The Effects of Sensory Stimulation on REM Sleep Duration

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Summary: Previous experiments have demonstrated that auditory (AS) and/or somatosensory (SS) stimulation can increase the duration of REM sleep periods in rats, cats and humans. The objectives of this study were to determine whether repeated AS stimulation causes habituation to the stimulus and whether any additive effects could be obtained with the simultaneous application of AS and SS. Three experimental procedures were used in this study. In experiment 1, animals were recorded for 4 consecutive days with AS, followed by a post-stimulus session. In experiment 2, they were recorded for 24 hours with AS applied at each REM period, followed by a subsequent 24-hours-post-stimulus recording. In experiment 3, animals underwent AS, SS stimulation, or simultaneous application of both in a random fashion at each REM period. The results of all experiments confirm previous findings showing that auditory or somatosensory stimuli significantly increase REM sleep period duration. In addition, AS—applied with different presentations during REM and throughout the sleep-wake cycle—are capable of increasing REM duration regardless of the manner in which they were presented. However, the effects of the stimuli were not additive. It is worth noting that although REM duration increased, REM period frequency decreased, resulting in no net change of total REM sleep through time. Furthermore, no changes were observed in other sleep-wake variables. These experiments clearly demonstrate that repeated auditory stimulation does not cause habituation, and there are no evident side effects on the sleep-wake cycle. These results confirm that the mechanisms involved in REM generation and maintenance can be modulated by sensory modalities.

Key words: REM sleep; sensory stimulation; auditory stimulation; somatic stimulation

PREVIOUS STUDIES have shown that rapid-eye-movement (REM) sleep can be regulated by several sensory modalities. Auditory stimulation (AS) has been shown to enhance REM sleep duration in rats,[1] cats,[2] and humans.[3,4] Similarly, somatosensory stimulation (SS) has also been shown to increase REM sleep duration in cats.[5] This increase in REM duration is unaffected by cholinergic blockage, and is independent from the enhancement in PGO spike density which occurs in parallel to this REM increase,[6] suggesting little involvement of cholinergic systems in the effects of sensory stimulation on REM sleep. On the other hand, kainic acid lesions of the pontine reticular formation (PRF) cells prevents the REM sleep period increase due to AS, without affecting the normal duration of REM sleep.[7] Since REM sleep increase induced by AS is associated with an increase in both single-unit activity frequency of PRF cells[8] and c-fos expression in several REM-on brain stem structures,[1,9] it has been suggested that the increment in REM sleep by AS is related to an enhancement of the excitability of a widespread neuronal network in the brainstem.

The application of AS during REM-sleep rebound after 24 and 48 hours of sleep deprivation induces a synergistic increase on REM sleep. However, after 96 and 102 hours of sleep deprivation, a ceiling effect was observed.[10] Other studies have shown that vago-aortic stimulation in cats, using a fixed paradigm alternating 1 hour with and 1 hour without stimulation throughout the sleep-wake cycle, increased REM sleep frequency.[11] In addition, auditory stimuli applied to old rats at fixed intervals of 10 minutes on and 15 minutes off[12] did the same. All of these studies

Accepted for publication November 1997

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Table 1.—Mean and SEM of waking and slow wave sleep variables for experiments 1 and 2. Note that there are no significant differences in total time during waking nor in both stages of slow wave sleep with respect to both experimental designs.

	WAKING TOTAL TIME (x ± S.E.M.)(in min.)	SLOW WAVE SLEEP I (x ± S.E.M.) (in min.)	SLOW WAVE SLEEP II (x ± S.E.M.) (in min.)
Experiment 1 (n=4)			
CONTROL	174.3 ± 64	76.7 ± 10	169.3 ± 62
AS - 1	142.1± 62	59.2 ± 11	189.5 ± 28
AS - 2	158.2 ± 61	63.8 ± 17	181.1 ± 39
AS - 3	154.2 ± 50	54.2 ± 18	176.6 ± 34
AS - 4	180.3 ± 62	48.1 ± 9.0	166.0 ± 40
PÒST S	179.7 ± 30	54.2 ± 8.0	161.9 ± 24
Experiment 2 (n = 4)			
CONTROL	443.2 ± 122	195.8 ± 34	556.4 ± 112
AS	571.1 ± 168	164.2 ± 77	466.9 ± 114
PÒST S	453.7 ± 204	169.0 ± 33	581.9 ± 122

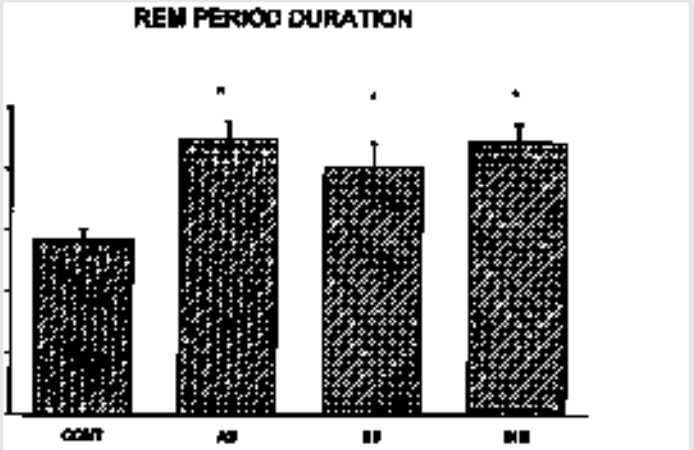

Figure 1.—The mean REM period duration in seconds (x± S.E.M.) of experiment 2 where signficant increases are seen in groups AS-1 through AS-4 (auditory stimulus applied for 4 consecutive days) when compared to control and post-S (post-stimulus recording for 24 hours) (*p < 0.05).

suggest that sensory stimulation has a modulatory effect upon the duration as well as the frequency of REM sleep. Therefore, among primary objectives of the following study was a determination of whether whether habituation occurs as a result of several days of repeated AS stimulation, and whether this type of stimulation causes alterations during the post-stimulus sleep-wake cycle. In addition, since AS and SS applied individually produce prolonged REM sleep periods, we wanted to determine whether simultaneous application of these stimuli has an additive effect.

MATERIALS AND METHODS

Fourteen cats of either sex, weighing between 2.5 and 3.5 kg, were used for this study. Under pentobarbital anesthesia (35 mg/kg), all animals were stereotaxically implanted for conventional sleep recordings with screw electrodes placed in the parietal bone for EEG, screw electrodes placed in the external canthus of the eye for recording EOG, electrodes implanted in the neck muscles for EMG, and tripolar electrodes implanted in the lateral geniculate nucleus for recording PGO spike activity. In addition, those animals which were programmed to receive somatosensory stimulation were implanted with silver wire rings in the skin of the neck. After 1 week recovery, the animals were placed inside a cage within a sound-attenuated room and allowed to habituate to their new surroundings for subsequent recordings of the sleep-wake cycle through a Grass Model 79D polygraph. Auditory stimulation (AS) was delivered through a stimulator designed in our laboratory. The AS was a 20-ms-duration, 90-dB, 2-kHz beep, every 20 seconds, applied at the beginning and throughout every REM period. Somatic stimulation (SS) was delivered by a Grass S88 stimulator through a constant current unit with an intensity which varied between 3 to 5 mA. Its duration was 5 ms and it was applied every 20 seconds at the beginning and throughout every REM period. Three separate experiments were conducted as follows:

Experiment 1

The animals were recorded under the following conditions (n=4): Day 1, control recording (CONT) for 24 hours (1000-1000 hours; days 2-5, recording with AS (90 dB) stimulus presented for 4 consecutive days (AS-1/ AS-2/ AS-3/ AS-4), 8 hours each day (1000-1800 hours) during every REM period observed; and day 6, follow-up recording without stimulus (post-S) for 24 hours (1800-1800 hours).

Experiment 2

The animals were recorded under the following conditions (n=4): Control recording (CONT) for 24 hours (1000 -1000 hours), with 3 days lapse at rest. Subsequent recording with AS (90 dB) stimulus was carried out as mentioned above for 24 hours (1000-1000 hours), and recording was continued for 24 hours (1000-1000 hours) without stimulus (post-S).

Table 2.—REM sleep values (mean ± S.E.M.) in experiment 3, where significant differences can be observed in the frequency of this sleep phase (* p < 0.05). PGO spike density is also significantly increased for all stimulus parameters in comparison to control (* p < 0.01). Note, total percent of REM sleep is not altered by any of the experimental designs used in this experiment.

Experiment 3 (n = 6)			
	REM PERIOD FREQUENCY (#) (x ± S.E.M.)	TOTAL REM PERCENT (x ± S.E.M.)	PGO SPIKE DENSITY (x ± S.E.M.)
CONTROL	23.3 ± 2.2	21.8 ± 2.6	46.9 ± 3.0
AS	13.8 ± 1.5 *	21.6 ± 2.9	61.3 ± 4.9 *
SS	13.6 ± 2.2 *	19.9 ± 3.3	65.3 ± 6.5 *
SIM	12.3 ± 1.9 *	17.7 ± 2.6	67.9 ± 3.4 *

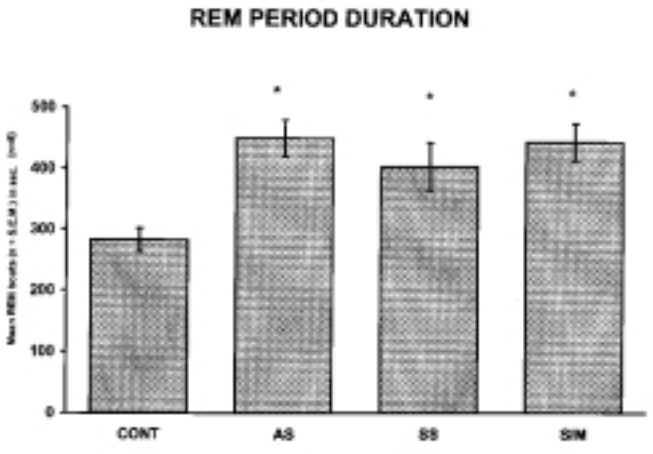

Figure 2.—The mean REM period duration in seconds **(x**± S.E.M.) of experiment 3 where significant increases are demonstrated for all conditions with respect to control (* p < 0.05).

Experiment 3

The animals were recorded for 8 consecutive hours (1000-1800 hours) (n = 6). Animals were subjected to the following conditions: Control recording (CONT), then random assignment to either a recording with auditory stimulus (AS), a recording with somatic stimulus (SS), or a recording with AS and SS applied simultaneously (SIM) every 20 seconds during each REM period observed.

All experiments were analyzed using a one-way ANOVA and a Duncan's test to determine significant differences between groups ($p < 0.05$).

RESULTS

In experiment 1, the results show significant increases in REM duration for all 4 consecutive days of stimulation where the mean (in seconds) was AS-1 (447.0±28.5), AS-2 (486.4±36.9), AS-3 (491.9±33.9), and AS-4 (472.6±31.5)(*p<0.05) as compared to control (266.5±26.4) and post S (224.2±10.4) (Fig. 1). There were no significant differences in any of the following REM sleep parameters: total REM sleep, mean REM percent and mean REM period frequency, except for post-S day (21.3±1.0), where the mean REM period frequency was significantly increased with respect to control (14.4±2.0) and stimulation days AS-1 through AS-4 (12.0±3.0, 11.2±2.0, 11.8±2.0, 10.8±4.0) (*p< 0.05). All other sleep-wake variables show no differences in this stimulus paradigm, as can be seen in Table 1.

The results for experiment 2, in a similar vein, show no differences, except in REM period duration for the stimulated day where the mean (in seconds) was 354.0±24.0 (*p <0.05), as compared to control (258.0±24.0) and post-S (276.0±12.0). All other variables of sleep-wake architecture remained unaffected by the stimulus paradigm used here (Table 1).

The results of experiment 3 show significant increases in REM period duration for all conditions with respect to control (*p<0.05) (Fig. 2 and Table 2). Furthermore, the mean frequency of REM periods is also significantly decreased for all the conditions, while the total percent of REM sleep does not change (Table 2). PGO spike activity also increased (*p <0.01) in all experimental conditions with respect to control, as previously reported with AS and SS stimulation[2,5] (Table 2). All other sleep-wake variables show no changes (data not shown), and are unaffected by the sensory stimulation conditions used in this experiment.

DISCUSSION

This study primarily shows that the one parameter largely affected by all experimental conditions is REM period duration.

The results of experiment 1 demonstrate that repeated auditory stimulation does not cause habituation to the stimulus, as can be seen in Fig. 1. Habituation can be generally described as a decrease in a behavioral response due to repeated presentation of a non-noxious stimulus. One way to determine whether habituation would have occurred due to repeated auditory stimulation would have been to show that the prolonged REM periods decreased to baseline duration levels across time in the distribution of sleep-wake architecture; however, this was not the case. All other REM sleep parameters remained unaffected by the stimulation, and on the post-stimulus day the mean duration of REM sleep returned to normal values. However, during the post-stimulus day, an increase in the number of REM periods was observed, which may suggest a possible compensatory feedback system related to the change in REM distribution due to the frequency decrease observed during stimulation days.

Another interesting finding is that total REM sleep time remains unaltered. This suggests that a decreased REM sleep frequency, even if not significant, compensates for the increased REM sleep duration. Additionally, the results of the second part of this study suggests that a quota of REM sleep is being maintained throughout the sleep-wake cycle. Up to 24 hours after the auditory stimulus is applied, no changes in any REM sleep parameters or other sleep architecture variables seem to be effected. There are no obvious side effects in the sleep-wake cycle with the use of the auditory stimulus.

The results of experiment 3 confirm previous findings,[1,2,5,9] but, in addition, show that the effects of applying both auditory and somatic stimuli simultaneously (SIM) also demonstrate a significant increase in mean REM duration, though these effects do not appear to be synergistic (Fig. 2). Perhaps the reason there are no additive effects is that the stimuli have a finite cell recruitment capacity for enhancing REM duration. In other words, assuming that the mechanism which induces the increase in REM sleep period duration is related to an increase in the number of cells that are excited, as shown by Merchant-Nancy et al,[1,9] it is conceivable that the stimuli recruit the same cells, and therefore the number of cells recruited does not change, which in turn causes no additive effects. Since the increase in REM sleep period duration in this study using simultaneous presentation is similar to the increase due to auditory or somatic stimulus alone,[2,5] it is possible that the mechanisms which modulate the REM increase are mutual to both sensory modalities. In fact, anatomical[13,14] and electrophysiological[15-17] studies have demonstrated that these pathways send projections to those reticular neuronal groups which have been proposed to play an important role in regulating REM sleep,[18,19] and it is precisely these areas which show an elevated level of c-fos expression following auditory stimulation.[1,9]

Extensive research over the years has demonstrated the various ways in which sleep architecture can be altered due to different manipulations of the sensory pathways.[20-22] Previous experiments using visceral afferent stimulation,[23,24] vestibular stimulation,[25] and vibratory stimulation[26] have been shown to affect various sleep parameters. These changes induced by sensory stimulation suggest that afferent pathways can exert an important influence on sleep.

The noninvasive techniques used in this study may have clinical applications in those situations in which it would be necessary to produce prolonged periods of REM without noticeable repercussions on the sleep-wake cycle or altered REM sleep due to pharmacological manipulations.

ACKNOWLEDGEMENTS

This work was supported in part by FIIRESIN, Fidecomiso UNAM and DGAPA UNAM-IN211294 to R.D.C. We wish to thank Mr. Jesús Mendez-Franco for his technical assistance and Mrs. Ma. Teresa Torres-Peralta for typing the manuscript.

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