Paper II

Effects of chronic mild stress on sexual behavior, locomotor activity and consumption of sucrose and saccharine solutions

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Abstract

Many symptoms of human depressive disorders are also observed in animals after exposure to unpredictable stressors. The chronic mild stress (CMS) paradigm was developed in order to better model the human situation by using chronic mild stressors over a longer period. It is claimed that the model induces anhedonia in the animals, a core symptom of depression in humans. Despite the fact that the CMS model has a high degree of face validity, there are a number of laboratories in which the establishment of the model is less reliably observed. We have examined behavior (sexual activity and open field activity) together with hedonic measures (sucrose and saccharine intake) after exposure to CMS. CMS decreased male sexual activity (e.g. reduced capability to ejaculate) and increased activity in an open field test. The hedonic measures showed diverging results after CMS in our laboratory. Sucrose consumption was reduced, while saccharine consumption did not show a comparable change. It is concluded that CMS induces comparable alterations to some depression-like symptoms in humans. Saccharine consumption is not a reliable indicator of the hedonic responsiveness to CMS.
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Keywords: Animal model of depression; Chronic mild stress; Sucrose intake; Saccharine intake; Sexual activity; Open field behavior

1. Introduction

A direct or indirect involvement of stress has been suggested in the development of human depression (e.g. [2,5]). In animals, unpredictable stressors have been shown to induce changes in a wide range of behavioral parameters, including changes in locomotor and explorative behavior, impairment of feeding, drinking and sexual behavior [38]. Such behavioral changes are often seen in human psychiatric disorders. A regime of uncontrollable stress has been

used extensively to model the deficits in motivation and reward. In DSM-IV (American Psychiatric Association 1994), anhedonia (loss of interest or pleasure in events that usually would be enjoyed) is defined as a core symptom of depression. Studying the behavioral and rewarding alterations, and the underlying physiological mechanisms in animal models of depression may be useful in obtaining more insight into human depression [3,8].

CMS involves exposure to unpredictable mild stressors over several weeks, designed to mimic the daily hassles that reportedly provoke the onset of depression in humans [2,19]. In the CMS model, the major symptom of human depression, anhedonia, is claimed to be reflected in the animals' decreased consumption of palatable solutions [40]. The intake or preference for sucrose solutions is the hedonic

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measure that has been most widely adopted. However, despite a high degree of face validity of the CMS model, its establishment is not reliably observed [4,21,27,39].

We have earlier reported that a chronic mild stress procedure in rats induced a decrease in sucrose intake per unit body weight, while sucrose intake in a non-stressed control group did not change [14]. The largest effect was obtained after 2 weeks of the stress protocol, and was attenuated thereafter. Also, CMS produced changes in both the structure and the continuity of sleep consistent with sleep abnormalities reported in depressed patients [14].

The main purpose of the present study was to explore whether CMS protocol could lead to other relevant behavioral changes. A decreased sexual drive is often seen in depressed patients. Also, a decrease or increases in spontaneous motor activity (e.g. psychomotor retardation or agitation) are common symptoms of depression in humans. Therefore, we expected that sexual behavior would be diminished and spontaneous activity would be altered in an animal model of depression. D' Aquila et al. [6] found a decreased sucrose intake together with a decreased sexual behavior in CMS rats. However, their animals were not screened for eventual non-copulators. The rats were interacting for the first time and only the mounting behavior was analyzed. In the present study, each rat underwent mating tests to screen for any non-copulators, and copulatory behavior was analyzed in greater detail for 30 min. One of the most frequently used and accepted variable for measure emotionality in rodents is to measure their locomotion in a novel field [13,28]. We used an open-field test to measure the locomotor activity to estimate if the CMS procedure affected the emotionality.

Another objective of the present study was to replicate in our laboratory the decreased sucrose consumption [14] and to test consumption of a different palatable solution. Sucrose and saccharine intake are both commonly accepted measures of anhedonia, and many investigators have reported that consumption is inhibited by CMS [23,25,26,40]. To our knowledge, this is the first study testing both sweet solutions in the same animals.

2. Materials and methods

2.1. Ethical evaluation

The experiment described in this article has been approved by the Norwegian Animal Research Authority and registered by the Authority.

2.2. Animal handling

Sprague–Dawley (Mol:SPD) rats (Møllegaard, Copenhagen, Denmark), 42 male and 24 females, were used in this experiment. On arrival, there were five animals in each transport cage. To minimize stress, they were allowed to

remain in the transport cage for 5 days before the males were separated and housed individually in conventional Macrolon type III cages. Females remained housed together in conventional Macrolon type IV cages in a separate room. The home cages were placed in a rack allowing visual, olfactory and auditory contact between animals.

The rats had free access to food (Rodent low protein diet, B and K Universal AS, Norway) and water, except when the CMS procedure required deprivation. Total food intake was not measured. The ambient temperature was 22 ± 1 °C with $52 \pm 2\%$ humidity. Male and female rats were kept on a reversed, controlled 12-h light/12-h dark schedule with gradually increasing lighting from 1800 h and lights fully on at 1900 for 10 days before the start of the experiment. Five to seven days have been seen to be a sufficient time period for the synchronization of spontaneous locomotor activity with a new circadian rhythm in male SPD rats [17].

2.3. Grouping

Male rats were divided into two main groups. The experimental group was exposed to chronic mild stress, whereas the control group was given ordinary daily care and housed separately in a different room. A subgroup of each main group was tested for sexual behavior. Thus, there were four groups in total: two control groups, tested/not tested for sexual activity and two CMS groups, tested/not tested for sexual activity. Before the start of the experiment, the groups and subgroups had similar levels of both sucrose intake and sexual behavior. All rats were tested for the open field activity.

2.4. Stress procedure

The CMS procedure (Fig. 1, bottom) was adapted from the procedure described by Willner and collaborators [40] with the addition of some stressors from Moreau and collaborators, e.g. empty bottle of water, restricted food [24], see Gronli et al. [14]. Each week consisted of one period (2 h) of paired caging, one period (3 h) of tilted cage (45°), one period of food deprivation (18 h) immediately followed by 1 h of restricted access to food (5 micropellets), two periods of water deprivation (18 h) immediately followed by 1 h exposure to an empty bottle, one 21 h period with wet cage (200 ml water in 100 g sawdust bedding) and one period with 36 h of continuous light. Thus, stressors were presented both during the rats' active (dark) period and during the inactive (light) period. Control animals were left undisturbed in their room and home cages.

2.5. Sexual behavior

The female rats used in the mating test were of the same strain as the males. They were ovariectomized at least 2 weeks before the test and brought into oestrus by subcuta-

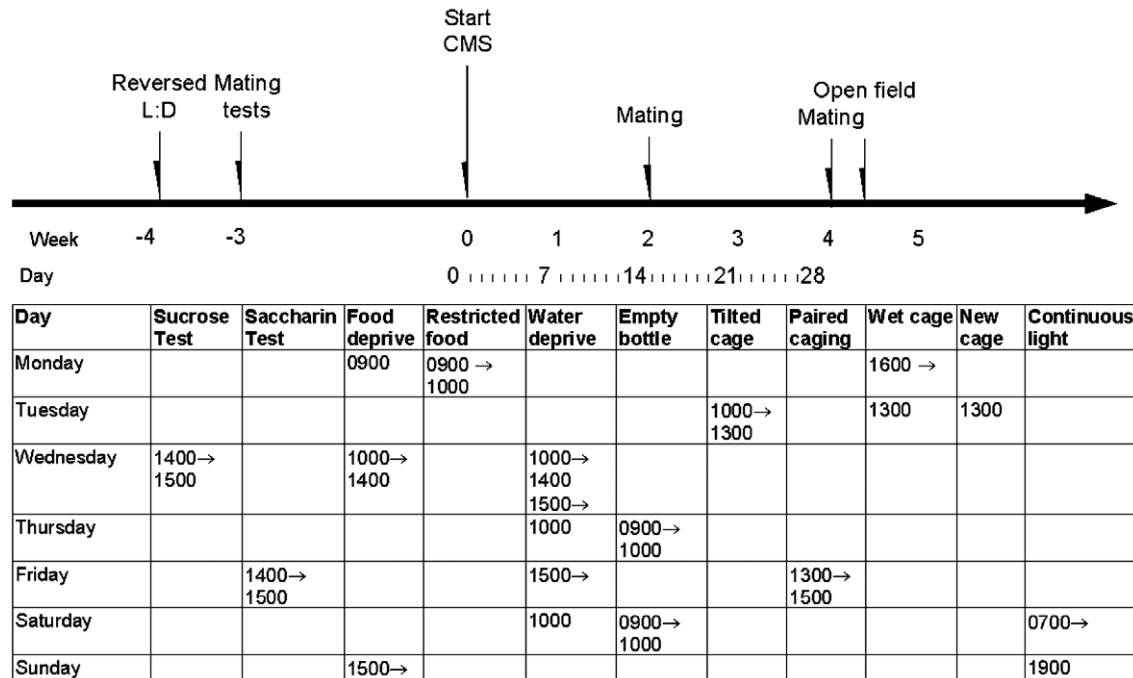| Day	Sucrose Test	Saccharin Test	Food deprive	Restricted food	Water deprive	Empty bottle	Tilted cage	Paired caging	Wet cage	New cage	Continuous light
Monday			0900	0900 → 1000					1600 →		
Tuesday							1000→ 1300		1300	1300	
Wednesday	1400→ 1500		1000→ 1400		1000→ 1400 1500→						
Thursday					1000	0900→ 1000					
Friday		1400→ 1500			1500→			1300→ 1500			
Saturday					1000	0900→ 1000					0700→
Sunday			1500→								1900

Fig. 1. An overview over the design for the experiment (top: with time course for CMS and post-CMS tests; bottom: CMS protocol).

neous injections of oestradiol benzoate (200 μg/rat in oil) and progesterone (0.5 mg/rat in oil), 48 and 6 h before the mating test, respectively.

Mating tests were carried out during the dark phase, the first rats starting 2 h into the dark phase. The room was lit by a dim red light. Food and water were removed from the home cage. Pre-experimentally, each male rat underwent 3 mating tests with a female in oestrus to identify any apparent non-copulator. A female was introduced into the home cage of the male and the behavior recorded by a video camera. The males were included in the main experiment if they had a total of three ejaculations during the training. Four males were identified as non-copulators and excluded from the experiment. The following measures of copulatory behavior were recorded online on an event recorder (Embla, Flaga, Iceland) for 30 min: *Mounting latency*: Time (sec) elapsed between introducing the female into the same cage with the male rat and the first mounting trial without intromission of the penis into the vagina. *Intromission latency*: Time (s) elapsed between introducing the female rat into the male cage and the first penetration of the penis into the vagina. *Ejaculation latency*: Time (s) elapsed between the first penetration and ejaculation. *Mount frequency*: Total number of mounts in the 30 min test. *Intromission frequency*: Total number of intromissions in the test. *Ejaculation frequency*: Number of ejaculations during the test period. Since sexually experienced rats may begin copulation with intromission, values for mount latency are given equal to the intromission latency. For rats that did not ejaculate during the test session, the latency to ejaculate is given as 30 min.

2.6. Open field test

The open field consisted of a base (100×100 cm) and black walls (20 cm) divided into 25 (5×5) identical sectors (20×20 cm) by white stripes. The squares were subdivided into peripheral and central sector, where the central sector included the 9 central squares (3×3) and the peripheral sector contained the squares close to the wall. The animals were placed in the central sector and their activity recorded for 6 min by a video camera and taped for further analysis. The open field arena was thoroughly cleaned between each test. The room was lit by a dim red light. Experiments were performed 2 h into the dark phase and no stressor was applied to the animals for at least 12 h before the test.

Open field activity was scored manually from a TV screen on an event recorder (Somnologica, Version 2.0.2, Flaga, Iceland). Motility was scored when an animal crossed a sector border with both its hind-limbs. The following activities were scored: *Peripheral activity*: the number of squares crossed in the peripheral sector. *Central activity*: number of central squares crossed. *Total activity*: the overall activity, in both peripheral and central areas during the 6 min test. *First minute activity*: the total activity in the first minute of the test.

2.7. Sucrose intake, saccharine intake and body weight

Sucrose intake (1% sucrose solution) and saccharine intake (0.1% saccharine solution) were measured once a week, on separate days, during a 1-h window after 4 h of food and water deprivation. Consumption was measured by comparing bottle weight before and after the 1-h window.

The intake was expressed in relation to the animals' body weight (g/kg). Baseline was measured less than 1 week before the start of CMS. The food and water deprivation period preceding sucrose/saccharin intake measurement may be considered as a further stress applied on top of the CMS protocol. However, control rats were also exposed to the food and water deprivation, as a part of the test.

2.8. Statistics

Statistica 5.0 (StatSoft, Inc.) was used for all statistical analysis.

Sucrose intake, saccharine intake and body weight: Analysis of Variance (ANOVA) for repeated measures was performed, with group (CMS or Control) as independent factor and time as the repeated measure. Subsequently, the effect of CMS or control procedures on sucrose intake over days was analyzed with one-way repeated measure ANOVA. Any difference between baseline consumption and day of CMS or control treatment was assessed by multiple comparisons performed by least significant deviation (LSD) post hoc test. Preliminary analyses indicated that, within each condition (CMS and Control), initial testing for sexual activity had no effects on sucrose or saccharine consumption. In subsequent analyses, therefore, the sexually tested and non-tested animals were combined.

Sexual activity and open field data: The non-parametric Friedman ANOVA was used.

One-tailed tests were used in cases where there were clear experimental hypotheses (e.g. reduced sexual activity; see Introduction). Otherwise, significance was accepted at $p < 0.05$, two-tailed.
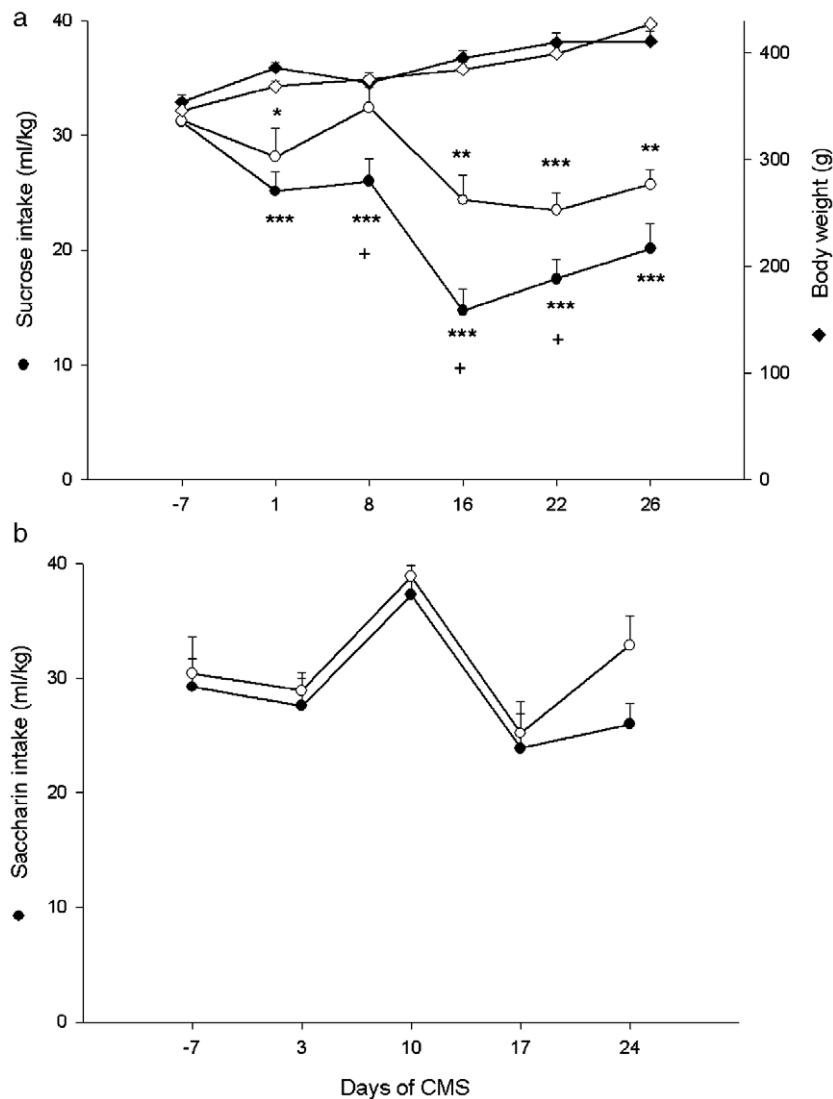

Fig. 2. Sucrose intake (ml/kg) and body weight (g) (a) and saccharine intake (ml/kg) (b) during the CMS period. Circles indicate saccharin or sucrose intake and diamonds indicate bodyweight. Open symbols indicate Control rats and filled symbols CMS rats. Results are presented as mean±S.E.M. *Indicates $p < 0.05$, **$p < 0.01$, ***$p < 0.001$ compared to their own baseline. [+]Indicates $p < 0.05$ compared to the Control group.

3. Results

3.1. Sucrose intake and bodyweight

Sucrose consumption in Control and CMS rats is shown in Fig. 2a. Chronic mild stress reduced sucrose intake. Tests of main effects for the period of CMS procedure (Day −7 to Day 26) showed differences between the two groups of animals ($F_{(1,34)}$=4.86, p=0.034). There was no significant interaction (group×day).

CMS animals showed a decreased sucrose intake over days ($F_{(5,85)}$=19.39, p<0.001). Also, Control rats reduced sucrose intake ($F_{(5,85)}$=8.65, p<0.001).

There was no difference of bodyweight between the two groups ($F_{(1,34)}$<1; Fig. 2a).

3.2. Saccharin intake

Saccharine consumption in Control and CMS rats is shown in Fig. 2b. Chronic mild stress did not change saccharine intake. Tests of main effects for the period of CMS procedure (Day −7 to Day 22) showed neither a difference between the two groups of animals nor an interaction between group and day, both F's $_{(1,34), (4,136)}$<1.

3.3. Sexual activity

After 2 weeks of CMS, the parameters of sexual activity changed compared to the control condition (Table 1). Both the latency to intromission ($\chi^2_{(10,1)}$=3.6, p=0.029) and latency to ejaculate ($\chi^2_{(10,1)}$=2.9, p=0.044), and the frequency of mounts ($\chi^2_{(10,1)}$=2.8, p=0.048) and ejaculations ($\chi^2_{(10,1)}$=2.7, p=0.049) were reduced in the CMS animals.

After 4 weeks of CMS, an effect on the latency to intromission ($\chi^2_{(10,1)}$=2.8, p=0.048) and ejaculation ($\chi^2_{(10,1)}$=2.7, p=0.049) was still present. There was a strong trend towards

Table 1
Latency and frequency of the different sexual activity parameters in Control rats and CMS rats: after 2 and 4 weeks exposure to Control condition or CMS

	Control (n=10)		CMS (n=10)	
	After 2 weeks	After 4 weeks	After 2 weeks	After 4 weeks
Latency				
Mount (s)	91±26	44±11	86±30	90±31
Intromission (s)	137±37	78±8	685±266*	644±287*
Ejaculation (s)	607±95	630±103	1079±221*	1076±210*
Frequency				
Mount	6±1	12±2	9±1*	15±3
Intromission	40±4	38±3	28±9	31±8
Ejaculation	2.3±0.2	2.1±0.3	1.4±0.4*	1.4±0.4

Values are expressed as means±S.E.M.
 * p<0.05, one-tailed t-tests compared to Control rats.

Table 2
Open field activity in Control rats and CMS rats after 4 weeks exposure to Control condition or CMS

	Control (n=20)	CMS (n=20)
Center activity	28±2	36±3
Peripheral activity	119±5	125±5
Total activity	140±4	161±5**
First minute activity	18±1	20±1*

Values are expressed as number of squares crossed in each sector. Values are expressed as means±S.E.M.
 * p<0.05 compared to Control rats.
 ** p<0.01 compared to Control rats.

decreased frequency of ejaculations ($\chi^2_{(10,1)}$=2.4, p=0.059) (Table 1).

3.4. Open field behavior

The CMS rats showed a higher total activity ($\chi^2_{(19,1)}$= 11.8, p<0.001) than Control animals. Locomotor activity was especially high during the first minute ($\chi^2_{(20,1)}$=5.0, p=0.025) of the 6 min recorded. There was a tendency to higher activity in the center squares ($\chi^2_{(20,1)}$=2.6, p=0.08) (Table 2).

4. Discussion

Chronic mild stress reduced male sexual activity and changed locomotor activity compared to control rats, indicating a behavioral consequence of CMS as predicted for an animal model of depression. Consumption of sweet solutions showed diverging results. CMS decreased sucrose intake per unit body weight, but did not affect saccharine intake.

The present experiment demonstrates that exposure to chronic mild stress decreases male sexual activity. CMS males had longer latencies to intromission and ejaculation, and achieved fewer ejaculations compared to control rats. These findings support CMS as an animal model of depression. However, the individual variability was high, especially within the CMS group, suggesting variations in the rats' response to CMS. Decreases in the number of animals achieving ejaculation and an increase in the intromission frequency or in the ejaculation latency are considered as signs of weakening of sexual activity [32]. Impairments in sexual activity have been found in other animal models of depression as well, e.g. Flinders sensitive and resistant line rats [12] and clomipramine treated rats [34]. In D'Aquila et al.'s CMS study [6], the sexual naivety of the experimental rats meant that sexual activity never proceeded beyond mounting, and the effects of CMS on the intromission and ejaculation activity were not evaluated.

When the rats were placed in a novel environment, an increase in spontaneous activity in the CMS rats was seen, especially in the first minute of the test. In addition, the rats tended to have higher activity in the center squares in the

open field. This may represent a model of the psychomotor agitation observed in some depressed humans. These results could also be interpreted as a possible increased response to the novelty of the test apparatus in the CMS rats. Low activity in an open field test has been used as an index of high emotionality in rats [18,31,35], but not all agree with this. Denenberg [9] showed that the Day 1 activity was uniquely different from the remaining days. On Day 1, high activity indicated high emotionality, while activity thereafter was indicative of low emotionality. Effects of CMS on Day 1 activity in an open field test are diverging. Harris et al. [16] showed more activity in the open field after CMS while others have shown enhanced locomotor activity only during the first minute, but an unchanged total activity [11], and even reduced activity [7]. Other stress induced changes in open field behavior also show divergent results [18,22,36]. Pijlman et al. [30] have shown that physical stress (repeated mild foot shocks) caused inactivity while emotional stress (witness to the foot shocks) caused hyperactivity. We only recorded the rats' activity in the open field on 1 day and we interpret the findings as indicative that the CMS model increases the emotionality in rats.

The criticisms of the CMS model have often focused on the inadequacy of the method to consistently produce a stress-induced anhedonia. Strekalova and collaborators [33] recently suggested that, chronic stress induces anhedonia in most animals (61% anhedonic subgroup) whereas fails to do so in the remaining animals (39% non-anhedonic subgroup). The authors argue that the predisposition for the stress-induced anhedonia is indicated by submissive behavior in a previous resident–intruder test. The difference suggested by Strekalova and collaborators may also be present in our CMS studies as suggested by the high inter-individual variability found in the CMS rats [14]. We interpret such variability as reflecting the individual appraisal and coping with the situation.

A maximum reduction of sucrose consumption was seen after 2 weeks exposure to the CMS protocol in both the present study and in our earlier study. However, the temporal aspects of the effect were not consistent between the two studies. In the present study, the maximum reduction in intake occurred after 2 weeks and the sucrose intake remained reduced throughout the 4 weeks of CMS exposure. In our earlier study, the largest effect was obtained after 2 weeks of the stress protocol, and then the effect was attenuated [14]. Although there was a significant difference on the sucrose consumption between CMS rats and Controls, we also saw variations in the sucrose consumption of Control animals that paralleled CMS rats' decrease (in particular a large drop during week 2–4 was seen in the present study). There is no obvious explanation to such spontaneous fluctuations, which also have been observed by others [21].

Reduced consumption of sucrose or saccharine solutions by CMS rats has been used as a commonly accepted measure of anhedonia, both are considered to have similar rewarding values [23,25,26,29,40]. To our knowledge, ours is the first study testing both sweet solutions in the same animals. In the present study, the sucrose intake (1% solution) was reduced while the non-nutritive saccharine intake (0.1% solution) was not. This is not in line with the above findings and suggestions [23,40]. The reason for this is not clear. Reduction of saccharine intake has been observed after several stressors, ranging from simple restraint to stress protocols lasting weeks [10]. Considering results from animal and human studies [15,20,37], one may hypothesize that depressive symptoms are predictive of lowered hedonic response to sweet stimuli. Contrary to this prediction, depressed patients gave similar pleasantness ratings to water and diluted sucrose solution as did a control group [1]. In terms of behavioral preference in rats, a 0.15% saccharine solution has been shown to be equivalent to a 1% sucrose solution [41]. It has been demonstrated that a saccharine reduction first appeared if the period of water deprivation was longer than 24 h preceding the test, and there was no effect when the rats were only mildly water deprived [16]. The latter finding might explain the lack of saccharine reduction in CMS animals.

In conclusion, the CMS protocol used in our laboratory changed the sexual and locomotor behavior in rats. The CMS effects on hedonic measures were divergent: CMS reduced sucrose consumption but had no effect on saccharine intake. This finding suggests that sucrose and saccharine consumption is not equally inhibited by CMS, and that saccharine consumption is not a reliable indicator of the hedonic responsiveness to CMS.

Acknowledgements

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