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## Mechanisms of seizure suppression during rapid-eye-movement (REM) sleep in cats

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REM sleep is the most antiepileptic state in the sleep-wake cycle for human generalized epilepsy, yet the neural mechanism is unknown. This study verified the antiepileptic properties of REM sleep in feline generalized epilepsy and also isolated the responsible factors. Conclusions are based on 20 cats evaluated for generalized EEG and motor seizure susceptibility before and after dissociation of specific REM sleep components. Bilateral electrolytic lesions of the medial-lateral pontine tegmentum created a syndrome of *REM sleep without atonia*. Systemic atropine created a syndrome of *REM sleep without thalamocortical EEG desynchronization*. Identical results were obtained in two seizure models, systemic penicillin epilepsy and electroconvulsive shock. (1) Normal REM sleep retarded the spread of EEG seizure discharges and had even more potent anticonvulsant effects. (2) Selective loss of 'sleep paralysis' (skeletal muscle atonia) during REM abolished REM sleep protection against myoclonus and convulsions without affecting generalized EEG paroxysms. (3) Conversely, selective loss of thalamocortical EEG desynchronization abolished REM sleep protection against generalized EEG seizures without affecting clinical motor accompaniment. These results suggest that the descending brainstem pathways which mediate lower motor neuron inhibition also protect against generalized motor seizures during REM sleep. Protection against spread of EEG paroxysms is governed by a separate mechanism, presumably the ascending brainstem pathways mediating intense thalamocortical EEG desynchronization during REM sleep.

Clues as to the nature of this differential mechanism are provided by the physiological characteristics of REM sleep.

### INTRODUCTION

REM sleep suppresses the electroencephalographic (EEG) and clinical (behavioral) signs of epilepsy. In fact, REM sleep is the most antiepileptic state in the sleep-wake cycle for generalized epilepsy<sup>27</sup>. Although localized brain epileptic discharges can continue in REM sleep at the same rate as during waking<sup>3,4,27</sup>, epileptic EEG discharge generalization is discouraged during stable REM, and the intact REM sleep state is even more resistant to motor seizure manifestations, such as tonic-clonic convulsions. Protection against generalized electroclinical seizures during REM sleep has been obtained in virtually all human clinical seizure types<sup>4,27</sup> and also in multiple animal models of primary and secondary generalized seizure disorders<sup>2,5,24,25,28</sup>. Animal models displaying suppression of seizure activity during REM sleep include electroconvulsive shock in rats and cats<sup>5,28</sup>, systemic penicillin epilepsy and temporal lobe kindling in *papio*<sup>5</sup>. Moreover, suppression of motor seizure events can persist even when generalized EEG seizures do occur during REM sleep<sup>24</sup>, suggesting differential mechanisms for EEG vs motor seizure suppression.

REM sleep is characterized by tonic components, notably intense thalamocortical EEG desynchronization and lower motor neuron inhibition (skeletal muscle atonia), and also by phasic component such as rapid eye movements and ponto-geniculo-occipital (PGO) spikes. The mechanisms underlying rapid eye movements and PGO spikes are thought to have epileptogenic properties<sup>5,7,34</sup>, whereas the generators of thalamocortical EEG desynchronization and atonia have been hypothesized to mediate seizure suppression<sup>5,20,21,27,34,38</sup>. Mechanisms underlying tonic REM sleep components are to some extent independent, as they can be selectively dissociated from REM sleep. For example, the forebrain components of REM sleep, such as thalamocortical EEG desynchronization, are abolished rostral to transection at the pontomesencephalic juncture<sup>14,39</sup>, whereas atonia is abolished only after transection at pontomedullary cats<sup>2,25</sup> as well as limbic status epilepticus in *Papio* juncture<sup>30</sup>. Differential regulation of these tonic REM

sleep components could provide a basis for differential suppression of EEG discharge generalization vs motor seizures in the REM sleep state.

Accordingly, we evaluated susceptibility to generalized

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EEG and motor seizures, separately, before and after dissociating the tonic components of REM sleep. A selective syndrome of REM sleep without atonia was created by bilateral electrolytic lesions of the medial-lateral pontine tegmentum<sup>11-13,17,22,31</sup>. A syndrome of REM sleep without thalamocortical EEG desynchronization was induced by systemic atropine<sup>35,37</sup>. Studies were conducted in two models of feline generalized epilepsy, systemic penicillin epilepsy and electroconvulsive shock. Consistent data in both epilepsy models supported our hypothesis by suggesting that thalamocortical EEG desynchronization during REM sleep discourages the propagation of EEG paroxysms selectively, whereas the lower motor neuron inhibition of REM sleep blocks only clinical motor accompaniment.

## MATERIALS AND METHODS

### *Surgery and baseline polygraphic recordings*

Conclusions are based on studies of 20 cats, weighing 2.0 to 4.3 kg. Each cat was stereotaxically<sup>32</sup> implanted under sodium pentobarbital anesthesia (35 mg/kg, i.p.) for chronic evaluation of sleep-waking states and generalized epilepsy before and after dissociation of REM sleep components. Electrode placements included: jeweler's screws threaded into the skull over motor cortex (A 27, L 8, 10) to record cortical EEGs; bipolar depth electrodes in ventral lateral (VL) thalamus (A 11, L 4, H + 2) to record thalamic EEGs; jeweler's screws threaded into the orbit of the frontal sinus to record eye movements (EOG or electrooculogram); bilateral tripolar electrodes in the lateral geniculate nucleus of thalamus (LGN: A 6, L 10, H +2.3, 3.0) to record PGO spikes; and stainless steel wires inserted into the nuchal musculature for an electromyogram (EMG). Following a two-week post-operative recovery, 12-h polygraphic recordings were performed to verify the presence of normal REM sleep before experimental epilepsy and REM sleep modification.

### *Experimental epilepsy before REM sleep modification (baseline)*

Two epilepsy models were studied in all 20 cats: (1) systemic penicillin epilepsy<sup>8,9</sup>, an acute model of primary generalized, myoclonic 'petit mal' epilepsy and (2) electroconvulsive shock (ECS), a model of primary generalized 'grand mal' epilepsy<sup>5</sup>. Both epilepsy models are characterized by generalized EEG paroxysms that can occur with or without clinical motor accompaniment. This dissociation permitted separate assessment of EEG vs motor seizure susceptibility during REM sleep. To verify antiepileptic properties of REM sleep, seizure activity was also studied during alert waking and slow-wave-sleep (SWS). Sleep-waking states were identified according to the criteria of Ursin and Sterman<sup>36</sup>.

Half the cats ( $n = 10$ ) had penicillin epilepsy trials first, and half ( $n = 10$ ) had ECS trials first. One week intervened between penicillin and ECS trials.

*Systemic penicillin epilepsy.* Systemic penicillin epilepsy was induced by 300,000 to 400,000 IU/kg sodium penicillin G, i.m.<sup>8,9,25</sup>. About 1 h after injection, generalized spike-wave paroxysms appear in the EEG and can be accompanied by bilateral myoclonus of the head and neck, the most common motor seizure manifestation of this model<sup>8,9</sup>. We assessed penicillin epilepsy for 6 to 12 h after onset of seizure manifestations in a single baseline trial. Dependent measures were: the mean number of spike-wave complexes and of myoclonic seizures per 20 s epoch during spontaneous periods of alert waking, SWS and REM sleep. All 3 states were evaluated during the first 6-h block of penicillin trials in all 20 cats. Only REM sleep was evaluated during the second 6-h block in the 12 cats who had 12-h penicillin trials.

ECS. Seizures were elicited by current (0.5 s 60 Hz sine wave) applied between frontal sinus and LGN electrodes. This approach appears less likely to disrupt sleep than conventional techniques and has been used in previous studies evaluating ECS seizures during sleep in cats<sup>5</sup>.

ECS stimulation elicits a generalized afterdischarge (AD) in the EEG, and the most common clinical seizure type in cats is a generalized tonic-clonic convulsion or GTC<sup>5</sup>. Thresholds (in mA) to generalized AD and to GTCs were established once per day during either alert waking, SWS or REM sleep, using a method of limits procedure adapted from our previous studies<sup>25</sup>. Initial stimulus intensity was set below the thresholds from the previous day and was increased by 1 to 2.5 mA increments until AD and, ultimately, a GTC occurred.

ECS thresholds were established during alert waking until three identical waking thresholds were obtained. At this point, a rotation was implemented in which a different sleep or waking state was evaluated every 24 h. At least 3 full rotations (i.e., a minimum of three thresholds per state) were completed per cat. Multiple rotations were employed to control for baseline shifts, interstimulus interval and the timing of stimulations after onset of a sleep stage.

*Baseline shifts.* A waking threshold was determined before and after each sleep test, and order of SWS vs REM sleep tests was partially counterbalanced. SWS was tested first in at least one series of thresholds, and REM sleep was tested first in at least one series of thresholds per cat.

*Interstimulus interval.* The interstimulus interval varied because of the need to evaluate thresholds during waking and sleep over comparable time frames and ranged from 1 min to 1.5 h. To control for this variable, the mean interstimulus interval during sleep trials was used to determine waking thresholds the next day in at least one set of thresholds per cat.

*Timing of stimulation after sleep onset.* ECS stimulation was applied between one and 5 min after onset of either SWS or REM sleep<sup>36</sup>. Stimulations were timed between the first and second minute after sleep onset in at least one SWS threshold and one REM sleep threshold test per cat. This procedure was adopted to control for two factors: (1) potential differences in threshold related to time of stimulation within a SWS or REM sleep epoch and (2) the anticipated shortening of REM sleep epoch durations after pontine lesions<sup>29,41</sup>.

Twelve-hour polygraphic recordings were repeated 24 h to one week after the last seizure trial. The objective was to determine whether the seizure models had lasting effects on sleep-waking state parameters prior to dissociation of REM sleep components.

### *Treatments to dissociate REM sleep components*

Animals were subdivided into two groups of 10 each according to whether they received atropine or a pontine lesion. To dissociate REM sleep components, we created neurologic models of either (1) REM sleep without EEG desynchronization (systemic atropine sulfate, 1.5 mg/kg, i.p.,  $n = 10$ ) or (2) REM sleep without atonia ( $n = 5$  atonia syndromes induced by 30 s, 3 mA electrolytic lesions of the medial-lateral pontine tegmentum<sup>32</sup> at P 1.5, 2.5, L 2,7, H -4.2). The remaining five cats sustained pontine lesions that did not affect REM sleep atonia (control lesion).

Twelve- to 18-h polygraphic recordings were performed to verify effects on REM sleep. Recordings were initiated within 10 min after atropine administration. Because pontine lesions require prior nembutal anesthesia, cats were allowed one to two weeks for recovery before post-lesion polygraphic recordings were obtained. After these recordings, epilepsy studies resumed.

### *Experimental epilepsy after REM sleep manipulation*

Susceptibility to generalized EEG and motor seizures was re-investigated in relation to each REM sleep manipulation. Both epilepsy models were studied in 10 cats during atropine trials and also in the 10 cats sustaining pontine lesions. Epilepsy procedures were similar to baseline, with two main differences.

First, atropine delayed REM sleep onset. Accordingly, all

atropine plus penicillin epilepsy trials were extended from 6 to 12 h to evaluate seizure activity during REM sleep. During baseline, 12 h recordings were obtained in only 60% of the penicillin trials. Atropine and REM sleep ECS trials were concluded within 10 h in 70% of the cases; this time frame was consistent with baseline ECS trials during REM sleep. However, 30% of atropine and REM sleep ECS trials were extended to 16 h. In these cases, the EEG synchronizing effects of atropine had not subsided.

Second, pontine lesions inducing REM sleep without atonia ( $n = 5$ ) markedly abbreviated the duration of REM sleep epochs, whereas 'control lesions' did not ( $n = 5$ ). Changes made to accommodate this difference were: (1) For penicillin epilepsy, we compared seizure activity in the first 2 min of the REM sleep epoch vs later in the REM sleep epoch in the two lesion groups. (2) For ECS trials, nearly all stimulations were timed between the first and second minute after REM sleep onset, regardless of whether the lesion affected REM sleep atonia. During baseline, only 50% of the REM sleep thresholds were obtained at this point during the REM sleep epoch.

#### Histology

At the end of the experiment, all cats were euthanized with an overdose of nembital (50 mg/kg), and their brain removed for histologic verification of stimulation and lesion sites.

#### Statistical analysis

Two major dependent variables were assessed: (1) generalized seizure susceptibility and (2) sleep-waking state parameters. The main focus of this report is susceptibility to generalized EEG vs clinical motor seizures during different sleep and waking states. Indices of EEG seizure susceptibility were the number of spike-wave complexes per 20-s epoch during penicillin epilepsy and AD thresholds obtained during ECS trials. Indices of motor seizure susceptibility were the number of myoclonic seizures per 20-s epoch during penicillin epilepsy and thresholds to convulsions during ECS trials. Sleep-waking state data derived from 12 h polygraphic recordings are described fully in separate papers<sup>29,35</sup>, but the measures pertinent to the epilepsy experiment are included here.

*Analysis of seizure data.* For global analysis of the population data, indices of EEG vs clinical motor seizures during penicillin epilepsy and GTC trials were averaged to yield a single value for each state per cat before vs after REM sleep manipulation. Repeated measures analyses of variance compared each EEG or motor seizure index as a function of state (waking, SWS or REM sleep) before and after REM sleep syndromes (REM sleep without EEG desynchronization vs REM sleep without atonia). Post hoc tests were independent or dependent *t*-tests.

*Other statistical tests.* Several additional statistical analyses were performed to assess other variables before vs after REM sleep syndromes: (1) Penicillin epilepsy: repeated measures analysis of variance compared penicillin seizure activity during REM sleep in the first vs second 6-h blocks of 12-h recordings and also in the first 2 min of REM sleep vs later in the REM sleep epoch; (2) ECS thresholds: repeated measures analysis of variance was used to assess GTC seizure threshold per state as a function of the following variables: order effects (e.g., SWS thresholds obtained before vs after REM sleep thresholds), interstimulus interval (e.g., stimulation at 1 min vs 1.5 h intervals), time of stimulation after sleep onset (<2 vs >2 min after sleep onset) and latency of REM sleep threshold after the beginning of the post-atropine polygraphic recording (4-10 h vs 10-16 h after atropine).

*Analysis of sleep-waking state parameters.* Repeated measures analysis of variance compared sleep-waking state parameters during 12 h recordings obtained at baseline, after epilepsy only and after REM sleep manipulation only. These parameters included: percent of total time spent in waking, SWS and REM sleep; the mean duration (min) of waking, SWS and REM sleep epochs; latency to SWS or REM sleep onset after initiation of the recording; and the integrity of polygraphic components in each state (e.g., power spectral analysis of cortical EEGs was performed to assess degree of synchronization per state").

## RESULTS

### *REM sleep before and after REM sleep syndromes*

Fig. 1 illustrates REM sleep before and after treatments to dissociate the EEG or motor components of REM sleep. Before these treatments, normal REM sleep was recorded in each cat. The top tracing depicts the cardinal polygraphic components of normal REM sleep<sup>36</sup>. REM sleep was defined by: EEG desynchronization in thalamocortical leads, rapid eye movements in the electrooculogram, PGO spikes recorded from the lateral geniculate nucleus of thalamus and atonia, indexed by silence in the nuchal electromyogram.

The middle tracing illustrates the syndrome of REM sleep without thalamocortical EEG desynchronization elicited by systemic atropine. As in rats<sup>37</sup>, atropine in cats induces a SWS-like thalamocortical EEG synchrony during both waking and REM sleep but does not augment synchrony in SWS<sup>35</sup>. REM sleep can readily be distinguished from waking and SWS because all other REM sleep components remain intact. Bursts of rapid eye movements and PGO spikes as well as atonia are clearly visible in the bottom 3 channels of the tracing. The combination of these components is unique to REM sleep<sup>31,36</sup>.

The bottom tracing depicts REM sleep without atonia following a lesion of the pons. This syndrome is quite selective, as three of the 4 polygraphic indices of REM sleep remain intact. Thalamocortical EEG desynchronization occurs in conjunction with the bursts of rapid eye movements and PGO spikes (top channels). The confluence of these polygraphic components occurs exclusively during REM sleep<sup>31,36</sup>. The only polygraphic component affected by the lesions is atonia; note the presence of muscle tone in the bottom channel of the tracing.

Adjacent to the bottom polygraphic tracing is a coronal section through the pons of this cat. The section illustrates the typical location and size of lesions inducing the syndrome of REM sleep without atonia. Lesions were located in a discrete region of the medial-lateral pontine tegmentum, as is well documented in the literature<sup>11-13,17,22,31</sup>. These lesions eliminate lower motor neuron inhibition during REM sleep, manifested by activity in the neck EMG channel and also by behavioral mobility. The animal typically bobs its head, and, with some lesions", moves about the chamber. Behavioral mobility in all our cats was confined to head bobbing.

### *Histology and lesion characteristics*

The parameters of pontine lesions which did and did not induce REM sleep without atonia are illustrated in Fig. 2. All lesions fell within the following stereotaxic coordinates<sup>1</sup>: AP 0 to P 4, L 1 to 3.0 and H 0 to -6.

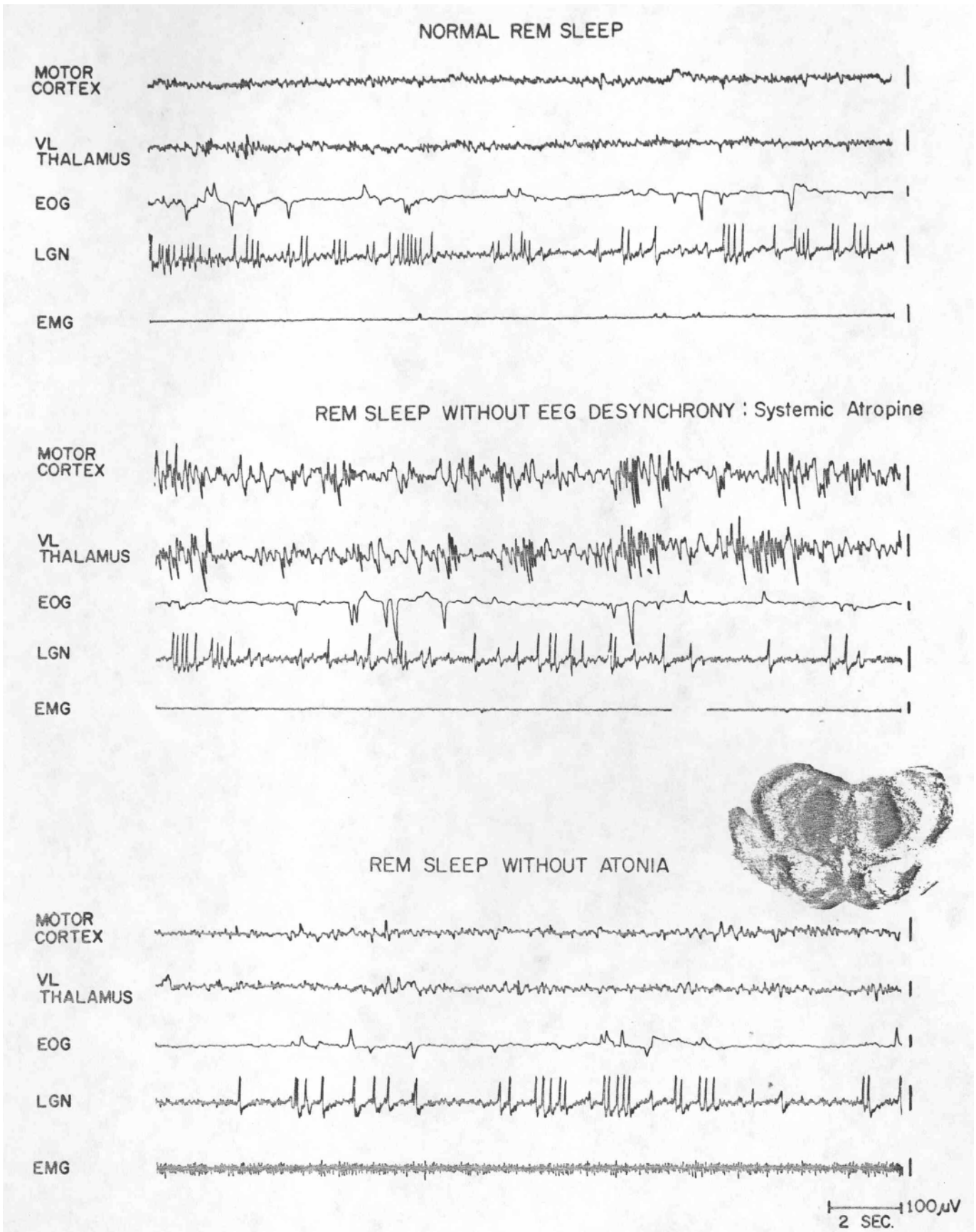


Fig. 1. Polygraphic characteristics of rapid-eye-movement (REM) sleep before and after REM sleep syndromes. The top tracing depicts normal REM sleep, defined by thalamocortical EEG desynchronization, rapid eye movements in the EOG, PGO spikes in the lateral geniculate nucleus (LGN) and atonia, reflected by silence in the neck EMG. The middle tracing shows a post-atropine REM sleep example, which had all REM sleep components except thalamocortical EEG desynchronization. The bottom tracing shows a post-lesion record, which had all REM sleep components except atonia.

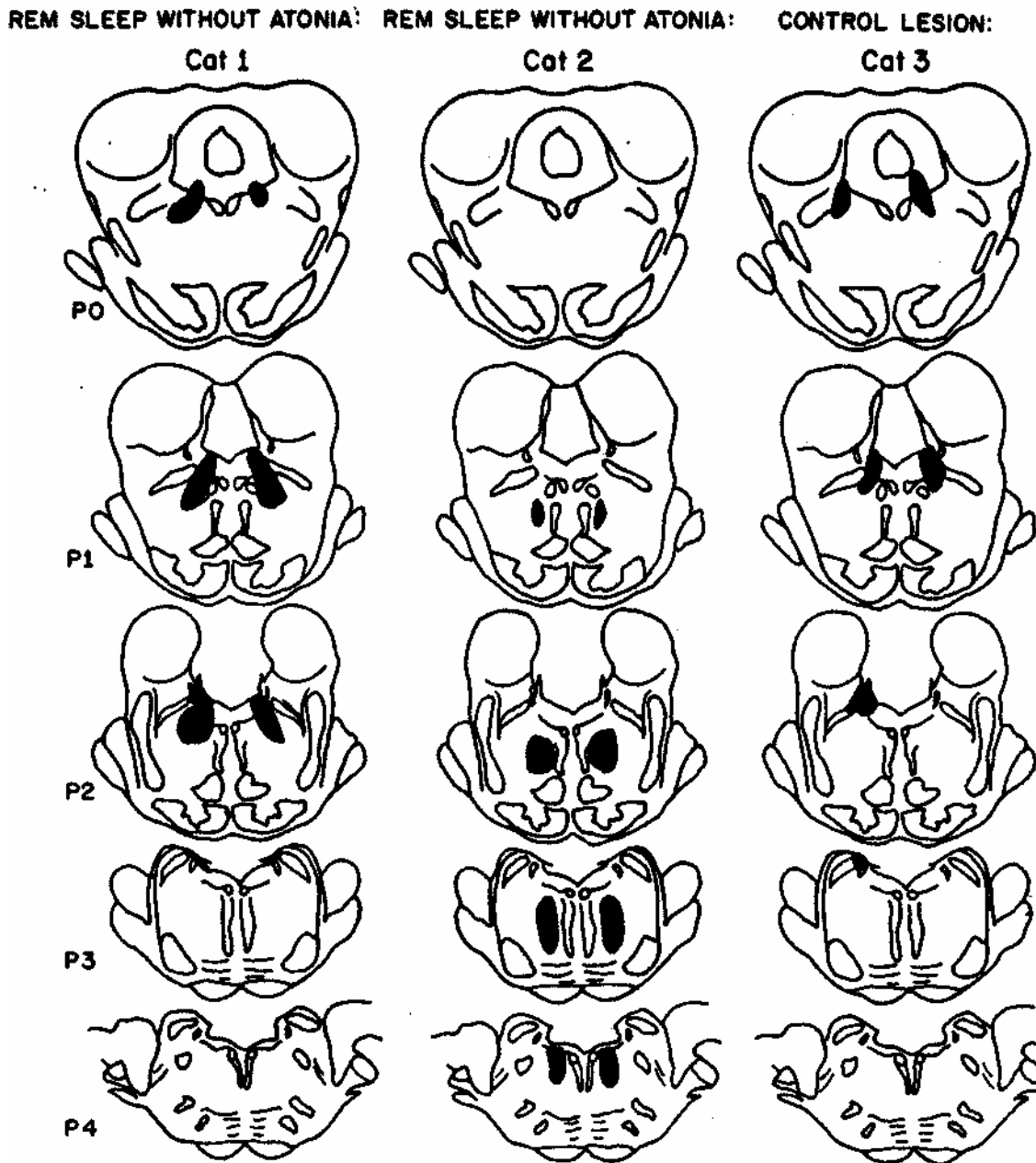


Fig. 2. Reconstructions comparing size and location of typical lesions which induced REM sleep without atonia and those which did not affect atonia in REM sleep. Extent of lesions are shown from APO through P4 in 3 cats. Coordinates correspond to Berman's atlas<sup>1</sup>. Reconstructions are shown to illustrate lesions in the medial-lateral pontine tegmentum that created REM sleep without atonia in two cats (left and middle). Lesions in control cats, who did not display REM sleep without atonia, are typified by the reconstruction on the right. Control lesions were smaller and situated more dorsolaterally in the pontine tegmentum than lesions which affected REM sleep atonia (see text).

Lesions occupying roughly 50% of this area produced the REM sleep without atonia syndrome. Some cats had lesions which were largest in the rostral, dorsolateral aspect of the pontine tegmentum (left), whereas other cats had lesions which were largest caudally and ventromedially (middle). Lesions in both of these regions have been shown to produce REM sleep without atonia<sup>11-13,17,22,31,41</sup>

Control lesions were defined a posteriori as those not affecting atonia in REM sleep and are illustrated by the reconstruction on the right side. Control lesions were

small and occupied the dorsolateral aspect of the lesions creating REM sleep without atonia.

#### *Epilepsy before and after REM sleep syndromes*

The contrasting effects of the two REM sleep syndromes on EEG and motor seizure manifestations are illustrated in Fig. 3A for penicillin epilepsy and Fig. 3B for ECS. The top tracing of Fig. 3A shows penicillin epilepsy before REM sleep syndromes. Spike-wave activity is prominent in SWS but is rare in REM sleep<sup>8,9,25</sup>. Myoclonus can accompany spike-wave paroxysms in SWS

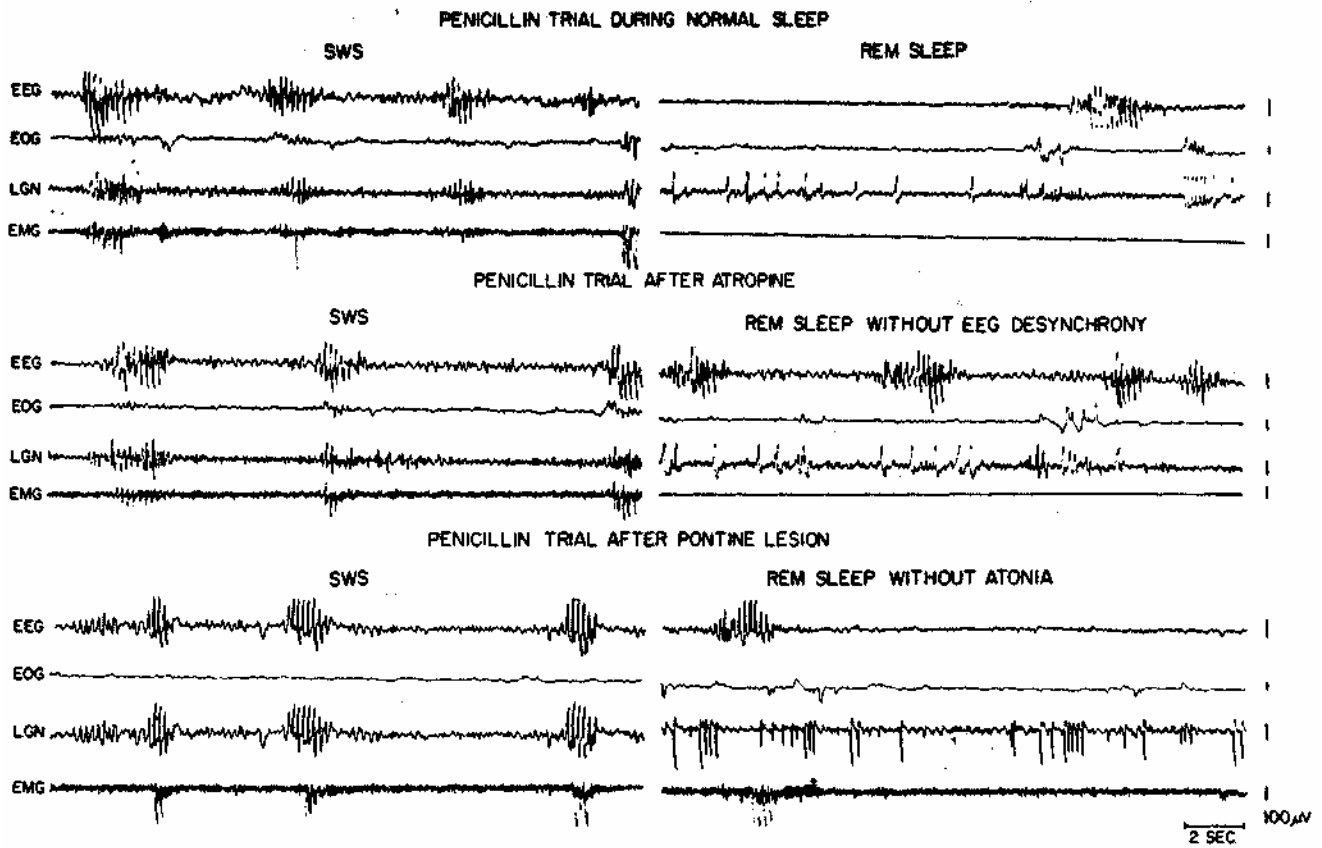


Fig. 3. A: systemic penicillin epilepsy during slow-wave-sleep (SWS) and REM sleep before and after dissociation of REM sleep component (pike-wave paroxysms are visible in the EEG tracing, and myoclonic seizures were associated with discharges in the EMG tracing in this ca

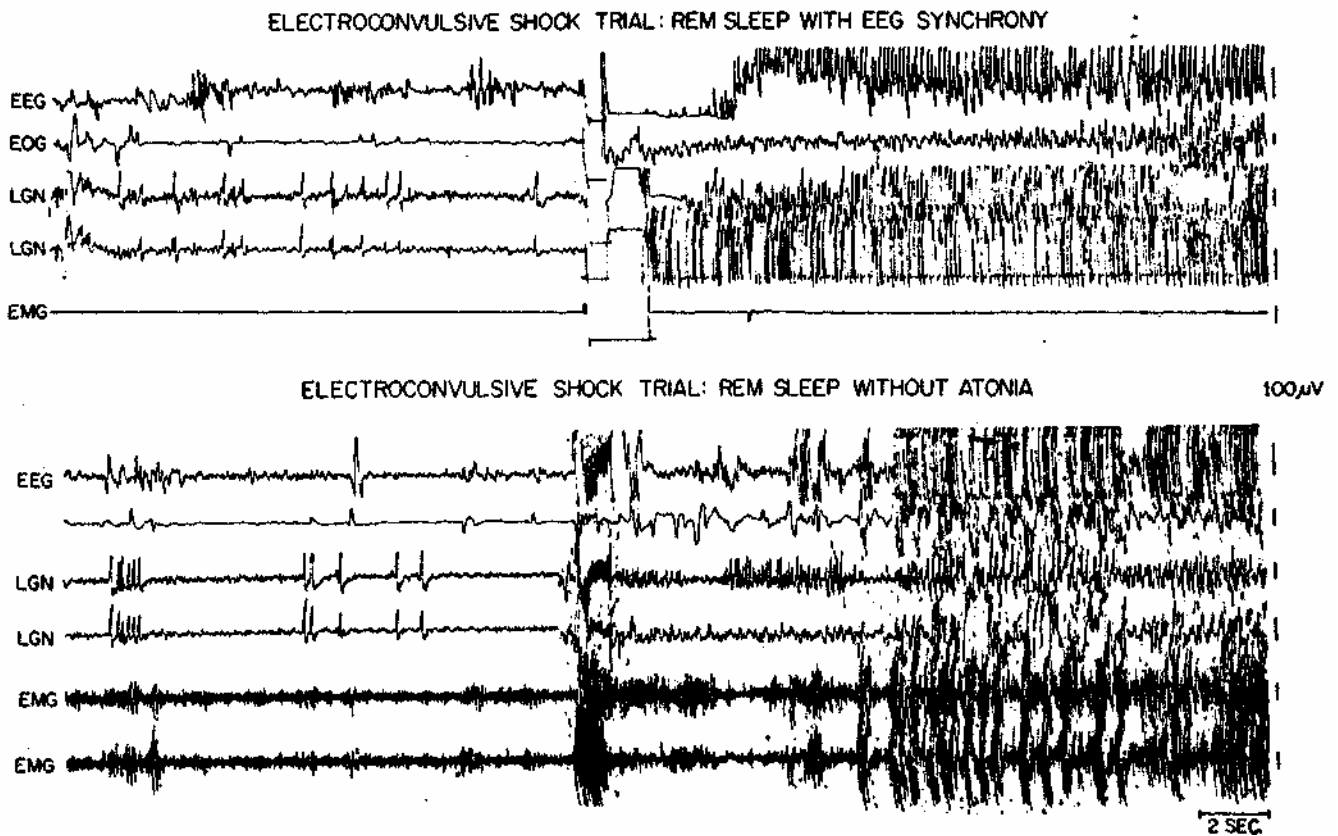


Fig. 3. B: electroconvulsive shock (ECS) trials conducted during REM sleep without thalamocortical EEG desynchronization (top) and during REM sleep without atonia (bottom). Stimulus artifact is visible in the middle of each tracing.

but never occurs during REM sleep with atonia (note EMG silence during REM sleep with EEG paroxysm). During atropine and penicillin trials (middle tracings), REM sleep had a SWS-like EEG, and spike-wave incidence in REM was identical to SWS; however, no clinical motor accompaniment occurred, evidenced by continued silence in the EMG. Opposite effects occurred during penicillin epilepsy after pontine lesions (bottom tracing). During REM sleep without atonia, penicillin spike-wave activity was as uncommon as in normal REM sleep, but when it did occur, it was often associated with myoclonus. The loss of lower motor neuron inhibition after medial-lateral pontine lesions appears to release epileptic myoclonus in the penicillin epilepsy model.

The same differential effect of these REM sleep syndromes was obtained in ECS trials (Fig. 3B). During REM sleep with atropine-induced EEG synchrony (top), ECS stimulation elicited an immediate generalized EEG seizure, which lasted 3 min in this cat. In spite of the massive EEG seizure, the animal never woke up, evidenced by continued atonia (note EMG silence), and there was no clinical motor accompaniment. In contrast, during REM sleep without atonia (bottom tracing), generalized AD was delayed (note late motor cortex involvement), but when the EEG seizure spread from LGN, there was immediate clinical motor accompaniment in the form of a convulsion. In this model, it is not possible to determine whether the pontine lesion increases susceptibility to motor seizures by reducing arousal threshold (the cat may awaken before the convulsion begins) or by releasing motor expression within REM. In either case, the absence of lower motor inhibition during REM sleep is conducive to clinical motor accompaniment.

Fig. 3A,B suggest that thalamocortical EEG synchrony abolishes protection against EEG but not clinical motor seizures; in contrast, loss of atonia during REM sleep abolishes REM sleep protection against motor but not EEG seizures. These and other effects were significant in the population data. Analyses of variance yielded significant  $F$  ratios for EEG and for motor seizure susceptibility, both with respect to the state factor (alert waking vs SWS vs REM sleep,  $P < 0.01$ ) and with respect to the REM sleep syndromes factor (baseline vs REM sleep without EEG desynchrony and baseline vs REM sleep without atonia,  $P < 0.05$ ). Fig. 4 summarizes the sleep-waking state dependency of generalized EEG (left) and clinical motor (right) seizure events before and after REM sleep manipulations; symbols indicate significant, post hoc  $t$ -test values. There are three main findings.

First, before REM sleep syndromes (Fig. 4A, clear bars), there was significant differences between waking.

SWS and REM in susceptibility to EEG and clinical seizures in both epilepsy models. During penicillin epilepsy (top), waking and particularly SWS are more vulnerable to electroclinical seizures than is REM sleep, as previously reported<sup>5,25</sup>. The same applies to ECS seizure susceptibility (bottom), presented as the *inverse* of threshold. REM sleep was least seizure prone and SWS most seizure prone, regardless of interstimulus interval, timing of stimulation after sleep onset and substantial fluctuation in day to day thresholds in some cats ( $P > 0.1$ ). Further, REM sleep is more resistant to motor than to EEG seizures in both epilepsy models ( $P < 0.001$ ).

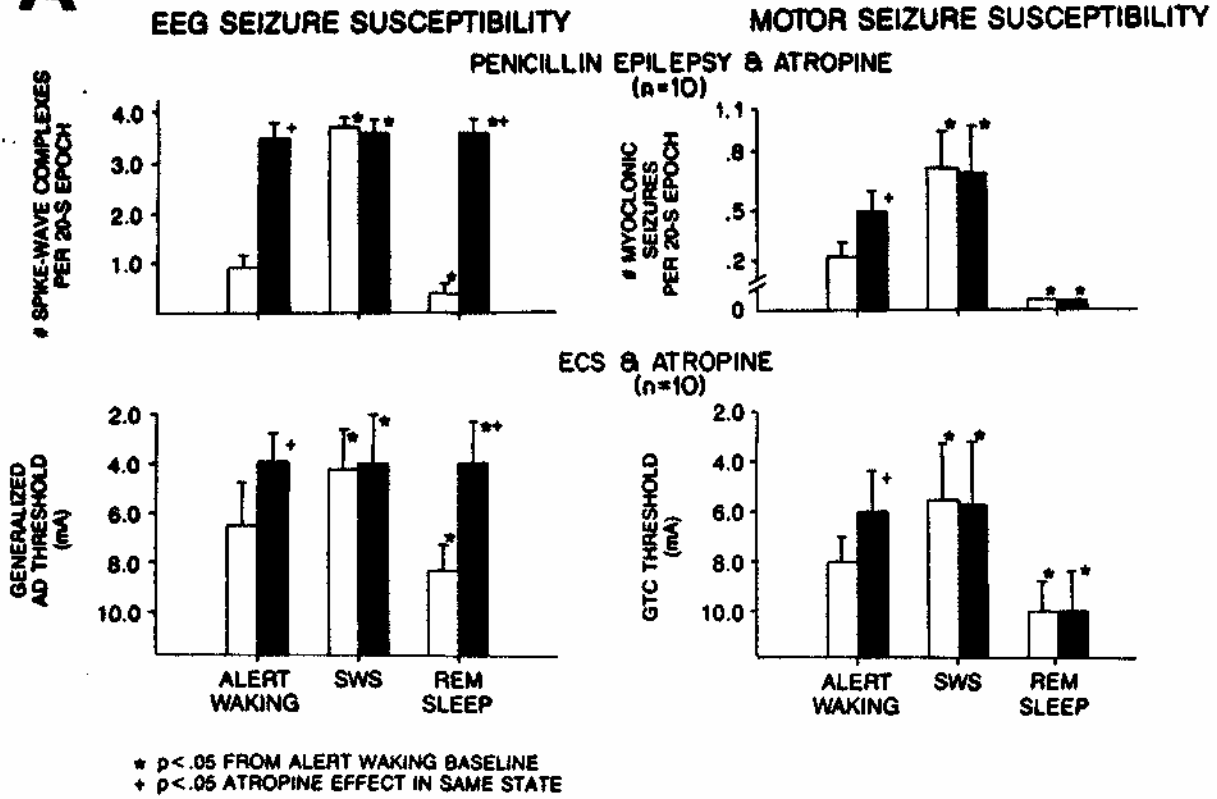
Second, atropine creates thalamocortical EEG synchrony in waking and REM sleep states<sup>37</sup> without augmenting synchrony in SWS<sup>35</sup>, and also increases susceptibility to EEG seizures in waking and REM sleep without elevating seizure activity in SWS (Fig. 4A, dark bars). In both epilepsy models, atropine erased differences in EEG synchrony between waking, SWS and REM sleep and also abolished sleep-waking state dependent differences in susceptibility to generalized EEG seizures (Fig. 4A, left). Atropine also increased vulnerability to clinical motor seizures (Fig. 4A, right) during waking but did not affect the anticonvulsant properties of REM sleep. Even though generalized EEG seizures were just as common in REM sleep as in any other sleep or waking state during atropine trials, clinical motor accompaniment was still suppressed.

Third, the lesion data can explain the continued suppression of clinical motor seizures during EEG seizures in REM sleep, as shown in Fig. 4B. Lesions which induced REM sleep without atonia released muscle tone in REM sleep, and, once an EEG seizure did occur in REM sleep, clinical motor accompaniment significantly increased in both epilepsy models (dark bars). In contrast, control lesions had no effect on REM sleep components, nor did they affect any measure of seizure susceptibility (clear bars). Although pre-lesion data are not shown for either seizure model, baseline seizure susceptibility parameters did not differ from the post-lesion data of control cats. Post-lesion data from cats showing REM sleep without atonia differed from baseline only in showing increased motor seizure susceptibility in REM sleep. Finally, neither pontine lesion affected polygraphic indices or seizure susceptibility parameters during waking or SWS when compared to pre-lesion baseline data.

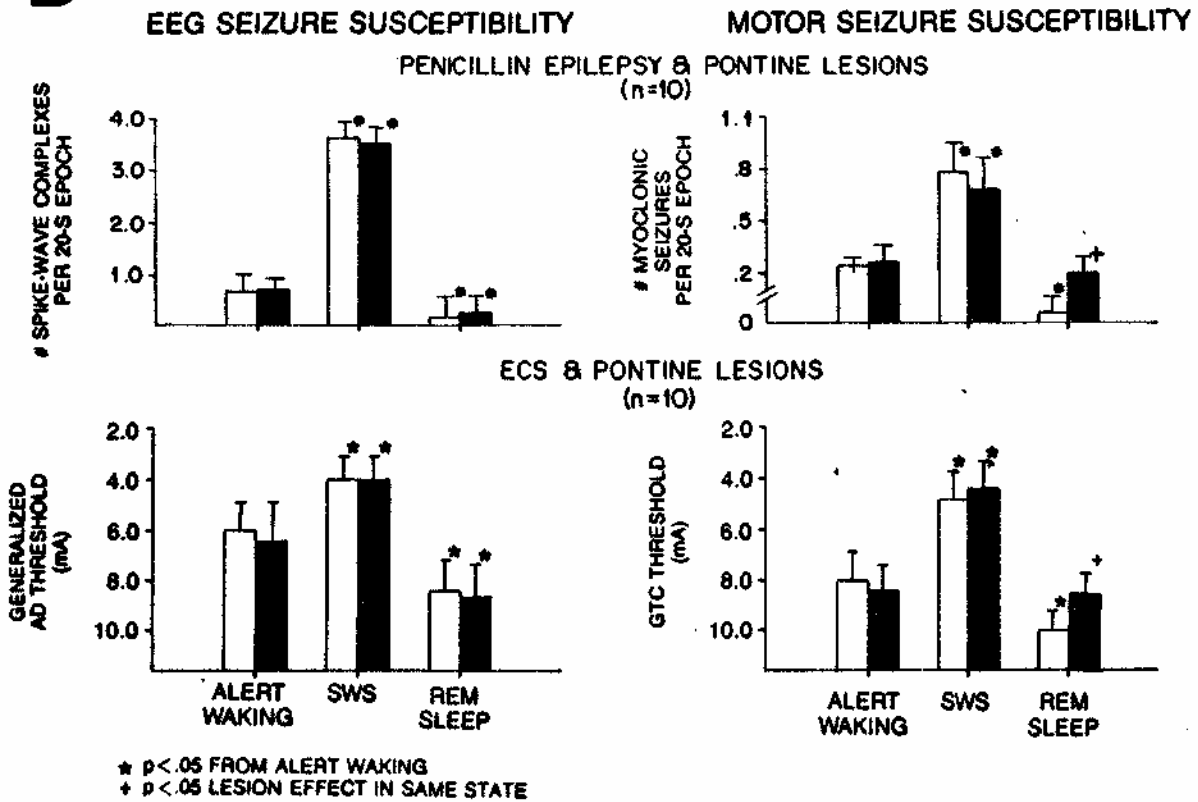
#### *Side-effects of atropine and pontine lesions*

The peripheral autonomic characteristics of atropine, such as pupillary dilation, are well known. Behaviorally, the cats are quiet and tend to be ataxic if they attempt to

**A**



**B**





move. REM sleep onset can be delayed 4 to 9 h at the dosage of atropine we used, especially in combination with penicillin and to some extent after ECS. Atropine synchronized the thalamocortical EEG for 12-18 h. Penicillin epilepsy persists for 12 to 24 h<sup>8,9,25</sup>. ECS stimulation is effective indefinitely. By extending the polygraphic recording time during atropine and epilepsy trials, sufficient samples of REM sleep without EEG desynchronization were obtained to study both epilepsy models during the altered REM sleep state. Extending the recordings did not affect the seizure results. For example, there was no significant difference between the first vs second 6 h recordings of penicillin seizure activity in REM sleep before atropine ( $P > 0.1$ ); there were also no significant differences between the first and second 6 h recordings after atropine, although REM sleep samples in the first 6-h block of atropine and penicillin trials were scarce. Differences in ECS threshold also could not be attributed to delayed REM sleep onset, since thresholds determined 4-10 h after atropine did not differ from thresholds established 10-16 h after atropine ( $P > 0.1$ ). The pontine lesion did not delay REM sleep onset, as latencies to REM sleep onset from initiation of 12-h recordings were not different before vs after lesions ( $P > 0.1$ ). However, lesions inducing REM sleep without atonia moderately, but significantly diminished REM sleep time ( $P < 0.05$ ) and substantially abbreviated the duration of individual REM sleep epochs ( $P < 0.01$ )<sup>29</sup>. Behavioral mobility during REM sleep without atonia appears to wake the animal prematurely during REM sleep epochs. We controlled for this complication during ECS trials by varying interstimulus intervals and by timing some ECS stimulations between the first and second minutes after REM sleep onset before and after the lesion. Interstimulus intervals were not a factor in REM sleep anticonvulsant effects ( $P > 0.1$ ). There was also no difference between thresholds obtained early (one to 2 min after REM sleep onset) vs later (>2 min after REM sleep onset) in the course of the REM sleep epoch, either before or after lesions ( $P > 0.1$ ). This issue also did not complicate penicillin epilepsy studies, as the penicillin seizure manifestations are equally uncommon in early and late segments of individual REM sleep epochs ( $P > 0.1$ ).

Other post-lesion behavioral complications were relatively minor, possibly because the lesions were comparatively small. Problems with ataxia were noted in nearly every cat but were more pronounced in cats displaying REM sleep without atonia. Symptoms disappeared in most cats 72 h post-lesion and were thus absent by the time post-lesion epilepsy studies began. Mild hindlimb ataxia persisted in other cats, regardless of whether atonia was present or absent during REM sleep. Thus, it seems unlikely that ataxia associated with pontine lesions was a factor in the seizure results reported here.

## DISCUSSION

Our study verified the antiepileptic properties of REM sleep and appears to have differentiated the components responsible for suppression of the EEG and clinical motor signs of epilepsy in this state. Lesions of the pontine atonia center eliminated 'sleep paralysis' and released clinical motor accompaniment during REM sleep. Atropine created thalamocortical EEG synchrony in waking and REM sleep states<sup>37</sup> without augmenting synchrony in SWS<sup>35</sup>, and also abolished protection against generalized EEG seizures during alert waking and REM sleep. Increased spread of EEG discharges during sleep and waking states with thalamocortical EEG synchronization is not a novel observation<sup>6,8-10,24-27</sup>; in fact, thalamocortical interactions have long been implicated in the integration of hypothalamic and brainstem sleep and waking mechanisms and also in the modulation of EEG discharge generalization during these states (e.g., refs. 8-10). Our findings are unique, however, in dissociating EEG seizures from clinical motor accompaniment and isolating the factors that might underlie this dissociation during REM sleep.

Before addressing this conceptualization in detail, two drawbacks to our study must be acknowledged. One is the absence of systematic control data with a peripheral cholinergic blocker at equivalent concentrations of atropine used here. We did test effects at comparable dosages of the peripheral muscarinic blockers, methylatropine bromide and nitrate, which are analogous to atropine but do not easily infiltrate the blood-brain barrier. In the two cats evaluated, we found that the peripheral anticholin-



Fig. 4. Susceptibility to generalized EEG vs motor seizures in a control condition (clear bars) and after treatments to dissociate REM sleep polygraphic components (dark bars). Each bar reflects the mean and S.D. from multiple measure of seizure activity during alert waking, SWS or REM sleep per cat. For example, penicillin seizure activity is indexed by the mean number of spike-wave complexes (left) or myoclonic seizures (right) per 20-s epoch of each state. ECS thresholds represent at least three thresholds to generalized afterdischarge (AD; left) or convulsions (GTCs right) per state in each cat. A: EEG and motor seizure susceptibility before atropine (clear bars) and after atropine (dark bars) in penicillin (top) and ECS (bottom) epilepsy models. B: EEG and motor seizure susceptibility after pontine lesions which did not affect atonia in REM sleep (clear bars) and lesions which eliminated atonia during REM sleep (dark bars). Data are provided for penicillin (top) and ECS (bottom) epilepsy models. ECS data: note that threshold is inversely related to susceptibility; the ECS ordinate is inverted to so that high bars reflect increased seizure susceptibility in both epilepsy models.

ergic agent does not affect EEG synchrony or seizure manifestations in either epilepsy model. This preliminary observation is consistent with other work comparing the effects of central and peripheral cholinergic antagonists at similar dosages<sup>19,42</sup>. Anticholinergic influences on EEG synchrony and epilepsy do not appear attributable to peripheral anticholinergic actions except at very high dosages<sup>19</sup>.

A second potential complication is REM sleep deprivation, which does exacerbate epilepsy<sup>5,27</sup>. Both atropine and pontine lesions suppress REM sleep, as do both epilepsy models<sup>5,12,13,17,25,26,35</sup>. The duration of sleep deprivation and/or magnitude of REM sleep loss seen after these manipulations is probably not sufficient to significantly affect seizures. In this experiment, maximum sleep loss was obtained after administration of both atropine and penicillin. This combination often delayed REM sleep onset for 4 to 9 h, whereas at least 24 h sleep loss is usually required to increase seizure activity in cats<sup>26</sup>. Pontine lesions and ECS also attenuate REM sleep<sup>12,17,41</sup> and/or prevent rebound<sup>5</sup>, but the magnitude of REM sleep loss is moderate in both cases, only 25 to 30% in the present study.

Several facts support our contention that REM sleep deprivation is unlikely to have contributed to our findings. First, sleep deprivation increases vulnerability to both EEG and motor seizures in all sleep and waking states<sup>26,27</sup>, yet our results were nearly always specific to seizure type (EEG vs motor) *and* to sleep or waking stage. After pontine lesions, effects were restricted to motor seizures in REM sleep. During atropine, increased seizure susceptibility was confined to waking and REM sleep and affected EEG, but not motor seizures during REM sleep. Furthermore, peak sleep deprivation effects on seizures occur during SWS<sup>26</sup>, yet we observed no increase in seizure susceptibility during SWS in relation to either atropine or pontine lesions. Finally, sleep deprivation is less effective in augmenting seizures during REM sleep than at any other time in the sleep-wake cycle<sup>26</sup>, a fact which further underscores the potent antiepileptic actions of this sleep stage.

Notwithstanding the above complications, our results suggest a dissociation of factors for suppression of EEG vs motor seizures in REM sleep. This conclusion is consistent with clinical literature indicating that generalized electroclinical seizures are uncommon during REM sleep except in severe epilepsy syndromes with disintegration of REM sleep polygraphic components<sup>6,20,21,27</sup>. Our data also have implications for seizure susceptibility in other sleep-waking states. Specifically, EEG discharge generalization is discouraged by EEG desynchronization in thalamocortical pathways during REM sleep and alert waking, whereas lower motor neuron inhibition appears

to suppress clinical accompaniment during REM sleep.

These speculations are compatible with abundant clinical and experimental evidence. Epileptic discharge generalization is attenuated during states of thalamocortical EEG desynchronization, such as alert waking and REM sleep, and is favored during states of thalamocortical EEG synchrony, notably SWS in infrahuman species or its human equivalent, non-rapid-eye-movement sleep<sup>6</sup>. The physiologic basis for the contrasting effects of thalamocortical synchrony and desynchrony on epilepsy is uncertain. However, large populations of thalamocortical and corticofugal neurons discharge synchronously in high frequency bursts during SWS (for a review see ref. 33) and could provide a natural mechanism for epileptic EEG discharges, whereas EEG desynchronization, with its asynchronous modes of neuronal discharge, does not<sup>5,8,10</sup>. EEG desynchronization is often but not always more intense in REM sleep than during waking, a fact which might explain the greater suppression of generalized EEG discharges usually seen in REM sleep than during waking and also other findings of equal suppression in the two states<sup>6,27</sup>. Moreover, the presence of muscle tone in waking and SWS<sup>36</sup> may be conducive to clinical accompaniment, whereas the coincidence of EEG desynchronization and active lower motor neuron inhibition is unique to REM sleep and could underlie REM sleep protection against both EEG and motor seizure manifestations.

A cholinergic mechanism has been hypothesized to mediate EEG desynchronization and its antiepileptic

creased EEG discharges during REM sleep after atropine. In this report and with reduced EEG discharges during REM sleep after carbachol in the Velascos' studies of the feline alumina cream preparation<sup>38</sup>. However, cholinergic hypofunction does not readily accommodate our data on REM sleep anticonvulsant effects. Atropine did not affect either atonia or motor seizures during REM sleep in this study. Our finding appears to conflict with extensive data suggesting that acetylcholine (ACH) provides the triggering mechanism for lower motor neuron inhibition in REM sleep. Carbachol infusion into the medial-lateral pontine tegmentum not only induces atonia but also blocks motor seizures in the feline alumina cream preparation<sup>38</sup>.

One explanation for the discrepancy is that ACH synapses were incompletely blocked at the dose of atropine we used. It is also possible that ACH is not the only neurotransmitter involved in REM sleep atonia. Cells from the medial-lateral pontine tegmentum are thought to hyperpolarize lower motor neurons via pro-

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- jections to the medulla<sup>4,31</sup>. The neurochemistry of the brainstem atonia generating system is poorly characterized, but it includes both cholinergic and noncholinergic cells<sup>18,23,31</sup>. Thus, either residual cholinergic or noncholinergic mechanisms could produce atonia and its anticonvulsant effects.

Collectively, our data are consistent with a cholinergic mechanism for thalamocortical EEG desynchronization and for suppression of generalized EEG discharges in both waking and REM sleep. Because REM sleep components have been localized to the brainstem<sup>15,31</sup>, protection against spread of EEG discharges might be mediated by the ascending cholinergic projection arising from the pontomesencephalic tegmentum<sup>13,40</sup>. Protection against motor seizures appears to be regulated by separate, descending brainstem pathways which mediate lower motor neuron inhibition in REM sleep.

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