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Mechanisms of Paradoxical Sleep as Revealed by Neurophysiologic and Pharmacologic Approaches in the Rabbit*

By

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With 5 Figures in the Text

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Introduction

The phenomenon of "activated" sleep in cats was described by DEMENT in 1958, and the report was quickly confirmed by JOUVET et al. (1959), HUBEL (1960), HUTTENLOCHER (1960, 1961), GRASTYAN and KARMOS (1961), ROSSI et al. (1961) and others. Characterized by a desynchronized cortical EEG, rapid eye movements, general loss of muscular tone and irregular respiration and EKG, this peculiar sleep pattern has gained the names "paradoxical", "rhombencephalic", "light" and "deep" as well as "activated" sleep. It has also been observed in the rat (MICHEL et al., 1961; SWISHER, 1962), the monkey (WEITZMAN, 1961), and man (DEMENT and KLEITMAN, 1957; JOUVET, 1962).

In the rabbit the behavioral manifestations and EEG patterns now recognized as paradoxical sleep (PS) were first observed in the female as a sequel to coitus (SAWYER and KAWAKAMI, 1959) and the EEG changes were described as part of an "EEG afterreaction". KAWAKAMI and SAWYER (1959a, b, 1962a) found that the peculiar pattern and associated behavior could be induced by low frequency electrical stimulation of hypothalamic-limbic areas and that the threshold of the response varied with the hormonal status of the rabbit. FAURE (1962) has made similar observations. During paradoxical sleep in the rabbit the EEG activity of the olfactory bulb is markedly depressed, but the theta rhythm of the hippocampus, central gray and reticular formation is increased in both amplitude and frequency, so much so that SAWYER and KAWAKAMI likened it to a state of hyperarousal and called it a phase

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of "hippocampal hyperactivity". More recently KAWAKAMI and SAWYER (1962b) have reported that induction of PS can be conditioned to a neutral tone. KHAZAN, KAWAKAMI and SAWYER (1963) have observed that spontaneous episodes of PS can be inhibited for many hours by CNS depressants as well as by a white masking noise (KHAZAN and SAWYER, 1963).

There is disagreement among authorities as to whether PS is light sleep or deep sleep. Although the EEG pattern during PS resembles the alert state, the threshold of behavioral arousal from either external stimuli or direct electrical stimulation of the reticular formation (RF) is elevated over the already raised level characteristic of spindle sleep. Evoked potentials of acoustic (HUTTENLOCHER, 1960; JOUVET, 1962) or optic origin, (HENDLEY, Personal communication, 1963), are generally depressed during PS. In man HODES and DEMENT (Personal communication, 1963) have observed that electrically induced reflexes involving the tibial muscles are diminished or abolished during PS. Loss of muscle tone is so constant a feature of PS that a silent EMG from the neck muscles is considered a positive criterion even in the absence of EEG records.

For these reasons a majority of investigators have considered PS as deep sleep, (ROSSI et al.; MAGOUN, 1963). However, unit activity in the cat brain stem (HUTTENLOCHER, 1961) or visual cortex (EVARTS, 1962), is accelerated to almost twice that during ordinary sleep and even in excess of the activity observed during the alert state. Since the role of the reticular formation in mechanisms of sleep-wakefulness is a familiar one (MAGOUN, 1952, 1963), and the cortical evoked response to reticular formation stimulation has been suggested as a guide to the state of arousal of the animal (TISSOT and MONNIER, 1959), the following experiments were carried out to study the CNS mechanisms involved in PS and to assess the relative degree of alertness during natural sleep and the influence of various psychoactive drugs.

Material and Methods

Adult ovariectomized New Zealand white rabbits, weighing 3—4.5 kg were anesthetized with pentobarbital and implanted stereotaxically (SAWYER et al. 1954) with chronic bipolar electrodes in the midbrain reticular formation and central gray substance, septum, dorsal hippocampus and olfactory bulb. A silver ball was placed on the frontal cortex at A10 and an indifferent screw electrode served as ground. Antibiotics were administered for a few days following surgery. The rabbits were permitted to recover for two weeks before testing. Extended observations over many hours were made of the behavior and the related EEG record in 15 rabbits.

The cortical evoked response to stimulation of the reticular formation was recorded in untreated animals in a soundproof room during the alert state, ordinary spindle sleep and PS while simultaneous EEG tracings and observations of behavior were being taken. Single 200—300 μA shocks of 0.1 msec duration were delivered to the reticular formation at the rate of two per second. The stimulus current was monitored continuously and the cortical evoked response was displayed on the Tektronics 502 oscilloscope. For each tracing one hundred responses were averaged on the Computer of Average Transients (Mnemotron CAT) and graphed with an X-Y plotter.

The effects of the following psychopharmacologic agents on the reticular formation — cortical evoked potential were assessed as well as their influence on the spontaneous incidence of PS: pentobarbital sodium, 10 mg/kg; morphine sulfate 1—2.5 mg/kg; chlorpromazine 2.5—5 mg/kg; atropine sulfate 1—5 mg/kg; reserpine 100 μg/kg; amphetamine sulfate 2 mg/kg; LSD-25, 50 μg/kg. The drugs were generally administered "remotely" via intravenous cannula permanently fixed in the jugular vein and polyethylene connector tubing from a syringe outside the sound-proof room. At autopsy the brains were fixed in formalin, sectioned by the freezing method and stained with thionin for histological confirmation of electrode sites.

Results

Both behavioral and EEG criteria were employed in analyzing sleep-wakefulness conditions. EEG patterns in three key channels are

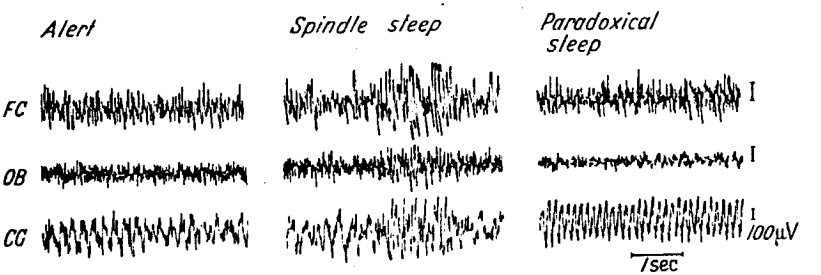

Fig. 1. EEG patterns in frontal cortex (*FC*), olfactory bulb (*OB*) and central gray (*CG*) during three stages of sleep-wakefulness in the rabbit

illustrated in Fig. 1. It is apparent that during PS the frontal cortex is desynchronized, and the olfactory bulb depressed while the central gray reveals a high amplitude rapid sinusoidal theta rhythm quite different from that seen in the alert state. Meanwhile the PS animal is stretched prone with its head resting on the floor, its ears depressed against the back of the neck and the eyelids closed.

Averaged evoked potentials recorded at the frontal cortex from stimulation of the midbrain reticular formation in each of these states are shown in Fig. 2. During the alert stage in each of the rabbits the averaged response consistently showed a major peak at a latency of about 20—25 msec. The more highly alert the animal's condition the greater the amplitude and the shorter the latency of the peak. During spindle sleep the averaged evoked response was practically a flat line, with complete disappearance of the peak. Surprisingly enough, during

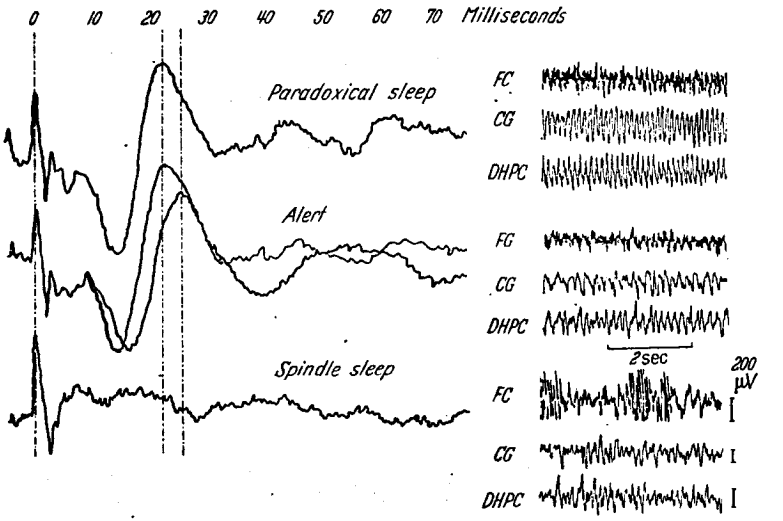

Fig. 2. Averages of 100 responses evoked in the frontal cortex by stimulating the midbrain reticular formation. Two averages are superimposed for the alert stage to show extremes of variation. Related EEG patterns in frontal cortex (*FC*), central gray (*CG*) and dorsal hippocampus (*DHPC*) accompany each of the three states. Recording conditions, including the settings on the Mnemotron Computer and the X-Y plotter, were maintained constant throughout an experiment. The evoked potential experiments, including the effects of drugs (Figs. 3 and 4), were repeated with essentially invariable results in five "chronic" animals

paradoxical sleep the response was almost identical to the more highly alert curve, with a sharp peak at 20 msec.

As a matter of fact the evoked response during PS differed from the normally alert record in a manner not unlike that observed following treatment with certain pharmacologic agents (Fig. 3). The psychotomimetic LSD-25, the sympathomimetic amphetamine and the tranquilizer reserpine all enhanced the cortical evoked responses, giving peaks of short latency and high amplitude. Amphetamine and LSD inhibited PS for 3-5 hours; their EEG records were characterized by continuous alertness and absence of sleep spindles. On the other hand reserpine not only permitted the appearance of spontaneous PS but the PS episodes actually appeared to be lengthened.

The averaged evoked responses during alertness and sleep in another rabbit are illustrated in Fig. 4. Also shown are the effects of four CNS

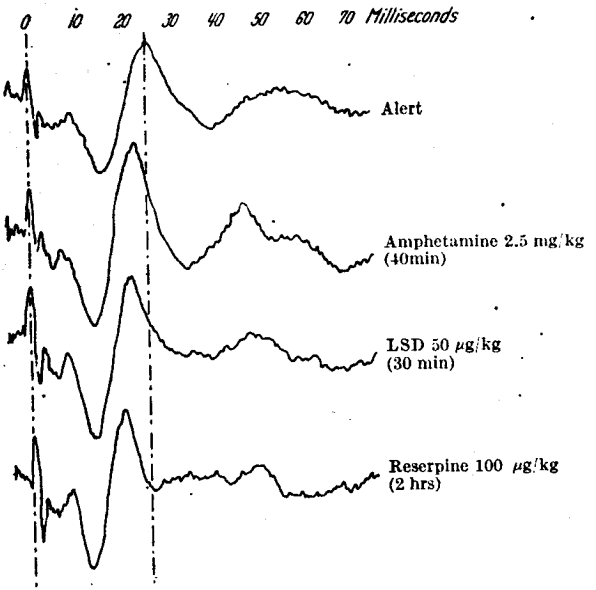

Fig. 3. Averaged evoked responses under treatment with agents which alerted the EEG record

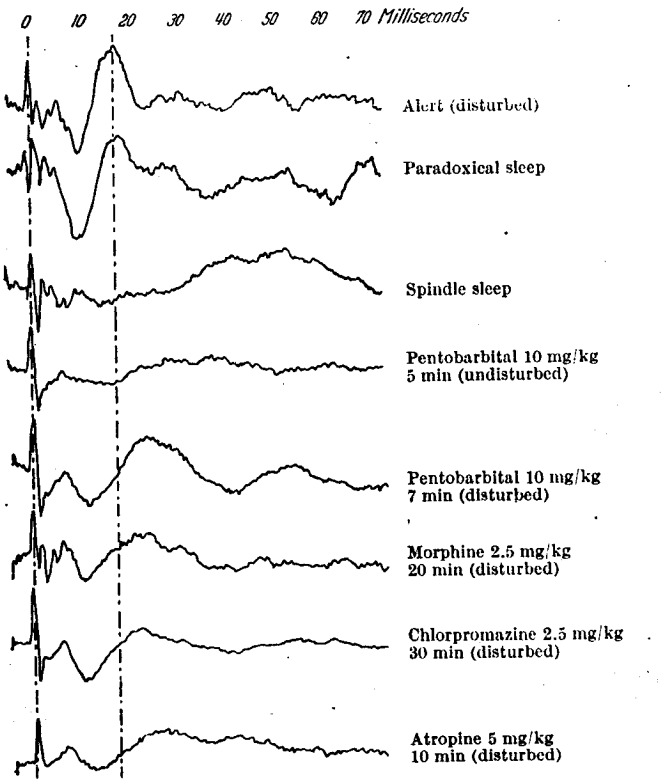

Fig. 4. Averaged evoked responses during natural sleep-wakefulness and drug-induced CNS depression. "Disturbed" indicates that attempts were made to alert the animal by a variety of noises which did arouse the untreated subject

depressants previously reported to inhibit PS in cats (JOUVET 1962) or the equivalent "EEG afterreaction" in rabbits (KAWAKAMI and SAWYER, see SAWYER 1962). In the undisturbed state under pentobarbital the evoked response was eliminated as it was during spindle sleep. With the rabbit partially wakened by a "disturbing" noise the peak was partially restored at lower amplitude and increased latency. Similar effects were observed following treatment with morphine, chlorpromazine and atropine in dosages which prevented the appearance of PS for three hours or more.

Discussion

The results of the present study indicate that paradoxical sleep is a highly active central nervous state, in many ways more closely related to a hyper-aroused condition than to deep sleep. The fact that the evoked responses during PS are of similar amplitude and latency as during the hyper-alert state makes it difficult to consider PS a sleeping stage of greater profundity than ordinary slow wave or spindle sleep. In PS the EEG is differentially activated with a high amplitude theta rhythm in the hippocampus and certain regions of the brainstem, a pattern called "hippocampal hyperactivity" by KAWAKAMI and SAWYER (1959). The rhinencephalic-hypothalamic system which these authors stimulated to induce PS in the rabbit is part of the more inclusive limbic-midbrain system of NAUTA (1958, 1963) invoked by JOUVET (1961, 1962) as the CNS circuit controlling PS in the cat. The latter system may well represent the complex circuit by which evoked potentials from the midbrain are conveyed to the frontal cortex in the current study.

The limbic midbrain system may be considered as part of the brainstem reticular system in its broadest sense (MAGOUN, 1963). However, evoked potentials emanating from the reticular formation are prevented from reaching the cortex by dosages of drugs which do not appear to elevate the threshold of EEG arousal. Similar dosages of CNS depressants such as pentobarbital, atropine, morphine and chlorpromazine do elevate the threshold of electrically induced PS in the rabbit (SAWYER, 1962; KAWAKAMI and SAWYER, unpublished observations) and inhibit the appearance of spontaneous PS in the cat (JOUVET, 1961, 1962). BRADLEY (1958) proposes that atropine blocks transmission rostral to the midbrain in the diffuse thalamic projection system, and KILLAM and KILLAM (1959) consider that chlorpromazine may exert inhibitory effects at this same level, a site at which behavioral arousal is divorced from EEG arousal. SAUL and SAWYER (1957 and unpublished observations) have found that atropine, morphine, chlorpromazine and pentobarbital (but not reserpine) all block hypothalamic or rhinencephalic activation of the adenohypophysis presumably by interrupting hypo-

thalamic synapses. Their depressant action at this level might well involve them as transmission blockers in the limbic midbrain system leading to the inhibition of PS.

Paradoxical sleep has still other claims of status as a hyperactive pattern in addition to the activated EEG and the facilitated cortical responses to the stimulation of the midbrain. HUTTENLOCHER (1961) reported that during PS in the cat most of the spontaneous midbrain units fired at more rapid rates than during the waking state. Eye movements were frequent during bursts of unit discharge. These and the other apparently involuntary facial movements so common to PS might be the result of direct activation of the motor nuclei of cranial nerves III—VII which lie in the brainstem adjacent to sites of augmented unit activity. EVARTS (1962) found similar increases in unit activity in the visual cortex during PS. Although PS is always entered through a state of ordinary spindle sleep, PS represents a return toward wakefulness rather than a progressively deeper sleeping condition; there is almost always a period of real alertness before the resumption of spindle sleep. The type of electrical stimulation of the brainstem employed by JOUVET (1961, 1962) and ROSSI et al. (1961) to induce PS during spindle sleep is the high frequency (300 cps) alerting variety ordinarily employed to evoke EEG and behavioral arousal.

Arguments proposed by previous workers suggesting that PS represents deep sleep include the extreme loss of skeletal muscle tone and the elevated threshold of behavioral arousal. However, there are at least two mechanisms consistant with hyper-aroused brainstem activity by which CNS may induce the ultra-flaccid condition of skeletal musculature resulting in loss of reflexes (HODES and DEMENT, Personal communication) and the quiescent EMG of the neck muscles (JOUVET, 1961, 1962): 1. Activation of the bulbar inhibitory system of MAGOUN and RHINES (1946) by the heightened brainstem excitation (see also MAGOUN 1950, 1963; LINDSLEY, 1952) with resultant active inhibition of lower motor centers, and 2. inhibition of afferent pathways by the activated reticular system (HAGBARTH and KERR, 1954). (See also HERNANDEZ-PEON, 1955, and FRENCH, 1958.) This type of inhibition would elevate the threshold of behavioral arousal and depress evoked potentials in the optic (HENDLEY, Personal communication), and auditory (HUTTENLOCHER, 1960; JOUVET 1961) systems as outlined earlier. The depression of activity in the olfactory bulb observed in the present study (Fig. 1) probably has a similar basis, for KERR and HAGBARTH (1955) found olfactory transmission inhibited by stimulation of the reticular system.

Our hypothesis concerning the stimulatory and inhibitory mechanisms at play in the brainstem during paradoxical sleep is summarized dia-

grammatically in Fig. 5. Projection rostrally from the midbrain reticular formation via the diffuse thalamic system and/or the midbrain-limbic system is facilitated. The results indicate that the hyperactive RF may stimulate the descending bulbar inhibitory system as a possible feature of the mechanism of PS: behavior during this phase suggests an active inhibition rather than a passive relaxation. The loss of reflexes and muscle tone could however be explained by the inhibition of afferent synapses. Probably both of the inhibitory mechanisms are brought into play by the activated reticular formation during this peculiar state.

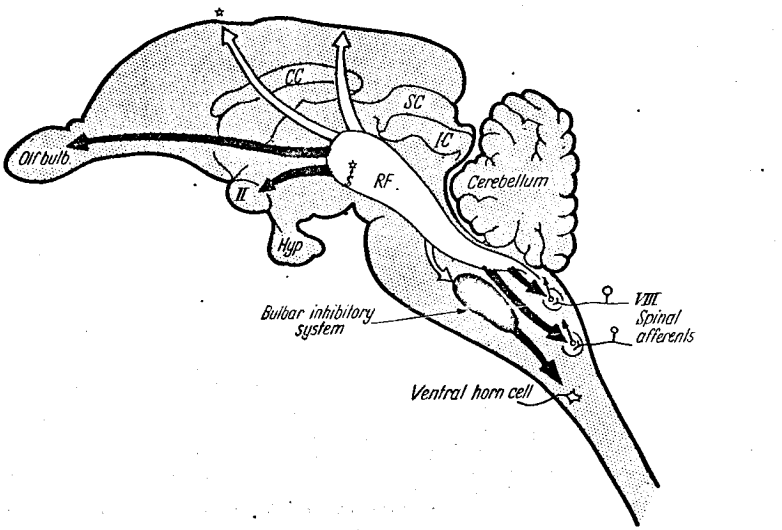

Fig. 5. Diagram of sagittal section of rabbit brain summarizing possible mechanisms involved in paradoxical sleep. The aroused reticular formation (*RF*) and stimulatory projections are shown in white; the inhibitory system and inhibitory projections from the *RF*, in black. Sites of electrical stimulation and recording of evoked responses are indicated as small open stars

Summary

With the use of the Computor of Average Transients a study has been made of evoked responses in the central nervous system (CNS) of rabbits during paradoxical sleep and other stages of sleep and alertness. In "chronically implanted" animals responses evoked in the frontal cortex by stimulating the midbrain reticular formation were averaged and used as a guide to the state of wakefulness. During the alert state the averaged evoked responses show consistently a major peak at a latency of about 20—25 msec, which disappears during ordinary sleep. During paradoxical sleep (PS) the peak reappears with a latency and amplitude comparable to the response evoked during hyperalertness.

Enhanced responses similar to the evoked potentials during PS are seen following treatment with amphetamine, LSD-25 and reserpine,

all of which induce an aroused EEG. Reserpine also fosters PS behavior. A group of central depressant drugs including pentobarbital, morphine, chlorpromazine and atropine all markedly reduce the amplitude and increase the latency of the evoked response in dosages which prevent the appearance of PS.

The conclusion is reached that PS is not a deep stage of sleep. The results suggest, rather, that PS represents a hyperactive condition of the limbic midbrain component of the brainstem reticular system and that the behavioral depression in PS results from an active inhibition of afferent synapses and/or enhanced activity in the descending bulbar inhibitory system.

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