Available online at www.sciencedirect.com
ScienceDirect
Journal of Nutritional Biochemistry 20 (2009) 948 – 955
Journal of Nutritional Biochemistry

Anxiolytic effects of short- and long-term administration of cacao mass on rat elevated T-maze test☆

Takashi Yamada[a], Yasushi Yamada[a], Yasuyo Okano[a], Takehiko Terashima[a,b], Hidehiko Yokogoshi[a,*]

[a]Laboratory of Nutritional Biochemistry, Graduate School of Nutritional and Environmental Sciences, University of Shizuoka, Shizuoka 422-8526, Japan
[b]School of Health Promotional Science, University of Hamamatsu, Hamamatsu 431-2102, Japan

Received 6 February 2008; received in revised form 11 August 2008; accepted 14 August 2008

Abstract

We demonstrated the effects of short- and long-term administration of cacao mass on anxiety in the elevated T-maze test, which is an animal model of anxiety. In the first study, we administered cacao mass (100 mg/100 g body weight) per os and immediately performed the elevated T-maze test. Short-term cacao mass significantly abolished delayed avoidance latency compared with the control but did not change escape latency. This result suggested that cacao mass administration reduced conditional fear-relating behavior. Short-term cacao mass administration did not affect the concentration of brain monoamines, emotion-related neurotransmitters such as norepinephrine, serotonin and dopamine, in the rat brain. In the next study, we fed a cacao mass-containing diet to rats for 2 weeks and performed the elevated T-maze test. Contrary to short-term administration, chronic consumption of cacao mass tended to increase avoidance latency and did not change escape latency. Brain serotonin concentration and its turnover were enhanced by chronic consumption of cacao mass. These results suggested that chronic consumption of cacao did not affect fear-related behavior but was involved in brain monoamine metabolism. In conclusion, we suggest that short-term cacao mass consumption showed an anxiolytic effect but chronic consumption did not.

Keywords: Cacao mass; Elevated T-maze test; Anxiety; Anxiolytic; Dopamine; Serotonin

1. Introduction

Anxiety disorders in modern society have a relatively high prevalence and are becoming a serious problem. Currently, the most widely prescribed medications for anxiety disorders are benzodiazepines; however, their clinical use is limited by their side effects, such as psychomotor impairment, potentiation of other central depression drugs, and dependence liability. Therefore, we considered that daily precautions against anxiety disorders are important and suggest that dietary nutrients are a good source to maintain equilibrium. Recent studies suggest that various nutrients affect the mind of mammals; administration of caffeine [1], succinic acids [2] and dietary soy phytoestrogens [3] improved anxiety in rats. Dietary amino acid imbalance is also involved in emotion [4]. Various active ingredients in food and drink have been identified as effective constituents for improving mood [5]. In this study, we examined acute and chronic effects of cacao mass, the raw material of chocolate and cocoa, on anxiety in a rat study.

Anxiety is a heterogeneous phenomenon including, among others, panic disorder (PD), obsessive-compulsive disorder (OCD), phobias, generalized anxiety disorder (GAD) and posttraumatic stress disorder [6]. These disorders are caused by conditional or unconditional fear. Various behavioral tests are used for anxiety and depressive conditions, and some reports have shown good correlation between the clinical efficacy of benzodiazepine anxiolytics in alleviating GAD and decreased indexes of fear/anxiety measured in conflict tests [7], the elevated plus-maze [8] and the social interaction test [9]; however, these behavioral tests could not differentiate conditional and unconditional fear. The elevated plus-maze test apparatus comprised two open arms and two closed arms and was elevated 70 cm above the floor. To estimate anxiety behaviors, the number of entries and time spend in the open and closed arms were measured;

☆ This work was supported in part by grants for scientific research from Shizuoka Prefecture, and the 21st Century COE program from the Ministry of Education, Culture, Sports, Science and Technology of Japan.
* Corresponding author. Tel.: +81 54 264 5558; fax: +81 54 263 7079.
E-mail address: yokogosi@fns1.u-shizuoka-ken.ac.jp (H. Yokogoshi).

0955-2863/$ – see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.jnutbio.2008.08.007

however, recent studies have suggested that the elevated plus-maze could not differentiate conditional and unconditional fear and could not estimate the anxiety-related behavior of animals accurately; thus, we performed the elevated T-maze test in this study. Based on the assumption that conditional fear is related to GAD, and unconditional, fear to PD, a new animal model of anxiety aimed at separating these two types of fear in the same rat has been developed [6,10]. This test, named the elevated T-maze, was derived from the elevated plus-maze [11] by sealing the entrance to one of the enclosed arms. In the experimental session, conditional fear is represented by inhibitory avoidance of the open arms of the maze and unconditional fear by one-way escape from one of the open arms.

With anxiety, various brain neurotransmitters and hormones levels change immediately. In particular, monoamines, such as norepinephrine, serotonin and dopamine, are involved in mood, stress and other physical homeostasis [12]. Serotonin and norepinephrine mainly regulate stress and negative mood in the mammalian brain, and their dysfunctions cause various mood disorders, such as social anxiety disorder and depression [13,14]. Dopamine also regulates mood and emotion-related behaviors and has a motivation/reward function and conditional fear responses [15,16]. Various anxiolytics and antidepressants aim at monoamine neurocircuitry, such as their receptors and transporters [17,18]. In addition, it has been observed that changes in brain monoamine concentrations are closely linked to the behavior of rodents in animal studies; therefore, some researchers measure brain monoamine concentrations in rat behavioral studies and use them as an index of emotion [19,20].

In this study, we performed the elevated T-maze test, measured brain monoamine concentration and estimated the acute and chronic effects of cacao mass consumption on anxiety in rats.

2. Materials and methods

2.1. Animals

Eleven-week-old male Wistar strain rats (270 g weight; SLC, Shizuoka, Japan) were kept in groups of three rats in a temperature- and humidity-controlled room (24°C and 55% relative humidity) under regular lightning conditions (12-h light:dark cycle). Rats were separated into 13 groups. Two cacao mass-fed groups, two control groups and a diazepam-injected group were included in the elevated T-maze test (n=12 in each group), and two cacao mass-fed groups and two control groups were used for the open field test (n=8 in each group). Four more groups (n=6–8) were used to measure brain neurotransmitter concentrations. Cacao mass powder was purchased from Ezaki Glico (Osaka, Japan). Diazepam was purchased from Sigma-Aldrich (Tokyo, Japan). In the short-term administration study, cacao mass [100 mg/100 g body weight (B.W.)] with saline or saline (control) was administered to rats per os and a typical anxiolytic, diazepam (0.1 mg/100 g B.W.), was injected intraperitoneally (i.p.) into rats 30 min before a behavioral test. In the chronic administration study, rats were given a 1% cacao mass-containing diet, and control group rats were given a control diet for 2 weeks. The experimental diet compositions are shown in Table 1. The test diets were prepared according to American Institute of Nutrition (AIN)-76, which refers to the guidelines of the AIN. Cellulose, casein, sucrose and starch were purchased from Oriental Yeast (Tokyo, Japan). Corn oil was purchased from Honen (Tokyo, Japan). Choline-Cl was purchased from Wako Pure Chemical (Tokyo, Japan). AIN-76 mineral mix and AIN-76 vitamin mix were purchased from Nihon Nosan Kogyo (Yokohama, Japan). This experiment was carried out in accordance with the Guidelines for the Care and Use of Laboratory Animals of the University of Shizuoka that refer to the American Association for Laboratory Animal Science.

2.2. Elevated T-maze test

The elevated T-maze device was made with reference to the methods of Teixeira et al. [6]. It was made of wood and had three arms of equal dimensions (50×9 cm). One arm, enclosed by clear plastic walls 40 cm high, was perpendicular to the two opposing open arms. To avoid falling, the open arms were surrounded by a 1-cm-high wood rim. The whole apparatus was elevated 70 cm above the floor (Fig. 1).

The animals were not handled until the test. All animals underwent pre-exposure to the closed arm, and unmoving rats were excluded 24 h before the test in the short-term cacao mass consumption study and 24 h before starting to feed the experimental diet in the chronic consumption study [21].

For the 2 min preceding the experiment, each animal was placed inside a plastic cage (28×18 cm), to which it had been habituated. The rat was then removed from the cage and placed at the distal end of the enclosed arm facing the intersection of the arms. The time taken by the rat to leave this arm with its four paws was recorded (Avoidance 1). The same measurement was repeated three times at 30-s intervals (Avoidance 2 and 3). Following the avoidance test (after a

Table 1
Composition of experimental diets

	Control diet	Cacao mass diet
Cellulose	5	5
Casein	20	20
Corn oil	5	5
Choline-Cl	0.3	0.3
Vitamins	1	1
Minerals	3.5	3.5
Sucrose	21.73	21.23
Starch	43.47	42.97
Cacao mass	0	1
Total	100 (%)	100 (%)

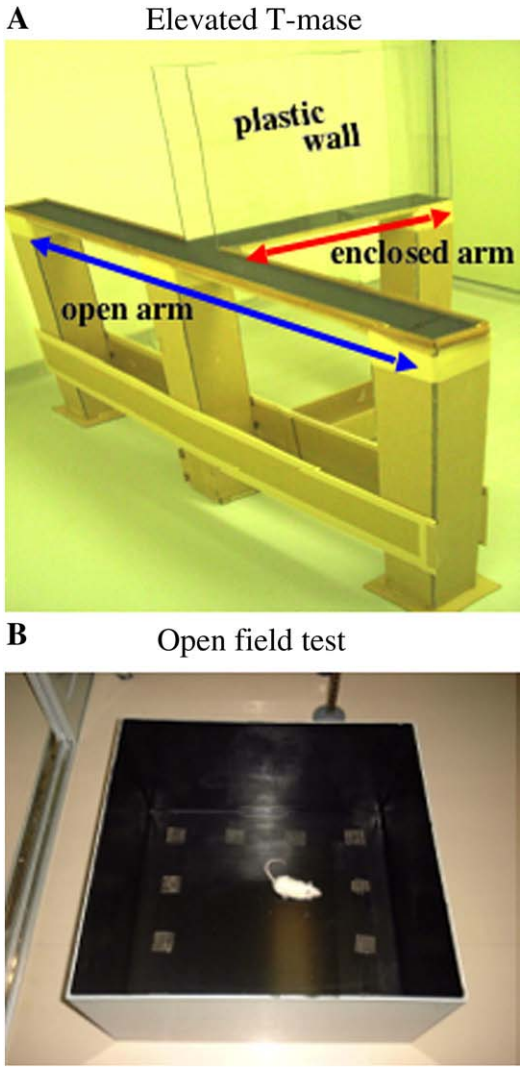

Fig. 1. Behavioral test apparatus. (A) Elevated T-maze: Each arm was 50×9 cm. The plastic wall was 40 cm tall. The whole apparatus was elevated 70 cm above the floor. (B) Open field box: the box was 70×70×70 cm tall.

30-s interval), the rat was placed at the end of the open arm and the time taken to enter the enclosed arm with the four paws was recorded (Escape 1). The same measurement was repeated after 30 s (Escape 2). During the 30-s intervals between trials, the animals were placed in the plastic cage.

2.3. Open field test

Motor activities of rats were measured by the open field test. The open field box was purchased from MUROMACHI KIKAI (Tokyo, Japan) and was 70×70×70 cm in height (Fig. 1). Animals were not handled until the test. The rat was placed in the open field box for 6 min, and its behavior was recorded by a DV-Track video tracking system, CompACT VAS/DV (MUROMACHI KIKAI).

2.4. Neurotransmitter concentration in the brain tissue

Neurotransmitter concentration was measured by the method of Okuyama et al. [22].

Short- and long-term cacao mass-administered rat brains were extirpated after the elevated T-maze and without any behavioral test.

Wet brain tissue was homogenized in two times the volume of 0.2 M perchloric acid buffer (pH 2) and kept on ice for 1 hour. Homogenates were centrifuged at 20,000 rpm for 15 min at 0°C and then filtered through a 0.45-μm cellulose acetate membrane filter. Neurotransmitters were detected by high-performance liquid chromatography (HPLC) under the following conditions: the mobile phase was 0.1 M sodium acetate citric acid buffer and 15% methanol containing EDTA-2Na and 1-octanesulfonic acid sodium salt. The HPLC system was equipped with a reversed-phase column (MA-5ODS, 4.6×150mm: EICOM, Kyoto, Japan) and an electrochemical detector (ED623: GLSCIENCE, Tokyo, Japan). Recording of chromatograms and all calculations were performed using an integrator (BORWIN: NIHONBUNKOH, Tokyo, Japan). We measured norepinephrine (NE), serotonin (5-HT), dopamine (DA) and metabolites 5-hydroxyindole-3-methoxyphenylacetic acid (5-HIAA), 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and 3-methoxytryramine. All chemicals used in the measurements were purchased from Wako Pure Chemical.

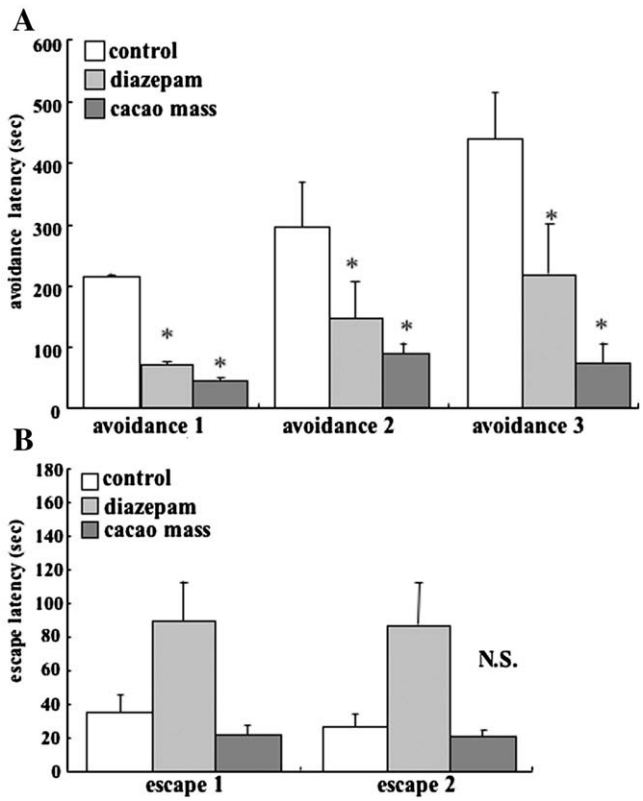

Fig. 2. Effects of short-term cacao mass and diazepam administration (acute effect) on avoidance latency (A) and escape latency (B) in the elevated T-maze test. Rats were i.p. administered cacao mass (100 mg/100g B.W.) (n=12), saline (control, n=12) per os, or diazepam (0.1 mg/100g B.W.) (n=12) 30 min before the test. Values are the means±S.E.M. *Significantly different from the control group (P<.05).

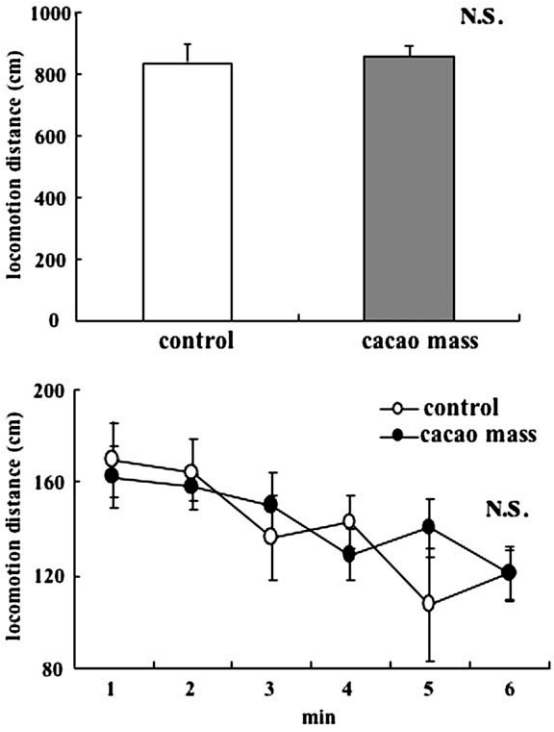

Fig. 3. Effects of short-term cacao mass administration (acute effect) on the locomotor activity of rats in the open field test. Rats were administrated cacao mass (100 mg/100g B.W.) (n=12), or saline (control, n=12) per os 30 min before the test. Values are the means±S.E.M. *Significantly different from the control group (P<.05).

2.5. Statistical analysis

Neurotransmitter concentration was measured by the method of Okuyama et al. [23]. Data for individual groups are expressed as the mean, and one-way analysis of variance was performed for statistical analysis. Student's t test was used to assess differences between the control group and cacao mass-fed group. The Tukey-Kramer test was used to assess differences between the three groups (control, diazepam and cacao mass). In all cases, P<05 was considered significant. Results are expressed as the mean±S.E.M.

3. Results

3.1. Effect of short-term cacao mass administration on anxiety

In the avoidance test, diazepam administration (0.1 mg/100 g B.W.) significantly abolished delayed avoidance latency in the three trials. In the escape test, diazepam administration did not affect escape latency significantly, but it tended to be delayed (Fig. 2). Cacao mass administration (100 mg/100 g B.W.) reduced delayed avoidance latency markedly but did not affect escape latency (Fig. 2).

In the open field test, cacao mass administration did not affect locomotor activity (Fig. 3).

3.2. Effect of long-term cacao mass consumption on anxiety

Two-week cacao mass diet (containing 1% cacao mass) consumption did not affect daily food intake and the body weight of rats compared with control rats (Fig. 4). The average amount of daily cacao mass intake was approximately 250 mg. In the avoidance test, long-term cacao mass consumption did not abolish delayed avoidance latency in the three trials, and contrary to the result of short-term administration, chronic consumption tended to delay avoidance latency. In the escape test, cacao mass consumption did not affect escape latency (Fig. 5).

In the open field test, long-term cacao mass consumption did not affect the total locomotion distance of rats but tended to increase the distance compared with the control group (Fig. 6).

3.3. Effect of short- and long-term cacao mass consumption on brain neurotransmitter concentration

We examined the effect of short- and long-term cacao mass consumption on neurotransmitter concentration after the elevated T-maze test (Tables 2 and 3; shown as "T-acute"

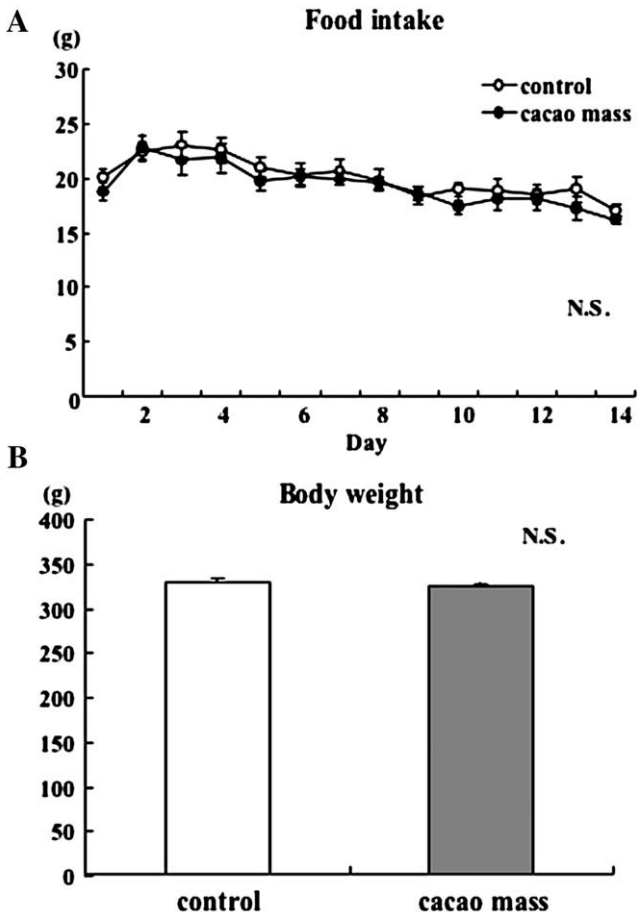

Fig. 4. Daily food intake of cacao mass-containing diet (A) and the effect of 2-week cacao mass intake on body weight (B). Rats were fed cacao mass diet (n=12), or control diet (n=12) for 2 weeks.

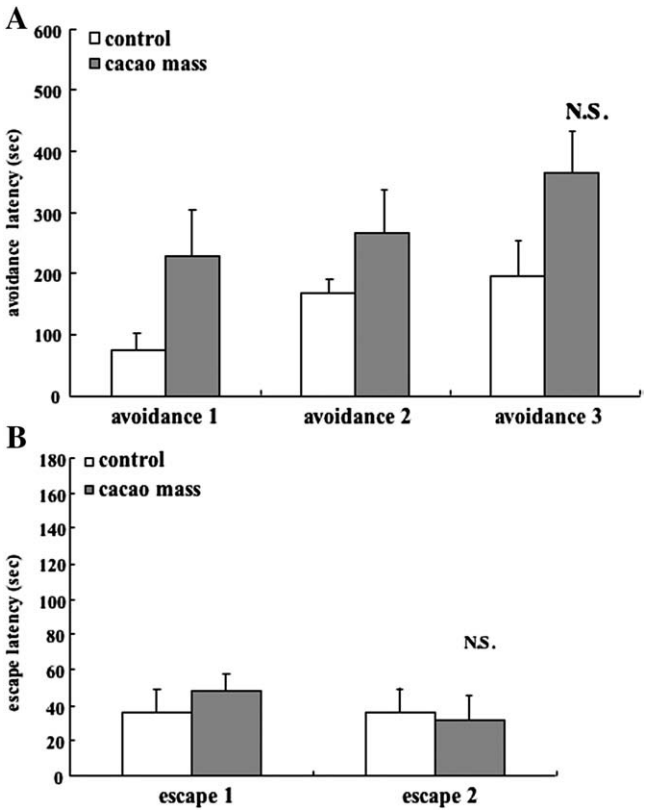

Fig. 5. Effects of long-term cacao mass consumption (chronic effect) on avoidance latency (A) and escape latency (B) in the elevated T-maze test. Rats were fed cacao mass diet (n=12), or control diet (n=12) for two weeks. Values are the means±S.E.M. *Significantly different from the control group (P<.05).

or "T-chronic") and without (before) the elevated T-maze test (Tables 2 and 3; shown as "acute" or "chronic"). Short-term cacao mass administration did not significantly affect brain norepinephrine, dopamine, serotonin and metabolite concentrations in several regions before the elevated T-maze test (Table 2). Long-term cacao mass consumption increased 5-HT concentration in the brain striatum and amygdala and increased 5-HIAA concentration in the striatum. After the elevated T-maze test, 5-HIAA concentration was increased in the cerebral cortex, hippocampus and amygdala. Serotonin turnover (5-HIAA/5-HT) was accelerated significantly by long-term cacao mass consumption in the hippocampus and tended to be promoted (P<.1) in the cerebral cortex. Long-term cacao mass consumption did not affect norepinephrine, dopamine and its metabolite concentrations in several regions (Table 3).

4. Discussion

Using the elevated T-maze test, composed of an avoidance test and escape test, we estimated the effect of cacao mass consumption on anxiety. The avoidance test evaluated conditional fear, and it was judged that shorter avoidance latency was more relieved than conditional fear. The escape test evaluated unconditional fear, and it was judged that longer escape latency was more relieved than unconditional fear [6]. Diazepam administration reduced delayed avoidance latency significantly and tended to delay escape latency in this study, consistent with a previous report [24]. Short-term cacao mass (100 mg/100 g B.W.) administration abolished delayed avoidance latency, but did not affect escape latency. On the other hand, it was considered that locomotor activity of rats influenced the evaluation of emotion-related behavior and the locomotor activity of animals was measured in emotion-related behavioral studies [25]. Thus, we examined the locomotor activity of cacao mass-administrated rats by the open field test and showed that cacao mass administration did not change the locomotor activity of rats. These results showed a particular effect on conditional fear without a change in locomotor activity. In the next study, we examined the chronic effects of cacao mass on anxiety in the same test. Chocolate contains approximately 30–60% cacao mass. We fed a cacao mass diet (containing 1% cacao mass) to rats for 2 weeks and performed the elevated T-maze test. The rats were fed approximately 250 mg cacao mass in a day, and the quantity was similar with that administered in the study that measured the acute effect. In contrast to short-term administration, chronic cacao mass consumption did not significantly affect

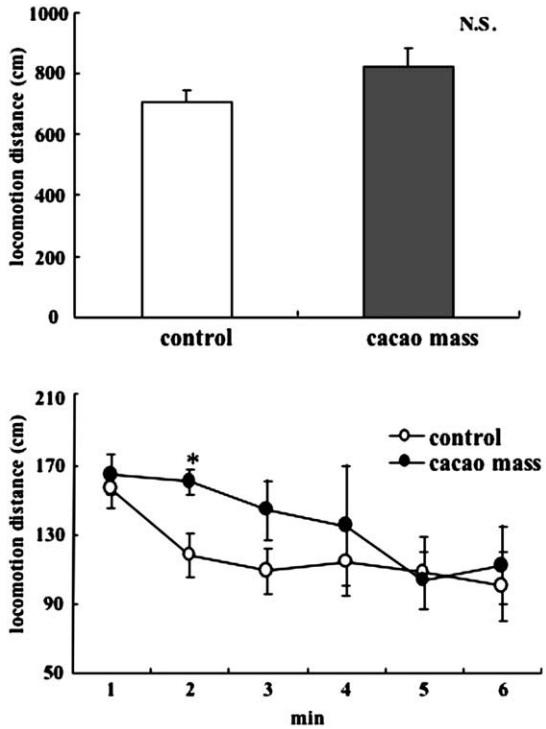

Fig. 6. Effects of long-term cacao mass consumption (chronic effect) on the locomotor activity of rats in the open field test. Rats were fed cacao mass diet (n=12), or control diet (n=12) for 2 weeks. Values are the means±SEM. *, significantly different from the control group (P<.05).

Table 2
Effects of short-term cacao administration (acute effect) on monoamine concentrations and their turnover rates in the rat brain

			NE	5-HT	5-HIAA	DA	DOPAC	HVA	3MT	5-HIAA/5-HT	DOPAC/DA	HVA/DA
Hypothalamus	Acute	Control	6.61 ±0.32	3.96±0.09	1.64±0.09	2.58±0.58	0.40±0.11			0.42±0.03		
		Cacao	6.97±0.26	3.86±0.10	1.63±0.10	1.85±0.09	0.27±0.03			0.42±0.03		
	T-acute	Control	13.45±±0.58	3.95±0.19	2.07±0.13	3.01±0.19	0.35±0.03			0.52±0.02		
		Cacao	12.46±0.27	3.75±0.08	1.90±0.09	2.89±0.07	0.30±0.02			0.51±0.02		
Cerebral Cortex	Acute	Control	1.31±0.07	1.39±0.07	0.65±0.02	0.22±±0.01	0.03±0.01			0.48±0.04		
		Cacao	1.20±0.05	1.39±0.05	0.62±0.03	0.21±0.01	0.04±0.00			0.44±0.02		
	T-acute	Control	1.36±0.03	0.78±0.02	0.64±0.02	0.20±0.02	0.04±±0.00	0.06±0.00		0.82±0.03		
		Cacao	1.39±0.03	0.77±0.02	0.60±0.01	0.18±0.01	0.04±0.00	0.06±0.00		0.79±0.03		
Hippocampus	Acute	Control	194±0.13	2.63±0.12	1.73±0.07	0.28±0.01	0.03±0.00			0.66±0.04		
		Cacao	194±0.10	2.62±0.15	1.59±0.05	0.26±0.01	0.04±0.00			0.62±0.05		
	T-acute	Control	4.16±0.06	2.52±0.06	2.49±0.07	0.49±0.05	0.07±0.02	0.08±0.02		0.99±0.03		
		Cacao	4.17±0.12	2.46±0.05	2.47±0.07	0.42±0.02	0.05±0.01	0.09±0.02		1.00±0.03		
Striatum	Acute	Control	2.57±0.10	2.60±0.14	1.87±0.14	52.88±1.48	7.92±0.83	3.79±0.28	1.84±0.08	0.72±0.03	0.15±0.02	0.07±0.01
		Cacao	2.40±0.05	2.55±0.13	1.82±0.09	54.04±1.70	7.78±0.75	3.64±0.12	1.77±0.04	0.72±0.03	0.15±0.02	0.07±0.00
	T-acute	Control	2.61±0.07	2.64±0.09	45.22±1.45	9.84±0.54	4.45±0.15	1.97±0.07	1.01±0.02	0.21±0.001		0.01±0.00
		Cacao	2.48±0.09	2.59±0.06	43.27±1.54	8.82±0.45	4.08±0.10#	1.87±0.05	1.05±0.03	0.21±0.001		0.01±0.00
Amygdala	Acute	Control	1.70±0.06	4.30±0.25	1.56±0.05	1.08±0.03	0.15±0.02			0.37±0.03		
		Cacao	1.62±0.06	4.34±0.21	1.51±0.06	1.15±0.06	0.16±0.02			0.35±0.03		
	T-acute	Control	3.24±0.11	4.26±0.17	2.24±0.06	1.33±0.07	0.16±0.03	0.70±0.06	7.38±1.18	0.53±0.02		
		Cacao	3.23±0.07	4.28±0.18	2.16±0.07	1.58±0.18	0.18±0.05	0.60±0.04	8.68±1.07	0.52±0.03		

Acute indicates rats administered cacao mass (100 mg/100g B.W.) or saline (n=6); T-acute, rats administered cacao mass (100 mg/100g B.W.) or saline and exposed to elevated T-maze test (n=12). Values are the means±S.E.M. #P<.1, *P<.05, **P<.01, significantly different from the control group.

either avoidance or escape latencies and tended to delay avoidance latency compared with the control. We expected that the different results occurred because of (i) differences in cacao mass component concentrations in the rat blood between short- and long-term cacao mass consumption when the behavioral test was performed and (ii) change in the effect of cacao mass because of sequential chronic administration. It was shown that chronic excess monoamine neurotransmission reduced the sensitivity of these receptors and reduced their neurotransmission [26]. Acute and chronic administration of imipramine, a tricyclic antidepressant, had conflicting effects on avoidance latency [6].

It is known that brain monoamines are important to express emotion and emotion-related behavior, and various anxiolytics and antidepressants aim at monoamine systems. It is suggested that GAD, a disorder caused by conditional

Table 3
Effect of long-term cacao mass consumption (chronic effect) on monoamine concentrations and their turnover rates in the rat brain

			NE	5-HT	5-HIAA	DA	DOPAC	HVA	3MT	5-HIAA/5-HT	DOPAC/DA	HVA/DA
Hypothalamus	Chronic	Control	8.38±0.21	3.55±0.10	1.40±0.09	2.55±0.18	0.32±0.03			0.40±0.03		
		Cacao	8.23±0.25	3.83±0.14	1.51±0.10	2.61±0.10	0.32±0.03			0.40±0.03		
	T-chronic	Control	7.84±0.29	3.73±0.08	1.99±0.06	2.86±0.12	0.47±0.02	0.56±0.02	0.11±0.01	0.54±0.02		
		Cacao	7.28±0.28	3.51±0.21	1.91±0.09	2.97±0.17	0.50±0.06	0.57±0.05	0.11±0.01	0.55±0.02		
Cerebral Cortex	Chronic	Control	1.44±0.03	1.28±0.04	0.63±0.02	0.37±0.01	0.06±0.00			0.49±0.03		
		Cacao	1.35±0.03#	1.25±0.04	0.60±0.02	0.37±0.01	0.05±0.01			0.48±0.02		
	T-chronic	Control	1.32±0.03	1.53±0.02	0.77±0.01	0.34±0.01	0.06±0.00			0.50±0.01		
		Cacao	1.35±0.03	1.51±0.05	0.82±0.02**	0.36±0.01	0.06±0.00			0.55±0.01#		
Hippocampus	Chronic	Control	2.50±0.07	2.48±0.07	1.61±0.05	0.53±0.03	0.04±0.00			0.65±0.03		
		Cacao	2.48±0.10	2.60±0.08	1.68±0.04	0.54±0.02	0.04±0.00			0.65±0.02		
	T-chronic	Control	2.29±0.03	1.86±0.04	1.99±0.04	0.37±0.02	0.08±0.01			1.07±0.02		
		Cacao	2.26±0.06	1.87±0.06	2.19±0.04**	0.37±0.02	0.07±0.00			1.18±0.04*		
Striatum	Chronic	Control		2.46±0.06	1.72±0.05	61.02±1.68	8.34±0.46	4.67±0.17	2.15±0.07	0.70±0.02	0.14±0.01	0.08±0.00
		Cacao		2.83±0.08**	1.96±0.06**	57.94±2.14	8.01±0.29	4.47±0.19	2.10±0.07	0.69±0.01	0.14±0.01	0.08±0.00
	T-chronic	Control	0.21±0.05	2.49±0.09	1.93±0.05	53.52±1.49	8.76±0.46	5.31±0.23	1.97±0.09	0.78±0.01	0.16±0.01	0.01±0.00
		Cacao	0.26±0.08	2.68±0.13	2.06±0.08	52.04±1.64	8.61±0.41	5.10±0.19	2.02±0.09	0.77±0.01	0.17±0.01	0.01±0.00
Amygdala	Chronic	Control	2.18±0.04	3.16±0.14	1.38±0.10	1.48±0.05	0.25±0.03			0.44±0.04		
		Cacao	2.11±0.08	3.71±0.12**	1.48±0.06	1.95±0.25	0.32±0.06			0.40±0.02		
	T-chronic	Control	2.00±0.05	3.66±0.13	1.88±0.03	1.40±0.06	0.32±0.03			0.52±0.02		
		Cacao	1.99±0.05	3.75±0.14	2.03±0.04**	1.64±0.13	0.35±0.04			0.55±0.02		

Chronic indicates rats fed cacao mass diet or control diet (n=8); T-chronic, rats fed cacao mass diet or control diet and exposed to elevated T-maze test (n=12). Values are the means±S.E.M. #P<.1; *P<.05; **P<.01, significantly different from the control group.

fear, is also involved in monoaminergic and GABAergic neurocircuitry [27,28]; therefore, we examined the effects of cacao mass consumption on monoamine concentration in several brain regions. Short-term cacao mass administration did not significantly change brain dopamine, serotonin and metabolite concentrations in several regions both before and after the elevated T-maze test. Chronic cacao mass consumption increased 5-HT concentration in the brain striatum and amygdala and increased 5-HIAA concentration in the striatum. After the elevated T-maze test, 5-HIAA concentration was increased in the cerebral cortex, hippocampus and amygdala. Serotonin turnover (5-HIAA/5-HT) was accelerated significantly in the hippocampus and tended to be promoted in the cerebral cortex. It was suggested that the reduction of serotonin stimulus caused depression, but excess serotonin neurotransmission in the amygdala and cerebral cortex caused conditional fear [29]. These results suggested that chronic cacao mass consumption enhances serotonin neurotransmission and induces anxiety-related behavior; however, we thought that chronic cacao mass consumption might act as a depressant, such as imipramine [6]. Because chronic cacao mass administration changed brain monoamine concentration in several brain regions, we suggested that cacao mass affects brain monoamine neurotransmission; however, short-term cacao mass administration did not change norepinephrine, serotonin and dopamine concentrations significantly in several brain regions but reduced anxiety-relating behavior in the elevated T-maze test. From these results, we expected that cacao mass affected the mood control system, such as monoamine receptors. Moreover, we expected that the acute effect of cacao mass on anxiety was caused by cacao mass components because cacao mass contains substances which greatly stimulate various properties and cannabinoids. It is known that chocolate has motivation/reward functions, but white chocolate, a milk- and cocoa-butter-containing chocolate with low cacao mass, does not affect mood [30] because recent studies showed that cannabinoid cacao powder affected mood and other physiological functions. Cacao mass contains caffeine, which has an anxiolytic effect [1], and cannabinoids such as anandamide, a brain lipid that binds to cannabinoid receptors with high affinity [31]; phenylethylamine, which acts in a similar manner to amphetamine [5] and theobromine, which has caffeine-like stimulant properties but does not affect locomotor activity [32]. It was suggested that anandamide stimulation had an anxiolytic effect, and cannabinoid receptor antagonists induced anxiety-like responses [33,34]. Chronic treatment with phentlethylamine affected the brain 5-HT system, and it was suggested that phentlethylamine worked like an antidepressant [35,36]. In addition, it was shown that cacao powder has *N*-acylethanolamines, which activate anandamide function in the brain [31]. Some studies demonstrated that these cannabinoids affected motivation and mood [32–37]; however, the cannabinoid concentrations in the cacao mass administered in this study were lower than in these reports. For example, the amount of caffeine administered in this study was approximately 250 μg per rat and that of anandamide was a few micrograms per rat [38,39]; therefore, we suggest that the anxiolytic effect of cacao mass shown in our study was due to synergic effects of these cannabinoids and other micronutrients in the cacao mass. On the other hand, dopaminergic neurocircuitry meditates serotonergic neurotransmission and maintains the balance of monoamine neurotransmission [16]. We suggested that the change in serotonin concentration by long-term cacao mass consumption was indirectly caused by its components; however, further studies are necessary to elucidate the detailed mechanism. Recent studies showed that functional foods and some nutrients in foods, for instance, soy phytoestrogens [3], essential oil from citrus [40] and alpha-lactalbumin [41], affect mood and have anxiolytic effects. We suggest that cacao mass and cacao mass-containing food or drink also act as functional foods that could improve mood.

In conclusion, we indicated that the short-term administration of cacao mass showed anxiolytic effects, but chronic consumption of cacao mass did not reduce anxiety-related behavior. The administration of cacao mass decreased conditional fear-related behavior but not unconditional-related behavior. We expected that cacao mass mediates brain monoamine neurotransmission because monoamines are closely concerned with mood; however, cacao mass did not affect monoamine concentration in the rat brain, while it had an anxiolytic effect in the elevated T-maze test. Therefore, we suggest that cacao mass components might influence the mood control system, such as dopamine receptors, because cacao mass has many components which affect brain function. We expect that cacao mass-containing cannabinoids are involved in the anxiolytic effect of cacao mass; however, the detailed mechanism is not clear yet and further experiments are necessary to prove the effects of cacao mass on anxiolytic-related behavior.

Acknowledgments

This work was supported in part by grants for scientific research from Shizuoka Prefecture and the 21st Century COE program from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

References

[1] Tang M, Kuribara H, Falk JL. Anxiolytic effect of caffeine and caffeine-clonazepan interaction: evaluation by NaCl solution intake. Pharmacol Biochem Behav 1989;32:773–6.
[2] Chen SW, Xin Q, Kong WX, Min L, Li JF. Anxiolytic-like effect of succinic acid in mice. Life Sci 2003;73:3257–64.
[3] Lund TD, Lephart ED. Dietary soy phytoestrogens produce anxiolytic effects in the elevated plus-maze. Brain Res 2001;913:180–4.
[4] Bechtholt AJ, Hill TE, Lucki I. Anxiolytic effect of serotonin depletion in the novelty-induced hypophagia test. Psychopharmacology 2007; 190:531–40.

[5] Ottley C. Food and mood. Nurs Stand 2000;15:46–52.
[6] Teixeira RC, Zangrossi Jr H, Graeff FG. Behavioral effects of acute and chronic imipramine in the elevated T-maze model of anxiety. Pharmacol Biochem Behav 2000;65:571–6.
[7] Sanger DJ, Perrault G, Morel E, Joly D, Zivkovic B. Animal models of anxiety and the development of novel anxiolytic drugs. Prog Neuro-Psychopharmacol Biol Psychiatry 1991;15:205–12.
[8] Rodgers RJ, Nikulina EM, Cole JC. Dopamine D1 and D2 receptor ligands modulate the behavior of mice in the elevated plus-maze. Pharmacol Biochem Behav 1994;49:985–95.
[9] File SE, Hyde JRG. Can social interaction be used to measure anxiety? Br J Pharmacol 1987;62:19–24.
[10] Greaff FG, Guimaraes FS, De Andrade TG, Deakin JF. Role of 5-HT in stress, anxiety, and depression. Pharmacol Biochem Behav 1996;54: 129–41.
[11] Handley SL, Mithani S. Effects of alpha2-adrenoceptor agonists and antagonists in a maze-exploration model of fear-motivated behavior. Naunyn Schmiedebergs Arch Pharmacol 1984;327:1–5.
[12] Blows WT. Neurotransmitters of the brain: serotonin, noradrenaline (norepinephirune), and dopamine. J Neurosci Nurs 2000;32:234–8.
[13] Stahl S, Briley M. Understanding pain in depression. Hum Psychopharmacol 2004;19:9–13.
[14] Brunello N, Blier P, Judd LL, Mendlewicz J, Nelson CJ, Souery D, et al. Noradrenaline in mood and anxiety disorders: basic and clinical studies. Int Clin Psychopharmacol 2003;18:191–202.
[15] Fricchione G, Stefano GB. Placebo neural systems: nitric oxide, morphine and the dopamine brain reward and motivation circuitries. Med Sci Monit 2005;11:54–65.
[16] Friedel RO. Dopamine dysfunction in borderline personality disorder: a hypothesis. Neuropsychopharmacology 2004;29:1029–39.
[17] Nutt DJ, Bell CJ, Malizia AL. Brain mechanisms of social anxiety disorder. J Clin Psychiatry 1998;59:4–11.
[18] Gutman DA, Owens MJ. Serotonin and norepinephrine transporter binding profile of SSRIs. Essent Psychopharmacol 2006;7:35–41.
[19] Dishman RK. Brain monoamines, exercise, and behavioral stress: animal models. Med Sci Sports Exerc 1997;29:63–74.
[20] Olson EB, Morgan WP. Rat brain monoamine levels related to behavioral assessment. Life Sci 1982;30:2095–100.
[21] Yacoubi EM, Bouali S, Popa D, Naudon L, Leroux-Nicollet I, Haman M, et al. Behavioral, neurochemical, and electrophysiological characterization of a genetic mouse model of depression. Proc Natl Acad Sci U S A 2003;100:6227–32.
[22] Okuyama S, Sawasaki E, Yokogoshi H. Conductor compounds of phenylpentane in *Mycoleptodonoides aitchisonii* myceliem enhance the release of dopamine from rat brain striatum slices. Nutr Neurosci 2004;7:107–11.
[23] Okuyama S, Lam NV, Hatakeyama T, Terashima T, Yamagata K, Yokogoshi H. *Mycoleptodonoides aitchisonii* affects brain nerve growth factor concentration in newborn rats. Nutr Neurosci 2004;7: 341–9.
[24] Graeff FG, Viana MB, Tomaz C. The elevated T maze, a new experimental model of anxiety and memory: effect of diazepam. Braz J Med Biol Res 1993;26:67–70.
[25] Knoll AT, Meloni EG, Thomas JB, Carroll FI, Carlezon WA. Anxiolytic-like effects of k-opioid receptor antagonists in models of unlearned and learned fear in rats. J Pharmacol Exp Ther 2007;323: 838–45.
[26] Gur E, Lerer B, van de Kar LD, Newman ME. Chronic rTMS induces subsensitivity of post-synaptic 5-HT1A receptors in rat hypothalamus. Int J Neuropsychopharmacol 2004;7:335–40.
[27] Ballenger JC. Overview of different pharmacotherapies for attaining remission in generalized anxiety disorder. J Clin Psychiatry 2001;62: 11–9.
[28] Stein DJ, Westenberg HG, Liebowitz MR. Social anxiety disorder and generalized anxiety disorder: serotonergic and dopaminergic neurocircuitry. J Clin Psychiatry 2002;63:12–9.
[29] Graeff FG, Viana MG, Mora PO. Dual role of 5-HT in defense and anxiety. Neurosci Biobehav Rev 1997;21:791–9.
[30] Michener W, Rozin P. Pharmacological versus sensory factors in the satiation of chocolate craving. Physiol Behav 1994;56:419–22.
[31] Tomaso E, Beltramo M, Piome D. Brain cannabinoids in chocolate. Nature 1996;382:677–8.
[32] Carney JM, Cao W, Logan L, Rennert OM, Seale TW. Differential antagonism of the behavioral depressant and hypothermic effects of 5'-(*n*-ethylcarboxamide) adenosine by theobromine. Parmacol Biochem Behav 1986;25:769–73.
[33] Gobbi G, Bambico FR, Mangieri R, Bortolato M, Compolongo P, Solinas M, et al. Antidepressant-like activity and modulation of brain monoaminergic. Proc Natl Acad Sci U S A 2005;102:18620–5.
[34] Navarro M, Hernandez E, Munoz RM, del Arco I, Villanua MA, Carrera MR, et al. Acute administration of the CB1 cannabinoid receptor antagonist SR 141716A induces anxiety-like responses in the rat. Neuroreport 1997;20:491–6.
[35] Dourish CT. Behavioral effects of acute and chronic beta-phenylethylamine administration in the rat: evidence for the involvement of 5-hydroxytryptamine. Neuropharmacology 1981;20:1067–72.
[36] Szabo A, Billett E, Turner J. Phenylethylamine, a possible link to the antidepressant effects of exercise? Br J Sports Med 2001;35: 342–3.
[37] Moreira FA, Aguiar DC, Guimaraes FS. Anxiolytic-like effect of cannabinoids injected into the rat dorsolateral periaqueductal gray. Neuropharmacology 2007;52:958–65.
[38] Stark T, Bareuther S, Hofmann T. Molecular definition of the taste of roasted cocoa nibs (*Theobroma cacao*) by means of quantitative studies and sensory experiments. J Agric food Chem 2006;54: 5530–9.
[39] Di Marzo V, Sepe N, De Petrocellis L, Berger A, Crozier G, Fride E, et al. Trick or treat from food endocannabinoids. Nature 1998;396: 636–7.
[40] Carvalho-Freitas MI, Costa M. Anxiolytic and sedative effects of extracts and essential oil from *Citrus aurantium* L. Biol Pharm Bull 2002;25:1629–33.
[41] Orosco M, Rouch C, Beslot F, Feurte S, Regnault A, Dauge V. Alpha-lactalbumin-enriched diets enhance serotonin release and induce anxiolytic and rewarding effects in the rat. Behav Brain Res 2004; 148:1–10.