Paper IV

Effect of chronic mild stress on hippocampal extracellular 5-HT levels in sleep and wakefulness

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Abstract

In rats, exposure of chronic mild stress (CMS) causes behavioral effects resembling some of the features of major depressive disorder, including anhedonia (loss of interest or pleasure). Specific alterations in electroencephalographic (EEG) sleep, including disinhibited rapid eye movement (REM) sleep and suppressed slow wave sleep (SWS) is a characteristic neurobiological change in human depression. One hypothesis of depression is a functional decrease of serotonergic neurotransmission. This neurotransmitter system is also involved in the modulation of sleep and wakefulness and its activity varies with sleep stage (W>SWS>REM sleep).

We investigated hippocampal serotonergic neurotransmission during sleep and waking in CMS rats using microdialysis while simultaneously recording sleep. Contrary to predictions, CMS did not change 5-HT levels in the hippocampus. However, there was a significant negative correlation between sucrose intake and 5-HT levels, the rats that consumed less sucrose were those having the highest levels of hippocampal 5-HT in SWS and REM sleep compared to the waking state. CMS also produced sleep alterations similar to those observed in depressed patients. Our findings support a possible common pathogenesis between depression-like behaviour and sleep abnormalities.

Introduction

Multiple neurotransmitters have been linked to the pathophysiology of mood disorders, including the serotonergic (5-hydroxytryptamin, 5-HT) system (Gold et al. 1988, Meltzer et al. 1989, Nemeroff 1998). The 5-HT system is one of the most widely distributed monoaminergic systems in the brain and thus has an impact on various brain circuits, among them limbic areas such as the dorsolateral prefrontal cortex and the hippocampus (Jacobs and Azmitia 1992). Both of these regions have been involved in the pathophysiology of mood disorders (Lopez et al. 1998). A decreased 5-HT activity has been suggested to be a key factor in depression (Mann et al. 1996, Sarrias et al. 1987), a hypothesis supported by an increased serotonergic transmission as a common denominator for antidepressant treatments (Blier, de Montigny & Chaput 1987).

The 5-HT system originates in the raphe nuclei. The most pronounced changes in serotonergic activity occur during sleep. The firing of serotonergic neurons in the dorsal raphe nucleus (DRN) and the extracellular levels of 5-HT at somato-dendritic and terminal level are state dependent. The neuronal activity decreases and loses its regularity from active to quiet waking, the activity further decreases during slow wave sleep (SWS) and falls almost to zero during rapid eye movement (REM) sleep (McGinty and Harper 1976) (Trulson and Jacobs 1979; Cespuglio et al. 1981; Guzman-Marin et al. 2000) A similar state dependent pattern is seen in the extracellular level of 5-HT in DRN and projection areas in cats and rats (Portas and McCarley 1994; Portas et al. 1998; Park et al. 1999).

Disturbed sleep is characteristic of patients with mood disorders (Benca et al. 1992; Kupfer 1995) and is one of the diagnostic criteria (Diagnostic and Statistical

Manual of Mental Disorders, DSM-IV, 1994). Changes in REM sleep (increased amount, higher frequency and shorter latency), sleep discontinuity and a reduction of SWS are common findings (Kupfer et al. 1990). The frequent coexistence of mood alterations and sleep abnormalities suggests that a common pathogenesis may be present. The observations of REM sleep alterations together with the REM sleep-suppressing capabilities of most antidepressants suggest that there is an enhanced REM sleep pressure in depressed patients (Vogel 1983). It is noticeable that serotonin directly inhibits REM-on neurons in the pedunculopontine (PPT) and the laterodorsal tegmental (LDT) nuclei (Sanford et al. 1994). Hence, a decrease of neuronal 5-HT may be a sufficient condition to trigger mood and sleep alterations.

Stressful life events are environmental factors that may play a role in the etiology of depression. In our laboratory, we have successfully established the chronic mild stress (CMS) model as an animal model of depression (Gronli *et al.* 2004; Gronli *et al.* 2005; Gronli J 2006). This model has a high degree of validity (Willner 1997) and mirrors daily hassles of human life. The animals are exposed to a series of mild stressors (e.g. soiled cage, tilting of cage, periods of food and water deprivation and grouping) for a short time (few hours to a day) over several (2-5) weeks (Willner et al. 1987). We have earlier reported that CMS induces a decreased sucrose intake per unit body weight (reflecting anhedonia, the major validation criterion for the CMS model [11]) (Gronli *et al.* 2004; Gronli *et al.* 2005), provokes behavioral and physiological disturbances typical in human depression (i.e. reduced sexual performance, increased activity in an open field test) (Gronli *et al.* 2005) and induces sleep changes similar to the ones reported in depressive patients (i.e. increased REM sleep, increased fragmentation and a reduction of the relative amount of deep sleep (Gronli *et al.* 2004)).

The hippocampus region is a key structure for studying the neurobiological substrates of stress and depression (Graeff et al. 1996; McKittrick et al. 2000), due in part to its involvement in the regulation of the hypothalamic-pituitary-adrenal (HPA) axis (Gomez et al. 1998). The hippocampus is rich in 5-HT receptors (Uphouse 1997) and the CMS protocol has been found to increase the binding to 5-HT_{1A} receptors in the hippocampus (Papp *et al.* 1994). This could suggest the occurrence of receptor up-regulation due to local decrease of 5-HT.

The aim of this paper is to replicate the CMS related sleep changes and to investigate serotonergic neurotransmission during sleep and waking.

In particular, we intend to investigate

a) whether CMS decreases hippocampal extracellular 5-HT-levels and affects the distinctive state-dependency of 5-HT.
b) whether there is a correlation between the level of hippocampal 5-HT and the CMS induced anhedonia (measured as sucrose-intake).

Materials and Methods

Ethical evaluation

The experiment has been conducted in accordance with the laws and regulations controlling experiments in live animals in Norway and The European Convention for the protection of Vertebrate Animals used for Experimental and other Scientific purposes.

Animals

Data were acquired from twenty male Sprague-Dawley (Mol:SPD) rats (Møllegaard, Copenhagen, Denmark). The same animals were also used in a study involving the measurement of specific protein levels in the hippocampus contralateral to the microdialysis site (Gronli J 2006). The rats were housed individually in conventional Macrolon type III cages. The rats had free access to food (Rodent low protein diet, B&K Universal AS, Norway) and water, except when the CMS procedure required deprivation. The ambient temperature was 22 ± 1°C with 52 ± 2 % humidity. Rats were kept on a reversed 12h light/12h dark schedule with gradually increasing lighting from 1800 h and lights fully on at 1900 h. The rats changed to the reversed L/D schedule 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 (Hillegaart and Ahlenius 1994).

Surgery

Nine weeks old rats, weighing approximately 300 g, were anesthesised by subcutaneous (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) diluted with distilled water. Animals were implanted with stainless steel screw electrodes for bilateral fronto-frontal and fronto-parietal electroencephalogram (EEG) recording and silver wires in the neck muscle for electromyogram (EMG) recording (see (Gronli *et al.* 2004) for further description). The rats were implanted with an intracerebral guide cannula (CMA 12 guide, CMA Microdialysis, Sweden), allowing easy insertion of the microdialysis probe in the hippocampus. The side for probe localisation was randomly assigned (left or right). Bregma coordinates for hippocampus were AP = -5.8, ML = ±5.0, DV = -8.0. Following surgery all animals received analgesic doses of buprenorphinum (Temgesic, Reckitt & Colman) (0.15 ml s.c.) twice a day for three days. At least two weeks were allowed for recovery and adaptation prior to start of experiments.

Design

Following an initial measurement of sucrose intake, the animals were randomly divided into two groups having similar average intake. One group was given ordinary daily care (Control rats, n = 6) and the other group was exposed to chronic mild stress (CMS rats, n = 12). The two groups of rats were housed separately in different rooms during the duration of the CMS procedure (paired caging, tilted cage, restricted access to food following food deprivation, exposure to an empty bottle following water deprivation, housing in a wet cage and continuous light). For further details see (Gronli *et al.* 2004; Gronli *et al.* 2005; Gronli J 2006). Sucrose intake was

measured once a week during a 1 h-window after 4 h food and water deprivation (as appeared in (Gronli J 2006)). The intake was expressed in relation to the animal's bodyweight (ml/kg).

Baseline sleep was recorded in both groups before the CMS procedure started. After 4 weeks of CMS/Control condition, sleep was recorded once more and extracellular dialysate was simultaneously sampled during the different sleep/waking stages. At the end of the experimental day, rats were decapitated. Brain tissue was dissected for histological verification of probe location.

Sleep recording conditions

The animals were adapted to the recording conditions for at least 6h during each of the three days preceding sleep recording and were not exposed to any of the described stressors the day before recording. During adaptations and sleep recordings, the animals remained in their home cages which were placed in sound attenuated recording chambers (430 x 280 x 620 mm) with constant light (15W electric bulb) and ventilation. Animals were connected to 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. This system gives free movement of the animals during adaptation and recording conditions. Animals had free access to food and water inside the chambers and the ambient temperature was 22-25 ^{o}C. Conditions were the same for Control and CMS animals. The recording started at 2000 h and lasted for a period of eight hours during which the animals were not disturbed.

Sleep recording and scoring

Fronto-frontal (FF) EEG, fronto-parietal (FP) EEG and neck muscle EMG were recorded (EMBLA, Flaga, Iceland) (for detailed information see (Gronli *et al.* 2004)). For visual display, the FF EEG was high-pass frequency filtered at 3 Hz and low-pass filtered at 35 Hz. The FP EEG was filtered at 1 Hz and 35 Hz, respectively. The EMG signal was filtered with high-pass at 5 Hz. All signals were filtered at 50 Hz to eliminate powerline artefacts.

Sleep and waking were scored manually in 10 second epochs with the support of power spectrum analysis acquired from sleep-dedicated software (Somnologica, Version 2.0.2, Flaga, Iceland). Sleep stages were classified as follows: Waking (W), SWS-1, SWS-2 and REM sleep according to the criteria given by Ursin and Larsen (Ursin and Larsen 1983) and previously used by Gronli et al. 2004 (Gronli *et al.* 2004). 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 the specific sleep stage.

Microdialysis and HPLC procedures

The microdialysis probes (CMA 12, CMA/Microdialysis, Sweden) had a diameter of 500 μm and a membrane length of 4 mm. Probes were inserted at least 16h before the experiment start to allow for equilibration of the extracellular environment around the probe tip. Relative recovery rate of the probe was in the range of 45 – 50%. Starting at about 1900 h, the probe was perfused with an artificial cerebral spinal fluid (aCSF) (147 mM NaCl, 4 mM KCl, 2.3 mM $CaCl_2$, and pH 7.2) and the flow rate maintained at 1.2 μl/min by a microdialysis pump (Univentor 864,

AgnThos, Sweden). Microdialysis sampling started 2h later. Samples were collected manually over periods of 20 minutes during polygraphic defined behavioral states (shorter samples were collected for REM sleep). W, SWS, REM sleep were identified as described above with the exception that SWS was not subdivided in SWS-1 and SWS-2. Samples were then frozen at –80^{o}C for later analysis. For each animal an average of 4 - 6 samples was collected during W and SWS and 1 - 3 samples during REM sleep. The difficulty in obtaining REM sleep samples is due to the short length of REM sleep episodes in rats which usually makes it necessary to add two or more REM sleep samples together to obtain enough dialysate. Seven µl of the dialysate sample was analysed for 5-HT.The rest of the sample was kept for further analysis of neurotransmitters. Separation of the chemical components in the dialysate was achieved with high performance liquid chromatography (HPLC) system (BAS, USA) using a microbore column (3 µm, ODS, 100 x 1 mm). The HPLC system was coupled to an electrochemical detector (EC) (UniJet LC-4C amperometric detector, BAS, USA). Mobile phase consisted of 0.6 mM ethylenediaminetetraacetic acid, 0.7 mM sodium octyl sulfate, 9.2 mM sodium chloride, 80 mM sodium acetate, 11% acetonitrile, pH was adjusted to 4.5 using acetic acid. Under these conditions 5-HT retention time was ~5 minutes. The potential applied to the glassy carbon electrode was 550 mV with respect to the reference electrode. The sensitivity was set to 1nA full scale. Chromatographic data were recorded and peak areas determined with an automatic integration system (ChromGraph 1.5.01, BAS, USA). Concentration of 5-HT in the samples was evaluated by converting peak area units into fmol using an external standard calibration curve method. Putative 5-HT peaks were identified by comparing retention times of 5-HT standards (Sigma-Aldrich) to retention times of sample peaks. Also, a known amount of 5-HT was added to a dialysate sample and

analyzed (‘spiked’ sample). This peak was identified by retention time as 5-HT and by a proportional increase. The detection limit for 5-HT was approximately 0.1 fmol. Raw chromatographic data showing the 5-HT peaks are illustrated in Fig. 1.

Histology

After the end of the experiment, animals were anaesthetized with Isofluran (Isofluran Baxter, Norway) and decapitated. The brain was rapidly removed and the hemisphere including the site of the microdialysis probe was stored in 15% sucrose / 0.1 M phosphate buffer until equilibration and then examined for histological assessment of probe location. All probes were successfully located within the hippocampus. The contralateral hemisphere was prepared for protein analysis (see Gronli et al., 2006). The side (left or right) for protein analysis or probe localisation was randomly assigned.

Statistics

Some animals were excluded from analysis: three animals died before or during the experiment, poor quality of the EEG/EMG signals (1 animal), or technical problems with HPLC-EC (1 animal). Additionally, some rats developed epileptic seizures during the recording. Rats showing more than 1 minute epileptic activity during the recording (3 animals) were excluded. Thus, 12 animals (CMS, n = 8 and Control, n = 4) were included for 5-HT assessment and 13 animals (CMS, n = 9 and Control, n = 4) for sleep analysis.

To test the normality of the distribution of the 5-HT data (absolute values, fmoles), we used the Shapiro-Wilk test (test of normality: W, $p = 0.0001$; SWS, $p = 0.007$; REM sleep, $p = 0.013$). Because this variance might be a function of differing

basal levels normally seen in individuals, the data were converted to percent of W mean value. The inter-individual variance was reduced (SWS, $p = 0.98$; REM sleep, $p = 0.11$). Subsequent statistical analyses were performed on the percentage level of 5-HT. Group effect was assessed by a two-way ANOVA (group x stage). Analysis of stage effects within the groups were performed with a one-way ANOVA. Least Significant Deviation (LSD) test was used for *post hoc* comparisons.

The statistical analysis relative to sucrose intake and body weight appear in another paper (Gronli J 2006). In the present study the individual measurements of sucrose intake were used in a correlation analysis (Pearson's statistics) to study the relationship between CMS induced anhedonia and state dependent extracellular 5-HT levels. Correlation analyses were performed on pooled data, from both control and CMS rats (n = 12).

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 × stage × 2 h period, and stage × 2 h period). Differences between 2 h periods were assessed by LSD post hoc 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 × number of episodes), difference between recording days was assessed by LSD post hoc test.

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

Significance was accepted at $p < 0.05$, two tailed.

Results

Hippocampal 5-HT level after CMS

Mean values of hippocampal 5-HT (expressed as fmoles) during waking and sleep are presented in Table 1.

There was no significant difference of hippocampal 5 -HT levels in waking and sleep between CMS and Control rats ($F_{(1,10)} = 2.27$, $p = 0.16$). A trend toward an increase of 5-HT in CMS animals in SWS and REM sleep is noticeable in Fig. 2.

In the Control group there was a state-dependent change in 5-HT levels ($F_{(2,6)} = 6.25$, $p = 0.03$). There was a 22% decrease of 5-HT level in REM sleep compared to W ($p = 0.02$) and compared to SWS ($p = 0.02$).

In the CMS group there was no state-dependent change in 5-HT levels.

Sucrose intake correlates with changes in hippocampal 5-HT in the sleep stages

CMS rats consumed less sucrose than Controls and gained weight at the same rate as Controls during the CMS protocol. These data have been presented in a separate paper (Gronli J 2006). Because of the lower sucrose intake the CMS rats as a group was regarded as anhedonic. To check for individual variations we correlated reward behavior (sucrose intake) and the hippocampal extracellular levels of 5-HT. There was a negative correlation between sucrose intake and 5-HT level in SWS ($r = -0.61$; $p = 0.034$) and REM sleep ($r = -0.74$; $p = 0.005$) compared to waking. In other words, the rats that consumed less sucrose solution were those having the highest levels of hippocampal 5-HT in SWS and REM sleep compared to their waking state (Fig. 3).

Sleep and waking alterations after CMS

Table 2 shows total sleep changes in Control and CMS conditions. Overall ANOVA showed a significant three-way interaction (recording day x sleep stage x 2h period) ($F_{(9,72)} = 2.36$, $p = 0.02$).

Baseline sleep: There was no difference between CMS and Control groups in baseline sleep ($F_{(1,15)} = 0.45$, $p = 0.51$).

CMS group: REM sleep was increased, especially in the initial 2h period following CMS ($p = 0.02$). SWS-1 was increased particularly in the second 2h period ($p = 0.004$). SWS-2 was decreased in the second and fourth 2h period ($p = 0.006$; $p = 0.007$, respectively).

Control group: There was no sleep change in the Control rats ($F_{(1,4)} = 0.29$, $p = 0.63$).

Sleep fragmentation: Following the CMS procedure rats showed a more fragmented sleep than Control rats ($F_{(1,11)} = 9.25$, $p = 0.01$) and a strong tendency to a changed duration of the sleep episodes ($F_{(1,11)} = 4.25$, $p = 0.06$).

The number of stage shifts was increased after CMS ($F_{(1,8)} = 5.83$, $p = 0.04$). The number of both REM sleep and SWS-1 episodes increased (Table 2). The latencies to enter REM sleep and SWS-2 did not change after CMS.

Control rats did not show any changes in sleep fragmentation.

Discussion

To our knowledge, this is the first microdialysis experiment to study the extracellular level of 5-HT during sleep and waking in an animal model of depression.

According to the monoamine hypothesis of depression (Maes M 1995) and considering CMS as an animal model of depression, we expected to find a lower level of extracellular 5-HT in CMS rats. Contrary to our prediction, CMS did not decrease 5-HT level in the hippocampus. If anything, a change toward increased 5-HT levels seems to appear in sleep as a consequence of CMS. This possibility is supported by the significant correlation we found between sucrose intake, a known measure of anhedonia in animals, and hippocampal 5-HT levels. The rats that consumed less sucrose were those having the highest levels of hippocampal 5-HT in SWS and REM sleep compared to the waking state.

These results suggest that selective neurotransmitter changes may occur as a consequence of CMS, but, due to large response variability in the animals such an effect only becomes significant when this variability is specifically addressed. This is better achieved by analyzing the correlation between different measures of depression-like symptoms instead of considering CMS as a homogenous group (Gronli *et al.* 2004; Strekalova *et al.* 2004; Gronli J 2006). Rats' individual variability possibly reflects the different capacity to cope with environmental challenges also present in humans. Not all humans become depressed after exposure to stress, and not all cases of depression results from stressful experiences (Anisman and Zacharko 1992). An epidemiological study reports that life events and perceived strain are positively correlated to depressive symptomatology (Aneshensel and Stone 1982). Thus, in our experience, correlation analyses are an important tool to identify CMS

sensitive rats and analyze selective behavioural and molecular responses associated to stress induced depression (Gronli *et al.* 2004; Gronli J 2006).

Studies of the serotonergic activity in hippocampus after CMS have shown contrasting results. Recently, Kang and collaborators (Kang et al. 2005) reported a reduction of the extracellular 5-HT (>20%) compared to Control rats, while in homogenized brain tissue an increase (Bekris et al. 2005), decrease (Li et al. 2003) and no change (Haidkind et al. 2003) have been described. Kang et al. did not include measurement of sucrose intake, the core marker of depression-like anhedonia in the CMS model (Kang *et al.* 2005), making it difficult to compare their findings to our study.

Serotonergic function is dependent on the availability of neurotransmitter in the synaptic cleft and on receptor binding activity and sensitivity (REF). There is a dense concentration of 5-HT_{1A} receptors in the hippocampus (Kia et al. 1996). Several studies have investigated the role of 5-HT_{1A} receptors in mediating the CMS response. The results are not univocal. An increase in the number of 5-HT_{1A} receptors in terminal areas, e.g. hippocampus has been reported (Papp et al. 1994). This up-regulation of 5-HT_{1A} receptors could imply that the amount of extracellular 5-HT is decreased in the synaptic cleft after CMS. In the dorsal raphe nucleus (DRN) it has been found that, in rats and mice, CMS induces functional desensitization of the inhibitory 5-HT_{1A} autoreceptors (Froger et al. 2004) suggesting that 5-HT output may increase as a result. A 5-HT_{1A} agonist, believed to be acting postsynaptically, has been shown to reverse CMS induced decreased sucrose intake in rats (Munoz and Papp 1999). In humans suffering from major depression, a desensitization of 5-HT_{1A} receptors was found in limbic areas (Drevets et al. 1999; Sargent et al. 2000) and a decrease in 5-HT_{1A} mRNA in prefrontal cortex and hippocampus has also been

reported (Lopez-Figueroa et al. 2004). Hence, it is possible that the effect of CMS on serotonergic activity largely derives from a direct action on 5-HT_{1A} receptors. The ambiguous effect of CMS on hippocampal 5-HT level suggests that CMS may affect the number or function (e.g. binding affinity) of the receptors rather than the availability of serotonin.

It is remarkable that the rats that consumed less sucrose were those having the highest levels of hippocampal 5-HT in SWS and REM sleep compared to the waking state. The REM sleep alterations typically associated with depression and replicated in the CMS model ((Gronli *et al.* 2004) and the present study) would suggest that more disinhibition of REM-promoting neurons may occur in depression. It is known that REM promoting neurons are under the inhibitory influence of 5-HT (Leonard and Llinas 1994) and REM sleep occurs when serotonergic output is lowest (Lydic et al. 1987). However, our results suggest that after CMS no decrease in REM 5-HT level takes place, and if anything an opposite trend appears. We measured 5-HT in the hippocampus, a representative serotonergic projection area. In this area, 5-HT levels have been shown to be state-dependent (e.g. W>SWS>REM sleep) and to parallel the state-dependent changes present in other serotonergic projection areas (including REM promoting regions of the pons) (for review (Portas et al. 2000)). In this view, we suggest that a dissociation between the level of hippocampal 5-HT and the occurrence of REM sleep may take place after CMS.

A reduction of SWS-2 and of the duration of SWS-2 episodes after CMS (alterations typically observed in human depression) were replicated in this study (Gronli *et al.* 2004). Serotonergic activity is known to be low during SWS (Lydic *et al.* 1987). However, in this study, the animals with the highest level of hippocampal 5-HT in SWS (as well as in REM sleep) were those more anhedonic. Hence, it

appears that CMS produces a paradoxical effect on serotonin in SWS and REM sleep. Neurochemically, the sleep/wake cycle is associated with interactions between aminergic and cholinergic neurotransmission (Steriade and McCarley 1990), the regulation of neuropeptides (Ehlers and Kupfer 1987) and other systems. GABAergic projections arising from the lateral preoptic area and the pontine ventral periaqueductal gray including the DRN itself strongly modulate the activity of the DRN serotonergic neurons during SWS and REM sleep, respectively (Gervasoni et al. 2000). A decreased GABAergic function has been found in humans affected by unipolar depression (Sanacora et al. 1999; Sanacora et al. 2004). This is also reported in other animal models of depression, e.g in learned helplessness (Sherman and Petty 1982), following olfactory bulbectomy (Jancsar and Leonard 1984) and forced swimming (Borsini *et al.* 1986). Hence, it is possible, while speculative, that the alteration of the serotonergic function observed in CMS animals may be related to GABAergic dysfunction. Unfortunately, no direct measures of GABA have yet been reported in the CMS model.

The sleep alterations in the present study parallel most of the findings from earlier CMS studies (Moreau *et al.* 1995; Cheeta *et al.* 1997; Gronli *et al.* 2004). REM sleep was increased and this increase was more pronounced in the first 2h period. However, REM sleep latency was not affected. Sleep fragmentation was increased and the amount of SWS-2 decreased as a consequence of CMS. These findings are consistent with many of the sleep disturbances reported in human depression (Benca *et al.* 1992; Kupfer 1995).

In conclusion, CMS did not decrease 5-HT level in the hippocampus and a trend towards an increase was present in SWS and REM sleep. In addition, a significant correlation between anhedonic behaviour and hippocampal 5-HT levels

during sleep was found. CMS also produced sleep alterations similar to those observed in depressed patients.

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Table 1. Mean hippocampal 5-hydroxytryptamine (5-HT) levels ± S.E.M. for each group.

*$p < 0.05$ compared to waking, † $p < 0.05$ compared to SWS.

	Waking	SWS	REM sleep
Control	1.64 ± 0.39	1.70 ± 0.54	1.35 ± 0.45*, †
CMS	2.76 ± 1.05	3.74 ± 1.47	3.49 ± 1.28

Table 2. Sleep parameters in control and CMS rats: baseline recording (before) and after a 4 week exposure to Control condition or CMS (after).

	Control		CMS	
	Before	After	Before	After
Sleep stages (min) *(total amount)*				
Wakefulness	103.7 ± 4.2	109.8 ± 15.6	120.4 ± 7.4	110.1 ± 9.5
SWS 1	186.8 ± 32.9	183.5 ± 29.3	175.8 ± 10.4	195.4 ± 10.0*
SWS 2	131.1 ± 19.1	110.0 ± 15.1	123.5 ± 11.7	97.4 ± 14.4
REM	58.6 ± 11.7	78.3 ± 22.0	61.0 ± 5.3	77.7 ± 7.2*
Fragmentation *(number of episodes)*				
Wakefulness	55.5 ± 5.9	55.3 ± 7.6	63.4 ± 3.5	63.8 ± 6.6
SWS 1	111.4 ± 3.6	91.8 ± 9.3	115.0 ± 3.9	134.2 ± 4.3**
SWS 2	58.2 ± 3.5	50.6 ± 8.5	57.4 ± 3.0	64.9 ± 7.8
REM	38.5 ± 7.8	38.5 ± 3.7	37.1 ± 2.7	49.9 ± 3.4***
Sum of stage shift	265.0 ± 15.2	222.7 ± 22.2	272.9 ± 8.4	312.8 ± 8.3*
(duration) (min)				
Wakefulness	1.8 ± 0.2	2.0 ± 0.3	2.0 ± 0.2	1.7 ± 0.1
SWS 1	1.8 ± 0.3	1.6 ± 0.2	1.5 ± 0.1	1.5 ± 0.1
SWS 2	2.1 ± 0.3	2.0 ± 0.3	2.1 ± 0.2	1.6 ± 0.1
REM	1.5 ± 0.1	1.8 ± 0.5	1.7 ± 0.1	1.5 ± 0.1
Latency (min)				
SWS 2	23.1 ± 6.1	24.3 ± 6.1	21.5 ± 4.0	15.0 ± 4.2
REM	57.3 ± 14.7	62.7 ± 15.5	66.1 ± 7.6	59.0 ± 21.0

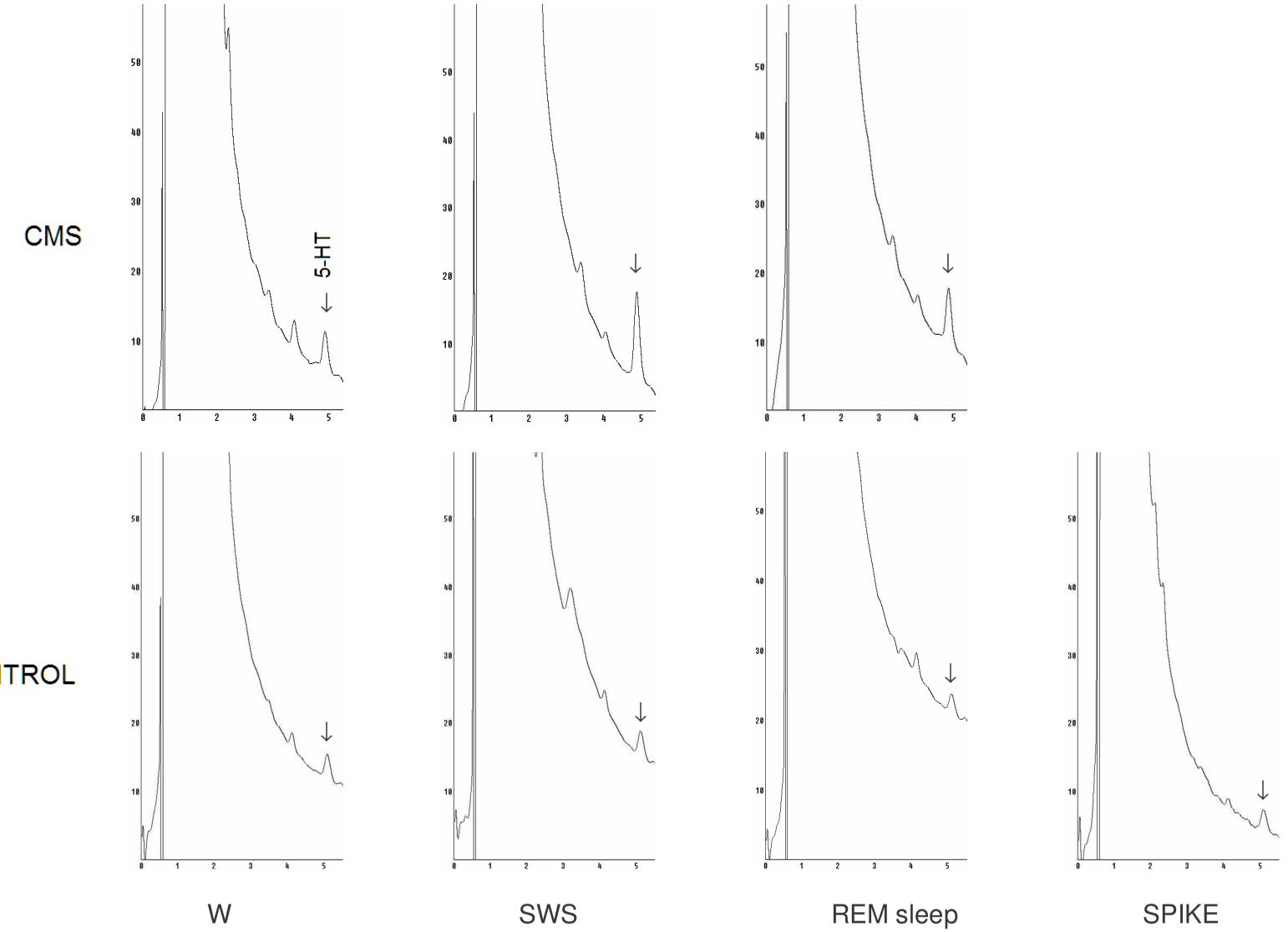
$^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ compared to baseline recording.

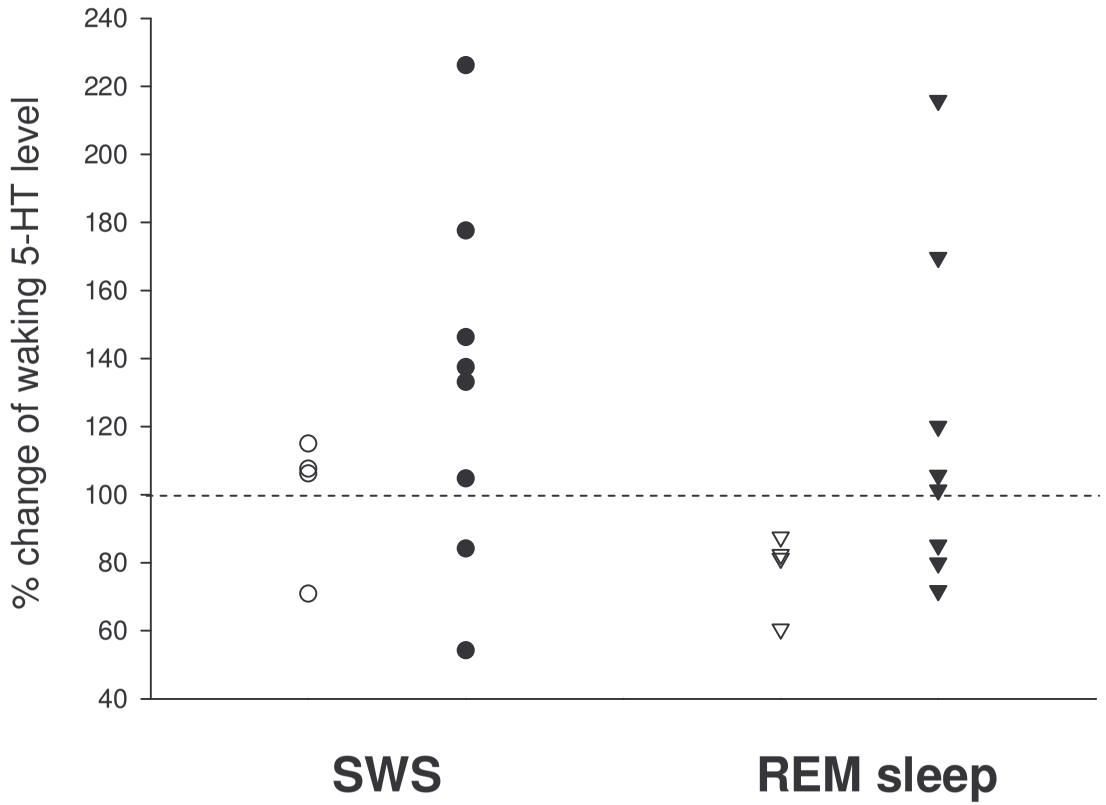
LEGENDS

Fig.1. Representative chromatograms in one CMS and one Control rat, each showing 5-HT levels (arrows) in dialysate collected during the specific behavioural state. Note the increase in amplitude of the 5-HT peak during sleep compared to waking in the CMS animal. The 'spike' chromatogram identifies 5-HT in the dialysate. The added amount of 5-HT standard solution results in an identical retention time with the peak in the dialysate. Abbreviations: *W*, waking; *SWS*, slow wave sleep; *REM* rapid eye movement sleep.

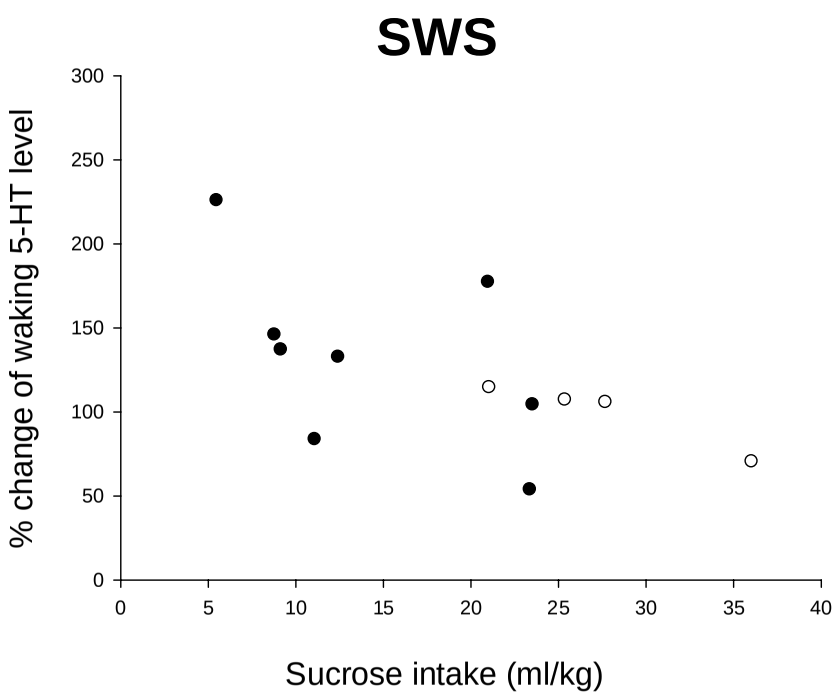
Fig. 2. The figure illustrates the intra-individual variability of extracellular 5-HT levels in hippocampus during SWS and REM sleep. Note the increase in the CMS group. Circles indicate SWS and diamonds indicate REM sleep. Open symbols indicate Control rats and filled symbols CMS rats.

Fig 3. Correlation between sucrose intake (ml/kg) and extracellular 5-HT levels in SWS (r = -0.61; p = 0.034; n = 12) (Fig. 3A) and in REM sleep (r = -0.74; p = 0.005; n = 12) (Fig. 3B). Open symbols indicate Control rats and filled symbols CMS rats.

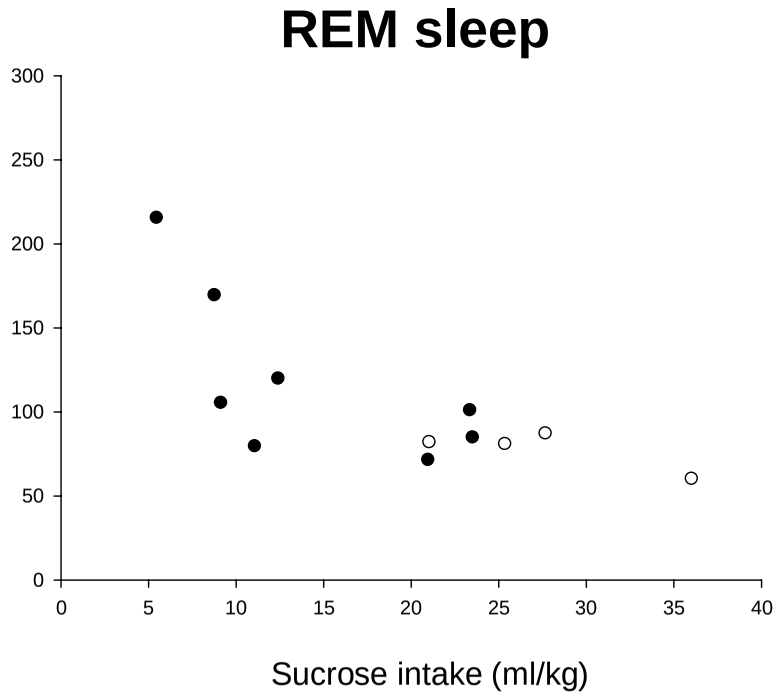

CMS
5-HT
CONTROL
W
SWS
REM sleep
SPIKE


% change of waking 5-HT level
240
220
200
180
160
140
120
100
80
60
40
SWS
REM sleep

A


SWS
% change of waking 5-HT level
300
250
200
150
100
50
0
0
5
10
15
20
25
30
35
40
Sucrose intake (ml/kg)

B


REM sleep
300
250
200
150
100
50
0
0
5
10
15
20
25
30
35
40
Sucrose intake (ml/kg)