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18. While this manuscript was in preparation, the sequence of the beta isolate was reported elsewhere [G. Bell *et al.*, *ibid.* **283**, 26 (1980)]. Our sequence differs at eight positions from that presented by these workers in both our alpha and beta isolates. The alpha isolate that we report matches the mRNA sequence (*3, 8*).
19. We thank R. Swanson, H. Noller, L. H. Kedes, P. H. Seeburg, H. Hendrickson, R. Gutell, and K. Katze for support and discussions. We thank S. Schmus, without whom this work could not have been completed. Carried out under P2 EK2 conditions in compliance with the NIH guidelines for recombinant DNA research.
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Rapid Eye Movement Sleep PGO-Type Waves Are Present in the Dorsal Pons of the Albino Rat

Abstract. *We have found rapid eye movement sleep central phasic activity in the form of episodic, repetitive, monophasic waves in the albino rat. This activity is recorded in discrete areas of the dorso-lateral pons, including the nucleus locus ceruleus. The vast majority of these waves occur during rapid eye movement sleep. Their distribution and electrophysiological characteristics are similar to those of ponto-geniculo-occipital waves in the cat.*

Rapid eye movement (REM) sleep is identified by the simultaneous appearance of characteristic phasic and tonic physiological events within behavioral sleep. The defining macroelectrode brain signal of REM sleep in cat and monkey is the repetitive ponto-geniculo-occipital (PGO) wave, which is readily recordable in pons, lateral geniculate nucleus, and cortex (*1, 2*). Studies in the cat suggest that PGO waves reflect a phasic discharge generated in the hindbrain during REM sleep (*3*). This PGO generating system has been viewed as essential in all mammals to the inception and maintenance of the REM sleep state (*4*). This hypothesis is consistent with the findings that (i) REM sleep episodes in the cat are always preceded and accompanied by PGO waves (*1*) and (ii) careful prevention of all PGO waves in a REM sleep deprivation procedure results in even greater REM sleep rebound after deprivation than does deprivation of REM sleep alone (*5*).

However, the necessity of PGO activity in the REM sleep process has recently been questioned because PGO waves have not been found during REM sleep in the albino rat in structures in which they are prominent in the cat and monkey, that is, the lateral geniculate nucleus and visual cortex (*6, 7*). Gottesmann (*6*) did show, in the rat, PGO-type waves recorded during REM sleep in other areas where they are found in the cat—the oculomotor nuclei and parasagittal pons. However, the distribution of these waves within the sleep stages was not presented. Further, the failure of the waves in that study to be affected by reserpine, which predictably alters the PGO-wave pattern in the cat, left the matter of PGO activity inconclusive in the rat. The lack of a clear demonstration until now of PGO activity in the albino rat has led some to the conclusion that such activity is not a fundamental component of REM sleep in mammals, but, rather, a visual system process appearing during REM sleep in some species (*7*).

We have hypothesized that systems other than the visual are also the recipients of REM sleep phasic activation. The expression of this activation by a macroelectrode event, the PGO wave, may be determined by the cytoarchitectonics of the investigated structures and also by species specialization. Given the aberrations found in the visual systems of albinos of some species (*8*), we reasoned that the search for REM sleep phasic activity in the visually poor albino rat would be more fruitful in brain regions that subserve other, highly utilized, waking state functions. We now report on the existence and distribution of REM sleep phasic activity monitored in the dorso-lateral pons of the albino rat.

Eleven male albino Sprague-Dawley rats (350 to 500 g) had electrodes implanted for long-term recording of electroencephalogram (EEG), electrooculogram (EOG), and electromyogram (EMG). In all rats, a twisted bipolar stainless steel electrode (0.20 to 0.25 mm in diameter), which was completely insulated except at the cross section of the tips, was aimed at the dorsal tegmentum of the pons, into the region of the nucleus locus ceruleus. After a 7- to 10-day recovery period, the subject was placed in a sound-attentuated cage and connected for polygraphic recording. After a 24-hour adaptation period, electrophysiological data were collected on an ink-writing polygraph (Grass 78). Monitoring sessions usually lasted 4 to 24 hours. The polygraphic recording time was divided into 30-second epochs for analysis. These epochs were characterized as either awake (AW), slow wave (SW) sleep, or REM sleep, according to conventional criteria in the rat based on EEG, EOG, and EMG activity. REM sleep was identified by EEG desynchronization, hippocampal theta, the loss of resting muscle tone, and the occurrence of rapid eye movements.

The electrophysiological output of the pontine placement was examined for evidence of discrete phasic activity associated with REM sleep. Since our hypothesis was that PGO waves can be recorded in the pons of the albino rat, the pontine tracings were initially explored for waves with characteristics similar to those of the PGO waves in the cat. In the cat, the waves typically (i) are monophasic, of the same polarity and having a duration of 60 to 120 msec; (ii) are present in every REM sleep episode; and (iii) occur primarily in REM sleep but also in the SW sleep immediately preceding REM sleep.

In 7 of the 11 rats, we found associated with REM sleep the unmistakable appearance of such wave forms in the pontine recordings. The waves were monophasic, always of the same polarity, 60 to 100 msec in duration, and 25 to 150 μv in amplitude. Figure 1A is an oscilloscope tracing of a representative phasic wave recorded in REM sleep from an electrode in the region of the nucleus locus ceruleus.

A majority of the waves occurred in REM sleep. Every REM sleep episode contained the pontine waves. Single

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waves began to emerge in SW sleep preceding REM sleep onset. Figure 1B is a 90-second recording of the transition from SW sleep to REM sleep. Single waves can be seen during the transition period; bursts of waves ensue once the REM sleep episode has become established. These bursts were simultaneous with eye movement clusters in the great majority of instances ($P = .80$) (*9*).

The central phasic waves were quantified independently of sleep stage scoring, and all clues to sleep stage were removed during this procedure. The waves were identified according to preset criteria that took polarity, duration, and amplitude into account to the exclusion of high-amplitude slow waves associated with SW sleep, background noise, and artifact. The temporal distribution of these waves was analyzed in four animals who had prominent pontine waves and at least 8 hours of continuous recording. This distribution is described with respect to sleep stage according to five mutually exclusive categories: (i) AW, (ii) REM sleep, (iii) SW sleep within 2 minutes of REM sleep onset (R-2), (iv) SW sleep within 2 minutes of a spontaneous awakening (AW-2), and (v) SW sleep other than R-2 and AW-2. Correlated *t*-tests were used to detect differences between categories. The percentage of waves appearing in REM sleep was significantly greater than in any other category ($P < .01$) (Table 1). Only 12.2 percent of the total recording time was spent in REM sleep, yet this stage accounted for 78.0 percent of the waves. The mean rates per minute of pontine waves in the five categories were: REM = 14.78, R-2 = 1.20, AW-2 = 0.72, SW = 0.64, AW = 0.36. The rate in REM sleep proved to be significantly greater than in every other category ($P < .05$) and the rate in R-2 was significantly greater ($P < .05$) than in AW-2, SW, and AW. Accordingly, as with PGO waves in the cat, the pontine waves in these four animals occurred most frequently in REM sleep and in the SW sleep segments within 2 minutes of the approach of REM sleep episodes (*10*).

Table 1. Percentage of time in the five sleep-wake categories and temporal distribution of the PGO-type pontine waves. Values are means and standard deviations, derived from 8 hours of continuous recording from four rats. Correlated *t*-tests were used to detect differences between categories.

Variables (%)	Category				
	REM	R-2	AW-2	SW	AW
Recording time	12.2 ± 2.5	9.3 ± 3.4	19.6 ± 2.9*	11.1 ± 6.1	47.8 ± 7.7*
PGO-type waves	78.0 ± 7.4†	4.7 ± 0.8	6.7 ± 4.0	2.8 ± 1.7	7.8 ± 4.2

*$P < .05$. †$P < .01$.

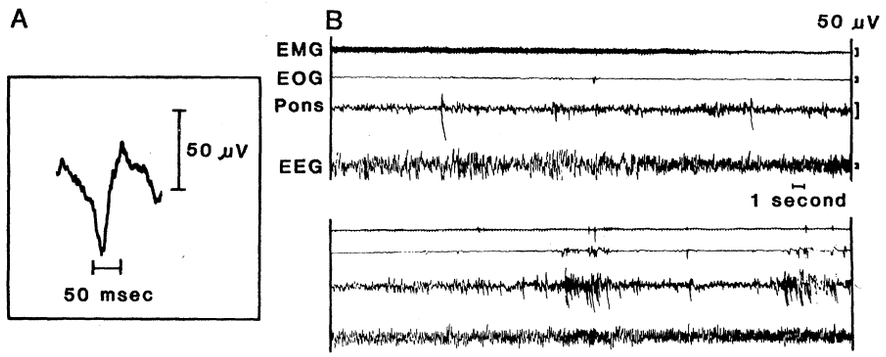

Fig. 1. (A) Oscilloscope tracing of a phasic wave recorded in REM sleep. (B) This 90-second polygraph record shows the transition from SW sleep to REM sleep. Single phasic waves appear in SW sleep (synchronized EEG and elevated muscle tone) just prior to the onset of a REM sleep episode (desynchronized EEG, theta activity, and muscle atonia). Bursts of phasic waves and single waves, often associated with rapid eye movements, are seen in the REM sleep episode.

All rats were anesthetized with sodium pentobarbital and perfused with normal saline followed by 10 percent Formalin solution. Frozen coronal sections, 40 μm thick, were stained with cresyl violet. The brain sites upon which the electrodes impinged were localized by two independent judges operating without information concerning the corresponding electrophysiological activity. In the seven rats from which REM sleep phasic activity was recorded, the electrode either directly impinged on the nucleus locus ceruleus ($N = 4$), straddled the locus ceruleus and the nucleus mesencephalic V ($N = 2$), or was located in the dorso-medial tip of the nucleus parabrachialis dorsalis ($N = 1$). In the four rats that did not show any REM sleep-related phasic activity, the electrodes were located in the superior cerebellar peduncle ($N = 1$), the dorsal tegmental nucleus ($N = 1$), or in the reticular formation ventral to the locus ceruleus ($N = 2$) (Fig. 2).

This study demonstrates that a REM sleep-specific phasic wave can be recorded from the dorsal pons of the albino

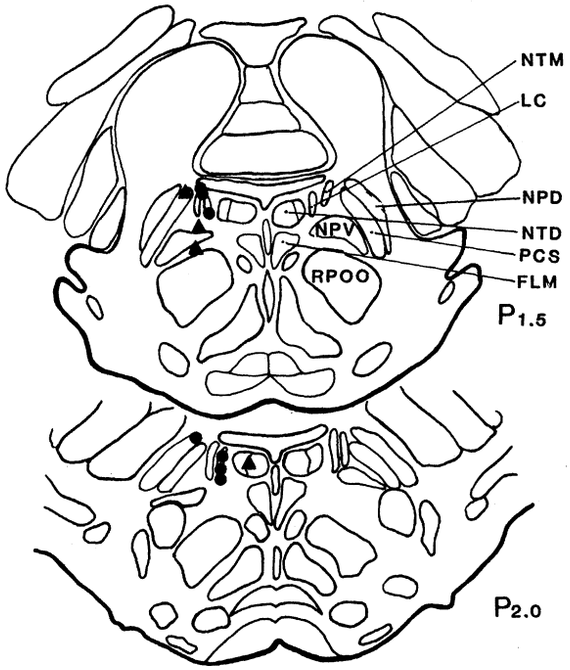

Fig. 2. The serial sections illustrate the pontine brain sites upon which the electrode tips impinged. (●) Sites where REM sleep phasic activity was recorded and (▲) sites where no REM sleep-specific phasic activity was recorded. Abbreviations: *FLM*, fasciculus longitudinalis medialis; *LC*, locus ceruleus; *NPD*, nucleus parabrachialis dorsalis; *NPV*, nucleus parabrachialis ventralis; *NTD*, dorsal tegmental nucleus; *NTM*, nucleus mesencephalic V; *PCS*, superior cerebellar peduncle; and *RPOO*, nucleus reticularis pontis oralis. [Adapted from maps in Palkovits and Jacobowitz (*14*)]

rat. In its electrophysiological characteristics and distribution, this pontine wave is similar to the PGO wave of the cat. Additional research in this laboratory (*11*) has shown that the administration of *dl*-parachlorophenylalanine (PCPA) as well as REM sleep deprivation have effects in the rat similar to the effects of these manipulations on the distribution of PGO waves in the cat (*4, 5, 12*). We suggest that the pontine phasic wave in the albino rat, like the PGO wave in the cat, is an electrophysiological expression of REM sleep central phasic brainstem activity.

The general process represented by PGO activity, that is, the generation in the pons of central phasic discharge in REM sleep (combined with its ascending and descending transmissions), consists of a complex set of relationships in which systems other than the visual also appear to participate. It has already been shown that auditory structures in the cat and human display phasic discharge during REM sleep in a manner similar to the discharge in the visual system (*13*). We believe that the pontine waves recorded in the albino rat represent this fundamental REM sleep process of central phasic activation. Further exploration for reflections of REM sleep phasic stimulation may yield positive results in structures of highly utilized sensorimotor systems (for example, the olfactory system). That the nucleus locus ceruleus—which in the rat (i) provides noradrenergic innervation to vast areas of the brain (*14*), (ii) participates in the mediation of waking functions (such as learning, positive reinforcement, hippocampal orienting responses) (*15*), and (iii) may play a role in neuronal maturation (*16*)—is a REM sleep phasic activity site supports the hypothesis that brain regions subserving highly relied upon functions are invested with phasic activation during REM sleep.

The extensive use of the albino rat in sleep experiments has been limited by the unavailability of an important variable—REM sleep central phasic activity. The findings reported here may have the additional practical value of establishing this relatively convenient and inexpensive laboratory animal as an appropriate subject for electrophysiological and pharmacological studies of sleep and phasic activity.

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Disruptive Coloration in Butterflies: Lack of Support in *Anartia fatima*

Abstract. *Experimental obliteration of high-contrast wing stripes of the neotropical butterfly* Anartia fatima *affected neither survival nor wing damage in a natural population over a 5-month period. There is no direct evidence supporting the hypothesis that so-called disruptive wing patterns function as protective coloration in butterflies.*

"Disruptive" color patterns, consisting of high-contrast markings that serve to break up the outline of an organism, are among the classical types of protective coloration common in the animal kingdom (*1*). Such patterns presumably protect their bearers by fragmenting the visual image into smaller units, by producing a conflicting outline that obscures essential body features, and/or by delimiting target areas that may direct attacks by predators away from vital organs. By whatever mechanisms they operate, disruptive patterns ought to improve the chances of survival of individuals bearing them, relative to concolorous individuals. We now report the results of a field experiment designed to measure directly the effectiveness of disruptive coloration in a butterfly.

The butterfly *Anartia fatima* (Lepidoptera: Nymphalidae) (Fig. 1) is commonly found in disturbed habitats throughout Central America (*2–4*). We studied the small, insular population located on Barro Colorado Island, Panama, from April through August 1978. Since *A. fatima* frequented only the disturbed site at the laboratory clearing, it was possible to capture and individually mark (*5*) nearly the entire population. Recapture rates were extremely high, often with many recaptures per individual. Hence it was possible to determine the minimum age of each butterfly at every capture and a minimum longevity for each individual in the population (*6*); in

Table 1. Longevity measured as difference between first and last captures (*6*).

Minimum age (weeks)	Experimental (No.)	Treated control* (No.)
0	37	38
1	24	26
2	20	25
3	9	6
4	6	1
5	1	1
Total	97	97

*Kolmogorov-Smirnov test: $D = 0.082$; $P > .10$.

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