Effects of sleep deprivation on extracellular serotonin in hippocampus and frontal cortex of the rat

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Abbreviations:

EEG, electroencephalogram; EMG, electromyogram; HPLC-ECD, High-performance liquid chromatography-electrochemical detection; 5-HIAA, 5-hydroxyindoleacetic acid; REM, rapid eye-movement; 5-HT, serotonin

Running title:

Sleep deprivation does not increase extracellular serotonin

Abstract

Sleep deprivation improves the mood of depressed patients, but the exact mechanism behind this effect is unclear. An enhancement of serotonergic neurotransmission has been suggested. In this study, we used in vivo microdialysis to monitor extracellular serotonin in the hippocampus and the frontal cortex of rats during an eight hour sleep deprivation period. These brain regions were selected since both have been implicated in depression. The behavioral state of the animal was continuously monitored by polygraphic recordings during the experiment.

Sleep deprivation produced a gradual decline in extracellular serotonin levels, both in the hippocampus and in the frontal cortex. In order to investigate whether the reduction in serotonin was due to other factors than sleep deprivation, i.e. time of day effect, another experiment was performed. Here animals were allowed to sleep during most of the recording period. This experiment showed the expected changes in extracellular serotonin levels: consistently higher levels in the awake, non-sleep deprived animals compared to during sleep, but no time of day effect. The reduction in extracellular serotonin during sleep deprivation may suggest that serotonin does not play a major role in the mood-elevating effect of sleep deprivation. However, since 5-HT levels are strongly behavioral state dependent, by eliminating sleep, there may be a net increase in serotonergic neurotransmission during the sleep deprivation period.

Key words: microdialysis, 5-HT, HPLC-ECD

Sleep deprivation has been shown to be a clinically effective antidepressive treatment. About 60-70% of depressed patients report improved mood following one night of total sleep deprivation (Wu and Bunney, 1990; Leibenluft and Wehr, 1992; Riemann et al., 1999; Wirz-Justice and Van den Hoofdakker, 1999). The effect is, however, short-lasting. Usually there is a relapse following the next sleep period, limiting the clinical usefulness of the intervention. Despite this, the effect of sleep deprivation has received much attention, since it indicates that it is possible to obtain a rapid antidepressant effect. This is in contrast to the delayed onset of action of antidepressant drugs, where several weeks of treatment are usually required to achieve a therapeutic response.

A large body of evidence suggests a dysfunction of brain serotonin (5-hydroxytryptamine, 5-HT) in major depression (Meltzer, 1989; Mann, 1999). Furthermore, most antidepressant drug therapies are thought to act by increasing serotonergic neurotransmission (Blier and De Montigny, 1994). It is unclear, however, whether the effect of sleep deprivation is due to an enhancement of serotonergic neurotransmission.

Tissue concentrations of 5-hydroxyindoleacetic acid (5-HIAA), the principal 5-HT metabolite, increase in the dorsal raphe nucleus area and the thalamus of rats after 24 hours of sleep deprivation (Toru et al., 1984), suggesting a possible role of serotonin in sleep deprivation. The ratio 5-HIAA/5-HT is often used as an index of 5-HT turnover. Sleep deprivation significantly increases this ratio in Wistar rats in the frontal cortex, the hippocampus, the hypothalamus and the brain stem, indicating increased 5-HT turnover in these areas (Asikainen et al., 1997). Furthermore, total sleep deprivation in cats increases mean firing rates of serotonergic neurons in the dorsal raphe nucleus by 18% (Gardner et al., 1997). These data suggest a role of serotonin in the effect of sleep deprivation.

In contrast, brain 5-HT levels do not differ from control immediately following 24 hours (Borbely et al., 1980) or 72 hours (Wesemann et al., 1983) of sleep deprivation. Furthermore, although 5-HIAA levels increase, the concentrations of 5-HT are reported to decrease after sleep deprivation, but the differences are not significant (Toru et al., 1984). Also, Asikainen et al. (1997) report that 5-HT concentrations are not affected by sleep deprivation, even though 5-HT turnover significantly increases. Moreover, total sleep deprivation for as long as 11-20 days fails to show any changes in 5-HT tissue concentrations in multiple brain areas, including the frontal cortex and the hippocampus (Bergmann et al., 1994). In fact, in that study prolonged sleep deprivation produces no changes in the ratio 5-HIAA/5-HT either (Bergmann et al., 1994). These latter data question the role of serotonin in sleep deprivation.

In the present study, we wanted to further explore the effects of sleep deprivation on serotonergic neurotransmission by using in vivo microdialysis. This technique enables us to directly follow the time course of extracellular 5-HT during sleep deprivation. We examined the extracellular 5-HT levels during an eight hour sleep deprivation period in two brain areas, the frontal cortex and the hippocampus. These two sites were chosen for two reasons: (i) both have been implicated in depression (Vijayakumar and Meti, 1999; Teneback et al., 1999; Bremner et al., 2000) (ii) they represent projection areas of both dorsal raphe (frontal cortex) and median raphe (hippocampus) (Casanovas and Artigas, 1996). To our knowledge, this is the first study that investigates the extracellular 5-HT levels during sleep deprivation in these brain areas.

EXPERIMENTAL PROCEDURES

Experimental animals and surgery

Male Sprague-Dawley (Mol:SPRD) rats (Møllegaard, Copenhagen, Denmark) weighing 250-300 g at surgery were used in these experiments. The animals were housed individually in conventional macrolone cages. They were kept on a controlled 12-hour light/12-hour dark schedule with the lights on at 06:00 hours and an ambient temperature 22° ± 1°C. The animals had free access to food (Rodent low protein diet, B&K Universal AS, Norway) and water *ad libitum*. To induce surgical anaesthesia animals were injected subcutaneously (s.c.) with 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 and fronto-parietal electroencephalogram (EEG) recording and silver wires in the neck muscle for electromyogram (EMG) recording. The frontal screw electrodes were placed epidurally 1-2 mm anterior to bregma and 1-2 mm lateral to the midline, and the parietal screw electrodes were placed 2 mm anterior to lambda and 2 mm lateral to the midline. The rats were also implanted with two intracerebral guide cannulas (CMA 12/guide, CMA Microdialysis, Sweden) allowing easy insertion of microdialysis probes into the ventral hippocampus (AP = -5.8, ML = -5.0, DV = -8.0) and frontal cortex (AP = +3.2, ML = -2.5, DV = -5.0) (Casanovas and Artigas, 1996).

Postoperative animal care and recording conditions

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 recording. During recording the animals in their home cages, with both water and food *ad libitum*, were placed into sound attenuated recording chambers (430 x 280 x 620 mm) with light (15-W electric bulb) and ventilation. The ambient temperature was 24-28° C inside the chambers during recording. Free movement of the animal was permitted using a flexible recording cable linked to a combined fluid and 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 microdialysis probes (CMA 12, CMA/Microdialysis, Sweden) had a diameter of 500 µm and a membrane length of 4 mm (hippocampus) and 3 mm (frontal cortex), and were inserted the day before the experiment to allow time for equilibration of the extracellular environment. The experiments were started by perfusing the probes with artificial cerebrospinal fluid (aCSF = 147 mM NaCl, 4 mM KCl, 2.3 mM $CaCl_2$, and pH 7.4) at about 08.00. Flow rates of 0.8 µl/min were maintained by microdialysis pumps (CMA 100, Sweden). Microdialysis samples of 20 minute epochs started one hour later, and were collected manually.

Experimental conditions

In the sleep deprivation study, the animals were kept awake from 07.30 in the morning until 16.00. Microdialysis sampling (20 min epochs) started at about 09.00. Following a stable baseline (2-3 samples), samples were taken at an hourly interval. Rats were kept awake by

gentle sensory stimulation, i.e. knocking on the plexiglas door, opening the door, gentle handling etc. To ascertain that the rats were fully awake, continuous polygraphic recordings of the animal were performed, in addition to visually inspecting the animal. Polygraphic recordings started at 09.00. Some rats developed epileptic seizures after hours of sleep deprivation. These rats were excluded in the further analysis.

In order to check whether the changes in extracellular 5-HT following sleep deprivation could be due to something else than the sleep deprivation, as for instance time of day effect or draining of extracellular 5-HT etc, another study was performed. Here another set of animals was allowed to sleep during a major part of the recording period. However, since extracellular 5-HT levels have been shown to vary according to behavioral state (Portas et al., 1998), baseline samples were all taken in the awake animal. Following a stable baseline (2-3 samples), the animals were then allowed to sleep undisturbed from 10.00 to 15.00. Samples were taken at an hourly interval. At 15.00 the animals were awakened and kept awake for three consecutive 20 minute samples, to again obtain comparable samples of extracellular 5-HT in the awake state.

In both studies, the experiment ended by injecting the selective 5-HT_{1A} agonist 8-OH-DPAT (0.05-0.10 mg/kg s.c.) in order to check that the measured peak in the chromatogram was in fact a 5-HT peak. Extracellular 5-HT was monitored for four 20 min epochs (four samples) following the 8-OH-DPAT injection.

High-performance liquid chromatography procedure

Microdialysis samples (10 µl of dialysate) were injected into a High Performance Liquid Chromatography (HPLC) System (BAS, USA). Separation was achieved using a microbore

column (3 µm, ODS, 100 x 1 mm). The HPLC system was coupled to an electrochemical detector (ECD) (Unijet LC-4C amperometric detector, BAS, USA). The potential applied to the glassy carbon electrode was 550 mV with respect to the reference electrode. The sensitivity was set to 1 nA full scale. Mobile phase consisted of 0.62 mM ethylenediaminetetraacetic acid (EDTA), 0.65 mM sodium octyl sulfate, 10 mM sodium chloride, 0.08 M sodiumacetat, 11% acetonitrile. pH was adjusted to 4.5 with acetic acid. Under these conditions 5-HT retention time was ~ 6 min. Chromatographic data were recorded and peak areas determined with an automatic integration system (WOW data system, Thermo Separation Products Inc., U.S.A.). Concentration of 5-HT in the samples was evaluated by converting peak area units into fmoles using an external standard calibration curve method. Putative 5-HT peaks were identified by comparing retention times of 5-HT standards (5-HT, Sigma Chemicals) to retention times of sample peaks. The technique of "spiking" was also used to confirm the retention time identification. In this technique a known amount of 5-HT was added to a dialysate sample and analyzed. The resulting chromatogram showed that the peak identified by retention time as 5-HT was increased and that the increase was proportional to the known amount of 5-HT used for spiking. The detection limit for 5-HT in the dialysate was approximately 0.2 fmoles/sample. Raw microdialysis records showing the 5-HT peaks are shown in Figure 1.

Sleep scoring

Fronto-frontal EEG, fronto-parietal EEG and neck muscle EMG signals were recorded using a paper velocity of 10 mm/sec. Scoring of the behavioral states (waking, slow wave sleep (SWS) and rapid eye-movement (REM) sleep) was done manually in 10 sec epochs

according to the criteria given by Ursin and Larsen (Ursin and Larsen, 1983), with the exception that slow wave sleep was not subdivided.

Drugs

(±)-8-hydroxy-2-(di-*n*-propylamino)tetralin hydrobromide (8-OH-DPAT), obtained from RBI (USA), was dissolved in saline and injected s.c. (0.05 – 0.1 mg/kg).

Histology

Following the termination of the experiment the animals were anaesthetized with 5 ml of chloral hydrate i.p., and the brains were perfused via the ascending aorta with saline followed by 10% formaldehyde in 0.1 M phosphate buffer. The brains were left in situ overnight in 4 degrees Celsius, and then blocked and placed in 20% sucrose (w/v) in 0.1 M phosphate buffer until equilibration. The placement of the probe in frontal cortex was visually verified. To verify the placement in the hippocampus, the brains were cut at a thickness of 40 µm on a freezing microtome. Sections were stained with Giemsa and observed under microscope. Only data from the animals with probes verified to be located within hippocampus and/or frontal cortex were accepted.

Ethical evaluation

All efforts were made to minimize the number of animals used and their suffering. The experiment described in this article has been approved by the Norwegian Animal Research Authority and registered by the Authority. The experiment has thus been conducted in accordance with the laws and regulations controlling experiments in live animals in

Norway, i.e. The Animal Protection Act of December 20th 1974, No 73, chapter VI sections 20-22 and the Animal Protection Ordinance concerning Biological Experiments in Animals of January 15th 1996. Norway has signed and ratified The European Convention for the protection of Vertebrate Animals used for Experimental and other Scientific purposes of March 18, 1986.

Statistics

Data were analyzed with StatSoft STATISTICA 5.5. Values are given as means ± S.E.M.

In the sleep deprivation study, data were analyzed by a one-way ANOVA for repeated measures. Due to technical problems with the HPLC-ECD system, data from some time points were missing. In these cases, the average of the preceding and consecutive values was substituted, in order to run the ANOVA statistics.

In the 'allowed to sleep' study, the average of the initial four waking samples, the average of the four sleep samples, and the average of the final three waking samples for each animal were analyzed by *t*-tests for dependent samples.

Significance was accepted at $p < 0.05$.

RESULTS

Sleep deprivation produced a gradual decrease in extracellular 5-HT levels, both in the hippocampus ($F_{8,16} = 18.3$, $p < 0.001$) and in the frontal cortex ($F_{8,16} = 10.8$, $p < 0.001$) (Fig. 2). At no point during the sleep deprivation period of about eight hours, did 5-HT

levels increase (Fig. 2). Actual 5-HT levels during baseline were 6.1 ± 1.6 fmol/sample, range: 1.5-13.4 fmol/sample (hippocampus) and 5.7 ± 2.0 fmol/sample, range: 0.9-16.1 fmol/sample (frontal cortex).

Even though the rats were under constant surveillance both visually and polygraphically, short-lasting sleep episodes were impossible to prevent. The rats became extremely sleepy during the deprivation. Sleep spindles in the EEG, indicating sleep, could at times be seen even though the animals appeared fully awake, i.e. standing on the hindlimbs. The sleep episodes during the deprivation period were short-lasting, but since we employ a 10 sec scoring epoch, some sleep was recorded (Table 1). In per cent of the total polygraphic recording period of seven hours, waking constituted 97.8% and slow wave sleep 2.2%. REM sleep was not seen.

In the experiment where animals were allowed to sleep, extracellular 5-HT levels showed the expected changes throughout the recording (Fig. 3). The average 5-HT value during the initial waking period was significantly higher than during the time when the animals were allowed to sleep (hippocampus: 99.3 ± 1.3% vs 56.8 ± 4.9%, $p < 0.001$; frontal cortex: 107.4 ± 8.4% vs 62.6 ± 11.6%, $p < 0.05$). When the animals were awakened at about 3 PM, average extracellular 5-HT levels in waking increased to levels significantly higher than during the time when the animals were allowed to sleep (hippocampus: 56.8 ± 4.9% vs 83.8 ± 6.9%, $p < 0.001$; frontal cortex: 62.6 ± 11.6% vs 114.8 ± 13.3%, $p < 0.01$). There were no significant differences between average extracellular 5-HT during the initial and final waking periods (Fig. 3). Thus, waking 5-HT levels were higher than levels where the animals were allowed to sleep, as expected. Actual 5-HT levels during baseline were

3.7 ± 1.0 fmol/sample, range: 1.6-10.0 fmol/sample (hippocampus) and 2.3 ± 0.8 fmol/sample, range: 1.0-6.4 fmol/sample (frontal cortex).

Table 2 indicates the vigilance states during the hours of polygraphic recordings. The animals slept most of the time when they were left undisturbed.

At the end of the experiments, a low dose of 8-OH-DPAT (0.05-0.10 mg/kg) was injected s.c. in order to verify that the measured peaks were serotonergic. Extracellular 5-HT levels decreased following the 8-OH-DPAT injection in all cases. In the sleep deprivation experiment, extracellular 5-HT levels in the hippocampus decreased from 51 ± 5% of baseline (before injection) to 19 ± 5% (lowest value after injection) and in the frontal cortex from 59 ± 12% of baseline (before injection) to 28 ± 9% (lowest value after injection). In the experiment where the animals were allowed to sleep, the reductions were from 90 ± 9% of baseline to 22 ± 4% and from 110 ± 5% of baseline to 20 ± 4%, in the hippocampus and frontal cortex, respectively. All these reductions in extracellular 5-HT levels following 8-OH-DPAT injection were significant ($p < 0.01$, *t*-tests for dependent samples). Figure 4 shows the time course of hippocampal extracellular 5-HT levels (in fmoles) during the whole experiment in one rat.

DISCUSSION

The present findings show that sleep deprivation does not increase extracellular 5-HT levels in the hippocampus or in the frontal cortex. In fact, 5-HT levels declined during the sleep deprivation period of about eight hours. These findings may seem surprising. Clinically,

sleep deprivation has profound and rapid effects on depressed mood in 60-70% of patients with affective disorders (Wu and Bunney, 1990; Leibenluft and Wehr, 1992; Riemann et al., 1999; Wirz-Justice and Van den Hoofdakker, 1999). A dysfunction of brain serotonin in depression is well-documented (Meltzer, 1989; Mann, 1999), and most antidepressant therapies are thought to act by increasing 5-HT neurotransmission. The present data of a reduction in extracellular 5-HT levels may suggest that serotonin does not play a major role in the effect of sleep deprivation on depression.

Several studies report an increase in 5-HIAA and in the ratio of 5-HIAA and 5-HT following sleep deprivation (Toru et al., 1984; Asikainen et al., 1997), suggesting a possible role of serotonin. However, no studies have found increases in tissue concentrations of brain 5-HT following sleep deprivation (Borbely et al., 1980; Wesemann et al., 1983; Toru et al., 1984; Bergmann et al., 1994; Asikainen et al., 1997). Serotonergic neurons in the dorsal raphe nucleus of cats increase their firing during sleep deprivation (Gardner et al., 1997). Such an increase in neuronal firing would presumably lead to an increase in 5-HT release, and thus to an increase in extracellular 5-HT levels. The increase in neuronal firing was however small, with a maximum increase of 18% after 15 hours of sleep deprivation (Gardner et al., 1997). Still, our data are not easily reconciled with these firing data.

One may argue that eight hours of sustained wakefulness are too short for an investigation of the effects of sleep deprivation. However, several factors indicate that our data are valid. Rats are nocturnal animals, which means they are active during the night and sleep during the day. Thus, the sleep deprivation period was during a time when the rats are mostly asleep (Tobler and Borbely, 1990). In addition, it is likely that the rats were mostly

awake during the night preceding the experiment. Hence, even though our experiment was an eight hour sleep deprivation study, the rats have probably been awake for a longer period. This possibility is supported by the fact that the rats were extremely difficult to keep awake, proving that they were indeed severely sleep deprived. Nevertheless, we cannot exclude the possibility that prolonging the sleep deprivation period might produce different results. Administration of selective serotonin reuptake inhibitors initially suppresses 5-HT neurotransmission, followed by an increase after several days of continued administration, presumably due to autoreceptor adaptation (Blier and De Montigny, 1994). These changes in 5-HT neurotransmission following reuptake inhibition parallel the clinical response of these drugs. Whether similar adaptive changes appear following sleep deprivation is unlikely, knowing the antidepressant effect seen in humans following one night of sleep deprivation.

It was surprising that the 5-HT levels decreased when the animals were kept awake during sleep deprivation. The reason for this is not clear. The spontaneous activity of serotonergic neurons throughout the brainstem is strongly dependent on the behavioral state, with activity being highest in waking, intermediate in slow wave sleep, and lowest in REM sleep (Jacobs and Fornal, 1991). Furthermore, recent microdialysis studies document the same behavior state-dependency in 5-HT neurotransmission. Extracellular 5-HT levels are highest in waking, lower in SWS, and lowest in REM sleep, in all brain regions studied so far, including the frontal cortex (Portas et al., 1998; Portas et al., 2000) and the hippocampus (Park et al., 1999). Thus, 5-HT neurotransmission is normally higher in waking than during sleep. By eliminating sleep, where 5-HT neurotransmission is decreased, a sustained discharge of serotonergic neurons and a corresponding tonic release

of 5-HT at postsynaptic receptors should enhance overall 5-HT neurotransmission, as discussed in Gardner et al. (1997). This is supported by the findings of increased 5-HIAA and 5-HT/5-HIAA ratio following sleep deprivation (Toru et al., 1984, Asikainen et al., 1997). With the increased metabolic demands of enforced wakefulness, however, the release of 5-HT may be impaired compared to spontaneous waking, as suggested by the present data. As mentioned above, several studies report no change in 5-HT levels in tissue extracts of various brain areas investigated immediately following sleep deprivation (Borbely et al., 1980; Wesemann et al.,1983; Asikainen et al., 1997), indicating that there is no depletion of serotonin during sleep deprivation. The present results, obtained by continuous monitoring of 5-HT in small areas of a living brain, may give a better picture of serotonergic function than analysis of tissue extracts, although they are not necessarily in contrast to the tissue findings. In any case, the mechanisms for the reduction of extracellular 5-HT during sleep deprivation in our study must be different from the well established reduction of extracellular 5-HT during sleep discussed above (Portas et al., 1998; Park et al., 1999), which is secondary to the reduction of serotonergic neuronal firing during sleep (Jacobs and Fornal, 1991). One might speculate that the reduction of 5-HT levels during sleep deprivation could be due to an intrusion of slow waves in the EEG during the enforced wakefulness, which is a well-established fact (Dijk 1995). However, the low amount of slow wave sleep scored during deprivation makes this explanation less likely.

It cannot be excluded that even if extracellular 5-HT levels decreased during the sleep deprivation period in the present rat study, overall extracellular 5-HT levels (total amounts of 5-HT per time unit) may be higher following sleep deprivation than during a

time period where the animals are allowed to sleep. However, the present data suggest that other explanations than increased 5-HT neurotransmission should be considered for the effect of sleep deprivation on depressed mood. Other neurotransmitter systems may be involved. Both noradrenergic (Delgado and Moreno, 2000) and dopaminergic (Ebert and Lammers, 1997) neurotransmission have been implicated in depression. In fact, Benedetti et al. (1997) have been hypothesizing that dopamine is involved in the antidepressant effect of sleep deprivation in humans. Thus, it is possible that the sleep deprivation effect on depressed mood is not primarily related to an enhancement of serotonergic neurotransmission. This hypothesis needs further investigation.

Finally, sleep deprivation has clear antidepressant effects on the mood of depressed patients, whereas it can make healthy non-depressed humans feel miserable and grumpy. This suggests a methodological problem when studying the effects of sleep deprivation in healthy animals. There are now different animal models of depression, and by using 'depressed' rats the effects of sleep deprivation on extracellular serotonin may differ from the present data.

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Table 1. Sleep and waking during the sleep deprivation period.

	waking	slow wave sleep	REM sleep
09.00-10.00	59.0 ± 0.4	1.1 ± 0.4	0
10.00-11.00	59.0 ± 0.6	1.0 ± 0.6	0
11.00-12.00	58.8 ± 0.5	1.1 ± 0.5	0
12.00-13.00	58.8 ± 0.3	1.2 ± 0.3	0
13.00-14.00	58.9 ± 0.3	1.1 ± 0.3	0
14.00-15.00	58.2 ± 0.6	1.8 ± 0.6	0
15.00-16.00	58.6 ± 0.5	1.4 ± 0.5	0

Data are expressed as means (in minutes) ± S.E.M. during the seven hours of polygraphic recording. n = 9.

Table 2. Sleep and waking for the control animals.

		waking	slow wave sleep	REM sleep
09.00-10.00	kept awake	58.6 ± 1.0	1.4 ± 1.0	0
10.00-11.00	allowed to sleep	26.2 ± 3.8	32.4 ± 3.7	1.3 ± 0.6
11.00-12.00	allowed to sleep	9.6 ± 4.8	43.6 ± 4.1	6.8 ± 2.1
12.00-13.00	allowed to sleep	5.9 ± 2.0	47,0 ± 3.0	7.1 ± 1.5
13.00-14.00	allowed to sleep	7.2 ± 3.4	42.4 ± 3.1	10.5 ± 2.5
14.00-15.00	allowed to sleep	9.4 ± 2.0	43.6 ± 2.1	7.1 ± 2.0
15.00-16.00	kept awake	59.3 ± 0.4	0.7 ± 0.4	0

Data are expressed as means (in minutes) ± S.E.M. during the seven hours of polygraphic recording. The animals were allowed to sleep from 10.00 until 15.00. n = 8.

Figure legends

Figure 1

Chromatograms showing 5-HT peaks in frontal cortex (FC) and hippocampus in the same animal. The arrow points to the 5-HT peak identified in a 'spiked' sample (dialysate + added amount of exogenous 5-HT, see methods). Note that the retention time of 5-HT was very constant over time (approximately 6.5 minutes). Small changes occurred within 1 second range.

Figure 2

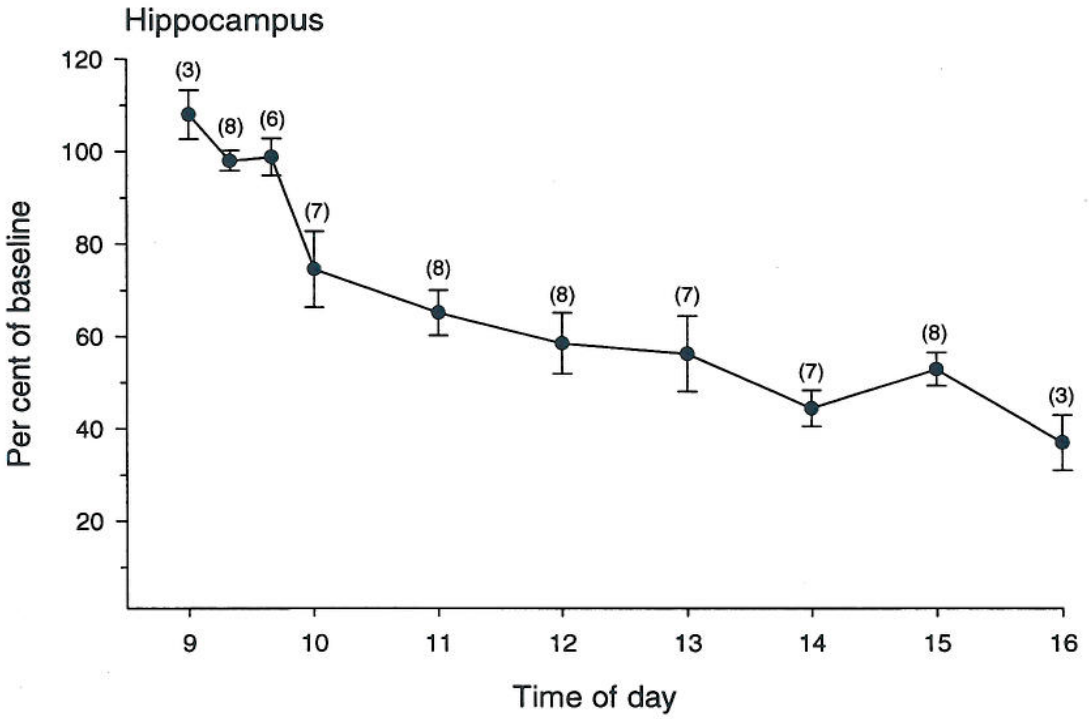
Extracellular 5-HT in the hippocampus and the frontal cortex following total sleep deprivation. Data are expressed as percentages of baseline sampling (means ± S.E.M.). n = 8 in both brain regions, although for some time points n is lower due to technical problems with the HPLC-ECD system. The actual number of animals at each time point is indicated in parenthesis.

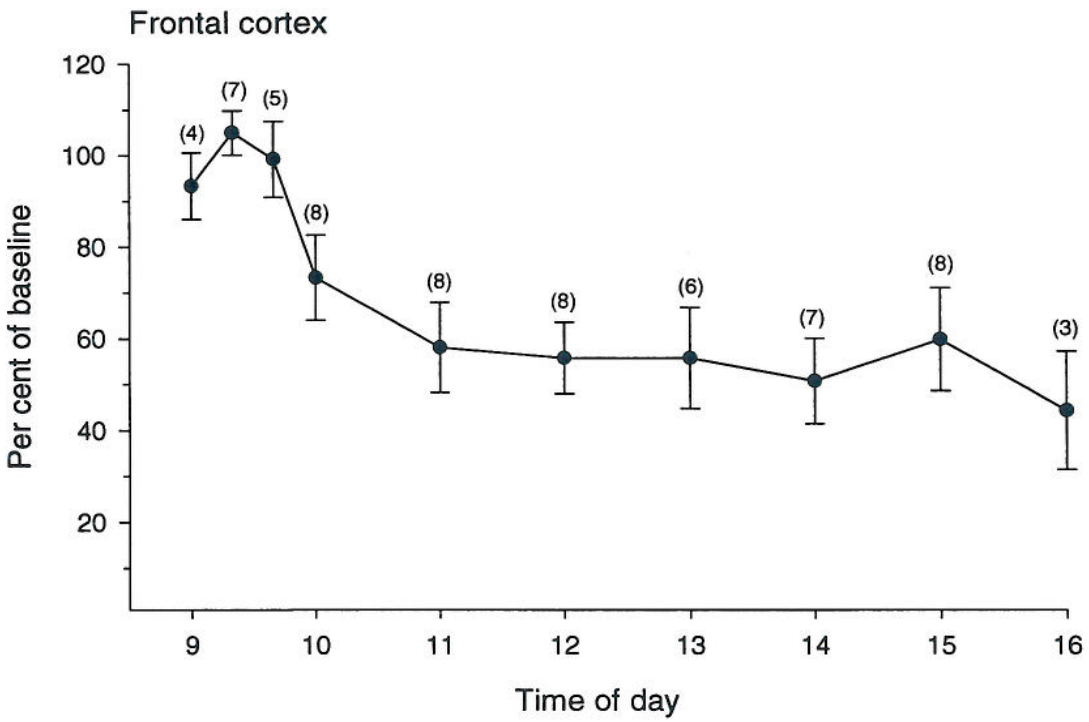
Figure 3

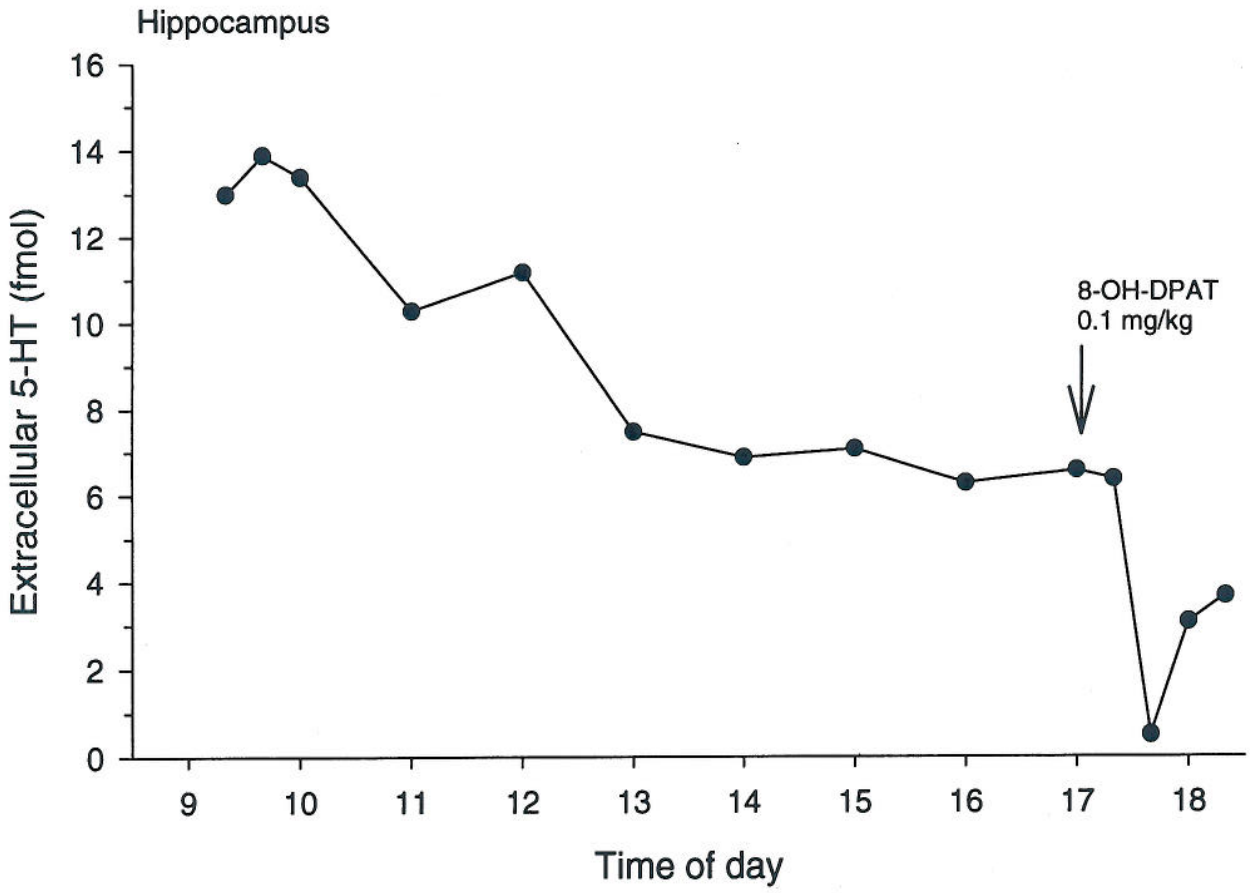
Extracellular 5-HT in the hippocampus and the frontal cortex. First four and last three values are collected in awake animals, whereas the animals were allowed to sleep during collection of the middle four values. Data are expressed as percentages of baseline sampling (means ± S.E.M.). n = 8 (hippocampus) and n = 6 (frontal cortex). In some rats individual time points are missing due to technical problems with the HPLC system. The actual number of animals at each time point is indicated in parenthesis.

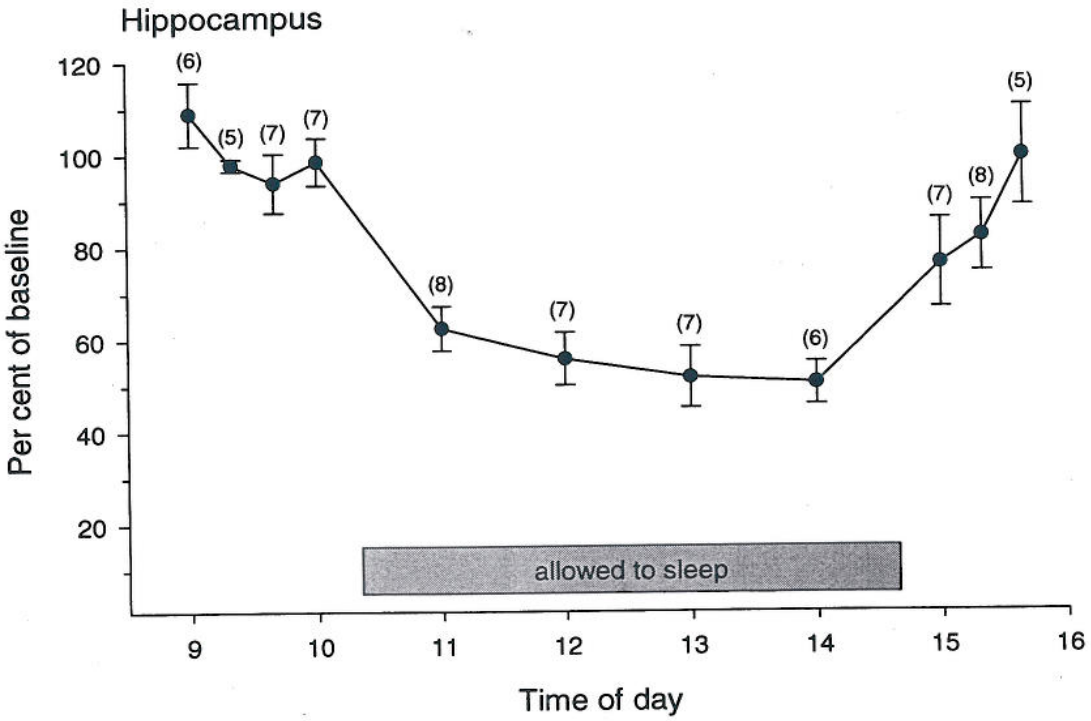
Figure 4

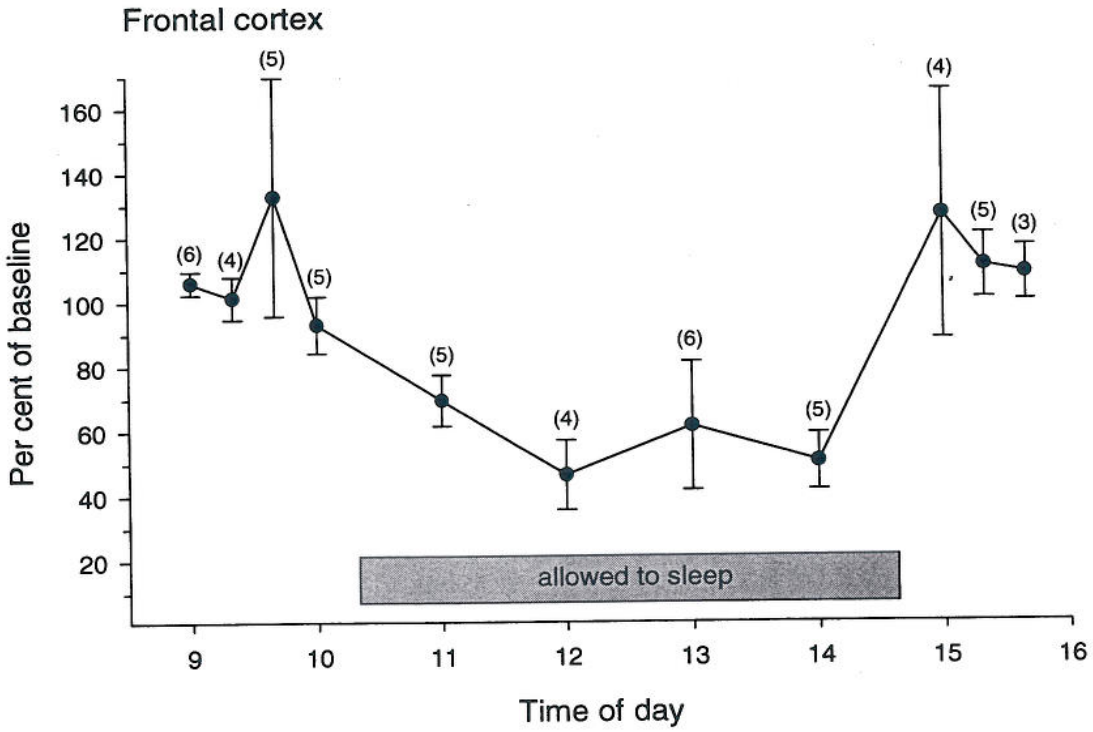
Extracellular 5-HT in the hippocampus following total sleep deprivation in one representative animal. Data are given in femtomol. 8-OH-DPAT was injected s.c. in a dose of 0.1 mg/kg, and extracellular 5-HT levels were followed for another four samples. Note that this rat was followed for one hour longer (until 5 PM) before 8-OH-DPAT was injected.


Hippocampus
Per cent of baseline
120
100
80
60
40
20
(3)
(8)
(6)
(7)
(8)
(8)
(7)
(7)
(8)
(3)
9
10
11
12
13
14
15
16
Time of day


Frontal cortex
Per cent of baseline
120
100
80
60
40
20
(4)
(7)
(5)
(8)
(8)
(8)
(6)
(7)
(8)
(3)
9
10
11
12
13
14
15
16
Time of day


Hippocampus
Extracellular 5-HT (fmol)
16
14
12
10
8
6
4
2
0
9
10
11
12
13
14
15
16
17
18
Time of day
8-OH-DPAT
0.1 mg/kg


Hippocampus
Per cent of baseline
120
100
80
60
40
20
(6)
(5)
(7)
(7)
(8)
(7)
(7)
(6)
(7)
(8)
(5)
allowed to sleep
9
10
11
12
13
14
15
16
Time of day


Frontal cortex
Per cent of baseline
160
140
120
100
80
60
40
20
(6)
(4)
(5)
(5)
(5)
(4)
(6)
(5)
(4)
(5)
(3)
allowed to sleep
9
10
11
12
13
14
15
16
Time of day