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AUDITORY DEPRIVATION MODIFIES BIOLOGICAL RHYTHMS IN THE GOLDEN HAMSTER

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INTRODUCTION

Locomotor activity pattern and the polyphasic wakefulness-sleep cycle are two remarkable biological rhythms in the Syrian hamster (Mesocricetus auratus). These, as well as many other rhythms, are controlled by a circadian oscillator located in the suprachiasmatic nuclei (SCN) (9, 12, 19, 20). These nuclei receive several types of regulatory signals. In particular, the light-dark cycle (LD) entrains circadian rhythms through a distinct neuroanatomical pathway from the retina to the clock itself (5, 12, 19). Light will phase-shift circadian rhythms when introduced during the subjective night. Light in the early subjective night will induce phase delays while in the later subjective night will induce phase advances. This pattern of phase shifts allows stable entrainment to changing photoperiods. The underlying mechanism of photic entrainment is thought to involve daily adjustments (phase shifts) of the endogenous oscillation.

While anatomical substrates over which cyclic environmental factors reach and affect the biological clock have been studied in detail, the entrainment pathways within the SCN are not well understood. In addition, non photic stimuli such as food (1, 11) or running on a novel wheel (13) can also phase-shift the circadian clock, or augment the amplitude of its oscillation, and little is known about the possibility that other sensory inputs, like the auditory one, may act as a Zeitgeber for locomotor activity or sleep/wakefulness rhythms (15, 16).

As far as the sleep/wakefulness cycle, a reciprocal relationship between sensory input and processing changes were found for many sensory modalities (23). The auditory system is the only telereceptor that remains "open" during sleep, and from the cochlea up to the auditory cortex, evoked potentials and unitary firing (both spontaneous and evoked) indicate the active processing of auditory information taking place during sleep in animals as well as in humans (4, 6, 8, 14, 16, 17, 18, 22). Auditory deprivation after surgical bilateral cochlea lesions of guinea pigs affected the wakefulness/sleep cycle by diminishing the total wakefulness (W)

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duration and by increasing the total time and the number of episodes of slow-wave sleep (SWS), and paradoxical sleep (PS) (15). In addition, a noisy ambient or sound stimulation also altered sleep structure (2, 3, 7, 21).

The present study was undertaken to get information on to what extent deprivation of a non-photic stimulus like sound or noise may affect the circadian clock in the Syrian hamster. To examine this, sleep/wakefulness and locomotor activity rhythms were studied in golden hamsters subjected to auditory deprivation by a bilateral cochlear lesion.

METHODS

Animals. - Adult male golden hamsters were housed in a sound-attenuated box with constant temperature (20-22°C) and water and food 'ad libitum'. The cage cleaning was done every day at 07:30 h. Animals were recorded after a surgical recovery period of 5 days. Experiments were conducted in accordance with national legislation, and with the European Communities Council Directive (86/609/EEC) and the National Institutes of Health Guide regarding the care and use of animals for experimental procedures to minimize pain or discomfort of the animals.

Wakefulness/sleep cycle study. - Eight adult male hamsters were chronically implanted under pentobarbital anaesthesia (35 mg/kg, i.p.), with electrodes for hippocampal electrogram (Hipp), parietal electrocorticogram (Cx) and neck electromyogram (EMG) recordings. Electrodes, placed bilaterally in the auditory cortices, were used to record auditory evoked local-field potentials in response to clicks. A second surgical procedure comprised the destruction of both cochleae through the tympanic membrane, also under pentobarbital anaesthesia. The evoked auditory cortical local-field potential was used as a pre- and post-lesion auditory electrophysiological test. The procedure produces a totally deaf animal, as demonstrated by the absence of electrical responses to continuous body internally generated sounds (24). The behavioural response to high intensity sounds was also used as a test for hearing loss. The total destruction of the lesioned cochleae were verified by post-mortem anatomical studies. The animals were studied no less than 10 days after lesion and only animals without signs of vestibular damage or weight loss were used. A total of 384 h of polisomnographic recording (computerized Polygraph, Nautilus Plus-PSG) were analyzed as follows: 192 h ($n = 8$) were used to establish the control sleep parameters and 192 h ($n = 8$) were employed for recording the effect of cochlear lesions. Recordings after cochlear lesions were compared with their own pre-lesions controls. The time spent in W, SWS and PS was computed into 20 s epochs. Sleep scoring was visually carried out by two individuals blind to the identity/condition of the animals. Twenty-four hour data was analyzed as a whole or in segments corresponding to the photo- or scotophase.

Locomotor activity rhythm study. - Eleven adult hamsters were used. Five animals received a bilateral cochlea destruction under pentobarbital anaesthesia while 6 animals were sham-operated. After recovery, the hamsters were individually housed in cages to assess wheel-running activity, recorded on-line in 10 min bins using a DataQuest System III (Data Sciences, Minneapolis, MN). The behavioural response to high intensity sounds was used as test of hearing loss. The total destruction of the lesioned cochleae was verified by post-mortem anatomical studies. After several days in 14:10 LD, animals were submitted to an 8 h phase-advance in their locomotor activity onset. Lastly, the hamsters were housed in constant darkness (DD). Phase-shifts were determined by eye fitting a line to the onsets of activity in LD cycle until the day of phase-advance and the second line from this time until the beginning in DD conditions. The other parameters studied were calculated using Circadia analysis system for Macintosh (Circadia Data Analysis Program, version 2.1.16, Behavioural Cybernetics).

Statistical analysis. - Student's t-test was used for comparisons between sham-operated and lesioned hamsters. When each animal was used as its own control, statistical analysis was performed by using paired t-tests. All data are presented as the mean ± S.E.M. ($p < 0.05$).

RESULTS

Figure 1 depicts typical polyphasic hypnograms of hamsters before and after cochlear lesions. Generally, an increase in the total sleep time and number of episodes was observed in lesioned animals, together with decreases in total time spent in W. In addition, the temporal organization of W/sleep episodes was different during the dark period as compared to the light phase.

As shown in Fig. 2A, a significant increase in the number of episodes for the three behavioural states was found in deaf animals. The effect of the lesion was seen during the light phase (Fig. 2B).

The results on percent of total duration of W and sleep stages are depicted in Fig. 3. In deaf animals, duration of W decreased and that of SWS increased during light. LD differences in duration were significant for the three behavioural stages in deaf hamsters, W duration being shorter, and SWS and PS duration being longer during the light phase of daily photoperiod. In controls, only PS duration increased significantly during the light phase (Fig. 3).

Results on locomotor activity rhythms are summarized in Table 1 and Fig. 4. Under LD conditions, a significant lower amplitude of locomotor activity was

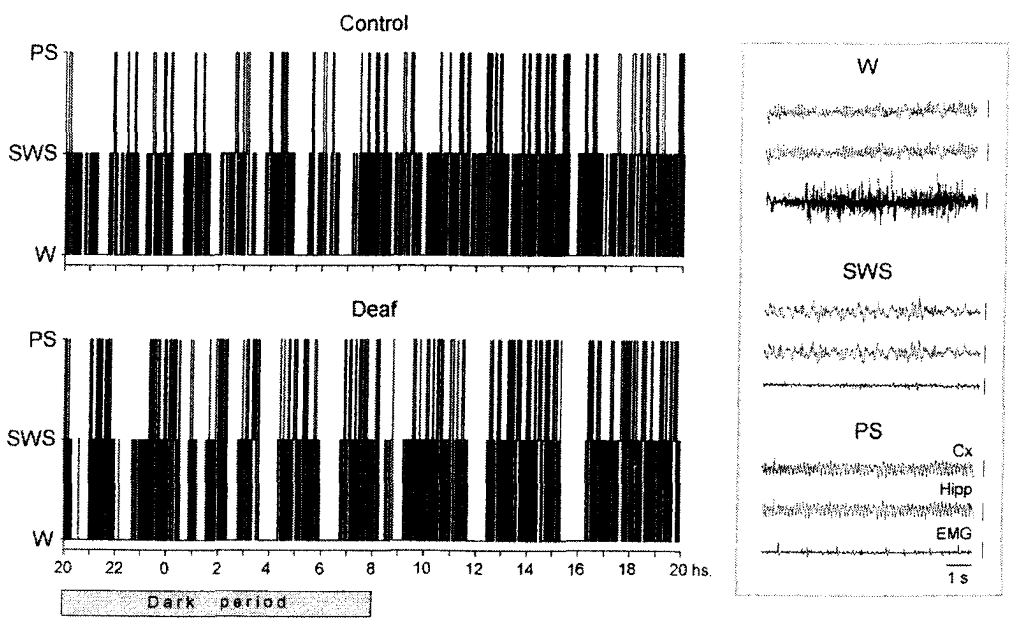

Fig. 1. - *Stage distribution during 24 h recording (hypnogram) in the same animal before (Control) and after cochlear lesion (Deaf).*

The inset shows raw data from active wakefulness (W), slow wave sleep (SWS) and paradoxical sleep (PS). Cx, electrocorticogram; Hipp, hippocampal electrogram; EMG, electromyogram. Cal: 1 mV; 25 mV.

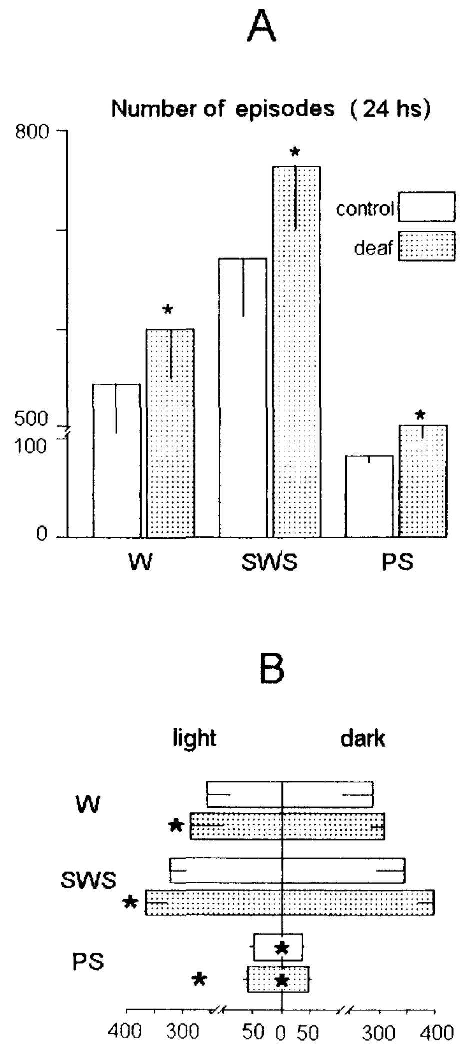

Fig. 2. - *Number of episodes in sleep and waking before and after cochlear lesion (24 h recordings; n = 8).*

A. The number of episodes augmented for all stages in deaf animals ($P < 0.05$). B. The number of episodes increased during both light and dark periods in deaf hamsters in comparison to controls, being statistically significant only during light periods. Differences in number of episodes between light and dark were significant during PS both in control and in deaf animals.

found in deaf hamsters. Likewise, a significantly different phase angle as far as the time of lights off was detected. After a phase-advance of 8 h, there were no significant differences in the resynchronization rate, i.e. the number of days needed to regain entrainment to the new LD cycle. between sham operated and lesioned animals. Differences observed in acrophases, i.e. phase of maximum value, did not

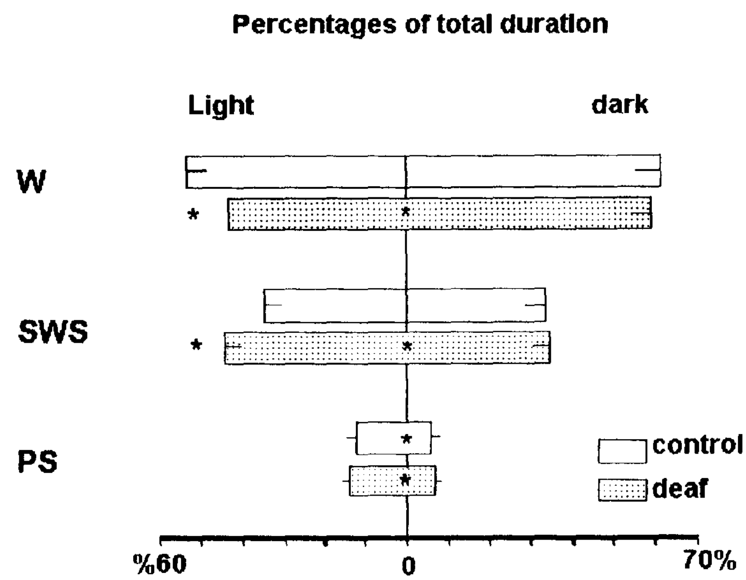

Fig. 3. - *Percentage of duration of behavioral states in deaf hamsters in comparison to controls.*

In deaf animals, wakefulness (W) decreased and slow wave sleep (SWS) increased significantly during light periods. Paradoxical sleep (PS) duration showed significant differences between light and dark periods but between control and deaf animals.
* Statistically significant changes ($P < 0.05$) between control and deaf (top of bars) and between light and dark periods (at the middle).

Table 1. - *Locomotor activity parameters (mean ± SEM) in sham and deaf golden hamsters. Amplitude was measured as wheel running turns, the phase was expressed in degrees, resynchronization rate in days, acrophase in degrees and period in hours.*

Light-dark (L:D; 14:10)

AMPLITUDE		PHASE		RESYNCRONIZATION RATE		ACROPHASE	
Sham	Deaf	Sham	Deaf	Sham	Deaf	Sham	Deaf
92.7 ± 9.0	31.4 ± 6.3***	-9.5 ± 4.6	53.2 ± 8.7***	9.3 ± 0.4	9.4 ± 1.0	-179 ± 77	-301 ± 28

Constant darkness (D:D)

AMPLITUDE		PERIOD	
Sham	Deaf	Sham	Deaf
53.8 ± 6.3 a	20.2 ± 6.3**	23.1 ± 0.04	24.1 ± 0.08

a, significantly different from sham in L:D ($p < 0.003$).
**, $p < 0.005$.
***, $p < 0.0001$, Student's Test.
Sham (n = 6).
Deaf (n = 11).

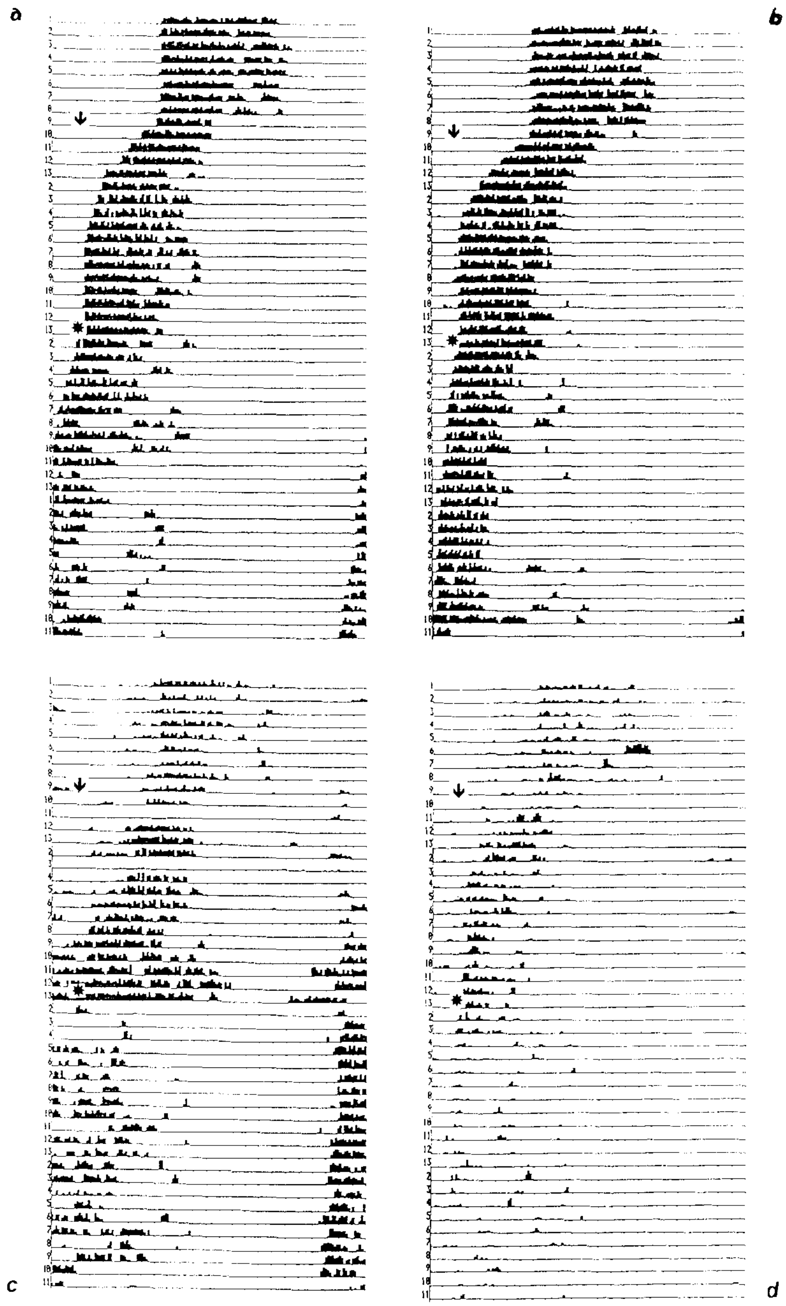

Fig. 4. - *Representative records of locomotor activity in golden hamsters.*

Each line represents wheel-running records. Successive days are plotted top to bottom. The arrow indicates an 8 h phase-advance in the light-dark cycle (LD, 14:10). Asterisks indicate beginning of constant darkness. Data were collected using the Mini-Mitter/DataQuest III system. (a, b) control animals; (c and d) deaf animals.

attain significance (Tab. 1; Fig. 4). Under DD conditions, a similar picture emerged, with a significantly lower amplitude in deaf hamsters. There were no significant differences in the period observed between groups (Tab. 1; Fig. 4).

DISCUSSION

The main conclusion emerging from the present work is that deafness affects the normal rhythm organization, specifically sleep/wakefulness and locomotor activities in hamsters. The most important change found in the wakefulness-sleep rhythm was an increase in the number of episodes and modification in duration of sleep and wakefulness. This demonstrate that the auditory deprivation altered the normal waking/sleep architecture in hamsters as previously reported in deaf guinea pigs (15). However, a greater individual variability was observed in hamsters as compared to guinea pigs.

The changes in wakefulness-sleep cycle observed in deaf animals were significant during the light period only, suggesting a sensory dependence or interaction between sound and light inputs. Moreover, the relative isolation from the outside world imposed by deafness could contribute to the W reduction and sleep lengthening, stressing the relevance of such sensory input for sleep organization. Although sleep is an active process, the sensory influences must not be disregarded, particularly for meaningful input normally open during sleep as the auditory one. The sleep-W normal balance may depend upon the coordinated function of multiple networks whose synaptic processes exhibit "cooperative interactions". Changing an input to a set of networks would introduce functional differences, that, when such input is lacking, an imbalance will be produced in the sleep-W cycle (15). The deafening method used is the only possible to obtain a deaf animal because of the many internally produced noises known that could lead to misleading results (24).

The study on locomotor activity rhythms in LD conditions showed that cochlear lesions affected amplitude and phase significantly, but not the resynchronization rate or acrophase values. Further supporting this, the amplitude of locomotor activity rhythm was lower without modifications on the period of the clock. Considering the fall in the amplitude, this parameter diminished either in LD or DD conditions in deaf animals. A similar picture emerge from aging studies in this species. Labyak *et al.* (10) showed that golden hamsters display age-related changes in the expression of circadian rhythms, reducing amplitude in 14:10 h light-dark cycles compared with young animals. On the other hand, it was possible to observe a significant difference between sham animals in LD and DD conditions, supporting the evidence of light as the main external Zeitgeber (12).

In conclusion, the present study indicates that the alteration in one of the sensory modalities (auditory) alters the normal architecture of the general rhythms by disturbing both sleep/waking cycle and locomotor activity rhythms. Our results suggest that the auditory deprivation may affect photic synchronization, rather than the clock mechanism itself.

SUMMARY

To assess to what extent auditory sensory deprivation affects biological rhythmicity, sleep/wakefulness cycle and 24 h rhythm in locomotor activity were examined in golden hamsters after bilateral cochlear lesion. An increase in total sleep time as well as a decrease in wakefulness (W) were associated to an augmented number of W episodes, as well as of slow wave sleep (SWS) and paradoxical sleep (PS) episodes in deaf hamsters. The number of episodes of the three behavioural states and the percent duration of W and SWS increased significantly during the light phase of daily photoperiod only. Lower amplitudes of locomotor activity rhythm and a different phase angle as far as light off were found in deaf hamsters kept either under light-dark photoperiod or in constant darkness. Period of locomotor activity remained unchanged after cochlear lesions. The results indicate that auditory deprivation disturbs photic synchronization of rhythms with little effect on the clock timing mechanism itself.

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