IN: J.A. Wada (Ed.), Kindling 4.
Plenum Press: New York,
1990, pp. 313-327.

BASIC MECHANISMS UNDERLYING SEIZURE-PRONE & SEIZURE RESISTANT SLEEP AND AWAKENING STATES IN FELINE KINDLED AND PENICILLIN EPILEPSY

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Epilepsy is a chronic neurological disorder which is manifested at some times and masked at others. Sleep-waking state physiology is one of the most well documented factors affecting the clinical expression or suppression of human epilepsy[1,2] . Specifically, non-rapid-eye-movement (NREM) sleep and the gradual process of awakening from NREM sleep are the most vulnerable periods for seizures, especially convulsions. Moreover, the type of epilepsy is an important consideration in the timing of convulsions. Temporal lobe epilepsy with secondary generalized convulsions is the most common pure sleep epilepsy, with convulsions occurring in NREM or the transition from NREM to rapid-eye-movement (REM) sleep in nearly 60% of the patients . In contrast, over 90% of patients with primary generalized, "petit mal" epilepsy display convulsions exclusively after awakening[2]. Finally, type of epilepsy is not a factor in the suppression of seizures during REM sleep. REM sleep is the most anti-epileptic state in the sleep-wake cycle for all generalized electrographic (EEG) and clinical seizures .

Our laboratory is identifying the brain mechanisms for these seizure-prone and seizure-resistant states in humans. The initial objective was to establish animal models of human generalized epilepsies with sleep or awakening convulsions.

FELINE MODELS OF SLEEP AND AWAKENING EPILEPSY.

The amygdala kindling model[3] of secondary generalized, temporal lobe epilepsy was used to study sleep-activated seizures. The systemic penicillin model of "petit mal" epilepsy[4] was used to study convulsions after awakening. These experimental models resemble human counterparts in the timing of seizures during the sleep-wake cycle, as detailed below.

Amygdala kindling model of sleep epilepsy.

Amygdala kindling is a viable model of "sleep epilepsy," based upon the timing of evoked and spontaneous seizures in the sleep-wake cycle.

Conclusions are derived from studies in amygdala kindled kittens (n=9) and adult cats (n=12). Table 1 summarizes the characteristics of kindling development in cats at different ages because subcortical kindling has never been described in kittens[5].

resembles
in the progression of seizure stages and kindling rates[3,6], but two
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adults
there are two main differences. First, initial and immediate post-kindling afterdischarge (AD) thresholds are much higher in 2.5 to 4-month-old kittens than in older kittens and adults. Second, the five youngest kittens (2.5 to 5 months) developed spontaneous seizures within three months of the first kindled convulsion. None of the older kittens (>5.5 months) or adults (>1 year) had spontaneous seizures.

One factor in the development of spontaneous seizures might be reduced capacity to inhibit seizure discharge in young animals. The three youngest kittens rarely had the normal post-ictal refractory period after elicited convulsions; rather, they relapsed into generalized seizures (stage 3 to 6) shortly after an evoked generalized tonic-clonic convulsion (GTC). Spontaneous seizures occurred one hour to several days after failure of post-ictal depression, mostly during slow-wave-sleep (SWS), the feline equivalent of human NREM sleep.

Kittens could have convulsions in the waking state, but the majority of waking seizures we observed were nonconvulsive. One kitten had unusual, nonconvulsive seizures which we have never seen before in a kindled cat. These events are called "catnip" seizures because the kitten purred and wagged its tail continuously. The kitten often assumed a stereotyped posture with dorsoflexion of head and hips, punctuated by jacknife-like jerks. Clinical signs accompanied bilateral amygdala spiking and spike-wave activity in thalamus and cortex. Symptomatology is consistent with a complex-partial seizure, although some aspects are reminiscent of West syndrome . Electroclinical events could persist 1.5 hours during waking;

Table 1. Kindling development in kittens and adult cats.

AGE at Initial AD	Initial AD Threshold (mA)	Kindling Rate (ADs to GTC)	Post-Kindling AD Threshold (mA)	Spontaneous Seizures
2.5-4.0 months (n=3)	15.3+ 0.6*	21.0+ 17.3	3.7+ 1.5*	74% GTCs in SWS; "catnip" seizures in waking
5 months (n=2)	1.3+ 1.1	23.5+ 7.8	0.7+ 0.2	100% GTCs in SWS
5.5-6.5 months (n=4)	1.2+ 4.0	24.3+ 6.7	1.3+0.3	NONE
>1.0 year (n=12)	1.1+ 0.7	22.0+ 8.7	0.9+ 0.5	NONE

p <.05 from adult cats (>1 year)

EEG seizures continued in SWS and could be accompanied by a convulsion; all EEG and clinical seieures were suppressed during REM sleep.

The timing of spontaneous convulsions during the sleep-wake cycle in young kittens (Table 1) corresponds to the threshold data in older kittens and adult cats. Table 2 shows that stable post-kindling thresholds were higher in kittens than adult cats, but state-dependent seizure patterns in evoked seizure susceptibility were the same, regardless of age. SWS, particularly the transition into REM sleep, is the most seizure prone state for both spontaneous (Table 1, Figure 1) and elicited (Table 2) convulsions. REM sleep is the least vulnerable state for spontaneous and elicited seizures.

Table 2. Timing of elicited convulsions, expressed as GTC thresholds, during the sleep-wake cycle in kindled kittens and adult cats. Threshold is the inverse of; seizure susceptibility. Susceptibility is highest during SWS, especially the REM transition, and lowest during stable REM sleep.

GTC Thresholds(mA)

Age at initial AD	Alert waking	SWS	SWS to REM transition	REM sleep
5 to 6.5 month old kittens (n=6)	.9± .4+	.8± .4*+	.7± .4*+	1.0± .5
Adult cats > 1 year (n=12)	.6± .3	.4± .3	.2 .2	0.7± .3

* p<.05 from alert waking baseline; +p<.05 from adult cats

The temporal distribution of kindled seizures in kittens and adult cats resembles the clinical literature for human temporal lobe epilepsy, where partial and generalized seizures can occur during waking, convulsions occur predominantly in NREM sleep, and all generalized seizures are rare in REM sleep * . We have kindled a spontaneous sleep epilepsy in kittens (<5.5 months) and shown that the older animals, although not developing spontaneous epilepsy, are still most vulnerable to elicited seizures during SWS and the transition into REM sleep.

Kindled sleep epilepsy vs. penicillin-induced awakening epilepsy.

Figure 1 shows the timing of generalized EEG and motor seizures during the sleep-wake cycle after amygdala kindling and during systemic penicillin epilepsy. The patterns of spontaneous seizures are clearly different. Whereas kindled kittens have SWS and REM transition seizures, penicillin epilepsy is most pronounced after awakening from SWS . 59% of kindled convulsions occurred in the REM sleep transition. Over 65% of generalized myoclonic seizures and tonic-clonic convulsions (GTCs) during penicillin epilepsy occurred during the extended drowsy period after awakening. Systemic penicillin epilepsy thus mimics its human counterpart, myoclonic petit mal epilepsy, in which 96% of myoclonus and convulsions occur exclusively after awakening . Finally, both epilepsy models are resistant to generalized EEG and motor seizures during REM sleep.

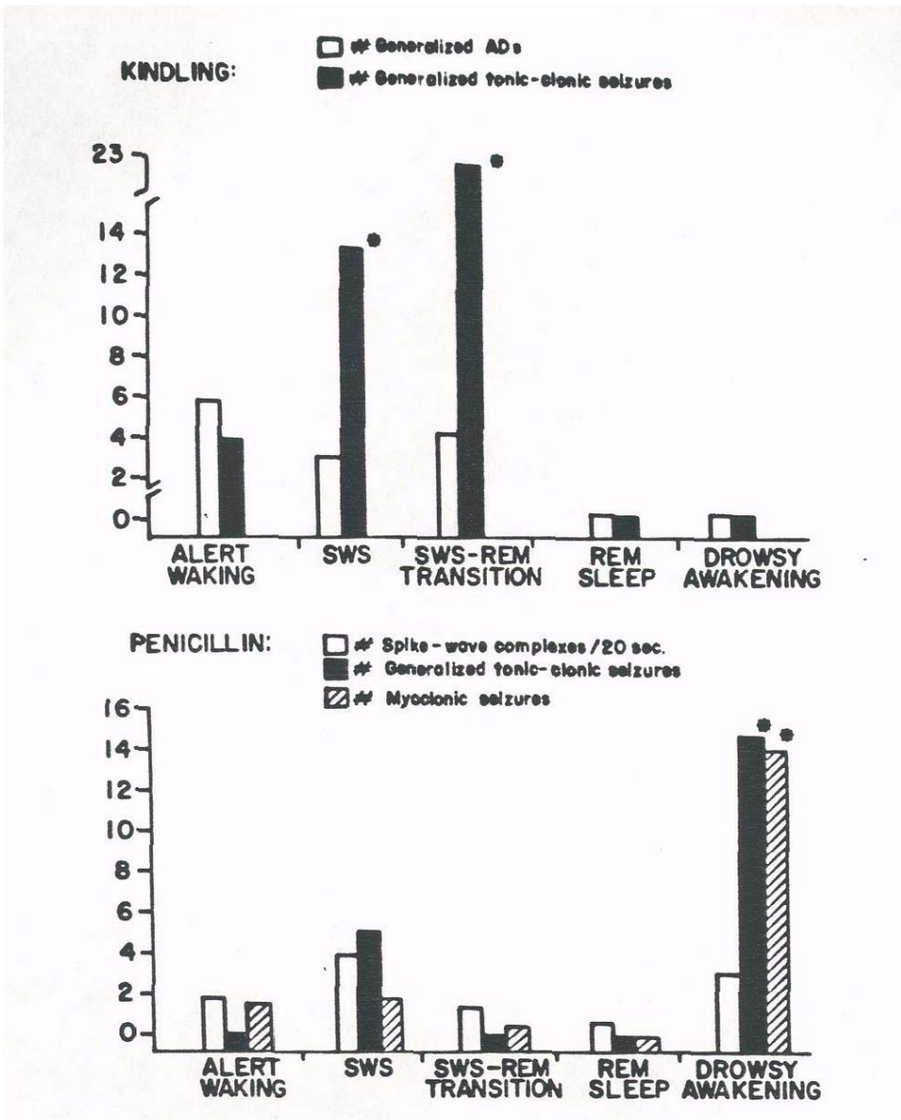

Fig. 1. Timing of spontaneous seizures during the sleep-wake cycle in nine kindled kittens (top) and 12 cats with systemic penicillin epilepsy (300,000-400,000 IU/kg; bottom).

Anatomical substrates of seizure prone and seizure resistant states.

Initial studies on the basic mechanisms that might explain these sleep vs. arousal activated seizure patterns used evoked potential methodology to study thalamocortical excitability in the two models. Many sleep-waking and sensorimotor functions are integrated at the thalamocortical level, making it a likely candidate as a final common pathway in the timing of convulsions in the sleep-wake cycle. We focused on the somatomotor pathway, in part because both models present motor seizures. Generalized motor seizures, especially convulsions, are more entrained to specific sleep and awakening states than any other focal or generalized seizure manifestation . Equally important, motor cortex and its thalamic relay nucleus, ventralis lateralis (VL) are modulated quite differently by hypothalamic and reticular inputs involved in sleep and arousal, and this modulation shows up well with evoked population amplitude measures * . Accordingly, it has been possible to distinguish sleep vs. arousal seizure mechanisms at this level of the neuraxis using evoked response amplitude measures

Evoked response studies (Figure 2) suggest that thalamocortical relays of the somatomotor system become hyper-responsive (hyperexcited)

throughout the sleep-wake cycle during the development of feline generalized epilepsy. However, it is important to note: 1) Thalamic cells are most hyperexcited during seizure-prone sleep states in kindled cats (A) and could combine with high or variable motor cortex excitability to trigger convulsions during SWS and the REM transition. 2) Motor cortex is most hyperexcited during seizure-prone states in penicillin epilepsy (B). Peak cortical response amplitudes correspond to frequent spike-wave activity during drowsiness and SWS and to frequent spontaneous convulsions during drowsy awakening; and 3) Thalamocortical cells are least hyper-excited during the seizure resistant state of REM sleep in both epilepsy models, seen as a minimal increase from baseline in thalamic response amplitudes for kindled epilepsy (A) and in cortical response amplitude for penicillin epilepsy (B). These findings suggested that the thalamocortical relay might be important in motor seizure generalization and might also provide a site for sleep-waking state modulation of motor seizures in both epilepsy models.

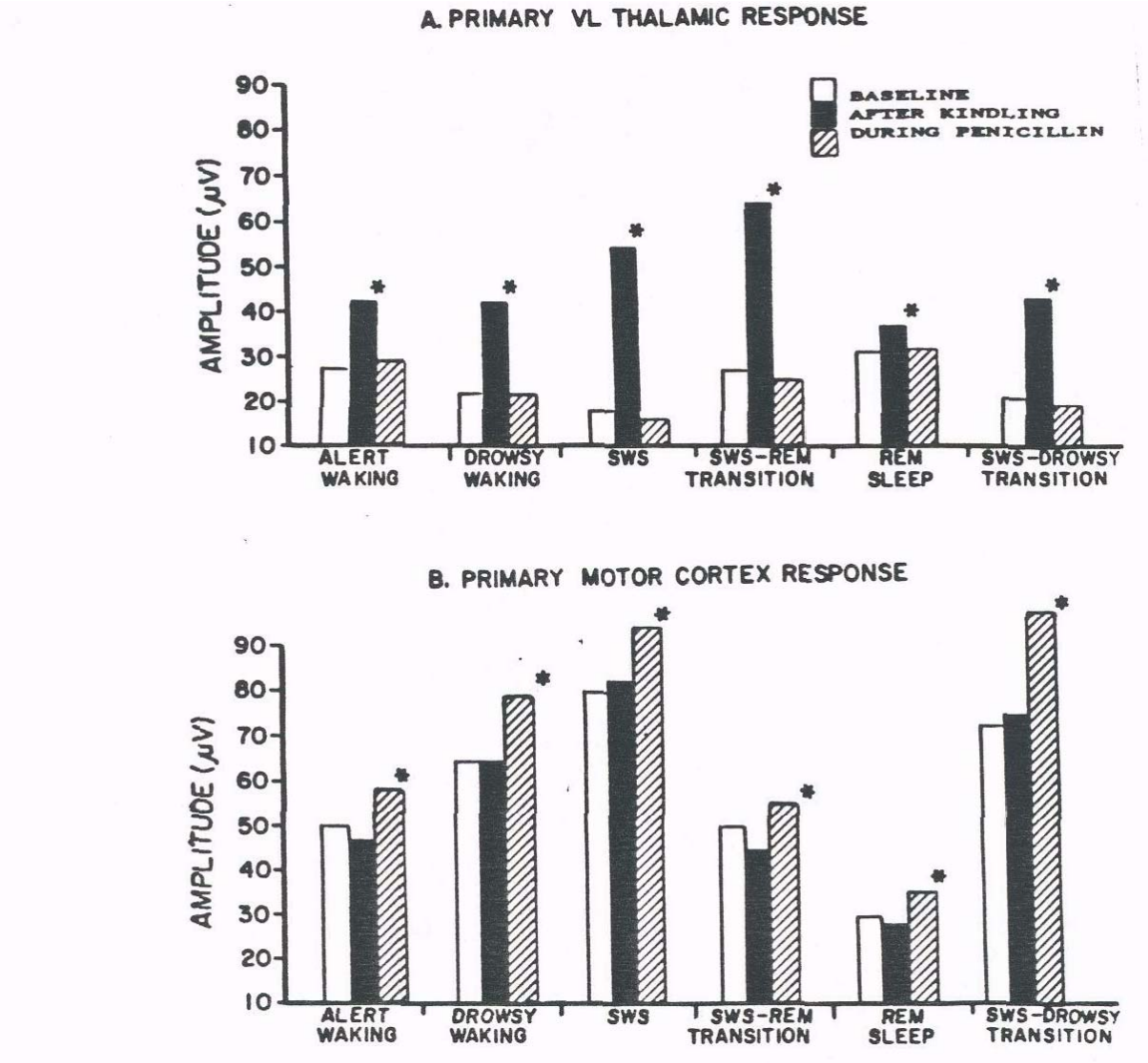

Fig. 2. Mean amplitude of primary evoked population responses (uV) in ventral lateral (VL) thalamus (A) and motor cortex (B) during baseline (n=8), after kindling (n=A) and during penicillin epilepsy (n=4). VL responses were elicited by stimulation of dentate nucleus. Motor cortex responses were elicited by stimulation of VL. Recurrent, low intensity stimulation of either dentate or VL was provided every 2.5 seconds. Evoked responses were sampled in each sleep or waking state (n=16 to 32 responses averaged per state in VL or motor cortex). Amplitude is an index of excitability. Kindling increased thalamic excitability. Penicillin increased cortex excitability. * = $p < .05$ from baseline

Follow-up studies used spontaneous and evoked extracellular unit and lesion methodology to examine the hypothalamic and reticular pathways thought to regulate thalamocortical excitability patterns as well as sleep and awakening states. Preliminary findings suggest testable anatomical models for seizure prone and seizure resistant states in kindled and penicillin epilepsy, as follows.

Kindling: Reticulothalamocortical pathways in sleep-activated epilepsy.

The timing of kindled seizures in the REM sleep transition, coupled with our previous finding that kindled cats show abnormal behavioral arousal at this time, suggest brain stem participation . It is well known that the ultradian REM sleep transition and REM sleep cycle are controlled by the brain stem, as they persist only in the brain stem after midcollicular transection . The specific mechanism for the REM sleep transition is obscure. However, a likely candidate for propagation of abnormal excitation at this time is the midbrain reticular formation and its rostral continuation, the reticular nucleus of thalamus. The thalamic reticular nucleus exerts a tonic excitatory influence on VL thalamus during shifts from states of EEG synchronization to EEG desynchronization, such as the REM transition * . Thus, ascending brain stem reticular influences increase VL thalamic excitability during the most seizure prone state for kindled convulsions.

Figure 3a shows that NMDA lesions of the midbrain reticular formation (MRF) and the thalamic reticular nucleus (TRN) block kindled GTCs during the REM sleep transition, whereas lesions of VL thalamus block kindled seizures in all states. These results are consistent with previous findings that MRF and thalamic reticular lesions suppress behavioral arousal and also amygdala kindling development[13,14] . Accordingly, we propose that the brain stem reticular formation propagates abnormal excitation to thalamocortical reticular and motor relays to trigger convulsion preferentially in the REM sleep transition.

The fact that VL lesions block kindled GTCs throughout the sleep-wake cycle is consistent with evidence that VL is chronically hyperexcited in kindled cats . VL lesions also block seizure generalization in other feline models of temporal lobe epilepsy as well as intractable seizures in humans . VL is a major relay to motor cortex for many extrapyrami-dal motor nuclei implicated in kindled seizure generalization and could elicit GTCs at any time in the sleep-wake cycle.

Penicillin epilepsy: Hypothalaroocortical pathways in awakening convulsions.

The timing of penicillin myoclonus and GTCs after awakening suggested forebrain regulation, as the "circadian" sleep-wake cycle persists only in the forebrain after midcollicular transection . The sleep-waking cycle has been further localized to the antagonistic divisions of the hypothalamus, where the preoptic basal forebrain induces SWS and the posterior hypothalamus induces awakening[17] . Recent evidence suggests that cells in the posterior lateral hypothalamus discharge in relation to EEG and behavioral arousal and might elicit spontaneous awakening by direct projection to neocortex * .

Preliminary lesion and extracellular unit studies suggest that this hypothalamocortical pathway could also mediate penicillin seizures after awakening. Figure 3b shows that lesions of the posterior hypothalamus (PH) block penicillin myoclonus and GTCs on awakening (Fig 3b), whereas lesions of the reticular and thalamic relays implicated in kindled seizures did not.

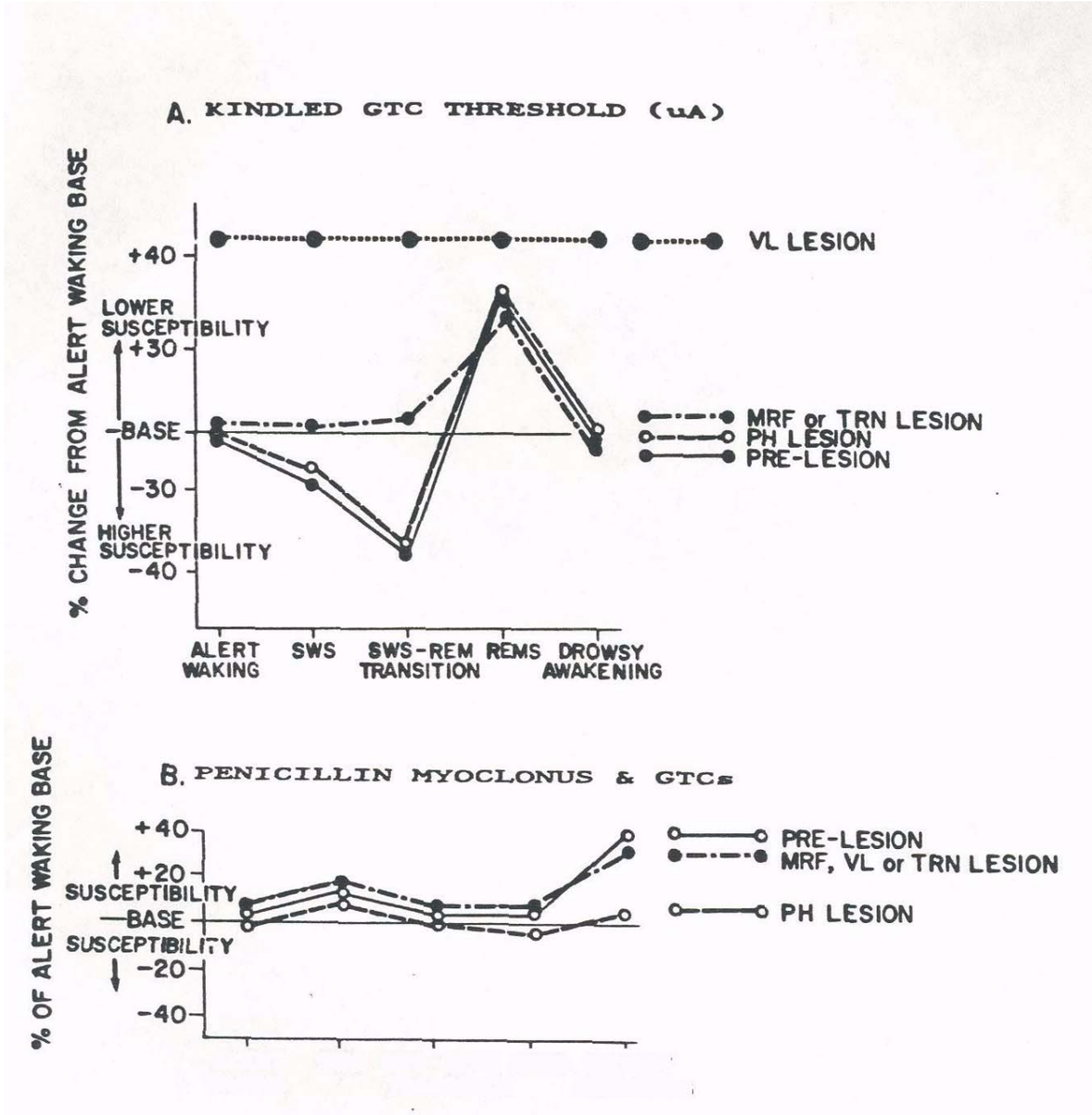

Fig. 3. **ALERT WAKING SWS SWS-REMS TRANSITION REMS DROWSY AWAKENING**

Motor seizure susceptibility before (n=24) and after NMDA lesions in one of four sites (n= 6 each): ventral lateral (VL) thalamus, the midbrain reticular formation (MRF), thalamic reticular nucleus (TRN) or posterior hypothalamus (PH). A: Kindling (n=12). Pre-lesion GTC thresholds are lowest (susceptibility is highest) in SWS and the REM transition. MRF and TRN lesions eliminated vulnerability only at these times. VL lesions protected against GTCs in all states. PH lesions had no effect. B. Penicillin epilepsy (n=12). Before lesions, myoclonus and convulsions peak during drowsiness after awakening. PH lesions eliminated vulnerability at this time, whereas the other lesions did not.

Figure A illustrates spontaneous discharge rates of a posterior lateral hypothalamic (PLH) neuron during and after awakening from sleep before and after a subconvulsive dosage of penicillin. PLH discharge rates normally increase during and after awakening (top), and awakening discharge is enhanced by penicillin (bottom). Figure 5 shows effects of penicillin on the spontaneous and evoked orthodromic response of a PLH neuron. Compared to pre-penicillin baseline (A), penicillin increased spontaneous discharge rates, enhanced evoked excitation and reduced the duration of post excitatory discharge suppression (B). Thus, penicillin could increase posterior hypothalamic cell activity by direct excitation and/or reduced inhibition.

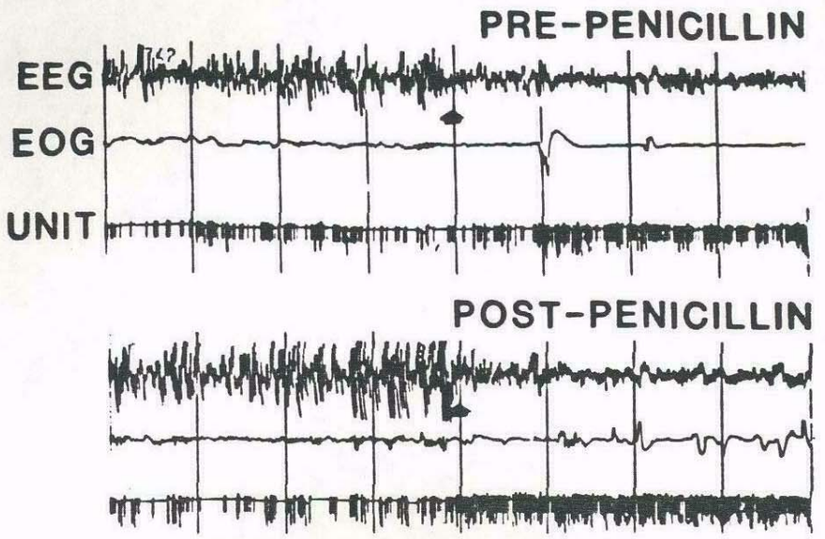

Fig 4. Spontaneous unit activity in a posterior lateral hypothalamic (PLH) neuron during SWS and at awakening (arrow) before and after a subconvulsive dose of penicillin (200,000 IU/kg).

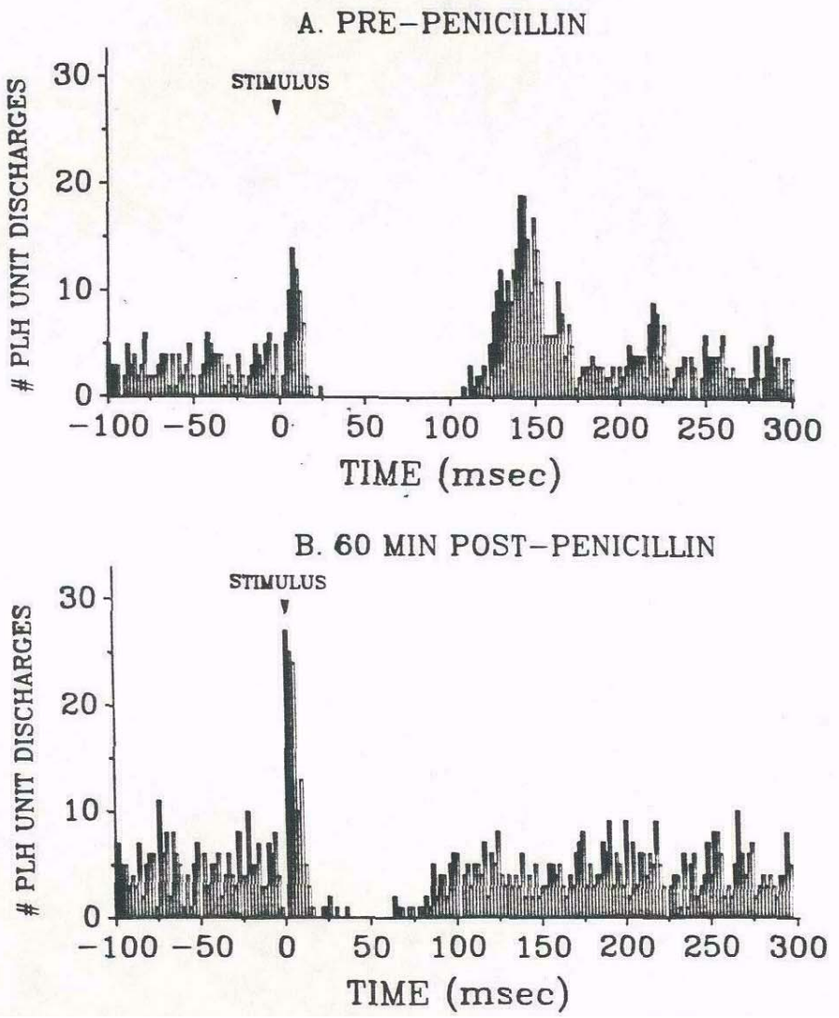

Fig. 5. Evoked orthodromic response in a PLH neuron, induced by stimulation of external capsule (100 pulses, .8 mA), before and after penicillin. Stimulus onset at time 0. Note increased excitation and reduced discharge suppression in B.

Collectively, the findings suggest that awakening convulsions might be triggered by abnormal discharges from the posterior hypothalamus, a region thought to underlie awakening from sleep in humans and animals. Discharges from this region are propagated to motor cortex, which is already hyper-excited in penicillin epilepsy. The interaction of hypothalamus and motor cortex could elicit penicillin GTCs preferentially after awakening.

REM sleep suppression of seizures *in* kindled and penicillin epilepsy.

REM sleep is the most antiepileptic state in the sleep-wake cycle for human generalized epilepsy, yet the neural mechanism is unknown . As in humans, REM sleep in cats retards the spread of EEG seizure discharges and has even more potent anticonvulsant effects[6,20] . The fact that generalized EEG seizures can occur without clinical motor accompaniment in REM sleep suggested differential mechanisms for EEG vs. motor seizure suppression. We have dissociated these mechanisms in both penicillin and kindled epilepsy. Thalamocortical EEG desynchronization protects against the spread of EEG seizures, whereas lower motor neuron inhibition of REM sleep blocks clinical motor accompaniment.

Figure 6 demonstrates differential modulation of seizure manifestations by REM sleep components. The top tracing shows penicillin spike-wave and myoclonic seizures during SWS and REM sleep before manipulation of REM sleep components. Spike-wave activity, the prominent EEG seizure manifestation of penicillin epilepsy, is common in SWS but rare during normal REM sleep. Epileptic myoclonus can accompany spike-wave activity in SWS but does not occur during normal REM sleep, which is characterized by profound lower motor neuron inhibition (note EMG silence during REM sleep with spike-wave paroxysm).

The middle tracing shows effects of a selective syndrome of REM sleep without thalamocortical EEG desynchronization, created by systemic administration of the anticholinergic agent, atropine . During atropine and penicillin trials, REM sleep has a SWS-like EEG, and spike-wave incidence during REM sleep is identical to SWS; however, no clinical accompaniment occurs, evidenced by continued silence in the EMG.

Opposite effects occur after a syndrome of REM sleep without lower motor neuron inhibition, called REM sleep without atonia. As indicated above, "sleep paralysis" (skeletal muscle atonia) occurs during normal REM sleep and is induced by descending pathways from the medial-lateral pontine tegmentum to the spinal cord . Lesions of the pontine "atonia" center disinhibit muscle tone in REM sleep[22,23](note activity in bottom right EMG channel), but all other REM sleep components remain intact. The animal is also mobile during REM sleep without atonia and appears to be "acting its dreams out." The bottom tracing of Figure 6 shows the effects of the pontine lesion on penicillin epilepsy. During REM sleep without atonia, penicillin spike-wave activity is as uncommon as in normal REM sleep, but when it does occur, it is 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 kindled cats, as shown in Figure 7. The top tracing shows a kindling trial during normal REM sleep. Normal REM sleep retards EEG discharge generalization from amygdala and further delays clinical motor accompaniment. The middle tracing shows a kindling trial during atropine-induced REM sleep without thalamocortical EEG desynchronization. Atropine facilitates EEG discharge generalization but does not affect clinical motor accompaniment, evidenced by continued atonia (EMG silence) during

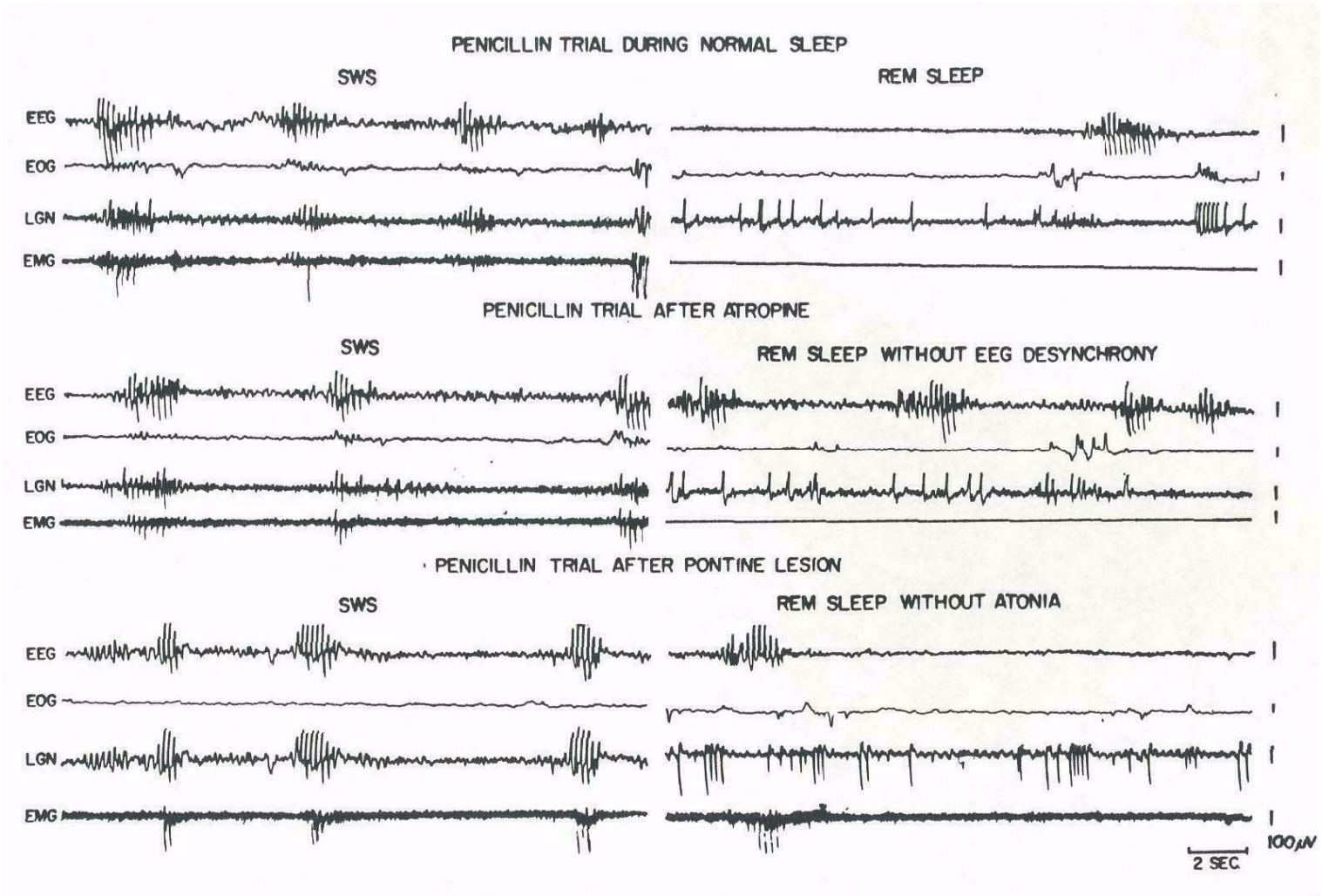

Fig. 6 Systemic penicillin epilepsy during SWS and REM sleep before and after dissociation of REM sleep components. Spike-wave paroxysms are visible in the EEG tracing, and myoclonic seizures were associated with discharges in the EMG tracing in this cat. TOP: Normal REM sleep suppresses EEG and motor seizures. MIDDLE: Atropine creates a SWS-like EEG in REM sleep and increases spike-wave activity during REM. BOTTOM: Pontine lesions induce REM sleep without atonia and release myoclonus in REM. From[20], with permission.

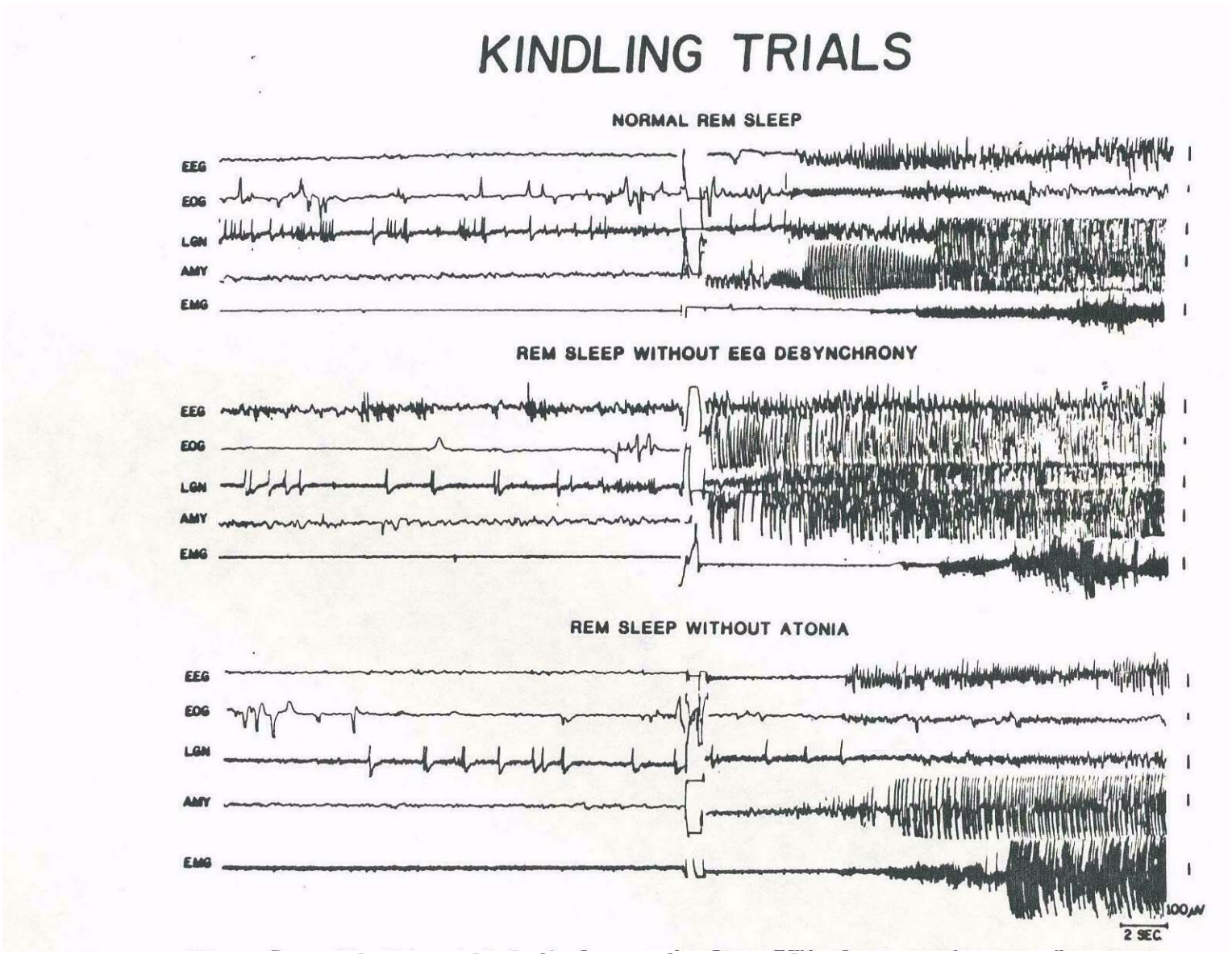

Fig. 7. Kindling **trials before and after** REM **sleep** syndromes. See text.

the first 10 seconds of generalized AD. This result is consistent vith preliminary findings that atropine facilitates EEG discharge generalization during the development of kindling in cats[24]. The bottom tracing shows the opposite effect during REM sleep without atonia. AD generalization is delayed as in normal REM sleep, but when the EEG seizure spreads from amygdala, there is immediate clinical motor accompaniment.

Figures 6 and 7 suggest that thalamocortical EEG synchrony abolishes protection against EEG but not clinical motor seizures; in contrast, loss of lower motor neuron inhibition during REM sleep abolishes protection against motor but not EEG seizures. These effects were obtained in 26 cats exposed to amygdala kindling (n=6), systemic penicillin epilepsy (n=10) or electroconvulsive shock (n=10) . Because REM sleep components have been localized to the brain stem, it seems likely that protection against the spread of EEG seizure discharges is mediated by the ascending brain stem pathways which induce intense thalamocortical EEG desynchronization during REM sleep[25,26] . Protection against motor seizures appears to be regulated by separate, descending brain stem pathways which mediate lower motor neuron inhibition during REM sleep.

SUMMARY

Amygdala kindled kittens and adult cats are most susceptible to spontaneous and elicited convulsions during SWS, especially the transition from SWS to REM sleep. Systemic penicillin epilepsy is associated with convulsions after awakening. Both epilepsy models are resistant to generalized EEG and motor seizures during REM sleep. Evoked population response, extracellular unit and lesion studies suggested the following anatomical pathways for seizure prone and seizure resistant states. For sleep epilepsy, abnormal excitation from the brain stem reticular formation may activate thalamocortical relays to trigger convulsions preferentially in the REM sleep transition. For awakening epilepsy, abnormal excitation from the posterior hypothalamus may activate motor cortex to elicit convulsions on awakening. Finally, we dissociated the factors for REM sleep suppression of EEG vs. motor seizures. Thalamocortical EEG desynchronization during REM discourages the spread of EEG seizures; in contrast, motor inhibition during REM sleep blocks only clinical motor accompaniment.

ACKNOWLEDGMENTS

This research was supported by the Veterans Administration and by PHS grants NS25629, NS14610, MH43811 and MH42903.

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