



## Effects of chronic administration of caffeine and stress on feeding behavior of rats

Leticia Ferreira Pettenuzzo<sup>\*</sup>, Cristie Noschang, Eduardo von Pozzer Toigo, Andreisa Fachin, Deusa Vendite, Carla Dalmaz

Departamento de Bioquímica, Instituto de Ciências Básicas da Saúde – ICBS, UFRGS (Saúde), Ramiro Barcellos, 2600, anexo. 90035-003, Porto Alegre, RS, Brazil

### ARTICLE INFO

#### Article history:

Received 12 December 2007

Received in revised form 5 June 2008

Accepted 5 June 2008

#### Keywords:

Feeding behavior

Caffeine

Stress

Obesity

### ABSTRACT

Anorectic effects of caffeine are controversial in the literature, while stress and obesity are growing problems in our society. Since many stressed people are coffee drinkers, the objective of the present study was to evaluate the effect of stress and chronic administration of caffeine on feeding behavior and body weight in male and female rats. Wistar rats (both males and females) were divided into 3 groups: control (receiving water), caffeine 0.3 g/L and caffeine 1.0 g/L (in the drinking water). These groups were subdivided into non-stressed and stressed (repeated-restraint stress for 40 days). During the entire treatment, chow consumption was monitored and rats were weighed monthly. Afterwards, feeding behavior was evaluated during 3-min trials in food-deprived and ad libitum fed animals and also in repeated exposures, using palatable food (Froot Loops® and Cheetos®). Chronic administration of caffeine did not affect rat chow consumption or body weight gain, but diminished the consumption of both salty (Cheetos®) and sweet (Froot Loops®) palatable food. In the repeated trial tests, stress diminished savory snack consumption in the later exposures [I.S. Racotta, J. Leblanc, D. Richard The effect of caffeine on food intake in rats: involvement of corticotropin-releasing factor and the sympatho-adrenal system. *Pharmacol Biochem Behav.* 1994, 48:887–892; S.D. Comer, M. Haney, R.W. Foltin, M.W. Fischman Effects of caffeine withdrawal on humans living in a residential laboratory. *Exp Clin Psychopharmacol.* 1997, 5:399–403; A. Jessen, B. Buemann, S. Toubro, I.M. Skovgaard, A. Astrup The appetite-suppressant effect of nicotine is enhanced by caffeine. *Diab Ob Metab.* 2005, 7:327–333; J.M. Carney Effects of caffeine, theophylline and theobromine on scheduled controlled responding in rats. *Br J Pharmacol.* 1982, 75:451–454] and caffeine diminished consumption of both palatable foods (savory and sweet) during the early and later exposures. Most responses to caffeine were stronger in females, and stress exposure influenced the effect. Neither chronic caffeine nor stress affected adrenal weight and plasma corticosterone levels of the rats. These observations suggest that chronic caffeine consumption may have sex-specific effects on palatable food ingestion.

© 2008 Elsevier Inc. All rights reserved.

### 1. Introduction

Caffeine is the most widely consumed behaviorally active substance in the world. Almost all caffeine consumed comes from dietary sources (beverages and food), most of it from coffee and tea [1]. The central effects of caffeine at consumed doses are due to the blockade of adenosine A1 and A2 receptors. These receptors are widely distributed through the brain, adenosine A1 receptors being present mostly in cortical layers, hippocampus and striatum, and A2a receptors being co-localized with DA receptors in the striatum [2,3].

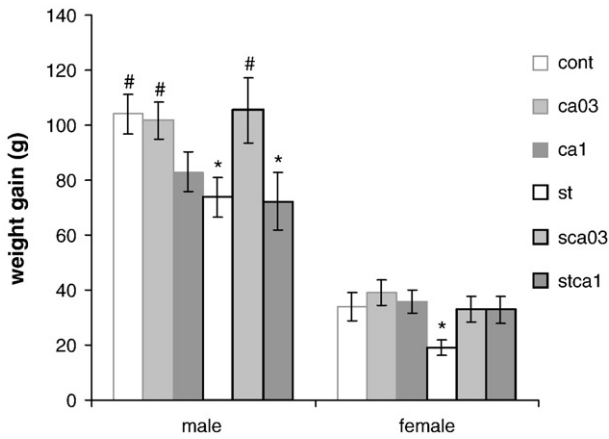
The anorectic affect of caffeine is controversial. Many central stimulants reduce the appetite, via mechanisms that are incompletely understood. Caffeine appears to have a small reducing effect on caloric intake [4–6]. Acute caffeine administration may exert a slight anorectic effect in men [4], and reintroduction of caffeine after a period of

abstinence in regular coffee consumers was found to reduce daily energy intake by decreasing the number of meals [7]. Additionally, caffeine disrupts operant responding in rats trained to press levers for food rewards, and tolerance develops to this effect [8]. However, it is not clear if this effect is due to altered cognition or motivation to eat, since the motivating stimulus used was food. This effect on operant conditioning is similar to, although less marked than, that observed after amphetamine [9]. It is possible that the decreased caloric intake might be an effect of acute rather than long-term caffeine use [1]. In addition, most of these studies concerning caffeine and food consumption were performed using standard rat chow.

Feeding control may be altered by different factors, such as biological status, available nutrients, and stress [10]. Additionally, previous studies from our laboratory have shown that repeated-restraint stress in adult rats leads to a greater ingestion of sweet food [11], which is reversed by acute diazepam administration before the test session, in a dose that does not affect feeding by itself [11], and by chronic administration of midazolam [12]. Although several studies

<sup>\*</sup> Corresponding author. Tel.: +55 51 33085530; fax: +55 51 33085540.

E-mail address: [leticiafpettenuzzo@yahoo.com.br](mailto:leticiafpettenuzzo@yahoo.com.br) (L.F. Pettenuzzo).



**Fig. 1.** Weight gain during eleven weeks of treatment for male and female chronically-stressed rats receiving caffeine. Data are expressed as means  $\pm$  SEM of the weight gain in grams. \*Different from respective sex-control group (non-stress, non-caffeine) and #different from stress, non-caffeine group ( $P < 0.05$ , Duncan multiple range test).

have been carried out with regard to how exposure to stress may affect food consumption, possible interactions between stress and caffeine on feeding behavior have not been studied.

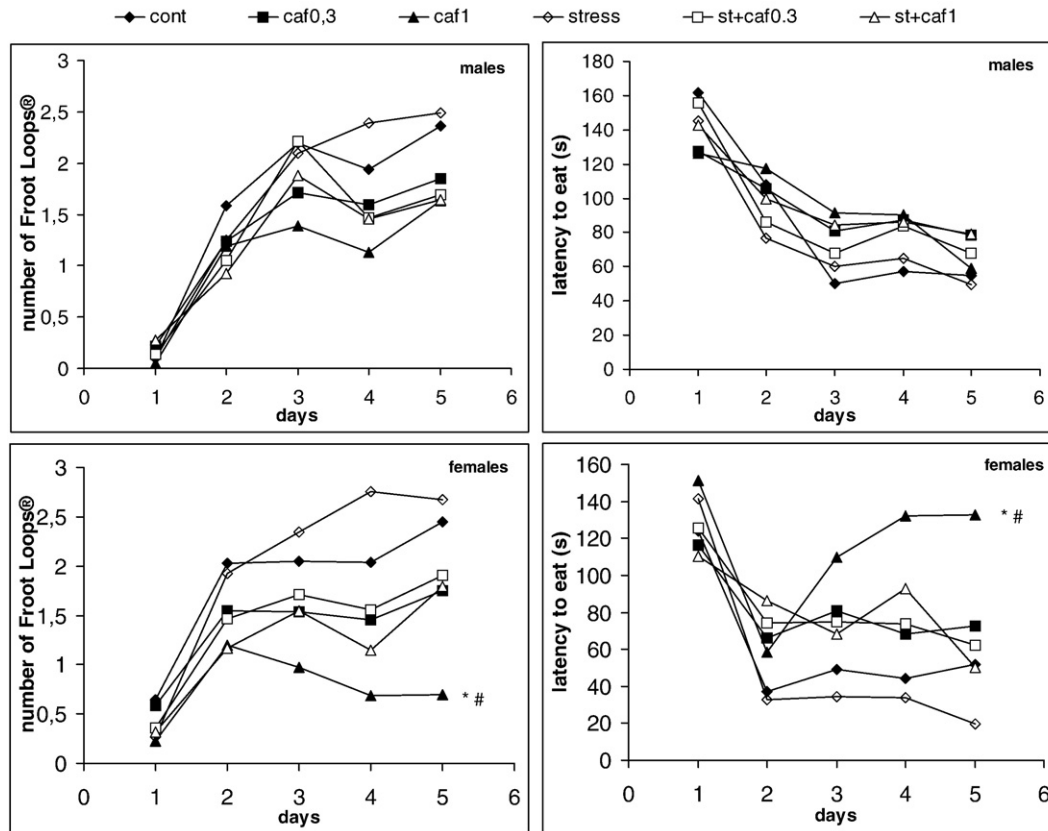
Several sources of data indicate that behavioral and physiological responses to stress are sexually dimorphic, including alterations in feeding behavior [11,13–15]. In humans, women are more sensitive to disturbances in feeding behavior than men [16,17]. These considerations emphasize the importance of studying stress effects on feeding behavior in both sexes.

Since obesity is a growing problem in our society and caffeine is widely consumed, the main objective of this study is to investigate the effect of chronic administration of caffeine on feeding behavior of male and female rats, subjected or not to chronic stress. Therefore, we evaluated the consumption of rat chow, sweet and savory snacks, as well as body weight in rats subjected to chronic stress and receiving caffeine, compared to their controls, and compared the effects observed in both sexes.

**2. Materials and methods**

**2.1. Animals**

For the present study, we used 58 adult male (7–13 per group) and 48 adult female (8 per group) Wistar rats (60 days of age at the beginning of the treatment), weighing 250–300 g (males) and 150–200 g (females). The animals were divided into 3 groups: control (receiving water), caffeine 0.3 g/L and caffeine 1.0 g/L (in the drinking water). These groups were subdivided into non-stressed and stressed (repeated-restraint stress for 40 days). The experimentally naive animals were housed in groups of three to five throughout the study, in home cages made of Plexiglas material (65  $\times$  25  $\times$  15 cm) with the floor covered with sawdust. They were maintained under a standard dark–light cycle (lights on between 7:00 and 19:00 h), at a room temperature of 22  $\pm$  2  $^{\circ}$ C. The rats had free access to food (standard rat chow) and water (or caffeine solution, see below), except for the stressed group, during the period when restraint stress was applied. All animal treatments were in accordance with the institutional guidelines and according to the recommendations of the International Council for Laboratory Animal Science (ICLAS), and all efforts were made to minimize animal suffering, as well as to reduce the number of animals.



**Fig. 2.** Consumption and latency to eat a palatable sweet food (Froot Loops®) during the habituation sessions (under food restriction) for male and female chronically-stressed rats receiving caffeine. Data are expressed as the mean of the number of Froot Loops® consumed and mean latency in seconds. \*Different from control group (non-stress, non-caffeine) and #different from stress, non-caffeine group ( $P < 0.05$ , Duncan multiple range test).

## 2.2. Stress model

Restraint was carried out by placing the animal in a 25×7-cm plastic tube, and adjusting it with plaster tape on the outside, so that the animal was unable to move. There was a 1-cm hole at the far end for breathing. The animals in the stressed groups were subjected to this procedure for 1 h/day, 5 days a week, for 40 days. After this period, they were habituated to the behavioral apparatus (see below, in task to new foods) and tested for food consumption. During the habituation period, which could last for 4 to 6 days (see below), restraint stress was maintained, and animals were stressed 1 h after the behavioral procedures. Control animals were weighed once a month.

## 2.3. Caffeine administration and rat chow consumption

Caffeine at doses of 0.3 and 1.0 g/L was administered in the drinking water as the only source of water during 40 days [1]. During the behavioral tests, animals continued receiving caffeine treatment. Control animals received tap water. The volume of water and caffeine solution consumed was measured each 48 h and replaced by a fresh one. Rat chow consumption was measured per cage and then divided by the number of animals per cage to determine mean consumption per animal. In the statistical analysis of amount of rat chow consumed, *n* represents the number of cages.

## 2.4. Habituation to the new foods

Starting on day 41 of stress and/or caffeine treatment, rats were habituated to a novel environment containing new foods. During this period, they were placed in a lightened rectangular box (40 cm×15 cm×20 cm) with floor and side walls made of wood and a glass ceiling. Ten Froot Loops® (Kellogg's® – pellets of wheat, cornstarch and sucrose) were placed in one extremity of the box. The animals were habituated to this environment during 5 days, 3 min each day, under food restriction (receiving about 80% of habitual ingestion of standard lab chow). After the last habituation session, the animals were fed ad libitum and were exposed to a 3-min test session, 24 h later. Time spent until the initiation of eating and the number of ingested Froot Loops® were evaluated in each trial and in the test session. A protocol was established so that when the animals ate part of the Froot Loops® (e.g., 1/3 or 1/4), this fraction was considered. For savory snack ingestion, five Cheetos® (Elma Cheeps – pellets of corn meal, cheese, and salt) were placed in the same apparatus. Habituation was established in 3 days because rats were already familiar with the environment, and testing was performed in the same manner as described above [18].

## 2.5. Repeated food intake to address satiety

One day after the test session described above, the animals were submitted to eight exposures to the box for evaluation of the number of Froot Loops® or Cheetos® eaten. After 3 min in the box, where they could eat the palatable food, rats were placed in a separate box for 30 s, then again in the box for the feeding evaluation; this cycle was repeated 8 times (total time: 28 min). This procedure was performed with the animals in the fed state. A protocol was established so that when the animals ate part of the Froot Loops® or Cheetos® (e.g., 1/3 or 1/4), this fraction was included. The test using each kind of food was performed on separated days [18].

## 2.6. Blood collection and adrenal dissection

One week after the last behavioral procedure, animals were killed between 12:00 and 14:00 h, and the trunk blood was collected into heparinized tubes, centrifuged at 4 °C at 1000 g, and plasma separated and stored at –70 °C until analysis. All animals were killed within this

interval of time in a random order with regard to stressed and non-stressed animals.

Adrenal glands were carefully dissected and weighed using a scale with a precision of 0.0001 g.

## 2.7. Corticosterone determination

For corticosterone determination, plasma was extracted with ethyl acetate, the extract evaporated and the residue suspended for evaluation of the hormone with an ELISA kit (Cayman Chemical Co., Ann Arbor, MI, USA).

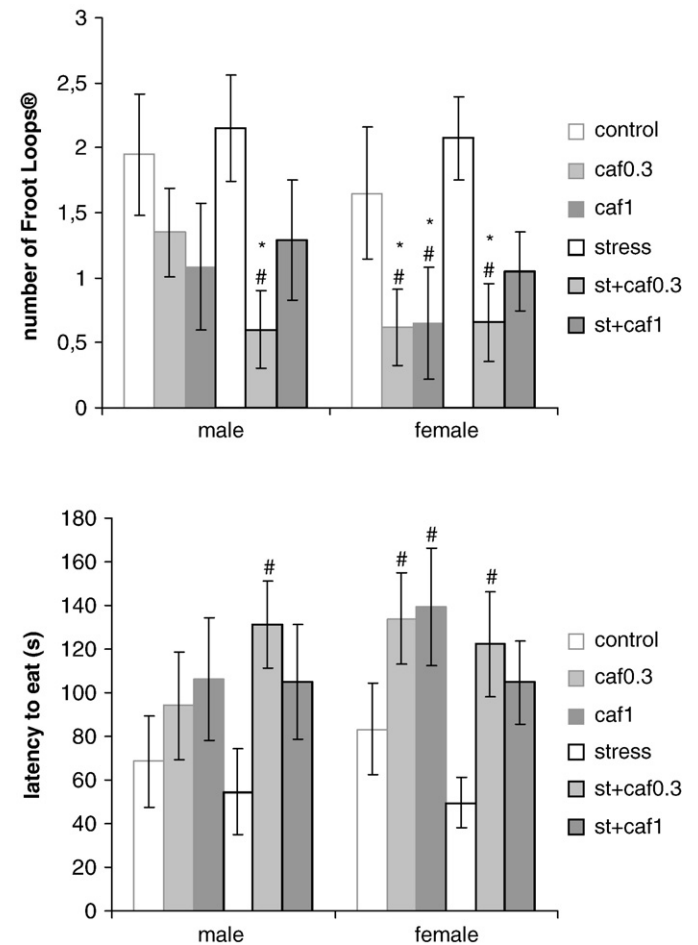
## 2.8. Statistical analysis

Data were expressed as means±SEM. Data were analyzed using four-way repeated measures ANOVA for analyses of the body weight and trials evaluating habituation for new foods and satiation, and three-way multivariate ANOVAs were used for analyses of the tests, adrenal weight and biochemical measurement. When indicated, a post-hoc Duncan multiple range test was performed.

## 3. Results

### 3.1. Body weight

Body weight was measured at the beginning of the treatment, and once a month afterwards. Four-way repeated measures ANOVA (time



**Fig. 3.** Consumption and latency to start eating the palatable sweet food during the test session (with animals previously fed ad libitum) for male and female chronically-stressed rats receiving caffeine. Data are expressed as mean±SEM. \*Different from respective sex-control group (non-stress, non-caffeine) and #different from stress, non-caffeine group ( $P < 0.05$ , Duncan multiple range test).

as within subjects factor and stress, caffeine and sex as between subject factors) showed that animals increased in weight during the treatment [ $F(2,186)=661, P<0.01$ ]; there was an interaction between sex and time of treatment, since males showed a greater increase in weight than females [ $F(2,186)=150, P<0.01$ ], an interaction between stress and time, since controls gained more weight than stressed animals [ $F(2,186)=5.16, P<0.01$ ], an interaction between caffeine and time, [ $F(2,186)=3.85, P<0.01$ ] and an interaction between stress, sex, time and caffeine [ $F(4,186)=2.52, P<0.05$ ], since caffeine prevented the reduced weight gain observed in stressed rats: in males only 0.3 g/L caffeine had this effect and, in females, both doses prevented the reduction in weight gain found in stressed rats (Fig. 1).

### 3.2. Standard lab chow ingestion and caffeine consumption

A three-way ANOVA showed that males ate more rat chow than females [ $F(1,16)=2.95, P<0.01$ ]. When we normalized the consumption of chow by the weight [energy intake/body mass to the 2/3 power [43]], the same result was observed [ $F(1,16)=6.90, P<0.05$ ] (data not shown). There were no statistical differences between stressed and caffeine-treated groups and their controls when comparing standard lab chow consumption in the home cages, and no interaction was observed between sex and group. There were no statistical differences between groups in drinking volumes consumed. Mean caffeine consumption did not differ between stressed and control animals, neither between males and females; rats receiving 0.3 g/L caffeine consumed about 40 mg/kg/24 h and rats receiving 1.0 g/L consumed about 100 mg/kg/24 h.

### 3.3. Sweet food ingestion

Four-way repeated measures ANOVA showed that all animals increased their consumption of Froot Loops® [ $F(4,376)=70.7, P<0.01$ ] and decreased their latency to eat this food [ $F(4,376)=38.8, P<0.01$ ] during the habituation sessions (under food restriction). There was an interaction between days of habituation and caffeine (0.3 and 1.0 g/L) treatment, since rats receiving tap water consumed more sweet food [ $F(8,376)=2.85, P<0.01$ ] and showed a lower latency to eat [ $F(8,376)=2.35, P<0.05$ ] than caffeine-treated rats (Fig. 2). Stress had no effect. The caffeine effect was more pronounced in females; analysis of individual groups indicated that females receiving 1 g/L caffeine consumed less sweet food and had a higher latency to eat this type of food than groups that did not receive caffeine 1 g/L (two-way ANOVA and Duncan test,  $P<0.05$ ; see Fig. 2).

Analyzing the test session (when animals were fed ad libitum during the last 24 h), a three-way ANOVA showed that caffeine-treated rats ate less [ $F(2,106)=8.12, P<0.01$ ] and had a higher latency to eat the Froot Loops® [ $F(2,106)=9.82, P<0.01$ ] (Fig. 3). Comparing individual groups (Duncan test), it was observed that, while caffeine decreased the ingestion of sweet food in stressed and non-stressed females, in males this effect was observed just in stressed animals.

### 3.4. Savory snack ingestion

Four-way repeated measures ANOVA showed that all groups increased their consumption [ $F(2,188)=117, P<0.01$ ] and decreased the latency [ $F(2,188)=18.4, P<0.01$ ] to eat this savory food (Cheetos®) during the habituation sessions (under food restriction). There was an interaction between consumption of Cheetos® along the days of

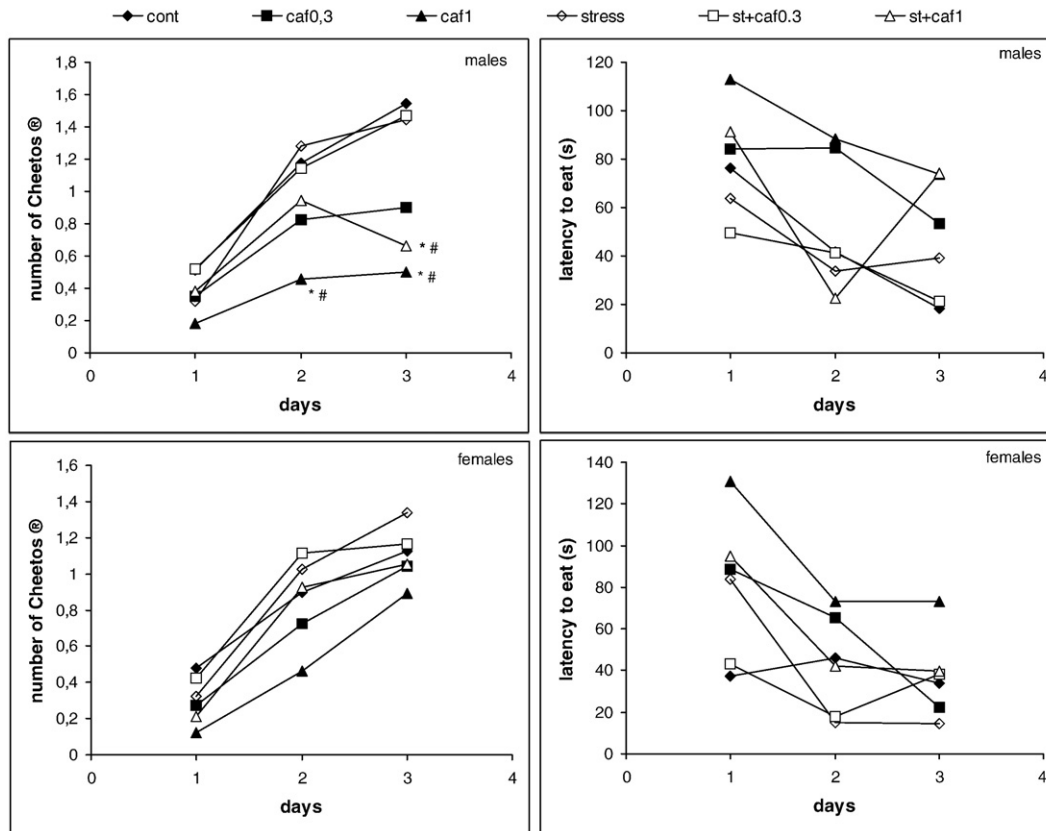


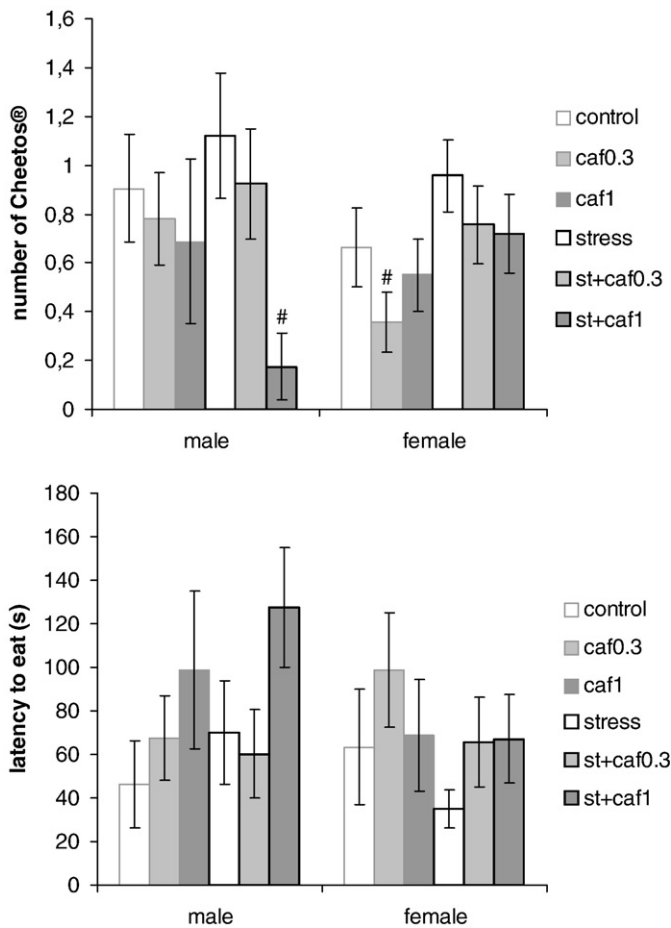
Fig. 4. Consumption and latency to eat a palatable savory food (Cheetos®) during the habituation sessions (under food restriction) for male and female chronically-stressed rats receiving caffeine. Data are expressed as mean number of Cheetos® consumed and mean latency in seconds. \*Different from respective control group (non-stress, non-caffeine) and #different from stress, non-caffeine group ( $P<0.05$ , Duncan multiple range test).

habituation and stress [ $F(2,188)=3.4$ ,  $P<0.01$ ], since stressed rats consumed more savory food along the habituation than non-stressed rats. Four-way repeated measures ANOVA also showed an interaction between consumption of Cheetos® along the days of habituation and caffeine [ $F(4,188)=2.5$ ,  $P<0.05$ ], since 1.0-g/L caffeine-treated rats consumed less Cheetos® than controls and 0.3-g/L caffeine-treated rats. There was also a trend towards an interaction between consumption of Cheetos® along the days of habituation, sex and caffeine [ $F(4,188)=2.4$ ,  $P=0.052$ ], since male rats treated with 1.0 g/L caffeine consumed less Cheetos® along the habituation days than females under the same treatment (see Fig. 4). Comparing individual groups (Duncan test), it was observed that, while caffeine at the higher dose decreased the ingestion of savory food in stressed and non-stressed males under food restriction, this effect was not significant in females.

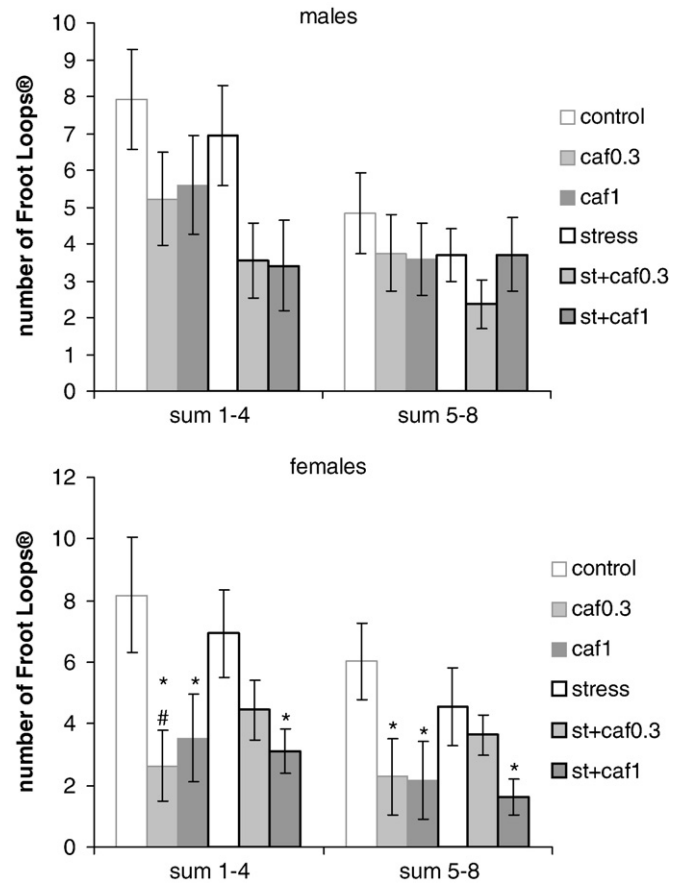
As displayed in Fig. 5, caffeine-treated rats ate less Cheetos® during the test session (when animals were fed ad libitum during the previous 24 h) [ $F(2,106)=3.19$ ,  $P<0.05$ , three-way ANOVA]. This effect was observed especially in chronically-stressed male animals, with the dose of 1.0 g/L (Duncan post-hoc test).

### 3.5. Satiation tests

The animals were fed ad libitum during the days preceding the satiation tests. A three-way ANOVA showed that caffeine-treated rats (0.3 and 1.0 g/L) ate less Froot Loops® during the early [sum of first 4 exposures to the food;  $F(2,105)=9.68$   $P<0.01$ ] and late [sum of last 4



**Fig. 5.** Consumption and latency to eat the palatable savory food during the test session (with animals previously fed ad libitum) for male and female chronically-stressed rats receiving caffeine. Data are expressed as means  $\pm$  SEM. #Different from stress, non-caffeine group ( $P<0.05$ , Duncan multiple range test).



**Fig. 6.** Consumption of palatable food (Froot Loops®) during the satiation test (with animals previously fed ad libitum; 8 exposures of 3 min, 30 s interval) for male and female rats chronically stressed and receiving caffeine. Data are expressed as mean  $\pm$  SEM of the sum of 4 exposures (1–4 or 5–8). \*Different from respective control group (non-stress, non-caffeine) and #different from stress, non-caffeine group ( $P<0.05$ , Duncan multiple range test).

exposures to the food;  $F(2,105)=5.27$ ,  $P<0.01$ ) satiation trials, and this effect was especially observed in females, as shown by post-hoc tests (Duncan multiple range test; please see Fig. 6).

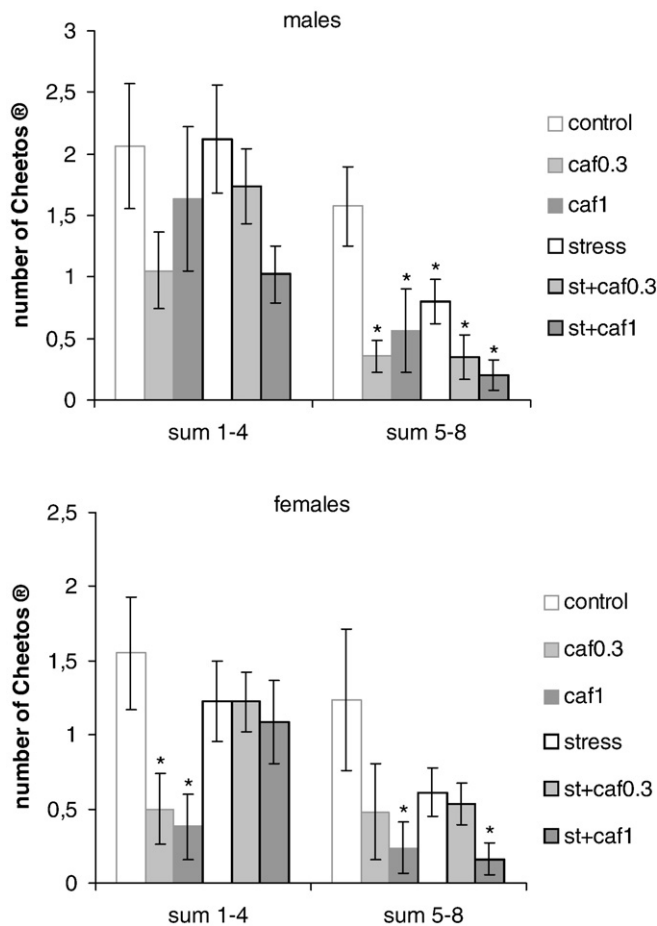
Additionally, caffeine-treated rats (0.3 and 1.0 g/L) ate less palatable savory food (Cheetos®) during the early [ $F(2,105)=4.45$ ,  $P<0.05$ ] and late [ $F(2,105)=11.46$ ,  $P<0.01$ ] parts of the satiation test (three-way ANOVA). Results also revealed that stressed rats consumed less Cheetos® in the latter (5th to 8th) exposures [ $F(1,105)=4.47$   $P<0.05$ ] and that males ate more than females during the early exposures to Cheetos® [ $F(1,105)=8.32$ ,  $P<0.01$ ] (Fig. 7). While caffeine-treated and stressed male rats consumed less savory food in the later exposures, females treated with caffeine showed reduced consumption of savory food in early and late exposures. Stress prevented the effect of caffeine on the early exposures (Duncan multiple range test).

### 3.6. Adrenal weight and corticosterone measurements

A three-way ANOVA revealed that females had higher corticosterone levels than males [ $F(58,1)=73.2$ ,  $P<0.01$ ]. There are no effects of chronic caffeine and/or stress on the basal corticosterone levels and adrenal weight of rats (data not shown).

## 4. Discussion

In this study, we verified an alteration in palatable food consumption in rats chronically treated with caffeine; this effect may be



**Fig. 7.** Consumption of palatable food (Cheetos®) during the satiation test (with animals previously fed ad libitum; 8 exposures of 3 min, 30 s interval) for male and female rats chronically stressed and receiving caffeine. Data are expressed as means  $\pm$  SEM of the sum of 4 exposures (1–4 or 5–8). \*Different from respective control group (non-stress, non-caffeine).

influenced by sex and by stress. However, neither rat chow consumption in the home cage nor body weight was decreased by caffeine treatment. It is important to note that these rats did not have free access to palatable food, since they could eat the palatable food just for short periods after chronic treatments: 3 min (during habituation or test sessions) or 28 min (on the occasion of the satiation test). Therefore, the reduced ingestion of palatable food induced by caffeine treatment in the present study could not lead to a decreased body weight. The decreased consumption of palatable food occurred when the animals were both under chow restriction and when they were fed ad libitum in the 24 h preceding the test, and this effect was more notable in females. We also observed that rats chronically treated with caffeine consumed less palatable food when they were exposed to it during a longer period (28 min). These data may indicate that the chronic administration of caffeine can alter the feeding behavior of rats with regard to palatable food, without modifying feeding behavior concerning standard rat chow in the home cage.

A lower weight gain observed in stressed animals has been previously reported [19,20], and may be related to the action of glucocorticoids, mobilizing energy stores and increasing hepatic gluconeogenesis [21,22]. Interestingly, caffeine treatment attenuated the effect of chronic stress on weight gain. We cannot precisely postulate the mechanism that resulted in a weight gain that was similar to that of controls in caffeine-treated animals, but it is possible that caffeine attenuates some stress responses. Although our caffeine-

treated animals did not show corticosterone or adrenal weight differences, differences in the stress response cannot be excluded.

Caffeine can also regulate serotonergic and noradrenergic neurotransmission [1]. Thus the effect of caffeine administration could be related to an increased serotonin content found in several structures (hypothalamus, hippocampus and striatum) in caffeine-treated animals, since serotonin is a neurotransmitter linked to decreased appetite [23]. On the other hand, it should be considered that feeding control is a complex mechanism that includes homeostatic and hedonic factors, and since no effect on body weight or rat chow consumption was observed, we could suggest that caffeine particularly decreases consumption of palatable food. Adenosine is known to modulate the action of neurotransmitters, including dopamine, in the nucleus accumbens [24,25]. Some studies demonstrate that a functional dopamine/adenosine interaction in the nucleus accumbens is necessary to induce the reinforcing effects of rewards [26], and that adenosine is involved in the sweet taste perception [27,28]. Therefore, this modulation could be involved in the present behavioral findings [29]. Since caffeine is an adenosine antagonist, this could mean that caffeine-treated animals had a lower perception of the rewarding effects of palatable food, due to a blunted dopaminergic tonus in the accumbens, i.e., the animals may decrease consumption of palatable food because the reinforcing effect is too small. Another possible explanation would be an increased cholinergic transmission on the accumbens. Cholinergic transmission is also related to feeding behavior, since it is a signal of satiation [30,31]. Caffeine can increase the release of acetylcholine in the nucleus accumbens and this effect is not altered after chronic treatment with caffeine [2], indicating that there is no tolerance. It is possible that an increased cholinergic transmission in our caffeine-treated rats may be related to the effects observed. However, this mechanism would probably also affect the rat chow consumption, which was not altered. Additionally, effects of caffeine treatment on sensorial perception cannot be excluded. Therefore, more studies are necessary to elucidate this mechanism.

We have previously observed that male rats subjected to chronic restraint stress consumed more sweet food, when this type of food is presented during short periods of time [11]. In the present study, however, no significant difference was observed. This lack of stress effect is probably due to some differences in the protocol used, especially with regard to the time when stress was applied. In that previous study Ely et al. [11] always applied restraint stress during the morning (when corticosterone levels were lower), while here the stress was applied in the afternoon, when corticosterone levels are increasing. These differences in protocol may result in different adaptation to stress.

In the present study, caffeine treatment induced more robust effects on females. In addition, when the animals are chronically caffeine treated, stress appears to increase the caffeine effect on palatable food consumption in males and decrease it in females. Previous studies have shown an interaction between hormonal status and response to stress in female rats, as well as different sweet food consumption [32] with high estrogen levels being associated with higher sweet food consumption, independently of the stress state [32,33]. Here, although estrous cycle was not monitored, tests of feeding behavior were conducted during several days, in such a way that females were tested under different hormonal profiles. Also, females are known to respond differently from males to stress. Females are believed to be more vulnerable to acute stress [34]. For example, the stress response to acute restraint is more intense in females, when assessed by corticosterone levels, [35], or by measuring the release of 5HT and dopamine in the amygdala [36]. Additionally, androgens are known to decrease, and estrogens increase HPA function [37]. This could explain the increased basal corticosterone levels observed in females in the present study, as well as in others [38,39]. However, behavioral responses to acute stress have been observed to be lower in females than in males [36], and it has been suggested that females may be endowed with a high ability to cope

with stress [36]. Therefore, adaptation to chronic stress may also be sex-specific [22,40]. Sex-specific adaptations to chronic stress may be involved in the observed prevention of some of the effects of caffeine in females, while it may potentiate the effect in males.

In addition, sex-differences in taste perception or in palatable food rewarding propriety could be part of the explanation for the differences observed here. Differences between male and female rats in the processing of sweet stimuli taste in the gustatory system have been reported, with females showing larger responses to sweet food [41]. Additionally, male and female rats show distinct food selection after stress exposure [34].

To our knowledge, the present study reported for the first time the sex-specific effects of chronic caffeine on the consumption of specific types of food (palatable ones). The anorectic effect of caffeine is controversial in the literature, and seems to be more remarkable in acute treatment than in a chronic model. Our data are in agreement with previous findings in the literature, since the consumption of standard lab chow was not altered in our model [1]. We observed a reduced consumption of sweet and savory snack food (palatable food) after chronic administration of caffeine, and this effect was also evident when animals were in the fed state; therefore, this is not a homeostatic need. In this study, we did not find an increased consumption of sweet food in stressed rats, as previously observed [11], but we observed an increased consumption of savory food during the habituation trials.

The present study showed that chronic administration of caffeine may diminish consumption of palatable food (sweet and savory snacks), and that tolerance did not occur for this effect, since the rats were treated for at least 40 days before the beginning of the behavioral measurements. Caffeine has been previously utilized in therapies for weight loss, due to its claimed action of amplifying the lipolytic effect of ephedrine [42], a treatment that has several side effects. The effect proposed here is different, since it is not related to lipolysis in the adipose tissue, but to behavior and motivation to eat palatable food. Thus, our results may indicate that caffeine, or an analog of caffeine, may be helpful in therapies for obesity or weight control, but also point to sex-differences, which should be taken into account.

## Acknowledgements

Supported by the National Research Council of Brazil (CNPq), FAPERGS-PRONEX and FINEP/Rede IBN 01.06.0842-00.

## References

- [1] Fredholm BB, Battig K, Holmen J, Nehlig A, Zvartau EE. Actions of caffeine in the brain with special reference to factors that contribute to its widespread use. *Pharmacol Rev* 1999;51(1):83–133.
- [2] Acquas E, Tanda G, Di Chiara G. Differential effects of caffeine on dopamine and acetylcholine transmission in brain areas of drug-naive and caffeine-pretreated rats. *Neuropsychopharmacology* 2002;27(2):182–93.
- [3] Richardson PJ, Kurokawa M. Regulation of neurotransmitter release in basal ganglia by adenosine receptor agonists and antagonists in vitro and in vivo. In: Kase H, Richardson P, Jenner P, editors. *Adenosine antagonists and Parkinson's disease*. Academic Press; 2000. p. 129–48.
- [4] Tremblay A, Masson E, Leduc S, Houde A, Despres JP. Caffeine reduces spontaneous energy intake in men but not in women. *Nutr Res* 1988;8:553–8.
- [5] Racotta IS, Leblanc J, Richard D. The effect of caffeine on food intake in rats: involvement of corticotropin-releasing factor and the sympatho-adrenal system. *Pharmacol Biochem Behav* 1994;48:887–92.
- [6] Comer SD, Haney M, Foltin RW, Fischman MW. Effects of caffeine withdrawal on humans living in a residential laboratory. *Exp Clin Psychopharmacol* 1997;5:399–403.
- [7] Jessen A, Buemann B, Toubro S, Skovgaard IM, Astrup A. The appetite-suppressant effect of nicotine is enhanced by caffeine. *Diab Ob Metab* 2005;7:327–33.
- [8] Carney JM. Effects of caffeine, theophylline and theobromine on scheduled controlled responding in rats. *Br J Pharmacol* 1982;75:451–4.
- [9] Foltin RW, Kelly TH, Fischman MW. Effect of amphetamine on human macronutrient intake. *Physiol Behav* 1995;58:899–907.
- [10] Silveira PP, Portella AK, Clemente Z, Bassani E, Tabajara AS, Gamaro GD, et al. Neonatal handling alters feeding behavior of adult rats. *Physiol Behav* 2004;80:739–45.
- [11] Ely DR, Dapper V, Marasca J, Correa JB, Gamaro GD, Xavier MH, et al. Effect of restraint stress on feeding behavior of rats. *Physiol Behav* 1997;61(3):395–8.
- [12] Silveira PP, Xavier MH, Souza FH, Manoli LP, Rosat RM, Ferreira MB, et al. Interaction between repeated restraint stress and concomitant midazolam administration on sweet food ingestion in rats. *Braz J Med Biol Res* 2000;33(11):1343–50.
- [13] Faraday MM. Rat sex strain differences in response to stress. *Physiol Behav* 2002;75:507–22.
- [14] Kelly SJ, Ostrowski NL, Wilson MA. Gender differences in brain and behavior hormonal and neural bases. *Pharmacol Biochem Behav* 1999;64:655–64.
- [15] Than TT, Delay ER, Maier ME. Sucrose threshold variation during the menstrual cycle. *Physiol Behav* 1994;56:237–9.
- [16] Kornstein SG. Chronic depression in women. *J Clin Psychiatry* 2002;63:602–9.
- [17] Oliver G, Wardle J. Perceived effects of stress on food choice. *Physiol Behav* 1999;66:511–5.
- [18] Silveira PP, da Silva BC, Ayres C, Pederiva FQ, Portella AK, Lucion AB, et al. Satiety assessment in neonatally handled rats. *Behav Brain Res* 2006;173(2):205–10.
- [19] Levin BE, Richard D, Michel C, Servatius R. Differential stress responsivity in diet-induced obese and resistant rats. *Am J Physiol Regul Integr Comp Physiol* 2000;279(4):R1357–1364.
- [20] Pecoraro N, Reyes F, Gomez F, Bhargava A, Dallman MF. Chronic stress promotes palatable feeding, which reduces signs of stress: feedforward and feedback effects of chronic stress. *Endocrinology* 2004;145(8):3754–62.
- [21] Dallman MF, Pecoraro NC, la Fleur SE. Chronic stress and comfort foods: self-medication and abdominal obesity. *Brain Behav Immun* 2005;19(4):275–80.
- [22] Torres SJ, Nowson CA. Relationship between stress, eating behavior, and obesity. *Nutrition* 2007;23(11–12):887–94.
- [23] Halford JC, Blundell JE. Separate systems for serotonin and leptin in appetite control. *Ann Med* 2000;32(3):222–32.
- [24] Krugel U, Schraft T, Regenthal R, Illes P, Kittner H. Purinergic modulation of extracellular glutamate levels in the nucleus accumbens in vivo. *Int J Dev Neurosci* 2004;22(7):565–70.
- [25] Quarta D, Borycz J, Solinas M, Patkar K, Hockemeyer J, Ciruela F, et al. Adenosine receptor-mediated modulation of dopamine release in the nucleus accumbens depends on glutamate neurotransmission and N-methyl-D-aspartate receptor stimulation. *J Neurochem* 2004;91(4):873–80.
- [26] Arolfo MP, Yao L, Gordon AS, Diamond I, Janak PH. Ethanol operant self-administration in rats is regulated by adenosine A2 receptors. *Alcohol Clin Exp Res* 2004;28(9):1308–16.
- [27] Schiffman SS, Gill JM, Diaz C. Methyl xanthines enhance taste: evidence for modulation of taste by adenosine receptor. *Pharmacol Biochem Behav* 1985;22(2):195–203.
- [28] Schiffman SS, Diaz C, Beeker TG. Caffeine intensifies taste of certain sweeteners: role of adenosine receptor. *Pharmacol Biochem Behav* 1986;24(3):429–32.
- [29] Hermanussen M, Garcia AP, Sunder M, Voigt M, Salazar V, Tresguerres JA. Obesity, voracity, and short stature: the impact of glutamate on the regulation of appetite. *Eur J Clin Nutr* 2006;60:25–31.
- [30] Kelley AE, Baldo BA, Pratt WE, Will MJ. Corticostriatal-hypothalamic circuitry and food motivation: Integration of energy, action and reward. *Physiol Behav* 2005;86(5):773–95 15.
- [31] Meister B. Neurotransmitters in key neurons of the hypothalamus that regulate feeding behavior and body weight. *Physiol Behav* 2007;92(1–2):263–71 10.
- [32] Gamaro GD, Prediger ME, Lopes JB, Dalmaz C. Interaction between estradiol replacement and chronic stress on feeding behavior and on serum leptin. *Pharmacol Biochem Behav* 2003;76(2):327–33.
- [33] Boswell KJ, Reid LD, Caffalette CA, Stitt KT, Klein LA, Lacroix AM, et al. Estradiol increases consumption of a chocolate cake mix in female rats. *Pharmacol Biochem Behav* 2006 May;84(1):84–93.
- [34] Diane A, Victoriano M, Fromentin G, Tome D, Larue-Achagiotis C. Acute stress modifies food choice in Wistar male and female rats. *Appetite* 2008;50(2–3):397–407.
- [35] Kant GJ, Lenox RH, Bunnell BN, Mougey EH, Pennington LL, Meyerhoff JL. Comparison of stress response in male and female rats: pituitary cyclic AMP and plasma prolactin, growth hormone and corticosterone. *Psychoneuroendocrinology* 1983;8(4):421–8.
- [36] Mitsushima D, Yamada K, Takase K, Funabashi T, Kimura F. Sex differences in the basolateral amygdala: the extracellular levels of serotonin and dopamine, and their responses to restraint stress in rats. *Eur J Neurosci* 2006;24(11):3245–54.
- [37] McCormick CM, Linkroum W, Sallinen BJ, Miller NW. Peripheral and central sex steroids have differential effects on the HPA axis of male and female rats. *Stress* 2002;5(4):235–47.
- [38] McCormick CM, Roberts D, Kopeikina K, Kelsey JE. Long-lasting, sex- and age-specific effects of social stressors on corticosterone responses to restraint and on locomotor responses to psychostimulants in rats. *Horm Behav* 2005;48(1):64–74.
- [39] Y. Lin, C. Westenbroek, P. Bakker, J. Termeer, A. Liu, X. Li et al. Effects of long-term stress and recovery on the prefrontal cortex and dentate gyrus in male and female rats. *Cereb Cortex*. 2008, Mar 20, in press.
- [40] Luine VN, Beck KD, Bowman RE, Frankfort M, Maclusky NJ. Chronic stress and neural function: accounting for sex and age. *J Neuroendocrinol* 2007;19(10):743–51.
- [41] Di Lorenzo PM, Monroe S. Taste responses in the parabrachial pons of male, female and pregnant rats. *Brain Res Bull* 1989;23(3):219–27.
- [42] Diepvens K, Westerterp KR, Westerterp-Plantinga MS. Obesity and thermogenesis related to the consumption of caffeine, ephedrine, capsaicin, and green tea. *Am J Physiol Regul Integr Comp Physiol* 2007;292(1):R77–85.
- [43] Heusner AA. Size and power in mammals. *J Exp Biol* 1991;160:25–54.