Extracellular Serotonin in the Rat Hippocampus during REM Sleep Deprivation

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Rapid eye movement (REM) sleep deprivation exerts activating effects in humans and rats. The mechanisms of action of these effects are not well understood. In the present report, we used \textit{in vivo} microdialysis in the rat to test the hypothesis that REM sleep deprivation increases extracellular levels of serotonin (5HT) in the hippocampus. Rats were REM deprived for 24 hours with the platform technique (rats on a small platform surrounded by water). Changes in dialysate 5HT concentration in these rats were compared with those found in stress-control rats (rats on large platform, surrounded by water), and cage-control rats. All rats were food deprived during the 24 hours of the experimental paradigm. No significant differences were found in 5HT levels in the hippocampus among the three groups of rats. There was a significant increase in 5HT levels after the rats were returned to the home cages, but again this increase was not different among the three groups, suggesting that REM sleep deprivation does not increase 5HT release. These results do not support the hypothesis that the activating effects of REM sleep deprivation are due to an increase in 5HT release.

CURRENT CLAIM: REM sleep deprivation does not change serotonin extracellular levels in the rat hippocampus.

Total sleep deprivation, as well as selective deprivation of rapid eye movement (REM) sleep, induces behavioral changes in humans and animals. In humans, both total and REM sleep deprivation have antidepressant effects (Pflug and Tolle, 1971; Vogel, 1975; Vogel et al., 1980; Wu and Bunney, 1990) and may trigger mania in patients with bipolar disorder (Wehr et al., 1987). In rats, REM sleep deprivation induces hyperactivity, irritability, and hypersexuality, which some authors consider a model for mania (Gessa et al., 1995). The putative underpinnings of the sleep-deprivation induced behavioral changes are not well understood. Most antidepressant drugs, which may also induce a switch to mania in bipolar patients (Bunney et al., 1970), are believed to cause their therapeutic effects by increasing serotonergic (5HT) activity (Blier and de Montigny, 1998). Therefore, it is reasonable to hypothesize that sleep deprivation exerts its activating effects also by increasing 5HT activity.

Changes in the 5HT system during total sleep deprivation are consistent with normal 5HT physiology during sleep and wakefulness: raphe unit activity and 5HT release are maximal during wakefulness, they decrease during non-REM sleep, and are minimal during REM sleep (Iwakiri et al., 1993; McGinty and Harper, 1976; Park et al., 1999; Portas and McCarley, 1994; Trulson and Jacobs, 1979; Wilkinson et al., 1991). Changes in the 5HT system induced by sleep deprivation are in accordance with maintained high levels of 5HT in the extracellular space during prolonged wakefulness, and thus augmented catabolism. These changes include: a) an increase in brain 5HT turnover (Asikainen et al., 1997); b) a decreased concentration of 5HT in homogenized brain tissue (Ramesh et al., 1999); and c) an increase in baseline raphe unit activity, probably as a result of desensitization/down-regulation of 5HT1a autoreceptors (Gardner et al., 1997).

In contrast, REM sleep deprivation, in which the animal is allowed to have prolonged episodes of non-REM sleep and hence a decrease in 5HT release during these episodes, does not strongly influence the 5HT system. REM sleep deprivation does not increase baseline raphe unit activity (Mauduit et al., 1996a, 1996b), and does not induce changes in 5HT concentrations in homogenized rat brain tissue (Stern et al., 1971) but, as in total sleep deprivation, it does increase 5HT turnover (Cramer et al., 1973; Hery et al., 1970; Vogel et al., 1980; Wu and Bunney, 1990) and does not induce changes in brain 5HT turnover (Cramer et al., 1973; Hery et al., 1970; Youngblood et al., 1997). Although the REM deprivation-induced increase in 5HT turnover may be suggestive of an increase of 5HT release, this is not necessarily the case since it has been reported that 5HT turnover is not a good indicator of 5HT release (Kuhn et al., 1986).

Microdialysis techniques, on the other hand, provide a better estimate of the synaptic overflow of 5HT (Sharp et al., 1989). However, to the best of our knowledge, no study has reported extracellular 5HT levels during REM sleep deprivation measured with microdialysis, and thus the effect of REM sleep deprivation on 5HT release is unknown. The present study was designed to clarify this unanswered question.

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by measuring 5HT levels with microdialysis in the posterior hippocampus. The hippocampus was chosen because the effects of 5HT in this structure have been proposed as playing a major role in the action of antidepressant drugs (Duman et al., 1999; Graeff et al., 1996; Jacobs et al., 2000).

METHODS

Experiments were performed on male Sprague-Dawley rats weighing 300-550 g. Animal studies followed the guidelines of the Animal Welfare Act and were approved by the Animal Research Committee of the University of California, Los Angeles. Rats were kept on a 12-h light/dark cycle with lights on at 08:00 AM. Surgery was performed under anesthesia with a combination of ketamine HCl (100mg/kg, i.m.), xylazine (4 mg/kg, i.m.) and acepromazine (0.75 mg/kg, i.m.). The animals were preoperatively treated with atropine 0.02 mg, s.c; Buprenorphine 0.01mg, s.c., was used immediately following the surgery and every 12 hours for 48 hours for additional post-operative analgesia. Animals were implanted with stainless steel screw electrodes for lateral (bregma coordinates AP=-1.0, L=3.0) and medial (AP=midpoint between bregma and lambda, L=1.0) cortical electroencephalogram (EEG) recording. Teflon coated stainless steel wires, stripped of the insulation for the last 3 mm, were placed in the neck muscle for electromyogram (EMG) recording. Bilateral intracerebral 20-gauge guide cannulae were implanted 4 mm above the target (AP=-5.8, L=5.0, H=-3.0) to guide and secure the microdialysis probe in the posterior hippocampus.

One week was allowed for recovery from surgery. Rats were connected to a polygraph (Grass Instruments, Model 8-16) with a flexible recording cable linked to a combined fluid swivel and electrical commutator. The ambient temperature in the chamber ranged from 24 to 28º C, and humidity from 40 to 60%. Rats had free access to water. However, during REM sleep deprivation, or at similar times for the two control groups (see below), food was not available because of technical difficulties. In order to allow for equilibration of the extracellular environment surrounding the microdialysis membrane, probes were inserted at least 12 h before sampling was started.

EEG and EMG were recorded using a Grass polygraph, Model 8-16, and digitized at 500 Hz/channel on a MacIntosh computer using SuperScope software. Behavioral states were scored in epochs of 15 sec, into four stages (see Figure 1): (A) Active waking (AW): gross body movements, cortical desynchronization, and medium-high EMG amplitude; (B) Quiet waking (QW): EEG desynchronization, and low amplitude EMG potentials; (C) Quiet sleep (QS): cortical and hippocampal high voltage slow waves and low amplitude EMG potentials; and (D) Rapid eye movement (REM) sleep or active sleep (AS): desynchronized cortical EEG, remarkably regular pattern of hippocampal theta activity recorded in the medial cortex electrode, and a very low amplitude EMG activity. Fifteen-second epochs were scored using an automatic scoring software based on a SuperScope platform. Automatic scoring was based on average EMG amplitude, power spectra from the delta band on the lateral cortex, and power spectra of the theta band of the medial cortex over the 15-second epoch. Next, several minutes of the record that contained examples of each of the behavioral states were manually scored in 15-second epochs. Average EMG amplitude, power spectra of the delta band of the lateral cortex, and power spectra of the theta band of the medial cortex electrode were obtained for each of the 15-second epochs. Then, we selected, in each of these three parameters, a threshold value that differentiated the several behavioral states (see below). The threshold values were then loaded into the software for automatic scoring. Active wakefulness was detected in those 15-second epochs that displayed an average EMG amplitude larger than the previously defined EMG threshold level. Quiet wakefulness was detected as averaged EMG amplitude and lateral cortex delta power spectra that were smaller than the previously defined respective threshold levels. Quiet sleep was defined as EMG activity below threshold and lateral cortex delta power spectra above threshold. REM sleep was defined as EMG amplitude below threshold and theta power spectra in the medial cortex electrode over threshold.

Animals were divided into three groups: 1) REM-deprived rats were placed on a 6 cm diameter platform in a tank containing water, 1 cm deep; 2) Stress-control rats were placed on a 14 cm diameter platform, also surrounded by 1 cm deep water. Rats belonging to these two groups were placed on the platform from 12:00 PM on Day One to 12:00 PM on Day Two; and 3) Cage-control rats were left undisturbed in their home cages, but food was withdrawn from 12:00 PM on Day One to 12:00 PM on Day Two. In some experiments, rats were video taped for the first hour after being moved to their home cages (Day Two), or at equivalent times in the cage-control group (Figure 2). Behavior was scored off-line by counting the seconds in which rats spent in each of the following behaviors: resting, rearing, grooming, exploring, eating, and drinking. Behavior was classified also as resting and active, with “active” defined as the summation of time spent rearing, grooming, exploring, eating, and drinking.

Microdialysis probes (CMA 11, 4 mm membrane, CMA/Microdialysis) were perfused with artificial cerebrospinal fluid (aCSF=125 mM NaCl, 2.5 mM KCl, 0.9 mM NaH2PO4-H2O, 5 mM Na2HPO4, 1 mM MgCl2_6H2O, 1.2 mM CaCl2_2H2O, and pH 7.4-7.6) at a flow rate of 2 µl/min. In all groups, dialysate samples were collected at 30 min intervals, from 9:00 AM to 8:30 PM on Day One, and from 9:00 AM to 5:30 PM on Day Two (Figure 2). Samples were stored at -70º C until high performance liquid chromatography (HPLC) analysis was performed.

HPLC with electrochemical detection was used for determination of 5HT in dialysates using a mobile phase consisting of sodium acetate (125 mM NaCl, 2.5 mM KCl, 0.9 mM NaH2PO4-H2O, 5 mM Na2HPO4, 1 mM MgCl2_6H2O, 1.2 mM CaCl2_2H2O, and pH 7.4-7.6) at a flow rate of 2 µl/min. In all groups, dialysate samples were collected at 30 min intervals, from 9:00 AM to 8:30 PM on Day One, and from 9:00 AM to 5:30 PM on Day Two (Figure 2). Samples were stored at -70º C until high performance liquid chromatography (HPLC) analysis was performed.

HPLC with electrochemical detection was used for determination of 5HT in dialysates using a mobile phase consisting of sodium acetate (75 mM), sodium dodecane sulphonate (0.75 mM), EDTA (10 µM), triethylenamine (0.01%), acetonitrile (12%), methanol (12%) and tetrahydrofuran (1%), pH 5.5, pumped at a rate of 200 µl/min (Shimadzu model LC-10AD) through a 100 x 2 mm column (3 um, Hypersil C18 resin). Under these conditions, 5HT retention time ranged from 7 to 9 minutes. The system was calibrated at regular intervals and provided a limit of detection of 20 pM for a 10 µl injection.
Figure 1. Examples of polygraphically-defined states of the sleep-wakefulness cycle. As explained in the text, a large part of the electrical activity recorded in the medial cortex is transmitted from the hippocampus by volume conduction. (A) Active wakefulness is characterized by desynchronized lateral cortex EEG, theta rhythm or low irregular activity in the medial cortex (the theta rhythm is generated in the hippocampus and transmitted to the medial cortex by volume conduction), and high to medium levels of EMG activity. (B) Quiet wakefulness displays desynchronized lateral cortical EEG, irregular activity in the medial cortex and low levels of EMG activity. (C) Quiet sleep is characterized by synchronized lateral cortex EEG, large irregular activity in the medial cortex, and low levels of EMG activity. (D) REM sleep is characterized by desynchronized lateral cortex EEG, theta rhythm in the medial cortex, and low levels of EMG activity.

Figure 2. Schematic diagram of the experimental paradigm. Samples were collected every half an hour, but dialyses 5HT concentrations within each rat were averaged in blocks, as represented in this Figure. No samples were collected from 8 P.M. Day one to 9 A.M. Day 2 (dotted line). The experimental manipulation occurred from 12 P.M. on Day one to 12 P.M. on Day two (gray bar). Groups of rats were classified depending on the experimental manipulation. Sleep deprived rats were placed on a small platform surrounded by water. Stress-control rats were placed on a large platform also surrounded by water. Cage-control rats were left in their home cages. All groups were food deprived during the experimental manipulation.
Following completion of the experiments, the rats were euthanized with an overdose of pentobarbital and perfused with saline and 4% paraformaldehyde. Fifty micron slices were stained with cresyl violet to verify the placement of probes within the hippocampus. Data obtained from probes outside the hippocampus were discarded.

Statistical analyses were performed using repeated measures two-way analysis of variance (ANOVA), in which animal group was the between subject factor, and time of collection the repeated measures factor. The dependent variable was 5HT dialysate concentration measured as percentage changes compared to baseline (baseline was the average of the first three hours). Behavioral analysis was carried out with one way ANOVA, in which the factor was the rat group, and the dependent variable the percentage of time the rats spent in each of the behaviors for the first hour after being moved to their home cages.

RESULTS

As previously described, rats on the small platform were totally deprived of REM sleep, whereas rats on the large platform were able to enter REM sleep (7.2±4.1% of the total time on the platform; mean±SEM in this and subsequent entries). Quiet sleep was 13.6±2.8% of the total time in the small platform group, and 17.6±9.3 in the large platform group. Sleep in the cage-control group was not measured.

REM sleep deprivation and stress-control experiments were performed six times. Stress-control experiments were performed on seven occasions. 5HT concentration in contiguous dialysate samples was averaged in each rat in blocks as follows (see Figure 2). Block 1: baseline (rat in home cage, undisturbed for three hours); Block 2: 4 hours following the placement of the rat on the platform, or at equivalent time in the cage-control rats; Block 3: 4 to 8 hours after placing the rat on the platform, or at equivalent time in the cage-control rats; Block 4: 3 hours preceding the return of the rat to their home cages; Block 5: 1 hour after the rat was moved to its home cage; Block 6: subsequent 4 hours in home cage.

As determined by repeated measures ANOVA, there was no significant experimental group effect ($F_{2,111}=1.25, p>0.05$), but there was an effect of the repeated measure factor, i.e., time ($F_{5,108}=8.97, p<0.0001$). This finding indicates that there was no overall difference among REM-sleep deprived, stress-control, and cage-control rats, but that the behavioral manipulation had an effect on 5HT levels. Bonferroni corrections for multiple comparisons showed that significant differences were obtained between Block 5 and Block 1 ($p<0.05$), and Block 5 and Block 6 ($p<0.05$) in the REM sleep-deprived group only (Figure 3 and Table 1). The stress- and cage-control groups displayed no significant differences among the various time blocks (Figure 3).

![Figure 3](image_url)

**Figure 3.** Bar histograms representing 5HT concentrations, expressed by changes from baseline. For explanation of the meaning of “block number,” in the horizontal axis, please see Figure 2 and text. Error bars represent S.E.M. The only statistically significant differences in the 5HT levels were obtained in the REM sleep deprived rats. These differences were found between 5HT obtained during one hour after the rat was returned to their home cages and both baseline (i.e., Block one) and recovery sleep (i.e., Block six).

*p<0.05. No other statistically significant difference was obtained, either between groups, or within groups.
After the first few experiments, we noted that 5HT increased during the first hour after the animals were returned to the home cage. Therefore, we decided to study the behavior displayed by the animals during this time frame to determine whether the increase in 5HT was due to behavioral factors. These behavioral analyses were not performed in three of the sleep-deprived experiments, and in one of the stress-control experiments.

Behavior was visually scored during the 60 minutes after the rats were returned to their home cage. Figure 4 displays the percentage of time spent by each group of rats on each of the behaviors, represented with bar histograms. The only behaviors that were different between the groups were grooming and eating (one way ANOVA, $F_{2,111}, p<0.05$). Bonferroni corrected simple effect comparisons showed that REM deprived rats spent significantly more time grooming ($33.9\pm3.7\%$) than either stress-control ($12.4\pm1.7\%, p<0.05$) or cage-control rats ($8.0\pm1.2\%; p<0.05$). There was no difference in the time spent grooming between the stress-control and cage-control rats. In addition, the REM-deprived group spent less time eating ($7.0\pm3.8\%)$ than the cage-control group ($50.1\pm8.1\%, p<0.05$). We collapsed the behaviors of exploring, rearing, grooming, eating, and drinking into one category: “active.” Rats in the REM sleep-deprived group were active $62.7\pm12.7\%$ of the 60 minutes ($n=3$), stress-control rats spent $70.6\pm9.4\%$ of the time active ($n=6$), and cage-control rats $76.3\pm9.9\%$ of the time ($n=6$). The percentage of time in which rats were active among the three groups was not significantly different (one factor ANOVA, $F_{2,111}=0.41, p>0.05$).

**DISCUSSION**

In the present study, no significant increase in extracellular 5HT was found after 24 hours of REM sleep deprivation. This result is consistent with that obtained by several authors who also failed to find changes in other indicators of 5HT activity, such as baseline unit activity of raphe neurons (Mauduit et al., 1996a, 1996b), or changes in 5HT concentrations in rat brain homogenized tissue (Stern et al., 1971). It may, however, be considered inconsistent with the results of other authors (Cramer et al., 1973; Hery et al., 1970; Youngblood et al., 1997), who reported an increase of tissue 5HIAA after REM sleep deprivation. Based on the assumption that 5HIAA is the product of the metabolism of 5HT that has been released from vesicles into the synaptic cleft, reuptaken, and oxidized in the cytoplasm, it has been suggested that 5HIAA is an indicator of 5HT release. However, it is not clear that all the 5HT that is metabolized to 5HIAA has been previously released into the synaptic cleft (Crespi et al., 1990; Kuhn et al., 1986). For example, microdialysis perfusion with a Ca++-free medium decreased 5HT output by 70%, whereas 5HIAA remained
constant (Kalén et al., 1988). In fact, it is known that newly synthesized 5HT can be metabolized to 5HIAA within the cytoplasm before being released (Kuhn et al., 1986). Therefore, an increase in 5HIAA after REM sleep deprivation might not reflect an increase in 5HT release.

Another possible explanation for our results is that 5HT release did change during REM sleep deprivation, but these changes were not robust enough to be detected by the microdialysis technique. For example, a slight increase in 5HT release might have been compensated for by rapid 5HT reuptake, preventing enough 5HT to reach the microdialysis probe. However, in our previous study (Park et al., 1999) and other studies (Rueter and Jacobs, 1996), it has been demonstrated that 5HT changes related to behavior can be detected with the microdialysis technique. Therefore, it would appear that increased 5HT release during REM sleep deprivation, if it occurs, is not of the magnitude that is observed during other behavioral changes.

When rats were returned to their home cages, the only group that displayed a statistically significant increase in 5HT was the REM sleep-deprived group. However, this increase is most likely not specific to the REM sleep deprivation procedure. First, although the increase in 5HT after rats were moved to their home cage was not statistically significant in the stress-control and cage-control rats, there was a clear trend. In fact, changes in 5HT levels during the duration of the experiment were not different among rat groups. Therefore, we cannot conclude, based on the results of the present report, that REM sleep deprivation induces changes in 5HT release. Rather, the increase of 5HT in the various groups of rats may be related to the behaviors in which the rats engaged after being moved to their home cages. The REM sleep-deprived group and the cage-control group spent a considerable percentage of time grooming and eating, respectively. It has been demonstrated that these behaviors correlate with an increase of 5HT activity. For example, 5HT is released during feeding (Hoebel et al., 1989; Orosco and Nicolaidis, 1992; Rueter and Jacobs, 1996; Schwartz et al., 1990). In addition, dorsal raphe neurons increase their discharge during the oral-buccal movements associated with grooming (Fornal et al., 1996).

Although the only significant change in 5HT dialysate concentration was found after REM sleep-deprived rats were returned to the home cage, there was a clear trend of 5HT increase over the course of the experimental manipulation. However, this trend was present in the three groups of rats, indicating that the cause of the increase is not the REM sleep deprivation procedure, but some intervention that was common to the three groups. A possible explanation for this increase is food deprivation. In fact, Ishida et al. (1997) reported that five hours of fasting in neonatal rats induced an increase in tryptophan levels in serum and 5HIAA in the striatum. Also, Stanley et al. (1989) observed a non-significant increase in paraventricular hypothalamus extracellular 5HT during food deprivation. These two studies suggest that fasting may cause an increase in 5HT release, thus supporting the hypothesis that the trend in 5HT increase in our data might be the result of food deprivation.

These results do not unequivocally eliminate the possibility that 5HT mechanisms are involved in the amelioration of depression induced by REM sleep deprivation. Experiments were performed in normal, healthy rats. REM sleep deprivation in normal volunteers induced minimal changes, including anxiety, irritability, and inability to concentrate, and these changes normally required more than one night of REM sleep deprivation (see Vogel et al., 1968). It is possible that the 5HT changes that sleep deprivation exerts depend on the previous state of the organism, and that these changes may be different in depressed subjects compared to healthy controls.

Another source of uncertainty is that the experiments were carried out in rats and not in humans. Other authors have argued that REM sleep deprivation exerts activating effects in rats, arguing that it is a good model of mania (Gessa et al., 1995), and hence, also a good model to study the antidepressant effects of REM sleep deprivation. However, there was no difference in the overall activity for one hour after the end of REM sleep deprivation among the three groups of rats, arguing against a specific increase in activity only in the REM sleep-deprived group. In addition, we found that the activity of REM sleep-deprived rats after the deprivation was orientated mainly to grooming. Rats may not groom properly when they are on the small platform, so it is reasonable to expect that they dedicate a significant part of their activity to grooming when they return to their home cage. Another possibility is increased grooming due to stress (van Erp et al., 1994). Again, our interpretation is that the activity of the rats was directed to satisfaction of drives that were not satisfied during the procedure, such as grooming in the sleep-deprived group, or eating in all of the groups, rather than the REM sleep deprivation, per se.

In conclusion, our results indicate that REM sleep deprivation does not induce changes in 5HT release, at least in the hippocampus. Suggestion for future study is to perform experiments using total sleep deprivation, which causes a more dramatic behavioral effect.

ACKNOWLEDGMENTS

We wish to thank Joyel Almajano, Oscar Freijoo and Adit Kirszenbaum for technical assistant, and Larry Ackerson for his assistant in HPLC analysis. Funding was provided by NIMH grant MH02012 K08 and NINDS grant NS02808.

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