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NEUROHUMORAL MECHANISMS OF SLEEP AND WAKEFULNESS

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ABSTRACT

Today it is well accepted that there exist at least two phases of sleep, which can be distinguished from each other both behaviorally and electrophysiologically. However, the problem of identifying the neurotransmitters responsible for sleep and wakefulness has not yet been solved. Present evidence indicates that serotonin (5-HT) is involved in the induction and maintenance of slow wave sleep, and that norepinephrine (NE) is involved mainly in the maintenance of fast wave sleep. In view of the fact that NE also been shown to be involved in wakefulness, it is thought that 5-HT regulates slow wave sleep, and that NE regulates both fast wave sleep and wakefulness. On the other hand there is evidence that acetylcholine and GABA, though the latter to a lesser degree, could have an important role in the regulation of one or several stages of the sleep-wakefulness cycle. In view of the fact that several neurotransmitters appear to have important roles in sleep, the experimental evidence showing that it is possible to extract either from blood, cerebrospinal fluid and/or brain tissue, substances which are capable of inducing sleep, is discussed. Possibly these latter techniques will allow characterization of the neurotransmitters involved in sleep and wakefulness.

RESUMEN

Hoy en día es bien aceptado que existen por lo menos dos fases de sueño, distinguible la una de la otra, tanto conductualmente como electrofisiológicamente. Sin embargo, el problema de la identificación de los neurotransmisores responsables de sueño y la vigilia aún no ha sido resuelto. Existe evidencia de que la serotonina (5-HT) es responsable de la fase de sueño de ondas lentas, y que la norepinefrina (NE) es responsable de la fase de sueño de ondas rápidas. Sin embargo, en vista de que la NE también está involucrada en la vigilia, es posible que la 5-HT regule la fase de ondas lentas y la NE regule la fase de ondas rápidas y la vigilia. Por otro lado, existe evidencia de que la acetilcolina y el GABA, aunque este último en menor grado, pueden tener un papel importante en la regulación de una o varias fases del ciclo vigilia-sueño. En vista de que existen varios transmisores que parecen jugar un papel importante en el sueño, se discuten los resultados experimentales que han mostrado que es posible extraer de la sangre, líquido cefalorraquídeo y/o tejido cerebral, substancias que son capaces de inducir sueño. Quizás estas últimas técnicas permitan caracterizar los neurotransmisores involucrados en el sueño y la vigilia.

THE BASIC ELECTROPHYSIOLOGICAL PATTERNS OF SLEEP

It is well accepted that mammals and primates present at least two basic patterns of sleep. Initially the behavioral attitude of sleep is characterized electro-

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encephalographically by the appearance of 14 to 18 Hz cortical spindles, which are later replaced by 2-4 Hz slow waves. At the same time high voltage (500-800 uv) sharp waves are recorded from the hippocampus, while the EMG decreases slightly. Usually after 30 to 40 minutes the cortical EEG is replaced by low voltage fast activity, a regular hippocampal theta rhythm (5-6 Hz), an isoelectric EMG, while bursts of rapid eye movements (REM) appear intermittently. Approximately half a minute prior to these latter events high voltage EEG spikes appear in the pontine reticular formation, the lateral geniculate body and in the occipital cortex. These PGO spikes have a fairly constant daily rate of about 1-4,000 in the cat (Jouvet, 1969), but have not been identified in human EEG's. For most of these events see Fig. 1.

Sleep events have been classed as phasic and tonic. Phasic events are represented by such activity as middle ear muscle contractions, cardiovascular irregularities respiratory changes muscular twitching, changes in pupil diameter, fluctuations in penile erections and PGO spikes, whereas tonic events are represented by EEG desynchronization, EMG suppression, temperature elevation, increased cerebral blood flow and so on.

Human EEG sleep stages are more differentiated and consists of 4 NREM sleep stages: Stage I, loss of alpha, Stage II, spindles and K complexes on a low voltage background activity, Stage III, high amplitude delta activity, Stage IV with over 50% of delta activity and one REM sleep stage which is characterized by low voltage theta activity, with no spindles or K complexes. There is an increase in the mean values and variability of the heart rate, respiratory rate and blood pressure, there are REM bursts, muscular twitching, areflexia of deep tendon reflexes, and loss of muscle tone.

In animals these stages of sleep have been variably called synchronized, NREM, forebrain or slow wave sleep, when referring to the initial stage, and REM, paradoxical, hindbrain, activated, desynchronized or fast wave sleep, when referring to the second stage. In both humans and animals the NREM period of sleep makes up about 70-80% of total sleep, whereas REM sleep makes up the remaining 20-30%.

NEUROHUMORS IN SLEEP AND WAKEFULNESS

Despite the great number of studies which have attempted to find the neurohumors responsible for the production and/or maintenance of sleep, knowledge about such substances remains today as elusive as when the search for them first began. Even a cursory review of the literature tends to indicate that there not only exists a wide variety of candidate neurotransmitters proclaimed to be hypnogenic but that most of the evidence is indirect.

Since the biogenic amines have been the principal focus of attention for the past several years in relation to a variety of behaviors, it is not surprising that they would also be thought of as being involved in sleep and wakefulness.

A body of rapidly accumulating evidence (Jouvet, 1969) has indicated that serotonin (5-HT) is possibly involved in the induction and maintenance of sleep and in particular slow wave sleep (SWS). Thus, in cats, destruction of the raphé nuclei, known to be made up mostly of 5-HT neurons (Dahlstrom and Fuxe, 1964) leads to a three-way correlation between extent of lesion, amount of sleep and serotonin in the forebrain. Injections in cats (Koella et al, 1968;

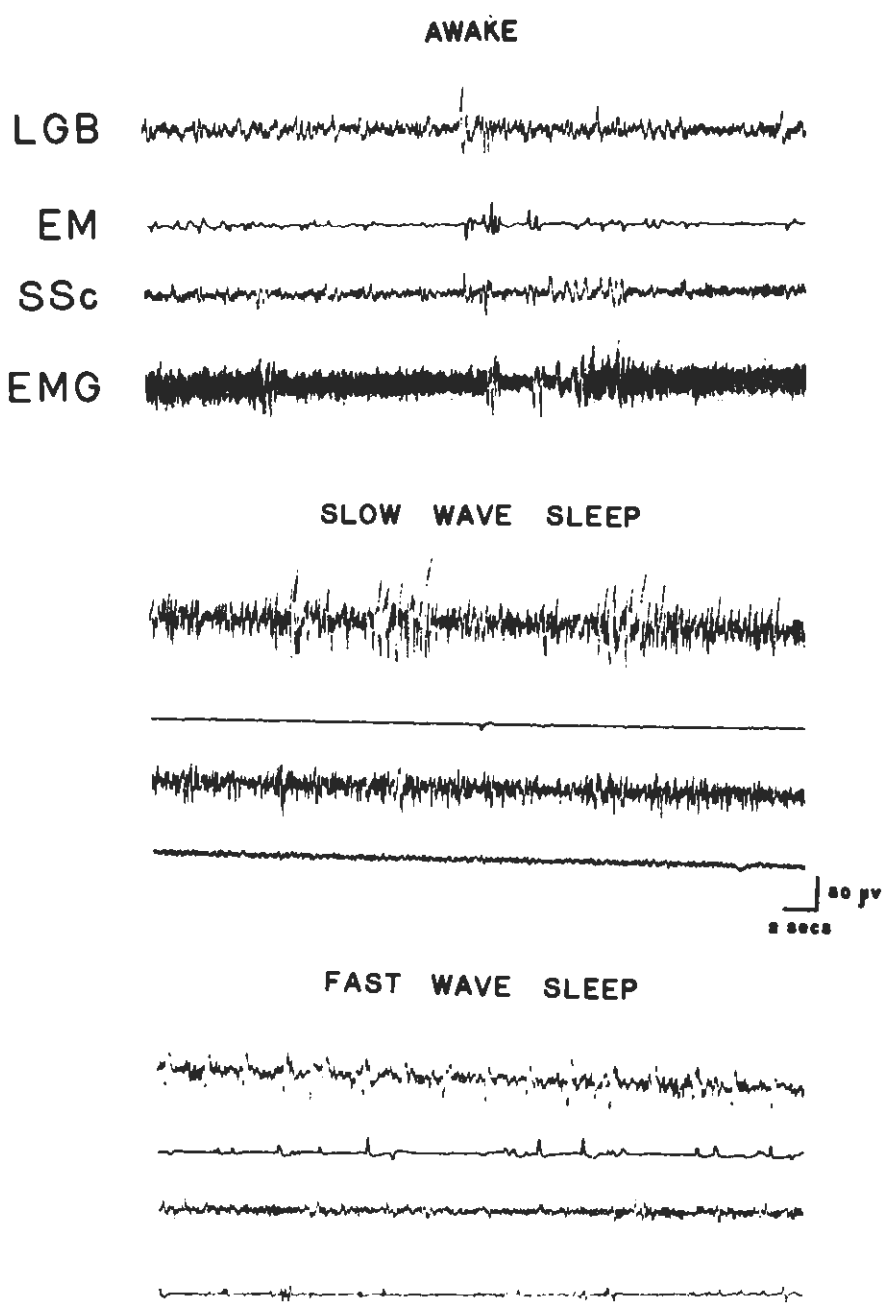

Fig. 1 EEG during wakefulness, slow wave sleep and fast wave sleep. Note during wakefulness the desynchronized EEG and the high amplitude EMG, the latter reflecting sustained muscular tonicity; during slow wave sleep the decreased muscle tonus and the appearance of high amplitude slow waves; and in fast wave sleep the total lack of muscle tonus, the PGO waves in the LGB and the desynchronized EEG similar to that of wakefulness. LGB: lateral geniculate body; EM: eye movements; SSC: supra sylvian cortex; EMG: electromyogram.

Delorme et al, 1966) rats (Mouret et al, 1967; Torda, 1967), rabbits (Florio et al, 1968) and monkeys (Weitzman et al, 1968) of para-chlorophenylalanine (PCPA) which depletes brain 5-HT by inhibiting tryptophan hydroxylation produces a prolonged period of behavioral and EEG state of wakefulness. In man, however, PCPA seems to reduce only REM sleep (Wyatt et al, 1969). The reduction of sleep by PCPA can be reversed by low dose injections of the serotonin precursor, 5-hydroxytrytophan (5-HTP), which circumvents inhibition of trytophan hydroxylation (Koella et al, 1968; Jouvet, 1969).

In addition to this, when 5-HTP is injected intravenously, for the following 5 to 6 hours, a state resembling SWS ensues (Jouvet, 1967). Rats on trytophan free or tryptophan-rich diets have significant changes in their SWS/FWS (REM) cycles, the former showing longer and the latter shorter cycles (Hartmann, 1967). Finally a single dose of reserpine suppresses SWS and REM for 10 to 12 hours, while 5-HTP administration following reserpine, restores SWS (Jouvet, 1967). On the basis of this evidence, it would seem that serotonin is essential for the appearance of SWS. However, there is some data that casts some doubt as to whether serotonin is a sleep transmitter. For example Dement et al (1969) have shown that even though serotonin content is kept at very low levels in the brain by repeated administration of PCPA, cats recover their normal sleep cycles after 7 to 8 days, and it has also been shown that repeated doses of PCPA to rats is incapable of blocking SWS during REM sleep deprivation (Drucker-Colín et al, 1971). Another difficulty in considering serotonin a hypnogenic neurotransmitter, is the fact that when lesioning the raphé system, the increase in waking time may be due to the interruption of ascending fibers originating in a "synchronizing" caudal brain stem system (Cordeau and Mancia, 1959; Mancia et al, 1968). or to destruction of raphé nuclei containing some unknown indolamine (Bjorklund et al, 1971).

Further complicating factors arise from Polc and Monnier's (1970) observations that both low and high frequency stimulation of the raphé magnus produced an EEG arousal effect in rabbits, thus suggesting that at least in this animal there exists an activating ponto-bulbar raphé system, an observation difficult to reconcile with the idea of a serotoninergic sleep inducing raphé system (Jouvet, 1969), unless we invoke species differences.

However, despite conflicting evidence, the bulk of observations tend to support the idea that serotonin is involved in sleep, mainly SWS, particularly if we take into consideration that local application of serotonin to the raphé shortens latencies of SWS and FWS (Drucker-Colín et al, 1972), and methysergide, a serotonin blocking agent, decreases sleep (Tabushi and Himwich, 1971).

Jouvet and his co-workers (1969) have reported that drugs which block norepinerphrine synthesis selectively block REM. Thus, α-methyl-p-tyrosine which blacks tyrosine hydroxylase (Spector et al, 1965), and disulfiram which impairs synthesis of nor-epinephrine at the level of dopamine β-hydroxylase (Goldstein et al, 1964), suppress REM sleep. In addition destruction of the catecholamine containing neurons (Dahlstrom and Fuxe, 1964) in the locus coeruleus reduces REM sleep. These observations added to the ones showing that nialamide selectively suppressed REM sleep and that during rebound of REM sleep deprivation there exists an increased turnover of nor-epinephrine (Pujol et al, 1968), suggested to Jouvet (1969) that REM sleep was nor-epinephrine dependent.

There are, however, several difficulties

in linking NE exclusively with REM sleep. First of all, intracerebral administration of NE or E either in solution or in the form of microcrystals induces a state of arousal with electrocortical desynchronization (Cordeau et al, 1963; Hernández-Peón, 1963; Rothballer, 1957; Yamaguchi et al, 1964; Torda 1968). Intraventricular administration of catecholamines, though having been reported in the earlier literature as producing a sleep-like state (Feldberg and Sherwood 1964; Leimdorfer and Metzner 1949), could not be confirmed in some recent experiments, and in fact was shown to produce opposite effects, mainly a prolonged period of arousal (Cordeau et al, 1971; Segal and Mandell, 1970). These observation agree well with those showing that intravenous administration of NE will desynchronize the "encephale isolé" synchronized activity (Bonvallet, et al, 1954, Rothballer, 1956, 1959). Although it has been argued that NE does not cross the blood-brain barrier the fact is that some brain areas escape the blood-brain barrier (Koella and Sutin, 1967). There are observations, however, that such arousal or cortical desynchronization is the result of the increased blood pressure concomitant to NE administration (Baust et al, 1957), though this has not been fully established (Mantegazzini et al, 1959, Rech and Domino, 1959).

Additional evidence indicating the possible role of NE in wakefulness, comes from experiments showing that intraperitoneal injections of DOPA is followed by a state of quiet wakefulness with mydriasis and cortical desynchronization lasting from 3 to 4 hours (Jouvet, 1967), and by experiments showing that inhibition of catechol-o-methyl-transferase (COMT) and monoamine oxidase (MAO) leads to continous waking, this arousal being much more intense when DOPA was added to such enzymatic inhibition (Jones, 1972). In general the bulk of evidence points towards NE as having a role in activities related to the waking state, particularly if we consider that the amphetamine induced excitation can be prevented by blocking NE synthesis (Bloom and Giarman, 1968), and by the observations that MAOI have strong amphetamine like effects which last some 6-10 hours. It is true, however, that after MAOI administration, the first effect is a rise in both 5-HT and NE, but that after some 5 hours NE levels begin to fall gradually, while 5-HT continues to increase (Green and Erickson, 1962). These latter results are a bit difficult to reconcile with a catecholamine arousal effect. Perhaps as has been suggested by Jones (1972) NE has a dual role, and thus the NE containing cells of the pontine and mesencephalic reticular formation are involved in waking, while those of the pontine tegmentum which contain NE and monoamine oxidase are involved in REM sleep through deaminated metabolites of NE.

In view of the fact that many of the lesions described by Jouvet include tegmental areas which are known to contain large amounts of cholinesterase (Lewis and Schute, 1967; Schute and Lewis, 1967), it is quite possible that Jouvet's results may in part be due to destruction of a cholinergic system. Furthermore several pieces of evidence indicate that acetylcholine (Ach) is in fact involved in sleep. Hernández-Peón and his colleagues (1963) have demonstrated the existence of a widespread ascending and descending cholinergic hypnogenic system, through studies showing that local application of microcrystals of Ach in various brain areas is followed in a few minutes by SWS and later by REM sleep. It has also been shown that during REM sleep there occurs an increased release of cortical Ach (Jasper and Tessier, 1971); this increase, however, is similar to the one occuring in wakefulness, and may thus be related specifically to cor-

tical desynchronization (Celesia and Jasper, 1966). In addition since eserine has been shown to decrease REM latency and increase RFM time (Iouvet, 1962) it seems possihle that Ach may he involved in all three stages of the wakefulness-sleep cycle.

Although it seems probahle that monoaminergic and cholinergic systems are involved in sleep and wakefulness, it seems just as probable that there are other amino acids involved. Despite the possible role of the GABA system in sleep, little work has been done in this area, if we compare it with the amount of effort expended on the biogenic amines. It has long been known that —hydroxybutyric acid (GHB) can induce sleep (Laborit et al, 1960). This effect on humans has been confirmed in mice, rats, rabbits and cats (Pérez de la Mora and Tapia, 1970), although in cats sleep induced by GHB differed from natural sleep in that there was no closing of eyelids (Drakontides et al, 1962), and in humans, sleep EEG was dissociated from behavior (Metcalf et al, 1966).

Furthermore it has been shown that ethanol potentiates the hypnogenic effect of GHB (McCabe *et al.*, 1971), this being possibly due to the increased stimulation of GABA synthesis by ethanol (Roth, 1970; McCabe *et al.*, 1971). In this respect it is interesting to note that GHB is a constituent of some wines, (McCabe *et al.*, 1971; Webb *et al.*, 1967; 1969) and as Pérez de la Mora and Tapia (1970) have suggested we migth speculate that the well known soportific effect of wine may be related to its GHB content.

Although all the above observations again only indirectly relate the GABA system with sleep, and a dubious type of sleep at that, there does exist however some evidence more directly suggesting a role for GABA in sleep. Thus Jasper et al, (1966) and Jasper and Koyama (1969) observed that brains of cats showing a sleep-like EEG pattern following midbrain transection, released 3 times more GABA from the cortex than aroused animals, the latter showing GABA release identical to controls. It may be important to note than in these experiments, aroused animals showed a higher release of glutamic acid than sleeping animals. Since GABA is formed almost exclusively from l-glutamic acid by the enzyme glutamic acid decarboxylase (GAD) (Cooper *et al.*, 1970; Robetrs and Kuriyama, 1968) Jasper's experiments may suggest that sleep may be dependent on GABA formation from glutamic acid accumulated during wakefulness. An experiment determining GABA synthesis during sleep deprivation may answer this, and is badly needed.

As can well be seen the "hypnogenic substance" is still at large, and most of the evidence linking a particular neurotransmitter with sleep is indirect.

Strangely enough, but possibly due to technical difficulties, few people have extended Legendre and Pieron's (1910, 1911, 1913) and Pieron's (1913) observations that cerebrospinal fluid (CSF) of experimentally fatigued dogs, induced signs of drowsiness and sleep when injected into the IV ventricle of non-fatigued dogs. Some 30 years later in a series of better controlled studies Schnedorf and Ivy (1939) confirmed these observations. However, it wasn't until some 20 years later that his research approach was reopered (Monnier *et al.*, 1969, 1964, 1965, 1971). These investigators by using a crossed circulation arrangement between two animals, have shown that sleep induced by electrical stimulation of the medial thalamus in a donor animal, releases a bloodborne factor capable of producing sleep in a recipient animal. This work extended earlier observations by Purpura (1956), who had shown in similar crossed circulation, arousal in

recipient cats following stimulation of donor cats.

In more recent years different approaches have been used in order to find whether a specific sleep substance can in fact be obtained. Pappenheimer and his group |Pappenheimer *et al.*, 1967; Fancl et al, 1971) have been able to demonstrate the existance of a low molecular weight (500) sleep promoting factor in CSF of sleep deprived goats, which is not species specific, since it can produce its effects in both rats and cats. Ringle and Herndon (1969), however were unable to demostrate such effect with CSF extracted from sleep deprived rabbits.

A yet different approach has been used by Drucker-Colín *et al.* (1970) and Drucker-Colín (1972), who have shown that it is possible to extract, through "pushpull" cannulas implanted in the midbrain reticular formation of sleeping cats, a substance which can induce sleep in a recipient awake cat. This "push-pull" system which has been successfully utilized by Myers and his group (1967, 1971; Yaksh and Myers, 1972) while studying eating, drinking and temperature regulation, may prove to be the most successful approach in the attempts to demostrate and characterize the hypnogenic substance, since this technique allows extraction of fluids directly from brain tissue where release of substances may more clearly reflect and be correlated with physiological functions. As can be seen from Fig. 2, such perfusates extracted from the midbrain reticular formation of a donor cat, when cross-perfused into recipient cats can induce physiological sleep within short periods of time.

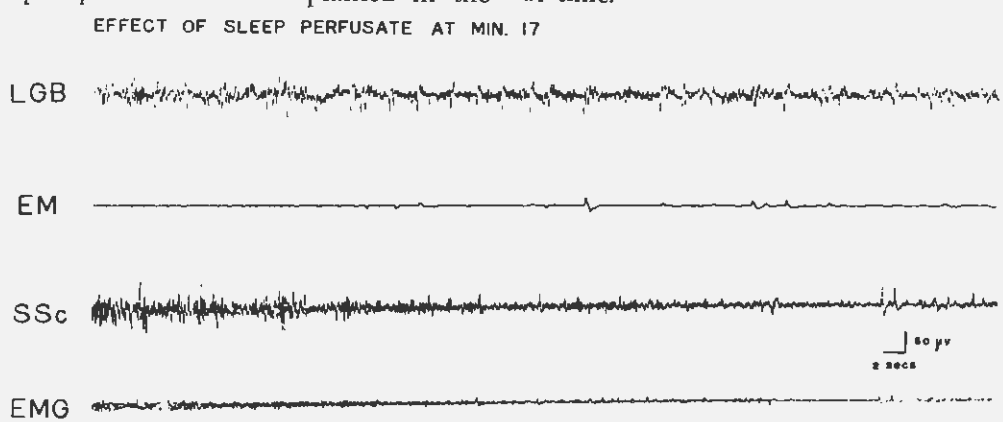

Fig. 2 EEG tracings from a recipient cat, at min 17 of reperfusion of donor's sleep effluent. Note PGO waves in LGB, eye movements, cortical desynchronization, and the isoelectric EMG, all characteristics of FWS, thus indicating that donor's sleep effluents induced natural sleep in recipient cats.

Such effect was much more dramatic in another cat to whom the sleep perfusate was injected into the raphé nucleus. This cat (Fig. 3) who 16 min. after the injection of the perfusate presented a state of hyperactivity, fell 5 sec. later and for 70 min. into slow wave sleep.

Although the physiological effects of the perfusates are quite clear, the most important question as to which may be the chemical nature of the "inhibitory transmitter" released at pre-synaptic terminals of the "hypnogenic neurons" necessary to produce hyperpolarizations of the post-synaptic "vigilance neurons", is still a mystery.

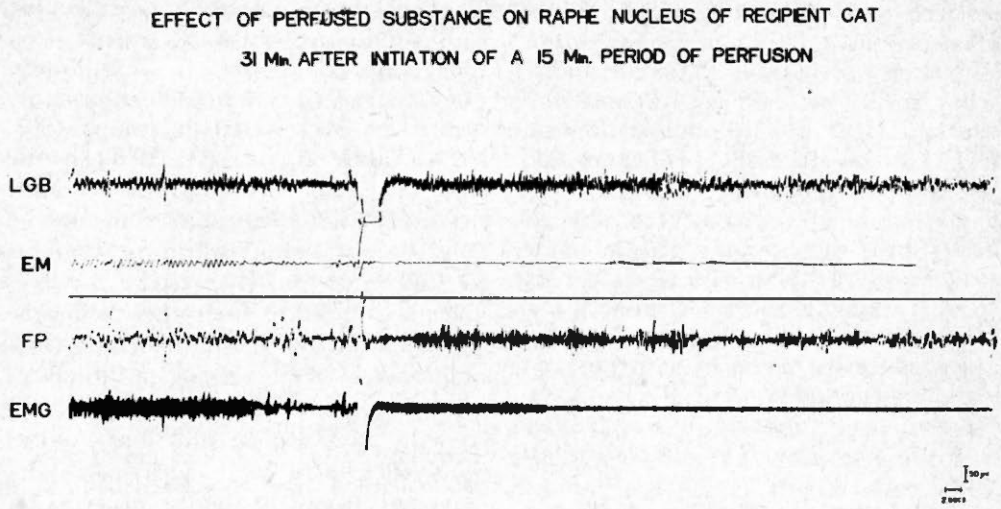

Fig. 3 EEG recordings of cat receiving sleep perfusate into its raphé nucleus. Note, at arrow, movement artefact produced when animal suddenly dropped his head, and appearance of SWS 5 sec later. It is also important to note that prior to these events the animal was extremely alert as can also be seen from the recordings, and in particular from the EMG and the eye movements.

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