

REM-sleep propensity accumulates during 2-h REM-sleep deprivation in the rest period in rats

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Abstract

Two-hour, highly-selective, rest-period, rapid-eye-movement (REM)-sleep deprivation (RD) was performed on rats to characterize the time-course of the homeostatic response to REM-sleep loss. RD caused a dramatic and progressive increase in the frequency of attempts to enter REM sleep, suppressed non-REM sleep EEG delta power, and (in late rest period trials) was followed by a rebound increase in REM-sleep expression.

Key words: REM-sleep homeostasis; Sleep cycle; Fourier analysis; Electrical stimulation

Selective rapid-eye-movement (REM)-sleep deprivation (RD) consistently produces two effects: an increase in the frequency of attempts to enter REM sleep over the course of the deprivation period and a rebound increase in REM-sleep expression during recovery sleep [8–10]. These findings suggest that a propensity for REM sleep accumulates during RD and persists until it can be discharged via REM-sleep expression. During RD, considerable amounts of both waking and non-REM (NREM) sleep are expressed, so that REM-sleep propensity could theoretically accumulate during either waking or NREM sleep. It appears that the vast majority of investigators have believed that the function of REM sleep relates to waking, hence that REM-sleep propensity accumulates during waking. Three prominent hypotheses regarding the function of REM sleep emphasize effects of REM sleep on waking behavior [7,13,16]; these (in part) have produced a large literature on the relationship between REM sleep and learning/memory (reviewed by [14,17]),

and on behavioral (waking-related) effects of REM-sleep loss (reviewed by [9,10,21]).

The alternative view, that the function of REM sleep relates to NREM sleep and that REM-sleep propensity accumulates during NREM sleep, has not been actively debated in the literature, even though no fewer than five investigators over 25 years have presented hypotheses concerning NREM sleep-related functions of REM sleep [5,11,12,20,22]. Recent work suggests that the timing of individual REM-sleep episodes is controlled homeostatically by accumulation during NREM sleep of a propensity for REM sleep [2]. We have therefore hypothesized that REM-sleep propensity accumulates during NREM sleep and that REM-sleep episodes occur at fairly regular intervals in sleep because REM-sleep propensity must be discharged periodically to permit optimal NREM-sleep expression (cf. [12]).

The dynamics of REM-sleep homeostasis in this (NREM sleep-related) model differ markedly from those in the traditional waking-related model. The normal interval over which REM-sleep propensity accumulates is the length of one NREM-sleep episode. In rats, this interval is only about 10 min long. In the waking-related model, REM-sleep propensity accumulates during the entire active period and is discharged over the course of multiple REM-sleep episodes. According to the NREM sleep-related model, if REM sleep is suppressed during

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Abbreviations: NREM, non-REM; RD, REM-sleep deprivation; NF, non-REM sleep fragmentation; NRT, NREM sleep-REM sleep transitions; ES, electrical stimulation; DRN, dorsal raphe nucleus.

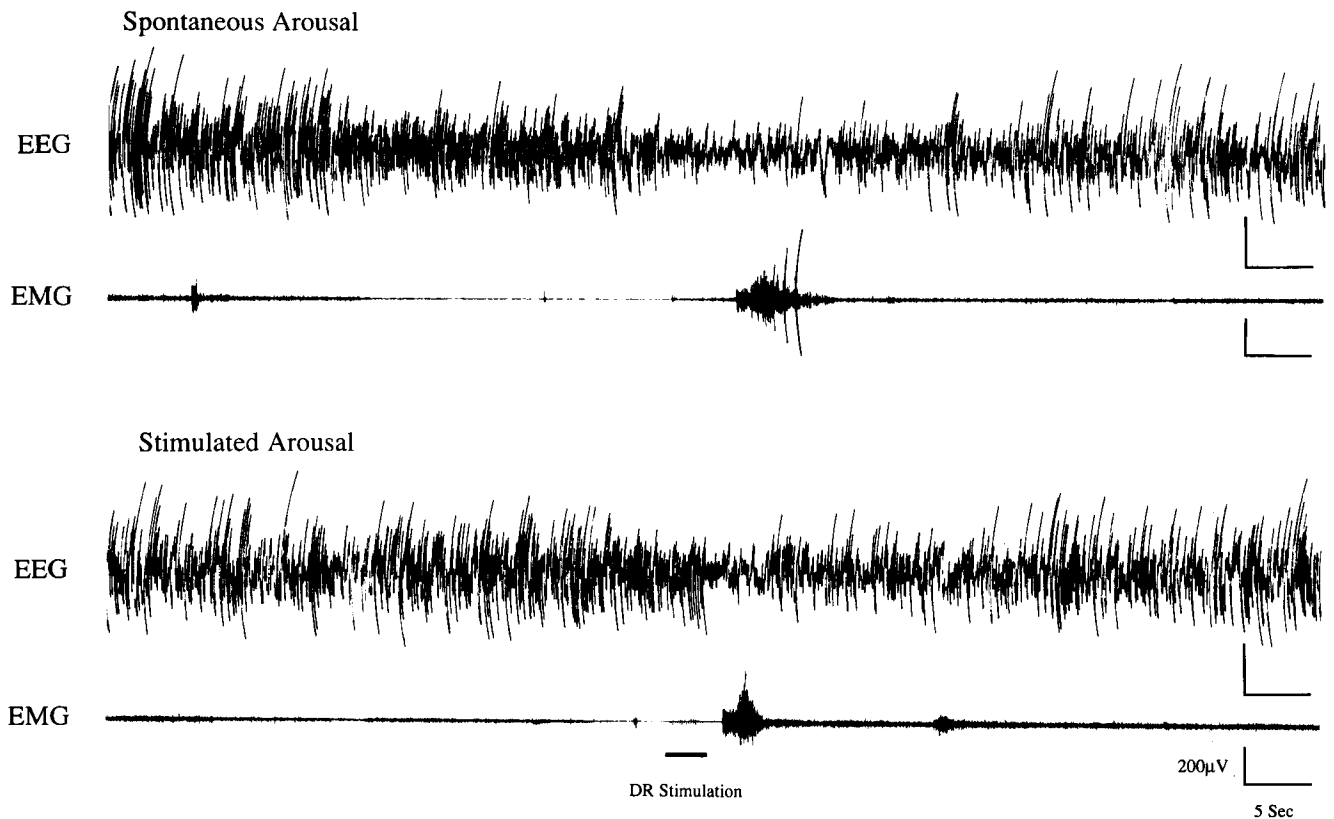


Fig. 1. Comparison of a spontaneous arousal from REM sleep with an arousal elicited by mild electrical stimulation of the DRN. Recordings are from the same animal. Illustrated are frontal-parietal EEG in one cerebral hemisphere and nuchal EMG. The DRN was stimulated at 20 μ A and 6 Hz for 3 s (horizontal bar).

sleep for a period of time that considerably exceeds the normal NREM-REM sleep cycle, then REM-sleep propensity will accumulate beyond the normal REM-sleep triggering threshold. According to the waking-related model, suppression of REM-sleep expression for a brief period during the rest period (the 12-h light period, when most sleep occurs) should not significantly increase REM-sleep propensity beyond the level accumulated during the active period.

We have therefore performed RD in rats for 2-h intervals during the rest period and characterized the effects of REM-sleep loss on the frequency of attempts to enter REM sleep (a measure of REM-sleep drive), EEG slow-wave activity in NREM sleep, and REM-sleep expression during the recovery period. Because the effects of very brief RD are the object of this study, a highly selective form of RD was needed to minimize disruption of NREM sleep during the early stages of RD. We have therefore utilized mild electrical stimulation (ES) of the dorsal raphe nucleus (DRN) at REM-sleep onset, using stimulus parameters that simulate the increase in serotonergic activity that naturally occurs at the termination of a REM-sleep episode [15,19].

Adult male Wistar rats were instrumented for electrographic recording as described elsewhere [4]. Electrodes for DRN stimulation were twisted, bipolar, 125 μ m

stainless-steel wire with teflon insulation except at the cross section of tip, having a 500–750 μ m longitudinal separation between poles. The recording environment, acclimation procedures, and methodology for obtaining electrographic recordings and performing Fourier analysis are described elsewhere [4]. Experimental sessions were preceded by acclimation of the animal to the experimenter's presence and the procedure of electrical stimulation. ES employed biphasic AC pulses (each pole alternating as cathode or anode) of 5–50 μ A, 4–6 Hz, a pulse width of 0.5 ms, in 3–4 s trains.

RD was begun at 10:00 h (early rest period) or 14:00 h (late rest period) \pm 30 min. RD was accomplished by applying DRN ES at the first signs of unambiguous REM sleep. The disruptive effect on sleep continuity of RD was controlled for by comparing RD results with time-matched 2-h sessions of NREM-sleep fragmentation (NF). The frequency of sleep interruption (per hour of NREM sleep) was 28.1 for RD vs. 29.1 for NF in early rest period and 39.4 for RD vs. 34.4 for NF in late rest period. Each animal ($n = 6$) received all four treatments in randomized order. Only one treatment was applied on any given day. Time-matched baseline recordings were collected from animals on days during which they were not disturbed.

Two-way, repeated-measures ANOVAs were calcu-

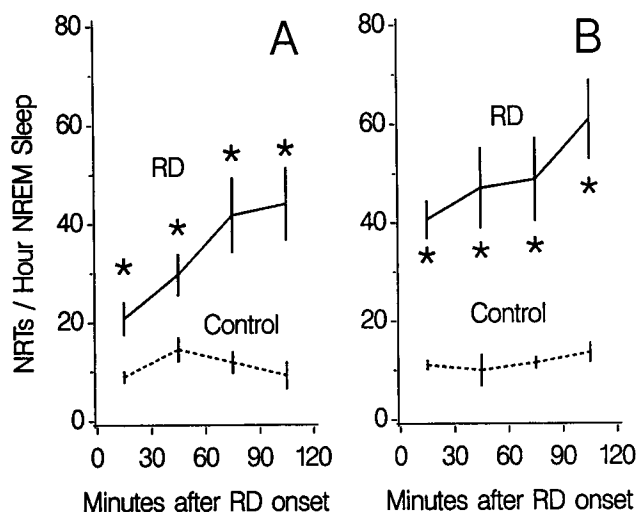


Fig. 2. The frequency of transitions from NREM sleep to REM sleep (NRTs) during RD. Plotted is the number of NRTs per hour of NREM sleep (\pm S.E.M.) in four 30-min intervals during RD and time-matched NF control sessions. Stars denote intervals that differ significantly from control ($P < 0.05$, Tukey's HSD test). A represents early rest-period data and B late rest-period data.

lated using SAS, version 6.03. Data from one animal were unusable because NREM-sleep time was 0 in the final 30-minute segment of RD, preventing calculation of NREM-sleep EEG delta power or NRT frequency. Data were lost from one animal for the period following RD, preventing calculation of REM-sleep rebound effects. Thus, $n = 5$ for all ANOVAs.

Mild DRN ES produces natural arousals from REM sleep and does not otherwise disturb the animal. The electrographic characteristics of a stimulated arousal from REM sleep are compared to those of a spontaneous termination of REM sleep in Fig. 1. In both cases, there is a brief desynchronization of the EEG followed by a burst of EMG activity. Return to NREM sleep occurs within 20–30 s. Spontaneous and stimulated arousals are also identical as regards behavioral effects. The animal twitches briefly, raises its head, and opens its eyes. Stimulated arousals are accompanied by no behavioral evidence of stress or abnormal excitation. Normal amounts of NREM sleep are expressed during the entire RD period. Animals express NREM sleep for 66.3% of the 2-h RD period in the early rest period and 64.8% in the late rest period. This compares with 64.5% and 62.8% for early and late rest period NF control, respectively.

Two hours of RD in either early or late rest period increases the frequency of attempts to enter REM sleep compared to NF control. By the end of the RD period, NRT frequency has increased from baseline levels of 12–18 per hour of NREM sleep to 44.1 per hour of NREM sleep in the early rest period and 60.9 per hour of NREM sleep in the late rest period (see Fig. 2). This effect is significant in both early and late rest period ($P < 0.001$). The effect of time within treatment is signif-

icant in early rest period ($P < 0.05$). RD decreases NREM-sleep EEG delta power versus NF control in both early and late rest period (see Fig. 3). The effect is progressive, being most pronounced in the last 30-minute segment of RD. The effects of treatment, time within treatment, and the interaction effect are significant in both early and late rest period ($P < 0.001$). REM-sleep expression is significantly elevated during the 4 h after RD in the late rest period but not in the early rest period (see Fig. 4, $P < 0.05$).

Two hours of RD during the rest period in rats produces a dramatic increase in the frequency of attempts to enter REM sleep, reduces NREM-sleep EEG delta power, and (in the late rest period) results in a small but significant REM-sleep rebound during the recovery period. Increasing frequency of attempts to enter REM sleep and REM-sleep rebound are the classic effects of longer lasting RD on which has been based the conclusion that REM-sleep propensity accumulates as a result of RD. The fact that the changes in the frequency of attempts to enter REM sleep and in NREM-sleep EEG delta power become progressively more pronounced during the 2-h RD period suggests that these are effects of accumulation of REM-sleep propensity. After just 2 h of RD, NREM sleep is severely fragmented. The behavioral and physiological effects of long-term, 'selective' RD may therefore result from disruption of NREM sleep rather than REM-sleep loss per se.

These immediate effects of RD are discernible because the technique used for interrupting REM sleep is highly specific and does not appear to reduce NREM-sleep expression or otherwise disturb the animal. Mild electrical stimulation of the DRN mimics the natural change in activity of serotonergic neurons that accompanies spon-

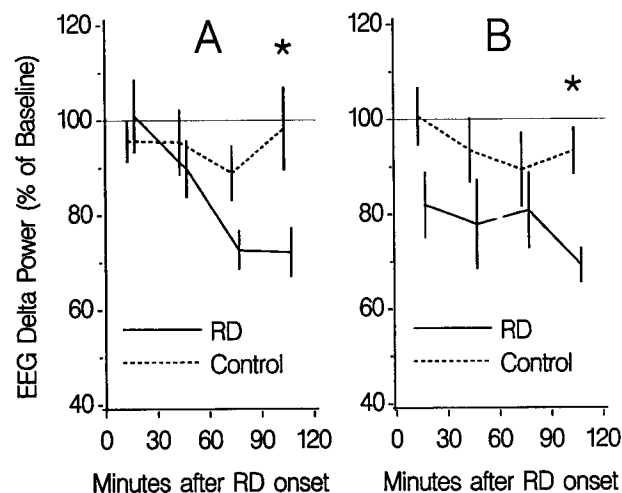


Fig. 3. EEG delta power in non-REM sleep during REM-sleep deprivation. Plotted is NREM-sleep EEG delta power as percentage of time-matched baseline recordings (\pm S.E.M.) in four 30-min intervals during RD and time-matched NF control sessions. Stars denote intervals that differ significantly from control ($P < 0.05$, Tukey's HSD test). A represents early rest-period data and B late rest-period data.

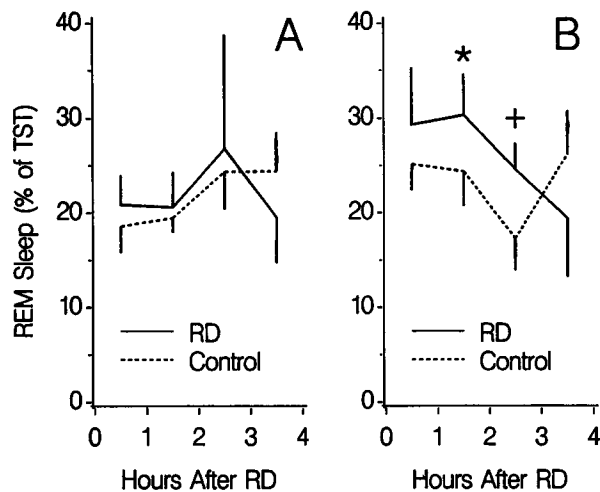


Fig. 4. REM-sleep expression during 4 h of recovery sleep following 2 h of REM-sleep deprivation. Plotted is REM-sleep expression as percentage of total sleep time (\pm S.E.M.) in four 1-h intervals of recovery sleep following RD and time-matched NF control sessions. Symbols denote intervals that differ significantly from control (* $P < 0.05$, * $P < 0.07$, Tukey's HSD test). A represents early rest-period data and B late rest-period data.

taneous termination of REM sleep [15,19]. Electrical stimulation of serotonergic neurons in this protocol does not considerably augment the endogenous activity of serotonergic neurons, as RD is achieved by only 20–25 stimulus pulses applied just 30–40 times per hour. Moreover, the effects of stimulus-induced serotonin release are controlled for in the NF condition, which entails an equivalent degree of DRN stimulation.

This study differs from previous RD experiments in that evidence is found of accumulation of REM-sleep propensity over a very brief interval. After 2 h of selective RD, drive to enter REM sleep appears to be dramatically elevated. By contrast, as much as 12 h of total sleep deprivation (comprising 18 times as much waking as in the 2-h RD intervals considered here) produces virtually no rebound increase in REM-sleep expression [18]. If REM-sleep propensity accumulates during waking, then the amount accumulated over the course of the entire active period should far outweigh the additional accumulation during the 40 min of waking expressed in the RD interval. If, on the other hand, REM-sleep propensity accumulates during NREM sleep and is largely discharged in each REM-sleep episode, then suppression of REM sleep for 2 h produces an accumulation of REM-sleep propensity that far exceeds the normal REM-sleep triggering threshold. The dramatic effects of brief RD on sleep structure are explicable only by this model.

Our findings are corroborated by 2 other studies. Beersma et al. [1] found that 5 h of RD in humans produces a significant REM-sleep rebound during 2 h of recovery sleep immediately following RD, and suppresses NREM-sleep EEG delta power during the RD period. Borbely et al. [6] found that the frequency of

attempts to enter REM sleep increases markedly during 2 h of RD in rats immediately following a 24-h total sleep deprivation period. If REM-sleep propensity accumulates during waking, the 24 h of total sleep deprivation in that study should have resulted in an accumulation of REM-sleep propensity far outweighing the additional increment accumulated during just 2 h of RD. The dramatic increase in the frequency of attempts to enter REM sleep during the 2-h RD interval suggests that accumulation of REM-sleep propensity during 24 h of total sleep deprivation (spent in waking) was small compared to accumulation during just 2 h of selective RD (spent mostly in NREM sleep).

The idea that REM sleep is functionally and homeostatically related to NREM sleep rather than waking has far-reaching implications. Conversely, a number of well-known phenomena are commonly taken as evidence that REM-sleep propensity accumulates during waking rather than NREM sleep. These include the occurrence (in laboratory animals but not humans) of a REM-sleep rebound after 24 h total sleep deprivation, the occurrence of sleep-onset REM-sleep episodes in narcoleptics, and the apparent predominance of REM sleep in the sleep of infants. This report does not allow space enough to consider these many issues. Both the implications of and findings for and against the hypothesis that REM-sleep propensity accumulates during NREM sleep are addressed in detail in a review and formal presentation of that hypothesis [3].

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