due to high levels of cocoa amounts again. Moreover, in the initial week of the experiment, the decreased trend of memory was slower in different types of chocolate diets than normal and sucrose diets. Moreover, the memory trend decreased in three chocolate diets in the second week. In other words, different types of chocolate prevented the reduction of memory in week one, but these diets did not have a severe effect on memory in the second week.

Based on another finding of the present study, brain electrical activity was changed by sugar cubes and different types of chocolate diets (Figure 6). A previous study indicated that the flavor of food can change mood and behavior through and after food consumption. In addition, it is a major factor that highly affects the food memory of consumers as other brain functions (Songsamoe et al., 2019). However, according to current electrophysiological findings, all brain waves non-significantly were changed by long-term consumption of sugar cubes diets (Figure 6). Oppositely, a study reported that sugar can increase theta, delta, and alpha brain waves; also, it can be able to impair the healthiness of subjects (Appleton, 2011). This paradox may be related to the type of sugar, the amount of sugar ingested, duration of feeding with sugar, sugar purity and type of sugar consumption, animal strain, age, and other factors.

Another present finding indicated that only beta waves decreased in the white chocolate diet in comparison with the normal diet. Whereas, beta waves respectively increased in milk and dark chocolate diets with respect to normal and white chocolate diets (Figure 6). Some previous studies explained that the beta wave plays the main role in attention, higher brain functions, conscious state, and bodily awareness, as a marker for sensitivity to food and dysfunctional disinhibition–inhibition mechanism (Hiluy et al., 2021; Songsamoe et al., 2019; Tammela et al., 2010). It seems that milk and dark chocolate diets similarly increased conscious state, bodily awareness and working memory. Hence, it is possible that milk and dark chocolate diets can be indicated as food preference on brain functions in comparison with the white chocolate diet. It is possible that elevated beta waves of milk and dark chocolate diets were related to cocoa flavor and their antioxidant properties. A previous study indicated that flavor and kind of food are the most important factors which impact consumer food product acceptability (Wexler and Thibault, 2019).

Another finding presented that only the dark chocolate diet improved alpha waves in comparison with other diets (e.g., normal, white and milk chocolate diets) in the present study. It was reported that alpha waves show cognitive performance and semantic information processing in rats (Kajic and Wennekers, 2015; Songsamoe et al., 2019). Hence, it seems that only dark chocolate diet could improve cognitive performance. However, increased alpha and beta wave activity caused neural circuit activation for enhancements of attention, sensory-motor integration and working memory (Campbell, 2009; Labbe et al., 2011; Murao et al., 2013; Songsamoe et al., 2019). Parallel to the current study, another study showed that beta and alpha waves were increased in the caffeine group according to the sugar group (Meng et al., 2017). Also, increases cerebral blood flow was indicated for cognitive improvement by flavonoids of the dark chocolate (Berk et al., 2017). It seems that having knowledge about these items is very useful for the development of the food diet field. However, in total, it is suggested that the dark chocolate diet can be indicated as the best portable diet for improvement of cognitive performance.

Moreover, it was noticeable that theta waves decreased in the milk and dark chocolate diets with respect to the normal, sucrose and white chocolate diets (Figure 6). A study demonstrated that acute intake of dark chocolate can decrease theta waves and increase alpha and beta waves especially in tempo occipital region (Santiago-Rodríguez et al., 2018). Theta waves indicated deep meditation and reduced consciousness (Songsamoe et al., 2019). Therefore, the current study showed increased attention and a reduction of deep meditation by milk and dark chocolate diets. It seems that milk and dark chocolate diets could decrease relaxation in comparison with normal and white chocolate diets. Finally, delta waves decreased in milk and dark chocolate diets in the present study (Figure 6). Some previous studies showed acute and sub-chronic consumption of dark chocolate and acute consumption of chocolate with 60% cocoa can decrease delta and increase beta waves (Montopoli et al., 2015; Santiago-Rodríguez et al., 2018). However, delta waves indicated the reduction of bodily awareness (Songsamoe et al., 2019). Therefore, the current finding confirmed that milk and dark chocolate increased bodily awareness again. Furthermore, the white chocolate diet caused a reduction of bodily awareness with respect to sucrose (Figure 6), it seems that it can be due to elevated calorie of white chocolate (with butter content), because a high-energy diet impairs brain functions (Kanoski et al., 2010). It seems that EEG data could be indicate positive or negative effects of food consumption on some brain functions. However, different paradoxical results may indicate the influence of treatment duration, material consumption manner, material dose, and the type of behavioral tests (Kalantarzadeh et al., 2020; Kaminska and Rogoz, 2016).

People mainly have a major interest in eating some palatable foods such as chocolate and sweet foods, and these materials are very available and effective on brain functions. Therefore, the importance of this study was the effects of different types of chocolate mono-diet on brain functions. Any issue on large scale can have significant economic and social effects, the issue of nutrition and its relationship to health is no exception. The consumption of palatable food increases and if they have a beneficial or harmful effect, it can cause significant changes in social health (impairment of brain functions and mood), also it can create an economical burden on society.

Conclusion

To sum up, unlike sucrose and white chocolate, dark chocolate (acute, sub-chronic and chronic consumption) and milk chocolates (acute and sub-chronic consumption) probably had beneficial effects on memory and nearly all electrical brain activity due to high levels of cocoa. Hence, these types of diets modified brain homeostasis and increased conscious state and relaxation reduction. However, further studies are needed to clarify the underlying physiological mechanism in addictive food diets on brain functions.

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Conflict of interest

The authors have no conflict of interest to declare.

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