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Neocortical Rhythmic Slow Activity during Wakefulness and Paradoxical Sleep in Rats

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Abstract. In the present study, we investigated the different types of neocortical rhythmic slow activity (RSA) during wakefulness and paradoxical sleep as well as their pharmacological modification. During wakefulness, the high-frequency (7–9 Hz) RSA_1 type, which is atropine-resistant, is accentuated by forebrain stimulation and is abolished by urethane, clonidine and alcuronium. These drugs induce the low-frequency (4–6 Hz) RSA_2 type that is atropine-sensitive and is activated by cholinergic agents and by some drugs such as tabernanthine, ibogaine, vincamine, SL 76.188-MS (10-chloro-hexahydrocanthinone methanesulphonate). The effects of pilocarpine and SL 76.188-MS on RSA_2 are antagonized by atropine and hemicholinium-3, which suggests the involvement of a cholinergic pathway in the neocortical RSA activation (as has been demonstrated for the hippocampal RSA). During paradoxical sleep, two types of RSA are also observed: RSA_T, of low frequency (5–7 Hz) present during its tonic components, and RSA_p, of high frequency (7–9 Hz) which is well correlated with phasic phenomena such as bursts of rapid eye movements generated, or controlled, by cholinergic mechanisms. Imipramine reduces phasic phenomena and the periods of neocortical RSA_p. Alcuronium does not modify RSA_p in paradoxical sleep-deprived rats and suppress RSA_1 during arousal, observations which would suggest that RSA_p and RSA_1 are regulated by two distinct central mechanisms. The EEG studies of neocortical RSA during wakefulness and paradoxical sleep allow the selection and the differentiation of pharmacological agents. Furthermore, this approach not only may represent a basis for the treatment of deficits in the regulation of vigilance and memory, but also a novel strategy for the analysis of RSA type of paradoxical sleep with respect to antidepressant and anxiolytic treatment.

In both wakefulness and paradoxical sleep, neocortical and hippocampal rhythmic slow activity (RSA; theta rhythm) occurs spontaneously in various species [1–4]. In rats, two distinct types of RSA can be observed in the neocortex as well as in the hippocampus [5–7]. During waking, the first type is of high frequency (7–9 Hz; RSA_1), is atropine-resistant, and is sensitive to ether and urethane anaesthesia. The RSA_1 is associated with locomotion and other voluntary movements. The second is of low frequency (4–6 Hz; RSA_2), is atropine-sensitive, ether-resistant, and may be present during behavioural immobility.

During paradoxical sleep (PS), two further types of RSA are also present [3, 7–11]. These are subdivided into tonic components (RSA_T) of low amplitude and frequencies (4–7 Hz), and phasic components (RSA_p) characterized by a large amplitude and high frequencies (7–9 Hz). RSA_p is concomitant with phasic phenomena such as bursts of rapid eye movements (REM bursts).

The present study considers the different neocortical RSA in rats and their pharmacological and experimental modification as investigated by sequential spectral analysis.

Methods

The animals and the surgical procedures used have been described elsewhere [9–12].

Chronic Preparations

In freely moving implanted rats, silver wire monopolar neocortical electrodes were soldered to a watchmaker's stainless steel screw (0.9 mm in diameter). Male Sprague-Dawley rats (250 g body weight) were anaesthetized with methohexital (Brietal®: 20 mg/kg

s.c.), the scalp retracted and the skull electrodes positioned on the area postcentalis oralis (sensorimotor area, SM) and area striata (visual cortex, Vis.). The deep monopolar electrodes (platinium-iridium, diameter 150 µm, uninsulated tip 0.2 mm) were implanted into the hippocampus: stratum moleculare (CA_1) and gyrus dentatus (GD). The electrodes were inserted into miniaturized connectors (Winchester, Litton) and fixed onto the skull of the rat with resin cement ('PD' Germicidal). The neocortical reference electrode was screwed into the interparietal bone (cerebellum).

Acute Preparations

The same implantation as for the chronic preparations was performed except that the connectors were not employed. Male Sprague-Dawley rats (Charles River, France, 220 g body weight) were anaesthetized with halothane 4% in a Plexiglas box, then muscular relaxation was achieved with alcuronium (5 mg/kg i.p.) and artificially ventilated with air using a mask over the muzzle. All pressure points and surgical incisions were infiltrated with lidocaine 2%. Body temperature was maintained at 37.5 °C. Electrocorticogram (ECoG) recordings were carried out after a 30-min control period.

Procedure

For each rat, the EEG continuously recorded on Grass polygraphs (Model 79), was simultaneously registered on FM magnetic tape using a Hewlett-Packard recorder (Model 3968 A). Sequential power spectra of 30-second EEG periods of wakefulness or PS were performed on SM or Vis. recordings, using a Berg-Fourier analyser. Power spectral densities were estimated at 0.25-Hz intervals from 1 to 16 Hz (filter: 1 and 16 Hz, 48 dB/Oct.).

The drugs studied were given via the intraperitoneal or oral route (in a volume of 5 ml/kg). Urethane (1 g/kg i.p.), clonidine (0.1 mg/kg i.p.) and imipramine (10 mg/kg i.p.) were studied in freely moving rats implanted chronically with electrodes. Increasing doses of drugs were also administered intraperitoneally at 30-min intervals in acute preparations (dose ranges are given in mg/kg i.p. in parentheses): oxotremorine (0.03–0.3), pilocarpine (1–3), physostigmine (0.3–1), tabernanthine (10–30), ibogaine (10–30), vincamine (10–30), *d*-amphetamine (1–3) and SL 76.188-MS (10–30;-10-chloro-hexahydrocanthinone methanesulphonate). Interactions with atropine sulphate (10–20 mg/kg i.p.), ethanol (1–2 g/kg p.o.), hemicholinium-3 (10 µg by intracerebroventricular route, i.c.v.) or yohimbine (1 mg/kg i.p.) were also examined.

RSA_1 type elicited by forebrain stimulation (diagonal band: 100 Hz; 0.1 ms; 250 µA) in immobilized animals was examined as well as the interaction of *d*-amphetamine (0.3 mg/kg i.p.) and haloperidol (0.3–1 mg/kg i.p.) on the stimulation-induced RSA_1.

Effects of 14 days imipramine (10 mg/kg i.p.) treatment on cortical RSA during PS were analyzed in chronically implanted rats. The influence of 72-hour paradoxical sleep deprivation on RSA activity during PS rebound was also examined as well as its modification by alcuronicum, a neuromuscular blocker, in artificially ventilated rats.

Expression of Results

The sequential spectral analysis summarizes a long experimental period (a few hours) and allows the rapid characterization and visualization of the effects of pharmacological agents (latency, intensity of action, etc. ...). Moreover, the reliability of this method (in acute preparations) to differentiate and evaluate different classes of central acting drugs [4, 12] is such that a limited number of animals (n = 4) are required. If sequential spectral analysis represents a robust visual method for the global analysis of an experiment, it does not allow statistical analysis under the conditions used which necessitate EEG quantification. Nonetheless, sequential spectral figures are more representative than discrete electrocorticographic tracings.

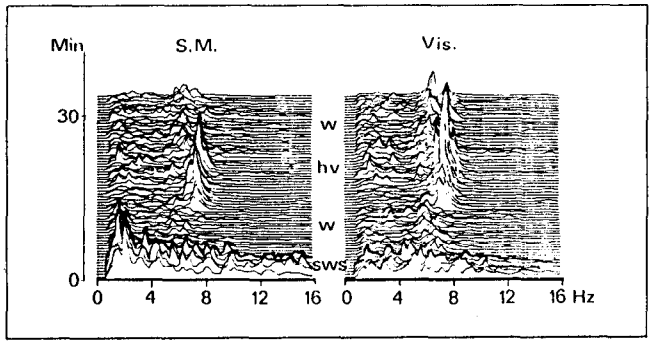


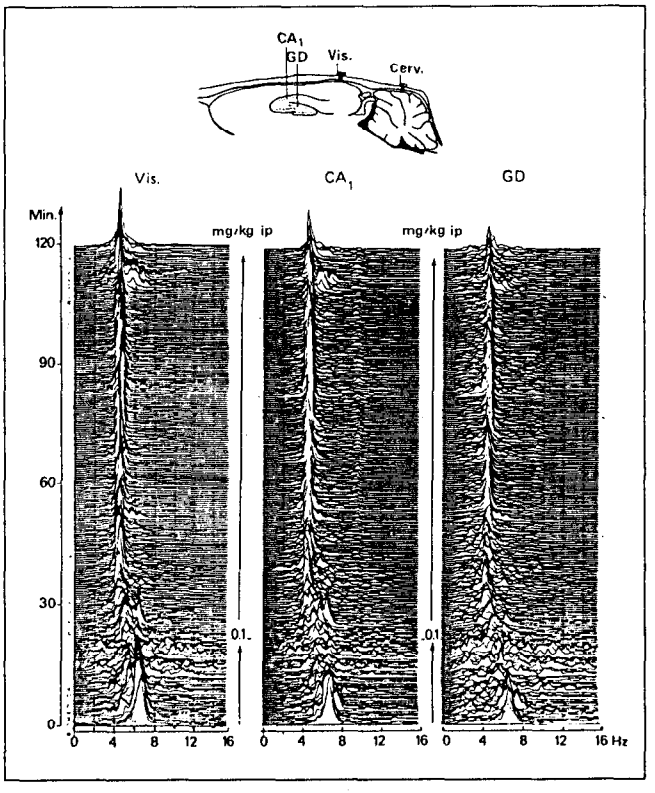
Fig. 1. Sequential spectral analysis of 30-second EEG epochs during wakefulness in a freely moving rat. w: Quiet wakefulness; hv: hypervigilance; sws: slow wave sleep.

Results

During the sleep-wakefulness cycle recorded in freely moving rats, sequential spectral analysis of 30-second epochs (fig. 1) shows different dominant peaks in the EEG power spectra according to the vigilance level. In quiet wakefulness, the maximum energy is seen in the frequency bands of 5–6 Hz (RSA_2 type) while it is present in 6.5–7.5 Hz frequencies (RSA_1 type) during hypervigilance.

The RSA_1 type, present during hypervigilance and voluntary movements, is abolished by administration of urethane (1 g/kg i.p.) and clonidine (0.1 mg/kg i.p.; fig. 2) which induce RSA_2 type. The RSA_2 type, present during behavioural immobility, appears continuously and with a stable activity (5–6 Hz) after treatment with alcuronium (5 mg/kg i.p.) in artificially ventilated rats (see the sequential spectral analysis of EEG control periods before the different drug administration of fig. 4–7). In this immobilized preparation, forebrain stimulation can elicit RSA_1 type (fig. 3) which is activated by *d*-amphetamine (0.3 mg/kg i.p.) and depressed by haloperidol (0.3–1 mg/kg i.p.). These data are not illustrated.

Furthermore, in alcuronium-treated rats, the RSA_2 type is depressed by amphetamine (1–3 mg/kg i.p.) [data not shown] and activated (increase of the theta-dominant peak) by clonidine (0.1 mg/kg i.p.), an alpha-2 adrenoceptor agonist (fig. 4), by pilocarpine (1–3 mg/kg i.p.; fig. 5) or oxotremorine (0.1–0.3 mg/kg i.p.), both cholin-



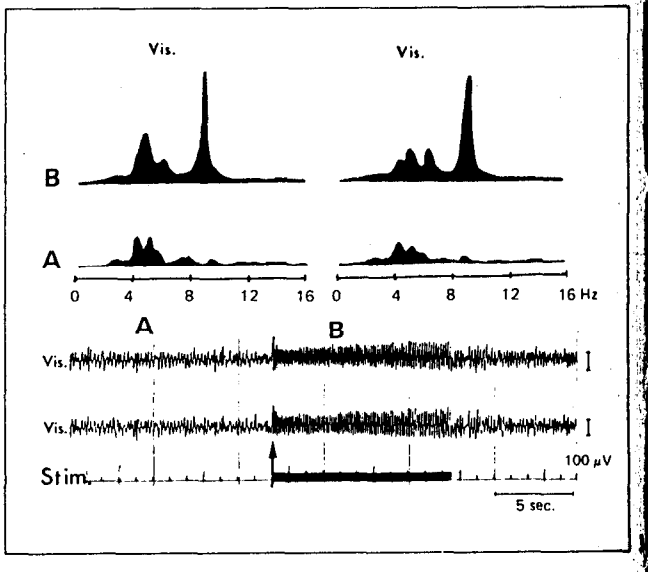


Fig. 2. Sequential spectral analysis (30-second periods) of the EEG recordings before and after clonidine (0.1 mg/kg i.p.) in a freely moving rat. Hippocampus: CA_1 and GD.

Fig. 3. Electrocorticogram and its absolute power density before (A) and after (B) forebrain stimulation (diagonal band; 100 Hz, 0.1 ms, 250 µA) in an immobilized rat (alcuronium: 5 mg/kg i.p.).

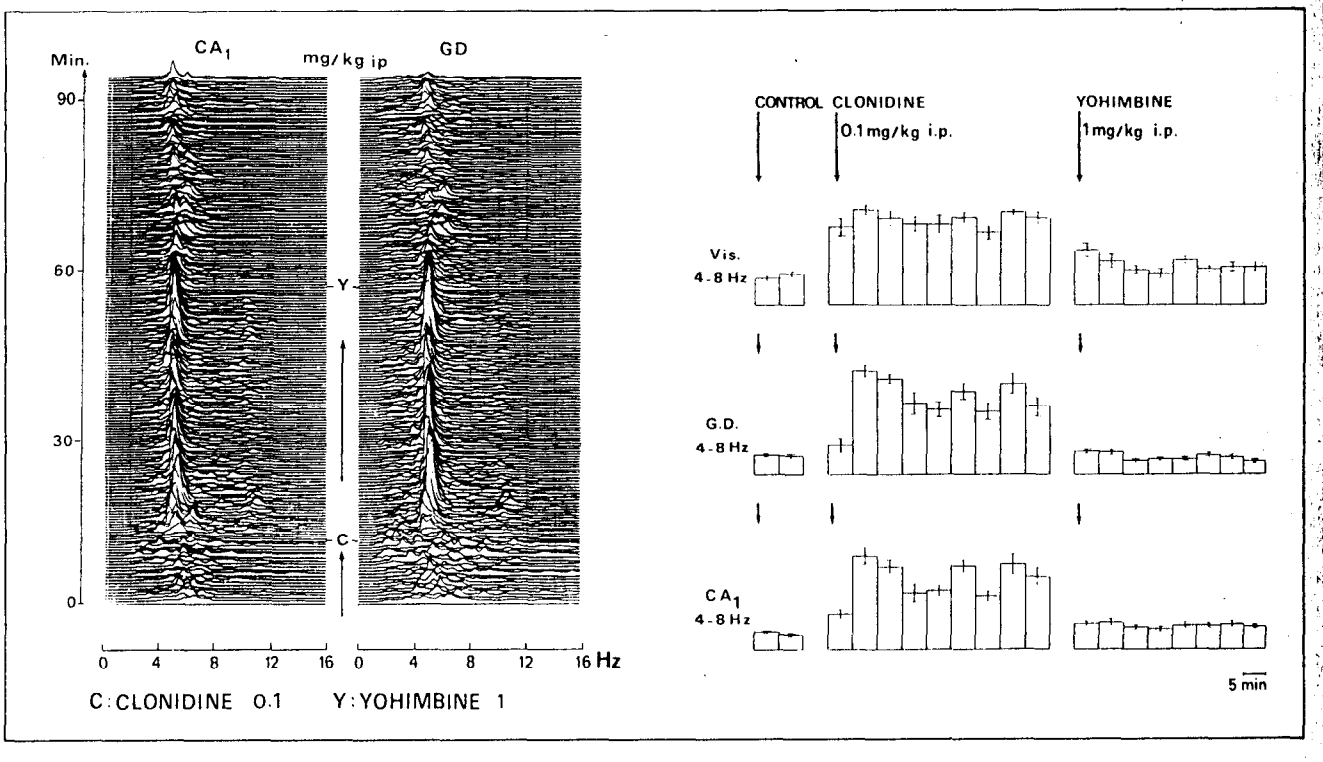


Fig. 4. Influence of clonidine (C, 0.1 mg/kg i.p.) and the interaction with yohimbine (Y, 1 mg/kg i.p.) on the rhythmic slow activity (RSA_2; 5 Hz) in an immobilized rat. On the left: sequential spectral analyses in the hippocampus. On the right: mean amplitude of the theta peak in 5-min epochs (arbitrary units).

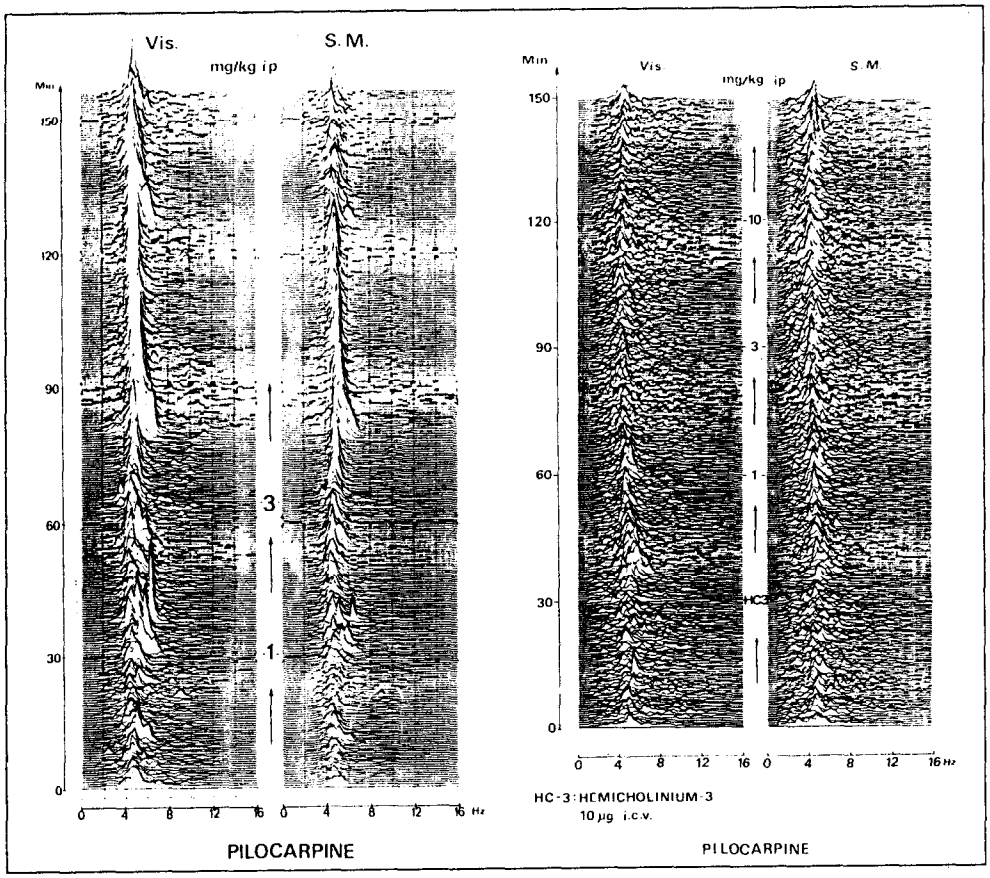


Fig. 5. Sequential spectral analysis of the influence on the low theta peak (5 Hz) of pilocarpine (on the left) and of its abolition of the pilocarpine effect by the prior i.c.v. administration of hemicholinium-3 (on the right) in artificially ventilated rats.

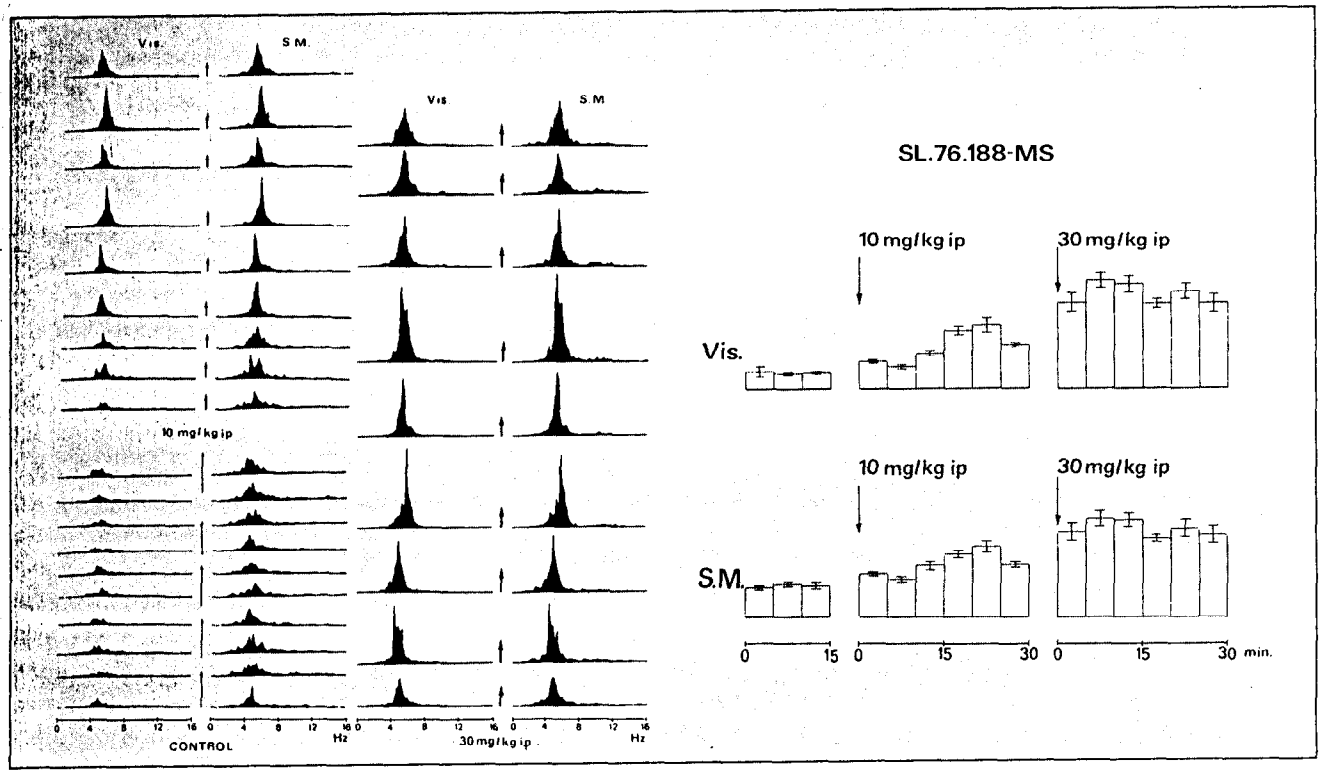
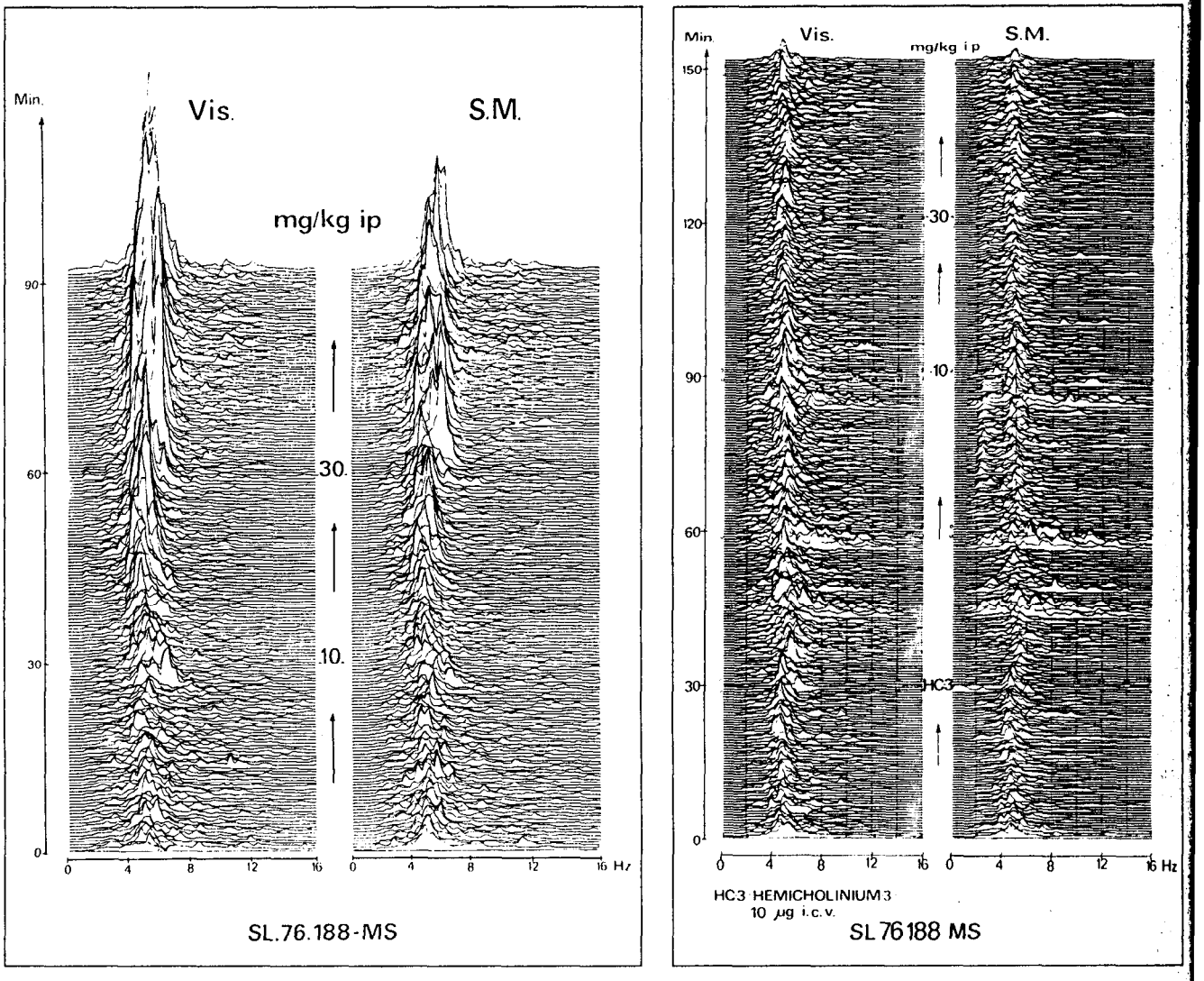


Fig. 6. Influence of increasing doses of SL 76.188-MS on the power spectra in an immobilized rat. Sequential power spectra (30-second EEG periods) on the left and mean amplitude of the theta peak (in 5-min epochs; 5 Hz; arbitrary units) on the right.

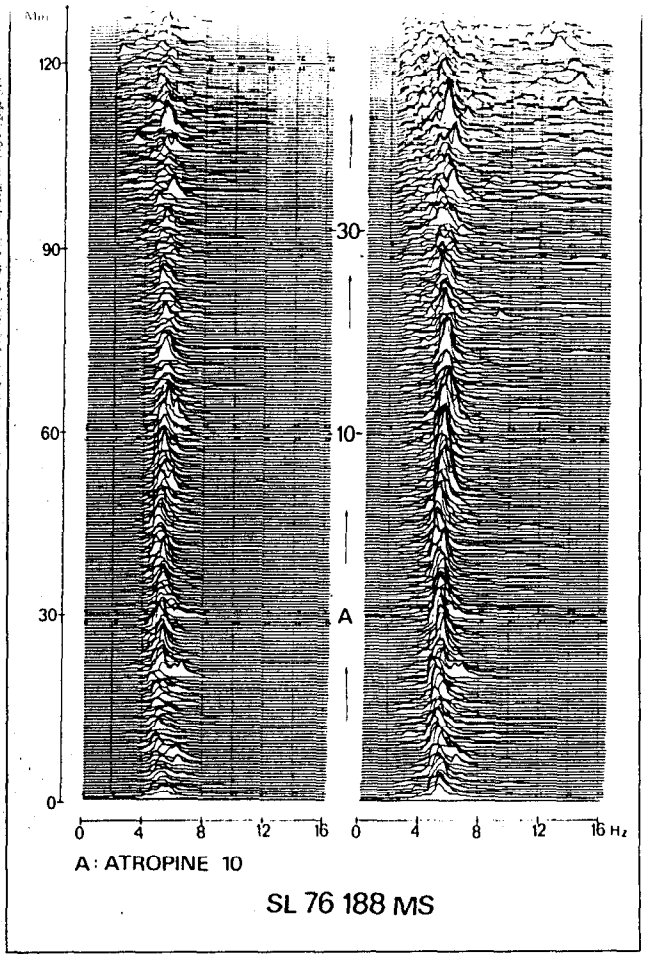


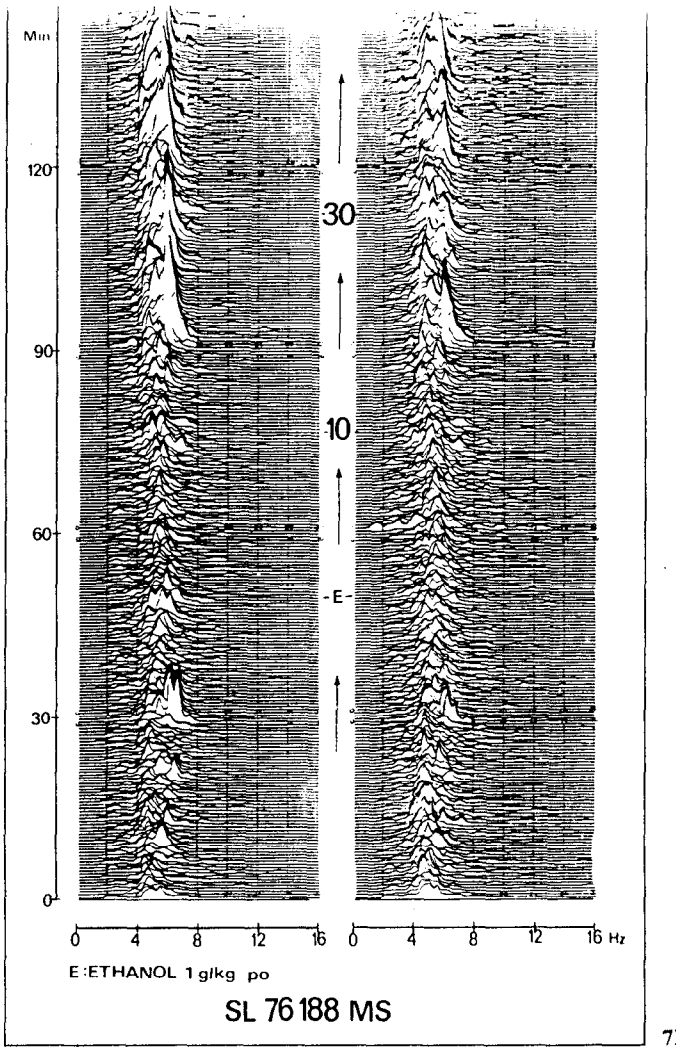
7A

ergic agonists, by physostigmine (0.3–1 mg/kg i.p.), an anticholinesterase inhibitor, and by some psychostimulant agents such as SL 76.188-MS (10–30 mg/kg i.p.; fig. 6, 7) as well as the alkaloids; tabernanthine, ibogaine (10–30 mg/kg i.p.), and vincamine (10–30 mg/kg i.p.).

The activation of visual and hippocampal RSA_2 activity by clonidine is antagonized by yohimbine (1 mg/kg i.p.), an alpha-2 antagonist (fig. 4). The RSA_2 activation by pilocarpine or by SL 76.188-MS is abolished by the systemic injection of atropine sulphate at doses of 10–20 mg/kg i.p. (fig. 7) or by the administration of hemicholinium-3 (10 µg i.c.v.; fig. 5, 7). In contrast, the oral administration of 1 or 2 g/kg of ethanol does not abolish the action of SL 76.188-MS on the theta peak (fig. 7).

In chronically implanted rats, two types of RSA are present during paradoxical sleep: RSA_T of low frequency (5–7 Hz) and RSA_p of high frequency (7–9 Hz) concomitant with phasic phenomena such as REM bursts. During paradoxical sleep, RSA_T type is reinforced by the chronic treatment with imipramine (10 mg/kg i.p.; fig. 8). During imipramine treatment, spectral analysis of 30-second periods of paradoxical sleep theta rhythm revealed a decrease in the dominant frequency compared to the control session (from 7 to 6 Hz), correlated with the reduction of REM bursts (and REM density) and the predominance of RSA_T type (fig. 8). In the 2 days after 14-day imipramine treatment withdrawal, the dominant theta peak during paradoxical sleep increased (from 7 to 9 Hz) as well as the REM density and REM bursts (fig. 8). The increase of the dominant peak of theta activity during paradoxical sleep is also observed after 72 h of paradoxical sleep





7D

Fig. 7 (A–D). Sequential spectral analysis of the effects of SL 76.188-MS as modified by hemicholinium-3 and atropine (though not by ethanol) on EEG recordings in alcuronium-treated rats.

deprivation (fig. 9). In fact, the comparison of power spectrum of 156 phases of paradoxical sleep in normal rats and 217 phases in 7 deprived rats shows that the dominant theta peak between 7 and 9 Hz represents 67% of the total episodes in deprived rats and 27% in normal rats ($p \leqq 0.05$, Student's t test). Moreover, the neuromuscular blocker, alcuronium, which induces continuous RSA_2 type during wakefulness has no significant effect on the increase of the dominant theta peak after paradoxical sleep deprivation. Indeed, the theta peak (7–9 Hz) calculated in 11 deprived rats, immobilized by alcuronium (5 mg/kg i.p.) and artificially ventilated, represents 57% of the total spectral sequences analyzed from 58 paradoxical sleep phases.

Conclusion

Previous studies have shown two distinct types of hippocampal RSA in rats as well as in rabbits [5, 13, 14]. The same is true for EEG recordings of the visual cortex (in which the cerebellum has been used as the reference electrode). In the present investigation, two types have been observed: RSA_1 type, abolished by alcuronium, urethane and clonidine; and RSA_2 type, atropine-sensitive and activated by cholinergic agonists. The neocortex possesses RSA patterns of activation that parallel those seen in the hippocampus. The blockade by atropine of the cholinomimetic EEG activation of RSA_2 type is not restricted to hippocampal activity but is equally ob-

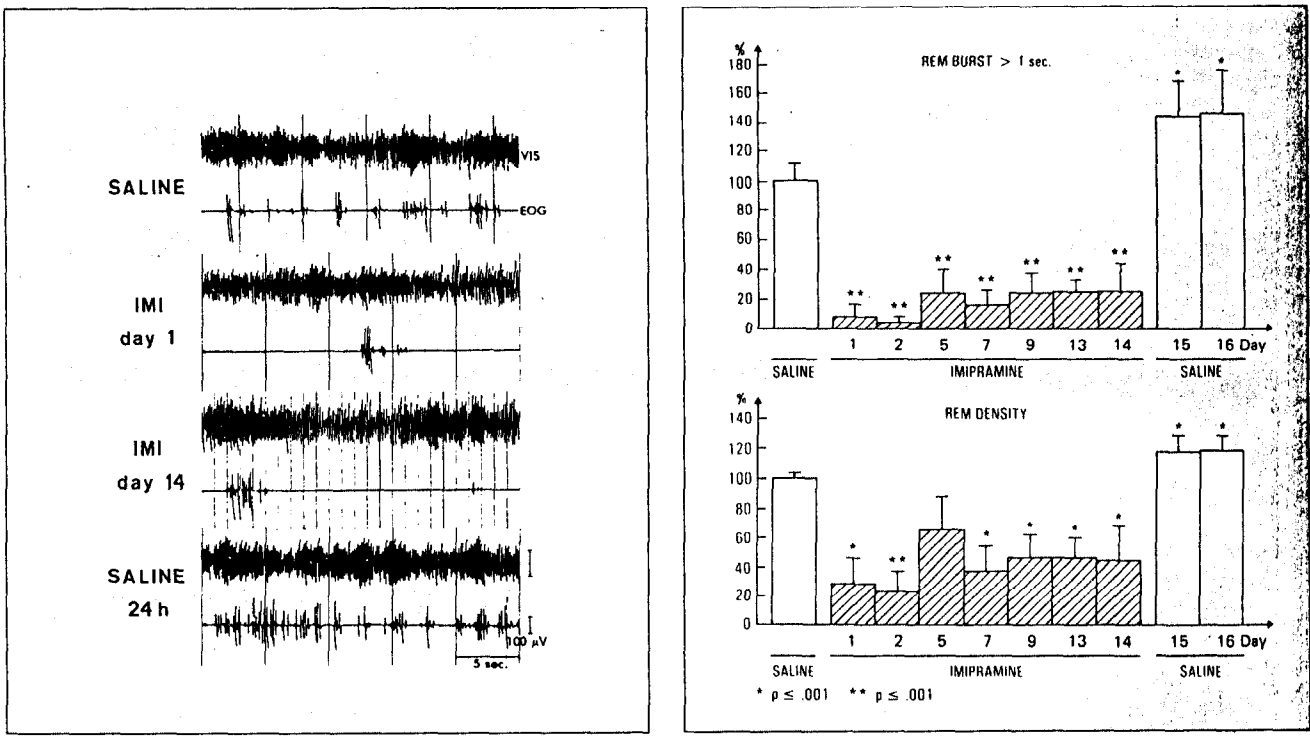


Fig. 8. Influence of 14-day imipramine (IMI) treatment (10 mg/kg i.p.) on the rhythmic slow theta activity of the Vis and on the REM density during paradoxical sleep in chronically implanted rats (n = 5). Electrocorticographic and electromyographic tracings after different treatment durations (on the left) and percent variations of REM bursts and REM density in comparison to saline (on the right). REM bursts and REM density are estimated each min and expressed in percent of the saline control values (100%). The paradoxical sleep phases were observed during the 5th and 6th h following imipramine administration while after saline, the 6-hour recordings (11.00 a.m. to 5.00 p.m.) were analyzed.

served in the visual neocortex. Likewise, clonidine, a sedative agent which induces sleep spindles in the sensorimotor cortex [9, 15, 16] increases the low theta peak in the visual cortex as well as in the hippocampus. These clonidine-induced effects are antagonized by alpha-2 antagonists such as yohimbine or piperoxane [16, 17]. The fact that clonidine-induced RSA_2 type is abolished by atropine suggests that this activity, largely dependent on the cholinergic septohippocampal pathway, can be modulated by alpha-adrenergic systems [18]. Moreover, from our investigation on EEG recordings in the rat during neuromuscular blockade with alcuronium, only RSA_2 type of 5–6 Hz appears. Nevertheless, the anticholinergic-resistant form of RSA (RSA_1) can be recorded during arousal in rats paralyzed by neuromuscular blockade after both fore- and midbrain stimulation [1, 19].

Thus, the stability of the EEG recordings in immobilized rats, as compared to normal rats, provides a means for the study of the central actions of drugs [4, 12]. Psychostimulant agents like *d*-amphetamine (or amantadine, AMPA, 5-HTP [4]) do not activate the RSA_2 while cholinergic agents and some drugs like ibogaine, tabernanthine, vincamine and SL 76.188-MS produce an increase in the energy of the theta band frequency (5–6 Hz). These latter drugs increase alertness: vincamine and SL 76.188-MS increase the duration of wakefulness in rats and the level of vigilance in man [20, 21]. The EEG activation of RSA_2 type is under the control of cholinergic pathways as is confirmed by the fact that the activation is abolished by the systemic administration of both atropine and hemicholinium-3 though not by the non-specific central depressant, ethanol. Hemicholinium-3 has been reported to disrupt cholinergic transmission by blocking choline uptake and thus by inhibiting acetylcholine synthesis in presynaptic terminals [22]. The present findings may be relevant to the investigation of new drugs that would counteract the effects of impaired cholinergic transmission. In fact, pharmacological studies in animals as well as in man support the hypothesis that central cholinergic activity affects learning and memory processes [23, 24]. Moreover, patients with Alzheimer's disease and senile dementia of the Alzheimer

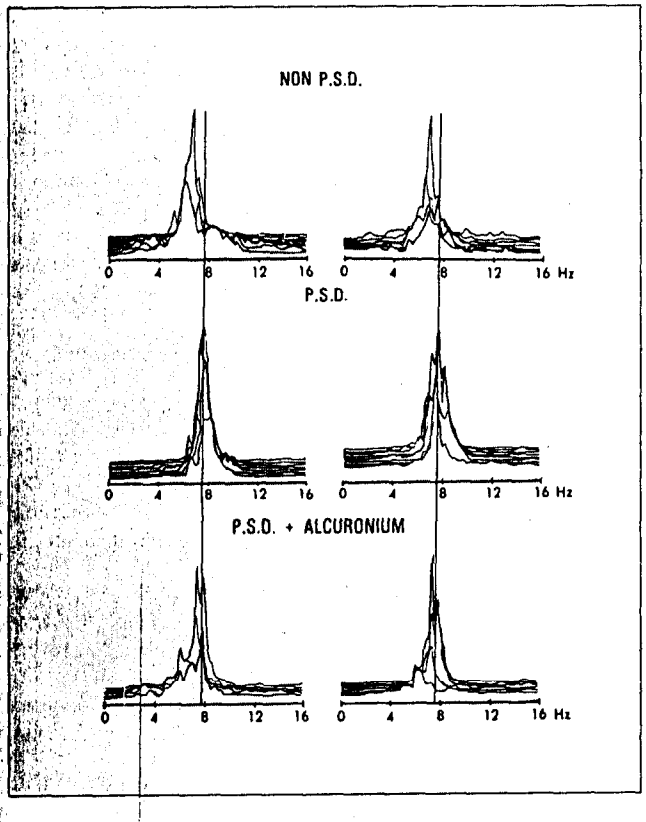


Fig. 9. EEG power spectra of paradoxical sleep episodes in normal rats (non PSD), in paradoxical sleep-deprived rats (PSD) and in paradoxical sleep-deprived, alcuronium-treated rats (PSD + alcuronium).

type show a decline in cholinergic markers in the cortex and hippocampus and a degeneration of cholinergic cells (medial septal nucleus, nucleus basalis of Meynert) that are correlated with the degree of cognitive and memory impairment [25–27].

During PS, two RSA types are also present corresponding to 'tonic' and 'phasic' components but they appear to be different from those observed during wakefulness: the amplitude of the theta rhythms is larger and neuromuscular blockade does not abolish RSA_P type. RSA_P type, of high frequencies and larger amplitude than RSA_T, is present during twitch periods of PS [8, 28] and is well correlated with REM bursts in rats [9, 11]. This correlation is confirmed by the fact that mean RSA frequency is significantly higher after imipramine withdrawal or after PS deprivation, two experimental situations well known to increase REM density [11] and phasic phenomena such as PGO spikes [29]. Electrophysiological and pharmacological studies have provided evidence that rapid eye movements and PGO spikes are controlled by cholinergic mechanisms [30, 32]. Eserine is known to increase phasic phenomena during PS such as REM bursts or PGO spikes which are reduced by atropine [33]. These findings support the idea that RSA_1 and RSA_p, both of high frequency, are in fact two pharmacologically distinguishable types of RSA. RSA_1, present during waking, is not activated by cholinergic agents and is atropine-resistant while RSA_p, present during PS, appears to be generated or controlled by cholinergic mechanisms. Imipramine, an antidepressant with anticholinergic activity, reduces the REM bursts as well as the episodes of RSA_p types. In patients with endogenous depression, their sleep shows an enhancement of PS latency and a decrease of PS duration as well as an inconstant increase in the frequency of REM bursts [34]. Thus, one could hypothesize that a modification in the dominant frequency of PS (increase of RSA_T episodes) and REM density (decrease of REM bursts) by imipramine are just secondary effects, but they might nonetheless contribute to the therapeutic action of this drug. Benzodiazepines, agents with anxiolytic or hypnotic properties, are also well known to induce low RSA during PS [7, 35]. The action of benzodiazepines may be mediated by a GABA system which appears to be an intermediate involved in the control of neocortical activation mechanisms during PS [7].

From our results, the existence of different generators of wakefulness and PS theta rhythms can be postulated. These findings supplement the results of previous studies [1–8, 13, 14], and support the idea that these anatomomical and physiological bases of RSA can be influenced differentially by pharmacological agents. Moreover, the EEG activation of cortical RSA allows the characterization of central drug actions and the selection of pharmacological agents which may represent a rational basis for the potential treatment of the deficits in the regulation of vigilance and memory which are particularly manifest in psychogeriatric patients.

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