must have been provided by a centrally generated corollary discharge. This experiment, while demonstrating that corollary discharge provides accurate eye position information, does not address the possibility that extraocular muscle proprioception may provide information for some oculomotor functions (*15*).

Although previous investigations have suggested that motor systems distribute corollaries of their output (*16*), none has unequivocally demonstrated a functional role for these internal signals. This study, however, clearly demonstrates that the oculomotor system generates a corollary discharge which accurately represents the intended motor act and is used in generating further movements.

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Subthreshold Excitatory Activity and Motoneuron Discharge During REM Periods of Active Sleep

Abstract. *A striking paradox of the rapid eye movement periods of active sleep, which are typically characterized by the exacerbation of somatomotor atonia, is the occurrence of muscle twitches and jerks. The purpose of this study was to examine the specific motoneuron membrane potential processes responsible for these myoclonic patterns of activity. In lumbar motoneurons, examined intracellularly in the cat prepared for long-term study, these processes consisted of recurrent depolarizing membrane potential shifts and spontaneous action potentials that were either full-sized or of partial amplitude. In addition, the invasion of antidromically induced spikes into the soma was often blocked. Hyperpolarizing potentials were evident in the intervals between spontaneous spikes. Hyperpolarization was also observed immediately before depolarization and spike activity, in contrast to the gradual depolarization of the motoneuron membrane potential that always occurred during wakefulness. Thus, during rapid eye movement periods, in conjunction with muscle twitches and jerks, a strong excitatory input is superimposed on a background of inhibitory input. The unique patterns of membrane potential change that arise thus seem to result from the simultaneous coactivation of excitatory and inhibitory processes.*

Certain patterns of motor activity seem, from a behavioral perspective, to be based on abnormal motor mechanisms. Specifically, the myoclonic twitches and jerks that predominate during the periods of rapid eye movements (REM's) of active sleep do not resemble normally controlled motor processes, nor do they fulfill any known purpose; they are, however, a constant and prominent feature of this state. We were therefore interested in determining the responsible motoneuron membrane potential processes that give rise to these "normally" occurring patterns of apparently "abnormal" motor activity.

To explore the underlying motoneuron membrane potential changes we recorded intracellularly, during sleep and wakefulness, from antidromically identified lumbar motoneurons in the unanesthetized, undrugged, normally respiring cat. The details of the procedures have been reported elsewhere (*1*).

Experiments were performed on six adult cats prepared for long-term recording. Glass micropipettes were used to record intracellularly from alpha motoneurons of the lumbar spinal cord. The micropipettes, which were filled with 2*M* potassium citrate, had tip resistances of 5 to 15 megohms. All recordings from the 47 motoneurons studied met established standards for monitoring intracellular activity (that is, antidromic identification; membrane potential ≥ 55 mV; spike height ≥ 55 mV; and data from cells recorded for at least 10 minutes and across a minimum of two behavioral states) (*1*).

We have previously described phasic episodes of postsynaptic inhibition, revealed by the presence of a depression of motoneuron excitability and a decrease in motoneuron input resistance, which occur during REM periods (*2*). These postsynaptic inhibitory processes are characterized by sequences of hyperpolarizing shifts in the membrane potential which are the result of the summation of discrete inhibitory postsynaptic potentials (*3*). However, during certain REM

periods excitatory phenomena, which are the specific focus of this report, predominate.

Various patterns of depolarizing events and spike activity were observed during REM episodes (Figs. 1 and 2). No single pattern predominated in any given cell, state of active sleep, or episode of REM's.

One of the most striking findings was that the majority of depolarizing potentials that eventuated in spikes were preceded by a well-defined hyperpolarizing shift in the membrane potential (Fig. 1, B′ and F′); gradual depolarization was the corresponding membrane potential change during wakefulness (Fig. 1, A′ and C′). In addition, many of the spontaneous action potentials during REM's were of partial amplitude (Fig. 2, B_1 and D_2) (*4*), even though full-sized spikes (those consisting of an initial segment and a soma-dendritic component) were also present (Fig. 1, A to F). Other characteristics of the action potentials during REM periods were the complete abolition of the afterhyperpolarization or a striking reduction in its amplitude and duration (Fig. 1, B′ and F′). Thus, action potential generation during REM episodes differed from that during wakefulness.

Different sequences of spike activity were present during REM episodes. In one of the most common patterns (Fig. 1, B, B′, F, and F′), spike doublets, triplets, and quadruplets arose in conjunction with each wave of depolarization. This characteristic of spontaneous action potential generation during REM's represents a conspicuous departure from the pattern during wakefulness (Fig. 1, A and C) (*4*). Other examples of motoneuron discharge during REM episodes are shown in Fig. 1, D and E, in which short bursts of spikes occurred at irregular intervals, and in Fig. $2D_1$, wherein a single isolated spike was generated.

An overview of the characteristic subthreshold excitatory events during REM episodes is presented in Fig. 2. Recurrent, "paroxysmal" depolarizing shifts were the most typical reflection of subthreshold excitatory input. The waveform of these depolarizing potentials varied, but they were typically of long duration (as long as 200 msec) and large amplitude (reaching 15 mV); they appeared to be the result of the summation of numerous small excitatory postsynaptic potentials.

The membrane potential threshold for spike generation was not always reached in spite of the relatively large amplitude of many of these potentials (Fig. $2A_1$). When a depolarizing bias was added during a cluster of REM's (by injecting 4 nA of depolarizing current via the impaling microelectrode), the depolarizing potential initiated motoneuron spike discharge (Fig. $2B_1$); these spikes were also of partial amplitude, as were many of those which arose spontaneously during REM periods (Fig. $2D_2$) (*5*).

The preceding excitatory events ap-

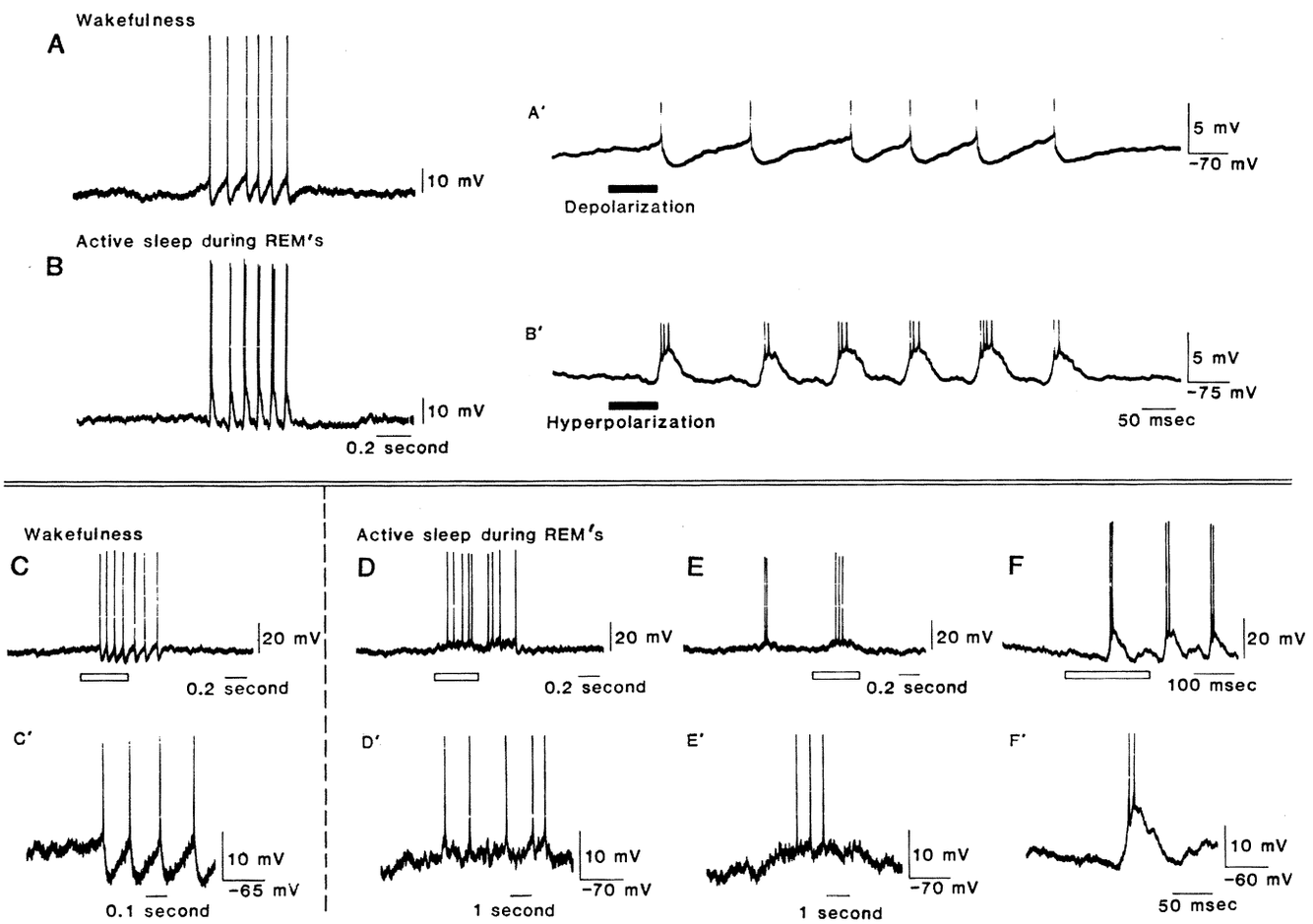

Fig. 1. Patterns of spike generation during REM periods of active sleep. (A) During wakefulness, depolarization (bar in A′) was the initial membrane potential event. (B) During REM periods, each depolarization shift was preceded by hyperpolarization (bar in B′) (see also F and F′). Full-sized spikes developed in both examples; in B doublets, triplets, and quadruplets accompanied each depolarizing shift. The open bars indicate the period of the traces shown in C′–F′. (C) Spike generation during wakefulness. (D) An irregular pattern of spike activity, (E) intermittent bursts and (F) spike doublets during REM periods. An increase in hyperpolarizing subthreshold synaptic activity during interspike intervals is present in D′ and E′ (*3*). (A) and (B) are records from a single tibial motoneuron and (C) through (E) from a single peroneal motoneuron; (F) is from another peroneal cell.

peared in conjunction with the concurrent presence of inhibitory phenomena. For example, inspection of high gain records obtained during spike activity (Fig. 1, D′ and E′ and Fig. 2, A_1 and B_1) revealed the presence of hyperpolarizing potentials, which were similar to the postsynaptic inhibitory potentials previously reported to impinge on spinal motoneurons during active sleep (*3*). The presence of inhibition during recurrent depolarizing shifts is indicated by these potentials as well as by Fig. 2C. During this particular REM episode, antidromic invasion of the soma (Fig. $2C_1$) was blocked even when it coincided with a depolarizing shift in the membrane potential (Fig. $2C_2$). The occurrence of a spontaneous partial-amplitude spike during a REM period accompanied by depolarizing potentials (Fig. 2, B and D) would also indicate the presence of inhibitory processes. Clearly, full-sized spikes were capable of being generated, for during the preceding and subsequent non-REM period of active sleep both antidromic (Fig. $2D_1$) and orthodromic (Fig. $2D_3$) action potential activation resulted in normal spikes (of 73 mV and 71 mV, respectively).

The site of origin of partial-amplitude spikes remains unclear; they may reflect action potential activity in dendritic compartments or in the initial segment (*5*). Regardless of their source, however, invasion into the soma region (which would result in full-sized spikes) was blocked, thus providing another indication that postsynaptic inhibitory processes (*2*) operate at the same time as the postsynaptic excitatory processes that initiate spike activity.

We wish to address three questions in our discussion of these findings: the first question is practical; the second is relevant to our understanding of basic motor mechanisms, but currently unanswered experimentally; and the third is of theoretical significance.

1) What cellular processes underlie the phasic patterns of motoneuron depolarization and spike activity during REM periods of active sleep? Depolarization and spike generation during REM periods are due to descending excitatory influences (*6*). But we have also shown that bursts of REM's are accompanied by an increase in postsynaptic inhibition (*2*) beyond that present during non-REM periods (*2*, *7*) and that postsynaptic inhibition is present even during the episodes of REM's characterized by depolarization shifts and spike generation. We suggest, therefore, that the unique patterns of membrane potential modulation that emerge during REM periods result from a complex interaction that develops when a strong phasic facilitatory drive encounters a motoneuron subjected to phasically enhanced postsynaptic inhibition.

2) What import is there to the coactivation of excitatory and inhibitory drives; why do they occur and what do they signify? The most parsimonious explanation for inhibition during active, as opposed to quiet, sleep is that it occurs to prevent motoneurons from discharging and initiating contraction of the peripheral musculature, which would presumably disrupt the active sleep state (*8*). But why should there be even greater inhibition during the REM periods of active sleep? It is well known that REM periods are accompanied by a striking increase in the activity of practically every motor facilitatory system (*6*); therefore, it is logical to assume that a compensatory increase in inhibition would be required to prevent the behavioral expression of this activity. Since an increase in postsynaptic inhibition apparently occurs during all REM periods, and motor activation arises in only a portion of them (*9*), we suggest that tonically increased motoneuron inhibition may be a protective measure that reduces or eliminates movements when there is the likely possibility of the onslaught of exceptionally strong excitatory input.

3) To what extent might the complement of excitatory and inhibitory processes, which affect spinal cord moto-

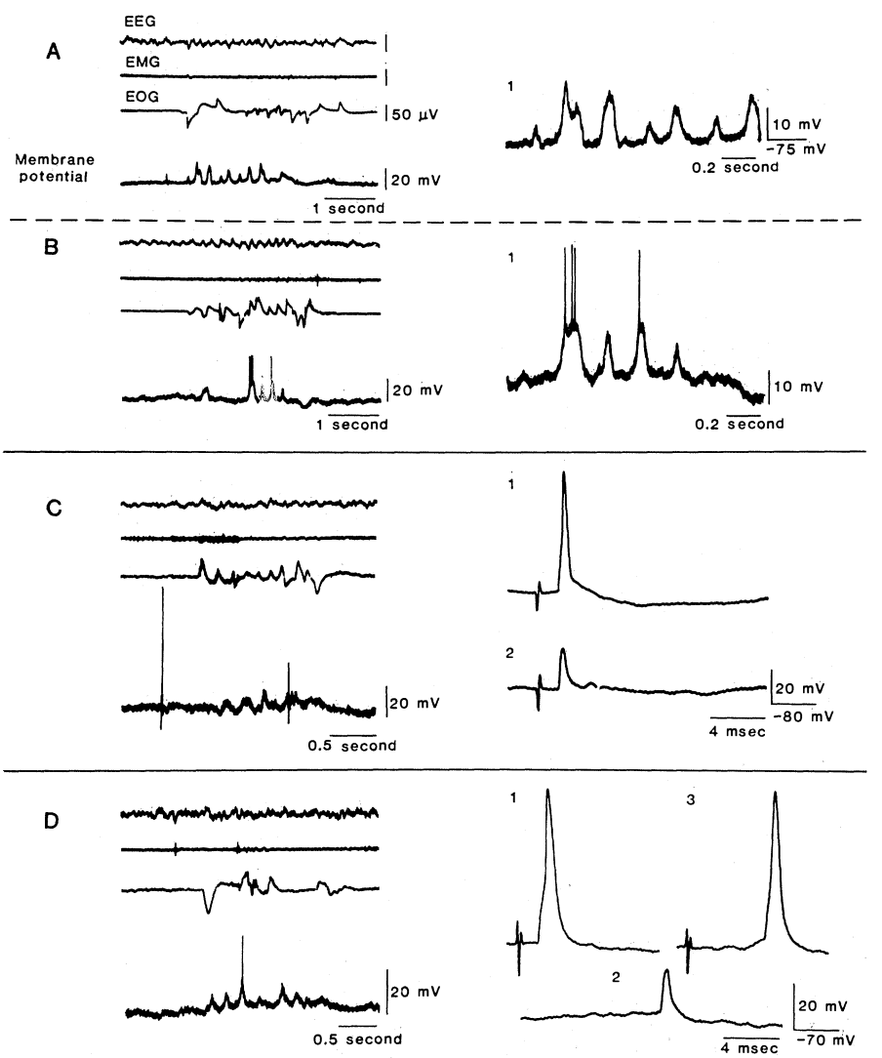

Fig. 2. Depolarizing potentials and partial amplitude spikes during REM periods. (A) Recurrent depolarizing potentials of variable amplitude and duration. During this period the potentials did not reach threshold. (B) In the same cell during another REM period the concurrent injection of depolarizing current (4 nA) led to spike generation. These spikes were of partial amplitude, indicating that the soma region was not activated (*5*). (The d-c membrane potential level in the figure is omitted because of distortion in its recorded value by the injection current.) An antidromic spike (C_2) and a spontaneously occurring spike (D_2) were also abbreviated with respect to (C_1) full-sized antidromic spikes initiated before REM's and (D_1–D_3) antidromic and orthodromic spikes initiated before and after the burst of ocular activity. (A) and (B) are records from a single tibial motoneuron; (C) and (D) are from two different sciatic motoneurons.

neurons during the REM periods of active sleep, act on other motor systems in a similar state-dependent way? The mechanisms responsible for the phasic contraction of the peripheral musculature during REM periods may reflect a general pattern that affects other somatomotor functions as well. For example, the striated muscles that move the orbits are active during REM periods. Despite the compelling heuristic value of the hypothesis that eye movements during active sleep are based on directed visualization of the dream experience, we believe there to be no convincing evidence that they are any more related to visual functions than the twitches and jerks of the limbs are to goal-directed movements. It is possible that the central neural areas that give rise to myoclonic activation of the limb muscles during REM periods also initiate a generalized pattern of twitches and jerks that affect all striated muscles. REM's are an example (*6*), as are irregular contractions of the middle ear musculature (*10*), and the erratic contractions of the respiratory muscles which result in irregular breathing patterns that are most prevalent during the REM periods (*11*).

We conclude that a strong motor facilitatory drive acts on spinal (and other) motoneurons during REM periods; when present, it encounters a somatomotor system subjected to enhanced inhibition. Consequently, during certain REM periods excitatory and inhibitory processes, both of which are postsynaptic, are simultaneously coactivated. We believe that, although paradoxical, these concurrently active but diametrically opposed processes could reflect proper adaptive responses to a widely activated nervous system; it seems logical for the organism to protect itself from the deleterious consequence of undirected and inappropriate movements when it is blind and unconscious.

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5. The cell soma is unlikely to be the source of partial spikes, because spikes originating in this region not only are greater in amplitude, but also are preceded by electrotonically conducted initial segment spikes (Fig. 2, C_1 and D_1) [L. G. Brock, J. S. Coombs, J. C. Eccles, *J. Physiol. (London)* **122**, 429 (1953)]. Partial spikes are also unlikely to represent activity generated in the dendrites, where motoneuron spikes occur only under conditions of abnormal hyperexcitability, for example, in the chromatolytic state after axotomy [J. C. Eccles, B. Libet, R. R. Young, *J. Physiol. (London)* **143**, 11 (1958); D. Purpura, in *The Neurosciences: A Study Program*, G. Quarton, T. Melnechuk, F. Schmitt, Eds. (Rockefeller Univ. Press, New York, 1967), p. 372]. We have also considered the possibility of damage to the neuron by microelectrode penetration which, theoretically, could result in partial spikes. This was not the case, because control spikes, initiated antidromically (Fig. 2, C_1 and D_1) or orthodromically (Fig. $2D_3$), were present during adjacent non-REM periods. Moreover, membrane potentials and the amplitude of the control spikes conformed to the most stringent standards for intracellular recording.
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An Opiate Binding Site in the Rat Brain Is Highly Selective for 4,5-Epoxymorphinans

Abstract. *In vitro binding studies have demonstrated the existence of multiple opiate receptor types. An additional site in the rat brain (termed the λ site) is distinct from the established types by its selectivity for 4,5-epoxymorphinans (such as naloxone and morphine). While the λ site displays a high affinity for naloxone in vivo and in vitro in fresh brain membrane homogenates, these sites rapidly convert in vitro to a state of low affinity. The regional distribution of the λ site in the brain is strikingly different from that of the classic opiate receptor types.*

The opiate receptor system consists of multiple types of binding sites, such as the well-characterized μ, δ, and κ sites. This receptor multiplicity may underlie the diversity of pharmacological actions of the opiates (*1*). Few opiates display specificity for any of the receptor types, which makes it difficult to evaluate the pharmacological significance of each individual type. For example, naloxone is considered to be a general opiate antagonist with affinity for most of the established receptor types. Therefore, naloxone antagonism often serves to distinguish opiate- from nonopiate-mediated actions. However, recent reports demonstrate pharmacological actions of naloxone that may be independent of opiate receptor blockade (*2*). These include attenuation of barbiturate anesthesia (*3*), alleviation of shock symptoms (*4*), analeptic actions (*5*), reversal of neurological deficit after stroke (*6*), and effects on the adrenal cortex (*7*). Moreover, some reports suggest an agonistic action of naloxone in several experimental systems (*8*). The possibility that naloxone may bind to unique sites in the rat brain was first suggested by Squires and Braestrup (*9*), who identified two binding site populations, type 1, with high affinity for naloxone, and type 2, with low affinity. It was subsequently shown that naloxone indeed has high affinity to the μ sites (type 1) and lower affinity to the δ and κ sites (*10*). However, the δ and κ sites apparently are not identical to the type 2 binding sites, since Squires and Braestrup (*9*) and Hewlett *et al.* (*11*) demonstrated that diprenorphine, which binds with equal affinity [binding affinity (K_d), $2 \times 10^{-10} M$)] to the μ, δ, and κ sites (*10*), was incapable of displacing [^{3}H]naloxone from its low-affinity binding sites.

During recent in vivo opiate receptor binding studies we reported that buprenorphine, an opiate with binding properties similar to those of diprenorphine, also failed to fully prevent [^{3}H]naloxone binding in intact rat brain (*12*). This suggested that the type 2 (*9*) sites do not represent an artifact of tissue homogenization. Furthermore, in vivo titration of the additional naloxone binding sites in the presence of a blocking dose of diprenorphine (*13*) revealed a K_d of 36 μg of naloxone per kilogram (that is, the dose that occupies 50 percent of these sites in vivo), which is only five times larger than the K_d of naloxone against μ sites in vivo (*13*). Thus, the relatively high affinity of naloxone for these diprenorphine-insensitive sites in vivo stands in contrast to the rather low in vitro affinity of type 2 sites reported in (*9*) and (*11*). The present report explains this discrepancy by showing that the additional naloxone binding site, which we now label the λ site (*14*), rapidly loses