

# Paper I

Research report

## Chronic mild stress affects sucrose intake and sleep in rats

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### Abstract

Depression in humans is associated with sleep abnormalities of three types: altered rapid eye movement (REM) sleep, fragmented sleep, and reduced delta sleep. In an animal model of depression, chronic exposure to mild stressors (CMS, e.g. periods of soiled cage, reversed light/dark cycle, grouped housing, food and/or water deprivation) causes behavioral and hormonal changes which, in humans, often are associated with depression. In the CMS model, a reduced sucrose intake has been defined as one of the core symptoms of depression, anhedonia, although this finding is not consistent among various laboratories. In the present study, we investigated if the CMS procedure, in our laboratory, would cause decreased sucrose intake and, also, give sleep changes similar to what is found in depressed patients.

Exposure to CMS decreased sucrose intake in our rats. The largest effect was obtained after 2 weeks of the stress protocol. CMS rats spent more time in REM sleep and showed more fragmented sleep compared to their baseline recording, while there were no changes in the control rats. Increased sleep fragmentation in CMS rats was particularly evident by increased number of arousals, and increased REM sleep and slow-wave-sleep-1 (SWS-1) episodes. The duration of sleep stage episodes was decreased. The amount of slow-wave-sleep-2 (SWS-2) was not decreased, however SWS-2 in percent of total SWS was reduced. Correlation analysis showed that animals that had less consumption of sucrose spent more time in REM sleep and had increased number of REM sleep episodes. In this study, CMS appears to be a model of depression.

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**Keywords:** Animal model of depression; Chronic mild stress; Sucrose intake; REM sleep; Corticosterone

### 1. Introduction

To provide a realistic analogue of the strains of everyday life Willner et al. [48] developed, based on Katz's procedure [23], the chronic mild stress (CMS) animal model of depression. In this model, rats are exposed sequentially to a variety of stressors. The procedure has been shown to produce several behavioral and hormonal disturbances which parallel to a large extent those found in depressed humans (see [47] for review). The core symptom is a reduction of sucrose intake, a symptom Willner et al. [48] equals to anhedonia, one of the core symptoms of depression as defined in the DSM-IV (American Psychiatric Association, 1994).

However, the validity of the CMS model has been questioned [17,19,20,27] because a decrease in sucrose consumption is not consistently observed following the stress procedure among various laboratories. Many attempts to develop and/or replicate the original model have failed (in a variety of laboratories). Stress-induced decrease in sucrose consumption varies between and/or within experiments, among laboratories, with animal strain used, according to a specific procedure, etc. [30].

The main purpose of this study was to test whether the CMS procedure when performed in our laboratory would induce anhedonia, defined as decreased sucrose intake. We also wanted to see whether the model would affect sleep and corticosterone level, two parameters commonly affected in human depression.

As many as 90% of patients with major depression will display some electroencephalogram (EEG) verifiable sleep disruption [6,16,37]. Polysomnographic recordings

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demonstrate three main types of sleep abnormalities in depressed patients: changes in rapid eye movement (REM) sleep (increased amount, higher frequency, shortened REM sleep latency), increased sleep fragmentation (more frequent stage shifts) and reduced delta sleep [5,24,25]. Alterations in the sleep cycle similar to those observed in depressed patients have been found in different animal models of depression, e.g. clomipramine-treated neonatal rats [44], prenatally stressed rats [14] and inbred stress-sensitive strains [15,40]. Disruption of REM sleep was also shown in chronically stressed rats [11,32]. However, these studies did not address some important issues. Moreau et al. [32] did not monitor muscle tone during sleep EEG recording, a measure that most researchers consider necessary to identify the occurrence of REM sleep. In addition, sucrose intake, the core measurement for the evaluation of the CMS model, was not measured in any of these studies.

The physiological response to depression includes an increased activity of the hypothalamic–pituitary–adrenal (HPA) axis (see [10] for review). Previous studies have shown that the inhibition of HPA axis by circulating glucocorticoids is impaired both in CMS rats [3] and in depressed patients [29,36].

Therefore, if CMS represents an animal model of depressive behavior, reduced sucrose intake should be associated with sleep alteration and, possibly, a change in HPA activity. In particular, we hypothesized that CMS would increase REM sleep, decrease REM latency, increase fragmentation (measured as increased stage shifts), reduce deep slow-wave-sleep and increase corticosterone level.

## 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

Male Sprague–Dawley (Mol:SPRD) rats (Møllegaard, Copenhagen, Denmark) 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 they were separated and housed individually in conventional Macrolon type III cages. The home cages were placed in a rack allowing visual, olfactory and auditory contact between animals, except during the adaptation to the sleep recording and the recording days. During these days the animals remained in their home cages which were placed inside the recording chambers.

The rats were kept on a controlled 12-h/12-h light/dark schedule with the lights on at 06:00 h and an ambient tem-

perature of  $22 \pm 1$  °C. They had free access to food (Rodent low protein diet, B&K Universal AS, Norway) and water, except when the CMS procedure required deprivation. Total food intake was not measured.

### 2.3. Surgery

Nine weeks old rats, weighing approximately 300 g, anesthetized by subcutaneously (s.c.) injection of a mixture of fentanyl, 0.05 mg/ml, fluanizone, 2.5 mg/ml, and midazolam, 1.25 mg/ml (Hypnorm, Janssen; Dormicum, Roche). The rats were implanted with stainless steel screw electrodes for bilateral fronto-frontal (FF) and fronto-parietal (FP) EEG recording and silver wires in the neck muscle for electromyogram (EMG) recording. The frontal screw electrodes were placed epidurally 2 mm anterior to bregma and 2 mm lateral to the midline, and the parietal screw electrodes were placed 2 mm anterior to lambda and 2 mm lateral to the midline [43]. All the electrodes were connected to a socket, which was secured to the skull with dental cement (Paladur, Kulzer & Co., Germany). Following surgery all animals received analgesic doses of buprenorphinum (Temgesic, Reckitt & Colman) (0.15 ml s.c.) twice a day for 3 days. At least 2 weeks were allowed for recovery and adaptation prior to start of experiments.

### 2.4. Design

Fig. 1 gives an overview of the design. Following an initial measurement of sucrose intake (described below), the animals were divided into two groups having similar average intake. One group was given ordinary daily care (control rats,  $n = 7$ ) and the other group was exposed to CMS ( $n = 7$ ). The two groups of rats were housed separately in different rooms during the duration of the stress procedure (see below). Sucrose intake was measured once a week. Baseline sleep was recorded in both groups before the CMS procedure started. Sleep was recorded again after 4 weeks of CMS/control condition. Following this, blood samples were taken for corticosterone analysis.

### 2.5. Stress procedure

The CMS procedure (Fig. 1, bottom) was adapted from the procedure described by Willner et al. [48], and some stressors were included from Moreau et al. (e.g. empty bottle of water, restricted food) [31]. 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 (five 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. Clock time and duration of the procedures are shown in Fig. 1. Thus, stressors were presented both during the rats' active

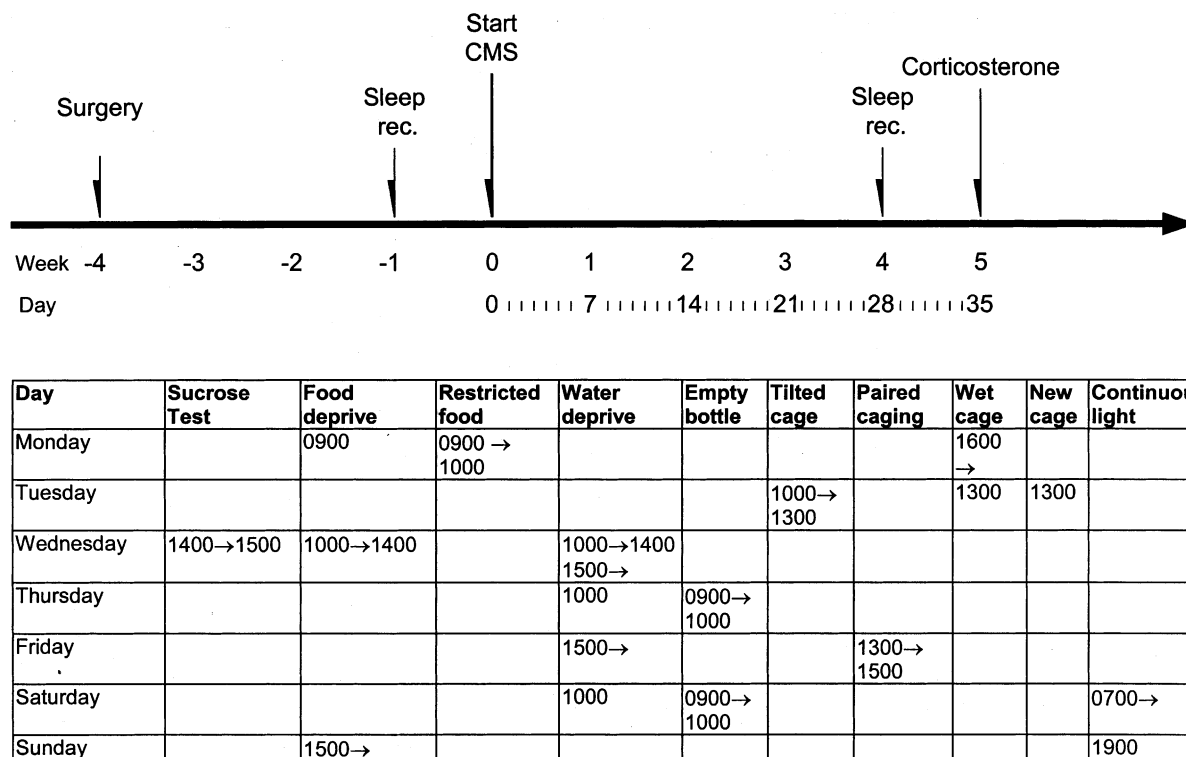


Fig. 1. An overview over the experimental design (top: with time course for CMS and post-CMS tests; bottom: CMS protocol).

(dark) period and during the inactive (light) period. Control animals were left undisturbed in their room and home cages.

### 2.6. Sucrose intake and body weight

Sucrose intake (1% sucrose solution) was measured once a week (on days 3, 10, 17, 24, and 28), during a 1-h window after 4 h of food and water deprivation. Sucrose consumption was measured by comparing bottle weight before and after the 1-h window. The intake was expressed in relation to the animal's body weight (g/kg). Baseline was measured 5 days before the start of CMS. The food and water deprivation period preceding sucrose 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 sucrose test.

### 2.7. Sleep recording conditions

The animals were adapted to the recording conditions for at least 6 h, during each of the 3 days preceding sleep recording and were not exposed to stress the day before recording. During sleep recordings the animals remained in their home cages which were placed into sound attenuated recording chambers (430 mm × 280 mm × 620 mm) with constant light (15 W electric bulb) and ventilation. They had free access to food and water. The ambient temperature inside the chambers was around 23–25 °C during baseline recording and 21–24 °C during recording after 4 weeks. Circumstances

beyond our control made the ambient temperature differ between recordings. Conditions were the same for control and experimental animals. Free movement of the animal was allowed by using a flexible recording cable linked to an electrical swivel (Alice King Chatham, Medical Arts, USA) fixed to a movable arm outside the chamber. A servomotor was connected to the swivel to allow easier rotation of the cable. The recording started at 08:00 h and lasted for a period of 8 h during which the animals were not disturbed.

### 2.8. Sleep recording and scoring

FF EEG, FP EEG, and neck muscle EMG were recorded and digitized at a sampling frequency of 100 and 200 Hz, respectively (EMBLA, Flaga, Iceland). For visual display, the FF EEG was high-pass frequency filtered at 3 Hz (3 dB/octave) and low-pass filtered at 35 Hz (3 dB/octave). The FP EEG was filtered at 1 and 35 Hz, respectively. The EMG signal was filtered with high-pass at 5 Hz (3 dB/octave). All signals were filtered at 50 Hz to eliminate powerline artefacts.

Sleep and waking were scored manually in 10 s epochs with support from power spectrum analysis from a sleep-dedicated software (Somnologica, Version 2.0.2, Flaga, Iceland). The following stages were scored: waking (W) with high frequency, low voltage activity in the EEG, and high/moderate activity in EMG; slow-wave-sleep-1 (SWS-1) with 11–16 cps spindles and <50% delta (0, 5–4 cps) wave activity per epoch; slow-wave-sleep-2

(SWS-2) with spindles and more than 50% delta activity; REM sleep with fronto-occipital theta activity and low neck muscle EMG [33,43]. The number of sleep episodes (expressing sleep fragmentation), duration and latency for each stage were computed using Somnologica. Latencies to SWS-2 and REM sleep were scored from the onset of sleep to the first epoch of that sleep stage. If rats were asleep when sleep recording started, sleep onset was set at 08:00 h.

### 2.9. Corticosterone measurements

On the sampling day (see Fig. 1), blood samples were taken from all animals between 09:00 and 12:00 h. Animals were transported to the sampling laboratory, removed from their home cages and placed in an anesthetic chamber. Anesthesia was induced with Isofluran (Isofluran Baxter, Norway). After muscle relaxation, the animal was removed from the chamber and placed in a dorsal position and a controlled amount of anesthetic gas was given through a mask covering mouth and nose. A 2–3 cm lateral incision was made on the right side of the throat, exposing the jugular vein. A 1 ml blood sample was collected using a 1 ml syringe with a 23 G needle. To be sure that anesthesia did not activate the HPA activity, the time used from moving the animals from home cages to completion of blood collection was less than 3 min. Cuts were closed by suture thread, and the animals placed in their home cages under supervision until full recovery from anesthesia. Samples were centrifuged at 3000 rpm for 20 min, and the plasma drawn off and frozen at  $-20^{\circ}\text{C}$  for later radioimmunoassay analysis of corticosterone (Count-A-Coat, Diagnostic Products Corporation, CA).

### 2.10. Statistics

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

Sucrose intake and body weight: ANOVA for repeated measures was performed on sucrose intake and body weight, with group (CMS or control) as independent factor and time as repeated measure. Subsequently, the effect of CMS or control treatment on sucrose intake over days was further analyzed with one-way repeated measure ANOVA. Difference between baseline consumption and day of CMS or control treatment was assessed by multiple comparison performed by least square deviation (LSD) post hoc *t*-test.

Sleep and waking stages: sleep data were entered into a four-way overall ANOVA for repeated measures, with group as independent factor and ‘recording day’ (baseline and post-CMS), ‘sleep stage’ and ‘2-h period’ as repeated measures. Subsequently, effects of recording day were further analyzed with within-group ANOVAs (recording day  $\times$  stage  $\times$  2-h period and stage  $\times$  2-h period, respectively). Differences between 2-h periods were assessed by Student’s *t*-test. Difference in latency to sleep stages between recording days was assessed by Student’s *t*-test. Fragmentation

and duration of sleep episodes were analyzed by two-way ANOVA (recording day  $\times$  number of episodes), difference between recording days was assessed by Student’s *t*-test.

Analysis of corticosterone measure was performed by means of Student’s *t*-test for independent groups.

Correlation: relationships between sucrose intake and different sleep parameters as well as corticosterone were assessed by Pearson’s correlation analysis. Correlation analyses were performed on pooled data, from both control and CMS rats ( $n = 14$ ).

One-tailed probability values were used in cases where there were strong experimental hypotheses (e.g. increased REM sleep; see Section 1). Otherwise, significance was accepted at  $P < 0.05$ , two-tailed.

## 3. Results

### 3.1. Sucrose intake and body weight

Sucrose consumption and body weight in control rats and CMS rats are shown in Fig. 2. CMS decreased sucrose intake. Tests of main effects for the period of CMS procedure (days 3–36) showed a difference between the two groups of animals ( $F_{(1,12)} = 5.54$ ,  $P = 0.036$ ). There was a strong tendency for interaction between group and day ( $F_{(5,60)} = 2.11$ ,  $P = 0.077$ ).

The rat’s body weight was also measured at the same time as sucrose intake. There was no effect on body weight by CMS ( $F_{(1,12)} = 1.44$ ,  $P = 0.253$ ; Fig. 2).

#### 3.1.1. CMS group

There was a clear effect of the CMS procedure on the sucrose intake ( $F_{(5,30)} = 26.18$ ,  $P < 0.0001$ ). Post hoc *t*-tests showed reductions in sucrose intake throughout the CMS procedure compare to baseline, day  $-5$  ( $P < 0.05$ ; see Fig. 2). The largest effect was obtained on days 3, 10, and 17 ( $P < 0.0001$ ), and the sum of these three measurements was defined as the Max Intake Response. After the second week, this effect was attenuated.

#### 3.1.2. Control group

No effect of day was found for sucrose intake ( $F_{(5,30)} = 1.18$ ,  $P = 0.343$ ).

### 3.2. Sleep and waking stages

An overall ANOVA (group  $\times$  recording day  $\times$  stage  $\times$  2-h period) showed an effect of group (CMS or control treatment) ( $F_{(1,12)} = 6.92$ ,  $P = 0.022$ ). There were significant interactions between recording day and stage ( $F_{(3,36)} = 13.10$ ,  $P < 0.0001$ ), between recording day, stage and 2-h period ( $F_{(9,108)} = 2.01$ ,  $P = 0.045$ ) and a trend towards an interaction between group, recording day and 2 h per ( $F_{(3,36)} = 1.99$ ,  $P = 0.100$ ). Table 1 shows total amount of

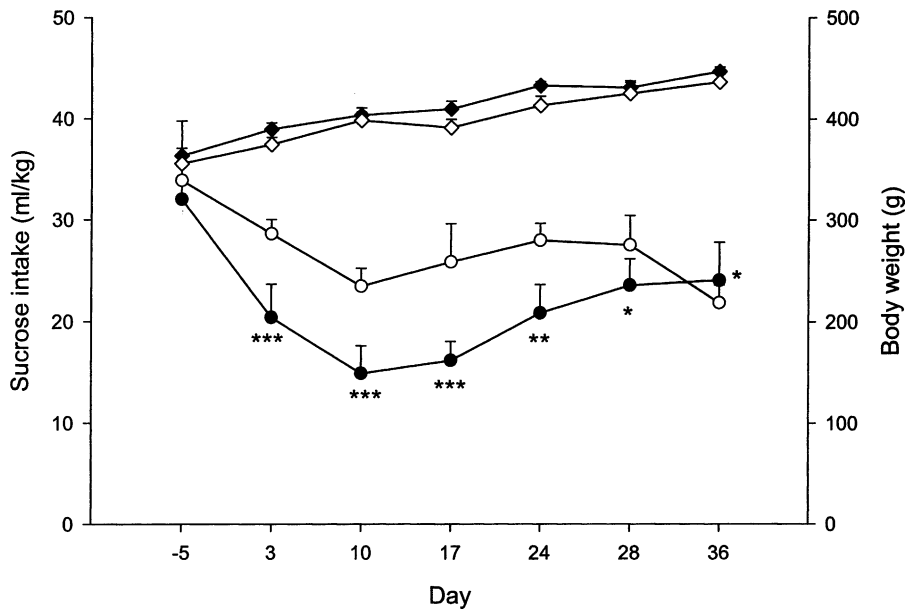


Fig. 2. Sucrose intake (ml/kg) and body weight (g). Circles indicate sucrose intake and diamonds indicate body weight. Open symbols indicate control rats and filled symbols CMS rats. Results are presented as mean  $\pm$  S.E.M. \*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$  compared to baseline recording (day -5).

sleep stages in the CMS and control condition. Fig. 3 shows changes in the different sleep stages.

### 3.2.1. CMS group

ANOVA (recording day  $\times$  stage  $\times$  2-h period) showed an effect of recording day (baseline and post-CMS;  $F_{(1,6)} = 6.08$ ,  $P = 0.049$ ). There were interactions between record-

ing day and stage ( $F_{(3,18)} = 11.08$ ,  $P < 0.001$ ), recording day and 2-h period ( $F_{(3,18)} = 3.90$ ,  $P = 0.026$ ) and stage and 2-h period ( $F_{(3,18)} = 3.52$ ,  $P = 0.002$ ). Waking was decreased in the post-CMS recording compared to baseline ( $F_{(1,6)} = 18.78$ ,  $P = 0.005$ ), and SWS-1 was increased ( $F_{(1,6)} = 20.54$ ,  $P = 0.004$ ). The amount of SWS-2 did not change significantly ( $F_{(1,6)} = 2.26$ ,  $P = 0.183$ ).

Table 1

Sleep parameters in control rats and CMS rats: baseline recording (before) and after 4-week exposure to control condition or CMS (after)

	Control		CMS	
	Before	After	Before	After
<b>Sleep stage (total amount)</b>				
Wakefulness	224.71 $\pm$ 13.55	166.00 $\pm$ 13.64*	219.98 $\pm$ 10.08	163.08 $\pm$ 15.43**
SWS-1	98.52 $\pm$ 14.12	142.29 $\pm$ 19.32	89.19 $\pm$ 5.84	154.95 $\pm$ 15.15**
SWS-2	137.93 $\pm$ 11.70	149.07 $\pm$ 26.14	144.43 $\pm$ 9.09	115.36 $\pm$ 17.38
REM	15.00 $\pm$ 3.37	15.83 $\pm$ 3.73	20.16 $\pm$ 3.33	29.67 $\pm$ 3.78*
<b>Fragmentation (number of episodes)</b>				
Wakefulness	80.57 $\pm$ 3.99	78.57 $\pm$ 17.16	75.29 $\pm$ 7.30	105.43 $\pm$ 15.31*
SWS-1	151.71 $\pm$ 14.45	150.57 $\pm$ 31.57	153.14 $\pm$ 14.19	207.86 $\pm$ 19.53***
SWS-2	74.29 $\pm$ 7.10	74.86 $\pm$ 13.09	88.29 $\pm$ 10.28	99.43 $\pm$ 10.49
REM	22.29 $\pm$ 5.60	18.43 $\pm$ 4.30	21.71 $\pm$ 2.64	33.14 $\pm$ 3.09*
Sum of stage shift	328.86 $\pm$ 27.96	322.43 $\pm$ 56.89	338.43 $\pm$ 30.52	445.86 $\pm$ 43.36**
<b>Fragmentation (Duration)</b>				
Wakefulness	2.85 $\pm$ 0.35	2.68 $\pm$ 0.58	3.19 $\pm$ 0.48	2.68 $\pm$ 0.58**
SWS-1	0.64 $\pm$ 0.07	1.11 $\pm$ 0.20*	0.61 $\pm$ 0.06	0.76 $\pm$ 0.06*
SWS-2	1.91 $\pm$ 0.22	2.25 $\pm$ 0.45	1.69 $\pm$ 0.10	1.18 $\pm$ 0.14**
REM	0.73 $\pm$ 0.11	0.93 $\pm$ 0.22	0.94 $\pm$ 0.10	0.89 $\pm$ 0.07
<b>Latency</b>				
SWS-2	14.21 $\pm$ 8.71	14.39 $\pm$ 8.69	8.19 $\pm$ 5.02	25.14 $\pm$ 13.25
REM	119.62 $\pm$ 22.13	197.71 $\pm$ 35.42	92.02 $\pm$ 31.44	111.48 $\pm$ 14.35

\*  $P < 0.05$  compared to baseline recording.

\*\*  $P < 0.01$  compared to baseline recording.

\*\*\*  $P < 0.001$  compared to baseline recording.

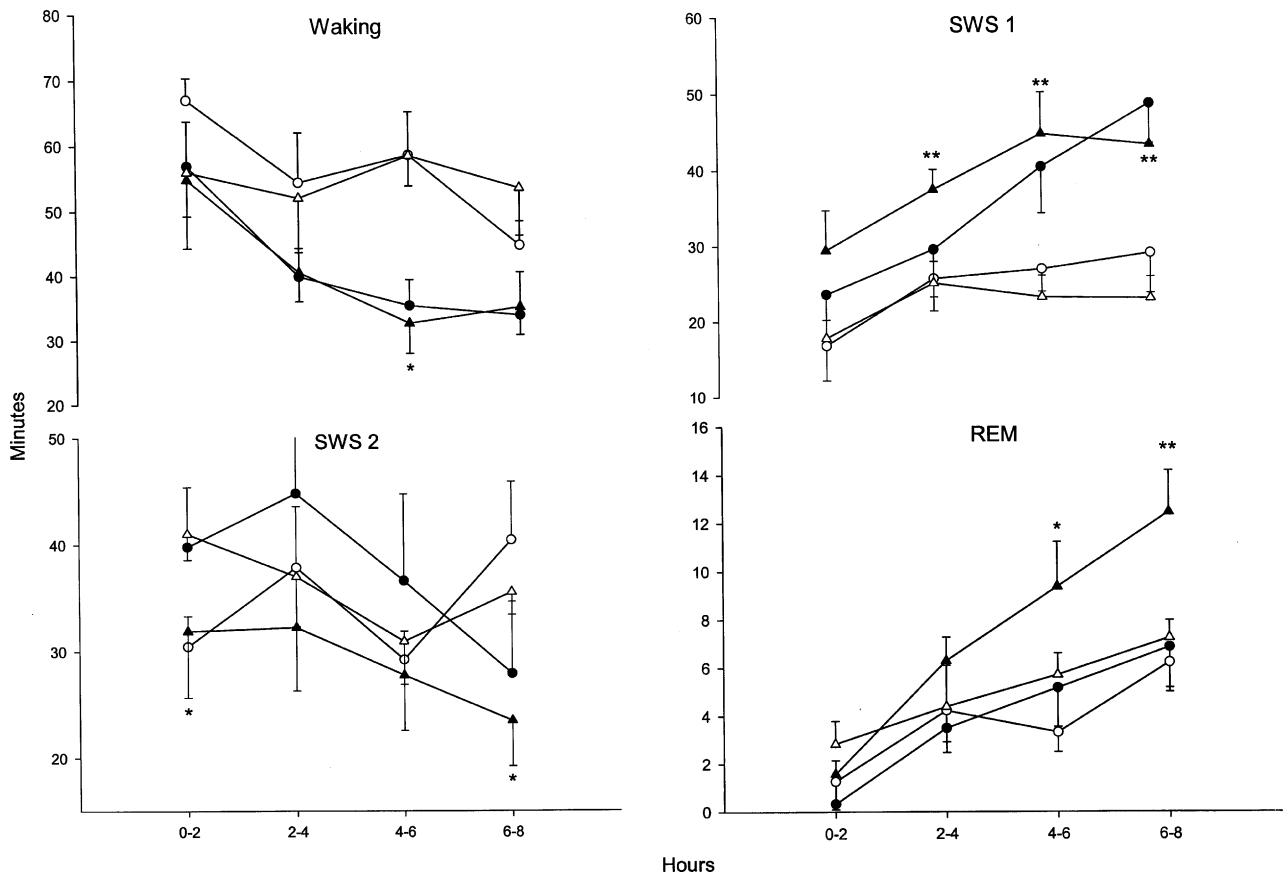


Fig. 3. Waking and sleep per 2-h recording, expressed as mean values  $\pm$  S.E.M. Symbols:  $\circ$  and  $\bullet$  indicate control rats, baseline and after 4 weeks control condition, respectively.  $\triangle$  and  $\blacktriangle$  indicate CMS rats, baseline and after 4 weeks CMS procedure. \* $P < 0.05$ , \*\* $P < 0.01$ , compared to baseline recording. See Section 3 for further details.

However SWS-2 in percent of total SWS was reduced ( $F_{(1,6)} = 12.32$ ,  $P = 0.013$ ). REM sleep increased after exposure to CMS ( $F_{(1,6)} = 11.20$ ,  $P = 0.038$ ), especially during the third 2-h period ( $P = 0.044$ , one-tailed) and the last 2-h period ( $P = 0.004$ , one-tailed) (Fig. 3). During the 8-h recording CMS rats spent 47% more time in REM sleep compared to their baseline recording, 68% more in the last half of the recording ( $P = 0.008$ ).

### 3.2.2. Control group

No effect of recording day was found ( $F_{(1,6)} = 0.18$ ,  $P = 0.686$ ).

### 3.3. Latency

#### 3.3.1. CMS group

Neither the latency to SWS-2 nor latency to REM sleep were changed in the post-CMS recording compared to baseline (see Table 1).

#### 3.3.2. Control group

Latency to SWS-2 and REM sleep latency was not significantly changed.

### 3.4. Sleep fragmentation

#### 3.4.1. CMS group

ANOVA (recording day  $\times$  number of episodes) showed an effect of recording day ( $F_{(1,6)} = 27.88$ ,  $P = 0.002$ ) and an interaction between recording day and stage ( $F_{(3,18)} = 6.29$ ,  $P = 0.004$ ). The number of REM sleep episodes increased (53% over the 8-h interval;  $P = 0.002$ ) as well as number of waking episodes (33%,  $P = 0.029$ ) and number of SWS-1 episodes (36%,  $P = 0.001$ ). The number of SWS-2 episodes was not changed.

The duration of stages was changed ( $F_{(1,6)} = 15.25$ ,  $P = 0.008$ ), and interaction between recording day and stage ( $F_{(3,18)} = 12.73$ ,  $P = 0.0001$ ) was found. In particular, duration of waking periods was reduced ( $-45\%$ ,  $P = 0.010$ ), SWS-1 periods were longer ( $+25\%$ ,  $P = 0.043$ ), while SWS-2 periods were shorter ( $-31\%$ ,  $P = 0.003$ ). The mean duration of REM sleep episodes was unchanged ( $P = 0.590$ ).

#### 3.4.2. Control group

Neither the number ( $F_{(1,6)} = 0.01$ ,  $P = 0.926$ ) nor the duration of stages ( $F_{(1,6)} = 0.43$ ,  $P = 0.534$ ) was changed between recordings.

### 3.5. Corticosterone secretion

Mean corticosterone levels in controls ( $172.43 \pm 34.58$  ng/ml) and CMS ( $155.43 \pm 17.25$  ng/ml) animals were not significantly different ( $P = 0.668$ ).

### 3.6. Correlation between sucrose intake and sleep parameters

There was a negative correlation between the Max Intake Response (the sum of sucrose intake in days 3, 10, and 17, data not shown) and the percentage change, calculated from the rat's own baseline value, of total amount of REM sleep ( $r = -0.54$ ;  $P = 0.044$ ), also between Max Intake Response and the change in number of REM sleep periods ( $r = -0.58$ ;  $P = 0.031$ ). A positive correlation between Max Intake Response and percentage change of SWS-2 duration was found ( $r = 0.50$ ;  $P = 0.083$ ). In other words, the rats with lowest sucrose intake were those that mostly increased the time spent in REM sleep and the number of REM sleep episodes, while they reduced the time spent in deep sleep. No correlation with REM latency was observed.

### 3.7. Correlation between sucrose intake and corticosterone

There were no significant correlation between Max Intake Response and level of corticosterone.

## 4. Discussion

The study shows that, in our hands, the CMS procedure in rats decreased sucrose intake per unit body weight, while sucrose intake in a non-stressed control group did not change. Also, the CMS-treated rats showed changes in post-CMS sleep compared to baseline sleep, not present in the control rats.

Some studies using CMS as an animal model of depression and anhedonia have raised the possibility that decreases in reward responsiveness, i.e. changes in intake of sweet solutions, may be related to loss of body weight [17,19,27]. In those studies CMS was not found to decrease sucrose intake per gram of body weight. In our study, we did not find any correlation between body weight and sucrose intake. Sucrose intake was reduced while body weight remained unaffected. We also found that the largest effect was obtained after 2 weeks of the stress protocol, this effect was attenuated afterwards. This trend towards recovery of normal behavior after long exposure to stress is not unusual. D'Aquila et al. [12] also observed a recovery of 'reward behavior' in the CMS model. Their findings suggest that in some cases only a temporary change of behavior accompanies the CMS procedure, possible due to an adaptation effect to the stressors.

To our knowledge, this is the first study where sucrose intake and sleep are studied simultaneously in CMS rats.

Exposure to mild unpredictable stress produced selective changes in both the structure and the continuity of sleep in rats after 4 weeks of CMS. In particular, we observed an increase in sleep fragmentation as well as in the amount of REM sleep following CMS. These increases are consistent with the sleep data seen in human depression. However, in human studies, the increase is usually seen in the first half of the night [37], whereas in CMS animals the strongest effect was observed in the last half of the recording period. Similar sleep findings in CMS rats were observed by Moreau et al. [32] and Cheeta et al. [11].

Increased sleep fragmentation in CMS rats was due to increased number of waking, SWS-1 and REM sleep episodes when compared to baseline recording. These results demonstrate that rats following the CMS procedure shift more rapidly from one vigilance state to another, indicating that they have a less stable sleep-wake pattern than before CMS treatment. This effect parallels the poor sleep maintenance often observed in depressed patients [25].

We found a clear increase of REM sleep, close to 50%, in the CMS animals compared to baseline. This was due to increased number of REM sleep episodes, while the duration of the episodes was not affected. An increase of REM sleep has also been reported in genetic rat models of depression; both in the rat strain Flinders Sensitive Line (FSL) [40] and Wistar-Kyoto (WKY) [15]. Prenatally stressed rats, which show depressive like behaviour as adults, also have increased amounts of REM sleep as well as increased sleep fragmentation [14]. Taken together with the present findings, these data indicate that an increase in REM sleep is strongly associated with depressive like behavior in rodents, whether the behavior is induced by environmental or genetic factors.

A decreased REM sleep latency is often seen in humans and in other animal models of depression. Moreau et al. [32] and Cheeta et al. [11] both found decreased REM sleep latency after CMS. Interestingly, in Cheeta et al.'s study, the REM sleep latency returned close to baseline levels after 5 weeks of stress. In our study, the REM sleep latency was not changed when we compared the two recordings of CMS rats, baseline and after 4 weeks of CMS. It is possible that our negative finding was due to the fact that we only recorded EEG sleep before starting the CMS procedure and 4 weeks later. Thus, only CMS-induced sleep abnormalities that lasted 4 weeks or longer could be detected in our sleep protocol.

At the present time the neurophysiological mechanisms underlying the REM sleep differences between CMS and control rats are unknown. However, both the noradrenergic and the serotonergic neurotransmitter systems are thought to play a role in REM sleep regulation [28,41] as well as in the pathophysiology of depression (see review; [34]), and therefore may represent the common basis for coexisting depression-like symptoms and sleep alterations.

Deep sleep (SWS-2) in CMS rats was also somewhat affected in that it was decreased when expressed as a percentage of SWS-2 relative to total SWS. This was also found in



prenatally stressed rats [14]. A transient decrease in SWS-2 has been reported in CMS rats [11]. A reduction of delta sleep is a consistent finding among depressed patients [21].

One might argue that stressors in the CMS protocol itself may change the sleep pattern, as most of the stressors were applied during the rats' rest period, and therefore may have disrupted sleep. Studies have shown EEG changes in all vigilance states after total sleep deprivation in rats [7]. Probably the most important change occurs in the delta activity. However, repeated sleep deprivation, in rats, in the dark period (activity-phase) for 5 successive days did not affect the amount of sleep stages, while in the light period (rest-phase) the amounts of all sleep states increased substantially relative to baseline and the delta activity was enhanced. The sleep episodes were lengthened in both cases [26]. The most prominent effect of repeated partial sleep deprivation in humans is an increase of the deep slow-wave-sleep (stages 3 and 4) and less fragmented sleep [9,13,45]. Brunner et al. have also studied the effect of repeated partial sleep deprivation on REM sleep homeostasis in humans [8], and found a delayed and prolonged REM sleep rebound. CMS animals did spend more time in REM sleep, but taken together with their increased fragmentation and reduced relatively amount of SWS-2, it is likely that the sleep changes were the result of the CMS procedure.

To see if CMS influenced the HPA axis, we measured corticosterone levels. Elevation of serum corticosterone, the endpoint of stress-induced activation of the HPA system, is frequently used as a stress indicator, and a convincing number of studies have found several measures indicative of a hyperactive HPA axis in depressed patients (for review see [36]). Our data could not confirm previous findings that CMS leads to an increase in plasma corticosterone compared to control rats [3]. This can be due to recovery of 'reward behavior' seen in CMS rats. Acute stress is known to cause hypersecretion of corticosterone whereas repeated exposure to several stressors can attenuate corticosterone responses to the same stress [42]. Katz's chronic stress model of depression, consisting of stressors of more severe character (such as footshock, tail pinch, and cold swim) has been shown to induce increased basal levels of serum corticosterone, indicating that adaptation does not occur [18,22,23]. However, other studies report unaltered corticosterone levels following this paradigm [2,39]. A similar inconsistency is found using CMS: a basal hypersecretion of corticosterone is found by some [3] and not by others [4,38,48].

The validity of the CMS model has been questioned [17,19,20,27] because a decrease in sucrose consumption is not consistently observed following the stress procedure among various laboratories. A number of potential confounding factors such as modification in the stress protocol, the sucrose consumption methodology and the way to analyse results have been examined (see [46] for review). In addition to the CMS protocol, we added an extra stressor, i.e. an empty water bottle, and food restriction. This may have had a reinforcing effect on the CMS protocol,

which otherwise was similar to what is used in previous studies. Also, Nielsen et al. [35] stress the need to consider inter-individual differences in response to stress in the CMS model. Our study showed high inter-individual differences of behavioral and physiological measures in CMS rats. In general, animals differ in their capacity to cope with environmental challenges [1]. If CMS represents an animal model of depressive behavior, and reduced sucrose intake is an indicator of depression in this model, then it is reasonable to assume that reduced consumption of sucrose solution correlates with the observed changes in behavior and physiology. In our study, this is consistent with our findings that rats consuming less sucrose solution spend more time in REM sleep, have more REM sleep episodes and shorter duration of the SWS-2 episodes. Thus, the animals more affected by what Willner defined as anhedonia-like symptoms develop more concomitant sleep abnormalities.

In conclusion, the CMS procedure performed in our laboratory decreased sucrose consumption in rats. It also induced sleep changes: increased REM sleep, increased fragmentation and relatively reduced deep SWS compared to the animals' own baseline values. In this study, CMS appears to be a model of depression.

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