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RESEARCH NOTE

Intracellular Recording of Lumbar Motoneuron Membrane Potential during Sleep and Wakefulness

FRANCISCO R. MORALES AND MICHAEL H. CHASE [1]

Brain Research Institute and the Departments of Physiology and Anatomy, University of California, Los Angeles, California 90024

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When mammals enter into the behavioral state of active (REM) sleep there is a dramatic reduction in tonic somatomotor activity together with an accompanying depression of monosynaptic and polysynaptic reflexes (3, 7, 10, 11). These phenomena reflect a decrease in discharge of final common pathway motoneurons. A first step in studying the cellular processes which are responsible for the depression of somatic activity during active sleep is a determination of the state-dependent changes in motoneuron membrane polarization. Direct information of this nature can be obtained by recording intracellularly from motoneurons.

We found previously that brain stem motoneurons become hyperpolarized and the amplitude of the brain stem masseteric reflex decreased when the animal (cat) shifted its state from quiet to active sleep (2, 3, 8). Subsequently, we became interested in determining the tonic membrane characteristics of spinal cord motoneurons during sleep and wakefulness. By studying the membrane characteristics of lumbar motoneurons during naturally occurring behaviors we hoped to obtain an intracellular correlate for extracellularly recorded state-dependent phenomena at this level of the neuraxis (10, 11). Another objective was to examine the membrane properties of these neurons in their natural state, rather than under the conditions in which all previous studies have been performed (e.g., tran-

Abbreviations: EEG—electroencephalographic, EOG—electroocular, EMG—electromyographic.

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section of the neuraxis, anesthetization, or immobilization with Flaxedil). We developed, therefore, a chronic cat preparation in which it was possible to record intracellularly from identified motoneurons of the lumbar spinal cord throughout naturally occurring states of wakefulness, quiet sleep, and active sleep.

Four adult cats were used. Initially, each cat was anesthetized to implant permanent electroencephalographic (EEG) and electroocular (EOG) electrodes in the standard manner for monitoring electrophysiologic activity in the chronic cat. In addition, two hollow tubes were placed within the acrylic mound covering the calvarium. The tubes were designed to receive calibrated steel bars that could be attached, a posteriori, to the stereotaxic frame (8). To fix the vertebral column in an immobile position, the surfaces of the lamina and laminar pedicles of the third through seventh lumbar vertebrae (L3–L7) were exposed. Two pairs of hard plastic (Dilrin) clamps, which had been previously sculpted to the contours of L3 and L7, were placed around the vertebrae, and steel screws were implanted in the vertebral surfaces. A small hole (diameter about 2 mm) was drilled in the caudal portion of the lamina of L5, 1.5 mm lateral to the midline. The dura was cut, and the hole was filled with bone wax. With the exception of the area surrounding the hole in L5, the clamps, screws, and exposed surfaces were covered with acrylic cement. In addition, two transversely oriented steel bars were placed in the acrylic mound for subsequent attachment to the stereotaxic frame. Chronic stimulating electrodes were placed around the sciatic nerve ipsilateral to the hole in L5.

After recovery from these surgical and implantation procedures, each cat was sleep-deprived for 36 h. It was then replaced in the stereotaxic frame by attachment of the lumbar bars and the bars which were inserted into the tubes overlying the calvarium. By these means the cat's head and lumbar vertebrae were held rigidly without pressure or pain and its movements restrained, but not abolished.

Pin electrodes were inserted into the neck and leg musculature to record electromyographic (EMG) activity. A reference electrode of Ag–AgCl wire was placed subcutaneously, lateral to the lumbar vertebrae, and surrounded by gauze soaked in 0.9% saline. The bone wax was removed from the hole overlying L5 to secure a path for a glass micropipet to reach the underlying motoneuron pool. Micropipets filled with 2 M K-citrate or 3 M KCl (tip resistances, 4 to 10 MΩ) were used to record intracellular activity. A characteristic field potential of 3 to 6 mV was observed in the vicinity of the motoneuron pool after single-pulse stimulation of the peripheral nerve (5, 9). Cellular penetration was accompanied by an abrupt shift of potential (see below); cellular identification was provided by the presence of a typical antidromic spike following excitation of the peripheral

nerve. Intracellular activity was passed through a standard high-impedance intracellular amplifier, displayed oscilloscopically, and recorded as low-gain DC and high-gain DC activity on a 10-channel polygraph and 14-channel FM tape recorder along with EEG, EOG, and EMG activity.

Recordings were obtained from the same animal for 2 to 3 consecutive days at intervals of approximately 1 week during a period of 1 to 2 months. After each recording session the animal was returned to its home cage. During the final recording session the region in the spinal cord where identified motoneurons had been penetrated was marked by passing current through a metal microelectrode. The animal was dispatched with an overdose of sodium pentobarbital, and the spinal cord underlying L5 was removed for histological analysis.

Intracellular activity was recorded from 26 identified lumbar motoneurons. All neurons were penetrated during wakefulness or quiet sleep; the mean potential upon penetration was 65 mV (SD $\pm$ 6.76), and the range was 50 to 75 mV. Antidromic spike amplitudes were from 60 to 95 mV. Neurons were not included in this study when their initial membrane potential was less than 50 mV, nor were records retained when neurons were held for less than 10 min. Recording periods were from 12 min to 2.5 h; the mean duration was 40 min. All records contained multiple transitions between wakefulness and quiet sleep. In addition, data were obtained during 10 transitions from quiet to active sleep and 10 from active sleep to wakefulness. In some motoneurons we were able to monitor the membrane potential throughout two to three consecutive cycles of active sleep.

During wakefulness, phasic movements of the ipsilateral leg were usually time-locked to periods of membrane depolarization (Fig. 1; e.g., at 27 and 34 min) and spike activity (which is not reproduced in the filtered polygraphic record). Against a background of spontaneous neuronal discharge, an increase in spike frequency was observed in conjunction with phasic leg movements. The transition from wakefulness to quiet sleep often was not accompanied by any dramatic change in the level of membrane polarization (Fig. 1). However, in many cases there was a slight, but discernable increase in the level of polarization, especially when the transition from wakefulness to quiet sleep was accompanied by a reduction in muscle tone (Fig. 1). Consequently, in accord with extracellular reflex studies which have indicated little or no change in induced somatomotor activity when wakefulness is compared with quiet sleep (2, 10, 11), the level of motoneuron membrane polarization did not exhibit dramatic fluctuations between these two states. Thus, our intracellular records provide a perfect reflection of extracellularly monitored patterns of somatomotor activity during wakefulness and quiet sleep.

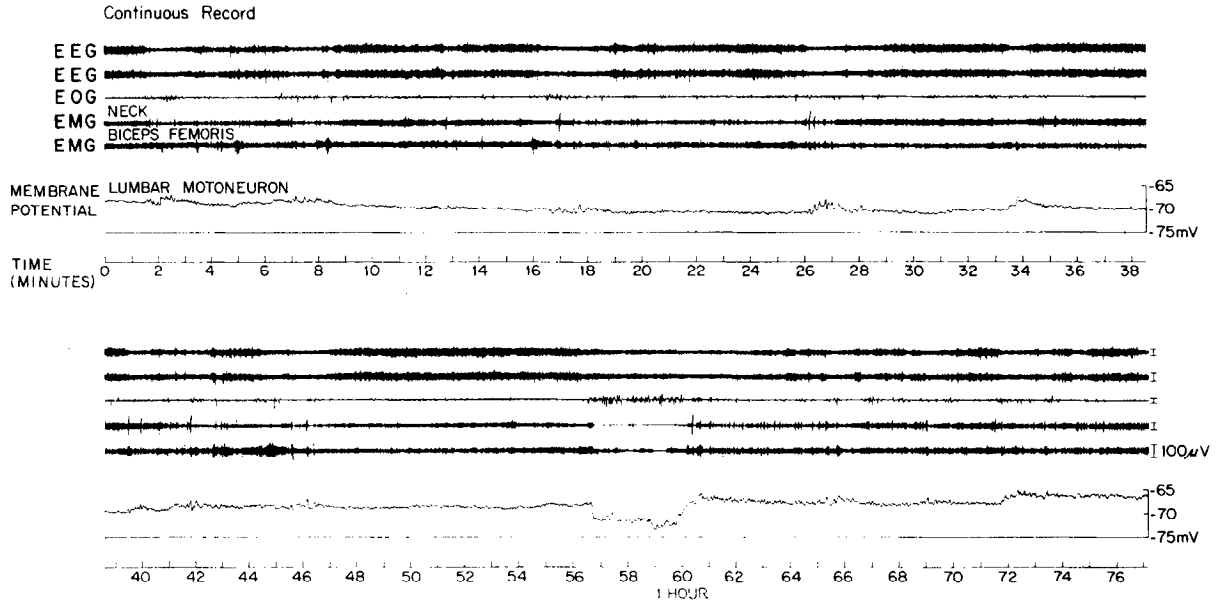

FIG. 1. Intracellular record from a lumbar montoneuron during sleep and wakefulness: correlation of membrane potential and behavioral state. This neuron was recorded from for 1 h and 17 min; during this period the animal exhibited numerous cycles of wakefulness and quiet sleep. There were no striking changes in the mean level of polarization between these two states except when phasic periods of membrane depolarization with accompanying spike generation occurred during wakefulness (spike activity is not evident because the DC record, for display purposes, was passed through a 0.1 cps high-frequency polygraphic filter). After recording 56 min the animal entered into a brief episode of active sleep which was accompanied by a corresponding period of membrane hyperpolarization. The intracellular record was obtained with a K-citrate-filled micropipet. The first and second polygraph traces are those of EEG activity recorded from left and right frontal–parietal cortex, respectively.

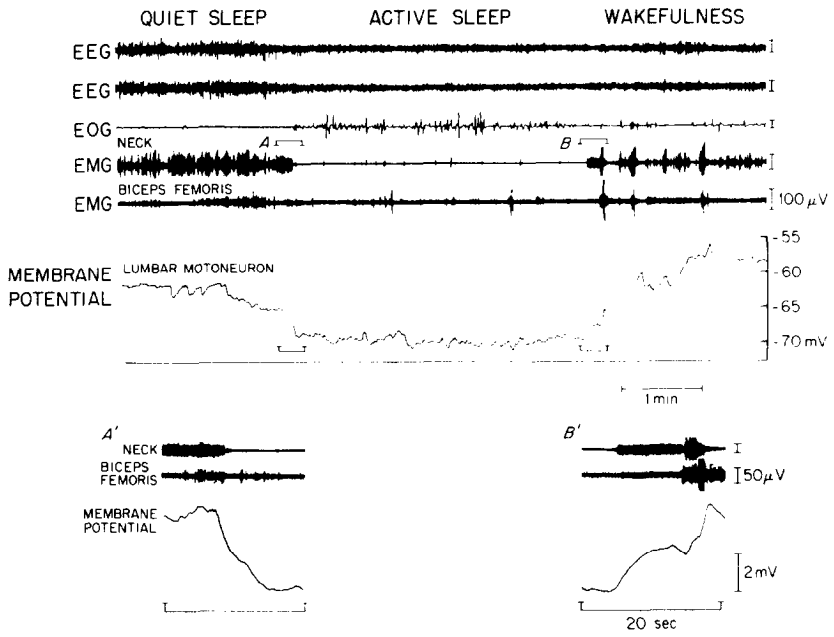

FIG. 2. Intracellular record from a lumbar motoneuron during sleep and wakefulness: correlation of membrane potential and behavioral state. This figure highlights membrane hyperpolarization which accompanies active sleep. Hyperpolarization commenced prior to the cessation of muscle tone, which was accompanied by a further and rather sharp increase in membrane polarization (*A*, and shown oscilloscopically at higher gain and expanded time-base in *A'*). At the termination of active sleep, the membrane depolarized coincident with the resumption of muscle tone and behavioral awakening (*B*, *B'*). Note the brief periods of depolarization during active sleep and wakefulness, which were accompanied by phasic increases in muscle activity (i.e., muscular twitches during active sleep and leg movements during wakefulness). Spike potentials often occurred during these periods of depolarization but are not evident in this figure because the DC record was passed through a 0.1-cps high-frequency polygraphic filter. This motoneuron was recorded for 28 min; the traces shown were obtained 12 min after the cell was impaled. The first and second polygraph traces are those of EEG activity recorded from left and right frontal–parietal cortex, respectively.

When the animal passed from quiet to active sleep there occurred, invariably, strong hyperpolarization of the motoneuron membrane (Figs. 1, 2). The average magnitude of this effect was 6.7 mV with a range of 4 to 10 mV. When the animal awoke from active sleep the level of polarization always decreased (Figs. 1, 2).

Motoneuron hyperpolarization has been hypothesized to be the basis for the depression of tonic somatic activity during active sleep. This concept emanated from an elegant series of extracellular stimulation and reflex

studies carried out by Pompeiano and co-workers (10, 11). However, it remained for information provided by intracellularly monitoring the level of membrane polarization, as described in this report, to demonstrate that, in fact, spinal cord motoneurons do become hyperpolarized during active sleep compared to wakefulness or quiet sleep. We can now report that at brain stem (8), as well as at spinal cord levels of the neuraxis, powerful factors operate during active sleep which result in robust hyperpolarization of final common pathway motoneurons.

The two basic mechanisms, individually or acting in concert, which may be responsible for motoneuron hyperpolarization are postsynaptic inhibition and disfacilitation of tonically active presynaptic elements. We are currently studying the extent to which postsynaptic factors participate in this process and preliminary data indicate that the hyperpolarization of active sleep is accompanied by a decrease in membrane resistance, a decrease in spike generation induced by the intracellular injection of depolarizing current, and the blockade of invasion into the soma of antidromically propagated spike potentials. These data, taken together, argue forcibly for the advent of postsynaptic inhibition during active sleep, which would contribute to the hyperpolarization of the motoneuron membrane that we report.

Because the level of membrane polarization of motoneurons in the brain stem (8) and spinal cord can be maintained tonically at values separated by as much as 10 mV during active sleep compared with other states, the tenet of a basic "resting" value of membrane potential may not be applicable in the chronic preparation. It is probable, in addition, that other classes of neurons will exhibit distinctly different levels of tonic state-dependent membrane potential. Accordingly, the resting level of polarization perhaps should be designated on the basis of the mean value attained for specific categories of neurons during specified behavioral states.

The wealth of electrophysiological descriptions of mammalian neuronal mechanisms and synaptic processes rests upon a foundation of data gathered from spinal cord motoneurons in acute preparations [reviewed in (1, 4, 6)]; these data may now be reexamined to determine the extent to which the cellular characteristics previously reported reflect the neuronal processes present during naturally occurring, physiologically normal states. For the present, we hope that this demonstration of intracellular recording from lumbar motoneurons will form the basis for future explorations of the cellular and synaptic state-dependent processes operating at the level of the spinal cord, not only during sleep and wakefulness, but during other behaviors as well.

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