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Diet influences cocaine withdrawal behaviors in the forced swimming test

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Abstract

The effects of drugs of abuse might depend on several environmental factors, among them the individual's feeding habits. It was our objective to study the influence of the diet on cocaine acute behavioral effects and during the first 5 days of withdrawal after prolonged treatment. Rats were fed a balanced diet, high-protein diet, high-carbohydrate diet or high-fat diet from weaning to adulthood. Adult rats were injected with 15 mg/kg cocaine 24, 5 and 1 h before the forced swimming retest or the drug was administered daily during 15 days and the animals were evaluated in the forced swimming test on five daily occasions after drug withdrawal. Diets alone did not induce significant behavioral differences in locomotion, immobility, swimming, climbing or head shakes. Acute cocaine reduced immobility during the forced swimming test and increased locomotion demonstrating a nonspecific antiimmobility effect related to hyperactivity. Acute cocaine reduced head shakes of rats fed high-protein and high-carbohydrate diets. After cocaine withdrawal, head shakes were decreased for rats fed any of the diets and rats were more immobile if fed a high-fat diet and were less immobile if fed a high-protein or high-carbohydrate diet. In conclusion, differences in the amounts of macronutrients in the diet may cause different behavioral outcomes after acute cocaine and during cocaine withdrawal.

Keywords: Protein; Lipid; Carbohydrate; Animal model of depression

1. Introduction

The behavioral effects of the psychomotor stimulants rely on dose, route of administration, type of preparation and frequency of use (King et al., 1993; Warner, 1993). Besides, individual differences such as genetic background, hormones, gender, age and environmental factors like feeding status also seem to influence the effects of stimulants (Brady et al., 1993; Kanarek and Marks-Kaufman, 1988).

The relationship between feeding behavior and its nutritional aspects in cocaine and other psychoactive drugs effects could be attributed, at least in part, to the role of some dietary amino acids that are precursors for synthesis of some neurotransmitters, particularly phenylalanine, tyrosine and tryptophan (Wurtman, 1982; Wurtman et al., 1980). In this respect, most studies concentrate on the drugs' effects on food intake or feeding behavior, such as nutrient selection. Amphetamine and fenfluramine induce a dose-dependent decrease of food intake of rats consuming high-carbohydrate and high-fat diets; fenfluramine induces greater reduction in food intake of rats feeding a high-fat diet in comparison to those fed a high-carbohydrate diet (Kanarek et al., 1991). The intake of protein, fat and carbohydrate diets is suppressed for 1 h in fasted female rats given an oral cocaine dose and for the following 2–6 h after cocaine, there is a compensatory increase in fat and carbohydrate intake, but not in protein consumption. Therefore, unbalanced diets, with higher carbohydrate or fat contents, might enhance anorectic effects of psychostimulants, which are further compensated, but no compensation is seen in individuals with higher protein contents in their diet (Bane et al., 1993). In fact, caloric and protein malnutrition occurs among human drug abusers (Santolaria-Fernandez et al., 1995). These results demonstrate that the great dietary variation between individuals, due to cultural or social differences, must be considered when evaluating the anorectic effects of psychostimulants. It might be interesting to uncover if there are changes in other behavioral effects of psychostimulants due to diversity of the diet.

There has been an increasing interest to study the effects of nutritional variables on drug intake, which is a determinant

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for drug dependence. It has been demonstrated that psychoactive drug self-administration might be altered by different factors related to feeding and nutrition. Mainly, food deprivation increases self-administration of several drugs, including etonizate, cocaine and phencyclidine (Carroll, 1985; Carroll and Meisch, 1984) Increased amount of food decreases drug self-administration (Carroll and Lac, 1998). However, food amount is not the only factor to change drug intake since higher palatability of food decreases the rate of acquisition and the number of rodents to achieve criterion on the acquisition of cocaine self-administration (Carroll and Lac, 1998). Sucrose availability alters oral consumption of morphine and amphetamine (as reviewed in Kanarek and Marks-Kaufman, 1988). Rats consume less amphetamine solution when given a choice to consume granulated sucrose in addition to the regular rodent chow (Kanarek and Marks-Kaufman, 1988). Therefore, different factors participate in the influence that variation of nutrients in the diet may have on drug self-administration.

Different diets may also influence the way individuals metabolize the drugs of abuse, increasing or decreasing its effects. Dietary factors are partially responsible for variations in plasma cholinesterase activity. Cholinesterase and other esterases metabolize cocaine to ecgonine methyl ester and as much as one third to one half of a given dose of cocaine may be metabolized via this pathway, as recently reviewed (Cahill-Morasco et al., 1998). Changes in esterases activity may cause variations on cocaine concentration in blood and tissues and correlate with cocaine-increased stereotypies. Indeed, protein and caloric malnutrition is associated with a reduction in plasma cholinesterase activity and enhanced cocaine toxicity in mice (Cahill-Morasco et al., 1998). Reduced plasma cholinesterase activity decreases the metabolism of cocaine, increasing cocaine plasma levels and, therefore, its behavioral toxicity, increasing the chances of a certain dose of cocaine significantly increasing sensitization to cocaine-induced stereotypy (Shumsky et al., 1997). Also, it is described that prenatal malnutrition of male rats leads to significantly more stereotypy and sensitization following cocaine (Shultz et al., 1999). It still remains to be clarified if there is a long-lasting deficiency in cholinesterase activity in adults who were prenatally malnourished. This area seems of interest because the changes in the behavioral effects of cocaine may be related to changes in its metabolism, which will need further debate.

Psychostimulants are occasionally proposed as adjuvant in the treatment of resistant depression (Santosh and Taylor, 2000) and are cited to be self-administered by individuals seeking relief for depressive mood (Lopes et al., 1996). In the forced swimming test, an animal model used to screen antidepressant agents, the psychostimulants, acutely decreases immobility of rats, as do most antidepressant agents, but also increases motor activity, which leads to the interpretation of a false-positive effect. The present study was planned to verify if acute and chronic cocaine behavioral effects would be influenced by diet. Therefore, acute cocaine behavioral effects in the forced swimming test and cocaine withdrawal behavioral manifestations were compared in rats consuming a well-balanced diet, a high-protein diet, a high-carbohydrate diet or a high-fat diet.

2. Methods

2.1. Animals

Sixty-day-old male Wistar rats from the breeding stock of the Animal House of Fundação Faculdade Federal de Ciências Médicas de Porto Alegre were used. Rats were maintained in polypropylene cages ($33 \times 17 \times 40$ cm), five rats per cage, under standard environmental conditions of illumination (lights on from 7 a.m. to 7 p.m.), humidity (55%) and temperature (22 ± 2 °C). The animals consumed the different diets prepared as described below from the 22nd day of life on. Food and water were given ad libitum. Breeding, housing and experimental procedures followed guidelines published in the NIH Guide for Care and Use of Laboratory Animals and obeyed current Brazilian laws.

2.2. Diets

Four different diets were prepared with different amounts of casein, cornstarch and soy oil to provide differential intakes from each nutrient source, as discriminated in Table 1. Rats were weighed every 7 days. Balanced diet (B) was composed of 20% of its calories from casein protein, 50% from cornstarch and 30% from soy oil and followed the recommendations of the Association of Official Analytical Chemists (1980). The high-protein diet (P) had 40% protein, the high-carbohydrate diet (C) contained 70% of calories from cornstarch and the high-fat diet (F) contained 50% of its calories from soy oil. Mineral salts (4%), vitamins (1.5%) and 1.0% of fiber (Pro-fiber; Kasdorf, Buenos Aires, Argentina) and 0.3% of methionine (Sigma, St. Louis, MO, USA) were added to all diets. Lactic casein (Labex, Porto Alegre, Brazil) contained 87% of protein. Minerals were of PA grade and were previously weighed and mixed to provide 1 kg in the following proportions: KH_2PO_4 (389.0 g), $CaCO_3$ (381.4 g), NaCl (139.3 g), $MgSO_4$ (57.3 g), $FeSO_4 \cdot 7H_2O$ (27.0 g), $MnSO_4 \cdot H_2O$ (4.01 g), $ZnSO_4 \cdot 7H_2O$ (0.548 g), $CuSO_4 \cdot 5H_2O$ (0.477 g), $CoCl_2 \cdot 6H_2O$ (0.023 g), KI (0.790 g). The vitamin

Table 1
Amount of macronutrients in 100 g of the different diets

Diet	Protein from casein (g)	Carbohydrate from starch (g)	Fat from vegetable oil (g)
Balanced	21.6 (20%)	53.7 (50%)	14.3 (30%)
High-protein	39.2 (40%)	39.2 (40%)	8.6 (20%)
High-carbohydrate	19.1 (20%)	66.6 (70%)	4.2 (10%)
High-fat	24.7 (20%)	37.0 (30%)	27.4 (50%)

Percent kilocalories of each component of the diets are represented in parenthesis.

mixture was prepared by the Department of Animal Nutrition at Roche (São Paulo, SP, Brazil) to provide vitamin A (2000 IU), vitamin D (200 IU), vitamin E (10 IU), menadione (0.5 mg), choline (200 mg), *p*-aminobenzoic acid (10 mg), inositol (10 mg), niacin (4 mg), Ca D-pantothenate (4 mg), riboflavin (0.8 mg), thiamine·HCl (0.5 mg), pyridoxine·HCl (0.5 mg), folic acid (0.2 mg), biotin (0.04 mg), vitamin B12 (0.003 mg) to 100 g of the diet. Dry components of the diets were weighed and mixed homogeneously. To avoid confusing the different chows, small amounts of red, blue, green or yellow food coloring were added to each diet. Enough water was added to the dry mixture to produce soft dough that could be spread in trays, cut in chunks and dried under constant ventilation at 22 °C. When ready, the diets were covered and stored in the refrigerator (4 °C) for use within 7 days.

2.3. Drugs

Cocaine hydrochloride (Merck, Germany) was solubilized in distilled water to a concentration of 15 mg/ml. Intraperitoneal (i.p.) administration of 1 ml/kg was chosen to avoid dosing variations due to different oral drug absorption (Kanarek, 1985).

2.4. Equipment

The forced swimming test is performed in an assemblage of four glass aquariums (25 × 25 × 40 cm) built over a table, as described previously (Nicilovitz, 2000). For visual isolation of each pool, a black cardboard surrounded the walls. The individual aquarium covers are transparent. The glass pools are linked to a water drainage system and to a warm and cold water faucet to facilitate entry of water at 25 °C. A camera hanging from 60 cm above the transparent covers allows videotape recordings from the four aquariums at the same time. The videotape recording system is connected to a monitor so the researchers follow the experiment from an adjoining room.

The studies were performed with clean water for each animal and the pool was full enough to prevent hind paws or tails from touching the bottom (27 cm). Dirty water was drained immediately after the release of the rat from pool. The pool was washed with some drops of nonperfumed liquid detergent and filled with cold water (25 ± 1 °C) immediately before another animal was introduced.

2.5. Procedures

Two different sets of experiments were performed. One experiment was done to study the acute effects of cocaine in the forced swimming of rats fed different diets. For this, 20 rats from each one of the four diet subgroups were observed in the forced swimming retest after treatment with cocaine. At this time, the classical forced swimming procedures were followed (Porsolt et al., 1977, 1978). Two swimming sessions 24 h apart were performed. The first session (test) lasted for 15 min and the second (retest) lasted for 5 min. The rats were randomly assigned to receive 1 ml/kg saline or 15 mg/kg cocaine intraperitoneally after the test session. The three doses of saline or cocaine (15 mg/kg/dose) were injected 24, 5 and 1 h before the retest (Porsolt et al., 1977, 1978). Videotape recordings were done for the retest session. After each swimming session, the rats were thoroughly dried with towels and warmed under a heat source. Both test and retest sessions were conducted between 1 and 4 p.m. in a room with natural illumination and with the room lights on. All rats were placed in the locomotion apparatus for 5 min immediately before the forced swimming retest as described previously (Gomez and Barros, 2000). The locomotion apparatus (73 × 30 × 30 cm) is equipped with three photoelectric cells 20 cm apart from each other that activate a digital counter whenever the light current is interrupted by the animal (ALSBARSCH, Porto Alegre, Brazil).

To study the influence of the diets on cocaine withdrawal behaviors in the forced swimming, 71 rats who were consuming the four different diets were used. When 60 days old, rats from each diet subgroup were randomly assigned to saline or cocaine treatment. Saline, 1 ml/kg, or 15 mg/kg/day cocaine was injected intraperitoneally once a day, between 4 and 6 p.m., for 15 days. From Days 1 to 5 after treatment, the animals' performance was evaluated daily in the forced swimming for 15 min in a protocol slightly modified from Abel and Hannigan (1992). The first swimming session was performed 24 h after the last dose. The different diets were given to the animals throughout the whole study. Videotapes were recorded on the five sessions.

2.6. Behavioral analysis

Behavioral analysis were conducted by a previously trained observer who had achieved similar rating performance in comparison to other trained researcher at the 95% confidence limit for each one of the behavioral parameters when repeatedly observing the same animal. The videotapes were analyzed through direct computer keyboard input to BASIC-written software. The observer depressed the key encoding the behavior being observed in the video as soon as the animal changed from one behavior to the other. The duration and frequency of immobility, climbing and swimming, and frequency of diving and head shakes were measured throughout the duration of the experiment. Immobility was counted when the animal was floating, with its nose just above the surface of the water and making only slight lateral movements with the front paws to keep from submerging. Climbing was considered when the animal was with the body in an upright position and moving front paws up and down while keeping very close to the aquarium wall. Swimming was counted when the animal's body was almost in a horizontal position, making vigorous front paws lapping movements while in the middle of the water or with one of its sides towards the wall. Diving was counted every time the

Table 2
Duration (in seconds) and frequency (number) of behaviors of rats treated with 15 mg/kg cocaine 24, 5 and 1 h before the forced swimming retest

Treatment	Diet	*n*	Immobility (s)	Climbing (s)	Swim (s)	Head shake (number)	Locomotion (number)
Control	Balanced	10	131.1 ± 10.8	164.7 ± 11.2	0.80 ± 0.61	53.9 ± 2.36	83.6 ± 14.9
Cocaine	Balanced	10	**18.4 ± 10.1**	**280.6 ± 10.0**	0.70 ± 0.70	57.0 ± 6.15 *	**105.9 ± 10.2**
Control	High-protein	9	99.4 ± 18.9	189.6 ± 20.1	*7.0 ± 2.84*	47.8 ± 4.24	80.1 ± 12.0
Cocaine	High-protein	10	**5.6 ± 4.49**	**285.2 ± 9.53**	*8.5 ± 4.71*	**20.7 ± 5.13** *	**124.5 ± 24.5**
Control	High-carbohydrate	10	113.1 ± 14.0	182.3 ± 13.8	0.80 ± 0.55	45.8 ± 5.89	85.1 ± 12.3
Cocaine	High-carbohydrate	10	**0.30 ± 0.30**	**299.7 ± 0.78**	0.70 ± 0.52	**28.4 ± 3.56** *	**86.3 ± 10.7**
Control	High-fat	10	109.5 ± 18.4	186.9 ± 18.8	0	36.0 ± 5.97	62.5 ± 13.8
Cocaine	High-fat	10	**11.3 ± 3.66**	**286.4 ± 3.71**	1.7 ± 1.7	38.7 ± 6.17 *	**123.1 ± 13.6**
F Treatment (1,71)			**158.1**	**148.8**	0.266	**7.09**	**9.54**
F Diet (3,71)			1.35	0.855	*5.58*	7.19	0.425
F Treatment × Diet (3,71)			0.347	0.401	0.115	4.24 *	1.57

Locomotion is the number of photocells crossed in an activity box immediately before forced swimming. Results were expressed as mean ± S.E.M. *n* is the number of rats in the group. Bold is used to represent the differences due to treatment. Italics is used to discriminate difference due to the diet in comparison to the balanced diet ($P < .05$).

* Represents interaction of treatment and diet ($P < .05$).

rats' head and body were totally under water. Head shakes were defined as abrupt horizontal movements of the head.

2.7. Statistical analysis

Results were expressed as mean ± S.E.M. of frequency and of duration (in seconds) of each behavior. For the evaluation of the cocaine acute effects, a two-way ANOVA test was performed considering the diet factor (balanced, high-protein, high-carbohydrate, high-fat) and the treatment factor (saline or cocaine). For the comparison of the study of cocaine withdrawal, a repeated-measures three-way ANOVA test (Diet, Treatment and Days of withdrawal) was used. Post hoc comparisons were performed with the Student–Newman–Keuls test. Significant statistical differences were accepted when $P < .05$. Videotape recording failed for one rat from the protein diet–saline treatment in the acute study.

3. Results

The results of experiment to analyze the acute cocaine effects on behaviors during the forced swimming retest are presented in Table 2. The type of diet given to the different groups of rats did not influence most behaviors in the forced swimming test except for swimming, which was longer for animals fed a high-protein diet. Treatment with cocaine did not alter swimming or diving behaviors. The duration of immobility was shorter and the duration of climbing was longer in animals treated with 15 mg/kg cocaine, independent of the diets they consumed after weaning. There was a treatment-and-diet interaction when the frequency of the head shakes was analyzed. Rats fed high-protein or high-carbohydrate diets had lower frequency of head shakes when treated with cocaine. Rats fed a balanced diet or high-fat diet did not present a different number of head shakes when given cocaine, in comparison to their controls.

The frequencies of behaviors of animals on cocaine withdrawal tested in the forced swimming on the first of the 5 days of observation are presented in Table 3. Diversity of diets does not significantly change the number of immobile and climbing behaviors. The frequency of immobile or climbing behaviors during the 5 days of forced swimming test was not significantly changed in animals that had received the prolonged daily treatment with cocaine in comparison to the controls (Table 4). Diving was observed mostly on the first day and was more frequent when the animals were under cocaine withdrawal. Animals that were

Table 3
Frequency (number) of behaviors in the forced swimming test on the *first day* of cocaine withdrawal of rats fed different diets

Treatment	Diet	*n*	Immobility	Climbing	Swim	Dive	Locomotion
Control	Balanced	9	17.2 ± 2.60	19.1 ± 2.46	2.4 ± 0.56	3.1 ± 0.81	22.5 ± 1.29
Cocaine	Balanced	9	17.9 ± 2.21	18.6 ± 2.73	3.8 ± 0.74	**5.0 ± 0.76**	**16.5 ± 1.32**
Control	High-protein	8	15.1 ± 2.88	17.2 ± 3.01	2.6 ± 0.50	3.1 ± 0.72	23.2 ± 1.59
Cocaine	High-protein	9	11.9 ± 1.68	13.9 ± 1.86	3.8 ± 0.60	**5.1 ± 0.81**	**17.0 ± 1.26**
Control	High-carbohydrate	8	14.6 ± 1.64	16.0 ± 1.49	1.6 ± 0.38	*1.6 ± 0.50*	19.8 ± 1.12
Cocaine	High-carbohydrate	9	16.3 ± 2.55	17.4 ± 2.91	2.0 ± 0.62	***2.7 ± 0.69***	**21.1 ± 2.54**
Control	High-fat	10	17.1 ± 2.00	17.8 ± 2.28	2.3 ± 0.42	*2.6 ± 0.50*	22.0 ± 1.26
Cocaine	High-fat	9	18.4 ± 2.84	19.4 ± 3.16	2.4 ± 0.53	***2.8 ± 0.52***	**16.6 ± 1.31**

The animals treated with 15 mg/kg/day cocaine or control solution, IP, for 15 days. Results were expressed as mean ± S.E.M. *n* is the number of rats in the group. Bold letters are used to represent the differences between treatment groups. Italics is used to discriminate difference due to the diet in comparison to the balanced diet ($P < .05$).

Table 4
Summary of *F* values of three-way ANOVA for behaviors in the forced swimming test and locomotion of rats fed different diets after 15 mg/kg/day cocaine withdrawal

Behavior	Treatment (1,315)	Diet (3,315)	Day (4,315)	Interaction Treatment × Diet (3,315)
Immobility frequency	0.070	2.25	**10.7**; 1>3,4; 2>4	2.34
Climbing frequency	0.094	2.70	**12.5**; 1>3,4; 2>4	1.93
Swimming frequency	**16.3 cocaine>control**	**6.30**; B,P>C,F	**64.8**; 1>2,3,4,5	**4.48 cocaine>control** in B and P
Dive frequency	**18.7 cocaine>control**	**8.62**; B,P>C	**94.2**; 1>2,3,4,5	**4.29 cocaine**: B,P>C,F; **control**: B,P>F>C
Head shake frequency	**12.8 cocaine < control**	1.80	**23.7**; 2,3,4,5>1	0.950
Immobility duration	3.27	0.230	**19.7**; 2,3,4,5>1;3,4>2	**5.02 cocaine**: F>B>P,C
Climbing duration	2.04	0.375	**14.2**; 1,2>3,4	**4.18** cocaine: P,C>B>F; **cocaine>control** in P,C diets
Swimming duration	**21.0 cocaine>control**	**6.82**; B,P>C,F	**79.8**; 1>2,3,4,5	**2.98 cocaine**: B>P,C,F; **control**: B,P>F>C
Locomotion	**45.1 cocaine < control**	1.82	1.20	**5.25 cocaine**: B>P < F

Balanced diet (B), high-protein diet (P), high-carbohydrate diet (C) and high-fat diet (F) were fed from weaning throughout the end of the withdrawal experiment. The numbers in parenthesis represent the degrees of freedom. Significant *F* values are in bold.

fed a high-carbohydrate or a high-fat diet dived and swam less frequently than rats fed a balanced or a high-protein diet (Tables 3 and 4). There was interaction between treatment and diet when frequencies of swimming and diving behaviors were considered. The animals fed a balanced or a high-protein diet showed higher frequency of swimming and of diving when on cocaine withdrawal.

Immobility lasted longer as testing was performed daily. Cocaine withdrawal or diet alone did not influence duration of immobility as isolated factors. As interactions were evaluated, it was verified that animals from the high-fat diet group were immobile for longer periods than the ones receiving balanced diet after cocaine treatment withdrawal. On the contrary, animals on high-protein diet were even less immobile than animals receiving balanced diet when abstaining from cocaine. Control solution-treated animals did not present differences in immobility time that could be related to which diet they were receiving (Fig. 1 and Table 4).

The time spent trying to climb the walls of the pools by rats during forced swimming shows almost a mirror effect to what is seen with immobility duration. Cocaine withdrawal or diet alone did not influence duration of climbing as isolated factors. Climbing behavior lasted less time as test-

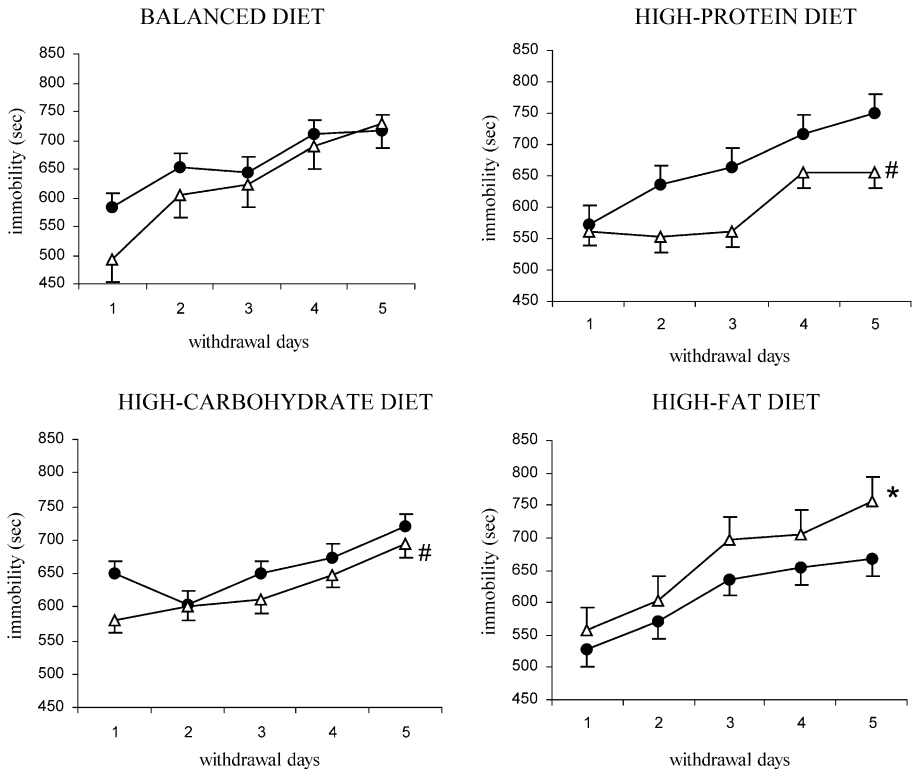

Fig. 1. Immobility duration in the forced swimming test of rats fed different diets was measured on the first 5 days of withdrawal after 15 days of 15 mg/k/day cocaine treatment. *Animals fed a high-fat diet presented significantly longer immobility duration during cocaine withdrawal when compared to animals fed a balanced diet. #High-protein and high-carbohydrate-fed animals reduced immobility duration during cocaine withdrawal. Results were expressed as mean ± S.E.M. (●) Represents controls and (Δ) represents cocaine-treated animals.

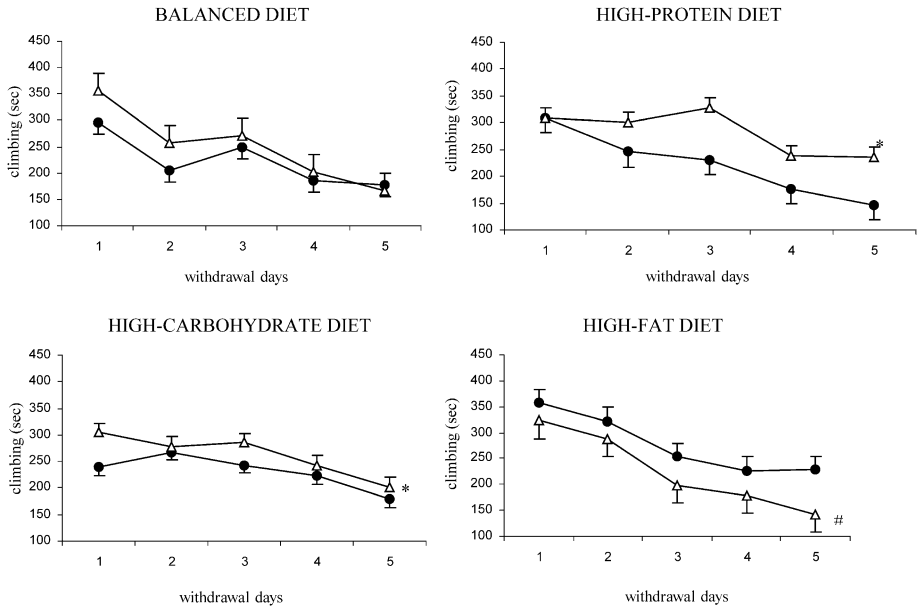

Fig. 2. Climbing duration in the forced swimming test of rats fed with different diets was measured on the first 5 days of withdrawal after 15 days of cocaine 15 mg/k/day treatment. * Animals fed a high-protein or high-carbohydrate diet significantly increases climbing time during cocaine withdrawal when compared to animals fed a balanced diet. #High-fat diet significantly reduced climbing during cocaine withdrawal. Results were expressed as mean ± S.E.M. (●) Represents controls and (Δ) represents cocaine-treated animals.

ing was performed daily; the duration of climbing on Days 3 and 4 was smaller than on Days 1 and 2. As expected, the duration of climbing (Fig. 2 and Table 4), showed an interaction between treatment and diet. Climbing was shorter for cocaine-treated rats fed a high-fat diet. After treatment with cocaine, longer duration of climbing was detected in animals getting high-protein or high-carbohydrate diet (Fig. 2 and Table 4).

The duration of the swimming in the center of the pool was short throughout the cocaine withdrawal experiment; however, it was significantly longer on the first day of testing. Also, a main treatment effect was detected, since animals treated with cocaine swim more in the center of the tank than the controls. Diet was also important to determine changes in swimming behavior. The animals fed balanced or high-protein diets swim more than those fed high-carbohyd-

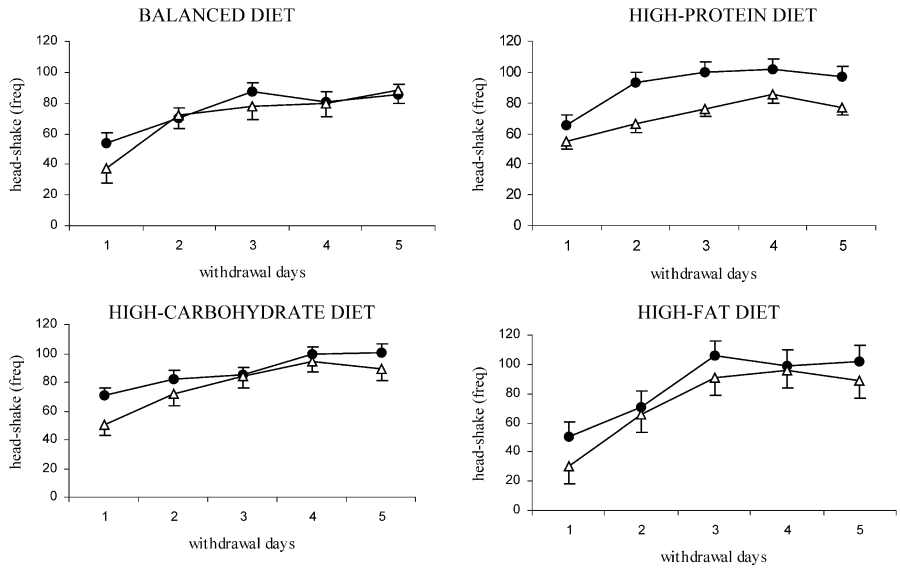

Fig. 3. Head shake frequency in the forced swimming test of rats fed different diets was counted on the first 5 days of withdrawal after 15 days of 15 mg/k/day cocaine treatment. The number of head shakes increases every subsequent testing day in the forced swimming test. Independent of the diet consumed, the cocaine-treated animals significantly decreased the number of head shakes. Results were expressed as mean ± S.E.M. (●) Represents controls and (Δ) represents cocaine-treated animals.

rate or high-fat diets. Additionally, there is a diet-and-treatment interaction. Animals fed a balanced diet will swim longer when cocaine treatment is withdrawn than the animals on the other diets, while a similar swimming behavior was seen among animals fed different diets after control treatment (Table 4).

The number of head shakes increased every subsequent testing day in the forced swimming test. Head shake behavior was not influenced by the different diets. There was an overall cocaine withdrawal effect since the cocaine treatment animals presented decreased number of head shakes, similar to what was detected after acute cocaine treatment (Fig. 3 and Table 4).

The results of locomotion on the first withdrawal day are discriminated in Table 3, and Table 4 presents the summary of the statistical analysis of locomotion according to diets, treatment and day of observation. It was observed that the rats from the cocaine withdrawal group had significantly less locomotion than the control animals and there was interaction between treatment and diet. The animals that consumed a high-protein diet had less locomotion during cocaine withdrawal than the ones fed a balanced or a high-fat diet.

Animal weights were always very similar for the animals receiving the different diets, whether they were treated or not with cocaine. All animals showed increased body weight from 50 g by 22 days of age to almost 300 g when the animals were 80 days old, when the withdrawal phase of the study was close to finish (results not shown). Blood samples were collected from 40 animals in the acute phase of the study, and measures such as plasma cholesterol, total protein in plasma and plasma albumin were not significantly changed in animals fed any of the diets. Triglycerides were significantly increased by carbohydrate-rich diet (77.9 ± 5.07 mg%) and were significantly decreased by fat-rich diet (28.3 ± 2.79 mg%) with respect to the controls (49.6 ± 4.00 mg%; $F(3,36) = 15.9$, $P < .05$). Therefore, none of the diets induced significant malnutrition in the animals.

4. Discussion

In this study, it is demonstrated that different diets: (1) only barely influence the acute behavioral effects of cocaine during forced swimming test, and (2) may influence the behavioral outcomes during cocaine withdrawal.

Acute cocaine treatment decreases immobility of rats in the forced swimming test, as expected (Lanziotti, 1996). Also, cocaine treatment increases locomotion due its motor-stimulating effect and the decrease of the immobility does not represent antidepressant effect. Similar results are observed with other psychomotor stimulants as amphetamine and caffeine (Porsolt et al., 1978). The animals from the different diet groups presented very similar immobile and mobile behaviors when exposed to cocaine. One would expect a more intense effect of cocaine in the group of animals fed a protein-rich diet, which has been associated with increased brain dopamine systems activity and more locomotor activity, hyperresponsivity to nociceptive stimuli and aggressive reactions to external stress (Brock and Prasad, 1991; Farooqui et al., 1994; Spring, 1986). An interesting effect seen in this study is that acute cocaine also decreased the number of head shakes during forced swimming. Interaction among the effects of acute cocaine and the diet the animals consumed was observed. Rats treated with cocaine decreased head shakes frequency when they were fed a high-protein or a high-carbohydrate diet. Head shakes may represent cocaine effects upon the serotonergic neurotransmitter system since it has been demonstrated that 5-HT_2 receptor stimulation increases head shakes frequency and 5-HT_2 antagonists block this effect (Berendsen and Broekkamp, 1990; Darmani et al., 1990; Yamada et al., 1995). However, it is also known that GABA agonists and morphine modulate the serotonergic system and decrease its influence on head shakes (Arnt et al., 1984). In case cocaine changes GABA or opioid neurotransmitter systems mainly on some individuals consuming special diets, rich in protein or rich in carbohydrates, there could be a smaller number of head shakes. Therefore, a complex interaction between neurotransmitters may be influencing the cocaine effects on head shakes and future studies need to be done to better explain the behavioral results seen here.

Depression is reported to happen very frequently to outpatients at the crash phase of cocaine withdrawal (Gavin and Kleber, 1986), or not to happen at all when inpatients are considered (Lago and Kosten, 1994). Experimental animals have shown higher intracranial self-stimulation threshold during cocaine withdrawal, representing lower motivation for hedonic events (Koob, 1995). There are controversial results with respect to cocaine withdrawal behaviors in the forced swimming test, an animal model of depression. Johnson et al. (1992) detected prolonged immobility of rats in the forced swimming, after 48 h of withdrawal of 10 mg/kg/day cocaine i.p. In our laboratory, in a previous study, immobility duration was not changed on the first days of oral cocaine self-administration withdrawal of rats fed a balanced commercial diet (Lanziotti, 1996). It seems possible that these controversies may occur due to individual differences, among them the diversity of intake of different macronutrients in the diet. If one considers the immobile posture to represent a depressive-type behavior, animals that consumed high-fat diet are more prone to depression during cocaine withdrawal, while high-protein and high-carbohydrate diet will induce rats to show less immobility than those fed a balanced diet during cocaine withdrawal. Therefore, diet may influence the behavioral outcome when prolonged treatment with cocaine is stopped. It is tempting to propose that diets rich in proteins and carbohydrates could have antidepressant-like effects during cocaine withdrawal. Anyway, these conjectures would need further studies in the clinical setting in order to establish their relevance for drug dependence treatments. Further studies will be necessary to determine if diet

differences also explain the high individual variability seen in the descriptions of depressive symptoms during cocaine withdrawal in the clinical setting (Gavin and Kleber, 1986; Lago and Kosten, 1994).

The neurobiological basis for the influence of diets on the depressive-type or antidepressant-type behavioral outcome of cocaine withdrawal may be related to the serotonergic system. Serotonin is formed after hydroxylation of L-tryptophan and tryptophan plasma levels correlate with its intake in diet (Biggio et al., 1974; Fernstrom, 1974; Fernstrom and Wurtman, 1971a) and insulin release (Fernstrom and Wurtman, 1971b, 1972). Hilakivi-clarke et al. (1990) observed that tryptophan administration decreases the immobility of animals in the forced swimming test. In the present study, the diet alone did not influence immobility, the parameter accepted as a marker for antidepressant effects. Behavioral modifications were the result of an interaction between diets and cocaine withdrawal. Therefore, different neurochemical effects are to be expected when animals fed different diets receive cocaine. For the sake of simplification, one may try to establish that the animals fed protein- or carbohydrate-rich diets have a higher level of serotonin during cocaine withdrawal and the animals from the fat-rich diet group would present lower serotonin levels during cocaine withdrawal. Once more, these are mere speculations not yet very well explained by literature findings that deserve further attention. The complexity of the subject might be related to the extension to which feeding affects brain tryptophan levels (Biggio et al., 1974; Fernstrom, 1974; Fernstrom and Wurtman, 1971a), depending on insulin secretion (Fernstrom and Wurtman, 1971b, 1972) and on intestinal absorption of amino acids that compete with tryptophan for blood–brain barrier transport system (Lehnert and Wurtman, 1993). Another research area that is presently very active is the one connecting high-fat diets, leptin and cocaine. High-fat diet increases plasma leptin and cocaine- and amphetamine-regulated transcript (CART) mRNAs (Ahima et al., 1999). Because of our results, it is admissible to propose that high levels of leptin and CART due to hyperlipemic diets do not participate in the acute effects of cocaine but may influence the depressive-type behaviors of the cocaine withdrawal state. Certainly, other studies should follow to clarify this initial observation.

In conclusion, we describe findings of the influence of macronutrients on the behavior of animals under cocaine treatment and during cocaine withdrawal. These results point to the importance of the interaction between dietary factors and psychoactive drugs and are relevant in the field of substance abuse. Further studies will be necessary to determine the clinical importance of these observations.

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