INTRODUCTION

Selective rapid eye movement sleep (REM) deprivation triggers a specific response. During the deprivation there are progressively more frequent attempts at transitions into REM. After the deprivation there is a REM sleep rebound. The increased number of attempts to enter REM can be interpreted to represent the buildup of a pressure that homeostatically regulates REM sleep (Benington et al. 1994a; Endo et al. 1997; Ocampo-Garcés et al. 1999). The REM sleep rebound can be interpreted as the expression of the homeostatic regulatory mechanism that compensates for REM deficit with respect to a reference level (Parmeggiani et al. 1980; Rechtschaffen et al. 1999).

It has been proposed that REM homeostasis expresses a specific relationship between REM sleep and non-REM sleep (NREM) (Benington and Heller 1994) so that the function of REM serves to reverse the consequences of some specific feature of NREM. Alternatively the regulatory mechanism may involve just the presence and absence of REM, being the REM need a function of the time elapsed without REM rather than the cumulated time in NREM or wakefulness (Endo et al. 1997, 1998b; Vivaldi et al. 1994a).

Previous long-term total-sleep-deprivation studies in the rat have suggested that drastic NREM reductions do not preclude a REM rebound (Endo et al. 1997; Rechtschaffen et al. 1999). The present study was designed to assess the interaction between REM homeostasis and NREM sleep at a much shorter time scale. We compared both the buildup of REM propensity and the REM rebound among three deprivation schedules, all of which involved 3 h of REM deprivation but differed in the coexisting NREM deprivation.

METHODS

Data acquisition

Twenty-two Sprague-Dawley rats weighting 300–350 g were implanted under deep chloraluminal 3 ml/kg ip anesthesia with four epidural and two neck-muscle stainless steel electrodes. Two of the epidural electrodes were placed in the midline for theta activity detection. Rats were housed in a 30 × 30 × 25 cm cage, placed within a sound-isolated cube, under a 12:12 light:dark schedule, ambient temperature 21–24°C, with water and food ad libitum. The data-acquisition program stored incidence of delta waves, sigma trains, and muscle spikes or movement artifacts in 15-s epochs. An off-line state scoring algorithm assigned each epoch to wake (W), NREM, or REM (Rocagliolo and Vivaldi 1991). The 3-h experimental sessions and their corresponding baseline hours presented in this report were additionally recorded on paper and visually analyzed by two independent scorers.

Sleep-deprivation procedure

Ten days after surgery, rats were adapted for 2 days to the cages before recordings. The deprivation periods started at hour 6 after lights-on and lasted for 3 h. Protocol 3R consisted of 3 h of specific REM deprivation. Protocol 1T2R consisted of 1 h of total sleep deprivation in the Rat. J Neurophysiol 84: 2699–2702, 2000. During specific rapid eye movement (REM) sleep deprivation its homeostatic regulation is expressed by progressively more frequent attempts to enter REM by a compensatory rebound after the deprivation ends. The buildup of pressure to enter REM may be hypothesized to depend just on the time elapsed without REM or to be differentially related to non-REM (NREM) and wakefulness. This problem bears direct implications on the issue of the function of REM and its relation to NREM. We compared three protocols that combined REM-specific and total sleep deprivation so that animals underwent similar 3-h REM deprivations but different concomitant NREM deprivations for the first 2 (2T1R), 1 (1T2R), or 0 (3R) hours. Deprivation periods started at hour 6 after lights on. Twenty-two chronically implanted rats were recorded. The median amount of REM during all three protocols was 1 min. The deficits of median amount of NREM in minutes within the 3-h deprivation periods as compared with their baselines were, respectively, for 2T1R, 1T2R, and 3R, 35 (43%), 25 (25%), and 7 (7%). Medians of REM rebound in the three succeeding hours, in minutes above baseline, were, respectively, 8 (44%), 9 (53%), and 9 (50%), showing no significant differences among protocols. Attempted transitions to REM showed a rising trend during REM deprivations reaching a final value that did not differ significantly among the three protocols. These results support the hypothesis that the buildup of REM pressure and its subsequent rebound is primarily related to REM absence independent of the presence of NREM.

Rapid Communication

Homeostasis of REM Sleep After Total and Selective Sleep Deprivation in the Rat

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Ocampo-Garcés, Adrián, Enrique Molina, Alberto Rodríguez, and Ennio A. Vivaldi. Homeostasis of REM sleep after total and selective sleep deprivation in the rat. J Neurophysiol 84: 2699–2702, 2000. During specific rapid eye movement (REM) sleep deprivation its homeostatic regulation is expressed by progressively more frequent attempts to enter REM by a compensatory rebound after the deprivation ends. The buildup of pressure to enter REM may be hypothesized to depend just on the time elapsed without REM or to be differentially related to non-REM (NREM) and wakefulness. This problem bears direct implications on the issue of the function of REM and its relation to NREM. We compared three protocols that combined REM-specific and total sleep deprivation so that animals underwent similar 3-h REM deprivations but different concomitant NREM deprivations for the first 2 (2T1R), 1 (1T2R), or 0 (3R) hours. Deprivation periods started at hour 6 after lights on. Twenty-two chronically implanted rats were recorded. The median amount of REM during all three protocols was 1 min. The deficits of median amount of NREM in minutes within the 3-h deprivation periods as compared with their baselines were, respectively, for 2T1R, 1T2R, and 3R, 35 (43%), 25 (25%), and 7 (7%). Medians of REM rebound in the three succeeding hours, in minutes above baseline, were, respectively, 8 (44%), 9 (53%), and 9 (50%), showing no significant differences among protocols. Attempted transitions to REM showed a rising trend during REM deprivations reaching a final value that did not differ significantly among the three protocols. These results support the hypothesis that the buildup of REM pressure and its subsequent rebound is primarily related to REM absence independent of the presence of NREM.
deprivation followed by 2 h of specific REM deprivation. Protocol 2T1R consisted of 2 h of total sleep deprivation followed by 1 h of specific REM deprivation. Each rat was subjected up to two times to a deprivation period. One day of recovery was left between trials. A total of 12 3R, 11 2T1R, and 8 1T2R obtained in seven, nine, and six rats respectively, were successfully run. One of the 3R cases was not fit for analysis of the 3 h after the deprivation period.

The appearance of the first unequivocal signs of NREM [increasing amplitude of cortical electroencephalogram (EEG) and sleep spindles] in total sleep deprivation or of REM (burst of spindles, theta activity, and lowering muscle tonus) in specific REM deprivation, prompted an intervention to interrupt the transition. The stimulus was the gentle movement of the cage that was located over a foam cushion by means of a mechanism connected to the outside of the isolation cube. Interventions were documented on polygraphic paper. In <20% of cases, REM ended spontaneously before the intervention, a situation that was counted as a missed REM event. To be counted as distinct events, transitions and missed REM had to be separated by at least one 15-s epoch. The transition epochs were added to total sleep time (TST).

Data analysis

The variables analyzed were the amount of the three states, the REM/TST ratio, REM transitions, and REM transition index. The REM transition index corresponded to the number of transitions per 10 min of TST. The variables REM/TST and REM transition index were calculated only if TST amounted to at least 3 min in a half-hour bin. Baselines were calculated for each hour of each rat by averaging the corresponding values of the two baseline days. Values from rats with two runs of the same protocol were similarly treated. Wilcoxon’s matched-pairs signed-ranks tests were performed to compare experimental results of a given deprivation period with its corresponding baseline. Kruskal-Wallis ANOVA by ranks test was applied among the protocols. The relationship between amount of NREM in the three experimental hours and of REM in the three consecutive hours was assessed by a Spearman correlation analysis.

RESULTS

Table 1 shows summary statistics for states in baseline and experimental conditions. As intended, during deprivation periods (hours 6–9) REM was always suppressed, and NREM was present in amounts that varied according to each protocol. The deficits in median amount of NREM in the 3 h as compared with their baseline, were 35 min (43%) in 2T1R, 25 (25%) in 1T2R, and 7 (7%) in 3R. The similarity of REM rebounds can be assessed by comparing the 3 h after deprivation periods (hours 9–12). The medians of REM rebound in minutes above baseline, were 8 (44%) after 2T1R, 9 (53%) after 1T2R, and 9 (50%) after 3R.

Time courses of NREM and REM during and after the deprivation periods

Figure 1 shows that during total deprivation NREM was markedly diminished (<5% of baseline) while REM was entirely absent. During specific REM deprivation, its value was <10% of baseline. There is a NREM rebound after the 2-h total sleep deprivation in 2T1R. The REM rebound is always present in the first hour post deprivation period (hour 9) without significant differences among protocols. It can be observed that at hour 9 the REM/TST ratio is significantly increased in all three paradigms, including 2T1R where at that time NREM and REM rebounds coexist.

Table 1. Median (interquartile range) of NREM, REM, and W before, during, and after the protocols and corresponding baselines

<table>
<thead>
<tr>
<th>Protocol</th>
<th>Time</th>
<th>Condition</th>
<th>NREM</th>
<th>REM</th>
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</tr>
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<td></td>
<td></td>
<td>1T2R, min</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td>3R, min</td>
<td></td>
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<tr>
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<td>Baseline</td>
<td>82 (25)</td>
<td>87 (18)</td>
<td>84 (16)</td>
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<td>97 (6)</td>
<td>94 (10)</td>
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<td>6–9</td>
<td>Baseline</td>
<td>82 (29)</td>
<td>100 (6)</td>
<td>97 (7)</td>
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<td>1 (2)*</td>
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<td></td>
<td>Experimental</td>
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<td>64 (8)</td>
<td>67 (14)</td>
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</table>

Time of day as hours after lights-on. Experimental and baseline data in 3-h groups: before (3–6), during (6–9), and after (9–12) protocol implementation. n = 9, 6, and 6 for 2T1R, 1T2R, and 3R, respectively. Numbers in parentheses are n values. NREM, non-rapid eye movement; W, waking; 2T1R, 2 h of total sleep deprivation followed by 1 h of specific sleep deprivation; 1T2R, 1 h of total sleep deprivation followed by 2 h specific REM deprivation; 3R, 3 h specific REM sleep deprivation. * P < 0.05 Wilcoxon’s matched pairs test of experimental vs. corresponding baseline condition. † P < 0.01 Kruskal-Wallis analysis of variance by ranks test among the experimental condition of the three protocols.

REM transition index

Figure 2 shows that in all protocols the REM transition index rapidly reaches significance over baseline when NREM is allowed. (This occurs at bin 1 in 3R, at bin 4 in 1T2R, and at bin 5 in 2T1R.) In 1T2R, the transition index is similar to that of 3R at bin 4 (the 2nd bin in which NREM is allowed). Transitions and transition index in bins 5 and 6 do not differ among the three protocols. It should be noted that for 2T1R, the transition index increases immediately after the four bins of total sleep deprivation and is not different from 1T2R or 3R regardless of the significant simultaneous enhancement in NREM.

REM expression as a function of preceding NREM sleep

Figure 3 shows pooled data relating the amount of NREM observed during the 3 h of the deprivation periods to the amount of REM sleep observed during the first 3 h of recovery. The nonparametric regression shows no relation between NREM in the deprivation period and later REM expression [Spearman’s rho = 0.1704, P = not significant (NS)]. The statistic analysis was performed on the whole pool, but Fig. 3 distinguishes with different symbols the three protocols just to illustrate their actual effect on NREM sleep.
DISCUSSION

The central question of this work was to clarify whether REM homeostasis interacts specifically with NREM sleep in the rat. We were interested in the operation of the homeostatic process within a shorter time scale than had been previously explored in deprivation studies. We assessed the buildup of REM pressure through a 3-h deprivation and its subsequent rebound, the main experimental factor being the amount of time in which the sleep deprivation was total or REM specific. The 3R, 1T2R, and 2T1R protocols allowed, respectively, 0, 1, or 2 hours of concomitant NREM deprivation within the REM deprivation schedule.

All experiments were performed at the hour 6 after lights on, the time when the REM expression is highest and NREM expression is declining (Franken et al. 1991; Trachsel et al. 1988; Vivaldi et al. 1994b). The deprivation period was extended for 3 h because it has been reported that a 2-h REM selective deprivation in the second half of the lights-on phase induces a consistent homeostatic response in the rat (Benington et al. 1994). The number of NREM to REM transition attempts was used as an indicator of REM propensity (Benington et al. 1994; Endo et al. 1997, 1998).

During 3R (specific REM deprivation only), REM reached levels below 10% of baseline values, while the amount of NREM was not reduced, confirming the selectivity of the procedure. Furthermore in 2T1R, a rebound in the amount of NREM was observed coexisting with the ongoing REM deprivation. As intended the amounts of NREM and wakefulness during deprivation were significantly different among protocols. On the contrary, REM rebounds and final indexes of attempted transitions to REM were not significantly different among protocols.

The question of whether REM pressure builds up differentially during NREM and wakefulness is of particular interest because of its implications to the issue of REM function. NREM has been considered to act either as a substrate, because...
REM would reverse some consequence of NREM (Benington and Heller 1999), or as a primer for REM because transition into REM involves NREM (Rechtschaffen and Bergmann 1999). Inversely, emphasis has been placed on REM being required for NREM to continue but without a dose-response effect (Feinberg 1974). Our experimental design is primarily relevant to the hypothesis of a substrate role for NREM in REM homeostasis.

Our results indicate that the buildup of REM homeostatic pressure throughout the first 3 h of REM deprivation and the REM rebound after them are independent of the cumulated time in NREM sleep. These findings are consistent with results obtained in long-term sleep deprivation protocols in rats (Endo et al. 1997; Rechtschaffen et al. 1999) and humans (Endo et al. 1998) and contradict the model of NREM-dependent REM homeostasis (Benington and Heller 1994).

To make compatible the results of long-term deprivation studies with REM dependence on NREM, it has been argued (Benington and Heller 1999) and counter-argued (Rechtschaffen and Bergmann 1999) that NREM like processes may be displaced into wakefulness. It should be noted that to account for our results, the transposition of NREM processes into wakefulness would now have to be postulated to start operating after an unsuspected brief period of wakefulness. The present results then give further support to the postulate that the build up of REM pressure is primarily a function of the absence of REM rather than the consequence of a specific effect of NREM on REM homeostasis.

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