Natural Waking and Sleep States: A View From Inside Neocortical Neurons

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Received 15 November 2000; accepted in final form 22 January 2001

Steriade, M., I. Timofeev, and F. Grenier. Natural waking and sleep states: a view from inside neocortical neurons. J Neurophysiol 85: 1969–1985, 2001. In this first intracellular study of neocortical activities during waking and sleep states, we hypothesized that synaptic activities during natural states of vigilance have a decisive impact on the observed electrophysiological properties of neurons that were previously studied under anesthesia or in brain slices. We investigated the incidence of different firing patterns in neocortical neurons of awake cats, the relation between membrane potential fluctuations and firing rates, and the input resistance during all states of vigilance. In awake animals, the neurons displaying fast-spiking firing patterns were more numerous, whereas the incidence of neurons with intrinsically bursting patterns was much lower than in our previous experiments conducted on the intact-cortex or isolated cortical slabs of anesthetized cats. Although cortical neurons displayed prolonged hyperpolarizing phases during slow-wave sleep, the firing rates during the depolarizing phases of the slow sleep oscillation was as high during these epochs as during waking and rapid-eye-movement sleep. Maximum firing rates, exceeding those of regular-spiking neurons, were reached by conventional fast-spiking neurons during both waking and sleep states, and by fast-rhythmic-bursting neurons during waking. The input resistance was more stable and it increased during quiet wakefulness, compared with sleep states. As waking is associated with high synaptic activity, we explain this result by a higher release of activating neuromodulators, which produce an increase in the input resistance of cortical neurons. In view of the high firing rates in the functionally disconnected state of slow-wave sleep, we suggest that neocortical neurons are engaged in processing internally generated signals.

INTRODUCTION

Previous studies conducted in brain slices and in animals under deep anesthesia have described the electrophysiological properties of neocortical neurons (Connors and Gutnick 1990; McCormick et al. 1985; Nuñez et al. 1993), their multiple ionic conductances (Crill 1996; Schwindt et al. 1988a,b, 1989), and their propensity to generate and synchronize a slow oscillation at 0.5–1 Hz (Steriade et al. 1993d,e). It was also shown that some firing patterns may be altered by setting into action generalized modulatory systems in anesthetized animals (Steriade et al. 1993a) or applying activating neurotransmitters in cortical slices (Wang and McCormick 1993). Similarly, firing patterns elicited by intracellular depolarizing current pulses could be transformed into different ones by synaptic activity in acutely prepared animals (Steriade et al. 1998a).

In view of these results, we started the present intracellular study in chronically implanted, naturally sleeping, and aroused animals with the hypothesis that synaptic activities during natural states of vigilance may have a decisive impact on the electrophysiological properties of neurons. We wanted to compare the firing patterns of various neuronal types and their incidence during natural wakefulness to those previously described in acute experiments. We also assumed that the condition of a chronically implanted animal would allow us to compare the intracellular characteristics of the slow oscillation during natural sleep to those previously recorded only under anesthesia (Contreras and Steriade 1995; Contreras et al. 1996; Steriade et al. 1993d,e). Finally, we hypothesized that, despite the increased synaptic activity during the alert state, the actions of some neuromodulators released by generalized activating systems may change the expected result of an increased membrane conductance.

The state of sleep with slow waves of brain electrical activity (SWS) was once thought to be associated with a global cortical inhibition that radiates to subcortical structures (Pavlov 1923). This would relegate the brain to complete inactivity and loss of mental processes during this sleep stage. With the advent of extracellular unit recordings in behaving animals (Jasper et al. 1960), it was shown that long-axonated neurons in the motor cortex of monkeys, which were antidromically activated from the thalamus, brain stem, and spinal cord, did not cease firing during SWS; however, their discharge patterns were different from those displayed by the same neurons in the two states of vigilance associated with an alert brain, waking and rapid-eye-movement (REM) sleep (Evarts 1964; Steriade et al. 1974). The rates and patterns of extracellularly recorded neocortical neurons have also been investigated in visual and association areas of chronically implanted cats (Hobson and McCarley 1971; Noda and Adey 1970; Steriade 1978). Until now, the mechanisms underlying the prolonged periods of neuronal silence during natural SWS have not been elucidated. To uncover the neuronal mechanisms underlying the physiological correlates of waking and sleep states requires intracellular recordings from identified neurons. In previous studies on waking and sleep states, spinal and brain stem motoneurons (Chase and Morales 1983; Chase et al. 1980; Glenn and Dement 1981), brain stem reticular neurons (Ito and McCarley 1984), and thalamic relay neurons (Hirsch et al. 1983) have

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been recorded intracellularly. Although intracellular recordings have also been used in neocortex for conditioning studies (Woody et al. 1978) and investigations on fast oscillations (Murthy and Fetz 1992) in alert animals, this method has not yet been used in the neocortex throughout the long periods of the natural waking-sleep cycle.

In the present study, we were interested in 1) the firing patterns of different cell types and their incidence in alert animals, as compared with our previous studies on similar cell types recorded under anesthesia; 2) the relation between fluctuations in membrane potential ($V_m$) and firing rates during wakefulness and sleep states; and 3) the state-dependent variations in the input resistance ($R_{in}$), a measure resulting from passive electrical neuronal properties and balanced changes in excitatory and inhibitory inputs from afferent (specific and generalized modulatory) pathways. Preliminary data have been published in abstract form (Steriade et al. 1999, 2000).

**METHODS**

**Preparation, recording, and stimulation**

Experiments were conducted on four adult cats. Surgical procedures for chronic implantation of recording and stimulating electrodes were carried out under deep barbiturate anesthesia (Somnotol, 35 mg/kg, ip), followed by two or three administrations, every 12 h, of buprenorphine (0.03 mg/kg, im) to prevent pain. Penicillin (500,000 units im) was also injected during 3 consecutive days.

The cats were implanted with one to three chambers allowing the intracellular penetrations of micropipettes [filled with 2.5–3 M potassium acetate (KAc) or 1.5–3 M potassium chloride (KCl), dc resistances 25 to 50 MΩ], field potentials recordings using coaxial macroelectrodes (with the tip in the cortical depth at about 0.8–1 mm and the ring placed at the cortical surface), and insertion of coaxial stimulating electrodes into different cortical areas and related thalamic nuclei for the antidromic and orthodromic identification of the input-output organization of recorded neurons. The antidromic identification of a callosal neuron, activated by stimulating the homotopic point in the contralateral cortical area, is shown in Fig. 12. The state-dependent changes in cellular responsiveness to antidromic and orthodromic volleys will be reported elsewhere. In different animals, the chambers were inserted over the pericruciate (motor) and anterior suprasylvian (association) gyri, or coronal (primary somatosensory) and posterior suprasylvian (visual association) areas. In addition, we recorded the electroencephalogram (EEG) from the vicinity of intracellular recordings as well as from distant cortical areas (to determine whether or not long-range synchronization between neuronal activity and EEG is present in natural SWS), the electro-oculogram (EOG) from pairs of electrodes placed in ocular cavities, and the electromyogram (EMG) from neck muscles.

The method used to keep the head rigid without pain or pressure during the recording sessions was similar to that described previously (Steriade and Glenn 1982). After surgery and 4–5 days of training to sleep in the stereotaxic apparatus, cats started to display normal sleep-waking cycles and, at that time, intracellular recordings began after small perforations in the dura were carefully made. The chamber was filled with warm sterile solution of 4% agar. As a rule, two to three recording sessions, each lasting for 1–3 h, were performed daily, and 7–10 days of recordings could be made in each chamber. The cats were not deprived of sleep between recording sessions. During recordings, the animals could move their limbs and they often made postural adjustments (see Fig. 1). The criteria for differentiating the three major states of vigilance (waking, SWS, and REM sleep) by EEG, EOG, and EMG are found elsewhere (Steriade and McCarley 1990; see also Figs. 4–5). The experimental protocol was approved by...
the committee for animal care in our university and also conforms to the policy of the American Physiological Society.

At the end of the experiments, the cats were given a lethal dose of pentobarbital.

RESULTS

Database and proportions of different cell classes

Stable recordings, lasting for at least 15 min, but up to 90 min, were obtained from 750 neurons recorded from primary somatosensory, motor, and association (visual and somatosensory) cortical areas. The stability of intracellular recordings was achieved even during periods of active waking, associated with numerous eye movements and phasic increases in muscular tone due to postural adjustments (Fig. 1). The intracellular activity could be investigated during the whole sleep-waking cycle in 34 neurons, while 320 neurons were studied in two behavioral states of vigilance with opposite features: SWS and REM sleep or SWS and waking.

FIG. 2. Electrophysiological identification of different cell classes. Left: responses of regular-spiking (RS), fast-rhythmic-bursting (FRB), fast-spiking (FS), and intrinsically-bursting (IB) neurons from area 4 to depolarizing current pulses (0.2 s, 0.8 nA). At right of each depolarizing current pulse, action potentials of each cell classes; note thin spikes of FRB and FS neurons, compared with those of RS and IB neurons. Right: width of action potentials (at half-amplitude) in a sample of 117 neurons (48 RS, 37 FRB, 24 FS, and 8 IB, corresponding to patterns depicted at left). See details in text.
Slow-wave sleep

Depth - EEG area 3

Depth - EEG area 7

-60 mV Intra-cell area 7

EMG

REM sleep

-57 mV

0.5 s

20 mV

Spontaneous

n = 234
firing rate = 11.7 Hz
int. between 20 to 100 = 96

30
20
10
0

0 5 10 15

interval duration (ms)

counts

Spontaneous

n = 298
firing rate = 14.9 Hz
int. between 20 to 100 ms = 187

50 ms
To determine the proportions of different discharge patterns of various neuronal classes in alert animals, we used a sample of 120 neurons that were selected because depolarizing current pulses could be applied during the steady state of quiet waking, without phasic motor events. In keeping with the results from previous in vitro studies (Connors and Gutnick 1990; Kawaguchi and Kubota 1997; McCormick et al. 1985; Thomson and Deuchars 1997) and in vivo experiments on acutely prepared animals (Gray and McCormick 1996; Nuñez et al. 1993; Steriade et al. 1998a), neurons were classified into four categories according to their responses elicited by intracellular depolarizing current pulses: regular-spiking (RS), intrinsically bursting (IB), fast-spiking (FS), and fast-rhythmic-bursting (FRB) (Fig. 2). The firing pattern was very similar to that previously described in vitro and in vivo under anesthesia. RS and IB firing patterns are typical of pyramidal cells recorded in previous studies and are therefore here taken to be indicative of excitatory cortical neurons. Conventional FS patterns are usually observed in inhibitory interneurons and cells with this behavior are generally assumed to be inhibitory. It was, however, reported that while some interneurons discharge like conventional FS cells, other local inhibitory interneurons fire like RS or bursting cells (Thomson et al. 1996). On the other hand, FRB neurons display fast (300–600 Hz), rhythmic (20–50 Hz) spike-bursts at given levels of depolarization, but below that level they exhibit RS patterns and above it they discharge like FS neurons (Steriade et al. 1998a). While some FRB neurons are pyramids located in layers II/III (Gray and McCormick 1996), other FRB neurons are deeply lying corticothalamic cells, as shown by their antidromic activation from the thalamus, and still other FRB neurons were intracellulary stained and found to be local-circuit, sparsely spiny, or aspiny neurons (Steriade et al. 1998a).

Out of 120 neurons tested with depolarizing current pulses during the steady waking state, we found RS patterns in 61 neurons (51%), FRB patterns in 25 neurons (21%), FS patterns in 29 neurons (24%), and IB patterns in 5 neurons (4%). Thus although the firing pattern of various cell classes was very similar to that previously described in slices maintained in vitro and in acutely prepared (anesthetized) animals, the proportions of FS and IB firing patterns were different from those previously found in anesthetized animals with intact cortex or small isolated slabs (Timofeev et al. 2000) and in cortical slices maintained in vitro (see Discussion).

We determined the duration of action potentials at half-amplitude in samples from all cortical cell types, namely 48 RS neurons, 37 FRB neurons, 24 FS neurons, and 8 IB neurons, tested in different (waking and sleep) states. Note more numerous neurons with IB patterns when also recorded during the sleep state \( n = 8 \), compared with the number found during waking \( n = 4 \); see also Fig. 3). Figure 2 shows the patterns of discharge elicited by depolarizing current pulses in each of these neuronal classes and the duration of their action potentials at half-amplitude. RS neurons showed a major mode between 0.6 and 0.75 ms, with a minor mode at 0.85–0.95 ms. By contrast, both FRB and FS neurons demonstrated much shorter action potentials, with a mode at \( \sim 0.3 \) ms. The very short action potentials of FRB neurons, similar to those of conventional FS (presumably GABAergic) neurons, were also observed in previous experiments on acutely prepared animals in which antidromically identified corticothalamic FRB cells (therefore glutamatergic and excitatory) displayed very short action potentials, like inhibitory FS neurons (Steriade et al. 1998a). The small proportion of neurons that displayed IB firing patterns during the alert state (4%) precludes accurate assessment of spike duration in this group.

With the exception of one IB cell, in which firing patterns were similar during all states of vigilance, in other IB neurons \( n = 4 \), which were analyzed during two states of vigilance with opposing characteristics (SWS and waking, or SWS and REM sleep), the bursting features on depolarizing current pulses or occurring spontaneously during SWS changed into an RS firing pattern during either waking or REM sleep. An example of such changes is illustrated in Fig. 3 showing 1) bursting patterns to depolarizing current pulses during SWS and single spiking in REM sleep, and 2) similar differences in the spontaneous firing of this neuron during these two states, with a mode of interspike intervals at 3–3.5 ms in SWS (lacking in REM sleep) and many more longer intervals (20–100 ms) during REM sleep (reflecting the single spike firing in the latter state).

**Relations between membrane potential and firing rates in different cell types**

The changes in \( V_m \) and firing patterns of an RS neuron and an FS neuron throughout the sleep-wake cycle are illustrated in Fig. 4. In the case of the RS neuron (Fig. 4A), SWS lasted for almost 20 min. During this state, neuronal activity was characterized by prolonged, cyclic hyperpolarizations that were associated with depth-positive field EEG potentials, whereas the neuron discharged tonically in both waking and REM sleep (see the expanded periods, from the three behavioral states of vigilance, below the upper panel). The FS neuron, recorded with a KCl-filled pipette (Fig. 4B), exhibited similar properties, namely tonic discharges during wakefulness, cyclic and prolonged hyperpolarizations during SWS, and again tonic but irregular firing during REM sleep.

The pooled firing rates in spontaneously discharging neurons, belonging to all four neuronal types, were \( 15.7 \pm 1.9 \) Hz (mean \( \pm \) SE) during waking, \( 11.4 \pm 1.2 \) Hz in SWS, and \( 17.9 \pm 3.4 \) Hz in REM sleep. We found no significant statistical difference between these firing rates in the three behavioral states (paired \( t \)-test 0.2 for SWS-REM sleep; 0.9 for SWS-waking; and 0.1 for REM-waking). However, when we calculated the firing rates for different cell types in a sample of 120 neurons, the state-dependent firing rates showed great differences among various neuronal types. An example of the relation between the membrane potential and firing rate is depicted in Fig. 5, during transition from SWS...
to REM sleep, for an RS neuron with a high discharge frequency during SWS.

The pooled analysis of relations between the mean membrane potential and mean firing rates showed that, at membrane potentials between $-55$ and $-65$ mV, during the states of waking and SWS, FS neurons discharged at much higher rates than RS neurons (Fig. 6, top). The firing rates of RS, FRB, and FS during all three major states of vigilance (waking, SWS, and REM sleep) are shown at the bottom of Fig. 6. These data also show that neurons with conventional FS firing patterns had a propensity for higher rates, compared with RS and FRB neurons, during all states of vigilance. Thus during the state of waking, neurons with FS and RS discharge patterns fired at $23.7 \pm 6.1$ and $9.4 \pm 1.7$ Hz, respectively; at $14.9 \pm 4.1$ and
FRB neurons discharged at 15.0 ± 2.5, 7.5 ± 1.9, and 5.4 ± 2.4 Hz in waking, SWS, and REM sleep, respectively; thus they fired at higher rates, compared with RS neurons, during the waking state. The increased discharge frequencies of FRB neurons during wakefulness is at least partially ascribable to the fact that they fired spontaneously with high-frequency (20–50 Hz) spike doublets and triplets during this behavioral state, similar to their responses elicited by depolarizing current pulses (Fig. 6). We do not provide the
mean discharge rates for IB neurons from that sample because of their small number during wakefulness and variations among different neurons.

SWS-related cyclic hyperpolarizations are obliterated in waking and REM sleep

Recordings of all electrophysiologically identified cortical cell types across the whole sleep-waking cycle demonstrated that the SWS state was distinguished from both waking and REM sleep by the presence of cyclic, long-lasting (0.3–0.5 s), high-amplitude (8–20 mV) hyperpolarizations during which neurons stopped firing. The mean SD of membrane potential during SWS was higher than in wakefulness (Fig. 7). However, the lowest values of SD were reached during SWS-related hyperpolarizing potentials. The increase in SD during SWS was associated with an increase in baseline fluctuations of membrane potential, thus suggesting the presence of high synaptic activity during brief periods of SWS.

The presence of prolonged hyperpolarizations in SWS was seen in all recorded neurons (Figs. 4–5 and 8–9), that is, not only RS neurons but also conventional FS (presumably GABA-ergic) neurons (Fig. 4B). The fact that none of the FS inhibitory neurons discharged during SWS hyperpolarizations suggests that these prolonged events are not mediated by GABAergic inhibition. This idea is consistent with the persistence of SWS hyperpolarizations in recordings with KCl-filled pipettes (see Fig. 4B) and the measures of input resistance during different epochs of natural sleep and waking (see Fig. 11).

The transition from SWS to either REM sleep, indicated by muscular atonia and EEG activation (Fig. 8), or wakefulness, indicated by EEG activation and increased muscular tone (Fig. 9), was invariably associated with the abolition of long-lasting hyperpolarizing potentials. This change was reflected in the disappearance of the hyperpolarizing tail (up to −80 or −85 mV) in the bimodal histogram of the $V_m$ during SWS and the appearance of a Gaussian-type histogram (Figs. 8–9). Overall, the mean membrane potential was $-62.1 \pm 0.5$ mV during the depolarizing component of the slow oscillation in SWS, $-71.7 \pm 0.7$ mV during the hyperpolarizing component of the slow oscillation in SWS, $-60.8 \pm 0.7$ mV in REM sleep, and $-62.5 \pm 0.6$ mV in wakefulness.
The obliteration of prolonged hyperpolarizing epochs with transition from SWS to brain-activated behavioral states was accompanied by more regular discharge rates, without brisk firing interrupted by silent periods, as shown by the sequential histogram of discharge frequencies (see Fig. 9, in which many 0.1-s bins display higher firing rates in SWS, compared with waking). The transition from SWS to waking initially occurred without visible changes in the membrane potential, which could depolarize by a few millivolts only a few seconds later (Fig. 9).

We investigated the evolution of a change in membrane potential with respect to the time 0 defining the onset of brain-activated states during transitions from SWS to either waking or REM sleep. Figure 10 illustrates the time 0 of EEG activation with a transition from SWS to REM sleep (top) and the evolution of $V_m$ (0.5-s bins in left plots, 5-s bins in right plots) in eight neurons, four of them analyzed during transition from SWS to REM sleep, and the other four during transition from SWS to wakefulness. The neurons showed a much higher dispersion of the membrane potential during SWS, compared with either REM sleep or waking, because of the succession of hyperpolarizing and depolarizing phases of the slow sleep oscillation. Obliteration of hyperpolarizing phases occurred at the very onset of brain-activated states (time 0) but in at least half of these cases (neurons $a$ and $b$ in transition to REM sleep, and neurons $b$ and $d$ in transition to waking), the overt depolarization followed time 0 by about 5–10 s (see also the neuron illustrated in Fig. 9).

Conversely, the first cellular sign in the transition from waking to SWS was the appearance of prolonged hyperpolarizations in all types of neocortical neurons. This was associated with depth-positive focal EEG waves, characteristic of the slow sleep oscillation, while the successive depolarizing phase was associated with a depth-negativity in EEG activity, on which thalamically generated spindles were superimposed. Similar to anesthetized preparations (Contreras and Steriade 1995; Steriade et al. 1993e; Timofeev and Steriade 1996), the synchronous discharges of neocortical neurons during the depolarizing phase of the slow oscillation are effective in triggering thalamic neurons to produce spindle waves (see Fig. 4B).

The input resistance of neocortical neurons is stable and higher during quiet waking than in other, phasically or tonically, depolarized states.

Although SWS was typically characterized by cyclic and prolonged hyperpolarizations accompanied by arrest in firing, the pooled discharge rates of RS neurons show only slight differences between waking and SWS (see Fig. 6). This was due to the fact that, during the depolarizing phase of the SWS slow oscillation, neocortical neurons discharged at rates...
equal to or even exceeding those found in the two brain-active states, waking and REM sleep (see Figs. 4–9).

We tested the apparent input resistance ($R_{in}$) of cortical neurons during all states of vigilance for two reasons. First, we wanted to compare the membrane conductance during the cyclic depolarizing components of the slow oscillation in SWS with that during the tonic depolarization in waking and REM sleep. Although the latter brain-active states are associated with an increased activity in afferent (thalamic and some generalized) systems and, thus, it would be expected that the $R_{in}$ in cortical neurons is lower than during the disconnected state of SWS, the release of some activating neuromodulators during wakefulness may change the situation (see DISCUSSION). Second, whereas the anesthetic state is relatively uniform, natural states of vigilance are much more diverse, with qualitatively different epochs even within the same state of vigilance. This is the case of the hyperpolarizing and depolarizing phases of the slow oscillation in SWS, or of the epochs without or with ocular saccades in REM sleep.

We measured the $R_{in}$ by applying intracellularly short (100

**FIG. 8.** Cyclic hyperpolarizations characterize neocortical neurons during SWS (S) and they are blocked during REM sleep. Intracellular recording of area 5 RS neuron, together with EEG from areas 3 and 7, and EMG. Periods marked by horizontal bars and arrows are expanded below. The bottom plots show the membrane potential during SWS (with a tail extending up to $-85$ mV) and a Gaussian-type histogram during REM sleep, around $-60$ mV. Note also slight depolarization on entering REM sleep, associated with EEG activation and muscular atonia.
Hyperpolarizing current pulses throughout the sleep-waking cycle, in 24 neurons. This provided consistent results, which are exemplified for one RS neuron in Fig. 11. 1) In the whole cellular sample, the $R_{in}$ was almost double during the hyperpolarizing phase of the slow sleep oscillation in non-REM (SWS) sleep ($30.8 \pm 4.3 \, \text{M} \Omega$) compared with the depolarizing phase of this oscillation that corresponds to the EEG depth-negativity ($16.8 \pm 2.3 \, \text{M} \Omega$). This further suggests that the prolonged hyperpolarization is not mediated by GABAergic events as the latter are associated with increased membrane conductance. 2) $R_{in}$ was higher ($26.4 \pm 2.1 \, \text{M} \Omega$) during tonically activated epochs, without ocular saccades, in REM sleep, compared with periods with ocular saccades ($15.8 \pm 2.4 \, \text{M} \Omega$). This indicates that an increased membrane conductance occurs during saccades and, indeed, as we reported elsewhere (Timofeev et al. 2001), FS interneurons impose GABAergic inhibitory potentials onto pyramidal neurons during ocular saccades. 3) In contrast to the two sleep states, the $R_{in}$ was

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**FIG. 9.** Transition from SWS to wakefulness is accompanied by obliteration of prolonged hyperpolarizations. The firing rate during the depolarizing phases of the slow sleep oscillation is as high as during waking. Five traces depict (top to bottom): depth-EEG from right area 7 and left areas 3 and 7; intracellular activity of RS neuron from left area 21; and EMG. Below: sequential histogram of firing rate (ordinate in Hz) during the whole period depicted in the top (0.1-s bins) reveals brief periods of high-frequency firing during SWS, interspersed with silent periods corresponding to the hyperpolarizing phases of the slow oscillation. Two epochs marked by horizontal bars (A and B) are expanded below (without EMG). Note phasic hyperpolarizations in area 21 neuron, related to depth-positive EEG field potentials, during SWS, tonic firing on awakening marked by EEG activation and increased muscular tone, and slight depolarization occurring only a few seconds after awakening and blockage of hyperpolarizations.
Sequential alterations in the $V_m$ during transition from SWS to REM sleep and wakefulness. Top: transition from SWS to REM sleep to indicate the time 0 taken for brain activation (arrow), as indicated by EEG changes. Three traces represent depth-EEG from area 21, intracellular recording of area 7 RS neuron, and EMG. The panel below shows the evolution of $V_m$ in 4 neurons (a to d) during transition from SWS to REM sleep. In all cases, each point in the left plots represents the peak in a histogram of membrane potential distribution for 0.5-s bins, while right plots show the same epoch in 5-s bins (median and SE). Ordinate represents mV and abscissa represents time (in s) before and after time 0. The same is shown below for the 4 neurons during transition from SWS to waking.
remarkably stable during the steady state of waking and it reached higher values (31.3 ± 2.4 MΩ) than in REM sleep or the depolarizing phase in SWS.

We also compared, in the same neuron and during all three states of vigilance, the neuronal excitability estimated by the number of action potentials elicited by depolarizing current pulses, every 0.5 s. The plot shows the dynamic changes of $R_{in}$ during the three states of vigilance, obtained from continuous recording throughout the sleep-waking cycle. Dots represent individual measurements of $R_{in}$; thick line and SD bars are the means of $R_{in}$ from every 10 consecutive measurements; thin line is the coefficient of variation from corresponding periods; circles indicate ocular saccades in REM sleep. Note that, during quiet wakefulness, $R_{in}$ increased and this increase was associated with a decrease in the coefficient of variation.

FIG. 11. Apparent input resistance of neocortical neurons during natural states of vigilance. Top: three periods of intracellular recording from the same RS neuron during SWS, REM sleep, and waking. $R_{in}$ was measured by applying 0.1-s hyperpolarizing current pulses, every 0.5 s. Bottom: averages of responses of this neuron during different epochs in the three states of vigilance (note differences between the hyperpolarizing and depolarizing phases of the slow oscillation in SWS and between epochs with and without ocular saccades in REM). The plot at bottom shows the dynamic changes of $R_{in}$ during the three states of vigilance, obtained from continuous recording throughout the sleep-waking cycle. Dots represent individual measurements of $R_{in}$; thick line and SD bars are the means of $R_{in}$ from every 10 consecutive measurements; thin line is the coefficient of variation from corresponding periods; circles indicate ocular saccades in REM sleep. Note that, during quiet wakefulness, $R_{in}$ increased and this increase was associated with a decrease in the coefficient of variation.
pulses, the $R_{in}$ measured by short hyperpolarizing current pulses, and the area of hyperpolarization associated with a period of spike suppression produced by a synaptic volley. As shown in Fig. 12, the number of spikes elicited by depolarizing current pulses did not change significantly as a function of the state of vigilance, but the $R_{in}$ was much higher during wakefulness than during depolarization of the slow sleep oscillation in slow-wave sleep. The area of hyperpolarization that followed a stimulus applied to the homotopic point in the contralateral cortical area was more stable during waking than during both sleep stages.

**DISCUSSION**

We found that 1) the proportions of FS and IB firing patterns, identified during natural wakefulness, are different from those previously found under anesthesia and in cortical slices or isolated cortical slabs in vivo; 2) compared with RS neurons, higher firing rates were reached by FS neurons during all natural waking and sleep states, and by FRB neurons during wakefulness; 3) despite the prolonged hyperpolarizations displayed by all neuronal types during SWS, their discharge frequencies during the depolarizing phase of the slow sleep oscillation were as high as, or even exceeded, those during the brain-active states of waking and REM sleep; and 4) the apparent $R_{in}$ was increased and more stable during quiet waking than during both sleep stages.

**Firing patterns and discharge rates in different cell-types during behavioral states**

We compared the proportions of different firing patterns recorded during waking in the present experiments to those found in our previous experiments on anesthetized cats, i.e.,
more than 1,000 intracellularly recorded neurons recorded from intact cortex (Contreras and Steriade 1995; Nuñez et al. 1993; Steriade et al. 1993a,d, 1998a) and 160 intracellularly recorded neurons from small isolated cortical slabs (Timofeev et al. 2000). Neurons displaying the firing patterns of conventional FS (presumably local GABAergic) neurons, defined by thin spikes and high rates of tonic discharges without frequency adaptation, were much more numerous in the present experiments on naturally alert animals (24%) than in previous experiments on the intact cortex of anesthetized animals (12%) or in small isolated cortical slabs in vivo (4%). On the contrary, neurons displaying IB firing patterns were presently found in only 4% of neurons of awake animals, whereas they represent 15% of neurons in anesthetized animals and reach 40% of neurons in isolated cortical slabs. The difference between the proportions of these firing patterns in any pair of our experimental conditions (namely, awake versus anesthetized animals; awake animals versus isolated cortical slabs; and anesthetized animals with intact cortex versus isolated cortical slabs) were highly significant ($P < 0.0001$, $\chi^2$ test).

These data showing quite different proportions of firing patterns in various cortical cell classes in different experimental conditions indicate that the intrinsic properties underlying firing patterns are modulated by the increased synaptic activities during the waking state. The results also suggest that one firing type may be transformed into another during natural shifts in the state of vigilance associated with changes in membrane polarization. Indeed, work in vivo showed that the same neuron may pass from the RS pattern to an FRB pattern, eventually reaching an FS pattern, by slightly increasing the frequency adaptation, were much more numerous in the present experiments on naturally alert animals (24%) than in previous experiments on the intact cortex of anesthetized animals (12%) or in small isolated cortical slabs in vivo (4%). On the contrary, neurons displaying IB firing patterns were presently found in only 4% of neurons of awake animals, whereas they represent 15% of neurons in anesthetized animals and reach 40% of neurons in isolated cortical slabs. The difference between the proportions of these firing patterns in any pair of our experimental conditions (namely, awake versus anesthetized animals; awake animals versus isolated cortical slabs; and anesthetized animals with intact cortex versus isolated cortical slabs) were highly significant ($P < 0.0001$, $\chi^2$ test).

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It is then tempting to predict that the firing patterns of RS neurons could develop into those of FRB neurons during activated states. Work in vitro has indeed shown that repeated direct depolarization of RS cortical neurons may eventually lead to FRB firing patterns (Kang and Kayano 1994). In view of its high-frequency spike-bursts repeated rhythmically at 30–40 Hz (Gray and McCormick 1996; Steriade et al. 1996a, 1998a), the FRB cell type may have a great impact on cortical and thalamic structures in the generation of fast oscillations which are characteristic for brain-activated states (Bouyer et al. 1981; Llinás and Ribary 1993; Murthy and Fetz 1992; Steriade et al. 1996a,b).

The FS (presumably inhibitory) neurons have been implicated in the generation of fast (20–40 Hz) rhythms (Buzsáki and Chrobak 1995; Llinás et al. 1991; Lytton and Sejnowski 1991; Traub et al. 1999), which characterize the spontaneous activity in the waking state and during high alertness. These states of network activity, accompanied by depolarized levels of membrane potential, may transform neurons with other firing patterns (i.e., FRB) into FS-type neurons (Steriade et al. 1998a). This would result in an increased proportion of neurons identified as FS. On the other hand, the strikingly diminished proportion of IB firing patterns in the alert condition is likely due to the relatively depolarized membrane potential, enhanced synaptic activity, and increased release of some modulatory neurotransmitters, all conditions that may transform IB into RS firing patterns (Steriade et al. 1993a; Wang and McCormick 1993). This suggests that a high degree of synaptic activity in the intact brain, which is lacking in brain slices, decisively modulates and may even overwhelm the intrinsic neuronal properties expressed by responses to direct depolarization.

The long-lasting hyperpolarizations that sculpt the cellular discharges during natural SWS were present in all types of neocortical neurons, including those identified as conventional FS neurons (see Fig. 4B). The arrest in firing of formally identified, intracellularly stained, inhibitory aspiny basket cells during the prolonged hyperpolarizations of the slow oscillation was also reported in anesthetized animals (Contreras and Steriade 1995). Together with the present demonstration that the prolonged hyperpolarizations are not affected in recordings with KCl-filled pipettes (Fig. 4B; see also Timofeev et al. 2001), these data indicate that these long-lasting sleep hyperpolarizations are not mediated by GABAergic events. Instead, they are likely due to decrease or cessation of excitatory input (disfacilitation) and accompanied by an increase in the apparent $R_{in}$ (Contreras et al. 1996; Timofeev et al. 1996).

The hyperpolarizations associated with the slow oscillation (<1 Hz) are the first intracellular sign with transition from waking to sleep (Fig. 7) and they are blocked with transition from SWS to either waking or REM sleep (Figs. 8–9). The increased firing rates during the transitions to both brain-active states resulted from the blockade of SWS hyperpolarizations and preceded in many instances the overt depolarization that occurred only later on (Figs. 9–10). This depolarization was probably a consequence of increased firing rates in thalamocortical neurons (Glenn and Steriade 1982) and afferents from generalized modulatory systems, such as nucleus basalis (Buzaáki et al. 1988) and brain stem neuronal aggregates (reviewed in Steriade et al. 1993c). Thus, in contrast to brain stem cholinergic neurons that display a precursor increase in firing by about 10–20 s before EEG activation (Steriade et al. 1990), neocortical neurons are followers of this increased activity in generalized systems, transmitted through thalamic synaptic relays.

Here only those cortical neurons are considered that are depolarized on awakening and increase their firing rates, compared with SWS. A smaller proportion of neocortical cells are hyperpolarized for a certain period on arousal from sleep (to be reported elsewhere). Indeed, earlier extracellular recordings of monkey’s precentral neurons showed a period of ~10–15 s, corresponding to the early awakening epoch, during which fast-conducting (>40 m/s) pyramidal neurons stopped firing, a phenomenon ascribed to disfacilitation because their antidromic responsiveness was increased during this period (Steriade et al. 1974). Intracellular recordings in acutely prepared midponsine pretrigeminal cats confirmed the hypothesis of disfacilitation, in view of an increased $R_{in}$ during the short period of hyperpolarization and arrest of firing on EEG activation from sleep patterns (Ezure and Oshima 1981; Inubushi et al. 1978).
Increased and stable membrane resistance during quiet wakefulness

The increased \( R_{in} \) during the steady depolarization of the waking state, compared with the depolarizing phase of the slow oscillation in SWS (Figs. 11–12), may seem surprising because of the high level of synaptic activity during waking, compared with the blockade of incoming messages from the outside world in SWS. The \( R_{in} \) measured in acutely prepared animals in vivo is reduced up to 70% during epochs associated with intense synaptic activity, compared with relatively quiescent periods, and increases by \( \sim 30–70\% \) after tetrodotoxin application in vivo, approaching the in vitro values (Paré et al. 1998). The explanation of the increase in \( R_{in} \) in the present experiments on anesthetized, naturally alert animals is probably the higher release of acetylcholine (ACh) in cortex during wakefulness (Jasper and Tessier 1971) and the ACh-induced increase in \( R_{in} \) of neocortical neurons (reviewed in McCormick 1992). The increase in apparent \( R_{in} \) during wakefulness may be related to earlier extracellular recordings showing an increase in antidromic and synaptic responsiveness of neocortical neurons during this behavioral state, compared with SWS (Steriade et al. 1974).

Implications of relatively high firing rates during the disconnected state of SWS

Taking into consideration the unexpected high firing rates of cortical neurons during SWS, a behavioral state when the brain is disconnected from the outside world, a reasonable hypothesis is that this sleep state, far from being associated with a complete annihilation of consciousness, may lead to plasticity processes due to the bombardment of target neurons by rhythmic spike-trains and spike-bursts associated with the slow sleep oscillation. This may play an important role in the consolidation, during SWS, of memory traces acquired during waking, a hypothesis advanced on the basis of intracellular recordings of neocortical and thalamic neurons during the slow sleep oscillation (Steriade et al. 1993b). A similar hypothesis was proposed (Buzsáki 1989) and tested experimentally in the hippocampal system (Qin et al. 1997; Wilson and McNaughton 1994). That short-term plasticity may occur during, and outlast, rhythmic volleys in the reentrant pathways of the thalamocortical loops was demonstrated by mimicking a landmark oscillation of early sleep stages, using rhythmic augmenting responses in the frequency range of spindle, \( \sim 10\ Hz \) (Bazhenov et al. 1998; Castro-Alamancos and Connors 1996; Steriade et al. 1998b). Dual intracellular recordings in anesthetized animals have shown that after such rhythmic thalamocortical events, as during spindles, neocortical neurons display self-sustained activities within the frequency range of the evoked response, a form of “memory” in this circuit (Steriade 1999), despite the fact the thalamus remained silent, under the hyperpolarizing pressure exerted by GABAAergic thalamic reticular neurons (Steriade et al. 1998b). Thus during SWS, neocortical neurons may be engaged in information processing of internally generated signals and may be implicated in plasticity processes related to operations performed during the waking state.

We thank P. Giguère and D. Drolet for technical assistance. I. Timofeev was a postdoctoral fellow; he is now a Fonds de la Recherche en Santé du Québec Scholar. F. Grenier is a Ph.D. student.

This work was supported by grants to M. Steriade and, more recently, to I. Timofeev from the Medical Research Council of Canada. The work was also supported by grants to M. Steriade from the Natural Sciences and Engineering Research Council of Canada, and Human Frontier Science Program.

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