Changes in pontine unit activity with REM sleep deprivation

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Short term rapid eye movement (REM) sleep deprivation produced a decrease in waking discharge rates of presumably noradrenergic pontine 'REM sleep-off' cells and an increase in waking discharge rates of pontine 'REM sleep-on' cells. These changes can be viewed as a correlate of increased REM sleep pressure. Slowing of REM sleep-off cells in waking is hypothesized to counteract the functional effects of REM sleep loss on noradrenergic receptor sensitivity This slowing and the resulting reduction in norepinephrine release may contribute to the loss of vigilance seen with sleep deprivation.

INTRODUCTION

Rapid eye movement (REM) sleep deprivation produces a number of behavioral changes including hyperphagia, hypersexuality[9,10,36], increased aggressiveness[15], increased activity levels[34], reduction of seizure threshold[8], and reversal of endogenous depression[37]. These changes are parallelled by alterations in evoked responses[18,27,35]. REM sleep deprivation may also produce shifts in receptor sensitivity[1,22,23,32]. However, most of these changes take as long as 2 weeks to develop, indicating that the brain is able to compensate for the effects of short-term REM sleep deprivation. Total REM sleep deprivation in rats is lethal after 16–54 days[19].

Despite the large number of studies on the behavioral effects of REM sleep deprivation, there have not, to our knowledge, been any studies of neuronal activity during sleep deprivation. The chronic microwire technique[14,31] is capable of maintaining stable single-unit recordings for periods of several days. We have taken advantage of this extraordinary stability to study neuronal activity during REM sleep deprivation using the platform deprivation technique[36]. Our target for these initial studies was the dorsolateral pons, an area known to be critically involved in generating REM sleep, and to contain neurons that selectively increase or decrease their activity during this state[29]

MATERIALS AND METHODS

Three mongrel cats served as subjects Surgical and recording procedures were as described previously[31].

Before surgery the cats were habituated to the experimental chamber and slept on a 'large' (30 × 35 cm) platform mounted 27 cm above the surface of a 5 cm deep pool of water. They were also habituated to a 'small' (12 × 12 cm) platform for 6 h a day for at least two sessions spaced at 1–2 day intervals When a unit with signal/noise ratio > 3/1 was discriminated, it was recorded for at least 1 h to confirm the stability of the signal The cat was then transferred to the large control platform. The platforms were in a well ventilated 55 × 58 × 85 cm chamber Baseline recordings with the cat on the large platform were continued until at least 3 REM sleep episodes were obtained

In order to classify cells into types on the basis of their sleep discharge pattern during baseline recordings, the discharge rate of neurons for 2 min continuously during REM sleep and equal periods during the preceding non-REM sleep and quiet waking[33] periods was determined Unit discharge during 3–8 episodes of REM and non-REM sleep for each neuron were counted using a MINC 1123 computer and compared by t-test A mean of 13 6 h of baseline data was collected for the determination of baseline sleep state distribution.

After the control recording was completed, the large platform was replaced, in the same chamber, by the small platform, where the cat was able to stand, turn and sit Though the cat could go into non-REM sleep on the small platform, it was unable to assume the side-down posture required for REM sleep During the deprivation procedure the cat was fed once a day during a 30 min period between 12.00 and 13 00 h For feeding, the cat was removed from the platform and given one can (160 g) of moist Kal Kan cat food and water ad lib The cat was under constant observation during the feeding period to be sure that no sleep occurred In two cases, polygraph recording was carried out during the feeding period to

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confirm the absence of sleep. The deprivation period lasted a mean of 28.8 h. At the end of deprivation, the smaller platform was replaced by the larger one used during the baseline recording, for the sleep recovery period, which lasted a mean of 32.4 h.

Stability of unit waveforms was assessed by oscilloscopic monitoring of the waveform at least once every 15 min, throughout the studies. The unit waveforms were periodically photographed for comparison with the initially observed waveform. Unit potentials from cells used in the study either showed no change or gradual change in signal-to-noise ratio throughout the experiment. Cells with abrupt changes in signal to noise ratio or waveshape were dropped from the study. The pattern of change in discharge rate with sleep state, the waveshape, and the behavioral correlates of discharge[31], were in every case the same after recovery from deprivation as they were during the baseline The duration of the recovery observation period was equal to the duration of the deprivation period, except for those cases in which the unit recording was lost. Once a unit was studied in the deprivation paradigm, a period of at least 7 days was allowed for recovery before any new unit was studied in the same cat.

Sleep states were scored, using the Ursin–Sterman criteria[33]. Scoring epochs were 60 s long. Four 1 h periods were independently scored by two investigators and interscorer reliability was 91% (97% for REM sleep, 97% for waking, 82% for slow wave sleep 1 (SWS1) and 74% for SWS2). To determine the effect of the REM sleep deprivation procedure on waking firing rate, unit firing rates from the last h of the predeprivation (baseline) condition, last h of deprivation and last h of the recovery period were counted. In each 1 h period, the first 5 episodes of quiet waking[33] were scored for unit discharge rate (average total duration of quiet waking samples in each 1 h period = 17.5 min) The significance of differences was determined with the BMDP statistical package. A $y = \log(x + 0.5)$ transformation was made to reduce skewness and increase variance homogeneity between cells and cell types A repeated measure, two-way analysis of variance (module 2V) was then used to determine the significance of observed changes.

Recording sites were labelled with iron deposition by passing a 15 μA current for 5 s at the bottom of each microwire track. Cats were sacrificed with an overdose of sodium pentobarbital, perfused with saline and 10% formalin and the brains were histologically analyzed with standard techniques.

RESULTS

Effects of platform on sleep

Confinement to the small platform completely eliminated REM sleep (Table I). The small platform restriction also reduced the total time in non-REM sleep, with the most marked effects on SWS2. While REM sleep is always accompanied by a complete loss of muscle tone, the nuchal muscles are often atonic in SWS2[29,31,33]. Thus this atonia, which would be impossible to maintain on the small platform, could account for the reduction in SWS2

along with REM sleep. Previous studies of the platform deprivation technique in cats have not separately scored for SWS1 and SWS2[27,36]. The hand awakening technique for human REM sleep deprivation, is known to reduce non-REM sleep time[38]. Thus while REM sleep deprivation techniques produce a disproportionate reduction in REM sleep, non-REM sleep stages are also altered. Since REM sleep-off cells show greatly reduced activity levels during REM sleep and the immediately preceding SWS2 state[5,17], our deprivation conditions greatly reduced both of the states during which locus coeruleus cells are inactive. REM sleep-on cells start discharging in SWS2 prior to REM sleep onset[28], and therefore this period of acceleration was also eliminated by the deprivation. Total time in REM sleep and SWS2, and proportion of sleep devoted to REM sleep and SWS2 increased to above baseline levels during the recovery period (Table I).

Effects of REM sleep deprivation on discharge rate

Sixteen cells were each held for an average of 74.8 h of continuous recording during which time REM sleep was deprived for an average of 28.8 h (Table II). During this period an experimenter was continuously present to monitor unit waveshape. Cells which could not be held for at least 48 h continuously, or which showed changes in unit waveform as described above, were dropped from the study. For purposes of data analysis, cells were divided into 3 groups on the basis of their baseline discharge patterns, 'REM sleep-off' ($n = 5$), 'REM sleep-on' ($n = 3$), and 'other' ($n = 8$) cell types. REM sleep-off cells were defined as those with significantly ($p < 0.05$, t-test) lower rates in REM sleep than in both non-REM sleep and active waking[33]. Two of the 3 tested REM sleep-off cells stopped firing during the scruff immobility reflex, as described by Reiner[26]. REM sleep-on cells were defined as those with significantly higher discharge rates in REM sleep than in both non-REM sleep and active waking. All cells not meeting the criteria for REM sleep-on or REM sleep-off cells were classified as 'other'. The most common pattern seen in the 'other' cells was elevated activity during both waking and REM sleep when compared to non-REM sleep. Recorded cells

TABLE I

Sleep state changes during deprivation and recovery in REM sleep-off cells in % total time

	%Awake	%Sleep	%SWS1	%SWS2	%REM	Hours
Baseline	41.3	58.7	27.2	17.0	14 6	6.8
Deprivation	78 7	21.3	17.8	3.5	0 0	20.4
Recovery	47.2	52.8	16 4	18.6	17.8	14.0

TABLE II

Duration of studies in hours

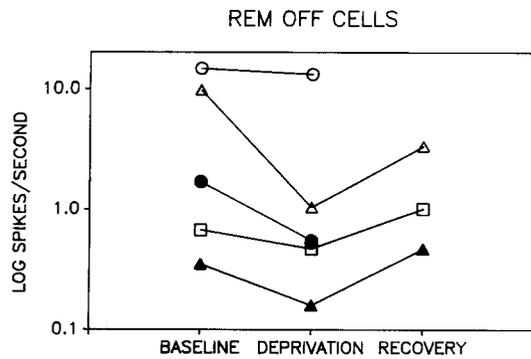|	n	Baseline	Deprivation	Recovery
REM sleep-off cells	5	6.8	20.4	14.0
REM sleep-on cells	3	15.7	41 7	39.0
Other cells	8	17.0	26.4	41.4
Overall	16	13.6	28.8	32.4

REM OFF CELLS

Fig. 1 Effect of REM sleep deprivation on waking discharge rates of 'REM sleep-off' cells. Each tracing shows the rate changes seen in a single unit during baseline, REM sleep deprivation and recovery. Deprivation reduced discharge rate in all recorded cells, with return towards baseline levels with recovery sleep.

OTHER CELLS

Fig. 3 Effect of REM sleep deprivation on waking discharge rates in 'other' cell types. No consistent pattern was seen in this group.

were distributed throughout the dorsolateral pons between P1 and P5, L1.5 and L3.0, H0 and H–5. REM sleep-on and -off cells were both located in the locus coeruleus and in the subcoeruleus region, as previously described[2,5,16,17,28].

Two-way ANOVA revealed no significant difference between waking discharge rate in baseline, deprivation and recovery periods when considering all cell types. There was also no significant difference between baseline and recovery periods for any single cell type. However, there was a significant interaction between cell type and the change induced by deprivation ($p < 0.002$, two-way repeated measure ANOVA, deprivation vs baseline + recovery/2 × cell type). This effect was due to opposite directions of change in REM sleep-off and REM sleep-on cells. Fig. 1 shows the response of REM sleep-off cells to REM sleep deprivation. Waking discharge rate was reduced during deprivation in all 5 cells. Rates returned towards baseline levels in all 3 cells that could be recorded after recovery sleep. The opposite pattern was
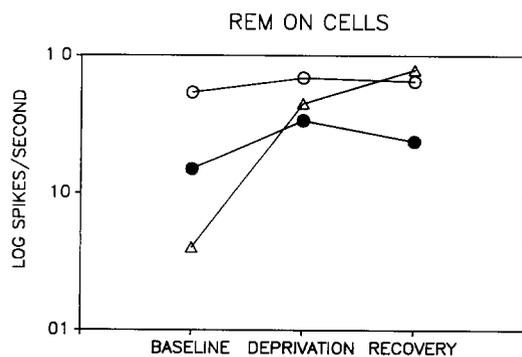
seen in two of the 3 REM sleep-on cells (Fig. 2). The third REM sleep-on cell did not show a return to baseline levels by the end of the recovery period. We were unable to find any variable that distinguished this cell from the cells whose waking discharge rates returned towards baseline levels after recovery sleep. As a group, the 'other' cell types showed no consistent change in discharge rate with deprivation (Fig. 3).

DISCUSSION

We have found that REM sleep deprivation alters waking firing rates in pontine REM sleep-on and REM sleep-off cells, while having no consistent effect on other cell types. In any sleep deprivation experiment, the issue of stress as a cause of the observed effects arises. In the present study, we have taken the approach of using a short period of REM sleep deprivation to minimize stress. We have also used a large platform control that produced minimal REM sleep deprivation, while providing a comparable behavioral environment. Recovery and baseline REM sleep occurred on the large platform, showing that the changes observed during deprivation were not secondary to platform restriction. Nevertheless, one could still argue that the small platform was more stressful than the large platform and that this accounts for the altered unit activity rates. However, the rate changes we observed in REM sleep-off units were not consistent with such an explanation. A number of studies have shown that stress tends to *increase* firing in REM sleep-off neurons, and to also increase norepinephrine (NE) release[2,3,4,5,11,12,17]. REM sleep-off cells in the dorsolateral pons are thought to be predominantly noradrenergic cells of the locus coeruleus complex[5,16,17,25]. In the present study we find that REM sleep deprivation produced a *decrease* in unit firing, the opposite of the change predicted by a stress explanation.

The discharge rates during baseline and deprivation

REM ON CELLS

Fig. 2. Effect of REM sleep deprivation on waking discharge rates of 'REM sleep-on' cells Deprivation increased discharge rate in all recorded cells. After recovery sleep, waking discharge levels decreased in two cells, but continued to increase in third
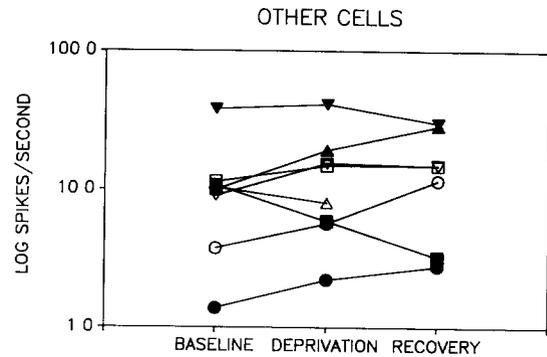
conditions were taken during desynchronized EEG conditions, utilizing the usual scoring criteria[33]. Thus the altered discharge rate could not result from increased sampling at times when the EEG was synchronized in the REM sleep deprivation condition. Nevertheless, one could view the REM sleep-off unit activity change as a *result* of a more subtle 'state' change during deprivation. That is, sleepiness could be hypothesized to produce a slowing of discharge in REM sleep-off cells without observable change in the EEG, in addition to the previously reported intrusion of 'microsleep' episodes accompanied by EEG changes[13]. Subtle changes in the EEG might be observable using power spectral analysis or other techniques. Conversely, one could view the altered activity in REM sleep-off cells as a *cause* of a subtle state change. There is ample evidence that NE release by the locus coeruleus is critically involved in the regulation of states of alertness[3,5,17]. The present data do not allow us to choose between these two alternatives. Indeed, since the locus coeruleus is in active interaction with adjacent cells of the cholinergic pontine nuclei in the regulation of level of arousal[4,6,7,20,21], it is not likely that the cause/effect distinction is useful in this case. In any event, the present results demonstrate that diminished activity in REM sleep-off cells and increased activity in REM sleep-on cells is a correlate of REM sleep deprivation.

One of the key early observations after the discovery of REM sleep was that REM sleep deprivation produced increased REM sleep 'pressure' that was manifest in more frequent attempts to enter REM sleep and a large REM sleep rebound when the deprived subject was left undisturbed[9,24,36]. The changes in unit activity that we report here can best be seen as a correlate of this increased REM sleep pressure. Thus, REM sleep-off cells are by definition those cells which decrease rate, and REM sleep-on cells those which increase rate, in REM sleep. We have found that during REM sleep deprivation, both cell types show rate changes in the direction of their normal rate changes during REM sleep. These changes can be seen as either a cause or as a result of the greater REM sleep pressure that develops during deprivation, as well as being a correlate of changes in arousal brought about by sleep deprivation.

Although we are seeing a shift in activity towards the REM sleep pattern in the REM sleep-on and REM sleep-off cell groups, we must emphasize that these changes are much smaller than those normally occurring in REM sleep. However, it is possible that longer-term REM sleep deprivation would produce even more marked changes in unit activity in these cells. We have recently hypothesized[30] that a key function of REM sleep is to reduce activity in noradrenergic REM sleep-off cells, and thereby upregulate or prevent downregulation of NE receptors.

Two strategies by which evolution would have compensated for the loss of the hypothesized REM sleep receptor regulation effect during sleep disruption can be envisioned. In the first strategy, a reduction in the waking discharge rate of noradrenergic cells during sleep deprivation, would tend to produce changes in receptors in the same direction as occur in REM sleep, albeit to a lesser extent. Such rate changes during deprivation would be the expression of an adaptive mechanism tending to attenuate the neural effects of REM sleep loss, by producing in waking some of the same reduction in NE release normally seen in REM sleep. This would slow the downregulation of NE receptors hypothesized to occur during waking. In emergency situations, an increased NE release would act on receptors whose sensitivity has been only slightly reduced. The disadvantage of this strategy would be a reduced level of tonic vigilance.

A second physiological strategy by which the organism might cope with the effects of REM sleep deprivation and the hypothesized NE receptor downregulation that would result, would be by increased tonic NE release. This would be mediated by increased discharge rates in noradrenergic cells and would tend to compensate for the reduction in NE receptor sensitivity. However, this strategy, by increasing NE availability would tend to accelerate receptor downregulation. Therefore, while this would initially be adaptive, by maintaining normal levels of vigilance, over a long period it would progressively reduce the responsiveness of the NE system to phasic release. Our data indicate that it is the first strategy which has evolved.

The present results demonstrate the feasibility of combining unit recording with sleep deprivation. Further studies using these techniques should help to shed light on the changes in neuronal activity and responsiveness resulting from sleep loss.

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