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Cortical long-axoned cells and putative interneurons during the sleep-waking cycle

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Abstract: Knowledge of the input-output characteristics of various neuronal types is a necessary first step toward an understanding of cellular events related to waking and sleep. In spite of the oversimplification involved, the dichotomy in terms of type I (long-axoned, output) neurons and type II (short-axoned, local) interneurons is helpful in functionally delineating the neuronal circuits involved in the genesis and epiphenomena of waking and sleep states. The possibility is envisaged that cortical interneurons, which are particularly related to higher neuronal activity and have been found in previous experiments to be more active during sleep than during wakefulness, might be involved in complex integrative processes occurring during certain sleep stages. Electrophysiological criteria for the identification of output cells and interneurons are developed, with emphasis on various possibilities and difficulties involved in recognizing interneurons of the mammalian brain. The high-frequency repetitive activity of interneurons is discussed, together with various possibilities of error to be avoided when interpreting data from bursting cells. Data first show opposite changes in spontaneous and evoked discharges of identified output cells versus putative interneurons recorded from motor and parietal association cortical areas in behaving monkeys and cats during wakefulness (W) compared to sleep with synchronized EEG activity (S): significantly increased rates of spontaneous firing, enhanced antidromic or synaptic responsiveness, associated with shorter periods of inhibition in type I (pyramidal tract, cortico-thalamic and cortico-pontine) cells during W versus significantly decreased frequencies of spontaneous discharge and depression of synaptically elicited reponses of type II cells during W compared to S. These findings are partly explained on the basis of recent iontophoretic studies showing that acetylcholine, viewed as a synaptic transmitter of the arousal system, excites output-type neurons and inhibits high-frequency bursting cells. Comparing W and S to the deepest stage of sleep with desynchronized EEG activity (D) in type I and type II cells revealed that: (*a*) the increased firing rates of output cells in D, over those in W and S, is substantially due to a tonic excitation during this state, and rapid eye movements (REMs) only contribute to the further increase of discharge frequencies; (*b*) in contrast, the increased rates of discharge in interneurons during D is entirely ascribable to REM-related firing. On the basis of experiments reporting that increased duration of D has beneficial effects upon retention of information acquired during W, the suggestion is made that increased firing rates of association cortical interneurons during REM epochs of D sleep are an important factor in maintaining the soundness of a memory trace.

Keywords: association cortex; motor cortex; interneurons; output cells; REM sleep; sleep-waking cycle

1 Why identify cells in sleep-waking studies?

The brain rests during sleep: This common sense view, an age-old belief, has persisted until very recently. The Pavlovian conception of sleep and inhibition being one and the same process (Pavlov, 1923) strongly suggested that during sleep, activity in cells of the cerebral cortex was extinguished, together with an irradiation of cortical mass inhibition to the whole cerebrum. In Sherrington's opinion, too, "the great knotted headpiece of the whole sleeping system lies for the most part dark. . . . Occasionally at places in it lighted points flash or move but soon subside" (1955, p. 183). The expectation was that, with the development of techniques allowing the recording of discharges from individual cells during the sleep cycle of behaving animals, investigators would find decreased rates of neuronal firing during sleep compared to wakefulness. Since another common sense view regards sleep as total obliteration of consciousness, diminished activity seemed a logical prediction, especially for cells of the neocortical mantle, site of the highest integrative processes. Instead, the pioneering work of Jasper et al. (1957), essentially devoted to conditioning and only incidentally related to vigilance states, reported that "when the animal was drowsy or asleep, . . . many cortical cells were found to be firing as actively as when the animal was alert" (p. 280).

The common procedure of subsequent experiments in this field generally consisted of picking up with microelectrodes the "spontaneous" spike activity of cells in various cortical areas, computing firing rates (number of spikes/sec) during different stages of the sleep-waking cycle, and, finally, pooling discharge frequencies of tens or hundreds of nonidentified neurons, *thus inevitably mixing cells with unknown input-output characteristics*. It is not surprising that without dissociation of different cellular classes, the only consistent conclusion of these studies was to confirm that cortical neurons do *not* cease to discharge during either of the two principal stages of sleep. Some authors reported quite equal firing rates in the cerebral cortex during wakefulness (W) and the sleep stage characterized by synchronized EEG waves (S); others observed equally increased discharge frequencies compared to S during W as well as during the deepest stage of sleep, characterized by EEG desynchronization (D); finally, most investigators found highest discharge rates in D, but with large discrepancies in results when the relationships between rapid eye movements (REMs) of D sleep and neuronal firing were analyzed (for details, see the recent

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monograph of Steriade & Hobson, 1976). Is this confusion ascribable to the uniqueness of neurons, to the fact that no two neurons are exactly alike, that "every neuron in the central nervous system has its dendritic field in a different place, and must have its own pattern of anatomical connectivity" (Horridge, 1969, p. 5)? Subsequent research developments give reason to believe that the above mentioned discrepancies in results reflected rather the lack of methodological maturity inevitable in the early stages of cellular studies of sleep. One consequence has been that some scholars have left this domain of research. Evarts (1967), confronted by the welter of "epiphenomenal data imbedded in which lie ... some hints on the nature of sleep," concluded that perhaps "some future investigator may discover the functional significance of sleep without studying sleep itself" (p. 545) and turned to the analysis of central correlates of movements.

A first step toward simplifying the complexity of neuronal events related to sleep and waking is to define the cellular type to which any recorded neuron belongs. The most parsimonious way to classify nerve cells according to their morphological characteristics stems from the discovery of the "reazione nera" by Camillo Golgi a century ago, which contributed the major tool for visualizing neuronal bodies and their emerging processes. The capriciousness of this method allows only one or a few cells to be visualized, but in all their splendor. Two cellular classes have been distinguished by the Golgi method (1886): *type I* neurons, often large, whose long axons leave the gray matter and constitute the major pathways reaching distant structures; and *type II* neurons, mainly small, whose short axonal processes, confined to gray matter, quickly break into fine branches distributed in the vicinity of the cell body of origin (Figure 1). Type I, or output, cells are executive cells that transfer the result of integration of afferent signals to distant structures. Type II cells, or local interneurons, mediate complex interactions between neurons within a center. Incoming signals may thus be amplified by means of an interposed set of local excitatory neurons. Conversely, and perhaps more importantly, other local cells reverse the sign of afferent excitatory messages and bring the relevant activity into focus by inhibiting neighboring elements whose activity should remain off the scene.

What a priori considerations predict differential alterations of type I and type II cells during sleep and waking, and what can be learned by identifying various classes and subclasses of neurons? In view of the presence within the same cellular pool of interneurons (*i*) inhibiting neighboring output neurons (*o*), it might be anticipated, for instance, that the increased firing rate of *i* cells would be associated with suppressed activity of *o* cells during the same stage of the sleep-waking cycle. And in view of presumed differences in electrical membrane properties of various cellular classes, it would not be surprising to find a particular type of input via a projection system having differential effects upon diverse categories of neurons in a target structure. If these various cellular types remain unidentified, work will continue to report that "some" units are more active, while "others" are less active at a certain level of vigilance, an inventory that can hardly lead to the formulation of further hypotheses.

By determining through physiological procedures the inputs of type I cells, the structures they project to, as well as the excitatory or inhibitory nature of these projections, one may (1) disclose one-way or reciprocal relations in a neuronal circuit with positive or negative feedback controls, (2) reveal the temporal course of a sequence of events, and (3) predict the occurrence of various phenomena arising within a system and its related subsystems. Accumulating evidence has in recent years suggested that activities of brain-stem neurons belonging to cholinergic and monoaminergic systems are involved in awakening or in the genesis of some sleep stages, but unfortunately the input-output organization and intrinsic operations of the brain-stem network were underestimated in electrophysiological

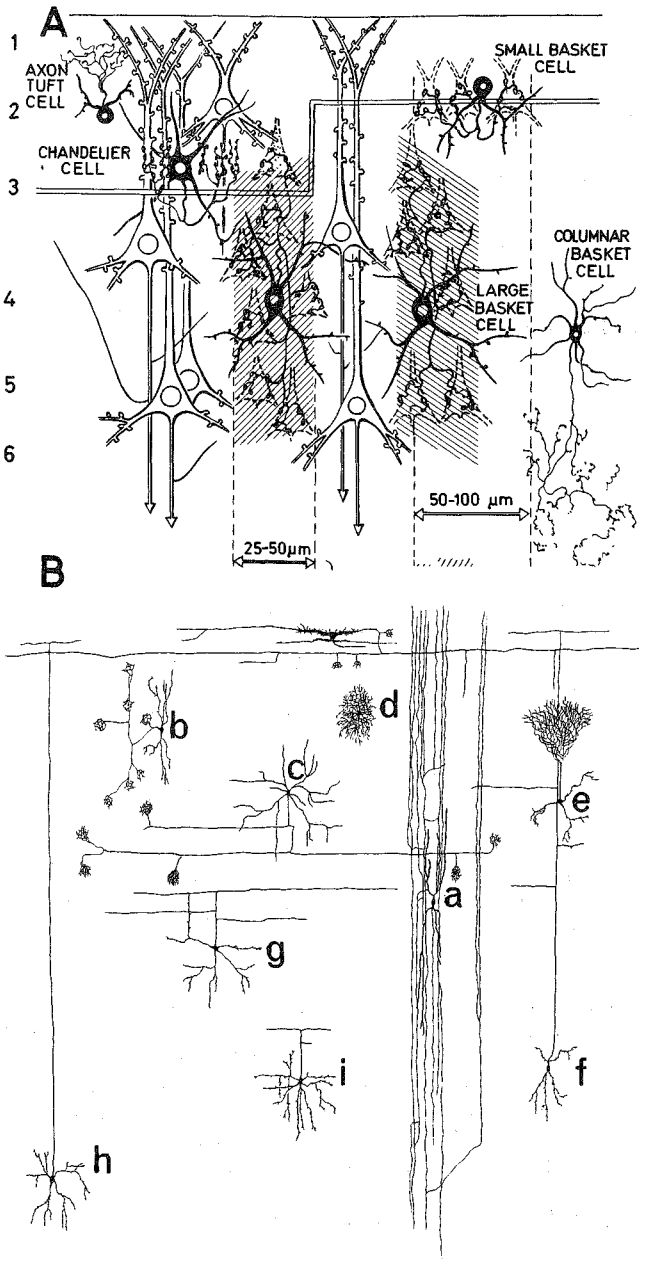

Figure 1. Morphological features of type I and type II cortical cells. A: diagram of a conventional vertical section to show pyramidal (type I) cells and putative inhibitory type II (large basket) cells that so far have been identified on the basis of the fine structure of their synaptic contacts (from Szentágothai, 1975, courtesy of Elsevier). B: free-hand composite drawing of various stellate (type II) cells described by Ramón y Cajal (from Colonnier 1966, courtesy of Pontificia Academia Scientiarum).

investigations (Krnjević, 1974). It is known, for instance, that ascending inputs from the rostral brain-stem reticular formation (RF) do *not* exert uniform effects on various types of cells in thalamic nuclei and cerebral cortex (Steriade and Hobson, 1976), but there are no more than hints concerning the pathways and mechanisms underlying the enhanced activity of type I and depressed activity of type II cortical cells during W (Steriade, 1976; Steriade, Deschênes, & Oakson, 1974). It is also known that some pontine neurons exhibit markedly increased firing rates during D sleep (Hobson et al., 1974), but lack of knowledge of the rostral projections and the nature of connections established by these pontine neurons leaves untouched the problem of their influence on different types of thalamic and cortical cells. The cerebral cortex is not a mere passive receiver of as-

cending impulses from brain-stem structures. Cortical cells may reinforce or dampen the development of sleep and waking states, once the latter are triggered in brain-stem prime movers. In order to disclose these corticofugal influences, projection cells should first be identified. Evarts (1964, 1965) was the first to investigate the spontaneous discharge of pyramidal tract (PT) output cells during sleep and waking. A decade later, the sleep-waking behavior of fast- and slow-conducting PT cells in the motor cortex of monkey was revisited by our microelectrodes, and the alterations undergone by these two subclasses of type I cells were found to differ fundamentally as a function of subtle changes in the vigilance level within the state of W (Steriade, Deschênes, & Oakson, 1974; Steriade et al., 1974). Data in this paper (part 3) suggest that, during the sleep-waking cycle, fluctuations in activity of identified output cells in motor and association cortical areas may be effective in influencing brain-stem and thalamic nuclei by their downward projections.

Attempts to identify type II cells may be rewarding not only for the question "how," regarding mechanisms, but also for the yet unanswered question "why," concerning the function of sleep. A decade ago, Moruzzi (1966), starting from the assumption that type II cells are particularly involved in higher neural activity, considered that, since plastic changes are expected to occur in these cells during W as a consequence of learning, the function of sleep would concern slow interneuronal recovery events. This expectation that interneurons "rest" during sleep, taken together with the fact that interneurons are especially viewed as exerting inhibitory actions on type I cells, was consistent with the inference by Evarts (1964) that recurrent inhibition of PT cells and inhibitory cortical interneurons are depressed during sleep. The premise in Moruzzi's hypothesis that interneurons play a decisive role in higher neural activity is supported by indirect findings based on the ontogenesis of nerve cells. Thus, there is enough information to make the general statement that type II cells develop later than type I neurons (Bodian, 1970; Jacobson, 1969; Mitra, 1955; Ramón y Cajal, 1955). Autoradiographic data in the cerebellum and hippocampus show that "microneurons" represent the last-developing component and suggest that the interposition of postnatally formed type II neurons between input and output elements may play a role in memory through the formation of new neural circuits (Altman, 1967). When, however, work in our own laboratory experimentally tested Moruzzi's and Evarts' attractive ideas on the mechanisms and significance of sleep, their hypotheses could not be confirmed. Instead, we found higher rates of discharge in type II cells during S sleep compared to W (Steriade, Deschênes, & Oakson, 1974; Steriade et al., 1974), and longer periods of inhibition in type I neurons during S compared to W (Steriade, 1976; Steriade & Deschênes, 1974). If, therefore, interneurons are effectively related to higher neural activity, the fact that they are more active during sleep than wakefulness leads to the supposition that type II cells are specifically involved in highly integrative processes occurring during certain sleep epochs. Data in part 3 actually reveal that, *in contrast with identified output cells, W and D sleep are polar states for interneurons: lowest rates of discharge occurring during W and highest rates selectively occurring during REMs of D sleep.*

Interchangeable terms will be used throughout this paper for type I, long-axoned, output, or projection cells; and type II, short-axoned, nonoutput, local, internuncial cells, or interneurons. This sharply opposed differentiation may raise some difficulties. Interneurons are not necessarily short-axoned: the axons of some intracellularly stained spinal cord interneurons (Jankowska & Lindström, 1972), leave the gray matter and run for long distances to distribute over several segments. They are not necessarily small: the commonly used term "microneuron" certainly does not apply to short-azoned cells in the auditory cortex described as "grandes ou même géantes (Ramón y Cajal, 1955, p. 625) or to large "stellate" (nonoutput) cells found in the primate motor and sensory cortices (Sloper, 1973). The tremendous morphological variety of cortical interneurons (Figure 1)[1] has not yet been related to their functional properties. The diversity of type II cells may be ascribed to a fact emphasized by Jacobson (1969), namely, that they remain uncommitted and modifiable until late in ontogeny, while the relative invariance of type I cells is the result of their development under tight genetic constraints. A classification of purely excitatory versus inhibitory elements within the type II group undoubtedly does not exhaust the heterogeneity of this neuronal class; in invertebrates, intracellular stimulation of an interneuron produces an excitatory postsynaptic potential (EPSP) in a follower cell and an inhibitory postsynaptic potential (IPSP) in another neuron. (Kandel et al., 1967). Still, the oversimplified dichotomy in terms of type I and type II cells is the best available at present to begin the functional delineation of cortical neuronal populations involved in the sleep-waking cycle.

The remaining two parts of this paper have different goals. Part 2 is a rather detailed exposition of attempts to differentiate type I and type II cells; I feel this part is necessary, since criteria are not always rigid and there are various potential errors to be avoided in identifying neuronal types. Part 3 deals with differential changes in cortical output cells and putative interneurons during the sleep-waking cycle, and discusses some behavioral implications of these findings.

2 Possibilities and limits in the identification of type I and type II cells

2.1 Stimulation and recording

The identification of cells and intercellular connections in the mammalian neocortex cannot lead to such unequivocal conclusions as are drawn by identifying a set of ten motoneurons that mediate the contraction of the mantle organs in mollusks (Kupfermann et al., 1974). Yet there are standard physiological procedures to determine the targets of *output neurons* and to infer (pending final histological confirmation) activity arising in locally operating *interneurons.*

The way to fully identify a neuron is to impale it with a glass pipette, disclose the converging inputs that excite or inhibit it, reveal the target structure(s) toward which its axon projects, make measurements of electrical membrane properties, and finally introduce *in vivo* a dye through the micropipette used for intracellular recording in order to identify the cell morphologically. This has been achieved in recent years for some cells in the mammalian central nervous system, for example, in the spinal cord (Jankowska, 1975; Jankowaska & Lindström, 1972), inferior olive (Llinás, Baker, & Sotelo, 1974), abducens nucleus (Gogan et al., 1973), and visual cortex (Kelly & Van Essen, 1974). No work on the cerebral cortex with the above procedures has succeeded in differentiating output cells versus interneurons according to discharge properties *and* morphological features, although, as will be shown later, partial evidence strongly suggests the coexistence of these two groups within the neuronal circuitry of motor, sensory, and association cortical areas. Since the distinguishing feature of type I versus type II neurons is their long-axoned versus short-axoned arborization, it is perhaps worthwhile mentioning the difficulties in revealing the trajectory of the axon with Procion Yellow (Jankowska, 1975) and the tendency to replace this dye by horseradish peroxidase. It goes without saying that such experiments are conducted on deeply anesthesized and/or paralyzed animals in order to preclude displacement of the intracellular recording-stimulating-injecting pipette, which would cause irreversible neuronal damage.

The study of neuronal activity during the natural sleep-waking cycle, with all the complexity of central and peripheral sleep phenomena, requires a nonanesthetized, nonparalyzed preparation. At present, the full range of procedures for neuronal

identification mentioned above, combined with intracellular recording throughout a sleep-waking cycle in a behaving animal and followed by intracellular staining, has not as yet been achieved. Recent studies have reported intracellular recordings from cortical neurons, in awake, nonparalyzed animals (Skrebitsky & Sharonova, 1976; Woody & Black-Cleworth, 1973). The elegant experiments of Woody and Black-Cleworth (1973) intracellularly analyzed neuronal events and membrane properties of sensorimotor cortical cells in the awake, chronically implanted cat during conditioning procedures. By having convincingly demonstrated that intracellular recordings are possible for short periods of time in previously conditioned animals, such pioneering studies certainly open new perspectives for neuronal correlates of learning. For the long (usually more than one hour) periods required to identify the input-output organization of neurons and to study the fluctuations in their activity during the unpredictable episodes of a natural sleep-waking cycle, the optimal approach would probably be extracellular unit analysis during a sleep-waking continuum or repeated sleep cycles, followed by an attempt to impale the investigated cell in order to resolve crucial points that cannot be dealt with extracellularly, and finally to identify the neuron morphologically by staining. (Since the completion of the present paper, Nakamura et al. (1978) have recorded intracellularly from trigeminal motoneurons of non-anesthetized, non-paralyzed cats during transition from S to D sleep).

Some aspects of sleep studies do not need intracellular recordings: measurement of the discharge frequencies (rate) and temporal distribution of successive interspike intervals (pattern). In fact, aspects having to do with "spontaneous" neuronal activity have been abundantly studied, but do not disclose underlying mechanisms, since in only analyzing "spontaneous" firing there is no possibility of dissociating effects generated by changes in afferents modulating the neuron from those generated by changes in its intrinsic excitability. To test the excitability of the neuronal body, it is best to avoid any synapse between the site of stimulus application and the responsive cell. Afferent fibers reach the neuron especially on its extensive dendritic branches and their unknown geometry may introduce complications in the interpretation of results. Instead, a long-axoned neuron may be fired *antidromically* following a shock applied to its distal axon. Some aspects of change in the probability of antidromic responses during sleep and waking can be measured with extracellular recordings. By studying the occurrence probability of synaptically elicited responses and the duration of suppressed neuronal firing following a testing volley, one may still obtain some evidence concerning the decreased or increased states of excitation and inhibition of the neuron. But it certainly cannot be known without intracellular recording *and* measurements of membrane conductance whether, for instance, the decreased rate of spontaneous firing or even complete neuronal silence in some periods of the sleep-waking cycle are ascribable to disfacilitation or to sudden rise in active influences of an inhibitory synaptic transmitter.

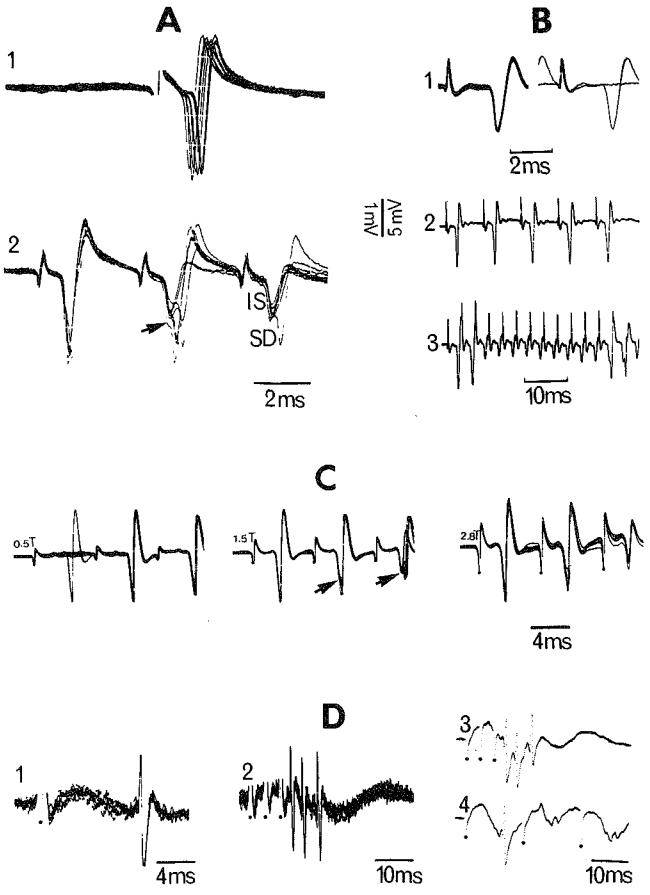

Figure 2. Antidromic and orthodromic activation of output (type I) cells. A: a neuron in the VL thalamus of cat; 1: monosynaptic activation following stimulation of the cerebello-VL pathway (note, in the superimposition of eight sweeps, the variations in latency of the evoked *single* discharges); 2: antidromic invasion following a three-shock train to the motor cortex (arrow indicates fragmentation between IS and SD spikes; modified from Steriade, Apostol, & Oakson, 1971). B: a slow-conducting PT cell in the precentral motor cortex of monkey, antidromically invaded following stimulation in the pes pedunculi; see collision in 1 (right); the cell could follow shocks at 110/sec without failure (2), but by increasing the frequency to 310/sec (in 3), only IS spikes were seen following the first two full spikes, with partial recovery (IS-SD split discharges) after 30 msec (modified from Steriade, Deschênes, & Oakson, 1974). C and D: antidromic activation of two output cells in parietal association cortex (areas 5 and 7) of cat following stimulation of the thalamic LI nucleus, the rostrodorsal part of the LP complex (unpublished experiments by Steriade and Kitsikis). In C, a jump decrease in response latency (from 3.3 msec to 2.2 msec) was elicited by increasing stimulation from subthreshold (0.5 T) to suprathreshold (1.5 and 2.8 T); note IS-SD fragmentation (arrows). In D, a very slow-conducting cell (10 msec antidromic response latency; 1.5 m/sec), which responded without failure at 265/sec shocks (2–3), but failed to respond following the first shock at much lower (65/sec) frequency (4). Fifty averaged sweeps in 3–4. The vertical bar (voltage calibration) in A-B represents 3.5 mV in C and 150 μV in D. In this and all subsequent figures, extracellular activity was recorded with a bandwidth of 1 to 10,000 Hz; positivity downwards.

2.2 Type I cells

Here not much is to be said. Most output cells are electrophysiologically identifiable by antidromic invasion following stimulation of the axon in target structures, and no supplementary (morphological) evidence is required to firmly ascertain their long-axoned feature. If antidromic responses are elicited in the same cell from different stimulated sites, this indicates that axon collaterals are distributed to various structures. Figure 2 depicts the criteria of antidromically elicited discharges: (*a*) fixed latency, but not necessarily short, since there are slowly-conducting axons within almost all projection systems; (*b*) cancellation of response when the antidromic impulse, travelling up the axon, collides with a spontaneously occurring or synaptically evoked orthodromic spike; (Darian-Smith, Phillips, & Ryan, 1963; Famiglietti, 1970; Fuller & Schlag, 1976; Paintal, 1959) and (*c*) ability to follow very fast (up to 600–700/sec) stimuli. The first two are *sine qua non* criteria; the last may be lacking in some instances when the cell is inhibited immediately after the first stimulus and thus becomes unresponsive to the subsequent stimuli. This inhibition may affect only the soma-dendritic (SD) membrane; in this case, an abortive spike, originating in the lower threshold initial segment (IS), may still appear (Figure 2, A–C; see also Figure 10). The differentiation between IS and SD spikes (Brock, Coombs, & Eccles, 1953) has been definitively es-

tablished by recording simultaneously from the same spinal motoneuron with extracellular and intracellular microelectrodes (Araki & Terzuolo, 1962; Terzuolo & Araki, 1961). A progressive fragmentation between the IS and SD components of a spike, with a delayed SD invasion, can be induced by artificial hyperpolarization of the neuron (Coombs, Eccles, & Fatt, 1955). We used this knowledge as a tool to study in behaving monkeys the changes from IS-SD split spikes during S sleep to unbroken discharges, with accelerated soma invasion, during W (Steriade & Deschênes, 1973; Steriade, Deschênes, & Oakson, 1974).

The relation between faster conduction velocity and a larger cell body, which was first inferred, has been confirmed by intracellular recording and staining of two types, small and large cortical output cells (Naito et al., 1969). Differential membrane properties have been demonstrated for slow-conducting (small) and fast-conducting (large) cortical PT neurons (Koike, Okada, & Oshima, 1968; Takahashi, 1965).

2.3 Type II cells

The profusion of intracellular studies establishing in great detail the properties of identified output cells contrasts with the scarcity of investigations aimed at recording the activity of interneurons. It should be stressed that, if the physiological procedure of antidromic invasion is sufficient for identifying an output cell, *unequivocal* recognition of an interneuron needs anatomical confirmation of its locally ramified axon. There is as yet no complete (electrophysiological *plus* morphological) evidence even on the simplest cortical or thalamic neuronal circuits involving type II cells. Thus wherever "interneuron" is specified, the actual designation should be "putative" or "presumed" interneuron.

The difficulties in impaling short-axoned cells and recording their activity intracellularly have been repeatedly mentioned. The task is particularly arduous in attempts to record such elements in the thalamus and allo- or neocortex. Difficulties arise not only with intracellular, but also with extracellular recordings. Many authors have been puzzled by the fact that interneurons have been recorded only infrequently, in contrast with the much larger numbers of short-axoned cells seen in morphological studies on the same structure (Shepherd, 1970). The possibility should also be kept in mind that some interneurons act by way of graded potentials ("nonspiking" interneurons) and these will obviously not be detected in extracellular unit recordings. It is thus likely that "the most interesting units . . . will be . . . omitted from preliminary classifications of . . . numerous types whose actions cannot be understood without them" (Horridge, 1969, p. 6).

2.3.1 Response characteristics and underlying mechanisms. To begin with, physiological identification of an interneuron should necessarily entail *absence of antidromic invasion* from all sites of stimulation, even at the strongest intensities used.[2] In some instances, when one is quite sure that the applied stimulus involves the total output of the structure (e.g., the emerging nerve of a motor nucleus in the brain-stem), this evidence might be decisive. But these are rare cases. A unit in the motor cortex should certainly not be considered an interneuron solely on the basis of lack of antidromic activation following stimulation of the main outflow, the PT, since there are many other projection systems. Stimulating electrodes would be required in multiple target structures and, in any case, could yield only negative evidence.

The synaptically elicited response of an interneuron usually consists of a *high-frequency repetitive (200–1000/sec, usually 400–600/sec) spike barrage* (Figures 3, 4, and 7). Such a burst response is by no means necessary, as there are some types of spinal interneurons that do not exhibit high-frequency barrages to an afferent (Eccles, Eccles, & Lundberg, 1960) or a ventral root volley (Willis & Willis, 1966). But these seem to be exceptions when considering the stereotyped burst patterns described, following the discovery of cord Renshaw cells (Renshaw, 1946) in the cerebellum (Eccles, Llinás, & Sasaki, 1966), inferior olive (Llinás, Baker, & Sotelo, 1974), brain stem RF (Hikosaka & Kawakami, 1976), VB (Andersen, Eccles, & Sears, 1964), and VL (Marco, Brown, & Rouse, 1967; Steriade, Apostol, & Oakson, 1971; Steriade, Wyzinski, & Apostol, 1972) thalamus, lateral geniculate (LG) (Burke & Sefton, 1966; Fukuda & Iwama, 1970; Sakakura, 1968), olfactory bulb (Shepherd, 1963b, 1970), hippocampus (Andersen, Eccles, & Loyning, 1964; Andersen, Gross, Lømø, & Sveen, 1969), and motor (Renaud & Kelly, 1974; Stefanis, 1969; Steriade, Deschênes, & Oakson, 1974; Steriade et al., 1974; Sukhov, 1968), somatosensory (Innocenti & Manzoni, 1972; Steriade & Yossif, 1977), and parietal association (Steriade, Kitsikis, & Oakson, 1978) cortices. The prolonged spike barrage

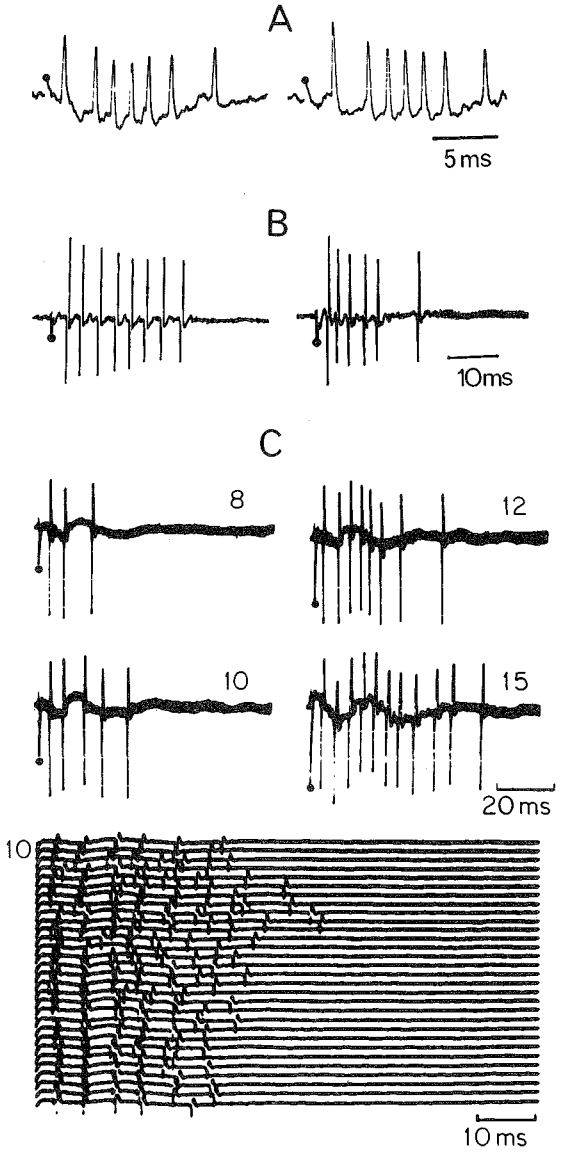

Figure 3. Synaptic activation of putative interneurons in VL thalamus and motor cortex of cat. Cells A and B: high-frequency barrage discharges elicited in VL thalamus by precruciate cortical shocks (modified from Steriade, Apostol, & Oakson, 1971; Steriade, Wyzinski, & Apostol, 1972). Cell C was recorded in the motor precruciate area and driven by VL shocks at increasing intensities (from 8 to 15 V); the 30-sweep sequence (with reduced gain) shows the responses to 10 V stimulation; note the progressive increase in number of spikes, shortening of latency and increased intra-burst frequency with increase in stimulation strength (modified from Steriade et al., 1974).

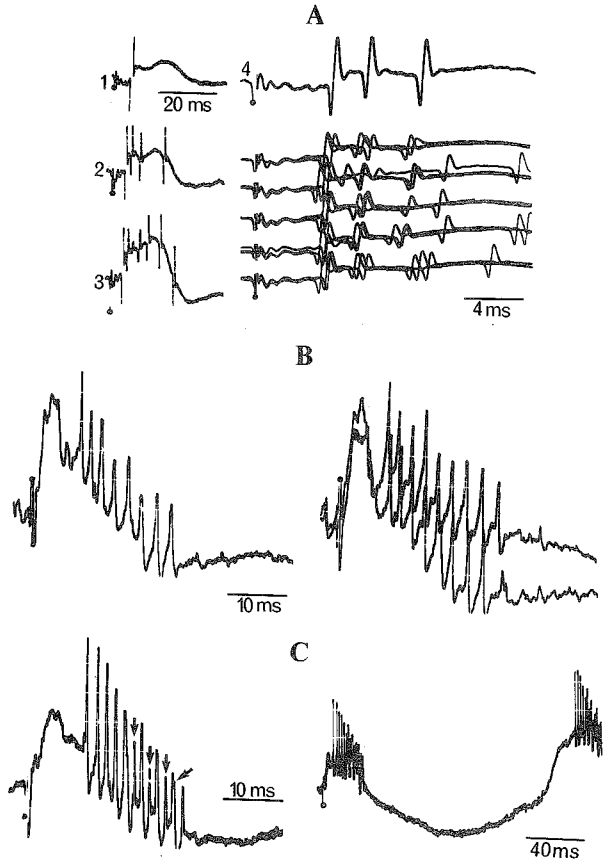

Figure 4. Response patterns of putative interneurons in primary somatosensory area (SI) and parietal association cortex of cat. Cell A was recorded in SI, stimulation applied to underlying white matter in animal with lesion in VB thalamic complex; stimulation in 1, 2, and 3 was 0.09, 0.1, and 0.12 mA, respectively; note marked spike inactivation in the middle of the evoked burst in 3; in 4, same intensity as in 2: single sweep and superimposed sweep sequences with reduced gain (modified from Steriade and Yossif, 1977). Parietal association neurons B and C were driven by stimulation of LP (B) and LI (C) thalamic nuclei; note spike inactivation and fragmentation (arrows in C) within the spike barrage; the lower speed sweep, with half gain, depicts both early and late events evoked by the LI shock; note also that spike barrages occurred immediately after the early depth-negative (depolarizing) wave that was associated with synaptically evoked discharges in identified output cells (modified from Steriade, Kitsikis & Oakson, 1978).

is often provoked by increasing stimulus intensity slightly above that eliciting a single spike response (Figure 4A). But other interneurons do not significantly increase the number of discharges as a function of stimulus intensity, and a prolonged barrage may occur at surprisingly constant latencies for each spike, even at low intensity levels (Figures 3C and 4). With respect to this, it was suggested by Shepherd (1970, p. 544; see his Figure 6B) that "impulse discharge is a very stereotyped affair, independent to a certain extent of the amount of depolarization impressed on the cell by the afferent volley." The spike amplitude may diminish in the middle of the high-frequency burst of an interneuron, and full discharge may even by reduced to abortive spikes (Figure 4C). This "inactivation" process, described in cerebellar cells as a consequence of strong depolarization (Granit & Phillips, 1956) suggests that protracted EPSPs underlie the repetitive spike activity of interneurons (Eccles, Eccles, Iggo, & Lundberg, 1961; Eccles, Fatt, & Koketsu, 1954; Willis, 1971).

Should the *high-frequency repetitive* spike activity be considered a distinct feature of interneurons, thus assuring a clear-cut differentiation from synaptically evoked *single* spike responses in output cells? An unequivocally positive answer to this question is not available and some reservations are needed. First, this distinction is valid in the analysis of responses to well synchronized (electrical) stimuli applied to central pathways, as peripheral (much less synchronized) stimulation may evoke repetitive responses in output neurons too. With this methodological requirement, there is common agreement that even if a small percentage of output cells (for example, dorsal spino-cerebellar tract (Kuno, 1969; Kuno & Miyahara, 1971) and cuneate neurons (Calvin & Loeser, 1975, Galindo, Krnjević, & Schwartz, 1968)) may discharge spike doublets or triplets in response to single shocks, prolonged burst discharges almost never occur. "No one has reported recording a burst discharge from a motoneuron" (Willis, 1971, p. 43). This applies not only to spinal cord motoneurons. Striking differences have also been observed between burst responses of interneurons (Shepherd, 1963b, 1970) and single orthodromic spikes (even after increasing the intensity strength) in mitral cells (Shepherd, 1963a) of the olfactory bulb. The same contrast has been repeatedly emphasized when investigating synaptically elicited responses in hippocampal (Eccles, 1969), LG (Burke & Sefton, 1966; Sakakura, 1968), VL (Steriade, Apostol, & Oakson, 1971; Steriade, Wyzinski, & Apostol, 1972), and neocortical type I versus type II neurons (Steriade, Deschênes, & Oakson, 1974; Steriade et al., 1974; Steriade, Kitsikis, & Oakson, 1978; Steriade & Yossif, 1977).

There are, of course, instances in which an output cell may

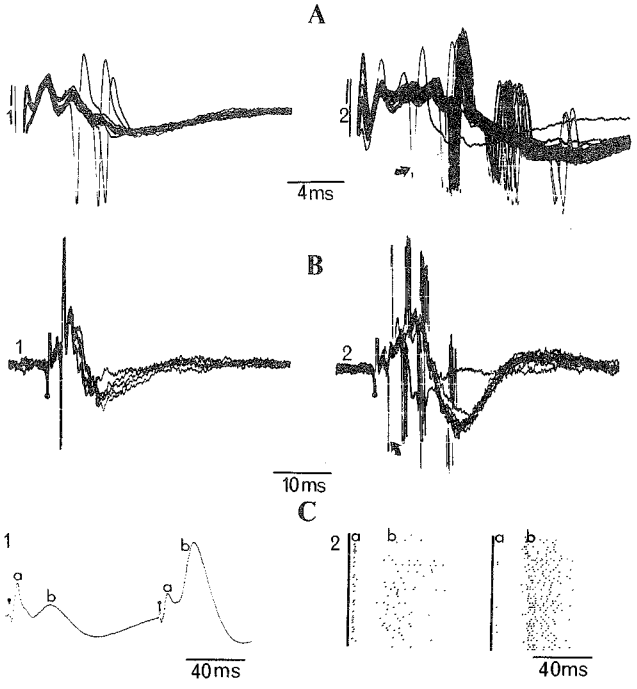

Figure 5. Incremental responses of augmenting type in cortical neurons of cat. A and B, two SI cells driven by VB thalamic stimulation at 1/sec (1) and 10/sec (2). Note: orthodromic repetitive discharges evoked by 10/sec shocks (with spike inactivation in A) occurring at longer latencies than single spike discharges to 1/sec stimuli; the response to the first shock in the 10/sec train is marked by arrow (modified from Steriade & Yossif, 1974). Neuron C was recorded from the parietal association cortex; two 100 msec-delayed shocks (10/sec stimulation) were applied to the LI thalamic nucleus, and focal slow waves (fifty averaged sweeps in 1) and unit discharges (fifty-sweep dotgram in 2) were recorded by the microelectrode; note that the response to the second shock had decreased amplitude of the early slow depolarizing wave (*a*,1) and, relatedly, decreased probability of firing in the first (*a*) part of the unitary response (in 2), simultaneously with increased amplitude of *b* slow wave and increased number of spikes in the secondary (*b*) repetitive firing (modified from Steriade, Kitsikis & Oakson, 1978).

exhibit repetitive discharges in response to central stimuli.

1. In some cases, the group of repetitive discharges occurs separately, a few milliseconds after the early, orthodromically, or antidromically evoked single spike (Steriade, 1976; Steriade, Deschênes, & Oakson, 1974; Steriade & Yossif, 1977). Two pathways may be envisaged in such types of responses, the burst probably reflecting activities in cortical excitatory interneurons engaged in parallel by the testing volley. This is supported by independent alterations of the early and late parts of the response in some experimental conditions (Steriade, 1976).

2. The augmenting responses of cortical output cells to 10/sec thalamic stimulation may also consist of several discharges at volleys following the first in a train (Figure 5), without, however, reaching the long bursts of interneurons (Steriade & Yossif, 1974). In this case, too, the peculiar susceptibility of type II cells to this kind of stimulation is probably at the basis of the spike doublets or triplets in type I neurons. The greater involvement of nonpyramidal, compared to pyramidal cells, in the mechanism underlying the transition from primary to augmenting responses was revealed in both the neocortex (Purpura, Shofer, & Musgrave, 1964) and hippocampus (Andersen, Gross, Lømø, & Sveen, 1969). One is tempted to speculate that the paradoxical finding of *increased latency associated with increased number of spikes* in response to the second and subsequent stimuli in a train of 10/sec shocks (Figure 5) would result from simultaneous blockade of direct thalamo-cortical excitation of output cells combined with increased activities of excitatory interneurons responsible for the secondary depolarization. This may well be supported by firing changes from the first to the second shock at 10/sec in those cortical cells that exhibit two distinct components of unitary responses; the second stimulus induces a decreased probability of firing in the first (monosynaptically evoked) discharge and, simultaneously, a spectacular increase of secondary, repetitive spikes (Figure 5C).

What structural features and mechanisms are responsible for the repetitive firing and underlying prolonged depolarization? Several nonexclusive possibilities may be entertained. One of them invokes regenerative excitation by positive feedback, involving transmission of both depolarizing slow potentials and spikes in a group of interconnected elements, leading to synchrony of the network. This mechanism, described for initiation and termination of burst activity in trigger-group neurons of mollusks, is intrinsic to nonrectifying electrotonic coupling, and may apply to some other coupled systems (Getting & Willows, 1974). Networks of interconnected interneurons are present in the LG of monkeys (Pasik, Pasik, & Hamori, 1976). Since evidence for such systems and mechanisms is still scarce in the mammalian brain, the discussion will call upon the following explanations. (*a*) Residual presynaptic action has been thought to determine the amount of repetitive discharges in Renshaw cells: when enzymatic destruction of synaptic transmitter is prevented, a single synaptic volley may induce, instead of the usual 50 msec-burst, a rhythmic discharge lasting as long as 2 sec (Eccles, Eccles, & Fatt, 1956). (*b*) The other explanation considers the intrinsic properties of the postsynaptic cell and arises from two types of observations: the membrane properties of Renshaw interneurons are altered for periods longer than that of the presence of the chemical transmitter (Longo, Martin, & Unna, 1960); and identical time courses of transmitter action may affect various postsynaptic neurons dissimilarly. This would imply that multiple spike discharges are characteristic for some types of cells and are possibly due to a smaller posthyperpolarization, absence of susceptibility to inhibitory action, or other similar factors. The reader is referred to Calvin's (1974, 1975) papers for elaborated models of multiple spike production. Differential properties have been found in two populations of cortical output cells, fast-conducting and slow-conducting PT neurons (Calvin & Sypert, 1976; Koike et al., 1970; Takahashi, 1965), but such a study has not yet been performed to distinguish long-axoned from short-axoned neurons.

2.3.2 Spontaneous firing. Reports on background discharge of interneurons are exceptionally few. A suitable analysis of fluctuations in rate and pattern of spontaneous firing requires a nonanesthetized animal, at best a chronically implanted preparation. Two papers describe spontaneous discharge of LG thalamic (Sakakura, 1968) and precentral motor cortex (Steriade, Deschênes, & Oakson, 1974) interneurons, as identified by repetitive responses to volleys setting in motion afferent and/or recurrent collateral pathways during sleep and wakefulness in behaving animals. While statistical analyses of discharge rates and patterns, indispensable in a study of spontaneous firing, are only anecdotal in both Sakakura's work (1968) and our own (Steriade, Deschênes, & Oakson, 1974), three findings deserve attention in these studies, namely the lower mean-rate of firing of interneurons compared to those of output cells (Sakakura, 1968), arrest of firing on arousal (Sakakura, 1968; Steriade, Deschênes, & Oakson, 1974), and a great proportion of very short (<10 msec or even <5 msec) and very long intervals regardless of changes in vigilance level (Steriade, Deschênes, & Oakson, 1974). All these characteristics rendering interneurons basically different from output cells have been confirmed in the statistical analyses of association cortex type I and type II neurons reported in the present article (see part 3).

2.3.3 "Injured" or "epileptic" cells? Since interneurons have been described as discharging in bursts with "inactivation" processes, much care should be taken to reject units mechanically damaged by the microelectrode, as cell injury creates conditions that may simulate the discharge pattern of an "interneuron." Fortunately, criteria for the recognition of a genuinely injured neuron are not lacking. Injury is certainly more frequently expected when impaling nerve cells than with extracellular recording. It is therefore not surprising that experimenters who use mostly intracellular electrodes (*a*) have more chances to miss small-sized interneurons, and (*b*) are susceptible to more anxiety when considering whether cells are "healthy" or "unhealthy." But it is obviously necessary to document clearly the "health" of units when describing interneuronal repetitive responses. This would imply rejection of elements exhibiting unusually high rates of discharge and illustrations with very fast sweeps, allowing one to detect other signs of injury such as notches on the first discharge in a high-frequency burst (the successive ones may sometimes be "normally" fragmented due to inactivation processes; see arrows in Figure 4C) and abnormal duration and configuration of spikes. An injured cell cannot sustain repetitive firing for periods longer than 10–15 min (Wyler & Fetz, 1974), while a study of changes in activity of a bursting interneuron during a full sleep-waking cycle requires long duration recordings (more than 1 hr). If these cells were "injured," it remains mysterious why they stopped bursting on awakening from sleep, continued to be silent for rather long periods of arousal (10 sec [Sakakura, 1968], 7–15 sec [Steriade, Deschênes, & Oakson, 1974]), and then progressively recovered the burst pattern with repeated transitions from W to S (see Figure 12). Besides these long-term recordings, an argument against the burst being a mere injury artifact is its modification by afferent driving (Calvin & Loeser, 1975). Furthermore, identified output cells do not exhibit burst responses, nor do they discharge spontaneously with spike bursts during W (Evarts, 1964; Steriade, Deschênes, & Oakson, 1974; Steriade et al., 1974) as interneurons do (Steriade, Deschênes, & Oakson, 1974; Steriade et al., 1974) (see also part 3). "Injury" is unlikely to be limited to interneurons.

Studies of the Seattle group (Calvin, Sypert, & Ward, 1968; Ward, 1969; Wyler, 1974; Wyler & Fetz, 1974; Wyler, Fetz, & Ward, 1975) devoted to epilepsy have reported bursting patterns in chronic alumina foci. As we did not attempt to render animals epileptic, our subjects did not exhibit spontaneously recurring focal and/or generalized motor seizures, nor did surface EEG or focal slow waves simultaneously recorded with unit discharges

from the cortical depth show EEG correlates of epilepsy. Nonetheless, it is possible that chronic experiments with multiple microelectrode penetrations might create conditions leading to increased glial forms and "partial denervation" of neuronal elements, which are thought to result in hyperactivity of epileptic cells (Ward, 1969). The histology of the explored cortical areas did not show evidence for this picture in our experiments. In fact, what seems sufficient to preclude structural epileptic changes in the recorded area is that *nonoutput bursting cells were recorded along the same track as antidromically identified, output, nonbursting cells.* In any event, there are several striking differences in rate and pattern between epileptic neurons and "healthy" interneurons. (*a*) The firing rate of epileptic cells (see Figure 10-2 of Ward, 1969) is incomparably higher than that of interneurons, and these recordings in epileptic monkeys "were carried out only when they were behaviorally awake" (Ward, 1969, p. 268), a state when interneurons are either silent or discharge at very low rates (see 3.1.2). (*b*) When comparing normal to epileptic cells, Calvin et al. (1968) found in the latter a "long first-interval" and a late mode due to the stereotyped silent periods between bursts, which are not defining features of interneurons.

There are certainly a few resemblances between interneuronal and epileptic bursts, and wyler et al. (1974; Wyler & Fetz, 1974; Wyler, Fetz, & Ward, 1975) have discussed our findings on cortical interneurons in relation to their own data on "epileptic" cells. Moreover, some authors have inferred a role for interneurons in the genesis of epileptic activity (Pollen, 1964), but, again, type II cells, believed to be perhaps the most important elements of the epileptic neuronal aggregate, remained inaccessible because of technical limitations in obtaining stable recordings from interneurons (Prince, 1972). I have reported (see 1973, 1974) that during drowsiness, when spontaneous firing and synaptic responsiveness of type II cells are enhanced, the spike barrages of about 1/5 of precentral interneurons in the normal, nonepileptic monkey become explosive as a consequence of VL thalamic stimulation, leading to development in the cortical depth of focal paroxysmal activity exhibiting a spikewave pattern, with poor or no reflection at the cortical surface. PT neurons recorded in the same region and even along the same track did not exhibit such paroxysms. Thus, "*normal* cells were brought into *epileptic* activities presumably as a consequence of both their membrane properties and their extensive connections favoring reverberation in closed neuronal chains" (Steriade, 1974, p. 259). The same conclusion was drawn in studies of cortical somatosensory interneurons (Steriade & Yossif, 1974) with respect to the progressive, unbroken transition between normal responses and pathologic, paroxysmal events, with a striking resemblance between configuration of responses in the final stage of stimulation and that of subsequently developing self-sustained, epileptic-like activity.

2.4 Output-interneuronal circuits

As discussed above, output cells, and, with much less certainty, interneurons can be identified by means of their response properties. These two cellular types reciprocally articulate to form complex circuits. The most extensively investigated interneuronal organization is that of the spinal cord, due to the work of the Swedish school in the last two decades. Yet, when asked what he means by "identified," Lundberg (1969, p. 245) emphasized that "great caution should be exercised in ascribing interneurons with a certain type of response as belonging to a given pathway." More recently, Jankowska (1975), who has contributed greatly to the morphological identification of intracellularly recorded spinal cord interneurons, has stressed the difficulties of axonal staining with Procion Yellow and concluded: "To define the role of these interneurons in a more complete way, however, requires fuller knowledge of their target cells and techniques allowing a detailed study of axonal projections" (p. 244).

If such is the case in the spinal cord, what will be found in the thalamus and cortex? The most popular circuit accounting for postsynaptic inhibition and rhythmically occurring spindle waves in specific thalamic nuclei (experiments done on VB) was based on excitation of local interneurons through the *recurrent collaterals* of thalamo-cortical axons, and synchronous IPSPs were believed to be elicited by ramification of interneurons acting back on thalamic output neurons through inhibitory synapses

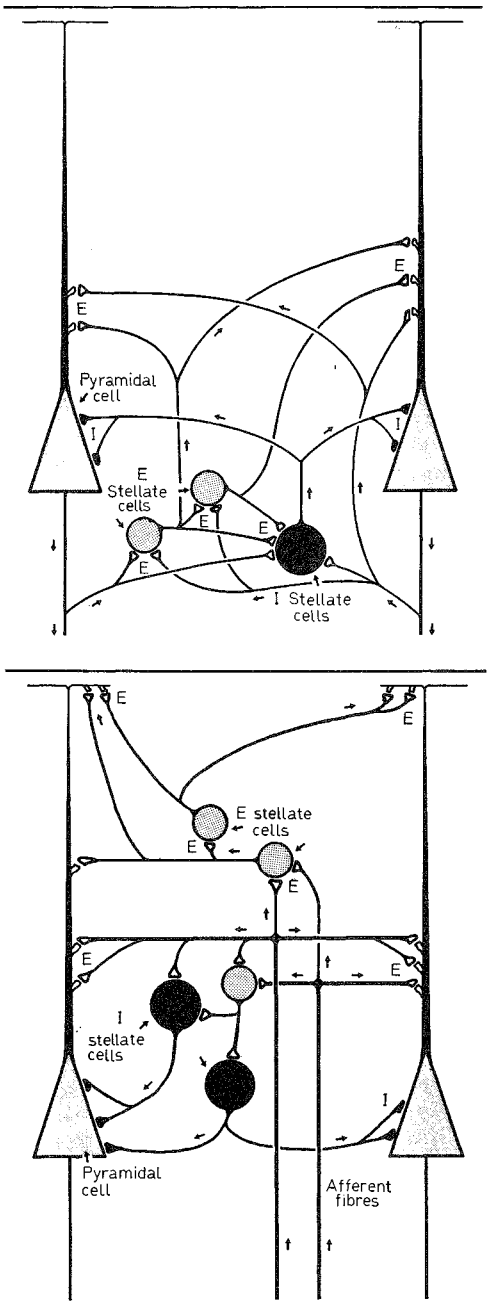

Figure 6. Diagrams showing postulated synaptic connections of axon recurrent collaterals of PT cells (top) and of specific afferent fibers (bottom). Inhibitory interneurons together with their inhibitory synapses on the somata of the PT cells are shown in black. All other stellate cells and the PT cells are assumed to be excitatory and are shown open. The arrows indicate directions of impulse propagation. Note that, as suggested by the experimental evidence, both the excitatory and inhibitory pathways can include interpolated excitatory interneurons (from Eccles, 1969, courtesy of Ch. C. Thomas).

on their soma (Andersen, Eccles, & Sears, 1964; Eccles, 1969). As far as the recurrent collaterals of thalamo-cortical axons are concerned, Scheibel et al. (1973) found them "in only 15 to 20 percent of all the cells . . . in the adult cat thalamus"; this would be "an inadequate substrate for the powerful recursive effects ascribed to them" (p. 302). In concurrent studies using Golgi technique in the lateralis posterior (LP) thalamic nucleus, "the initial collaterals of projection cells could be traced occasionally to Golgi type II interneurons; however, regarding the sparseness of this collateral arborization, it has little significance in the neuronal connectivity" (Hajdu, Somogyi, & Tömböl, 1974, p. 93). The same difficulties for the recurrent collateral inhibitory pathway arose from morphologic studies on the LG, which rather supported the feed-forward type of inhibition, with retinal afferents presynaptic to dendritic appendages of Golgi II cells (Pasik, et al., 1973).

The diagrams used to depict synaptic connections within output-interneuronal neo-(Eccles, 1969) and allocortical circuits (Spencer, 1969) almost invariably postulate type II cells excited by recurrent collaterals of pyramidal (type I) neurons. This can be seen in the operational diagrams of Figure 6, which illustrate the neocortical basic circuitry according to Eccles (1969). While the importance of recurrent collaterals of thalamo-cortical axons has been questioned, especially following the discovery of dendritic synapses established in thalamic nuclei by type II cells in almost all combinations (Famiglietti, 1970; Morest, 1971; Pasik, Pasik, & Hamori, 1976; Ralston & Herman, 1969; Scheibel, Davies, & Scheibel, 1973), the richness of synaptic contacts made by recurrent collaterals of PT neurons has been repeatedly mentioned, since Ramón y Cajal (1955), continuing with Lorente de Nó (1938) and other contemporary neuroanatomists (Scheibel & Scheibel, 1970; Szentágothai, 1975; Tömböl, 1975). The emphasis is on engagement of interneurons by recurrent collaterals of output cells, and on pericellular basketlike nests made by type II (inhibitory) synapses around the somata of type I neurons (Colonnier, 1966; Szentágothai, 1975). The shortest latency IPSPs are produced via a bisynaptic pathway, with an intercalated inhibitory interneuron, but, generally, inhibition is achieved by longer pathways, with excitatory interneurons preceding inhibitory ones (Figure 6).

Activity of *excitatory* interneurons activated by recurrent collaterals of PT axons and acting back on PT cells was thought to result in reexcitation of the latter (Chang, 1955). Unit recordings actually revealed "extra impulses" elicited at a few (6–10) msec following the PT-evoked antidromic spike (Li, 1958). However, without precautions to prevent spread of current from the PT to ascending pathways, the "extra impulses" might be ascribed to contamination of lemniscal fibers. The activity of excitatory interneurons is probably responsible for a period of increased responsiveness, lasting about 20–30 msec following an antidromic testing stimulation of the pes pedunculi, provided that this was achieved in a preparation with a transected medial lemniscus (Steriade & Yossif, 1977). It should be stressed that all these facts are suggestive only of the existence of excitatory type II cells interposed in the basic cortical circuit. The crucial evidence will come from experiments utilizing cellular stimulation and recording of cortical excitatory interneurons and output cells belonging to the same neuronal pool.

The same is valid for *inhibitory* interneurons. The close time-relation between high-frequency repetitive discharges of interneurons and a focal positive wave, reflecting summated hyperpolarizing potentials in the neuronal pool, is commonly regarded in the literature as indicative of the inhibitory nature of the recorded bursting interneurons. Since, however, *there is as yet no criterion to differentiate electrophysiologically excitatory from inhibitory type II cells, and as the former represent the most powerful source of activation of the latter,* some of the bursting cells might be considered as excitatory local neurons, driving the immediate progenitors of postsynaptic inhibition. Renaud and Kelly (1974) recorded from pairs of neighboring PT and non-PT cells and suggested, on the basis of reciprocal changes in these two neuronal types, the inhibitory nature of the latter. The latencies in their negative cross-correlograms ranged from 1.9 msec to 7.3 msec. If short latencies (<2 msec) are taken to imply monosynaptic inhibition of PT cells by non-PT cells, in the case of longer latencies non-PT elements may be regarded as type II excitatory cells driving inhibitory interneurons. The connections of excitatory with inhibitory type II cells certainly do not exhaust the yet undeciphered complexity of cortical neuronal circuits.

When looking at Eccles (1969) diagrams in Figure 6, one may be convinced that different interneurons are required for the feedback and feed-forward collateral mechanisms. The goal of those diagrams however is pedagogic. In fact, experiments conducted on precentral motor cortex of primates have revealed that *the same type II cell may subserve both recurrent and afferent collateral pathways* (Steriade, Deschênes, & Oakson, 1974). The two interneurons illustrated in Figure 7 could be driven from the pes pedunculi at latencies (<1.3 msec in A, <1 msec in B) indicating monosynaptic activation through recurrent collaterals of fast PT fibers, and both were also activated at slightly longer latencies (1.5–2.3 msec) from the specific thalamic nucleus. Greater latency of thalamically elicited repetitive discharges (compared to those evoked by peduncular shocks) precludes spread of current to the internal capsule.

Summing up: It is possible to identify cortical type I cells by

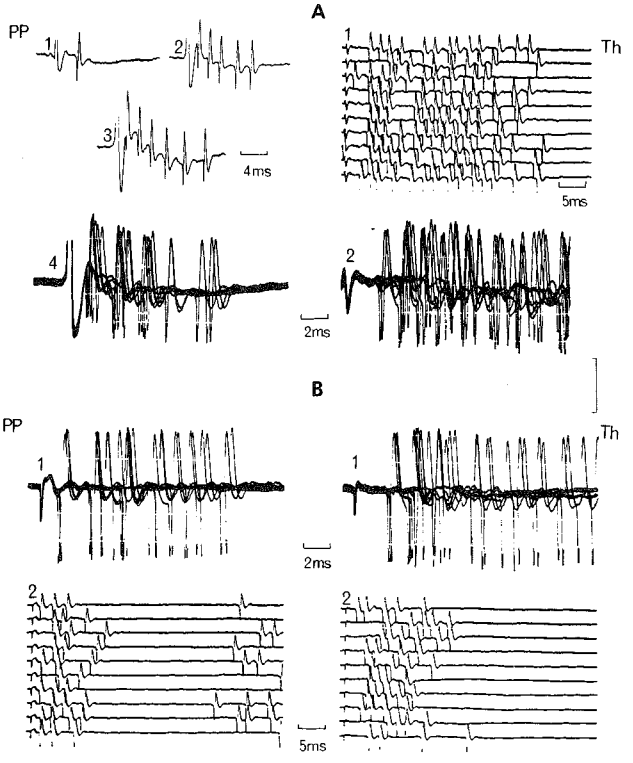

Figure 7. Monosynaptic activation of putative interneurons in precentral motor cortex of monkey by antidromic stimulation in the pes pedunculi (PP) and by thalamic (Th) stimulation at the border between VB and VL nuclei. A and B: two different interneurons. Left column in both cases is PP stimulation; right column, Th stimulation. A: the PP testing shock was delivered in 2 and 3 at a double and triple intensity relative to the liminal one used in 1; intensity for superimposition of several traces in 4 (spikes depicted with gain double of that in 1–3) was the same as in 2. Same unit (A) was driven from thalamus (Th); 10 successive sweeps (with reduced gain) and, below, superimposition of several traces. B: PP-evoked (left) and thalamically elicited (right) responses depicted in the top superimpositions (1) and, below, in 10-sweep sequences (2). Vertical bar (at right): 0.8 mV for A, 2 mV for B (modified from Steriade, Deschênes, & Oakson, 1974).

antidromic invasion, to know some of their target structure(s), and to estimate their size on the basis of the conduction velocities of their axons. Without morphological confirmation, the features of evoked and spontaneous firing are not sufficient to recognize unequivocally cortical type II cells; however, when *all* electrophysiological criteria are met, it can be strongly inferred that a locally ramifying neuron has been encountered. There is as yet no definitive sign to differentiate electrophysiologically an excitatory from an inhibitory type II cell in the cerebral cortex.

3 Cortical output cells and interneurons during the sleep-waking cycle

Our initial observation of the strikingly dissimilar alterations in the firing rate of cortical output cells and interneurons as a consequence of changes in vigilance level was made during experiments on *encéphale isolé* cats. An "arousing" stimulation of the

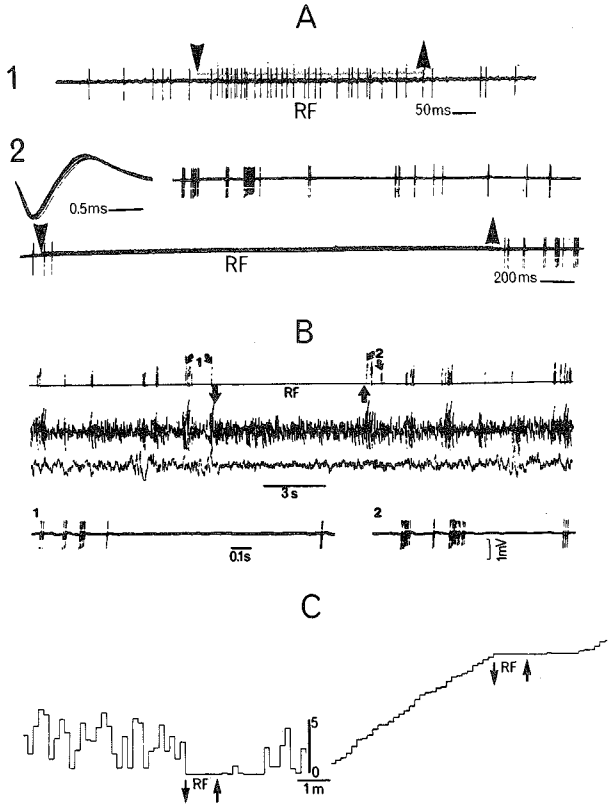

Figure 8. Opposite effects of brain-stem reticular formation (RF) stimulation on spontaneous activity of output cells and interneurons in motor, somatosensory and association cortices of cat. A: pyramidal tract (PT) cell (1) and putative interneuron (2) recorded from the precruciate motor cortex; RF stimulation (between arrows) increased PT spontaneous discharge from 25/sec to 75/sec and stopped interneuronal spike bursts; in 2, configuration of spikes in a burst were depicted (with double gain and at a very fast speed) to show that the bursting pattern was not due to injury of the cell (from Steriade et al., 1971). In B and C, effects of reticular-elicited EEG activation on spontaneous firing of cortical interneurons recorded from SI (B) and area 7 (C). The polygraph traces in B represent: unit spikes (periods 1 and 2, between small oblique arrows, correspond to those in the oscilloscopic traces below), focal slow waves recorded by the microelectrode, and surface EEG waves; note complete suppression of burst firing during high-frequency stimulation of the RF (unpublished data by Steriade and Yossif). The same suppressing effect of RF stimulation can be seen for the parietal association interneuron in C; it is illustrated in both forms of sequential mean rates (left) and cumulative histogram (right); note the after-effect of RF stimulation (unpublished data of Steriade, Kitsikis, and Oakson).

mesencephalic RF significantly increased the spontaneous discharge of pericruciate PT cells, but completely stopped high-frequency spike bursts of interneurons in the same area (Steriade et al., 1971) (Figure 8A). The enhanced excitability of cortical type I cells was further demonstrated by increased probability of antidromic invasion in PT cells during RF-elicited EEG activation compared to periods of EEG synchronization (Steriade, 1976). On the other hand, the arrest of firing in type II cells during RF stimulation was subsequently confirmed in primary somatosensory and parietal association areas (Figure 8, B–C). The observation that EEG activation is associated with *selective obliteration of VL-evoked discharges in PT cells at latencies of 5–10 msec, leaving intact the early (<5 msec) responses* also supported the hypothesis of depressed activity, during arousal, in those interneurons that relay the VL-fugal impulses before they reach the PT neurons (Steriade, Wyzinski, & Apostol, 1973).

The RF effects in acute experiments encouraged us to pursue these findings in behaving animals. For the last six years, research in our laboratory on chronically implanted primates and cats has shown the essentially different or even opposite changes in activity of output (PT, cortico-pontine and cortico-thalamic) and nonoutput neurons during the natural sleep-waking cycle. The next two sections (3.1 and 3.2) in this paper will summarize the results of our experiments on activity of type I and type II cortical cells recorded from motor and association areas during W, S and D. Since, prior to this, only Evarts (1964, 1965) has undertaken experiments on identified PT cells during sleep and waking, his results will be discussed in section 3.1, in relation to our own findings on type I cells in the precentral motor cortex. Other studies, dealing with nonidentified cortical cells, will be compared to our results at the beginning of section 3.3, before final comments on the mechanisms and possible significance of these changes in cortical cellular activity during the sleep-waking cycle.

3.1 Opposite behavior of type I and type II cells in wakefulness versus synchronized sleep

3.1.1 Type I cells. In his study on the precentral motor cortex of macaques, Evarts (1964) reported for fourteen investigated units that all PT neurons showed a reduction in frequency of spontaneous discharge in S compared to W. Although fast- and slow-conducting PT cells were not yet dissociated in that initial paper, it is likely that both neuronal subclasses were recorded and, in view of the microelectrode bias for recording large neurons, the majority of units found to be more active during W would be fast-conducting (large) cells. Surprisingly, a year later, when Evarts split his population according to antidromic response latencies, he reported that short-latency (therefore large PT) cells are "relatively inactive during W and speed up with S, whereas the reverse relationship was observed in the longer latency units" (1965, p. 224).

According to our own findings, the discrepancies in Evarts' results may be explained by significant differences seen in fast-conducting PT cells during "excited" versus "relaxed" epochs of W (Steriade, Deschênes, & Oakson, 1974; Steriade et al., 1974). Actually, all thirty-six of our slow-conducting cells and twenty-six of our thirty-one fast-conducting PT cells in the arm area of the monkey's motor cortex discharged during the *quiet* state of steady W at rates from 1.7 to 4 times higher than the values found in the same neurons during S (Steriade, Deschênes, & Oakson, 1974). However, when we analyzed the short-lasting period of *arousal* from S and phasic periods of *excited* W due to orienting reactions, changes in spontaneous firing rates of fast- and slow-conducting PT cells contrasted with one another. Fast-conducting units stopped firing or markedly decreased their rates during the first 10 sec of arousal, while slow-conducting PT cells increased firing rates upon arousal from S (Figure 9A). The sudden silence or decreased firing in fast-conducting PT cells upon

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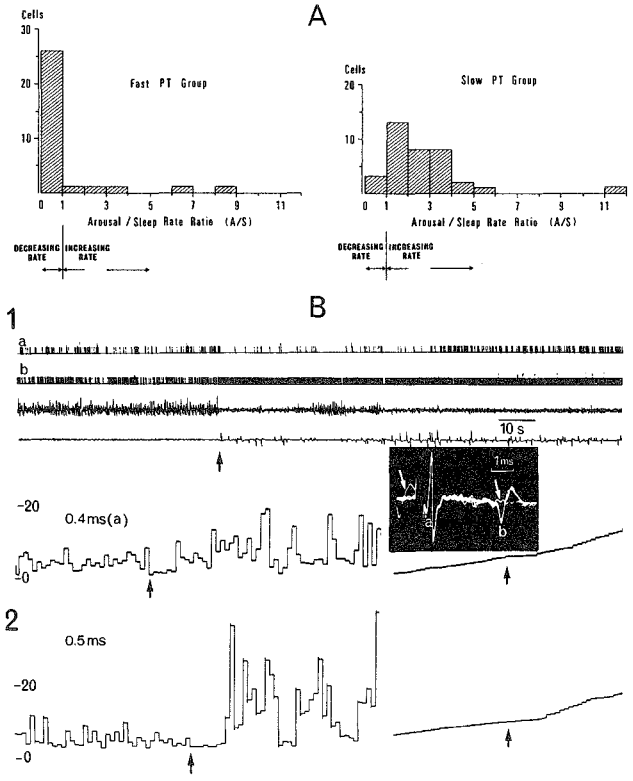

Figure 9. Changes in spontaneous discharge of 67 PT neurons in motor cortex of monkey on arousal from slow sleep. The histograms in A show the contrast between fast-conducting PT neurons (antidromic response latencies: 0.3–0.8 msec) and slow-conducting PT units (latency of antidromic responses: 0.9–5 msec), as far as the arousal/sleep (A/S) ratios were concerned. A/S ratios were computed in each neuron from spontaneous discharges during the first 10 sec of arousal and during the 10 sec of sleep immediately preceding arousal. Of 31 fast-conducting cells, 13 stopped firing completely and 13 exhibited A/S ratios between 0.02 and 0.51, while A/S ratios exceeding 1 (usually 1 to 4) were found in 33 of 36 slow-conducting PT cells. In B, sequential mean rates (left) and cumulative histograms (right) of two (1 and 2) fast-conducting PT cells of monkey during slow sleep, arousal (arrows), and subsequent steady state of quiet waking. Values on the ordinate indicate number of spikes per second; 10-sec bins. Antidromic response latencies indicated in each case at left. In B_1, the fast-conducting PT cell *a* (0.4 msec antidromic response latency) was simultaneously recorded with the slow-conducting PT cell *b* (arrows in the insert indicate a spontaneous discharge of the *b* unit and the corresponding lack of its antidromic response). The upper polygraph traces represent spontaneous firing of separated discharges of units *a* and *b*, EEG waves, and ocular movements. Note: increased firing of unit *b* from the very beginning of arousal (arrow); much lower mean rate of discharge in unit *a* during the first minute following arousal (compared to sleep) but, afterwards, during steady waking, increase of its firing rate over the value seen during sleep, as can also be observed below in sequential mean rate (left) and cumulative histogram (right). Similar events in the fast-conducting PT cell (0.5 msec latency) of B_2 (modified from Steriade, Deschênes, & Oakson, 1974).

arousal could continue for a longer period (1–2 min) when the experimenter deliberately kept the animal very alert (Steriade, Deschênes, & Oakson, 1974). Afterwards, during quiet W, the rate of spontaneous firing of fast-conducting PT cells (like that of slow-conducting units) was higher than during a preceding period of S (Figure 9B).[3] These findings suggest that the decreased firing rate during W, as reported by Evarts (1965) in fast-conducting PT neurons, is attributable to alertness in his preparations.

The above results were corroborated by investigating *evoked discharges* in output neurons. *An enhanced probability of antidromic invasion was seen during W compared to S in all subclasses of output cells* in monkey's precentral motor cortex: fast or slow PT neurons, and cortico-thalamic cells (Steriade, 1976; Steriade, Deschênes, & Oakson, 1974; Steriade et al., 1974). Moreover, significant alterations could be observed in the pattern of antidromic spikes, with IS-SD split discharges during S sleep and full recovery of the first spike in the train, together with accelerated soma invasion, during W (Steriade & Deschênes,1973; Steriade, Deschênes, & Oakson, 1974). Testing synaptically evoked responses to paired shocks revealed very effective inhibition in PT cells during W, but with much shorter duration compared to S (Steriade & Deschênes, 1974). *Deep but short inhibition during W shows that this state provides neuronal organization leading to accuracy in the analysis of excitatory inputs and to ability to follow rapidly recurring activity.*

The choice of the motor cortex as the first region in which identified cortical output cells were investigated was not essentially dictated by theoretical considerations concerning sleep and waking, but by methodological and technical considerations, namely, the ease of implantation of a stimulating electrode in a compact bundle of fibers in the pes pedunculi or the medulla (PT), allowing antidromic invasion of the main source of cortical outflow. The picture of type I cell activity in motor cortex, as noted above, could not, however, necessarily be extrapolated to cortical fields outside the classical motor area without direct evidence, implying identification of output cells and investigation of their spontaneous and evoked activities during the sleep-waking cycle. The association areas, whose loss may lead to a deficit in acquisition and/or retention of complex learning tasks (Iversen, 1973), seemed particularly interesting to explore in order to throw some light on sleep-waking behavior of different types of cells involved in highly integrative processes (see part 1). Since there is as yet no available study on association cortical neurons identified antidromically from various target structures, we first studied the input-output organization of parietal association areas 5 and 7 in the cat. Two principal cortico-subcortical circuits have been thus disclosed (Figure 10).

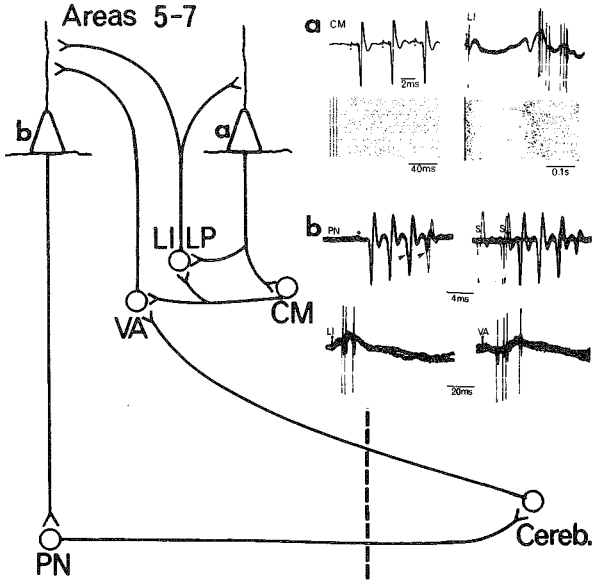

Figure 10. Cortico-thalamic and cortico-pontine circuits involving output cells in parietal association areas 5 and 7 of cat. Neuron *a* was antidromically invaded from the CM thalamic nucleus (three-shock train) and synaptically excited from the LI thalamic nucleus; note in dotgrams much more powerful suppression of firing following afferent LI stimulation. Neuron *b* was antidromically invaded by stimulating the pontine nuclei (PN) with a four-shock train (first shock marked by dot) and convergently modulated from the LI and ventralis anterior (VA) thalamic nuclei; note IS-SD breaks and IS spikes in isolation (arrows) and collisions with preceding spontaneous (S) discharges (modified from Steriade, Kitsikis, & Oakson, 1978).

In one of them, afferents from the LI-LP thalamic complex reach mainly cortical cells projecting to the CM thalamic nucleus (Steriade, Kitsikis, & Oakson, 1978); the LI or LP-cortico-CM pathway is completed at thalamic levels by direct linkages between CM and LI or LP neurons (Steriade et al., 1977). In the other circuit, afferents from the VA thalamus or converging inputs from VA and LI nuclei articulate with cortico-pontine neurons (Figure 10) (Steriade, Kitsikis, & Oakson, 1978). The proportion of antidromically identified corticofugal cells in areas 5 and 7 (101 of 239 responsive cells) was unexpectedly high, especially for association cortex, suggesting that the functions of parietal association areas depend greatly on downstream projections to the integrative CM and LI-LP thalamic nuclei, and to the ponto-cerebellar system.

Cortico-thalamic and cortico-pontine (type I) cells of areas 5 and 7 discharged at significantly higher rates ($P \leq 0.0001$, Wilcoxon unpaired rank test) compared to nonoutput (type II) cells recorded from the same association areas. The majority of output neurons discharged at rates between 8 and 32/sec in W and S, while the majority of interneurons discharged, in both W and S, at less than 1/sec.

We computed weighted pooled mean rates for each of the output neurons by summing the number of spikes for all epochs in a given state and dividing by the sum of the epoch durations. The nonweighted sample mean rate for each state was then computed by averaging all cell mean rates in that state. Ten of twelve output cells decreased firing rates in S compared to W. The mean rate of output cells was 12.97/sec in W and 10.35/sec in S (median 11.00/sec in W, 9.53/sec in S), the change from W to S being significant ($P \leq 0.05$) and, as shown below, opposite to that of type II cells (Steriade, Oakson & Kitsikis, 1978).

The decrease in discharge frequency of output cells during S compared to W was even more dramatic during transitional periods from W to S (Figure 11). The first, distinct EEG spindle

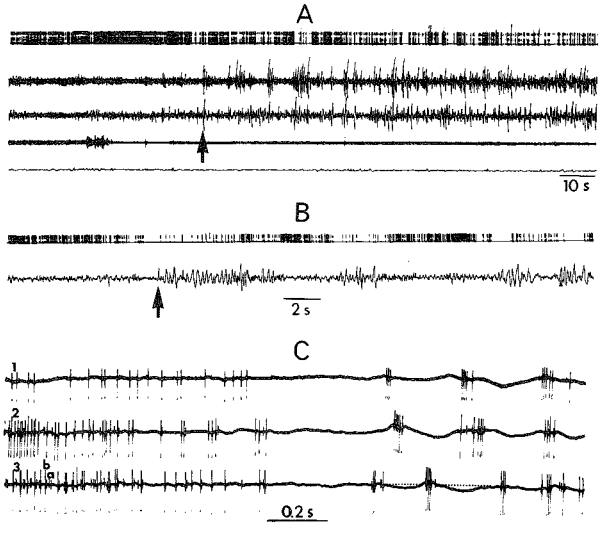

Figure 11. Changes in neuronal firing of three parietal association output (A to C) cells with transition from W to S. Latencies of antidromic invasion of cortico-CM (A, and cells *a* and *b* in C) and cortico-LI (B) neurons: 1 msec in A; 3.5 msec in B; and 2 msec (neuron *a*) and 0.9 msec (neuron *b*) in C. The five polygraph traces in A depict unit discharges, focal slow waves simultaneously recorded by the microelectrode, EEG from the depth of the visual cortex, EMG, and eye movements. In B, polygraphic unit spikes and simultaneously recorded focal slow waves. In C, two (small, *a*, and big, *b*,) simultaneously recorded discharges during three (1 to 3) W→S transitions in successive sleep-waking cycles; note increased amplitude of both discharges from periods 1 to 3, separated by about 70 minutes; dotted line in 3 tentatively indicates the base line. Positivity downwards. Vertical arrows in A and B mark the first sharp wave in a series of spindles at EEG synchronization onset. Further description and comments in text (modified from Steriade, Oakson, and Kitsikis, 1978).

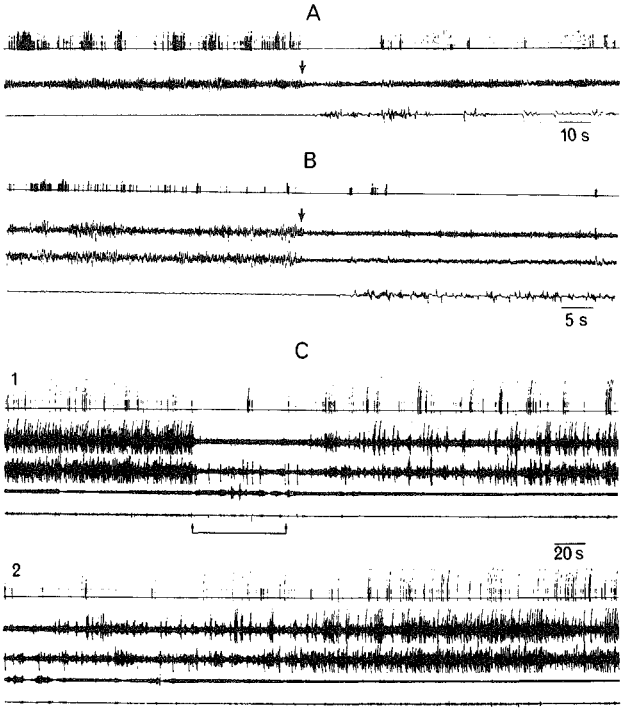

Figure 12. Arrest of firing of precentral motor cortex (A-B) and parietal association (C) interneurons of monkey and cat on behavioral arousal from synchronized sleep. The four polygraph traces in B represent from top to bottom: unit spikes (displayed on the oscilloscope and used to deflect the pen of the EEG machine; deflections exceeding the lowest level represent bursts of high-frequency discharges), EEG focal waves recorded by the same microelectrode and adequately amplified, surface EEG perirolandic waves, and ocular movements. In A, only surface EEG waves are depicted. Neuron A was driven by peduncular stimulation producing a barrage of 3–4 spikes at 500/sec, the shortest latency being 2.0 msec. Neuron B was driven by both peduncular and specific thalamic stimulation producing a barrage at 350/sec occurring at 2- to 3-msec latencies. In both cases, the vertical arrows indicate arousal elicited by the experimenter. Note: very frequent spike clusters during slow sleep; arrest of spontaneous discharge on arousal; decrease in background firing could also be seen subsequent to short-lasting arousal, when the two units resumed their activity, but at a significantly lower level than that seen during sleep (A and B: from Steriade, Deschênes, & Oakson, 1974). C: an interneuron recorded in area 5 of cat, driven by LP thalamic stimulation with a 500/sec barrage at a latency of 6 msec. The five polygraph traces depict: unit discharges, focal EEG waves, surface EEG waves, EMG and ocular movements. Note in 1: striking diminution of spontaneous firing during arousal from slow sleep elicited by auditory stimulation (between arrows); in 2, progressively increased occurrence of spike bursts with transition from wakefulness to slow sleep (modified from Steriade, Oakson, and Kitsikis, 1978).

sequence over a background of desynchronized EEG was *preceded* (median value in twenty-one desynchronization-synchronization transitions: 1.75 s) by decreased firing rate (slowing to 1/4–1/7 of the level seen in W), leading to a short period of complete neuronal silence (Figure 11B). Thereafter, high-frequency repetitive discharges occurred on the negative component of the focal EEG spindle waves, followed by suppressed firing during the long-lasting depth-positive shift (Figure 11C).

The change in firing rate of identified output cells during W and S is, therefore, similar in motor (Steriade, Deschênes, & Oakson, 1974; Steriade et al., 1974) and parietal association cortices (Steriade, Oakson & Kitsikis, 1978). Higher firing rates of slow- and fast-conducting output cells during W were accompanied in association areas, as in motor cortex, with sustained discharge, resulting in a distribution of interspike intervals exhibiting a lower probability of short and long intervals than

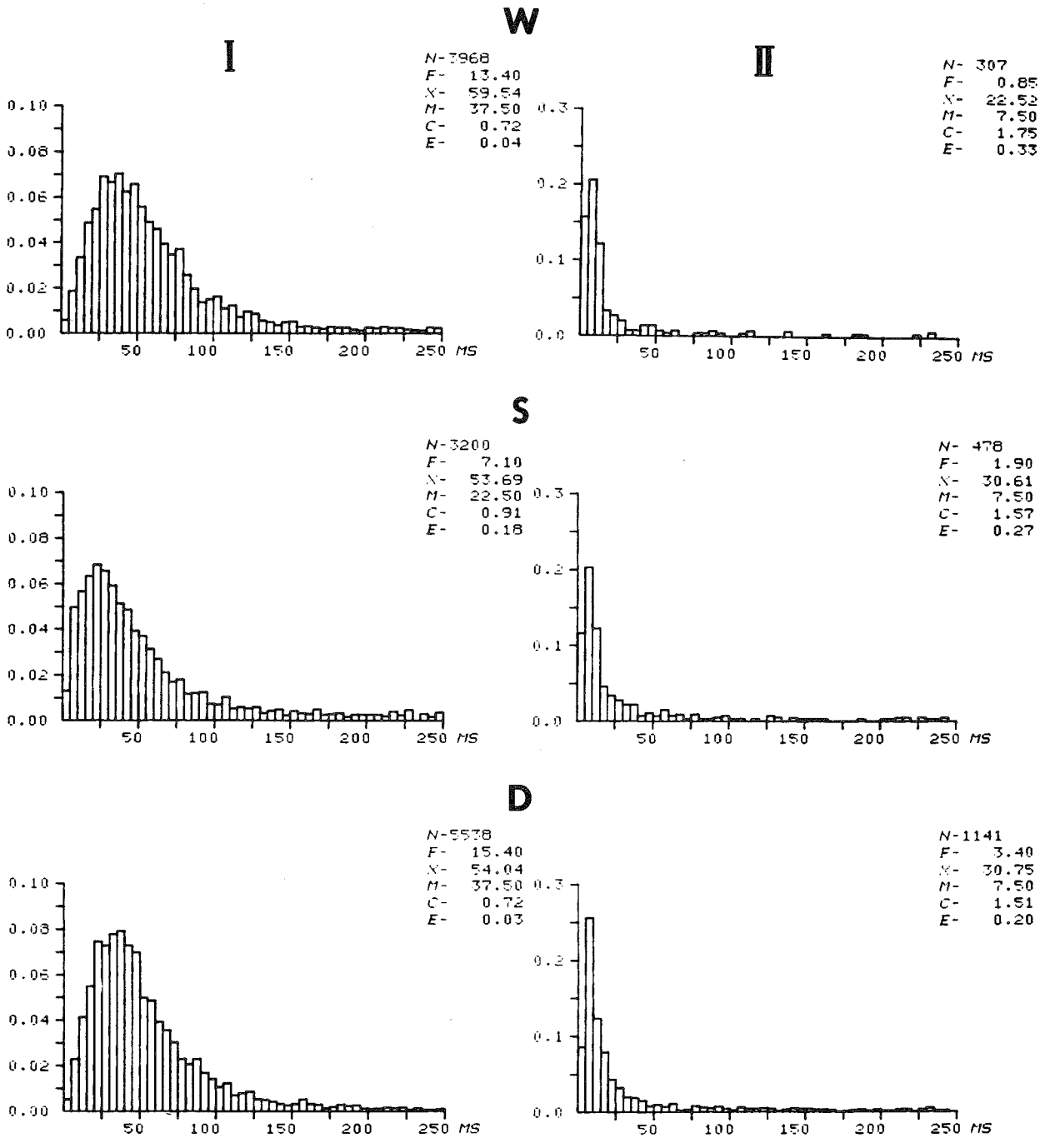

Figure 13. Distribution of interspike intervals in a cortico-thalamic cell (I) and an interneuron (II) recorded from the cat's parietal association cortex during the sleep-waking cycle. Ordinate shows probability of different classes of intervals, as indicated, in milliseconds (5 msec-bins) on abscissa. Symbols: N = number of intervals; F = mean-frequency (spikes per second); X = mean interval; M = modal interval, C = coefficient of variation; E = intervals in excess of the depicted time range. Note that the output cell exhibited 4.5 to 6 times more intervals in excess of 250 msec in S than in W or D; also note that, in contrast with the output cell, the interneuron had about a half of its total number of intervals in the 0–15 msec bins, and much greater probability of long (>250 msec) intervals during all states (unpublished data by Steriade, Oakson, and Kitsikis).

seen during S (Figures 13–14). The change from W to S in the mean modal interval for a group of PT cells recorded from the monkey's precentral motor cortex (Steriade, Deschênes, & Oakson, 1974) was found to be qualitatively similar to that of cortico-thalamic and cortico-pontine cells in the cat's association areas (Steriade, Oakson, & Kitsikis, 1978). Statistical testing of differences between firing patterns of output cells in W and sleep states, as well as between firing patterns of type I and type II neurons in the same state, was performed on parietal association cells using a method developed in our laboratory by G. Oakson and derived from the Wilcoxon rank tests (Steriade, Oakson, & Kitsikis, 1978). These pattern differences are discussed in section 3.1.2 for W and S states, and in section 3.3.3 for some aspects of the D state.

3.1.2 Type II cells. In spite of the great heterogeneity of interneurons (see part 1), we have found these bursting cells to be surprisingly homogenous with regard to rate and pattern changes during the sleep-waking cycle. Spontaneous or sensory-elicited arousal was invariably associated with complete arrest of

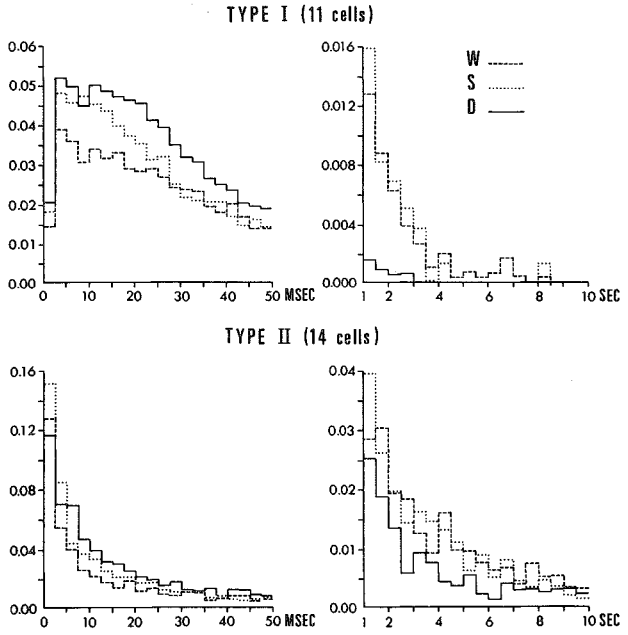

Figure 14. The mean interspike interval histograms as computed on a bin-by-bin basis from individual cell histograms are shown for 11 type I and 14 type II cells. The mean probability of interspike intervals is indicated on the ordinate. Abscissae at left indicate bin widths of 2.5 msec (covering a range from 0 to 50 msec) and at right bin widths of 0.5 sec (covering a range from 1 sec to 10 sec) (unpublished data of Steriade, Oakson, and Kitsikis).

background firing, lasting up to 30 sec, in precentral motor cortex (Steriade, 1976; Steriade, Deschênes, & Oakson, 1974) and in parietal association areas (Figure 12). Simultaneously, the synaptically evoked high-frequency bursts showed, upon arousal, a striking reduction in the number of spikes and a progressive lengthening of response latencies, compared with those seen during a prior period of S sleep (Steriade & Deschênes, 1973; Steriade, Deschênes, & Oakson, 1974). When the steady state of quiet W followed the short-lasting period of arousal, spontaneous firing rates were lower than those observed during S. Thereafter, with transition from W to S, interneurons showed a progressively increased occurrence of high-frequency spike bursts (Figure 12). In a sample of twenty-three interneurons recorded from association areas 5 and 7 during at least one complete sleep-waking cycle, twenty units increased firing rates in S compared to W (Steriade, Oakson & Kitsikis, 1978). The mean firing rate of the sample was 0.33/sec in W and 0.66/sec in S (median values: 0.21/sec and 0.58/sec). Compared with the significant *decrease* in firing rate of parietal association output cells from W to S ($P \leq 0.05$), interneurons *increased* their firing rates spectacularly from W to S ($P \leq 0.001$).

The essentially opposite direction of change in firing rates of type II cells during W and S, compared with type I neurons, was associated with strikingly dissimilar firing patterns in these two cellular classes. While, as a rule, output cells were found to discharge in a sustained manner during the activated state of W (and D), the spontaneous firing of interneurons was characterized by high-frequency bursts separated by very long periods of silence (0.5 – 10 sec) regardless of changes in the vigilance state. We made this observation first in the precentral motor cortex of the monkey (Steriade, Deschênes, & Oakson, 1974) and have recently extended it, with statistical analyses, to interneurons recorded from areas 5 and 7 of the cat (Steriade, Oakson, & Kitsikis, 1978). From the latter sample of type II cells, some discharged in stereotyped bursts at 400–600/sec during W and both S-D sleep stages (See Figure 15), exhibiting the majority of interspike intervals in a single 1 msec-bin, the mode (2.5 msec) reflecting the high intraburst frequency; other interneurons had less structured and lower frequency bursts (100–300/sec), with 25–50 percent of the intervals between 0 and 15 msec (Figure 13). These concentrated distributions were never seen in output cells. The mean interval modes in cortical association interneurons were 4.9 msec in W and 4.1 msec in S, much lower than those for output cells ($P \leq 0.02$, Wilcoxon unpaired rank test).

The pooled mean probability of interspike intervals on a bin-by-bin basis in 11 type I and 14 type II cells of parietal association cortex (Figure 14) showed (*a*) higher probability of short (<20 msec) and long (300–1500 msec) intervals in type I neurons during S compared with W and (*b*) higher probability of very short and very long intervals during both S and W in type II compared with type I cells: 8–9 times more intervals shorter than 2.5 msec and 3 times more intervals between 1 and 3 sec.

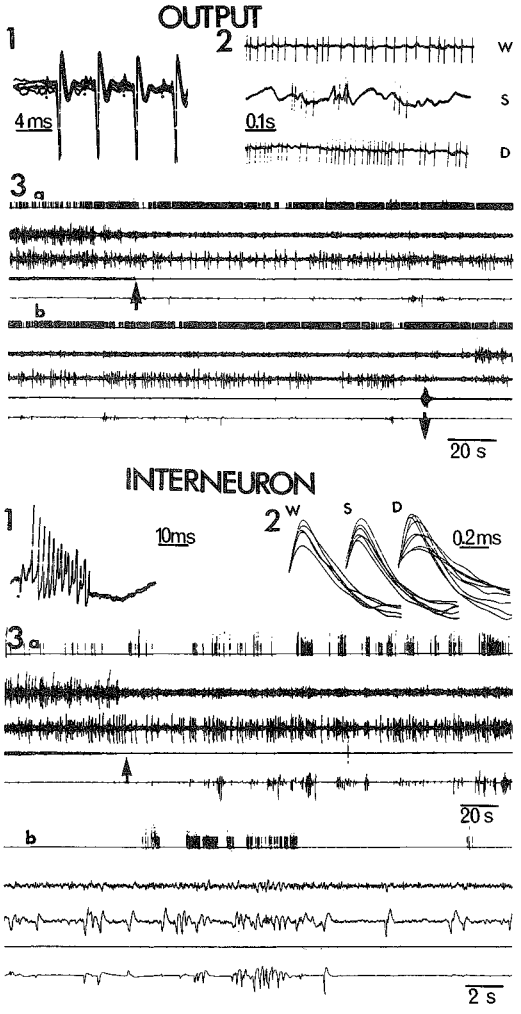

Figure 15. Discharge features of output cells and interneurons recorded from the parietal association cortex of cat. In both cases, 1: response patterns, 2: spontaneous discharge patterns, and 3: rate changes in D sleep. The five polygraph traces in 3 represent from top to bottom: unit spikes, slow waves simultaneously recorded by the microelectrode ("focal" EEG), EEG recorded from the depth of the visual cortex, EMG, and eye movements. An output cell was antidromically activated from the center median thalamic nucleus (1); note sustained spontaneous firing in W and D (2); 3a is separated from 3b by a nondepicted period of 140 sec; onset of D sleep in 3a and awakening in 3b are indicated by arrows; *note increased firing rate during the whole D period.* An interneuron was synaptically driven from the lateralis posterior thalamic complex with a high-frequency barrage (1); note stereotyped spike bursts during W, S and D (2); *note closely REM-related firing in D sleep* beginning at the arrow (3); in *b*, a detail at faster speed during saccadic REMs (unpublished data by Steriade, Kitsikis, and Oakson).

3.2 Type II cortical association cells increase their discharge in D sleep selectively during REMs

All previous studies on nonidentified cortical and thalamic cells have unequivocally reported increased firing rates during D sleep, over those during W and S. Some investigators did not find prominent REM-associated discharge in cortical units, but in most cases the high D sleep firing rates were *in part* a function of REM-related discharge (Steriade & Hobson, 1976). This would imply that phasic excitation during REM epochs is superimposed against a tonic background of ascending activation during the whole D stage.

We analyzed in the parietal association cortex of the cat eight type I (cortico-thalamic and cortico-pontine) cells and twenty-three type II neurons during at least a complete sleep-waking cycle, including D for which REM data were available, and catalogued all REM and non-REM (including poor-REM) epochs having durations greater than 5 sec. Figure 15 depicts typical examples of a type I (fast-conducting cortico-CM neuron) and a type II (LI-synaptically driven) bursting cell. It is obvious that the output neuron increased its firing rate about 20 sec before complete EMG suppression and exhibited a tonically increased discharge frequency throughout the D stage,[4] apparently independent of phasic ocular events. In contrast, the interneuron discharged during D in strikingly close temporal relation with REM epochs and was silent in most non-REM or poor-REM epochs.

The rate differences between D+ (REM) and D− (non-REM), and between each of these substates and W or S, were tested for significance for each cellular type (Wilcoxon paired signed-rank test). In addition, the two cellular types were compared statistically with regard to percent change of rate in the various state pairs (Wilcoxon two-sample rank test). The salient features of rate changes between cellular types shown in Table I (Steriade, Oakson & Kitsikis, 1978) are the following: (*a*) output cells increased while interneurons decreased firing rate from S to D−, the difference between these cellular types being highly significant ($P \leq 0.004$); (*b*) both output cells and interneurons increased firing rate from D− to D+, but the difference between percentage rate changes in these two cellular types was highly significant ($P \leq 0.005$).

Summing up: The increased firing rates of output cells in D over those in W and S are substantially due to tonic excitation during this state, and phasic ocular events only contribute to a further increase of discharge frequencies; in contrast, the increased rates of discharge in interneurons during D is entirely ascribable to REM-related firing.

Table 1. *Rate change and percent rate change data of 8 output cells and 23 interneurons in D sleep*

	Output		Interneurons		
State pairs	N	MED %	N	MED %	$P_{MED} \leq$
D−→D+	8/8↑	17	21/23↑	153	0.005*
W→D−	7/8↑	48	16/23↑	74	>0.5
S→D−	8/8↑	97	15/23↓	−22	0.004*
W→D+	8/8↑	85	20/23↑	364	0.09
S→D+	8/8↑	143	18/23↑	75	0.2
W→D	8/8↑	63	19/23↑	225	0.2
S→D	8/8↑	129	14/23↑	21	0.05*

State pairs compared are not contiguous transitions. N denotes fraction of cells changing rate when comparing first with second state in state pair. MED is median of percent rate changes calculated for all cells (negative sign denotes decreasing rate in second state of pair). P_{MED} give significance level for comparison of percent rate changes between the two cellular classes (Wilcoxon two-sample rank test).
*Significance level: $P \leq 0.05$.

3.3 Comments on possible mechanisms

3.3.1 Comparison of our data with previous studies on nonidentified cortical neurons. Before commenting on possible mechanisms of differential alteration undergone by type I and type II cells, I feel it necessary to discuss data from studies by Noda and Adey, Hobson and McCarley and Evarts et al. on sleep-waking behavior of nonidentified cortical cells recorded from nonmotor areas, which are particularly relevant to our recent experiments on the association cortex. Other sleep-waking studies on nonidentified cortical cells have been reviewed in a recent monograph (Steriade & Hobson, 1976).

In a series of four papers (1970a, b, c, 1973), Noda and Adey have presented analyses of firing rates and patterns of neurons in the cat's suprasylvian gyrus at a level that corresponds to our own recordings. They reported that an impressive majority (85 percent) of neurons decreased their firing rates from W to S, and about 95 percent increased the discharge rate from S to D (1970c). (Similar changes in direction have been found in parietal association cortex of the monkey [Desiraju, 1972].) In Figure 5 of Noda and Adey's paper (1970c), showing the discharge rate of single cells during various behavioral states, the group mean ($\bar{X}$) of the distribution of mean discharge rates is 13/sec in W, 10/sec in S, and 22/sec in D. These values are almost identical to those found in our identified type I neurons (see section 3.1.1). That Noda and Adey (1970b) probably recorded the activity of type I neurons is also indicated by the interspike interval histograms showing a greater probability of long (>200 msec) intervals during S than during either W or D (see Figure 2 in their paper), which corresponds to the temporal pattern of discharge found in our output, cortico-thalamic and cortico-pontine cells (Figures 13-14). *What is the reason for such homogenous changes, reflecting in fact the activity of type I cells, reported by Noda and Adey?* The answer may be found in their own papers, which indicate that a great number of units were discarded because their discharge rate was very low ("extremely inactive, discharge rate 0.1-1/sec" [1970c, p. 272]). Their technique, therefore, "yielded samples of neurons which were biased in favor of more spontaneously active cells" (1970c, p. 272). In the very slowly discharging units, firing "pattern was characterized by prolonged bursts of high frequency regular firings alternating with long periods of silence" (1970c, p. 266; 1973, p. 246). In other words, *the discarded units were probably type II cells.* It is perhaps worth mentioning that this conclusion seems to be supported by the fact that Noda and Adey "were not successful in sampling these units because of difficulty in approaching them with a microelectrode without irreparable damage" (1970c, p. 272). This is actually the difficulty experienced by most investigators in recording activity of interneurons (2.2).

The studies by Evarts et al. (1962) and Hobson and McCarley (1971) concern neuronal activity in the visual (postero-lateral) cortex and, in Evarts's (1962) paper, the suprasylvian gyrus, too. Comparing our results with their data, we might assume that "fast discharging" and "slow discharging" neurons described in their studies behave like our output and nonoutput cells, respectively, since "fast discharging" cells decreased firing rates from W to S, whereas the opposite was observed for neurons discharging at low rates; and the increased rate of "slow discharging" cells from S to D was entirely accounted for by the increased firing accompanying phasic ocular events in Hobson & McCarley (1971).

3.3.2 Reciprocal changes in type I and type II cells during W and S. A simple hypothesis to explain the opposite behavior of type I and type II cells in W and S would be that type II cells inhibit output neurons. This explanation, which would perpetuate the habit of equating interneurons and their inhibitory attribute, may have the advantage of accounting for (*a*) reciprocal changes in spontaneous firing of type II versus type I cells during S and W and (*b*) longer periods of thalamically elicited inhibition of

synaptic responsiveness in cortical output cells during S (Steriade & Deschênes, 1974; Steriade et al., 1974) when interneurons are more active than in W. These correlations are in line with the study by Purpura et al. (1966) showing reticular-induced blockade of IPSPs in VL thalamic cells, which suggests "inhibition of inhibition" on arousal, and are consistent with the observations of Jasper et al. (1965) on gamma-aminobutyric acid (GABA), one of the best candidates for cortical inhibition: the rate of release of GABA from the cerebral cortex being three times higher during S than during W.

However, some other findings prompt us also to consider the diminished activity of *excitatory* interneurons upon arousal from S: (*a*) selective obliteration, during spontaneous or reticular-induced EEG activation, of responses evoked by thalamic or cortical stimuli at latencies suggesting that they were interneuronally mediated, leaving intact monosynaptically evoked discharges (Steriade, 1969, 1973, 1976; Steriade, Wyzinski, & Apostol, 1973); and (*b*) arrest of spontaneous firing in fast-conducting PT cells upon behavioral arousal, simultaneous with their increased antidromic responsiveness (Steriade & Deschênes, 1973; Steriade, Deschênes, & Oakson, 1974). As to the latter point, the phasic silence of large PT neurons upon arousal can be ascribed neither to postsynaptic inhibition, since the same neurons exhibit enhanced susceptibility to be backfired, nor to disfacilitation along the specific thalamocortical excitatory pathway, since arousal is associated in VL output cells with strikingly increased firing rates even exceeding those seen during the subsequent state of steady W (Lamarre et al., 1971; Steriade, Apostol, & Oakson, 1971). The suggestion has been made that arrest of firing in fast PT cells may result from removal, during arousal, of powerful depolarizing pressure exerted by cortical excitatory interneurons (Steriade, Deschênes, & Oakson, 1974). The large PT neurons, with lower membrane resistance than small-sized PT cells (Takahashi, 1965), might indeed be particularly susceptible to this removal of excitation since they require repetitive discharges of local interneurons to be set in motion.

The possibility is therefore open that arousal from S, or any form of phasically increased level of vigilance, is associated with a decrease in high-frequency repetitive discharges, a pattern that may characterize both inhibitory *and* excitatory type II cells. Some findings support this suggestion. It is known that acetylcholine is released effectively during arousal and EEG desynchrony induced by stimulating the brain-stem RF (Celesia & Jasper, 1966; Krnjević et al., 1971; Phillis, 1968; Szerb, 1967). Knowledge of the intimate mechanisms of ACh excitatory action in the cerebral cortex comes from studies combining intracellular recording with extracellular iontophoresis and were summarized by Krnjević (1974, pp. 437–39). But inhibitory actions of ACh have also been described following brain-stem stimulation, and these have mainly been found in the upper cortical layers (Jordan & Phillis, 1972; Krnjević et al., 1971). Krnjević (1974) has recently observed that this postulated inhibitory cholinergic system particularly affects "cells that tend to discharge spontaneously in high-frequency bursts" (p. 440), whereas ACh excitation is exerted on deeper cortical, PT-type, neurons (Phillis & York, 1968). The RF influence, probably mediated by ACh, is actually exerted in an opposite way on PT cells and bursting interneurons (Figure 8A). Two years ago, Ben-Ari et al. (1976a, b) convincingly demonstrated that iontophoretic ACh excites neurons in the VB thalamic nucleus but inhibits almost every spontaneously active unit in the thalamic reticularis (Re) nucleus. The exclusively inhibitory action on Re cells could be elicited by a short, low amplitude pulse of ACh and developed with a latency that was very much shorter than that of ACh-induced excitation in VB neurons. It is well known that Re cells discharge with long-duration, high-frequency spike barrages (Frigyesi, 1972; Lamarre, Filion, & Cordeau, 1971; Massion, Angaut, & Albe-Fessard, 1965; Purpura & Cohen, 1962; Schlag & Waszak, 1971; Steriade & Wyzinski, 1972; Steriade, Wyzinski, & Apostol, 1972; Waszak, 1973), like some inhibitory interneurons.

The Scheibels (1966, 1970) hypothesized that the Re nucleus exerts widespread inhibitory effects on various thalamic nuclei. Since the axons are distributed outside the field of the Re nucleus (Minderhoud, 1971; Scheibel & Scheibel, 1966), this would be an inhibition induced by a long-axoned element, like that mediated by some spinal cord interneurons (Jankowska, 1975) or by cerebellar Purkinje neurons (Ito & Yoshida, 1966). One might alternatively conceive that the spike bursts of some Re neurons may exert these effects by exciting inhibitory interneurons in specific thalamic nuclei (see Steriade and Hobson 1976, pp. 224–27). In any case, the studies by Ben-Ari et al. (1976 a,b) strongly indicate that the same chemical agent, commonly viewed as a synaptic transmitter of the "arousing" reticulofugal fibers, excites VB cells of relay type and inhibits high-frequency discharging Re cells. This evidence, together with the already mentioned data on ACh-inhibition of cortical bursting cells (Krnjević, 1974) may well be an explanation of the opposite effects of arousal on type I and type II cells and constitutes a promising line for future investigation.

It goes without saying that, aside from the above factors, the increased activity of type I cortical cells during W compared to S is also attributable to enhanced excitation in thalamo-cortical projection fibers. During EEG activation, antidromically identified output cells in the VL (Lamarre, Filion, & Cordeau, 1971; Steriade, Apostol, & Oakson, 1971; Steriade, Wyzinski, & Apostol, 1972) and LP (Steriade et al., 1977; Steriade, Oakson, & Diallo, 1977) thalamic nuclei actually exhibit higher rates of spontaneous firing, enhanced antidromic responsiveness and shorter periods of inhibition than during EEG synchronization periods. The increased firing rate of cortical output cells during W compared to S may thus be partially ascribed to tonic activation along their major source of afferents. Conversely, disfacilitation in cortical areas is likely to occur during S, when the excitatory impingement arising in the waking brain-stem RF is cut off. Experiments in our laboratory demonstrated that a high-security, excitatory pathway links the rostral activating RF with LI-LP thalamic nuclei, which project directly to the parietal association cortex (Steriade et al., 1977), and that depolarizing focal responses and unitary discharges are elicited with very short latencies (<5 msec) by RF stimulation in anterior suprasylvian cortes (Kitsikis & Steriade, 1975). There are also excitatory projections from the upper RF to the VL thalamus (Purpura et al., 1966; Steriade & Hobson, 1976). The withdrawal of the reticulofugal ascending impulses with transition from W to S will result in disfacilitatory phenomena at the level of lateral thalamic nuclei and corresponding cortical fields. Striking reduction in discharge rates or even complete silence in background firing was actually observed in thalamic targets of RF excitatory projections (Steriade, Apostol, & Oakson, 1971; Steriade, Wyzinski, & Apostol, 1972) and in cortical association areas (Steriade, Oakson, & Kitsikis, 1978) as a precursor of EEG synchronization. The overt effect of disfacilitation and active inhibitory processes seen in relay thalamic nuclei as direct and indirect consequences of reticular deactivation is a powerful inhibition exerted on the synaptic transmission of ascending impulses, which are indeed blocked at sleep onset, in spite of the unchanged presynaptic input (Steriade, Iosif, & Apostol, 1969). This deafferentation in sensory and motor thalamic relays contributes to the maintenance and further development of S sleep.

In identified output cells of the cerebral cortex, W and S are distinguishable by both rates and patterns of discharge. The disclosure of similar types of activity in PT cells of the motor cortex and cortico-thalamic or cortico-pontine cells of the parietal association cortex (see section 3.1.1) allows some generalization on corticofugal projections and suggests further investigations of their possible influences on distant structures primarily involved in the genesis of wake-sleep states. (Besides more significant influences on pontine structures, the increased firing rates of

cortico-pontine neurons during W and D, over those in S (3.1.1), should also be considered as an important input to account for changes in the cerebellar cortex during the sleep-waking cycle [Hobson & McCarley, 1972].) One line of research arises from the assumption that collaterals of corticofugal axons to brain-stem activating structures induce excitation of their targets and contribute to the maintenance of the W state; the disfacilitation of thalamic and, consequently, cortical output cells at sleep onset will reduce the tonic impulses along PT and cortico-pontine axons and, thus, add to reticular deactivation; this may be tested by interrupting various corticofugal fiber systems in order to detect their effects on postsynaptic elements of the waking reticular core. (Reversible cryogenic blockade of cortical sensori-motor areas resulted, in experiments by Buser et al. (1969), in a considerable depression of mesencephalic RF cell responses to peripheral somatic stimulation, but a cortical effect at the spinal cord level was not eliminated.) Another line of future investigation would be to consider the spike clusters that, during drowsiness and S, follow long periods of silence (Steriade et al., 1974), as an effective factor in triggering the various structures involved in the development of synchronizing processes, thus contributing to further development of S sleep. Impulses in cortico-thalamic pathways actually trigger inhibitory-excitatory rhythmic sequences in thalamic neurons, with the appearance of EEG spindle waves and unit bursting (Steriade, Oakson, & Diallo, 1977; Steriade, Wyzinski, & Apostol, 1972). One of the two major output channels of areas 5 and 7, the cortico-CM pathway (Steriade, Kitsikis, & Oakson, 1978), may project clustered discharges of cortical neurons to the medial thalamus, which, in turn, is at the origin of rhythmic and long-lasting inhibitory potentials in lateral thalamic cells (Purpura, 1972), with the consequence of deafferentation in thalamo-cortical systems. Spike bursts of PT cells at S onset may reinforce the activity of certain hypothesized synchronizing areas in the lower brain-stem (Puzillout & Ternaux, 1974). Both lines of research may be envisaged, in spite of their apparently different premises, as there exist many inferences concerning the role of the cerebral cortex in maintaining activation patterns of W or synchronizing activity of S (Jouvet, 1967), but intimate knowledge is still lacking concerning the nature of corticofugal influences upon those structures that initiate waking and sleep states.

3.3.3 Tonic excitation of output cells during D sleep taken as a whole and selectively REM-related increased discharge rates of interneurons. At present, the best candidates to account for the increased mean rates of thalamic and cortical cells during D are neurons located in the pons. Jouvet's (1967) lesion experiments have revealed that pontine structures are sufficient for the periodic appearance of the major EEG and behavioral signs of D. Unit recordings by Hobson et al. (1974b) disclosed that the increased firing rate of neurons in the pontine gigantocellular tegmental field (FTG) during D sleep is almost entirely related to REMs, and that FTG cells exhibit much higher selectivity ratios (relative to rates in S and W) than in other brain-stem structures, or cerebellar, thalamic, and cortical neurons. The maximally increased firing rate during D sleep found in all investigated thalamic nuclei and cortical areas (Steriade & Hobson, 1976) could be attributed to the dramatic excitation of executive D-neurons in the brain-stem. Pontine FTG neurons, for instance, increase their rate in S→D transition 10 sec before a group of cells recorded in the postero-lateral cortex (Hobson et al., 1974a). The same pontine-cortical relationship could also account for some aspects of firing changes in association cortical neurons reported in the present paper. It is necessary, however, to search for brain-stem structures other than the pontine FTG to explain the *tonic activation*, seen in cortico-thalamic and cortico-pontine cells of areas 5 and 7, that occurs during the *whole D sleep (REM and non-REM) and throughout the W state*. We tentatively suggest that direct projections from the pontine to the mesencephalic reticular core transfer the ascending excitation of pontine origin to midbrain neurons that add their intrinsic features to the continuing transfer of activity to thalamic and cortical areas. In collaboration with Ropert and Oakson we are currently investigating midbrain reticular cells, physiologically identified as receiving multiple converging inputs and/or projecting to medial thalamus or subthalamic fields. Some of these elements increase their firing a few seconds in advance of S→W transition, and most of them are tonically active during both W and D sleep, with firing rates much higher than during S sleep. Such changes in midbrain reticular cells may reasonably be regarded as being behind the alterations in firing rates of cortico-thalamic and cortico-pontine cells in areas 5 and 7. Some ascending projections to the association thalamo-cortical systems support the above assumption. Actually, short-latency (<5 msec) synaptic excitation was disclosed in cortical cells of areas 5 and 7 following RF stimulation (Kitsikis & Steriade, 1975); this is a bi- or oligosynaptic pathway relayed in LI-LP thalamic nuclei (Steriade et al., 1977).

The finding that interneurons selectively increase their discharge rate during REM epochs of D sleep prompts us to envisage an active role for interneurons during specific functions of D sleep. The very low discharge rate of cortical interneurons during W, as well as the lack of temporal relation between eye movements in W and spike bursts of interneurons, preclude the high selectivity of interneuronal discharge during REMs of D sleep as being simply a consequence of eye movements. Increasing evidence supports the idea of the beneficial effect of sleep upon the retention of information acquired during W: selective deprivation of D produces a retention deficit without affecting subsequent relearning (Bloch & Fischbein, 1975), and the total duration of D is augmented following learning (Lucero, 1970), due to an increase in the number of D epochs but not in the average duration of each phase (Leconte, Hennevin, & Bloch, 1973). Experiments on rodents in Bloch's laboratory showed that the increase of D is related to the degree of learning achieved, but when performance reaches the asymptote of the learning curve, the time spent in D returns to control levels.[5] Research on human subjects relating D to intellectual level (Bloch & Fischbein, 1975) also corroborates the idea that D sleep plays a role in the fixation of the memory trace.

If we accept the idea that D sleep is important for the consolidation of information acquired during W, then *the increased firing rates of association cortical interneurons during REM periods of D could indicate that these cells are preferentially involved in maintaining the soundness of a memory trace.*[6,7] This is in line with the role in memory ascribed to type II cells, on the basis of morphological and developmental arguments (Altman, 1967; Jacobson, 1969; see part 1). As interneurons are selectively active in D sleep during REMs, one would expect that the retention of information particularly increased the duration of REM periods. Some studies indicate positive correlations between intellectual functions and the amount of REMs during D in man (Bloch & Fischbein, 1975), but the studies on animals that report increased duration of D following learning are not as yet directed to dissociate REM from non-REM periods (Bloch and Leconte, personal communication). Experiments could be designed to test whether an instrumental conditioning paradigm is effective in increasing REM-epochs during D and to relate the development of a learning task to activities in output and nonoutput cortical association cells.

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NOTES

1. See Colonnier's (1966) comments on multiple varieties of "stellate" cells and subvarieties of the "cellule à double bouquet dendritique." Seven interneuronal types were recently described by Jones (1975) in the somatosensory cortex of the squirrel monkey. In a study on the visual cortex, with a technique allowing one to determine the physiology *and* morphology of neurons, Kelly and Van Essen (1974, p. 542) disclosed numerous types of "stellate" cells, but "the functional significance of the striking differences between spine-free and spiny stellate cells remains uncertain. We also have no real understanding of why there are so many unusual cell types, such as double bouquet cells and crescent cells."

2. Of course, this general statement is not valid for spinal interneurons in laminae VIII and VII, which form long propriospinal pathways. They may be antidromically invaded from ventral white matter of the cord, but not following stimulation of the ventral root (Willis & Willis, 1966). In fact, any interneuron can be antidromically invaded by stimulating its target structure. This was recently achieved for inhibitory Golgi type II cells in the brain stem (Hikosaka & Kawakami, 1976; Nakamura et al., 1976). If antidromic invasion of interneurons is possible in instances when the targets of type II cells are at a certain distance from the latter, this becomes impossible when interneurons belong to the same cellular pool as type I neurons. This is most often the case in the neocortex and, as this paper deals particularly with cortical interneurons, lack of antidromic invasion following stimulation of distant corticofugal pathways is an obligatory criterion to specify cortical type II cells.

3. I should emphasize that, when investigating differences between phasic arousal and tonic W or between "excited" and "relaxed" W, these sub-states were recognized from behavioral observations combined with estimation of the overall amount of the recorded eye movements (see Figure 5, $A_{2\text{-}3}$ in Steriade, Deschênes, & Oakson, 1974), but we still lack a precise method to quantify the level of arousal.

4. In this context, I would like to discuss the suggestion that there is an increased burst-silence pattern (more short and more long interspike intervals) of cortical cells in passing from W to S and a still further increase in D (Evarts, 1964). We actually found a greater probability of short (<20 msec) intervals in identified output cells of areas 5 and 7 during D (compared to W), but this was associated during this state with virtual lack of long intervals (Figure 14), which resulted in a higher rate of discharge. Increased short intervals in D are not, therefore, related to long periods of neuronal silence, and they are ascribable to the tremendous excitation along corticopetal pathways during D. On the other hand, more numerous short intervals in S (again compared to W) are associated with significantly greater probability of long intervals, which results in a decreased firing rate. Spike bursts in S are known to occur with brief depolarizing events immediately following long-lasting hyperpolarizing shifts (Steriade & Deschênes, 1974; Steriade et al., 1974) (see Figure 11C). In other words, the significance of short intervals in S and D is different. Since there are almost ten times more long intervals (1–3 sec) in S compared to D, and also a greater probability of long intervals in S compared to W (Figure 14), it can be concluded that the firing pattern in D is *not* an extreme type of S.

5. Some clinical observations, such as the lack of memory or learning disturbances in a case with total insomnia lasting several months (agrypnic syndrome in Morvan's disease, Fischer-Perroudon et al., 1974), are, however, arguments against the theory of D role in memory consolidation. Confronted with this problem of relations between D and learning processes, Jouvet stated (1973, p. 574): "ainsi même s'il est possible que le sommeil paradoxal joue un rôle favorisant dans la mémoire et l'apprentissage, *c'est au niveau de la mémoire de l'espèce, et donc la programmation des instincts qu'il faut sans doute rechercher ses fonctions.* En fait, si le rêve constitue l'étape d'organisation des comportements innés, il y a peu de chance que des troubles caractéristiques soient décelés chez l'individu adulte."

6. A first difficulty arises from the observation that the quantity of memorized information increases and the duration of memory consolidation is shortened by increasing the level of arousal with RF electrical stimulation (Bloch & Fischbein, 1975), an experimental condition that, in our hands, led to arrest of firing in interneurons (see Figure 8).

7. As our nonoutput cells also discharged at higher rates during S sleep than in W, we wonder whether this state is also involved in some integrative processes, usually attributed exclusively to D sleep. We should, however, mention that the percentage increase in firing rate of interneurons in D sleep with REMs is much higher than in S sleep, the difference between these two states being highly significant ($P \leq 0.005$).

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Acetylcholine: synaptic transmitter of the arousal system? In this paper Steriade has reviewed some of the elegant electrophysiological studies he and his colleagues have performed in order to elucidate the events that occur in thalamo-cortical neurons in relation to the sleep-waking cycle. With regard to the conclusion reached by the author, it may be worth stressing the following general comments:

1. As attractive as the hypothesis may be that views acetylcholine as the arousal-system transmitter, several controversial issues should be cleared up, and additional lines of evidence obtained. More specifically, concerning the reticular nucleus, there is only limited neurochemical information on the distribution of cholinergic markers. In contrast to the intense cholinesterase staining (Shute & Lewis 1970), Brownstein et al. (1975) have found relatively small concentrations of choline acetyl transferase, and studies aimed at tracing the putative cholinergic pathways to the reticular nucleus have not been performed. Furthermore, appropriate electrophysiological evidence on the putative cholinergic structures that project to the reticular nucleus is crucial to test the hypothesis and complement the data on the powerful inhibitory effects of micro-iontophoretically-applied acetylcholine on reticularis neurons (Ben Ari et al., 1976b, *op. cit.*). Dingledine and Kelly (1977) have not obtained such evidence concerning a putative monosynaptic cholinergic inhibitory projection from the MRF to the reticular nucleus; stimulating the same structures, which in Steriade's hands strongly inhibited the cortical type II neurons, these authors obtained a long latency inhibition that was resistant to a large amount of iontophoretically-applied atropine. Thus it may be that other structures are responsible for the putative inhibitory cholinergic innervation.

2. When micro-iontophoretically applied on reticularis neurons, acetylcholine induces marked changes in pattern of discharge, in addition to inhibiting both spontaneous and glutamate-induced activity (Ben-Ari et al. 1976b, *op. cit.*). The bursting discharge elicited by acetylcholine has been observed in other brain structures (see Krnjevic 1974). Such changes may be of particular relevance with regard to the suggestion of Steriade that control interneurons are preferentially involved in "maintaining the soundness of a memory trace" during REM sleep. Rather than the increase in the mean rate of discharge per se, it may well be that during the longer silent periods that separate consecutive bursts of spikes in REM sleep, local integrative events occur that are of primary importance with regard to consolidation of information.

NOTE

In the discussion, the functional significance of D sleep with respect to memory is controversial and should have been discussed in relation to studies other than those of Bloch and Fishbein (1975 *op. cit.*). The author quoted the work of Fischer-Perroudon (1974) on man but did not quote the work on mice by the school of Jouvet, and particularly those of Valatx (e.g. Kitahama et al., 1976) which are not so affirmative.

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Sleep and waking and two populations of neurons. How is the general organization of cortical activity affected by the sleep-wake cycle? How do changes in cortical functioning affect the brain-stem neurons primarily responsible for sleep and waking? These questions seem rather simple, but it is a fact that many decades of intense and often brilliant experimental and speculative efforts have not provided definite answers to them. In recent years Dr. Steriade and his coworkers have amassed an impressive amount of data on the activity of single cortical neurons in normal sleep and waking. Their experiments have been guided by two sound principles, i.e. 1) neurons studied during sleep and waking must be categorized as precisely as possible on the basis of their input-output characteristics, and 2) processes of neuronal excitation and inhibition correlated with the sleep-wake cycle must be demonstrated by direct tests of excitability, rather than inferred from changes in spontaneous activity. In the course of their work, Steriade and collaborators have convincingly disconfirmed two hypotheses that had originated from previous work of Evarts, *op. cit.* They have shown that a) inhibition of corticofugal neurons is certainly not less in sleep than in waking, and b) the pattern, as well as the rate, of discharge of corticofugal neurons during deep sleep is much more similar to that of waking than to that of light sleep. Taken together with much evidence on subcortical activity (see e.g. Mukhametov et al., 1970), the latter finding suggests that the pyramidal tract neurons previously reported by Evarts, *op. cit.*, to discharge in bursts during both light sleep and deep sleep were exceptional. Most corticofugal and subcortical neurons appear instead to discharge in a rather continuous manner during deep sleep, as well as during waking.

Steriade reiterates the proposal, strengthened by a good deal of fresh evidence, that changes in cortical activity during sleep and waking can be satisfactorily described and interpreted by resorting to a binary classification of neurons. Binary classifications are characteristically appealing to the minds of scientists, and the history of the neuro-sciences can certainly offer countless examples of them.

Starting with Golgi (1886, *op. cit.*),[1] neurons have been assigned to one or the other of two categories on the basis of many distinctive morphological or functional characteristics such as: presence or absence of a myelin sheath on the axon; size of cell body (large or small); length of axon (long or short); appearance in ontogeny (early or late); stability of morphology (invariant or variable); stability of function (genetically-determined or environment-dependent); synaptic action (excitatory or inhibitory); role in neuronal circuitry (projection neuron or interneuron); and so on. The suggestion has been made that many, if not all, of these dual categorizations are correlated, so that one can speak of two neuronal archetypes (see e.g. Hirsch and Jacobson, 1974).

Steriade seems to be fully aware of the oversimplifications implicit in this categorization of neurons. He discusses some of the difficulties that one encounters when applying this categorization to the study of the neural mechanism of sleep and waking. However, I think he fails to provide a precise definition of a cortical interneuron. In terms of their role in cortical circuitry, some long-axoned cortical neurons cannot be differentiated from short-axoned ones. I have in mind here some callosal neurons of the visual cortex whose functional role is that of extending receptive fields across the vertical midline of the visual field (Berlucchi and Rizzolatti, 1968). Functionally speaking, these neurons are fully comparable to locally-projecting cortical neurons, which participate in the constitution of similar receptive fields not extending across the vertical midline. Some of these callosal neurons may even be stellate cells (Shatz, 1977), as are most of the locally-projecting neurons. If we make reference to the functional organization of the cortical circuitry, it is hard to separate these callosal neurons from locally-projecting neurons; and if we call interneurons the locally-projecting neurons, we must give the same name to the callosal neurons. A similar argument may apply to at least some of the long intrahemispheric cortical association neurons. If, on the contrary, we make reference to distinctive features other than role in functional cortical wiring, such as axonal length, area of membrane surface, protoplasmic volume, and related metabolic characteristics, then it is obvious that callosal and associational cortical neurons, on one hand, and locally-projecting cortical neurons, on the other, fall into different classes.

In his present work, Steriade makes no reference to the behavior, during sleep and waking, of callosal and long associational neurons. He and his coworkers (Steriade, Deschênes, Wyzinski, and Hallé, 1974 *op. cit.*) have previously reported that callosal neurons of anterior cortical regions behave during sleep and waking in a way similar to that of pyramidal tract neurons. I believe that for Steriade's theory at least some of the callosal neurons should, on the contrary, behave like his interneurons. Obviously we need some discussion on this point.

Another point which calls for further explanation is the finding of a REM-associated increase in discharge of interneurons during deep sleep. If it is assumed that the net action of interneurons on corticofugal neurons is inhibitory in nature, then one would expect a REM-associated decrease in activity of corticofugal neurons in deep sleep. Since the opposite is true, one must postulate some uncoupling between interneurons and corticofugal neurons during deep sleep.

Finally, a weak point in all theories of sleep and waking, which assume a rather simple fundamental mechanism, is their general inability to deal with the fact that, because of their heterogeneity, both sleep and waking must correspond to an enormously high number of states of cerebral organization. Steriade acknowledges this when he admits that a distinction between corticofugal neurons and cortical interneurons on the basis of spontaneous activity may be possible only during relaxed waking, because during active waking both interneurons and large corticofugal neurons tend to be silent. In this connection one should of course be aware of the possibility that changes in neuronal activity may be associated with movement or sensory stimulation occurring during waking or even sleep [cf. Vanderwolf, this *Commentary*] rather than with the states of sleep and waking per se. This possibility applies not only to the motor and sensory areas, but also to associational cortical areas (see e.g. Mountcastle et al. 1975). In speculating on the possible functional significance of sleep, Steriade accepts Moruzzi's (1966 *op. cit.*) idea that sleep may be required for allowing the occurrence of synaptic and interneuronal processes in some way related to learning and memory. However, while Moruzzi argued for a rest state of cortical interneurons during sleep, Steriade maintains that during sleep, and particularly deep sleep, cortical interneurons become active for the fixation and consolidation of memories. Superficially, it may be objected that these hypotheses are clearly beyond the limits of fruitful speculation, and that in terms of heuristic value they are no more productive than, say, Cajal's suggestion that short-axoned neurons are the reservoirs of nervous energy (Ramon y Cajal, 1901). I disagree with this objection. On the one hand, these hypotheses may provide the impetus for an essential qualitative leap in the fine analysis of cortical organization in the free-behaving animal. This analysis must ultimately be based on specific and detailed assumptions, but the attainment of a fertile conceptual background for experimental operation is often more related to earlier vague and simplistic ways of thinking than is evident in retrospect. On the other hand, the fact must be faced that virtually nothing is known of the functional significance of sleep. Our understanding of the brain will never be complete if this problem remains unsolved, and its solution is certainly not aided by disparaging all conjectures about it.

NOTE

1. I find it amusing and somewhat ironical that nowadays Camillo Golgi is credited with the categorization of neurons on the basis of axon length. Since he believed that there was a protoplasmic or fibrillar continuity in the cerebral neuropil, he never admitted that axons end in any place, and therefore he never discussed axonal length! He defined as neurons of the first type those whose axon "retains its individuality" and which constitute the core of a medullated fiber that eventually becomes continuous with the body of another neuron or with the neuropil. Neurons of the second type were defined as those whose unmedullated axon loses its individuality, at an undetermined distance from the cell body, to become inextricably enmeshed in the neuropil. Thus, in a sense, it can hardly be said that the axons of the Golgi second-type neurons are short, since they can be considered as long as the whole neuropil! We owe to Ramon y Cajal (1955, *op. cit.*) and particularly to Tanzi (1893), who both rejected the diffuse nerve net theory, the distinction between short-axoned and long-axoned neurons.

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Active wakefulness and paradoxical sleep: common mechanisms? The central theme of Steriade's review paper is the difference in discharge pattern between two classes of cortical neurons in relation to vigilance states. Steriade's review constitutes an impressive attempt to bring law and order into an unruly and confusing mass of experimental data. Neuronal discharge patterns are analyzed in his paper mainly with respect to differences between sleep and wakefulness (W), and between the two substates of sleep, slow wave sleep (SWS) and paradoxical sleep (PS; active sleep), whereas less emphasis is placed on variations occurring within the waking state. In the following I shall point out the usefulness of the dichotomy of wakefulness into "active wakefulness" (aW) and "quiet wakefulness" (qW), which reveals a close similarity between aW and PS.

Electrophysiological studies. The majority of neurons in the association cortex of the cat show a decrease in their discharge rate with the transition from W to SWS, and an increase with the transition from SWS to PS (Noda and Adey, 1970b, *op. cit.*). Thus both W and PS exhibit higher discharge values than SWS. A further subdivision of W into aW and qW has revealed that the coefficient of variation of individual units, as well as the correlation between the temporal firing patterns of two neighbouring cells, were smallest during aW and PS (Noda and Adey, 1970a *op. cit.;* 1973 *op. cit.*). Data presented in Steriade's review also indicate that output cells in the parietal association cortex increase their firing rate in the transition from SWS to aW as well as to PS. It is likewise interesting to note in this context that a disfacilitation may underlie both the sudden arrest of firing in fast-conducting PT cells upon arousal (Steriade and Hobson, 1976, *op. cit.* p. 249) and the motoneuron hyperpolarization during PS (Nakamura et al., 1978).

Recordings from brainstem neurons have provided more direct evidence for a close relation between aW and PS. Thus the firing rate of neurons in the pontine reticular formation was shown to be particularly high during both aW and PS (Siegel and McGinty, 1977; Siegel et al., 1977; Vertes, 1977), the high discharge rate during aW being closely related to motor activity (Siegel and McGinty, 1977). Common electrophysiological features of the two states are evident also in the hippocampus, where the theta rhythm in the EEG and a high firing rate of hippocampal "theta cells" typically occur during voluntary motor activity and during PS (Vanderwolf, Ranck, this *Commentary*). Multiunit activity in the dorsal hippocampus and the brainstem reticular formation undergoes similar changes during aW and PS (Koranyi et al., 1977). Finally, results from lesion studies are relevant in this context to support the assumption that an intense covert motor activity prevails during PS. Thus, lesions in the pontine tegmentum of the cat result in a dramatic "hallucinatory" motor activity during PS episodes, probably "released" by the destruction of pathways mediating descending motor inhibition (Jouvet and Delorme, 1965; Henley and Morrison, 1974).

Behavioral studies. Motor activity and PS exhibit striking similarities with respect to their circadian pattern. In both man (Kleitman, 1963; Webb, this *Commentary*) and rat (Borbély, 1978; Borbély and Neuhaus, 1978), PS relative to total sleep as well as motor activity (restlessness) have both been shown to increase progressively during the daily rest period, whereas SWS decreases. In mice maintained under constant light or darkness, the circadian rhythm of SWS and W exhibits a temporary damping, whereas both motor activity and PS tend to maintain their rhythmicity (Mitler et al., 1977). In rats kept under constant light or constant darkness, the circadian amplitudes of motor activity and PS are less reduced than those of W and SWS (Borbély and Neuhaus, in preparation). Finally, light-dark transitions induce a shift from SWS to PS in the sleeping rat, and a shift from low activity to high activity in the awake animal (Borbély, 1976).

Taken together, the results strongly indicate that aW and PS represent closely related states, not only in terms of the similarity of neuronal firing patterns or EEG, but also with respect to circadian rhythms and reactivity to environmental influences. The studies underscore the importance of carrying out electrophysiological sleep studies in completely-unrestrained, behaving animals. Finally, the data summarized in this commentary suggest that the exploration of the substates of wakefulness may further our understanding of the substates of sleep, and may eventually contribute to the solution of one of the most tantalizing problems in sleep research: why do we need active sleep in addition to active wakefulness?

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Electrophysiological differentiation between output cells and interneurons: an alternate methodological proposal. The excellent paper of Steriade, synthesizing several years of experimental work by the author and his group, is mainly concerned with the effectiveness of differentiating long-axoned, projecting cells (for output (O) cells) from short-axoned, Golgi type II cells (or interneurons (I) cells) within the cerebral cortex. I should like to suggest a much simpler alternative method for identifying and, thereafter, for studying the characteristic activities of homogenous cell populations of the cerebral cortex in behaving animals, which would, at least to some extent, enable us to avoid some constraints in chronically-implanted preparations.

Basically, the proposed method is a combined recording system: integrated recording from large populations of fibers in a projecting tract, in addition to simultaneous recording from single units belonging to cortical areas from which the fiber tract originates.

As is well known, the method of integrating spike activities from large populations of neural elements, originally described by Arduini and Pinneo (1962), has been employed many times, both in acute and chronic experiments (see Arduini and Corazza, 1977). Owing to some general and theoretical assumptions concerning its applicability, the method is mainly concerned with levels of activity defining statistically relevant behaviors of a neuronal population, particularly but not exclusively under steady-state conditions. Even in the steady-state condition, however, integrated recordings, just because they are "integral" operations in time, cannot give any thorough information about the sequential temporal order of impulses within trains of spikes, nor about the temporal succession of occasionally occurring bursts. For this purpose we really need simultaneous combined recordings of single unit activity in the cell population from which the fiber tracts originate (O cells).

But how does one recognize the O cells? First of all, let us consider the simplest case. It is represented by recordings from a cortical area in which the projecting cells (O cells) compose a homogeneous functional population – i.e. their activities are never divergent from one another when detected in the absence of sensory stimulation or motor performance. Then, if this is the case, the only possible detectible difference arises from the activity characteristics of projecting and nonprojecting cells. By comparing the level of activity measured in the fiber population and in the cortical single units during the steady-state condition at each stage of the sleep-waking cycle, we can very easily detect all the homogeneous activities. These represent, at the cortical level, the O-cell behaviors, and every other type of activity must be ascribed to nonprojecting I cells. Even in the case of homogeneous O-cell populations, however, we may find a more complicated situation. This could happen if and when I- and O-cell activities resemble each other during the whole S-W cycle. Nevertheless, the occurrence of such a situation is highly improbable and up to now has never been clearly observed, as far as I know.

Let us now consider the most difficult and, unfortunately, more frequent situation when the projecting cells do not constitute a homogeneous functional population, since their related activities may exhibit differential behaviors even in the absence of sensory stimulation and overt motor performance. Cell identification by means of simple recording techniques like the ones we are referring to here may prove to be a rather intricate operation in this case, but still within the possible range of our combined method. The most pertinent example of functional heterogeneity, in the sense defined above, would be a case of opposite behavior exhibited during arousal from sleep by fast-conducting (large) versus slow-conducting (small) PT cells. As Steriade reported, however, it was only "during the first 10 sec of arousal," or time-locked with only "phasic periods of excited wakefulness due to orienting reactions," or "when the experimenter deliberately kept the animal very alert" for periods as long as 1–2 min that "changes in spontaneous firing rates of fast- and slow-conducting PT cells contrasted with one another." Nevertheless, both slow- and fast-conducting PT neurons showed the same reactions during steady, relaxed wakefulness; and in fact in this stage their firing rates were consistently "higher than the values found in the same neurons during slow sleep." During maintained states of sleep and waking, all PT cells behave as if they belonged to a homogeneous functional population, and their levels of firing rates should be entirely comparable with the levels of integrated activity recorded from large populations of pyramidal tract fibers (cf. the results of Steriade et al., 1974 on PT units, and of Arduini et al., 1963, on pyramidal integrated activity). Once a PT cell has been identified because its firing rate (level of unitary activity) and the level of pyramidal fiber activity were changing in the same way when measured during maintained states of sleep and waking, that PT neuron may further be characterized according to its responses during the very phasic periods of arousal from sleep and/or during epochs of excited wakefulness. Finally, identification of I cells within motor cortex areas can be easily achieved, since every unit displaying a change in firing rate different from that recorded from the pyramidal fiber tract during steady-state periods of S and W can only be regarded as a nonprojecting I unit. Even if the converse may not always be true (I cell behaving as O cell might indeed be assumed), this combined method seems to be helpful in solving problems such as those so keenly studied by Steriade and his group.

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Physiological measurements and the "programming" hypothesis for the function of REM sleep. As a nonphysiologist, I find it very difficult to make detailed comments on the experimental contribution of Steriade. Leaving that to other commentators, I wish instead to discuss an alternative experiment that has yet to be done and that may have the effect of greatly enhancing the implications of Steriade's findings.

Dewan (1970) suggested that REM sleep is necessary for the establishment of new functional pathways when neurons are physically damaged. In that paper an experiment was proposed consisting of a modification of the classic study of Adametz (1959), which had shown that if a brain lesion is made in two steps separated by an interval of time lasting several days, then the resulting functional damage to the animal is smaller than when the lesion is made in a single step. The interval of time between lesions, thus, is very important for the animal's recovery of function.

One way to interpret Adametz's finding is to suppose that, during the time between lesions, the damaged part of the brain is repaired by some sort of reorganization of the neuronal pathways, and that this restructuring process can make use of information stored in the part of the brain where the second lesion will be made. If both sites are destroyed simultaneously, then this process would be to a large degree prevented.

It is certainly possible to explain Adametz's result in alternative ways. Nevertheless, I would like to explore the consequences of it when viewed in the context of Adametz (1959), who assumed that restructuring or "reprogramming" cannot occur in the absence of REM sleep. This suggests the following modification of Adametz's procedure. Let there be two control groups, one with the lesions made in two steps and the other with the lesion made in one step. In contrast, the experimental group would have the lesions made in two steps but with maximal feasible REM deprivation in between. The prediction is that the REM-deprived animal would resemble the animals with the lesion performed in a single step and would thus experience a great loss of function. In other words, without REM sleep the animal's brain would not manufacture the new pathways, and hence, at the time of the second lesion it would have no redundancy to fall back upon.

Greenberg and Dewan (1969) describe an experiment conducted along similar lines. In that study statistical evidence is presented implying that patients exhibiting signs of recovery from brain damage spend more time in REM than those with comparable damage but showing little recovery.

If the above modification of Adametz's experiment were to give a negative result, it would refute the (original) interpretation of the data of Greenberg and Dewan (1969) or at least make that interpretation very dubious. In addition, it would imply that REM sleep was not generally necessary for the repair of brain damage. On the other hand, a positive result would have far-reaching consequences for both research and the practice of medicine. For example, one would certainly avoid medication that suppressed REM in patients recovering from brain damage. In the area of research it would, for example, raise the question of whether or not *interneurons* play a role in the repair of brain damage.

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Cortical interneuron activation, D sleep and memory. A primary goal of psychobiology is understanding the biological bases of behavior. The contribution of Steriade and his group in Quebec is one example of a team of researchers pushing back this frontier by examining cortical activity at the cellular level and relating it to the sleep-waking cycle. The team has been exploring the spontaneous and evoked discharges of long-axoned output neurons and the activity of association interneurons upon which all higher mental processes are dependent. Most important of all is the discovery that interneurons selectively increase their discharge firing rate during the REM epochs of D sleep – considerably augmented above W and S. This specific discovery has led Steriade to suggest an active role for interneurons in promoting important aspects of memory storage processes during D sleep, a hypothesis that has gained considerable momentum in recent years and has taken its place alongside a variety of other views about the function of D sleep (for a review of this literature see Fishbein & Gutwein, 1977).

The hypothesis that the neurophysiological and neurochemical events occurring during D sleep play an important role in the process of memory consolidation has raised a variety of questions; Steriade's observations provide answers to at least some of them. One crucial issue to the D sleep-memory hypothesis is the observation that human subjects completely deprived of D sleep (either by experimental manipulation or disease) show no serious learning or memory disturbance. It is also evident from animal and human studies that memory-storage processes persist whether D sleep occurs or not. Steriade's findings add to the evidence that D sleep is a biological state that is part of a continuous neuronal-activation cycle. Cortical activation is a necessary condition underlying memory-related macromolecular events, and, as such, the development of total amnesia is dependent on the level of interneuronal activity throughout the sleep-wake cycle. There is no reason to expect that animal or human subjects solely deprived of D sleep should display serious memory disturbances, because D sleep occupies only a fraction of the activation cycle, even though it is an exceedingly intensified part.

On the other hand, data from several laboratories make abundantly clear that D sleep deprivation (or for that matter any protracted suppression of neuronal activation (Flood et al., 1977)) prolongs the period during which a recently consolidated memory remains highly susceptible to disruption when the brain is assaulted with an appropriate amnesic agent (Wolfowitz & Holdstock, 1971; Fishbein et al., 1971). The accumulated evidence so far suggests that enhanced forebrain activation occurring at any time during the sleep-waking cycle serves to promote: 1) the conversion of a learned response from STM (short-term memory) to LTM (long-term memory), and 2) the normal active maintenance of the stability of a consolidated memory trace. Since D sleep is an important part of a natural and periodically occurring activation system (Hobson et al., 1975), it is not surprising that its disruption alters the memory trace and places it in an unstable condition.

Another issue that Steriade raises is his finding that experimental electrical stimulation of the RF (reticular formation) leads to arrest of firing in interneurons – a procedure which in the hands of Bloch et al. (1970) leads to facilitation of memory consolidation. While this commentator cannot provide an easy reconciliation for this seeming paradox, there are several lines that should be explored. For example, substantial axon projections from the LC (locus coeruleus), which is linked to cortical activation associated with both wakefulness and D sleep (Jones et al., 1973), terminate in the neocortex and on pyramidal cells throughout the hippocampal formation (Ungerstedt, 1971). Jones et al. (1975) have reported that damage to the LC results in 85% depletion of endogenous norepinephrine in the neocortex and hippocampus, found on biochemical assay 21 days after lesioning. The lesions produce immediate and permanent alteration in tonic and phasic components of D sleep, with PGO (ponto-geniculo-occipital) spikes reduced in frequency by 50% of controls. In short, it is quite necessary to know the precise projection system into which the RF stimulating electrodes are placed and the extent of current spread produced by the stimulation before any definitive conclusion can be made.

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A time for inhibitory neurons to rest. The hypothesis. Getting information out of Steriade's publications is a lot like getting oil from shale – hard work is required but the results are worth the energy expended. The opening paragraph demolishes the idea that the purpose of sleep is to rest the brain. Calling this the common sense view, Steriade feels that the failure of sleep to markedly decrease neuronal discharge rates brushes this concept into science's wastebin. After 12 thousand words and 15 pictures of little slashes across an endless line, Steriade presents his own concept relating interneuronal discharge to memory consolidation during the D state.

Much evidence relates D sleep to memory (reviewed in detail by Hartmann, 1973). I think the clearest such correlation is the report of Empson and Clarke (1970), who monitored pairs of students in the sleep laboratory. After both were asleep one was randomly designated to be deprived of D sleep; whenever he entered the D state (as defined by ongoing polygraphic measurements) both sleepers were awakened. The next morning the D-deprived subject had less recall than did his partner, who had been aroused the same number of times, for verbal material that both had learned the previous evening.

Since interneurons have a greater discharge rate during D and since D is somehow related to memory, perhaps memory is "consolidated" by the discharging cells. In Steriade's words, "cortical interneurons during . . . D . . . are preferentially involved in maintaining the soundness of a memory trace." One wonders why these cells so actively involved in memory during D are so quiet during wakefulness. An unspoken assumption of this hypothesis avers that a cell that fires more often is more likely to be involved in cognitive function; this reminds me of the granting agencies that measure research productivity by counting publications.

Another hypothesis. I wonder if we have too easily rejected the common sense view that the brain somehow rests during sleep. Suppose that this "rest" requires a decrease in neuronal firing rate in a specific neuron type in order for that cell to build up stores of neuromodulator substances and energy metabolites. If this hypothesis were correct, Steriade's data would suggest that neocortical output neurons "rest" during S and neocortical inhibitory interneurons "rest" during D. A decreased firing rate during S of the long-axon output neurons is measured directly. A decreased firing rate of inhibitory interneurons during D is inferred from the increased firing rate of the many uninhibited neurons.

A "rest" of inhibitory cells could not continue for a period of minutes. A group of neurons which, when activated, produces a muscular contraction must be inhibited most of the time. When this inhibition disappears, the contraction occurs. Prolonged "rest" of this inhibition would produce continuous movement (I wonder if this movement would be in the form of epileptic seizures or choreoathetotic writhing). If this inhibitory "rest" is real,

then it must be discontinuous; this discontinuity creates the eye movements and jerky muscular twitches that so characterize the D state.

This "rest" hypothesis and Steriade's putative relation of interneuronal firing bursts to memory consolidation are not, of course, mutually exclusive. The former seems more easily testable. Special attention could be paid to those maverick interneurons that do *not* increase firing rate during D (three of 23 interneurons from association cortex). The "rest" hypothesis predicts that these are inhibitory.

The basic axiom of neuroscience holds that organized behavior results from impulse transmission through complex neuronal networks. The study of neuronal firing rates builds the foundation for an understanding of what Uttal (1975) calls "the cellular basis of human experience." Steriade has taken a step on the long road to that understanding by relating the activity of specific types of neurons to sleep behavior. One can only hope that the translation of mental experience into neuronal properties will involve more esthetic displays than a series of tiny crosshatches resembling empty clothespins on a laundry line.

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Neuronal identification and classification strategies. In contrast to the usual limited experimental procedures, Steriade and his coworkers have characterized recorded units in sleeping animals relatively extensively. Provided that his experimental results are reproducible in other laboratories, we hope the efforts and findings of his group will stand as an example of the advances possible through their approach.

The author has divided his cell population into two clearly separate groups, defined primarily by their discharge patterns. One question concerns the possible existence of cells with intermediate properties. For instance, cat hindlimb motoneurons have often been divided into two groups, tonic and phasic (Granit et al., 1957). This distinction has usually been based upon motoneuronal discharge patterns in response to excitatory input such as stretch of innervated or agonistic muscles. Tonic and phasic motoneurons were thought to have different ranges of size and after-hyperpolarization duration. However, even though muscle units have been classifiable into rather distinct and nonoverlapping populations, the conduction velocity of the motoneurons that innervate these muscle units have not (Burke, 1967). On the average, the motoneurons for the different muscle-unit types do have different size ranges. However, overlap among the two ranges is so large that a size index alone does not allow prediction of muscle-unit type. In addition, under different conditions of anesthesia, transection level, or deterioration of a preparation, some tonically-discharging cells have been found to exhibit phasic patterns, while some originally-phasic cells have switched to tonic ones (Henneman et al., 1965). In his clearcut cellular classification of relay and intrinsic neurons, Steriade does not discuss the possible existence of cells with mixed properties ("intermediate" cells). It would have been of interest to know if some cells burst only partially and were not antidromically activated. If such cells were encountered, what was their percentage of the total sample? If no "intermediate" cells were ever recorded, a quantification of the two groups would have been useful. An index of interspike variability during NREM sleep correlated with the ratio of discharge for wakefulness to NREM sleep or for isophasic to multiphasic REM could, for example, serve such a purpose. Steriade's hypothesis, obtained from his elegant experimental work, would not be as strong if only two well-defined populations had been considered without taking into account units with intermediate properties. An indication of the number and properties of cells that were discarded, if any, would also render the experiments of his group more easily reproducible.

Interestingly, the interneuronal discharge during multiphasic REM observed by Steriade in the precruciate and parietal cortices is also found in lamina IV through VIII of the lumbosacral spinal cord. During the past eighteen months we have consistently observed such a correlation during cellular recordings performed in unanesthetized, uncurarized cats during sleep and wakefulness.

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The cortex finds its place in REM sleep. Research in neurophysiology is a long way from demonstrating many clear relationships between neural events and higher mental activity. The processes of memory storage or organization, for example, can be described on a psychological level, or the activity of certain neurones or pathways can be shown to be involved when such processes are assumed to be taking place. In effect, however, our efforts to understand the physical basis for much mental activity is actually a search for correlations between events studied at different levels and at different times. Often observations at one level give us clues about where to look or how to interpret other, seemingly parallel findings. In a way, we try to fit together pieces of a 3-dimensional jigsaw puzzle. Steriade's paper provides us with an elegant description of the relation between discrete neuronal activity and behavior of the nervous system in different states of arousal. In his closing paragraphs he attempts to relate these findings to studies suggesting that REM sleep is related to certain memory processes. I am not qualified to comment on the elaborate neurophysiology in this paper (I assume other commentators will do so), but I would like to focus my commentary on the amazing consistency of these findings with many other studies of sleep, and to show how they might help resolve the controversy about the relative roles of the pons and the cortex in REM sleep and its associated mental activity – dreaming. In effect this will be an attempt at an elaboration of the final paragraphs of Steriade's paper and an expansion of the correlations between neuronal events during REM sleep and psychological processes that seem related to REM sleep. As will be seen, there is a sense of things fitting together when one includes all the information, but this has not always been done.

A certain amount of controversy has revolved around the idea of REM sleep being pontine sleep. This is epitomized in Hobson & McCarley's (1977) recent discussions of the implications of their single-unit studies in the pons. In essence, they suggest that the FTG (giant tegmental field) cells in the pons are the generators of REM activity, and that the process of dreaming is an attempt by the cortex to make sense of the random firing from below. The pons is therefore seen as the prime mover in the process of REM sleep. This conclusion is consistent with some of the earlier studies that showed that destruction of structures in the dorsal pons could abolish REM sleep (Jouvet, 1962).

While Jouvet's early studies, along with Hobson & McCarley's (1977) work, show that certain structures in the pons are necessary for the development of REM sleep, other research suggests that the labeling of REM sleep as pontine sleep does not necessarily do justice to the whole process. The fact that dreaming is associated with REM sleep, and that the direction of eye movements is associated with dream content (Roffwarg et al., 1962), has suggested a greater role for the cortex. This idea has led to a study revealing that humans with attentional hemianopias caused by lesions of the visual-association areas showed an absence of REM in the direction of the disturbed field (Greenberg, 1966). Two decorticate patients were shown to have normal REM periods, except for the fact that eye movements were reduced to single isolated movements instead of the normal bursts (Jouvet, 1961; Greenberg, 1966). At the same time, Jeannerod et al. (1965) showed that destruction of the visual-association areas in cats led to a marked reduction of eye movements during REM periods. Thus, while there is evidence that the pontine cells' firing precedes eye movements, the evidence just cited suggests that cortical areas also play a role. We must wonder, then,

whether there is an interaction between cortical and pontine structures, perhaps, as Steriade suggests, with certain thalamic structures as intermediaries in a process of both reciprocal inhibition and excitation. Villablanca (1974) discusses in detail the possible role of the thalamus in sleep and how destruction of the thalamus leads to dissociation of EEG-behavioral manifestations, and he suggests a decoupling of rostral and caudal sleep-regulating mechanisms. Villablanca also makes the very cogent point that while certain activities may occur when higher centers are damaged, this does not mean that lower centers are functioning alone when the brain is intact.

If we follow this path, the suggestion that REM sleep is involved in certain kinds of information processing, which we have reviewed for both animals and humans (Greenberg and Pearlman, 1974), begins to make more sense. The activity of the cortical interneurones, which Steriade suggests may be related to "higher neural activity," is most prominent during a phase of sleep that has been shown to be involved in the integration of experiences that have occurred during waking. It is hard to believe that such responses are merely passive events secondary to the firing of pontine cells. Rather they must be part of an integrated activity of the brain during REM sleep that results in integration of information. It would be of interest in this regard if Steriade could provide data showing the relative timing of the increase in firing of the interneurones in relation to REM and to FTG cell firing. Do they precede, coincide precisely, or follow? The pontine cells may then be seen as a trigger for such activity, just as the pontine reticular formation is necessary for the arousal associated with waking behavior. The brain stem reticular formation cannot be considered the source of waking perceptions and behavior, and the pontine FTG cells cannot be seen as responsible for the information processing occurring in REM sleep. Instead, we must consider that Steriade's demonstration of interneuronal activity during REM sleep shows more clearly how the cortex may be involved in this process. We can say, at a minimum, that there is a clear correlation between a particular kind of cortical neural event, a phase of sleep that is associated with information processing, and a mental activity (dreaming), which may provide some clues as to the nature of the information processed. This certainly suggests that the cortex plays an active role in the development and function of REM sleep.

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The cellular substrates of state. It is becoming increasingly evident to the physiologists of state that, in order to develop more functional constructs of sleep and waking, a better understanding is needed of the cellular events subserving these behaviors. It is the heterosynaptic interaction of these cellular events that not only generates the behavioral states but also gives rise to the specific electrophysiological concomitants that state physiologists use as criteria to define wakefulness, slow-wave sleep, and rapid-eye-movement sleep. Early studies of spontaneous neuronal discharges across sleep/wakefulness cycles did little more than to dispel the naive notion that neurons are silent during sleep. However, recent neurophysiological studies, emphasizing more rigorous identification of the type of neuron recorded, have described the diversity of cellular activity across states. The approach used over the last six years by Dr. Steriade and his colleagues should stand as a model for neurophysiologists interested in the analysis of the cellular basis of state. It will be through the rigorous characterization of input-output relationships of individual neurons, a method utilized by Steriade, that the elucidation of the substrates of state will become possible.

Unfortunately, those brain areas particularly implicated in providing the substrate for the genesis of sleep and waking states – i.e. tegmento-reticular systems and possibly basal forebrain mechanism – are so anatomically heterogeneous that specification of neuronal type becomes difficult. Nevertheless, the use of anatomical as well as electrophysiological techniques to specify as much as possible the neuronal subtypes in a particular area should now be considered a minimal prerequisite for the investigation of the role of neuronal activity in the generation of sleep/waking states.

Steriade argues in his paper that, as a first step, it has been appropriate to investigate state alterations in the input-output relationships of the mammalian neocortex in lieu of well-specified systems in the brainstem, the neocortex being a structure specified in greater detail and, in addition, one that provides corticofugal influence on state-generating mechanisms in the brainstem. I was particularly appreciative of the didactic treatment given by Steriade (section 2) to the process he has used in identifying the neuronal types from which he has recorded and which he discusses in the subsequent section. His elegant presentation of the technical approach used to specify the neuronal types, his discussion of possible artifacts and misinterpretations, and his thoughtful explanation for the variation in results obtained by other investigators all serve to bolster his admittedly speculative proposals concerning the role of cortical interneuronal networks in mnemonic mechanisms.

His intriguing observation of the apparent electrophysiological dichotomy between output pericruciate pyramidal neurons and local circuit neurons across states of arousal demonstrates spontaneous heterosynaptic interactions heretofore only suggested. It was particularly surprising to read of the apparent homogeneity in the spontaneous activity of the morphologically-diverse type II interneuronal elements. One would have predicted far more diversity in activity across states. Steriade is quick to recognize the possibility of recording bias, particularly with this elusive neuronal population, and this could be a possible reason for such homogeneity. Even more intriguing was the observation of the parallel between the spontaneous activity in output and interneuronal elements across states in pericruciate and association cortex. The data argue for a rather general cortical pattern of state-dependent discharge, a situation not anticipated when one considers the apparent functional diversity of cortical regions. Does this mean that corticofugal interaction with subcortical regions may have a somewhat uniform output-operating system?

Steriade presents evidence for reticular mechanisms in the regulation of cortical neuronal excitability and discharge during waking and sleep. Clearly, a multiplicity of subcortical inputs influence output as well as interneuronal elements in cortical structures. With the new data presented on the virtually inverse relationship of discharge patterns between output and local neurons in cortex, the time would seem ripe for the investigation of possible specificity of putative neurotransmitters in producing alterations in discharge patterns across states. The technique of micro-iontophoresis lends itself well to this type of investigation and could be employed to further this end. For example, recent evidence suggests that both norepinephrine (NE) and serotonin (5 HT) cell bodies generally discharge more slowly in sleep than during arousal, and during REM sleep they are virtually silent. Are alterations in cortical output discharge rates across wake/sleep the result of direct NE (and/or 5 HT) postsynaptic action or are they indirect, through interneuronal networks, probably utilizing gamma-aminobutyric acid (GABA) as a mediator? Micro-iontophoretic application of GABA antagonists such as bicuculine to identified output neurons during different sleep/wakefulness states could be fruitful in answering this question. Further, is the atropine-sensitive acetylcholine excitation of identified cortical pyramidal cells state-specific or altered during state changes in the unanesthesized preparation? With the judicious application of putative neurotransmitters, now known to be present in abundance in these cortical areas, one could help resolve the question of the interaction between discharge patterns of output and interneuronal cortical elements observed during changes of state.

The state of REM sleep is unique, and the data presented by Steriade support this electrographic observation. Of particular importance would be data supporting the proposition that output and interneuronal elements in cortex respond differentially to REM sleep processes. Apparently, as others have reported, the increase in the mean firing rate of cortical-output neurons during REM sleep is brought about by both tonic and phasic increases in firing patterns. The astounding new piece of evidence is that the interneuronal increase in discharge rate is entirely due to an increase in spontaneous bursting associated with phasic components of REM sleep. However, the dual increase in discharge rate of these normally reciprocal sets of neurons also suggests a curious uncoupling of the normal waking and sleeping relationship of cortical-output cells and interneurons during REM sleep. This would seem to suggest that reticulopetal efferents to neocortex adhere to highly state-specific hodological principles.

In a more speculative vein, Steriade suggests that in addition to the neocortical cellular response to state changes, association neocortical output neurons may well participate in the development of state changes. A mechanism is suggested whereby output neurons of cortical areas 5 and 7 may, by clustered bursting patterns, influence medial thalamic mechanisms intimately involved in the development of synchronized spindle bursts characteristic of sleep. Mechanistically, this is very appealing. Although largely forgotten, the role of cortical and/or basal forebrain mechanisms influencing alterations in state is experimentally established. Doubtless, it is within the brainstem that the basic mechanisms generating the alterations in state reside. However, as sleep is not strictly temporally obligatory, forebrain and cortical mechanism must have tremendous influence on these brainstem generating mechanisms. Data presented in this paper support such a possible mechanism of interaction. It is also now appreciated that phasic events of REM sleep, in particular the ubiquitous ponto-geniculo-occipital (PGO) waves, although generated in the pontine reticular formation, are nonetheless under strong modulatory control from cortical sites. Future investigation of these events with a cortical, and particularly frontal, level of neurophysiological analysis, may lead to a more complete understanding of the mechanism underlying these events.

Finally, the experimental observation that neocortical interneurons specifically increase their discharge rate during REM sleep leads Steriade to speculate on the possible role of this local circuit neuronal population (and the state of REM sleep) in memory consolidation. Evidence is cited indicating that increased REM sleep time occurs in both humans and experimental animals following learning tasks. However, one wonders why, if there is such a preeminent relationship between memory processing and REM sleep, is there such a well-established inverse relationship between ontogenetic development and REM sleep? Furthermore, the specificity of the interneuronal bursting in REM sleep appears to be incomplete, as slow-wave sleep also exhibits weak bimodal interval characteristics associated with dramatically increased discharge rates. These facts tend to weaken the proposal of a REM sleep state-specific role for association neurons in mnemonic mechanisms. What seems intriguing, however, is the apparent uncoupling of reciprocal interaction between output and interneuronal cortical systems during REM. Perhaps this process, taken as a whole rather than considering the local circuit neurons independently, may uniquely subserve the consolidation of information acquired during waking. Perhaps these mechanisms, or similar processes occurring in allocortical structures, may ultimately describe mechanistically the unique information-retrieval paradox of REM sleep. It remains a fascinating question why the vivid memory for oneiric phenomena occurring during REM sleep episodes is precipitously lost following induced arousal. This argues for a unique REM state process for dealing with a memory-retrieval mechanism. Using the techniques outlined by Steriade, the investigation of these processes across states seems feasible at the neuronal level.

In the final analysis, the schema described for the alterations in discharge patterns of neocortical neurons is admittedly simplified. However, the data concerning the unexpected reciprocity of output and interneuronal elements across state are extremely provocative, and, if generalizable to other cortical and subcortical neuronal systems, provide a rich new conceptual framework for the process of state. One wonders how the observations of Steriade's group will fit with the observation of specific state-spanning neurons identified in the basal forebrain, with the possibility that nonspiking interneuronal networks may participate in state processes, and with the recent recognition that various putative transmitter substances may have unexpected diversity in their time constants of postsynaptic action. Of particular interest in the latter category may be the recently identified hypothalamically-derived endorphins with extensive innervation of not only medial thalamic structures but also of the brainstem catecholamine and indoleamine systems thought to be involved in the regulation of state. Despite the increasing complexity of the generation and elaboration of the sleep/waking continuum, the data presented in this paper suggest a highly successful methodological strategy for the analysis of state mechanisms and offer a descriptively-intriguing neurophysiological conception of the neocortex as a structure more than passively involved in the alternation of behavioral states.

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On the proportions of identified output cells and putative interneurons in the precentral arm area of the monkey's motor cortex. It was both informative and a pleasure to review the paper by Steriade on the behavior of efferent cells and presumed interneurons in motor and parietal association areas of the cortex during the sleep (S)-waking (W) cycle. This investigator's emphasis upon the anatomic identification of cells that are observed during the S-W cycle would appear to be not only an important methodological advance in this particular area of research, but a practice that should be used in all studies in which attempts are made to correlate central neuronal activity with various behavioral states. Clearly, the results of any correlative study of this type can be interpreted functionally only when the projection targets of various behaviorally-defined categories of cells are also known.

Steriade's review consists essentially of three sections: first, a discussion of the criteria that may be used to identify cortical neurons as either output cells, or as putative interneurons; second, a discussion of principally his own findings with regard to the behavior of efferent cells and interneurons in motor and parietal areas of the cortex in relation to the S-W cycle; and third, the presentation of a provocative but empirically less well-grounded hypothesis concerning the role of association cortex interneurons in consolidation of memory traces during REM stages of sleep.

This commentary will be directed principally at sections one and two. In discussing these sections, data will be presented from recent studies on the cellular composition of the monkey's precentral gyrus, which may allow the reader to assess with somewhat better perspective some of the more subtle implications of Steriade's methods and findings.

Criteria for identification of cortical output cells and interneurons. Steriade's discussion of the criteria for antidromically identifying cortical output cells is excellent. Clearly, of the criteria that are used, those of collision between spontaneous and evoked spikes, and of near-invariant response latency during *both* low- and high-frequency stimulation of a particular output pathway, are the most definitive. As has been noted previously (Humphrey 1968; Steriade *et al* 1974 *op. cit.*), the somatodendritic (SD) portion of a cell may fail to respond faithfully during high-frequency antidromic activation if it is also subjected to recurrent inhibition. Moreover, monosynaptically-activated cells have been observed in other systems to respond faithfully to stimulation rates in excess of 200–300 pulses/sec (Jankowska and Roberts 1972; D. R. Humphrey, unpublished observations on the responses of rubrospinal tract neurons to stimulation of nucleus interpositus).

The criteria that are proposed for identification of cortical interneurons – namely, absence of antidromic activation, high-frequency burst response to orthodromic activation, and a preponderance of short and long intervals in the spontaneous interspike interval histogram – are clearly less definitive. Failure to meet the first criterion may mean only that the appropriate output pathway has not been stimulated. Moreover, as noted by Steriade, output cells may also respond with multiple spikes to particular synaptic inputs. On the other hand, the third criterion may be too restrictive, for it fails to admit the possibility of interneurons that fire with more regular (non-burst) patterns. At present there appears to be insufficient evidence available on the discharge properties of clearly-identified interneurons in the unanesthetized nervous system to evaluate the proposed criteria adequately. In the absence of such information the criteria proposed by Steriade are perhaps appropriate as a first *approximation,* for they are clearly defined, have led to observations of interest already, and can easily be modified as required by future findings.

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Which output cells are identified and which are missed when the cerebral peduncle and specific thalamic nuclei are stimulated? Although it has long been known that the motor cortex projects to a variety of subcortical structures, the prevailing view until recently has been that many of these projections are composed principally of pyramidal tract (PT) axons (see, e.g., Endo *et al* 1974). Moreover, the precentral gyrus of the monkey, or at least its middle and deeper layers (III-VI), has been viewed principally as an output structure. Thus it was reasonable to assume that by studying identified PT cells within the motor cortex (and in particular within the arm area), one could in fact characterize the behavior of the majority of output neurons within this particular cortical zone.

Recent evidence has begun to suggest, however, that the various projections that issue from the precentral gyrus are derived principally from separate neuronal populations. The corticorubral projection has been found, for example, to originate almost exclusively from small *non*-PT cells, which lie just superficial to corticospinal tract cells within layer V, and which are about half as numerous as PT neurons (Humphrey and Rietz 1976; Jones and Wise 1977). Corticobulbar, corticostriatal, corticopontine, and corticothalamic projections from this zone are also thought to arise from separate populations of non-PT cells (Kitai *et al* 1976; Jones *et al* 1977; Catsman-Berrevoets and Kuypers 1976; Humphrey and Corrie 1978). Thus the PT neuron system, though substantial, appears to account for a smaller fraction of the output cells within the precentral gyrus than was originally supposed. In fact, using cell counts from single-unit recording (Humphrey and Rietz 1976; Humphrey and Corrie 1978) or retrograde-cell-labeling experiments (Jones *et al* 1977; Jones and Wise 1977; Humphrey, Gold, and Rowinski, unpublished data on the corticospinal and corticorubral projections), it is possible to estimate the relative densities of each of these cell systems within layers III-VI of the monkey's precentral arm area, and thus to arrive at an order-of-magnitude estimate of the relative numbers of output cells, as opposed to presumed interneurons, within this particular cortical zone.

The major intralaminar locations of these various neuronal output populations are shown in Figure 1, along with a qualitative ranking of the modal soma-dendritic size of each neuron type. Estimates of the average packing density of each cell group within its layer of residence are shown in Table I. These estimates were computed by measuring the volume occupied by the layer of residence on a 50-micron thick section through area 4, and by counting the number of retrogradely-labeled cells within this volume on representative sections through the same zone (either those from our own labeling experiments or from the published figures of Jones *et al* 1977, and

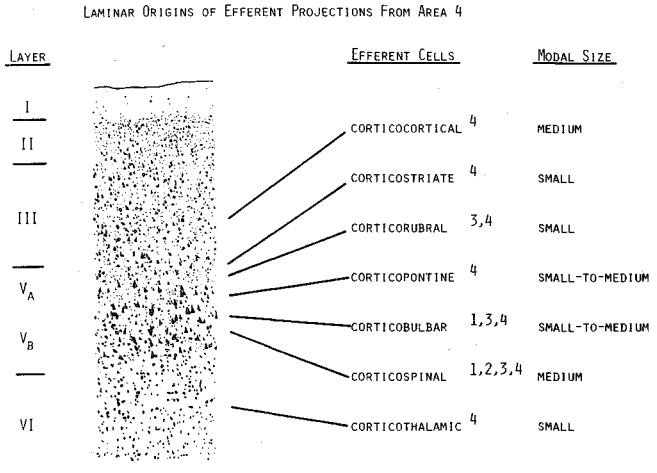

Figure 1 (Humphrey). Intralaminar locations of identified populations of cortical output cells, as identified by single-unit recording and retrograde-cell-labeling studies. A camera-lucida tracing of a Nissl-stained section from the precentral arm area of the monkey's motor cortex is shown to the left, in order to illustrate the general sizes and relative densities of its cells, and the approximate locations of the laminar boundaries. Numbers to the right of each labeled projection system indicate the studies from which data leading to this summary figure were drawn. 1: Catsman-Berrevoets and Kuypers 1976; 2: Coulter et al. 1976; 3: Humphrey and Rietz 1976; Humphrey and Corrie 1978; 4: Jones et al. 1977, Jones and Wise 1977. The relative modal size of each type of cell is also indicated.

Table 1. *Estimated average packing densities of identified efferent cells within cortical layers III, V, and VI of the monkeys precentral gyrus (arm area).*

Cell System	Layer	Average packing density in cells/(0.1 mm)³	Packing density assuming only ¼ of cells are labeled	% of cells in that layer[a]	Packing density estimates from microelectrode studies (cells/(0.1 mm)³)
Corticocortical	III	0.61	2.44	12.0	—
Corticostriatal	V	0.08	0.32	1.5	—
Corticorubral	V	0.20	0.80	3.8	1.00 (4.8%)[b]
Corticopontine	V	0.10	0.40	1.9	—
Corticobulbar	V	0.10	0.40	1.9	0.40 (1.9%)[c]
Corticospinal	V	0.50	2.00	10.0	2.40 (11.4%)[c]
Corticothalamic	VI	2.00	8.00	38.0	—

[a]Computed from the preceding column, using the estimate of 21 cells/(0.1 mm)³ for the average neuronal packing density within layers III-VI of this area of the monkey's precentral gyrus (Tower and Elliott 1952). [b]Humphrey and Rietz 1976. [c]Humphrey and Corrie 1978.
The remaining estimates were obtained from the figures published by Jones *et al.* (1977), Jones and Wise (1977), and our own studies of retrogradely-labeled corticorubral and corticospinal cells.

Jones and Wise 1977). In order to take into account the facts that the horseradish peroxidase (HRP)-diamino benzidine method may be less sensitive than newer techniques (Mesulam 1977; Hardy and Heimer 1977), and that only a portion of the cells may be labeled following relatively discrete injections of HRP, a column of estimated densities is also presented, in which the observed densities were multiplied by a factor of four; the percentage estimates shown in column five of Table I were computed with the use of these inflated values.

The general implication of these estimates is clear. Even if it is assumed (a) that each of these output cell populations is completely separate, and (b) that only an average of one fourth of the cells in each are labeled by localized HRP injections into the appropriate target structures, they would still appear to account for only 12% of the cells within layer III,[1] 19% within layer V, and 38% within layer VI of this major efferent zone of the *motor* cortex! Thus, assuming that other projection systems of substantial size have not been overlooked, some 62-81% of the cells within layers III-VI, and an even greater proportion of those within layer II, would appear to be local interneurons.

But what implications do these estimates have for the methods and results reported by Steriade in his studies of the behavior of motor cortex cells during the S-W cycle? As this commentator sees it, there are two.

First, it must be noted that Steriade's method of antidromically identifying presumed PT cells by stimulation of the cerebral peduncle was in fact a perhaps fortuitous, but nonetheless excellent, choice. Although many of the neurons fired antidromically from this site are most probably *not* PT cells, the method nonetheless allows one to more thoroughly sample several subcortically-projecting cell systems (corticorubral in part, corticopontine, and corticobulbar) than would be the case if the electrodes were placed more caudally within the pyramidal or corticospinal tract. Moreover, by stimulating also within the thalamus, another major output population was identified and sampled. Thus, though many were no doubt non-PT cells, Steriade's assumption that stimulation of the peduncle would allow one to observe a significant portion of the output neurons within the precentral gyrus appears to this commentator to be justifiable, in the light of recent anatomical data. This point adds strength to his general conclusions concerning the behavior of cortical output cells within the precentral-arm area in relation to the S-W cycle. Moreover, since the majority of these output cells are of the small and slowly-conducting type (Humphrey and Rietz 1976; Jones and Wise 1977), with fast-conducting PT cells accounting for only 20% of the PTN population in this cortical area (Humphrey and Corrie 1978), it may be further deduced that the majority of subcortically-projecting output cells in this zone will perhaps fire in relation to the S-W cycle in the manner described by Steriade *et al.* (1974 *op. cit.*) for slow PT (or peduncular) cells.

The second implication of the estimates shown in Table I is somewhat less supportive. If these order-of-magnitude estimates are correct, then it is clear

that a substantial portion of the cells within the precentral gyrus may be interneurons, and in particular those within layers II and III. Yet, whereas the sample of units reported by Steriade *et al.* (1974 *op. cit.*) in their studies of the monkey motor cortex contained some 134 peduncular cells and 10 corticothalamic units, observations were obtained from only 14 putative interneurons; to this we may add Steriade's present additional 23 interneurons from the parietal lobe of the cat. Clearly, this failure to observe interneurons in more substantial numbers was due in part to their small size, the difficulty in observing them uninjured over the entire S-W cycle, and the known selectivity of micro-electrode sampling procedures for large neurons (Towe and Harding 1970; Humphrey and Corrie 1978). Moreover, it may have been due in part to the known difficulty in recording from superficially-located cortical cells after compressing and penetrating the tough and thickened dura of a chronic recording preparation with a micro-electrode. But it may also have been due in part to criteria for identification of interneurons that were too selective, that would omit cells not firing spontaneously in widely-separated bursts or not responding with burst-like patterns to orthodromic inputs.

Whatever the reasons, it seems to this commentator that the apparently substantial interneuronal populations within the motor and parietal areas have not as yet been sampled in sufficiently large and representative quantities to allow firm conclusions about their *general* behavior in relation to the S-W cycle, or their role in the consolidation of memory traces.

But Steriade's hypotheses are nonetheless provocative and a stimulus for further research. Perhaps additional evidence for or against them might be obtained from (1) studies in which single-unit recordings are obtained during the S-W cycle from *histologically-confirmed* sites within the more superficial layers of the cortex, where the proportions of interneurons would appear to be greatest; and (2) studies in which stimulating electrodes are placed within the internal capsule, allowing for identification of a greater proportion of cortical output cells, and also within the *cortex* near the site of recording, allowing one to drive and identify cortical interneurons that might not fire spontaneously during the unit-search procedures, and which could, therefore, be missed in the sampling process.

NOTE

1. Earlier electrophysiological studies suggested that layer III of the motor cortex of the cat also contained substantial numbers of slow PT cells (Towe *et al.* 1963). More recent electrophysiological (Humphrey 1968; Humphrey and Corrie 1978) and retrograde cell labeling studies (Groos *et al.* 1978; Jones and Wise 1977; Humphrey and Gold, unpublished observations) have failed to confirm this conclusion, however, and have shown that the PT projection originates exclusively from layer V in both cat and monkey.

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Sleep-waking studies on the lateral geniculate nucleus and visual cortex. In his paper Steriade emphasizes that to understand neuronal events related to sleep and waking, it is of primary importance to specify the cell types; otherwise all the data are simply pooled, providing no clear insight into the neuronal mechanism of sleep-waking. We believe that classification of cell types is not only advisable in sleep-waking studies but in all fields of neurophysiology.

Steriade cites an earlier work by Sakakura (1968 *op. cit.*) from this laboratory, which was designed to reveal sleep-waking-related changes of neuronal activity in the cat lateral geniculate nucleus (LGN). While studying LGN neurons, Sakakura classified them into two types: P cells, which are relay cells identifiable with antidromic stimulation of the visual cortex, and I cells, which respond with repetitive discharges to stimulation of the optic nerve and the visual cortex. This classification was first introduced by Burke and Sefton (1966 *op. cit.*) for the rat LGN with the assumption that I cells are inhibitory interneurons for P-cells. Obviously, P and I cells correspond to Steriade's Type I and II neurons, respectively.

It is clearly seen in Sakakura's data that, in transition from synchronized sleep to either quiet waking or desynchronized sleep, P cells increase firing rates. This is consistent with the behavior of Steriade's Type I cortical neurons. Concerning I cells, which correspond to Steriade's Type II neurons, Sakakura did not draw any definitive conclusion, but from a detailed examination of the data he suggested that I cells would be less active during quiet waking than during synchronized sleep, indicating opposite behaviors for P and I cells. Although Sakakura reported that P cells were excited by REMs during synchronized sleep, no explicit descriptions were given of how firings of I cells were affected by REMs. The sleep-waking studies on cortical neurons by Steriade and associates are valuable, in that they succeed in making detailed observations on Type II neurons.

In the past 10 years there has been an enormous expansion of our knowledge on functional and morphological properties of LGN cells. The principal points are as follows:

1. Both functionally and morphologically, retinal ganglion cells are classifiable into three types: Y, X, and W types. Similarly, P cells of the cat LGN are differentiated into Y, X, and W types according to their respective retinal inputs (Wilson et al. 1976).

2. It has been shown in cats and rats that I cells, once believed to belong to LGN, are in fact neurons of the perigeniculate reticular (PGR) nucleus (Dubin and Cleland 1977; Sumitomo et al. 1976). In rats evidence has been presented that PGR neurons are a source of inhibition for P cells (Sumitomo et al. 1976).

3. In cats and rats a species of LGN cells has been identified electrophysiologically (Dubin and Cleland 1977; Sumitomo and Iwama 1977) as corresponding to the morphologically-established interneurons believed to be inhibitory cells from the configuration of synaptic vesicles contained (Kriebel 1975).

Given these new pieces of evidence, we feel that the simple dichotomy of LGN cells that was useful in previous experiments may not be satisfactory in future studies. For example, we are interested in knowing whether the three types of P cells show different behaviors during the sleep-waking cycle, because Foote et al. (1977) have reported that in acute experiments the excitatory effect from the brainstem reticular formation on X-type P cells was found to be stronger than on Y-type P cells. Also, it seems worthwhile to examine whether the effects of sleep-waking are different, comparing neurons of PGR and interneurons of LGN, both presumed to be inhibitory neurons for P cells (Burke and Sefton 1966 *op. cit.*; Dubin and Cleland 1977; Sumitomo et al. 1976) and found to receive inhibition from the brainstem reticular formation (Fukuda and Iwama 1971; Sumitomo and Iwama, 1977).

Finally, brief mention should be made of the visual cortex. It was about 20 years ago that, from differences in response properties to visual stimulation, neurons of the visual cortex were classified into three types: simple, complex, and hypercomplex cells (Hubel and Wiesel 1957). Their morphological counterparts are now known (Kelly and Van Essen *op. cit.* 1974), and a question as to their relations to the three types of LGN P cells has been answered at least in part (Wilson et al 1976). On the other hand, Toyama and associates (1974) have succeeded in identifying three species of projection neurons (associative, commissural, and cortico-geniculate or -tectal) in the visual cortex by means of antidromic stimulation; to explain synaptic effects of specific visual afferents upon these projection neurons, they postulated the existence of a variety of excitatory and inhibitory interneurons. Only with experiments designed along these lines of new evidence can one resolve divergences in opinion about the sleep-waking-related behaviors of neurons of the visual cortex (Steriade and Hobson 1976 *op. cit.*).

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Steriade's current monograph dispels a great deal of confusion with regard to the activity changes of cortical neurons during the sleep-waking cycle. By careful, painstaking studies he has successfully distinguished between cortical output cells and local or interneurons and has convincingly shown that these two cell populations behave in an opposite manner in the transition from wakefulness (W) to synchronized sleep (S). According to this finding, the ambiguity of earlier studies, which showed heterogeneous changes among individual cortical neurons, is rectified to reveal a constant and organized change in neuronal activity in two functionally-distinct cell groups. Secondly, the inverse changes, which occur as an increase in interneuronal activity compared to a decrease in output neuronal activity in S with respect to W, suggest that the interneurons may be responsible for the decrease in output neuron activity during S. Such an inhibitory influence of the interneurons on the output neurons would represent a very important basic mechanism for the maintenance of sleep. This hypothesis is supported by Jasper and his colleagues' finding (Jasper et al. 1965 *op. cit.*) that GABA, known to be an inhibitory transmitter, is released from the cortex in greater amounts during S than W. Unfortunately, however, as Steriade points out, a uniquely inhibitory influence of the interneurons upon the output cells cannot be inferred during sleep, since both inhibitory and excitatory local neurons are recorded and may be active in this state. Furthermore, in terms of rate changes, the inverse relationship of interneuronal and output activity is not very marked, since the mean rate for interneurons is 200% greater, while that for output cells is only 20% less during S than that during W (calculated from the mean rate reported by Steriade for W and S of interneurons: 0.33/sec and 0.66/sec, and for output neurons: 12.97/sec and 10.35/sec, respectively). Such a small change in spontaneous firing rate evinces the same surprise and confusion that it did in earlier studies. These findings suggest once again that in the explanation of the drastic state changes that occur in the transition from quiet W to S, the *spontaneous* rate of firing may not be of great significance. On the other hand, the change in excitability of the output cells, which Steriade describes as a great decrease in S compared to W, may be the most crucial functional change. Steriade's careful studies of multiple parameters in the activity of distinct populations of cells permit a great step to be taken in beginning to understand basic mechanisms of the sleep-waking cycle through correlates of neuronal activity.

But the ultimate question in this field of research concerns the generating mechanism of sleep. Steriade has presented a scheme by which interactions of cortical interneurons with output cells, and output cells with brain-stem neurons, may maintain the state of sleep. Structures necessary for the initiation and primary maintenance of synchronized sleep have been localized to the lower brain stem by lesion and transection studies (see, for review, Moruzzi 1972 and Jouvet 1972). Yet recording of neuronal activity throughout the brain stem has not revealed single-unit activity that correlates with the onset or maintenance of synchronized sleep. So one wonders, as did researchers ten years ago, whether spontaneous neuronal activity during the sleep-waking cycle can reveal the basic mechanisms of that cycle.

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Toward an understanding of the basic mechanisms of the sleep-waking cycle. As Steriade recounts in his introduction, the original studies of neuronal activity during the sleep-waking cycle initially produced great surprise, due to the unexpected finding that cortical cells did not cease or dramatically decrease firing during sleep, as had been expected from the popular theory that sleep subserved the function of rest.

Continued studies provoked a certain confusion, due to the heterogeneity in the pattern and rate change among individual neurons' activities in the transition from waking to sleep. Indeed, although the approach of single-unit recording during a normal sleep-waking cycle has described neural activity correlates for many different cells in many brain regions, this approach has not as yet illuminated the basic mechanisms of the dramatic state changes that occur in this cycle.

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Cortical interneurons and paradoxical sleep. Despite (and probably also due to) the fact that I have reached the highest level of incompetence concerning the recent developments of unit recordings, I have read Dr. Steriade's paper several times with the utmost interest.

In most cases, indeed, the description of unitary activity during the sleep-waking cycle does not provide much information about the mechanism or the function of sleep. However, the discovery of opposite-unit behavior in output cells and cortical interneurons has great heuristic value for the understanding of D sleep or paradoxical sleep (PS). For this reason I will limit my commentary to some problems raised by Steriade in the field of PS mechanisms.

1. "FTG" neurons as selective executive PS cells. The selective increase of activity of FTG (giant tegmental field) cells during PS, as com-

pared to W and S, has been obtained in cats habituated to immobilization in a head holder (Hobson et al 1974 *op. cit.*). Subsequent experiments on freely-moving cats have shown that FTG cells can discharge at the same high rate during waking, and that "PS selectivity" was an artifact (Vertes 1977; Sakai et al. 1978). Thus, FTG cell activity is probably related to the central motor events occurring either during W or PS. Although this does not totally rule out the possibility that some FTG cells might be involved in executive mechanisms of PS, it is probable that most of them are output cells receiving inputs from the executive mechanisms of PS. Moreover, the use of the term FTG (or FTC) is unfortunate and ambiguous. The recent histochemical or neuroanatomical techniques have made possible the delineation of several different sybsystems in the pontine or mesencephalic reticular formation. Even if the latest results of histo- or immunofluorescence are not taken into consideration, the still instructive cytoarchitectonic classification of Taber (1961) should be used. The distinction of only two subdivisions, FTG or FTC in the brain stem RF, is quite useless, since it would signify that those two systems are intrinsically homogenous, which is not the case according to lesion studies (Jouvet 1962).

These criticisms do not apply to the main core of Steriade's hypothesis, since the exact location of the "executive neurons" of PS does not need to be known in order to test his theory further.

2. Mesencephalic "FTC" and cortical activation during W and PS. I am less optimistic than Steriade concerning the role of those FTC cells in cortical activation during W *and* PS, since: a) rostral lesions of the mesencephalon may suppress cortical activation during W but not during PS (Jouvet 1962); this indicates either different mechanisms or pathways responsible for fast cortical activity during W or PS; and b) almost total transection of the brainstem does *not* suppress cortical activation during PS, provided that a small bridge of neural tissue is left, dorsally or ventrally; this suggests that neurohormonal or neurohumoral effects are more likely to be involved than classical neural transmission.

3. Selective REM-related increases in discharge rates of interneurons. It is now well established that REMs are causally related to so-called PGO activity (Cespuglio et al. 1975). This activity is generated, through the interplay of complex inhibitory and excitatory phenomena in some clusters of cells located outside the so-called FTG area in the nuclei parabrachialis lateralis, dorso lateralis tegmenti, Kolliker fuse. These neurons project either to the premotor area for the oculomotor nuclei or to a relay located in the brachium conjunctivum (X region), which projects directly to the lateral geniculate nucleus (Sakai and Leger 1977).

Cortical interneurons are thus selectively influenced by the PGO generator (and not by REM itself). The following experiments could strengthen this interesting hypothesis.

a) Does lesioning (or reversible cooling) of the last relay of ascending PGO activity in the isthmus (which selectively abolished the PGO wave in the lateral geniculate without altering other components of PS – i.e. cortical activation or REM, (Sakai et al. 1976)) suppress the phasic increase of interneuron discharge during REM? In such a case, cortical interneurons should be silent during PS, while output cells should probably continue to produce high *tonic* activity.

b) It is possible to induce discharges of PGO waves *during waking,* either by pharmacological or lesional alterations of monoaminergic neurons, which may have some inhibitory role upon the PGO generator (injection of reserpine, lesioning of the Raphé dorsalis or the locus coeruleus proper). This permanent discharge of PGO activities has been called "reserpinic" or "pseudoreserpinic syndrome" (see review in Jouvet 1972). Thus it would be important to know whether cortical interneurons are phasically active in relation to induced PGO activity during W. This would permit deciding whether cortical interneurons are selectively activated by PGO *only during PS,* or are strictly dependent upon ascending PGO activity (whatever the state of the animal, W or PS).

These two experiments would be of great heuristic value in demonstrating the possible independence of Golgi type-II neurons from output neurons.

c) If a positive answer to these questions is furnished, then Steriade could investigate further concerning the function of cortical (and possibly subcortical) interneurons.

i) It has been shown that the PGO (or REM) patterns could have some genetic components, at least in mice (Cespuglio et al. 1975).

ii) Moreover, when inhibition of muscle tone does not appear during PS (after destruction of locus coeruleus alpha, the main inhibitory control center of muscle tone during PS located outside the FTG area), dramatic species-specific behavior occurs during PS. This "oneiric behavior" is related directly or indirectly to the occurrence of PGO activity (Sastre and Jouvet 1977).

It may accordingly be possible that Golgi type-II cells are responsible for the organization of this behavior by their action on output cells. Thus, cortical (and subcortical) interneurons may be the executive neurons of some genetic (endogenous) programming that invades the brain during PS (Jouvet 1978). If this hypothesis is true, the phasic activation of cortical interneurons would be related more to genetic memory than to putative epigenetic memories related to learning. It should be recalled here that the relationship between PS and learning is still not established (Vogel 1975). Since PS is much increased during ontogeny, it should be very interesting to study the ontogenetic evolution of the selective discharge of cortical interneurons during REM in kittens – which begins first: tonic activity of output cells or phase increase of "maturing" Golgi type-II cells?

iii) Finally, I should also address one last question to the skillful methods of Steriade: Are there interneurons in cortical perceptual areas that phasically increase their activity during REM? Would it be possible that they impinge upon "innate releasing templates"? There are neurons which are selectively excited in monkeys during waking by distress calls – powerful *innate* and biologically-significant stimuli. Are there similar neurons in the cat cortex? If these neurons are selectively activated by Golgi type-II cells during PS, then we could possibly test the hypothesis of a genetic programming by these interneurons whose unit behavior has been so well unveiled by Steriade.

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Cholinergic control of excitability in the sleep-waking cycle. A basic question that is raised by a reading of this article is: Should one try to understand sleep? A case could well be made that there have been too many fruitless attempts and too much elaborating of "epiphenomena" (cf. Evarts, as quoted by Steriade here), and that until the key issues have been brought to light, it

is pointless to try to answer questions that cannot even be formulated adequately.

I believe that Steriade's approach is a real advance, since it uses a methodology that can be applied to large numbers of cells and permits relatively prolonged observations of single units, whose behavior can thus be correlated with changes in the state of vigilance. On the basis of a simple classification as "output" and "non-output" cells, Steriade has been able to show systematic differences in the behavior of these two populations. Together with a simple test of excitability provided by an examination of antidromic invasion, this has been sufficient to resolve some of the confusion caused by the apparent lack of any regular pattern of firing behavior of cortical units during the sleep-waking cycle. Moreover, his observations have led him to propose a coherent scheme of reciprocally-organized cortical output cells and inhibitory interneurones.

Cholinergic control of output neurones. A key factor in this scheme is a cholinergic innervation, ascending from the brain stem (probably from the midbrain reticular formation). It may therefore be useful to summarize the salient features of this postulated cholinergic system.

A significant point is that ACh (acetylcholine) excites predominantly (and perhaps exclusively) deep cortical neurones, and particularly such output neurones as the cells of origin of the pyramidal tract (PT cells) (Krnjevic & Phillis, 1963). The cholinergic system thus appears to have a biasing function at the output from the cortex.

The characteristics of this muscarinic excitatory action are as curious and specific as its localization. It is relatively slow and is typically associated with long after-discharges. Moreover, it is accompanied by what at first seems like a paradoxical *rise* in cell resistance, probably due to a diminution in potassium (K) conductance (Krnjević et al. 1971 *op. cit.*).

Unlike some other excitatory actions, this would initiate a rapid depolarization only if there is already a substantial background of high sodium conductance; in other words, it is most effective as a facilitator of other excitatory inputs. By raising the membrane resistance, it also increases the space constant along the cell and its dendrites and should therefore increase the effectiveness of synaptic inputs arriving at relatively distant dendritic sites. A further possibility is that the diminished shunting effect of the reduced K conductance may cause dendrites that normally act as passive conductors to become capable of initiating propagated action potentials (for an analogous situation in certain invertebrate cells see e.g. Hagiwara et al. 1969). Thus it may be useful to think of many central neurones as being in one of two possible states: one in which there is a high resting potassium conductance that makes the cell insensitive to excitatory inputs, and another characterized by a low potassium conductance (induced by ACh), and therefore a high degree of responsiveness to even small inputs.

As emphasized by Steriade, inhibitory interneurones in the cortex may be *inhibited* by ACh, so that the ascending cholinergic pathway could act in a push-pull manner, causing both direct facilitation and disinhibition of the output cells. Unfortunately the evidence for the latter is much less plentiful or convincing. Only relatively few "bursting-type" neurones have been tested with ACh and have shown a clear inhibitory effect; and, of course, we do not know with certainty that these cells really inhibit the output cells. One reason why there is relatively little information about their responses to ACh is that cells of this type are not very easily found. This may be a serious problem from the viewpoint of Steriade's hypothesis. Only a large number of inhibitory cells can explain the powerful inhibitory control that is so evident in the cortex. So why are discharges in bursts rather inconspicuous? Inevitably one wonders whether most inhibitory cells are not of a different type, possibly non-spike-generating, in which case the "bursting" neurones may not reflect the behavior of the inhibitory "system."

A possible involvement of calcium in sleep. Under normal conditions the free calcium (Ca) concentration in the cytoplasm of cells is probably at most 10^{-7} M (Baker 1972). Thus the electrochemical gradient tending to drive Ca inward is much steeper than the corresponding gradient of any other ion. Accordingly, much more work must be performed (per unit of ion) to "remove" free Ca from the cytoplasm. Yet there is increasing evidence that substantial amounts of Ca enter nerve cells during the action potential (Baker, 1972). For cerebral neurones that discharge almost continuously, often at quite high frequencies, this must impose a high expenditure of energy.

How, in fact, do nerve cells preserve the normal, very low internal level of free Ca? Much of the Ca^{2+} that enters is immediately buffered by various normally-available, negatively-charged molecules such as ATP and perhaps high-affinity Ca-binding proteins. And, of course, mitochondria (and possibly other internal particles) can take up large amounts of Ca. In the long run, however, even all these buffers cannot accumulate Ca indefinitely, and the excess of intracellular Ca can be disposed of only by outward pumping at the cell membrane.

Another protective mechanism may also help to prevent excessive influx of Ca. In a variety of cells free intracellular Ca tends to raise the membrane K conductance and thus lowers excitability (Krnjević & Lisiewicz 1972). Thus, if free intracellular Ca rises significantly because of excessive firing, an insufficient energy supply, or hypoxia, this causes a fall in excitability, reduces firing, and therefore slows down the Ca influx. If a state of high neuronal responsiveness, associated with much repetitive activity and dependent on a low K conductance maintained by a cholinergic input is indeed an essential feature of consciousness (Krnjević, 1974), its disappearance should conversely result in a loss of consciousness. It does not seem too far-fetched to suppose that as a result of prolonged activity during wakefulness the Ca-buffering capacity of brain neurones progressively decreases, and that sleep at least partly reflects the need for a period of reduced responsiveness and activity, during which the Ca-buffering capacity is restored by energy-consuming processes (ATP formation, Ca outward pumping, etc). The reduced neuronal responsiveness may be directly inactivated by a rise in internal free Ca, as well as indirectly by a diminished ascending cholinergic drive (the latter effect being perhaps reinforced by feedback from the cortex, cf. Dudar & Szerb, 1969).

The obvious weakness of this scheme is that firing in the cortex does not vary with the sleep-waking cycle as much as it would lead one to expect. On the other hand, according to Steriade's evidence, arousal is accompanied by increased excitability of all PT cells, as indicated by the facilitation of antidromic invasion. Therefore the fact that fast-conducting PT cells do not show more rapid firing must be due to either a disfacilitation (owing to depression of an ongoing excitatory input) or direct inhibition through inhibitory synapses on relatively distant dendrites (where they could block certain excitatory inputs without affecting antidromic invasion). Clearly another important modulating influence is that of inhibition.

An observation that is particularly emphasized by Steriade is the greatly-increased firing during sleep of bursting-type neurones. If these are indeed inhibitory neurones, as the author believes on the basis of mainly circumstantial evidence, such increased inhibitory activity would undoubtedly agree with Jasper & Koyama's (1969) finding of augmented GABA release in the cortex during sleep. Moreover, Jasper & Tessier (1971) further observed an increased release of ACh during REM sleep, which thus seems to be characterized by the coexistence of an enhanced facilitatory input and a high level of inhibition. The latter is presumably sufficient to prevent increased firing of output cells. A relatively high level of cortical activity disassociated from a correspondingly-enhanced output may result in a modified form of consciousness – dreaming.

Steriade's proposal that the firing of inhibitory interneurones reinforces memory traces is undoubtedly a useful hypothesis. Perhaps the increased discharge of inhibitory interneurones is tightly linked to specific afferent inputs, thus providing the basis for an internal representation of the outer world. A selective replay of the activity of these cells during REM sleep may result in a more permanent change in their responsiveness. Thus one would have imprinted in a subset of inhibitory cells an image which can be reproduced by appropriate scanning of the total population.

While most of this is speculative, I believe we are significantly nearer to understanding what happens in the cortex during sleep, thanks to Steriade's painstaking and imaginative analysis of unit behavior.

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Output neurons, interneurons, and the mechanisms and function of sleep.

Sleep: how does it happen and why do we need it? After two decades of the modern era of sleep research we are much further along in answering the question of how, and some important progress in this area is the real news of Dr. Steriade's paper. The path into the unknown country of the why of sleep is, we fear, not much advanced.

For the nonspecialist it may be helpful to emphasize what Steriade has done and why it is important. In finding out what is happening in the brain during sleep, it is obviously necessary to poll the constituent members of the vast neuronal assembly. Early studies emphasized recording neurons in particular regions, now accomplished for a large number of brain areas. What Steriade has done, and what had not been done before him, is to make the enormous technical advance of differentiating between cell populations within a brain region. In the parietal association area Steriade has distinguished between the output cells, which project outside this area, and the interneurons, which are likely to be concerned with affairs only within this area.

Although Steriade is the first to report on any population of interneurons during sleep, predictions about their activity have been historically important. The pioneering investigations of Evarts (1964, 1965, 1967, *oper. cit.*) into the activity of output cortical cells during sleep failed to give any real hint as to either generation mechanisms or the function of sleep, and, in particular, it did not give any support for the common-sense notion that neurons rested in sleep. Evarts raised the possibility that other smaller cells, possibly interneurons, might be the ones to show rest in sleep, and if they were inhibitory interneurons, this rest might account for the changes in output cell discharge pattern in synchronized (S) and desynchronized (D) sleep. With the results presented here Steriade lays to rest the interneuron-rest hypothesis, and in so doing, he closes an era in sleep physiology.

Steriade's discussion of the technical and logical problems in asserting that certain neurons are "interneurons" is admirably conservative and cautious (for convenience, we will omit further use of quotation marks around interneurons; the reader may make his own decision whether to insert them mentally). Steriade is, of course, absolutely correct that negative evidence can never be accepted as positive proof: because he was unable to find projection of his interneurons to certain areas does not mean that he can rule out that projections may exist, or that future improvements in stimulation technique will not yield antidromic activation. The characteristic discharge pattern of these cells adds suggestive weight to their identification as interneurons but cannot prove it. (Our subjective probability estimate is that it is indeed likely that the "putative" interneurons are bona fide interneurons.) Steriade is also commendably cautious in not wishing to commit himself on whether his population of interneurons might be excitatory or inhibitory or, as one should add, mixed – including some of each.

The real meat of the paper comes in the comparison of 8 output cells and 23 interneurons in parietal association cortex, recorded during all phases of the sleep cycle. For convenience we have tabulated the mean and median discharge rates in Table 1, and we are grateful to Steriade for allowing inclusion of these data. In Steriade's mind the output cells are principally distinguished by tonic changes in their discharge rate over the sleep cycle, especially by a tonic discharge rate increase in D sleep (with very little change occurring during the runs of rapid eye movements, REMs). He bases his conclusion on the presentation of mean discharge rate changes during REM-rich and REM-poor portions of D sleep. Further documentation that the D-sleep increase in tonic could have come through presentation of a tonic discharge signature of flat autocorrelograms.

What causes this tonic increase? Steriade believes that state-related change in discharge rate is the result of tonic changes in mesencephalic reticular formation input that is excitatory to output cells, and for brevity we shall call this hypothesis the MRF hypothesis. From the historical point of view, we note that Steriade is now in a position to test, at the cellular level, this and other implications of the Moruzzi-Magoun theory. It appears to us, however, that Steriade has missed an important opportunity to strengthen his case for the MRF hypothesis, and, in so doing, to see if state-related changes in discharge pattern are dependent upon or independent of rate changes in these states. The logic in this regard is straightforward: If indeed changes in tonic input level are the principal cause of alterations in discharge of output cells over the sleep cycle, then one would expect the discharge pattern of these output cells to show an orderly alteration that was dependent on the current level of tonic input – i.e. discharge patterns should be a function of the level of the tonic excitatory drive, which should in turn be measureable as an alteration in mean discharge rate. Thus, a demonstration that the discharge pattern of the output cell was a function of mean discharge rate would suggest that the best model for output cell change was the simple alteration of tonic excitability and would thus favor the MRF hypothesis.

Table 1. *Population discharge rate statistics for 8 output neurons and 23 interneurons recorded in waking (W), slow-wave sleep (S), desynchronized sleep (D), D with (D +),* and D without (D –) rapid eye movements. [After Steriade, target article, this *Commentary*]

	W	S	D –	D +	D
Mean Rate (s/sec):					
Output Neurons	11.1	9.3	17.2	21.9	20.2
Interneurons	.33	.66	.61	1.69	1.12
Median Rate (s/sec):					
Output Neurons	9.9	8.5	16.3	22.1	20.6
Interneurons	.20	.58	.32	.82	.54

Unfortunately, Steriade appears to dispute his own MRF hypothesis in his apparent retention of a persistent but supported notion: that change of discharge pattern in slow-wave sleep is a function of state and not a function of altered mean discharge rate. Methods for testing this hypothesis, and results showing that the discharge pattern of cerebellar Purkinje cells is dependent on discharge rate and not state, exist in the literature (McCarley and Hobson 1972). The point is of theoretical importance for a model of the changes in discharge pattern during the sleep cycle. It will be remembered that Evarts postulated that changes in motor cortex output cell discharge pattern might result from disinhibition. However, the data provided by Steriade in this paper clearly contradict the hypothesis of interneuronal rest and the possibility of disinhibition; thus one must turn to other mechanisms for discharge rate and pattern change in S, and to this end the MRF hypothesis is both parsimonious and testable. Documentation of pattern changes over the sleep cycle that could be related to mean discharge rate changes would provide important support for the MRF hypothesis.

The changes in discharge rate of the interneuron population over the sleep cycle are remarkable. The three-fold increase in the median discharge rate as the state changes from waking to slow-wave sleep is, as far as we know, unparalleled by any other neuron type yet recorded. (We prefer to focus attention on changes in the median discharge rate as a better indication of population changes than the mean – inclusion of population-rate histograms would have been a useful descriptive statistic.) Within D sleep there are changes according to whether REMs are present or not (increases during REM flurries and decreases with no REMs), but these population changes are less dramatic than those noted on passage to slow-wave sleep from waking, and changes of this magnitude are common on other neuronal populations.

Having noted these facts, there is, alas, very little more that can be said with certainty at this time, simply because at present we are unable to identify how the recorded interneurons fit into the local circuitry, including their relationship with the output cells. The changes in interneuron discharge rates cannot be described as reciprocal to the relay cells, for while they are in the opposite direction during S, they tend to follow the same tonic course in D sleep, and thus a simple kind of reciprocal interaction between the two populations seems to be ruled out. Although the recording techniques are technically difficult, cross-correlational studies might indicate whether the discharges of the interneurons bear any consistent relation to those of the relay cells. Finally, as Steriade notes, HRP injections into the putative

interneurons would be of greatest benefit in removing any questions about local or distant projections. There thus remains much to be learned about the specific dynamic relationships between the discharge of interneurons and relay cells.

After his initially cautious attitude toward the significance of his finding, and after his detailed and critical examination of the possible uncertainties in his data, Steriade in the final portion of the paper chooses to make what can only be called a Kierkegaardian leap of faith when he asserts that the interneurons might have something to do with memory. While this certainly may be true, the data presented do not seem to indicate that this is necessarily so, or even that we should think it more likely after knowing his results than before. For our part, we think the results *an sich* are enough, and it is our conclusion that Steriade's study will constitute a notable landmark in sleep physiology as the first documentation of the activity of an interneuron population over the sleep cycle.

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Why do cortical long-axoned cells and putative interneurons behave differently during the sleep-waking cycle? Dr. Steriade's paper is a very useful summary and updating of one of the more productive attempts to understand sleep at the cellular level. Because it treats a single approach, this review may make the main features of Steriade's recent work more accessible to the general neuroscientist or sleep researcher than does a previous, more comprehensive review of cellular studies in sleep (Steriade and Hobson 1976 *op. cit.*).

One of Steriade's important contributions has been his attempt to classify extracellularly-recorded neurons as either Type I or Type II. The two types are, respectively, output cells and interneurons; in general, they are distinguishable by firing patterns and presence or absence of responses to antidromic stimulation. Sections 1 and 2 of the paper provide good discussions of the rationale, procedures, and difficulties in making these discriminations. In spite of these limitations, pointed out by the author, all arguments against the procedure are made moot by the demonstration that differentiations made on these bases are systematically related to independently-defined behavioral states: the two types of cells behave differently in quiet wakefulness, slow-wave sleep, and paradoxical (D) sleep.

Data presented for the first time in this paper show that Type I neurons in the parietal association cortex of cats decrease their firing rate from waking to sleep, then tonically increase again during D sleep. There are also phasic increases accompanying REMs. Type II neurons, on the other hand, increase their firing rate during sleep relative to wakefulness, and then show only phasic increases during D sleep in association with REMs. Identical patterns had been previously described by Steriade and his colleagues for neurons in the motor cortex during waking and slow-wave sleep (Steriade, Deschênes, and Oakson 1974 *op. cit.;* Steriade et al. 1974 *op. cit.*). Steriade argues, apparently on a firm basis, that other workers (Noda and Adey 1970a, b, c, 1973 *op. cit.;* Evarts 1964, 1965 *op. cit.;* Hobson and McCarley 1971 *op. cit.*), who had not distinguished cellular types, reported enough information in their studies to suggest that they had observed the same patterns in primary visual sensory cortex and the suprasylvian gyrus. This consistency is remarkable, not only because it includes such diverse brain regions, but because it also seems to apply within the regions to heterogeneous cell populations of the two types (see section 3.1.2).

Such a strong finding raises two obvious types of questions: what controlling mechanisms underly these changes, and what might be the functional significance, if any, of this kind of cellular behavior in the cortex?

This work goes far in specifying what we ought to expect from purported sleep mechanisms. In the first place, the fact that Type I and Type II cells are different from each other, but not consistently reciprocal, suggests that some mechanism outside the cortex affects both types of cells independently, at least at some times. The need for a widespread phasic mechanism has been recognized for some time, and a comprehensive model has been proposed (McCarley and Hobson, 1975). However, assuming that the Type II samples included both excitatory and inhibitory interneurons, it is difficult to imagine a simple regulatory circuit that has cortical interneurons as a principle component, with these opposite types behaving similarly throughout behavioral states. Thus, it appears that the mechanisms that affect the neurons during slow-wave sleep and tonically increase Type I rates during D must also act in parallel rather than in series on the two classes of cells. Unfortunately, control circuits for these types of mechanisms have not been worked out as thoroughly as the phasic system. We can hope that continued application of Steriade's methods and the introduction of intracellular recording techniques during sleep (Nakamura et al. 1977) will soon help provide testable models.

It is probably expecting too much for an extracellular electrophysiological technique to answer the basic question of why an animal sleeps or has REMs. Still, this question has defied so many experimental manipulations that it seems that, for now, we will have to make inferences from a large number of diverse correlations. In this context, Steriade's suggestion that the bursting of Type II neurons during REMs may be involved in memory processes is intriguing. There is a large behavioral literature implicating paradoxical sleep in memory consolidation (see Fishbein and Gutwein 1977 for a recent review). It seems arbitrary, however, to assign a specific role to the bursting of interneurons. Many cells in addition to cortical interneurons, including the cortical output cells, are very active during REMs independently of the eye movements. In addition, the interneurons are not active only during REMs, but also during slow-wave sleep. It might even be suggested that slow-wave sleep is the period when interneurons are uniquely active, as opposed to a general increase in activity of most cells during REMs. Nevertheless, the proposal to study the behavior of cortical neurons during a learning experiment seems worthwhile, although the REMs themselves are probably not sensitive enough to indicate changes, and PGO spikes should be monitored instead.

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The problem of determining selectivity of neuronal firing during different behavioral states. Steriade's work performs a very real service to sleep research, or state physiology, for he rightfully stresses the need to determine the connectivity of the neurons under study if one is to predict accurately the functional significance of firing patterns in various behavioral states. Furthermore, his discussion of the important criteria to be considered in determining the interneuronal character of a particular cortical neuron is of considerable educational value.

Steriade reports a selective increase of interneuronal firing in cortical areas 5 and 7 associated with REM during D sleep and notes that there is a very low discharge rate during W and no temporal relation between eye movements and activity of these units during W. Because there is some evidence that one function of D may be to consolidate information acquired during W, he suggests that the increased firing during D may indicate a role for these neurons in preserving the memory trace in this state.

There is, however, a simple alternative explanation for the REM selectivity which ought to be tested. The state selectivity of the area 5 and 7 interneurons may be a function of head restraint during recording (I am assuming head restraint was used on the basis of an earlier description of the author's technique (Steriade & Hobson 1976 *op. cit.*). Siegel et al. (1977) have recently demonstrated that the apparent selective activation of the giant neurons of the pontine reticular formation during D that others had reported (Hobson et al. 1974b *op. cit.*), was due to the fact that the latter has imposed such head restraint. Siegel et al. found that when cats were free to move during W, these neurons discharged in relation to specific head, neck, and forelimb movements. Prior to the cat's becoming habituated to such restraint, the pontine neurons were observed to discharge in relation to struggling movements of the head and neck during W (movements of these parts frequently accompany D REM bursts in cats). Once the cats became accustomed to the

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restraint and no longer moved, however, only a low level of neuronal activity was recorded in W.

One wonders what the activity of interneurons in areas 5 and 7 might be in totally-unrestrained cats, or in those trained to do specific tasks. Mountcastle et al. (1975) have found that neurons in areas 5 (11%) and 7 (33%) of monkeys fire "when the animal projects his arm or manipulates with his hand within the immediate extrapersonal space to obtain an object he desires. . ." (p. 904). It is reasonable to suspect that similar cells exist in the homologous area of the cat's cortex and that one might expect them to fire in relation to exploratory movements of the forelimbs, head, and neck. But what relevance might this have to the high rate of cortical neuronal discharge recorded during D REM bursts? Hendricks et al. (1977) have observed that cats lacking the normal muscle atonia of D following specific pontine lesions exhibit a considerable amount of "orienting" and "searching" behavior involving head, neck, and forelimb movements during REM bursts. Thus, systems involved in these behaviors during wakefulness appear to be particularly active during D as well. Because interneurons of areas 5 and 7 fire when these behaviors are occurring during REM, the possibility exists that they would also be active in W in the appropriate behavioral situations.

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Cortical unit activity and the functional significance of the sleep-wakefulness cycle. There is no doubt that after the publication of this review a large body of data will become more comprehensive, data according to which there is no strict consistency in the dynamics of unit activity in the sleep-wakefulness cycle; on the other hand, new experimental possibilities will be brought up as well. The following are some comments that I would like to share with the author.

Perhaps the study of neocortical unit activity in the sleep-wakefulness cycle could be more fruitful in elucidating the informational processes that may occur in various phases of this cycle. Steriade is concerned with the importance of different phases of sleep in the organization of memory and the processes underlying learning. In particular, he suggests that hyperactivation of cortical interneurons during eye movement in the paradoxical phase may be associated with consolidation of memory traces. At the same time he refers to data that describe, on the one hand, an increase in the appearance of paradoxical phases under the influence of learning (Hennevin & Leconte 1977; Lucero 1970 *op. cit.*) and, on the other hand, impairment of fixation of memory traces upon deprivation of the paradoxical phase (Kruglikov et al. 1975; See also Pearlman, this *Commentary*). If one considers, as established, that the phase of consolidation is a necessary link in the organization of memory, (although this has recently been called into question), even then the significance of the paradoxical phase in memory consolidation remains doubtful. First, after learning during wakefulness, the sleep cycle generally starts with a rather long slow-wave phase followed by a paradoxical phase; one would expect the consolidation process (if it occurred at all) to be terminated before the onset of the paradoxical phase. Second, it has been demonstrated that during the paradoxical phase (at least in cats and rats, on which the majority of the authors studying the relation of learning to paradoxical sleep conducted their experiments) brain activity attains a level characteristic of emotional wakefulness during the performance of affective behavioral acts (Oniani 1977). It is doubtful that a similar activation of the brain is a good background for consolidation processes. Would not similar activation rather exert an interfering influence? In this respect the optimal background for consolidation would seem to be provided by the slow-wave phase rather than the paradoxical phase. Nor does the activity pattern of interneurons contradict this conclusion. Are they not activated during the slow-wave phase? The fact that interneurons are activated during eye movements in paradoxical sleep requires a special explanation that can hardly be associated with a specific functional significance of the paradoxical phase of sleep. If the paradoxical phase has anything to do with organization of memory, then this seems to be realized through reproduction of memory traces and not by their consolidation.

Debate as to which of the sleep phases is deepest has arisen ever since the discovery of the paradoxical phase. At the present time there is no clear basis for regarding the paradoxical phase to be the deepest, as Steriade does. As has been pointed out above, according to most existing evidence, brain activity in the paradoxical phase approximates the level of emotional wakefulness. This has been demonstrated in our laboratory by determination of electrical stimulation thresholds of various structures for the production of isolated EEG arousal (see Oniani 1977 for references), as well as by recording multi-unit activity in mesodiencephalic structures during the sleep-wakefulness cycle (Oniani et al. 1978). An impression that the paradoxical phase is the deepest has arisen based on the study of electrical stimulation thresholds of mesencephalic structures for behavioral arousal (Rossi et al. 1961). However, thresholds for behavioral arousal in the paradoxical phase are high, due to an active inhibition of spinal reflexes under descending brain-stem influence (Pompeiano 1967), and not due to an attenuation of excitation in the activating structures of the brain.

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Intracellular activities of cortical laminae I-III neurones during EEG arousal. Transected brain preparations could provide a useful model for studies of the synaptic mechanisms underlying a labile cerebral state such as EEG arousal. In this commentary I offer some new information on the behavior of presumptive cortical interneurones during transition from the state of cortical slow-wave and spindle bursts to EEG arousal, occurring spontaneously or induced by high-frequency stimulation of the midbrain reticular formation. The data to be described are taken from a part of our recent results, of which full account will be given elsewhere (Inubushi et al., 1978a, b.)

Acute experiments were performed on 9 encéphalé isolé and 6 pretrigeminal cats. Intracellular recordings were made with single glass microelectrodes filled with 2M KCH_3SO_4 (resistance, 30-60 MΩ) from 13 cells in laminae I and II (vertical depths from the cortical surface, 100-400 μ), and 24 cells in the upper half of lamina III (depth, 400-800 μ) of the precruciate cortex in area 4. These cells were not antidromically activated by stimulation of the cerebral peduncle. The resting potential ranged between −35 and −80 mV. Spontaneous depolarizing shifts of the membrane produced action potentials. The spikes showed the so-called IS-SD inflection in the rising phase, indicating that the recording was made from the soma. Hyperpolarizing or depolarizing current steps of 0.5-1 nA were injected through a bridge circuit to measure the effective resistance of the cell membrane (R_m) at various phases of recording.

Upon EEG arousal, all the laminae I-II cells were depolarized by several

mV with a concomitant increase of the firing rate and a decrease of the R_m. Therefore, these cells were identified as the recipients of postsynaptic excitation upon EEG arousal. Seven of the 24 upper lamina III cells showed depolarizing responses similar to those in laminae I-II cells, whereas the remaining 17 upper lamina III cells were hyperpolarized by 2-15 mV with concomitant decreases of firing rate and R_m. The former cells are thus excited and the latter inhibited postsynaptically upon EEG arousal.

The mean values of effective R_m were measured in the cells of each response type, selected for high resting potentials, ranging between −50 mV and −80 mV. The laminae I-II cells had an R_m of 13-66 MΩ (mean and S.D., 26 ± 17 MΩ, n = 8) during the cortical slow-wave phase and 11-59 MΩ (23 ± 16 MΩ) during EEG arousal. The upper lamina III cells receiving excitation showed an R_m of 22 – 50 MΩ (29 ± 11 MΩ, n = 5) during the slow-wave phase and 20 – 47 MΩ (27 ± 10 MΩ) during EEG arousal. The cells with the inhibitory response had an R_m of 14 – 49 MΩ (25 ± 10 MΩ, n = 11) during the slow-wave phase and 11 – 36 MΩ (18 ± 8 MΩ) during EEG arousal. These R_m values were all considerably higher than those obtained previously in fast and slow pyramidal tract cells and would be related to smaller sizes of laminae I-III cells.

When a weak reticular stimulation (a train of pulses of 0.1 msec width, 30-100 μA, 100 Hz, and 3-7 sec total duration) was used to induce EEG arousal, the latency of excitation could be measured in some trials with reasonable accuracy from the onset of stimulation. Its mean value was calculated as 230 ± 120 msec for 12 laminae I-II cells and 380 ± 120 msec for 6 upper lamina III cells. The mean latency of inhibition was 560 ± 280 msec for 13 upper lamina III cells. This slow development of depolarizing or hyperpolarizing responses could be explained by summation of postsynaptic potentials via mono- or oligosynaptic relays. It is therefore suggested that EEG arousal starts with direct excitation of laminae I-II cells, followed by indirect excitation or inhibition of upper lamina III cells. Perhaps some of the laminae I-II cells are excitatory and some others are inhibitory interneurones. Their respective actions would be conveyed to the cells in lamina III or deeper cortical layers. There is also some evidence that the upper lamina III cells, as interneurones, relay an indirect excitation or inhibition to the cells in laminae V and VI (Inubushi et al., 1978a, b). Unfortunately, with regard to the high-frequency repetitive spike activity as a criterion necessary to identify the inhibitory interneurones in the recurrent inhibitory pathway, we have no information on these cells, since no intracellular sampling has been made.

The behavior of laminae I-III cells can be taken as evidence that both excitatory and inhibitory responses of cortical interneurones are involved during the EEG arousal response. This information may be helpful in possibly providing one missing link in our present knowledge, as reviewed by Dr. Steriade, as well as in contributing to our understanding of the cerebral state of arousal as a reorganization of cellular activity in terms of both spatial and temporal patterns (cf. Jasper 1958).

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On the possibility of a third category of neurons in cortical circuitry. Steriade's attempt to segregate by physiologic means at least two neuronal populations in the cerebral cortex and describe their different activities in the waking and sleep states is undoubtedly a step in the right direction in trying to understand the complex cortical functioning at various levels of alertness. The author quite rightly recognizes, however, that the classification of nerve cells into Golgi types I and II may be an oversimplification, particularly when one considers the many exceptions to the criteria utilized to assign a certain cell to the Golgi type II group. These difficulties have been amply discussed in a recent monograph (Rakic 1975), and the limitations thereby imposed apply in all strength to "corticalized" structures such as the cerebellar cortex, superior colliculus, and cerebral cortex. It is by now well recognized that the central nervous system is organized in terms of neuronal networks rather than in sequential neuronal chains (Szentágothai & Arbib 1975). Although the components of most networks have been described in terms of output cells and interneurons, such arrangement is characteristic of certain subcortical structures – e.g. the relay nuclei of the thalamus – where the input to the structure impinges directly upon the output cell, the activity of which is modified by the interneurons in a more or less complex fashion (Hámori et al. 1974). In cortical systems, however, a third functional and perhaps morphologic type of neuron can be recognized, and that is the cell which receives the main thrust of the input to the structure. The clearest example of these input elements is the granule cell of the cerebellar cortex, which behaves as a true relay neuron and not as an interneuron for local processing (Llinás & Hillman 1969). Although its axon, which gives rise to the parallel fiber, remains within the structure, (i.e. the cerebellar cortex), this neuron can hardly be considered as a Golgi type II cell. The small stellate cells of layer IV in the cerebral cortex may also belong to such a third type of neurons, namely the input cells. Their axons can travel considerable distances and are most probably of excitatory nature (Szentágothai 1973). Similar morphologic-functional properties can also be found to some extent in the neostriatum (Pasik et al. 1976, 1977). The existence of a third category of neurons may help to segregate from the bulk of putative interneurons described by Steriade those with responses that do not conform to the general pattern of high-frequency repetitive spike barrages.

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Neurophysiologic implications of information processing during D sleep. In the ongoing attempt to understand the mechanisms of engram formation, the restricted role of REM sleep in information processing may be a fruitful area for neurophysiologic exploration. As noted by Steriade, no obvious disturbances of memory or learning in humans have been observed, even with several months of total insomnia. Demonstration of the deleterious effect of D (dream) deprivation requires special circumstances involving unusual or creative adaptation by the subject (McGrath and Cohen 1978). In rats this deficit is most apparent with complex tasks such as serial reversals and cooperative behavior, or with information not directly related to immediate reinforcement such as latent learning. Thus, although RF (reticular formation) stimulation has been noted to facilitate consolidation of many tasks, and even to abolish the post-training elevation of D sleep with a Lashley-III maze, it did not completely counteract the learning impairment caused by brief post-training D deprivation in this situation (Bloch et al. 1977). Steriade's cortical interneurons, which are active during D but suppressed by arousal, are logical candidates for this special consolidation mechanism. The close connection between activity of these neurons and rapid eye movements is consistent with the emphasis by Greenberg (1966) on the importance of cortical mechanisms in the D state.

Perhaps RF activation is sufficient for simple learning such as position

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habits or one-way avoidance and the initial stages of more complex tasks. The REM-consolidation mechanism may be crucial for a click between divergent images (an Ah Hah! experience) in the final stage of complex learning. This process was exemplified by a classical conditioning experiment during which cats were allowed to fall asleep after each exposure to the CS (conditional stimulus) (Maho 1977). Increased D sleep followed maximal arousal in reaction to the CS just preceding stable establishment of the conditioned response. Thus, the alteration of D seemed clearly linked to the animal's realization of the nature of the task. This situation might be suitable for further exploration of neuronal consolidation mechanisms during D sleep.

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Possible functions of sleep – memory consolidation? Steriade addresses the puzzle as to what might be the function of brain cells that seem to be inactive during waking and become extremely active during sleep – especially deep sleep and paradoxical sleep. On the basis of work done by Bloch & Fischbein (1975 *op. cit.*) and others, he suggests that the activity of the short axon (and axonless) cells reflects memory consolidation, which presumably occurs during sleep.

I believe that the issue may be more complicated. As Steriade correctly notes, Bloch's studies have not as yet been addressed to a dissociation of what occurs during paradoxical (REM) and slow-wave deep sleep. McGaugh (1976) has shown that the consolidation of memory is a multiple-stage process not amenable to a single-factor (e.g. a Golgi Type II burst) interpretation. Further, if the bursting activity of the Golgi II cells is responsible for memory consolidation, what then is the function of the spiking of Golgi I cells, which is also enhanced during desynchronized sleep, but not during the slow-wave portions?

Steriade makes his interpretations about memory (and they are few, clearly labelled, and not critical to the body of his review) on the basis of the burst activity recorded from Golgi Type II neurons. However, intracellular recordings (e.g. Purpura 1964 *op. cit.*, 1972 *op. cit.*; Rakic 1976; Shepherd 1974; Benevento et al. 1972) have shown that the great majority of the activities of such cells is graded, and that their effective interaction is most often by way of hyperpolarization – i.e. inhibition. I propose therefore that these cells, as well as the Golgi Type I cells, do have a function during waking. For Golgi Type II cells this function is most probably mediated by inhibition.

What then might the functions of Golgi cells be during waking? Perhaps that of *organizing* input and the consequent memory trace. This organizing function would be maintained by both Golgi Type I and Golgi Type II cells during slow-wave deep sleep.

Finally, during the paradoxical phases of sleep the burst activity of the Golgi Type II cells might indicate that organization has been consolidated and that the system is ready to *re*organize. Established inhibitory interactions are wiped out, the system has been cleared and is prepared for a new round of computations.

In support of this proposal are Steriade's findings concerning arousal. Bloch and Fischbein (1975 *op. cit.*) had shown electrical stimulation of the reticular formation (arousal) to increase the quantity of information that can be memorized and to shorten consolidation time. This same procedure led, in Steriade's hands, to an *arrest* of burst production by interneurons (Fig. 8). As he notes, this poses a difficulty for his own proposal – but not for the one suggested here. Perhaps the model entertained in this commentary can, therefore, be considered as a tentative next step, based on the fascinating work of both Bloch and Steriade.

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Two basic neuronal configurations in the cerebral cortex. In the always adventurous task of correlating the physiological properties of individual neurons with their morphology, Steriade's work deserves close examination. It is indeed interesting that it should be possible to speak of two large families of cortical neurons showing radical differences in their behavior during sleep and wakefulness.

It also appears legitimate to reach such a simplified classification from the morphological point of view. In spite of the large variety of morphological types described by Ramon y Cajal (1911 *op. cit.*), Retzius (1893), Martinotti (1890), and others, it appears certain that there are two basic neuronal configurations in the cerebral cortex that recent authors have described as pyramidal and "stellate." Some qualifications are necessary however: the term "stellate" is not a felicitous one. As opposed to pyramidal neurons, stellate neurons include a large number of configurations, some of which do not even look stellate in shape. It is also erroneous to equate this neuronal type with the so-called "Golgi II type" cells, or short-axon cells. To make matters even more complex, there are reasons to believe that the latter may also have very different functional significance, depending on their location. For example, the short-axon cells of the cerebellum do not appear to be involved in dendro-dendritic or axo-axonal contacts, whereas those of the thalamus are often supposed to participate in this type of synaptic arrangement. If a Golgi II axon is defined as one that ramifies within the dendritic field of the neuron to which it belongs, this is a valid statement for many neurons of the cerebral cortex, thalamus, and striatum, but not so for those of the cerebellum. Ironically, these were the cells that Golgi used to endorse his original classification. Furthermore, since the work of Ramon y Cajal (1911 *op. cit.*) and Lorente de No (1938 *op. cit.*), it is now generally accepted that a spectrum of short-axon neurons can be found within the cerebral cortex, ranging from those that meet the above specification of having an axon that ramifies within the dendritic field, to those that have axons reaching distances of up to 1 mm. One further source of confusion is the term "granule cell." Brodmann (1912) used this term for the cells of the cerebral cortex. However, he was working only with Nissl-stained material, and with this technique one cannot differentiate among a number of cell types which have in common only the fact that "they do not look pyramidal." Brodmann used the term granule cells to describe certain small-sized neurons, mainly located in the second and fourth layer of the cerebral cortex (accordingly called the external and internal granular layers). But when stained with the Golgi method, most of the cells present in the external granular layer are simply typical, though small, pyramidal cells, whereas the cells of the internal granular layer are of the stellate type and correspond to the "star cells" that, according to Lorente de No, are located in the middle of the specific afferent plexus. This location could imply that many of the so-called stellate neurons are not typical intrinsic neurons of the cerebral cortex, since they could constitute one of the primary targets of the thalamocortical fibers.

In spite of the above considerations, there are some reasons that justify a dichotomous classification of cortical neurons. It would be perhaps less misleading to distinguish simply between *pyramidal and nonpyramidal neurons*. It is very likely that the physiologist's "projection neuron" is always a pyramidal neuron, although the converse is not true: not all pyramidal neurons are projection neurons. Many of them, particularly the small-sized ones located in the second layer, may constitute intrinsic neurons, insofar as their axons remain within the cerebral cortex. We are inclined to distinguish between pyramidal and nonpyramidal neurons on the basis of two main criteria: (1) the general geometry of the neuron, and (2) the relative richness in dendritic spines. Also, the work of Peters (1971) appears to indicate that a third criterion could be added: the relative richness of axosomatic synapses.

The geometry of a deep pyramidal neuron is unmistakable. It is provided

with: (1) an apical dendrite oriented towards the pia, to which they are perpendicular; (2) basal dendrites, preferentially oriented along a plane parallel to the pia; and (3) an axon oriented towards the white matter. In the case of the most superficial pyramidal neurons (Brodmann's external granule cells), it is difficult to distinguish between apical and basilar dendrites, because the proximity to the pia removes the necessary space to develop an apical dendrite. However, their axon is always oriented towards the white matter, and it is easy to realize that there is a continuous spectrum of transitional forms between these small external pyramidal neurons and the large ones located mainly in the fifth layer.

The second criterion, richness in dendritic spines, is a puzzling one. All pyramidal neurons, including those of the second layer, are extremely rich in dendritic spines. By contrast, all nonpyramidal neurons, regardless of any other differential characteristics, are provided with relatively bare dendrites (Ramon-Moliner 1961). We cannot put forward any theory to explain why cells that are otherwise so variegated should share this relative dendritic bareness. In any case this finding is interesting because it seems to indicate that between pyramidal and nonpyramidal cells there is a difference involving more than the spatial arrangement of the processes. Additional support for this view is provided by Peters' (1977) finding that stellate cells, as opposed to pyramidal neurons, have perikarya rich in axosomatic synapses.

Steriade's observation that interneurons exhibit their lowest discharge rate during waking is an intriguing one. If these interneurons turn out to be nonpyramidal neurons, this could mean that, generally speaking, the input to dendritic spines plays a diminished role during sleep, while the input on perikarya could be enhanced. By contrast, the higher activity observed in output neurons during wakefulness may imply that dendrites are then the main source of neuronal input, while signals reaching the perikarya may play a lesser role. The involvement that Steriade attributes to intrinsic neurons in memory consolidation is more difficult to correlate with histological data. One should not forget that the cerebellar cortex is very rich in interneurons, yet the cerebellum appears to play no role in any kind of memory consolidation. Admittedly, the interneurons of the cerebral cortex could be, if not the "site" of memory, at least one of its material requirements. It is a well-known fact that in the course of phylogeny these cortical interneurons become increasingly abundant, and the greater capacity for learning found in higher vertebrates could be related to this fact.

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The importance of neuronal interaction patterns. It is useful to know the rates and patterns of firing of the neurons in a part of the brain during different states and behaviors, and it is also useful to know the electrophysiology and connections of those neurons. However, to have these two kinds of information on the *same* cell increases the value of the information many times. Indeed, such combined data on the same neuron are among the kinds of information it is *necessary* to know in order to work out the cellular mechanisms of behavior. The work of Steriade and his associates over the past ten years (much of which he summarizes in his article) is perhaps the best single example of the use of this important strategy.

To know only the relation of the firing of a neuron to a behavior, without knowledge of electrophysiology of the neuron or its inputs or outputs, does not allow us to work out how the neuron works in the brain as a whole, or even its relation to other neurons in the same area. It is "microphrenology." It is the interaction of neurons that produces information processing. In order to build an idea of how some neurons interact as a group, we must know their interconnectivity. Indeed, we must know this before we can talk about the function of individual neurons or the group.

While Steriade's work has been primarily in sleep, the strategy he uses can be generalized to the study of neurons in many different behaviors. The study of the behavioral states of sleep is wise, not only because sleep is interesting and important in itself, but also because these states are reproducible and occur continuously over a long enough time so that one can obtain quantitative data. There are not many other behaviors for which this is true. I would hope that others will follow Steriade's lead in the use of this approach. In my laboratory we use a similar approach in the study of the hippocampus. The recently developed technique of intracellular injection of horseradish peroxidase would be an important addition to studies of this sort.

There is another advantage to the electrophysiological identification of neurons with known relations to behavior. It is sometimes useful to study neurons in a part of the brain in a decerebrate or anesthetized animal or in a slice of brain *in vitro* (as is done so successfully now in hippocampus). When one has an electrophysiologically-identifiable class of neurons that have a known relation to behavior, one can identify that class even if behavior is not possible in the preparation used – i.e., electrophysiological characteristics can be used to translate from a behaving preparation to a nonbehaving one.

Steriade is appropriately cautious in describing "putative interneurons." It seems very likely that these are indeed interneurons, but even if they are not, they are a distinguishable class of cells. It is remarkable how many other putative interneurons have similar electrophysiology, especially high-frequency repetitive spike discharge in response to electrical stimulation input. These observations should spur others to look for the same thing. Steven Fox in this laboratory has found the same response (high-frequency repetitive spike discharge) in putative interneurons ("theta cells") in hippocampus. However, these hippocampal theta cells have high rates of spontaneous firing, rather than the low rates Steriade sees in putative neocortical interneurons.

Steriade's definition of various waking behaviors is weak. "Excited" and "relaxed" epochs of wakefulness are poorly defined, and questionably reproducible in the same laboratory, not to mention, other laboratories. The use of poorly-defined waking states is characteristic of most studies of unit firing in the sleep-wake cycle; this tradition should be changed. The commonly used category of "quiet wakefulness" is just not good enough. There are only a few well-defined, readily-reproducible waking behaviors that can either be maintained long enough or elicited frequently enough to yield quantitative data. Working with rats in this laboratory, we have found that running a treadmill and drinking water from a water spout with a small hole do meet these criteria. Even with its head immobilized, an animal could surely engage in drinking and probably also treadmill-running.

We just do not know on which neurons these putative neocortical interneurons synapse; investigation with horseradish peroxidase would help, but I know of no unequivocal way to decided these issues. A knowledge of the firing patterns of different groups of interconnecting neurons in specific behaviors can give clues, sometimes strong ones, about the excitatory or inhibitory nature of the interaction. However, a given group of neurons has many inputs, and unless we know how all of them are firing in a given behavior, we cannot push this line of reasoning very vigorously.

Before one can seriously suggest, or state with testable clarity, the function of a neuron, one should know its firing pattern in a wide variety of behaviors. Many neurons presumably have functions in many behaviors, and some of these functions may be different in different behaviors. Lacking this information, Steriade's suggestions about the functions of the putative interneurons are premature. Extending these observations to additional behaviors would be an important next step.

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The role of interneurons (Golgi type II) in memory. The major point of my review will be to comment on the relation of the sleep process to memory, raised at the end of Dr. Steriade's review. Before doing so, it is important to emphasize that the significant contributions made by Steriade in this review involve the electrophysiological differentiation between output neurons and interneurons in the mammalian central nervous system and the importance of such differentiation in relation to function (e.g. sleep – memory). Too often do investigators looking at function collect "unit data" without characterizing the topography of the unit's firing pattern. Steriade's review makes it clear that

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this should be an essential picture of any study which has as its goal the study of the relation of cellular activity of the functional state of the organism.

However, this distinction has implications far beyond the methodological and informative advantages discussed. In a recent review (Routtenberg 1978) I have argued that the relation between intrinsic and extrinsic neurons confers on individual brain structures (a) redundancy, and (b) self-monitoring functions. Redundancy, because the interneuron, whether inhibitory or excitatory, is logically related to the extrinsic neuron and hence can carry much the same information (depending on the efficiency of the matching or mismatching). In addition to helping understand why redundancy may be demonstrated, the relation between intrinsic and extrinsic neurons confers a self-monitoring function on individual nuclear groups in the brain. The ability of a wide variety of interconnected brain structures to monitor their own activity, in a collective sense, was defined as the mechanism of consciousness. These lofty speculations notwithstanding, it is clear to this commentator that the implications of the distinctions that Steriade has demonstrated are, indeed, very important and may well be crucial for understanding brain mechanisms in relation to function.

This commentator has been concerned with sleep mechanisms (Routtenberg 1973), as well as the anatomical (Routtenberg 1975) and biochemical (Routtenberg and Kim 1978) substrates of memory formation. Of particular interest is the proposition suggested earlier (Routtenberg 1968: see 1975) that hippocampal theta indicates the memory-consolidation process. Direct experimental support for this view has been obtained (see Landfield et al. 1972).

It is interesting, with regard to Steriade's hypothesis that consolidation occurs during the D state, that this state is accompanied by high-density theta activity. Thus, taking the view that theta activity represents the consolidation process, one could conclude from completely different lines of inquiry than those reviewed by Steriade, in fact, that the D state is important for memory consolidation. When such independent lines of reasoning lead to the same conclusion, one can be encouraged, at the least, to give additional credence to the hypothesis.

In sum, Steriade has performed a valuable service in emphasizing the distinctive properties of interneuron and extrinsic neuron, particularly as it enables one to re-analyze and re-interpret previously reported single-unit data. For this commentator the distinction made has certain important implications for understanding the fundamental organization of the mammalian central nervous system.

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Possible reticular mechanism underlying altered activity of cortical neurons during sleep. Steriade's dichotomy model for the change in activity of cortical neurons during the sleep-waking cycle is surprisingly clear-cut. His type II neurons (interneurons) are also strikingly homogeneous in terms of spike discharges. So we are confronted with the apparent paradox that neural functions of the highest order are determined by an interneuron network, all of whose elements are operating in a rather similar manner. This may not be paradoxical, however, if we suppose that the interneurons picked up by Steriade were confined to a particular type, probably due to functional characteristics of the electrodes used. This seems especially likely when we are dealing with small cells such as interneurons, which behave delicately according to the electrode used and are very difficult to hold for a long time in the chronic preparation. The definitive conclusion must await intracellular staining of the interneurons, which could be penetrated with a procion yellow-containing glass pipette after successful extracellular recording.

It would be possible to record simultaneously a pair of neurons with excitatory or inhibitory connections. This kind of analysis would contribute significantly to our understanding of both the mechanism determining the discharge pattern of individual neurons and the functional significance of each element in the cortical neuron circuit. Several ways to produce multipolar microelectrodes have been worked out, among them the photoengraved method (Prohaska et al. 1977).

My colleagues and I have recently performed natural-sleep experiments on changes in activity of lower brainstem neurons during the sleep-waking cycle in the cat (presented at the Annual Meeting of the Association for the Psychophysiological Study of Sleep, 1978). In our experiment one of the so-called sleep-wakefulness centers was electrically stimulated with a chronically-implanted electrode and single-neuron spike responses to the stimulation were picked up from other centers. The structures studied were the mesencephalic reticular formation, raphe dorsalis, raphe magnus, locus coeruleus, gigantocellular tegmental field, and solitary tract nucleus. By recording the background discharge rate, the number of spike responses, and the duration of the response period measured from the PSTH (poststimulus time histogram), the effectiveness of a center to drive or inhibit the neurons in other centers could be expressed as an index. When the cat passed from wakefulness (W) into slow-wave sleep (S), the behavior of the index showed no particular tendency; a similar number of neurons were found to have greater, equal, or smaller index. The suggestion derived from these results would be that S is not a simple reduction of the activity level of W; rather, there occurs a reorganization of functional connections between neurons belonging to sleep-wakefulness centers. The reorganization at the centers would inevitably lead to the altered discharge pattern of the cortical neurons found by Steriade.

During REM sleep (D) almost all neurons in the sleep-wakefulness centers had a very small index as compared to W and S. The reduction in the index was generally associated with a marked augmentation in the background discharge rate. These results seem to indicate that effective communication between sleep-wakefulness centers is greatly impaired during D, and as a consequence each center is allowed to run at its own pace, providing enormously increased outputs to the target neurons in the lower and higher central nervous system, including the cerebral cortex. A high discharge rate of cortical neurons during D would thus be accounted for by an increase in excitatory inflow from the mesencephalic reticular activating system, which cannot be appropriately counteracted by the inhibitory system. The REM-related bursts of cortical neurons may be explained in the same way; periodically-occurring burst activity in the dorsolateral pontine tegmentum (Laurent and Guerrero 1975) is not prevented from propagating into various structures where phasic events characterizing D can be recorded.

Steriade has suggested a possible feedback control by cortical neurons of the reticular core in the lower brainstem. This feedback control is conceivable during W, but it is uncertain whether the control is also operating during S, when interneuronal connections appear to be reorganized, resulting in a functional state quite different from W. During D the feedback control would not be operating at all, because sleep-wakefulness centers are almost unresponsive to inputs from other centers and specific sensory pathways (Huttenlocher 1961).

Monoamine-containing neurons located in the raphe nuclei and the locus coeruleus are considered to play an important role in the manifestation of S and D. Synaptic effects at the axon terminals of these monoamine neurons are mostly inhibitory (Sasa et al. 1975), though excitatory effects have also been reported for the raphe nuclei (Olpe & Koella 1977). Existence of monoamine nerve terminals in the cortex has been demonstrated (Anden et al. 1966). Monoamine neurons show altered spike activity during the sleep-waking cycle. It has been reported that their discharge rate is decreased during S and further diminished during D (McGinty and Harper 1976; see also McCarley & Hobson, this Commentary). An increased discharge rate during D has also been reported (Sheu et al. 1974). Precise histological information about monoaminergic innervation of cortical neurons is still lacking. In view of the widespread innervation by the monoamine nerves covering almost the entire central nervous system, it is important to take into account the monoaminergic influence, especially when we are dealing with a function such as sleep. What is of great interest is the differential action on cortical

neurons of the two diffuse projection systems, namely the reticulocortical system, which might be cholinergic, and the ascending monoaminergic system.

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On the significance of observations about cortical activity during the sleep-waking cycle. Steriade's work offers a contrast between the sophistication of the methods used and the simplicity of the assumptions on which the study is based. It is no small feat to record single units from chronic animals long enough to cover various phases of the wakefulness-sleep cycle and to run all necessary tests to identify these units. This requires skill, expertise, and patience. Steriade's careful approach allows him to draw the maximum information from his data. If there are difficulties in interpreting the latter, if sometimes the conclusions are not entirely convincing, it is probably due to the limitations of the techniques rather than to deficiencies in their application.

The detailed discussion of tests used for neuronal identification and of the significance of these tests is by itself already a significant contribution of this paper. One certainly can argue on the choice of the means to reach the objectives, but I would like to comment more particularly on the objectives themselves.

This investigation revealed rather consistent features of neuronal activity in forebrain structures with respect to the stages of wakefulness and sleep. At least three assumptions underlie the chosen approach: (1) that our understanding of sleep stages is sufficient to know what the brain is doing during these stages (what part of the circuitry remains functional and how?); (2) that the categories of output cells and interneurons are homogeneous to the point that they can be treated as functional entities; and (3) that frequency of firing is a meaningful index of, at least, something in particular.

Steriade's success in showing correlations among these variables seems to be an a posteriori justification of the soundness of his approach. This is why the data are interesting. They offer a coherent picture to replace vague notions of what cortical neurons are doing during sleep. The data also raise questions, but are they able to provide answers? Probably not at the present stage, because the study deals with three unknowns: what is going on in the brain during sleep; what is common either to all interneurons or to all output cells; and what spontaneous neuronal activity, in general, means. There are inherent, fundamental uncertainties in interpretations on all three points. Whereas intercorrelations among them can be established, it is somewhat doubtful that much light could come from such a correlation. It is not easy to explain an unknown on the basis of another unknown.

To those who are not familiar with the progress of Steriade's work prior to this report, the results may appear surprising. Indeed, throughout the wakefulness-sleep cycle the apparently consistent changes of activity within output-cell groups and interneuron groups is hard to conciliate with the view that these neurons play a specific processing role very probably related to some form of behavior. The discussion in the paper concentrates only on the alternative that interneurons are either excitatory or inhibitory. But these interneurons are themselves within complex networks, where they receive a variety of inputs. Why then should they behave in a stereotyped manner irrespective of their position in the circuitry? Why should the length of their axons be a more relevant factor?

In general, one wonders whether frequency of cell discharge averaged over long periods is an opportune choice among parameters of nervous activity that can be utilized in this type of study. The frequency of firing is known to change with a variety of experimental variables (behavioral, hormonal, thermic, etc); therefore, the cause of a change is hard to trace. Measurements of frequency tend also to reduce observations to single figures, thereby eclipsing other possibly significant features such as changes in patterns of firing. The paper provides information with respect to these patterns. Yet, the abstract stresses the frequency aspect, which alone probably has limited significance.

There seems to be a gap between electrophysiological studies that report results in terms of mass excitation-inhibition and other studies that actually deal with the same neuronal populations, but in specific behavioral situations, which stress the individual role of single neurons in performing definite operations on nervous signals. This is not simply a matter of "lumper" versus "splitter" philosophy; it is a question of concept and language. How do we go about translating statements from one language into the other one? To understand the significance of increased and decreased levels of activity, we would need to know what the signals are for the cells studied when they perform their function in the awake animal. One way to assess the effect of sleep would be to test, throughout the stages of sleep, the performance of neurons of which the involvement in a specific operation is relatively well-defined. Thus, for example, it would be a first step to determine whether the cells bursting during REM episodes have an activity related to some motor behavior. Otherwise, one may be limited to pure guesses in assessing the significance of these bursts.

Steriade's results show that changes of firing affecting output cells and interneurons are not consistently related (i.e. similar or reciprocal) throughout the states of wakefulness and sleep. Therefore, no easy deduction can be made on interneuron-output cell interactions. This problem remains a matter of speculation, and the number of possible hypotheses to consider is staggering.

It is now clear that the brain is not shut off when it sleeps. It would be too simple and we know better. Even the picture that Steriade presents appears a little bit too reductionistic to be true. Is the sleeping brain that much different from the awake brain?

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Postsynaptic potential influences upon postsynaptic impulse generation. Complementary to this interesting paper is the question of the precise effects exerted by excitatory or inhibitory postsynaptic potentials (IPSPs, EPSPs) upon the intensity of postsynaptic firing; this, I feel, could be commented on further, summarizing earlier reports (Segundo 1970; Segundo and Perkel 1969; Segundo et al. 1976). It is permissible, on the one hand, to accept that a neuron will discharge less or more when receiving IPSPs or EPSPs, respectively, than when they are absent; this is generally true, though exceptions exist (e.g. Figure 160B in Segundo and Perkel 1966). It is not permissible, on the other hand, to accept, for IPSPs or EPSPs, that consequences are restricted to either slowings or accelerations, respectively, nor that faster arrivals necessarily mean less or more intense postsynaptic firing, respectively. Indeed, contradictions to the latter alleged rules are encountered experimentally about as commonly as agreements, and magnitudes are comparable. Commonplace and influential features are, for example, "rebound"-rate increases after IPSPs, "paradoxical" accelerations with IPSP acceleration or EPSP slowing, "hysteretic-like" dependencies on presynaptic discharge changes, and "saturation-like" consequences of extreme rates. The form of the pre-to-postsynaptic transfer is subordinated to numerous factors, and any summary description is conditional to the extant circumstances. Furthermore, it seems reasonable to believe that at least some of the neuronal transactions of the S-W-D cycle involve modulations of

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ongoing activity and not just the extreme passages from activity to inactivity and vice versa. "Modulations" imply increases and/or decreases in activity, both being equally valid signs of involvement.

The above considerations could be helpful when conjecturing as to the neuronal mechanisms that underlie the input-output relations described in this paper.

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Interneurons and memory consolidation. Steriade is to be complimented for presenting convincing evidence for the differential behaviour of long-axoned (or so called "output cells") versus short-axoned interneurons in the cortex of monkeys and cats in various stages of the sleep-walking continuum. It is particularly gratifying to see that physiological analysis of cortical function has reached a stage where the hitherto elusive interneurons can be recognized and data about their behaviour can be collected with almost as much confidence as in the case of the long-axoned cells identified by antidromic activation.

There is very little to argue against in most of the impressive factual material collected and most of the elegant reasoning presented by the author. Also, as an old hand engaged in the pursuit of various types of interneurons for over twenty years, this commentator is certainly far from wishing to deny the crucial role of interneurons in lower as well as higher CNS function. However, if as a neuroanatomist I may be privileged to comment on the final conclusion at which this paper appears to be driving, a word of caution should be introduced. The idea that short local interneurons (often referred to also as "microneurons," a mistake correctly pointed out also by Steriade) might be the real structural substrates of higher neural function dates back to the great classical neuroscientists (among many others, to Ramón y Cajal himself), and it has been taken up in various modifications ever since. (Since space limitations prevent entering into a discussion of the several forms of this hypothesis, we shall also refrain, in due fairness to the various authors, from specific citations.) It should also be added that Steriade does not directly adopt this hypothesis, but his final conclusion might easily be misunderstood as a revival of the idea, and it is this misunderstanding against which the following comments are principally directed.

Interneurons, however important, do not function by themselves but are driven synaptically by an input, which in most cases arises from some other part of the nervous system, and is hence conveyed by long-axon cells. Let us for the time being neglect the possibility that the local neuronal network is driven by spontaneously active (perhaps even by "pacemaker") neurons. Logically, we would therefore have to deal with an ensemble of interneurons and long-axon cells, even if under certain circumstances the activity of some of the "output neurons" was suppressed. There is nothing in Steriade's present paper that would speak against this view of neural organization. The type I or "output" neurons identified in his very elegant studies all led to some subcortical structure: the spinal cord, the pontine nuclei, and to some of the thalamic nuclei. These neurons, were hence true "output" neurons, in the sense that they mediated the output of the cortex. It is well known today that all these neurons consist of pyramidal or spindle cells located in laminae V and VI. Some of these may have collaterals entering the corpus callosum and reaching the opposite cortex. But what about the very rich cortico-cortical connections? At this point we realize the ambiguity in the term "output neuron." The "output" neuron at one end of a cortico-cortical connection is the "afferent" in the cortical region at the other end. Or should the term "output neuron" be reserved for cortical neurons sending their axons to other parts of the CNS? Either way we get into difficulties.

The vast majority of the ipsilateral cortico-cortical connections consist of pyramidal cells located in lamina III (Klór and Majorossy 1977). We do not know whether the same cells also send collaterals to the contralateral cortex via the corpus callosum; certainly some of the callosal terminals originate from lamina III. But neither of these cells sends an axon (as far as we know) to the subcortex. The callosal cortico-cortical connections may arise from all layers from II to VI, although the majority come from lamina III. (Some of the recent literature has been summarized in my 1977 Ferrier Lecture [Szentágothai 1978].)

Nothing about this vast majority of long-axon (type I) cortical cells emerges from the studies of Steriade and his co-workers. Should these be called "output" cells, or should that term (as already mentioned) be reserved for the cells projecting to the subcortex? Both are pyramidal cells, and some – the V and VI laminar callosal cells – may subserve both cortico-subcortical and cortico-cortical relays. How do the true cortico-cortical cells behave in various stages of sleep and wakefulness? Until these questions are clarified it might be better to refrain from drawing far-reaching conclusions about the mechanism of memory consolidation during certain stages of sleep.

If one may be permitted to add some loose speculations, it would be attractive to visualize the working of the brain as a continuous back-and-forth switching between the various columnar cortical modules, involving the actions of billions of both pyramidal (i.e. type I) cells and interneurons. The difference between sleep and wakefulness would then be, essentially, that the majority of the long-axon cells of the deeper cortical layers would be turned off temporarily, while the cortico-cortical mechanisms might become even more active in certain stages of sleep.

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Classification of cortical interneurons on the basis of Golgi impregnation. The basic method for the morphological identification of cortical neurons has been established for more than a hundred years; the "reazione nera" technique reveals the most delicate neuronal details and makes it possible to classify the cells in terms of types. The two main types and associated subtypes were described by the classics of neurohistology and have been verified by recent investigations both morphologically and physiologically. The recent descriptions, however, diverge somewhat as to the classification of the neurons–i.e. there has been an endeavor at simplification. Above all, general ideas based on distinctive neuronal features should be considered and applied in any classification scheme; these should be valid at the various levels of the CNS, and correspond to functional requirements.

The first main group of neurons consists of output, "type I," or long-axon neurons. Their axons leave the region where their cell bodies are located and enter the white matter to establish distant contacts; in investigations such as Steriade's it is important to identify these distal target structures, since connections seem to constitute the basic factor in further classification, determining the three associated subtypes: projective, association, and commissural neurons. For example, in the visual cortex, output neurons in layer VI project to the lateral geniculate body, while those in layer V send their axons to the optic tectum; such cells belong to the projective subgroup of type I. Other output neurons from layers II and III establish association and commissural connections, constituting the association and commissural subgroups of output neurons.

Interneurons or "type II" cells are local, intrinsic neurons within circumscribed regions of the CNS; *their axons never enter the white matter.* The axons of the interneurons ("intrinsic cells," according to Shepherd 1974) may follow a longer course in the parent area, but they always remain in the grey matter.

At different levels of the CNS the interneurons differ in type and number. The greatest variety of types occurs in the cortex. An attempt has been made to classify cortical interneurons in the rabbit, cat, and monkey, and on the

basis of relevant morphological features these may be classified into five groups:

1. *Short-axon cells.* Neurons in this group have dendritic and axonal fields of similar extent in all directions, and the cells are confined mostly to one layer, although they may also occur at the border of two layers. Golgi type II neurons and neurogliform neurons are in this group. They can be found in all layers of the cortex, slightly varying in the different species. Golgi type II neurons most often occur in layers II, V, and VI, while the neurogliform neurons are predominantly in layers I, II, IV, and V.

2. *Cells with medium-range horizontal axons.* The horizontal cells of Cajal in layer I and the fusiform horizontal cells in layer VI belong to this cell group. Both the dendrites and the axon of the horizontal cells follow their courses in the parent layer. Although the dendrites of these neurons are long, especially those of the fusiform neurons, the axon extends even beyond the dendritic tree. It may establish countless contacts in the parent layers both with its several branches and with its numerous spine-like side-branchlets.

3. *Cells with medium-range vertical axons.* The neurons of this group are also found in layers I and VI, like the horizontal neurons; they are the multiangular neurons in layer I and the Martinotti cells in layer VI. These neurons connect the first and sixth layers and vice-versa.

4. *Vertically-arranged neurons.* These cells are in layers III and IV, but their dendrites and axon branches can invade layers II and V, respectively. Different subtypes of horse-tail neurons belong to this cell-group, and their distinction is based on the horizontal dimension of axon arborization. This can be extremely narrow (60–70 μ) or several hundred microns wide, although even the widest is insignificant compared to the vertical size of the dendritic and axonal fields. The contacts of the horse-tail neurons seem to be established by neuronal elements arranged in the vertical direction.

5. *Omnidirectional cells with medium-range axonal and dendritic fields.* On the basis of their axon arborization, two subgroups must be distinguished in this class: basket neurons (subgroup I), and the chandelier neurons (subgroup II). The size of the basket neurons is different, some being extremely large. Their axons extend in all directions, and their dendrites and axons pass through more layers. The larger neurons penetrate several layers in the depth of the cortex and also have significant expansions in the sagittal and frontal directions.

Neurons of both subgroups can be found in layers II, III, IV, and V. Axon arborizations may enter layers VI and I as well. Regarding the vertical direction of the axon terminals of group-V neurons, very probably they contact mostly the apical dendrites of pyramidal neurons of various layers and/or the cell bodies of pyramidal neurons. But all the nearly-vertical dendrites and axons or the cell bodies can be the target of the axon terminals of basket neurons and chandelier cells.

The five groups of cortical interneurons may represent five functionally-different classes. The short-axon neurons, with their relatively small, well-circumscribed extension, may be either excitatory or inhibitory. We are inclined to think the neurons in groups 2, 3, and 4 are excitatory, while the neurons in group 5 are most probably inhibitory interneurons. The basket neurons of different size may produce collateral inhibition, and the chandelier neurons, with their vertical terminals, may inhibit the pyramidal neurons through their apical dendrites, or axons.

The five groups of type II cells (or interneurons) with their subgroups, as distinguished in Golgi preparations, are in good agreement with Steriade's statements: "The connections of excitatory with inhibitory 'type II' cells certainly do not exhaust the yet undeciphered complexity of cortical neurons circuit." And in this complexity every kind of type II cell (interneuron) may well have its own characteristic function.

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A comment on wakefulness as a reference state in sleep studies. In many sleep studies involving an analysis of the neuronal discharge rates in wakefulness (W) and in both sleep states (S and D), the definition of W is unsatisfactory. When the animal awakens, its isolation from the outer world disappears and is replaced by the variegated state of wakefulness. The accompanying behavior is typical for the species and is modified by learned habits and the actual level of habituation. It is not sufficient in these studies to divide W into "attentive and quiet W" or "W with and without detectable gross movements." To standardize W and to give a true description of it is naturally difficult, but such an attempt can be made, principally in two ways: 1) Precise specification of the experimental conditions (e.g. Was the cage dark or illuminated? Was the animal moving freely and thus able to show the motor adjustments of arousal, orientation, and falling asleep? Was the arousal "spontaneous" or provoked by the person carrying out the experiment?), and 2) Analysis of the neuronal activity of the behaving animal in different experimental conditions (e.g. in relation to conditioned motor activity) and a comparison of such behavioral correlates of neuronal activity in W with changes in neuronal firing in sleep.

In his study Steriade describes a large increase in the neuronal firing rate of type I neurons in a state pair "W→D+" (Table 1). In addition, he points to the very low discharge rate of these putative interneurons in W and says: ". . . then the increased firing rates of association cortical interneurons during REM periods of D could indicate that they are preferentially involved in maintaining the soundness of a memory trace." The immediate reaction of the reader here is to ask: Since these putative interneurons have been given such a high functionality, why, in these experiments, are they relatively silent in W? This question emphasizes the importance of analyzing the same neuron population in different behavioral conditions of W. One might also ask how such a highly *discontinuous* process as the occurrence of D+ periods together with the discharges of type I neurons could be critically significant with regard to the apparently strictly *continuous* process of memory consolidation?

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What does cortical electrical activity have to do with sleep? The paper by Steriade consists of a summary and discussion of the author's own recent efforts to relate neuronal physiology to the study of the sleep-waking cycle in mammals. Level of arousal or vigilance is classified into three basic states: waking (W), slow-wave sleep (S), and active or rapid-eye-movement sleep (D, REM sleep). It is assumed by Steriade, as by nearly all workers in the sleep-research field, that this subdivision is fundamental and appropriate to the study of the generalized physiology of the brain. I believe that this fundamental assumption is invalid. If I am correct, then we must reconsider the significance, not only of Steriade's work, but of sleep and behavioral-electrophysiological research in general.

A basic contention of the conventional view of sleep is that the generalized slow-wave patterns (electroencephalogram, EEG) of the neocortex exhibit an important correlation with sleep and waking, or with the level of arousal, consciousness, vigilance, or attention. Twenty-five years ago it was generally held that the presence of waking or consciousness was accompanied by low-voltage fast activity (LVFA, desynchrony) in the neocortex, while sleep or coma was accompanied by large-amplitude slow activity (synchrony). The discovery of active sleep led to the hypothesis of a third state of consciousness to account for the paradoxical fact that LVFA is often present during deep sleep. In fact, such sleep is often called "paradoxical sleep" to emphasize (probably) the puzzling nature of this phenomenon from the point of view of the original theory. The paradoxical converse of this sleep state occurs when waking animals, thoroughly alert from a behavioral point of view, display large-amplitude slow-EEG patterns while standing motionless, drinking milk, shivering, or washing their faces (Vanderwolf, in press). This phenomenon might be dismissed as due to drowsiness or inattention were it not for the fact that tests of reaction time given during periods of waking synchronization in cats (Rougeul et al. 1972) or alpha rhythm in man (Larsson 1960) suggest that high levels of vigilance are possible at such times. Further difficulties for the conventional view of EEG-behavior relations result from the well-known fact that animals given atropinic drugs display a great deal of large-amplitude slow activity but do not become comatose or go to sleep. Conversely, following inhalation of gases such as diethyl ether or chloroform, LVFA coexists with anesthetic coma. Many other examples of a failure of EEG phenomena to correlate with the presumed level of arousal, vigilance, or consciousness can be cited.

It is apparent that one can maintain that there is a correlation between EEG phenomena and the level of arousal or consciousness (as conventionally understood) only at the expense of ignoring a large part of the available data.

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Two conclusions seem possible: 1) The conventional concepts of arousal, consciousness, vigilance, etc., remain of prime importance in understanding CNS function, but the EEG is an unreliable guide to such function. 2) Concepts such as consciousness, arousal, vigilance, etc., as presently conceived, are invalid as correlates of cerebral physiology and are misleading as guides to further investigation. I favor alternative #2 since, provided that one abandons the hypothesis that brain slow-wave activity is related to arousal, consciousness etc., it is possible to demonstrate a number of consistent, easily-verifiable relations between brain slow-wave activity and behavior. Descriptions of these relations are provided in a series of papers (see Vanderwolf et al. in press). In brief, the results indicate that many features of brain electrical activity are organized in terms of motor output rather than the presumed level of arousal or consciousness. It appears that there is a noncholinergic component of the ascending reticular formation which produces LVFA in the neocortex and is active if, and only if, an animal is performing a behavior of a class which includes head movements, manipulation of objects, changes in posture, and all types of locomotion (Type I behavior). Although it might initially seem appropriate to relate these behaviors and the correlated brain events to inferred processes such as arousal, consciousness, or attention, further investigation has shown this to be inappropriate. Inferred psychological processes such as consciousness, arousal, etc. are usually considered to occur during immobility in the waking state, yet the system that produces LVFA in correlation with Type I behavior is not active during such immobility. Therefore, there is no consistent relation between Type I behavior (and its central correlates) and the hypothetical processes of arousal, consciousness, and attention (cf. Black 1975).

In addition to a noncholinergic component there is a second, probably cholinergic, component of the ascending reticular formation. The latter system appears to be responsible for all LVFA occurring during waking immobility and during ether or chloroform anesthesia. The presence of activity during both waking and comatose states suggests that this system, too, is unrelated to arousal or vigilance.

Both components of the ascending reticular formation can be inactive during the waking state (leading to EEG synchronization in a waking animal), and both are normally inactive during slow-wave sleep. Slow-wave sleep, then, can be regarded simply as a type of prolonged behavioral immobility. Both systems are active during active sleep, as in the waking state, and the noncholinergic system is active then if, and only if, phasic muscular twitches occur. In fact, data from both slow-wave and unit-recording experiments are strongly supportive of the view that higher-level motor-control systems are in vigorous activity during active sleep and would result in violent movement except for the fact that the spinal motor neurones and their reflex afferents are strongly inhibited (Robinson et al. 1977; Siegel et al. 1977; Vertes 1977). Thus, claims by Steriade and others (Hobson et al. 1974b *op. cit.*) that various aspects of brain activity are uniquely correlated with active sleep are invalid, since the correlates of vigorous movement in the waking state were not studied.

The hypothesis that active sleep is a state in which brain motor systems are in intense activity is relevant to the question of the function of sleep, a topic which is also discussed by Steriade. According to one recent reviewer, some theorists, including Steriade, regard sleep as a "nightly repairman who fixes things," while others view it as a "strict nanny who demands quiet behavior simply because it keeps us off the streets and out of trouble" (Allison 1978). Steriade ignores the papers of Meddis (1977), who has summarized much evidence that sleep is a means of conserving energy and/or avoiding predation, which is used by animals whose habitat and way of life provides security while they lie immobile and unreactive. Thus, sleep has a clear ecological function, and it is probably unnecessary to search for some obscure physiological function for it. As suggested by the previously mentioned electrophysiological-behavioral data, sleep is simply one of several motionless behaviors and is not, in itself, a fundamental category of CNS function. Viewed in this way, the hours spent in sleep every day require a prolonged shutting-down of the movement-generating machinery of the brain. Mammals seem to have opted for a strategy like that chosen by diesel-tractor operators working in the arctic winter. The "motor" is allowed to run at intervals with the "clutch" disengaged (accomplished by inhibiting spinal motor systems). However, the biological clutch is only partially successful in uncoupling the "motor" from its effectors, giving rise to the phasic muscular twitches of active sleep.

Steriade is concerned that we lack a precise method by which to quantify arousal. I suggest that any attempt to quantify an inferred process such as arousal is essentially an attempt to observe something which is not observable or even definable. If it is true, as I argue, that brain activity is organized largely in terms of motor output, our task is very much simplified. Overt behavior can easily be recorded in objective fashion, and a variety of methods exist for doing so (Hutt & Hutt 1970). Systematic use of such methods will, I believe, lead to many advances in the brain-behavior field.

Finally, I would suggest that the answer to the question posed in the title of this commentary is "Nothing in particular!"

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Memory-consolidation hypothesis of REM sleep. The behavioral significance of the firing patterns of type-1 and type-2 neurons outlined by Steriade remains to be explored. The major thesis presented by Steriade is that type-1 neurons (cortical long-axoned output cells) have high rates of firing in wakefulness, decreased firing in sleep, and increased firing in REM sleep. The firing rate in REM sleep for these neurons increases proportionally to eye movement occurrence. On the other hand, type 2 neurons (short-axoned putative interneurons) exhibit low firing rates during wakefulness, increased burst rate during sleep, and highest burst rate during REM sleep with eye movements rather than REM sleep without eye movement. Steriade suggests, on the basis of animal learning data, that the behavioral significance of the type-2 neuron activity during REM sleep is memory consolidation.

Memory and memory consolidation are concepts inferred from learning studies. It is presumed that, after a learning session and intervening time or task, presentation of the learned stimuli will result in conditioned responses. The percentage correct (or some other measure) at this second session reflects the inferred memory. If REM sleep is associated with the memory consolidation process, then changes, either increases or decreases, should affect the learning criteria. Studies with rats as subjects have indicated that REM does affect the learning-memory dimension. Pearlman and co-workers (see e.g. Pearlman & Becker 1974) reported that REM deprivation (REMD) immediately following a learning session disrupted retention, while REMD three hours later did not. They also observed that REMD retarded the learning of the discrimination problem but did not affect the learning of a position problem and indicated that REMD affected difficult tasks much more severely than simple tasks. Tasks in ascending order of REMD decrements included bar pressing in an operant situation, DRL 20 performance, serial

reversal and probability learning, and cooperative behavior. Thus cooperative behavior, a very difficult task for rats, was never learned following REMD.

Data on humans is not nearly so clear cut. Efforts to demonstrate that REMD, slow-wave sleep deprivation, or total sleep deprivation affect learning or performance have not been very rewarding. Chernik (1973) attempted to modify the retention of learned paired associates or serial trigrams by REMD. Her study was unsuccessful, which may be due to the relative lack of complexity of the learning task. Costello & Ogilvie (1974) found that 3 nights of REMD produced omission errors in a vigilance task. However, one night of REMD did not. Their results are similar to the effects of total sleep deprivation on vigilance and are therefore not definitive. Greenberg et al. (1972) did obtain REMD effects on learning a complex task, while no effects were observed for performance of an overlearned behavior.

Another approach to demonstrating the relationship between learning and REM is to determine whether REM increases as a function of successful learning trials. Lucero (1970 *op. cit.*) found that when rats learned where food was placed in a labyrinth, increased amounts of REM time relative to total sleep time were observed. The ratio of slow-wave sleep to total sleep time was not affected. Food deprivation without learning trials did not result in a similar REM effect. The work of Leconte et al. (1973 *op. cit.*) explored the relationship between REM and task mastery across 4 daily sessions. The important finding of this study was that the amount of REM sleep and the percentage of correct responses to the two-way avoidance task increased in the same linear fashion across days up to day 4, where learning reached an asymptote. Following that session, REM percentage dropped to the original prelearning level. The augmentation of REM appeared primarily in the first half hour of the 3-hour sleep period. Fishbein & Kastaniotis (1973) obtained similar REM augmentation, but throughout a 24-hour recording session. Procedural differences may account for the discrepancies. These data suggest that memory consolidation may be a function of increased REM activity rather than level of REM activity per se.

If the activity of type-2 neurons is associated with memory consolidation in rats, then during a learning session in which increased REM is obtained, increased type-2 activity should also be observed. After the learning session, when REM returns to basal level, type-2 activity should also decrease. Eye movement activity should show a similar pattern. If it is presumed that it is only type-2 neurons that are involved in the consolidation process, then the activity of type-1 neurons must be partialled out.

The question then becomes: What is the function of the remaining REM sleep? Perhaps REM levels reflect the maintenance of memory or degree of conditionability, while REM increase reflects the updating of memory. Pagel et al. (1972) demonstrated that mice who learned complex tasks (two-way avoidance and Lashley Type III maze) exhibited significant REM increase throughout the night more quickly than poor learners. Rats who did not learn in the Leconte et al. (1973 *op. cit.*) study exhibited lower REM/total-sleep ratios than animals that were conditioned. (No statistical comparisons were made.) Feinberg et al. (1967) observed that patients with severe memory loss show large deficits of REM sleep rather than slow-wave sleep, in comparison to age-matched control subjects. Feinberg (1975) reported that the correlation between REM% and verbal performance I.Q. on the WAIS was 0.72. These data reinforce the observations of Pagel et al. (1972). If the memory maintenance or conditionability notion of REM is correct, type-1 plus type-2 activity should correlate with amount of material in storage (I.Q., for instance) or ease of acquiring any new S-R relationship (learning set).

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by **A. C. Webb**

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How important is the mean frequency of neuronal discharge? I have no intention of presenting an exhaustive critique of the paper under discussion. Instead, I should like to examine an assumption which seems to me to underlie both the design of Steriade's experiments and his interpretation of the results of those experiments. This is the premise that the number of action potentials emitted by a nerve cell is the most important aspect of its behavior, and that mean frequency of discharge is directly related to the amount of information conveyed.

If one looks first at his decision to hunt for two classes of cell, his argument may be paraphrased in the following way: "Various people have examined the changes in discharge rate which accompany alterations in an animal's degree of alertness. No consistent findings have emerged. Consequently, we must either despair, or assume that this apparent chaos is due to the indiscriminate pooling of results from neurones which belong to fundamentally different populations. It therefore behooves us to look for subsets of cells having different physiological characteristics."

This is a very reasonable argument, provided that one shares the conviction that mean frequency of discharge is the only aspect of cellular activity worth considering. If one lacks this conviction, there is a third, rather more parsimonious, alternative. It is possible that discharge rate is not essentially related to the observed behavioral changes, and that some other measure of neural activity should be considered. Evarts (1964 *op. cit.*) first proposed this alternative, reporting that, in monkey motor cortex, consistent alterations occurred in the *temporal pattern* of neural discharge whenever the animal fell asleep. Several subsequent sets of experiments have confirmed his impression that this is a far more reliable electrical index of an animal's general state than a simple measure of discharge frequency.

The temporal pattern of discharge produced by an individual neurone is usually displayed in graphical form, as an "interval distribution." To make such a graph, a train of action potentials is recorded, the duration of each interspike interval is measured, and the graph emerges with "duration of interspike interval" as abscissa, and "number of intervals of each duration" as ordinate. Burns and Webb (1976) showed that when such a histogram is plotted on a logarithmic abscissa, it conforms satisfactorily to a normal curve. Technically, this is a useful fact, implying, as it does, that the temporal-discharge pattern of each neurone can be described in terms of the two defining parameters of a normal distribution. It soon became clear that the values of the two parameters were directly related to the state of the animal, and that mean frequency of discharge was a derivative and irrelevant measure.

By that time we had also developed a technique for recording the activity of single cells from the cortices of completely unrestrained cats (Burns, Stean, & Webb 1974). This enabled me to take the further step of trying to place sleep and its attendant neuronal events in the wider context of other changes in the animal's state of alertness. I could startle a cat and ask whether the changes in discharge pattern which I then observed were qualitatively similar to those produced by the more familiar transition between sleeping and waking. The results of these experiments suggested that vigilance could reasonably be considered a neurophysiological continuum, and that information about the animal's fluctuating state of alertness was present in the activity pattern of each of the cells from which I recorded (Webb 1976a & b).

Following my own basic assumptions, I have been led to the belief that information about an animal's general state can be detected in the discharges of a large proportion of cortical neurones, and that this information always takes the same basic form, regardless of each cell's more specialized functions. Steriade, acting upon his assumptions, has concluded that there are two distinct classes of neurone, each differently affected by the animal's level of alertness, their differences being in some way determined by their other functions.

Assumptions also influence one's interpretation of results, and again, Steriade's major premise is his confidence in the physiological importance of a high rate of discharge: "If, therefore, interneurones are effectively related to higher neural activity, the fact that they are more active during sleep than wakefulness leads to the supposition that type-II cells are specifically involved in highly integrative processes occurring during certain sleep epochs." Such a supposition must be largely a matter of faith, since evidence is scarce concerning the nature of events which accompany the transmission of biologically significant information. The proposition that such information is signalled by a high-frequency burst of action potentials has rarely been tested (Burns et al. 1972; Hocherman et al. 1976), and the results of the few existing experiments are equivocal. Meanwhile, it now appears possible that work done by the nervous system may be inversely related to the degree to which neighboring nerve cells fire in unison (Noda & Adey 1970a; *op. cit.,* Webb 1977; Burns & Webb, in preparation). If this were so, the single cell could not properly be regarded as an independent functional unit – its usefulness would lie in its contribution to a matrix of activity, and questions regarding the significance of a given discharge rate recorded from a given cell would become very complex indeed.

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by **C. D. Woody**

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Identification and functional characterization of electrophysiologically studied cortical neurons. Cortical neurons must be involved in a variety of functions. How they are specifically involved is a matter for physiologic investigation. It is generally agreed that such investigations must be conducted at the level of single units. This is because attempts to study the function of large numbers of units, en masse, have been unsatisfactory, physiologically.

As Steriade indicates, single-unit studies require identification and characterization of each unit. The question is how to identify and characterize the cell in a way that will be physiologically relevant. This commentary concerns means for doing so in the mammalian cortex.

I. Identification: a. Antidromic stimulation has been the major technique used for *in vivo* identification of cells. It has several limitations. These include:

1. Anatomical inaccessibility. If a locus can't be found at which stimulation is effective, the cell cannot be identified. Thus, *absence* of an antidromic response to pyramidal stimulation does not allow identification of a cortical interneuron. The cell could instead be a corticobulbar output cell.

2. Spread of stimulus current. If afferent as well as efferent fibers are activated by the stimulus current, orthodromic activation may obscure the effects of antidromic activation (cf. Humphrey, this *Commentary;* Fuller and Schlag 1976 *op. cit.*). The same may occur if axonal *recurrent* pathways of the cell studied are activated. High rates of following and a fixed response latency favor direct, antidromic activation (cf. Steriade, Fig. 10; also Fig. 5 in Darian-Smith et al. 1965). A variable response latency does not (cf. Steriade, Figs. 2A and 7). Satisfaction of still other criteria (Fuller and Schlag 1976 *op. cit,*) may be required to exclude orthodromic activation completely.

3. Locus of cell. Though activated antidromically, the locus of the cell body is still not established. In the cat this may not prove a problem for cortical PT cells. Naito et al. (1969 *op. cit.*) have found *all* PT cells to be located in layer V. In the monkey this localization is thought to be more ambiguous (Humphrey, this *Commentary*). If so, this would constitute a species difference, since the results in the cat have been confirmed (Sakai, H., personal communication).

b. Intracellular marking is used increasingly to identify electrophysiologically-studied neurons (Naito et al. 1969 *op. cit.;* Kelly and Van Essen 1974 *op. cit.;* Sakai et al. 1978). Dyes or enzymes such as HRP are injected intracellularly by iontophoresis or pressure. This is done *after* studying the cell. Thus, the ejection does not interfere with date retrieval.

As Steriade points out, two possible limitations arise with this approach. They are 1) sampling bias of the electrode, and 2) cell injury upon penetration.

1. Sampling bias. This is small, at least for intracellular recordings from cortical neurons (Kelly and Van Essen 1974 *op. cit.;* Sakai et al. 1978). *All* cell types commonly found *in situ* can be penetrated, injected, and recovered. Cells with small somas are penetrated as easily in layer III as cells with larger somas. Seventy percent of the neurons penetrated in motor cortex are pyramidal cells from layers III and V. The slight bias towards the larger pyramidal cells appears to be due to their extensive dendritic arborization. At least 25% of all penetrations are of dendrites.

2. Functionally-significant injury can be avoided. This has been demonstrated by the ability to record equivalent PST (poststimulus time) histograms of spike activity from populations of units sampled intracellularly and those sampled extracellularly (Woody and Black-Cleworth, 1973 *op. cit.*). Moreover, there are means available, such as the injection of ramp currents (Woody and Gruen 1976), that permit some significant injuries to be recognized, when present.

II. Characterization: All penetrated cells should be characterized functionally. This can be done with respect to activity, excitability, pharmacologic response, motor projection, and sensory receptivity. The characterization may be expected to change as a function of behavioral state.

a. Spike activity profiles. Techniques for evaluating patterns of spontaneous and stimulus-evoked spike activity are well established (cf. Segundo, this *Commentary*).

b. PSP activity. Techniques for evaluating characteristic synaptic inputs and identifying cells orthodromically are also well known (e.g. Lux and Schubert 1975).

c. Excitability. The excitability of the cell can be measured as the level of extracellular (EC)- or intracellularly (IC)-injected current required to produce repeatable discharge (see Woody 1977 for detailed description).

1. μA EC stimulation. Used with metal electrodes (Woody 1974) results can be equivalent to those of intracellularly-injected current (Woody and Black-Cleworth 1973; *op. cit.;* Brons et al. 1978).

2. nA EC stimulation. Used with glass micropipettes to provide a different index of neural excitability (cf. Woody 1977).

3. nA IC stimulation. Used with glass micropipettes; can reveal postsynaptic excitability change.

4. Antidromic stimulation. Provides measurable excitability changes as indicated by Steriade, but may not avoid recurrent synaptic excitation. This approach is conceptually problematic in view of the safety factor associated with normal spike propagation (Tasaki 1959). The technique should be relatively insensitive to small excitability changes *within* the cell and more susceptible to changes in the surrounding subliminal fringe.

d. Pharmacological response. Techniques for evaluating the response of single cells to extracellularly – or intracellularly-applied pharmacologic agents are readily available (e.g. Krnjevic 1974 *op. cit.*).

e. Motor projection. The pauci-synaptic projection of some cortical units to the peripheral musculature has been demonstrated and confirmed (Woody and Black-Cleworth 1973 *op. cit.*). This procedure permits functional classification of single units according to their ultimate motor projection (Woody 1974).

f. Sensory receptivity. The techniques described in a and b (above)

permit the classification of a unit according to its ability to respond (or not) to particular sensory stimuli e.g. Woody 1977).

Studies of the sort attempted by Steriade are needed and may be advanced by application of the above techniques. The results, in mammals, should permit conclusions as "unequivocal" (cf. Steriade) as those in molluscs (Kandel 1977).

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Author's Response

by **M. Steriade**

Cell classification and changes in neuronal activity with shifts in vigilance state

The reader who, for time-saving or fun, bypasses my paper and turns directly to the commentaries may be overawed by the vast fields covered, extending from the estimation of cellular density in various cortical layers and sophisticated methods for physiological characterization of neurons to general theories of sleep function, memory, and consciousness. Although a wide sample of brain sciences is represented, each commentary is rather specialized, and this leads me to circumscribe my own domain. Bewildered by the diversity of brain cells, the maze of pathways, and the ingenuity of some researchers who record signals from neurons without trying to know their synaptic inputs and projections, I duly attempted to identify the functional organization of thalamic and cortical neurons and to study their activities as modulated by a continuously-reproducible behavior. This behavior happened to be sleep and waking, perhaps because I am Bremer's pupil. I hope to have made it clear that, until quite recently, I have not worked on structures involved in the *genesis* of waking and sleep states, but rather on state-dependent changes in identified neurons of thalamic nuclei and cortical areas believed to play some role in high-order integrative processes. The "why" of sleep has always seemed to be a too-complicated problem for me to embark upon. Most of my paper is therefore devoted to criteria for cell identification and to differential changes undergone at various levels of vigilance by two cortical cell types. I am less concerned with the psychological speculation on the possible significance of REM-related interneuronal discharges, confined to the last two paragraphs in my paper, so I shall not respond to the multitude of related and alternative hypotheses advanced by commentators concerning the sleep-memory story; let the reader consider each of these on its own merits. I shall, however, take the opportunity of referring the reader to a paper by Doty (1975), discussing the assignment of a mnemonic function to specific classes of cortical neurons. Some questions, like many existential problems, must remain unanswered; the most dramatic is obviously **Schlag's** final sentence. To stay on more solid ground, let me comment on several methodological points.

Two cellular classes. I was under the impression that I had sufficiently underscored the oversimplification involved in the dichotomous classification of cortical elements into long-axoned cells and interneurons and would not need to re-emphasize this point in my response. With one exception, all commentators realized that I am fully aware that these two classes lack homogeneity, but that many (morphological, functional, developmental) arguments support the categorization into two neuronal archetypes (see **Berlucchi, Ramón-Moliner,** and others), especially when first beginning to clear a field. The exception was **Schlag,** who thought that one of the assumptions underlying my approach was "that the categories of output cells and interneurons are homogenous." The simplest way to resolve this misunderstanding would be to refer to my footnote 1, Figure 1, and the corresponding text, in which the tremendous morphological and functional variety of interneurons is repeatedly stressed. But I now feel this to be an excellent opportunity to go into this point, all the more because some commentators have added important data to it.

To begin with, the division into two cellular classes behaving in opposite ways with changes in vigilance level resulted from a chance observation (Steriade *et al.* 1971 *op. cit.*, Fig. 8A). I was puzzled that, along the same microelectrode track, PT cells increased their firing rate, while bursting non-PT elements (putative interneurons) stopped firing during an "arousing" reticular stimulation. Obviously, I wanted to see if this was a more general rule, and I explored several cortical areas and specific thalamic nuclei in cat and monkey. Puzzlingly enough, it appeared that the differentiation between two neuronal populations was present at all the above levels during behavioral arousal (or reticular stimulation) and synchronized sleep. The most striking finding was the arrest of interneuronal firing on arousal from sleep. I am quite sure that this unexpectedly homogenous behavior of interneurons, a cellular class that is heterogeneous by definition, is partially ascribable to the criteria I used for their identification, which are, as **Humphrey** remarked, perhaps *too selective, thus omitting non-bursting interneurons*. The existence of interneurons that do not exhibit high-frequency barrages was mentioned in my paper (2.3.1), but I deliberately decided not to include such elements in order to avoid the contamination of the interneuronal group by output neurons that could not be invaded antidromically. In my opinion, this deliberate simplification à la Claude Bernard is a necessary first step in any experimental approach and does not preclude further improvement. In other words, the data presented here should be taken as applying to *bursting* interneurons. I am confident that anyone who has fine microelectrodes, who does not reluctantly look at spike bursts as necessarily coming from sick neurons, and who is fortunate enough to have a stable preparation in order to follow a particular cell in repeated sleep-waking cycles, will be able to predict the arrest of firing of that interneuron each time the animal wakes up.

I agree with **Humphrey** that, because of the difficulties encountered in recording from superficial layers (thickened dura in chronic experiments), our cells came mostly from deeper layers. It is therefore possible that recordings directed to explore the upper laminae will pickup *non-bursting* non-output cells which, if identified by staining, will disclose the diversity of mor-

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phological features (see the **Pasiks, Ramón-Moliner,** and **Tömböl**), membrane properties, and sleep-waking behaviors of these internuncial elements. I am glad that **Oshima's** commentary offers the first data in this direction. Unfortunately, as he did not record elements discharging with high-frequency barrages, a comparison with our data on bursting cells is difficult. Moreover, the latencies he reported for reticular-induced excitation and inhibition of non-output cells (ranging from 230 msec to 560 msec) are much greater than the latencies of reticular-elicited inhibition found in our bursting interneurons. Figure 8A in my target article actually shows that arrest of interneuronal firing occurred less than 100 msec after reticular stimulation; even shorter latencies characterize the reticular-elicited excitation of PT cells. Neurons in areas 5 and 7 are driven synaptically by a midbrain reticular volley at latencies as short as 3–5 msec (Steriade, Kitsikis, & Oakson, 1978 *op. cit.*). It is hard to imagine what happens in the cortex during the several hundreds of milliseconds required to get the effects reported by Oshima. In fact, many possibilities exist including excitation of non-output cells through recurrent collaterals of previously activated PT neurons, or as yet uninterpretable interneuronal affects.

The existence of cells with mixed or "intermediate" properties (**Glenn & Guilleminault**) may be related to the case of callosal neurons (raised by **Berlucchi**), which, like corticothalamic, corticopontine, or corticospinal cells, increase their overall discharge rate during waking compared to long periods of synchronized sleep, but, like interneurons, exhibit striking bursts of discharge during periods of high-amplitude spindles and slow waves, with frequencies far exceeding those seen during waking (see Steriade, Deschênes, Wyzinski, & Hallé 1974 *op. cit.*). These "intermediate" features may be common to callosal and some intracortical associational neurons. The case of giant stellate cells (described by Ramón y Cajal 1960 *op. cit.*, in the visual cortex of man, cat, and dog) whose axons leave the gray matter ("leur cylindre-axe, épais, . . . descend presque verticalement à travers toutes les couches sous-jacentes pour se transformer en un tube de la substance blanche," p. 606), should be recalled in this context. I am aware that a simplification involved in my paper is that, due to the use of antidromic identification of corticofugal cells projecting to the thalamus, pons, and spinal cord, the behavior of intracortical association neurons (some of them true long-axoned elements, as linking distant areas in the ipsilateral hemisphere) escaped my analysis. **Szentágothai**'s commentary is particularly useful in noting the ambiguity of the term "output" neuron and the fact that this term should *not* be reserved for cortico-subcortical cells. The interest of the data referred to by Szentágothai (showing that pyramidal cells yielding cortico-cortical connections are mostly found in layer III) resides in the very different locus of these elements compared to the deeper sites of neurons projecting to subcortical structures. It is possible that further analyses will show that intracortical association and callosal elements behave in some respects more similarly to short-axoned interneurons than to cortico-subcortical cells, in support of the final suggestion of Szentágothai. This would not greatly change my speculation about the *intrinsic* cortical mechanisms of memory consolidation during certain stages of sleep.

Concerning possible mechanisms underlying the simultaneously-occurring increased firing of output cells and depressed firing of bursting interneurons with arousal, my current preference is for the acetylcholine (ACh) account (3.3.2), commented on by **Krnjević**. In their commentary **Ben-Ari & Naquet** draw my attention to some controversial issues in the recent paper by Dingledine and Kelly (1977 *q.v.*), published after completion of my manuscript, and they interpret their findings as "having not obtained such evidence concerning a putative monosynaptic cholinergic inhibitory projection from the mesencephalic reticular formation (MRF) to the reticular (thalamic) nucleus" and "having obtained a long latency inhibition that was resistant to large amounts of iontophoretically-applied atropine." A further reading of the same paper by Dingledine and Kelly reveals that "in a number of neurons the latency for the MRF-evoked inhibition was compatible with the fastest conduction velocities reported for ascending reticular fibers" (p. 145–146), and "at least some of the inhibitions were mediated *monosynaptically*" (p. 150). Other inhibitions were of longer latency (mean: 13.7 ± 3.2 msec). Now, as far as the relatively weak potency of atropine to block the MRF-evoked inhibition is concerned, Dingledine and Kelly advance several factors to explain it (inhibitory cholinergic synapses located on distant dendrites, etc.) and wisely recall that "even in the habenulo-interpeduncular . . . and septo-hippocampal . . . pathways . . . shown to contain all the biochemical properties expected to cholinergic tracts . . . iontophoretic atropine has failed to block the responses to electrical stimulation" (p. 181).

The firing criterion. Two commentators (**Webb** and **Schlag**) do not believe that frequency of firing should be regarded as a meaningful index of cell activity. I am sure they have no doubts about the usefulness of this criterion when dealing with central neurons involved in motor command, in which case a succession of physiological events (increased discharge frequency → movement) is, as a rule, regarded as implying a causal relation [cf. Kupfermann & Weiss: "The Command Neuron Concept" *BBS* 1 (1) 1978].

Webb put the problem in simple alternative terms: she claims (curiously overlooking pattern data in my Figures 13 and 14, the corresponding text, and the discussion on discharge patterns in footnote 4 of my paper) that I measure only the mean *rate* of discharge, while she favors *patterns*. After having summarized her results, which revive prior findings of the sixties on state-dependent changes in cortical cell temporal patterns, she concludes "that mean frequency of discharge was a derivative and irrelevant measure." By way of contrast to this lapidary postulate, I would refer the reader to a diametrically-opposite view in the commentary by **McCarley & Hobson,** who do regard changes in discharge patterns as dependent on changes in discharge rate. *Both* rate and pattern should be presented (as is the case in this paper and in all other articles coming from our laboratory), and the relation shown by McCarley & Hobson (1972 *op. cit.* by M & H) in the cerebellum should be checked in many other cerebral structures before a definite conclusion is drawn on whether pattern is a function of rate or of behavioral state, and whether the rate-dependent pattern in simple Purkinje cell spikes is the rule for all neuronal types or, as I would guess, only for tonically-discharging cells. In any case, the "hope of finding some simple and universally applicable way of describing the series of spontaneous action potentials" (Burns & Webb 1976 *op. cit.* by Webb) should be abandoned when dealing with a complex structure like the cerebral cortex. In one of her papers Webb (1976 *op. cit.* by Webb) is confident that "the modal interval of every cortical neurone shortens when a cat falls asleep" (p. 235), and that "interval distributions with *modal intervals which are shorter than 20 msec appear to be characteristic of neural activity recorded from a sleeping cat*" (p. 225, italics mine). Her results apply to nonidentified cells recorded by means of semimicroelectrodes (impedances lower than 500 kΩ). Unfortunately, for interneurons of the suprasylvian gyrus (an area from which she recorded), statistical analyses have shown the mean modal interval to be 4.9 msec in wakefulness (see also Steriade, Oakson, & Kitsikis 1978 *op. cit.*), indicating that "intervals shorter than 20 msec" may not be a sufficient criterion for the sleeping state. Let me also add that data in my paper, comparing output cells to bursting interneurons, show that modal intervals are significantly lower for interneurons in *both* waking and slow-wave sleep, a fact that emphasizes differences between cell types rather than between behavioral states.

Criteria for waking and the question of movement. I stated in my paper (footnote 3 and Figure 9) that there does not exist a

precise method for quantifying level of alertness during the waking state (see also **Ranck**). This lack becomes even more problematic with the change in direction of my research to the midbrain reticular formation, subthalamus, and medial thalamus, where we currently record activities of rostrally-projecting cells thought to generate activation processes in the forebrain (see below). **Vanderwolf** is breaking down open doors in saying that EEG signs fail to indicate level of arousal and concluding that the EEG is "an unreliable guide" for defining various states of vigilance. I wonder who, among sleep researchers, exclusively regards EEG without looking at the EMG, eye movements, and the general behavior of the animal. Faced with our difficulties in finding good criteria for *quantifying* behavioral arousal, Vanderwolf proposes a solution based on the motor-nonmotor alternative: "sleep is simply one of several motionless behaviors"; during waking, animals move; thus "our task is very much simplified. Overt behavior can easily be recorded in objective fashion . . . ." That is reasonable, provided that we are convinced that all waking processes are organized in terms of motor activity. But if one ventured to stray from this line of exclusive reasoning, one could conceive that, in wakefulness, *ascending* reticular systems may modulate highly-complex activities of the forebrain, of which motor behavior is only an ancillary aspect. There is, for instance, a large body of evidence (especially in the geniculostriate system) indicating that integrative processes are facilitated during waking with a background of EEG desynchronization (Burke & Cole 1978), without being related to motor output. While Vanderwolf's experimental strategy (of exploring the relations between various EEG hippocampal waves and struggling or biting by the rat) may be commendable to those who are interested in these vital phenomena, the search for intimate cellular mechanisms underlying the increased sensory discrimination at some levels of alertness is rather difficult to perform when the animal is fighting. The interesting data that **Iwama & Fukuda** bring up in their commentary concerning reticular influences upon neuronal events in the visual system could not have been obtained under such experimental conditions.

I now come to the point concerning how the animals were restrained, which seems to have constituted a great source of disquiet for some commentators. As **Jouvet** recognized, the now popular stage-play with restrained versus unrestrained actors (which on this continent has generated an east-west struggle, now four years old, with occasional European participation) concerns neither my findings nor my hypotheses. But this allows me to answer some questions and to make my entry into this fray. Concerning **Valleala**'s and **Morrison**'s questions on our experimental conditions: except for the head, which is restrained, the animals move freely and show motor adjustments on arousal, orientation, and falling asleep. Contrary to **Vanderwolf**'s assumption, "vigorous movements" in the waking state *were* recorded, and they were associated in many instances with phasic rises of discharge rates of identified or nonidentified cortical cells. Such events are depicted for a cortico → CM neuron in Figure 1A of a recent paper from our laboratory (Steriade, Oakson, & Kitsikis 1978 *op. cit.*). We did *not*, however, include such periods in the analysis of mean discharge rate, because (unless one carefully analyzes movement-related discharge with the specific aim of determining the temporal relations between central neuronal events and peripheral ones) we cannot ascertain whether a given cell is a premotor neuron or if it is driven by proprioceptive feedback [Roland: "Sensory Feedback to the Cerebral Cortex During Voluntary Movement in Man" *BBS* 1(1) 1978]. As all of us know, one can record anywhere in the brain and find signs of "motor" activity in regions having nothing to do with the elaboration of movements, simply because the neuronal discharge is elicited through collaterals of somatosensory *afferent* systems. This is why I am so reluctant to look at gross, undifferentiated movements. In current experiments at the level of the subthalamic areas (namely zona incerta) and midbrain n. cuneiformis and FTC area (in collaboration with A. Kitsikis, N. Ropert, and G. Oakson), we have observed that, for some neurons, discharge rate actually increases in periods of waking accompanied by movements compared to motionless wakefulness. But at present we do not know what this means and, obviously, cannot jump to the conclusion that such findings favor the idea of a mechanism that organizes and initiates motor acts. If we feel that a given neuronal class underlies some motor phenomena characteristic of the waking state, we will certainly design a protocol to simplify the event and to investigate the temporal relation between the changes in unit firing and the appearance of movement. Otherwise, nothing is convincingly "demonstrated" (I feel that people use this word too often), and one may speak about "occurrence in association with," which, in physiological terms, does not suggest causal relations.

Morrison wonders (in relation to recent papers on monkey parietal association cortex) what the activity of interneurons in areas 5 and 7 might be in animals free to perform exploratory movements. The interneurons from which we recorded, in animals completely free to move their limbs, fired at very low rates (<1/sec) during the waking state, and their spike bursts did not increase in relation to limb or trunk movements. That this low rate of discharge is not due to head restraint, but to some peculiar, unknown characteristics of this neuronal class, is shown by the high discharge rate of cortico-pontine and cortico-thalamic cells during the same behavioral state, in the same preparation. As stated in my paper, the lack of interneuronal firing during waking eye movements also excludes the possibility that the increased discharge rate during the REMs of D sleep are a proprioceptive effect of such movements.

Interesting hypotheses and many experimental designs were proposed to **Jouvet** to test the relation between cortical interneurons and some genetic (endogenous) programming, but, as I have since moved our investigations into the rostral reticular core, all this is now well behind me. I hope other people will be stimulated by such a project. To Jouvet's questions in point (iii), I have no answer, as my experience with interneurons and REM sleep does not transcend areas 5 and 7 of cat.

Present endeavors. Let me finally go beyond the data presented in this paper and stress an idea mentioned in section 3.3.3, namely, that *changes in rate and pattern of midbrain reticular cells may underlie the tonically-increased firing rates of thalamic and cortical long-axoned neurons during the activated states of both waking and D sleep.* This idea was the starting point in the recent shift of my research. In collaboration with some of my colleagues I am now testing this hypothesis in the midbrain reticular formation and related subsystems. In fact, this represents a cellular investigation of some implications of Moruzzi & Magoun's (1949) reticular-activation concept. Some data will be delivered in November 1978 at the St. Louis symposium *The Reticular Formation Revisited* (Steriade, Ropert, Kitsikis, & Oakson 1979). This is not the place to present a complete summary of our findings, but what I can say is that **Jouvet**'s pessimism about the role of midbrain reticular cells in cortical activation during W *and* D sleep (point 2 in his commentary) seems unjustified in view of our results. In this context I would also reassure **Fishbein** (citing an older paper by Jones and others) concerning the contamination of fibers arising in the locus coeruleus during electrical stimulation of the midbrain tegmentum eliciting EEG desynchronization. In a more recent paper Jones *et al.* (1977) bilaterally destroyed the majority of the locus coeruleus region in cat, with the consequence of a significant reduction of noradrenaline (NA) content in midbrain, thalamus, and cortex, but they failed to observe any changes in EEG desynchronization. These results indicate that the lesions in the previous (1973) study (cited by Fishbein) affected *non-NA* neurons and fibers, most probably *involving the midbrain reticular formation.*

I am tempted here, in advance of the St. Louis Symposium, to champion the role of the rostral reticular core in forebrain activa-

tion processes during *both* W and D sleep on the basis of the following findings. (*1*) The increased firing rate of midbrain reticular cells from S sleep to either state of W and D sleep is highly significant. This increase persists even when comparing S to W without movements ($P \leq 0.003$) or to D without REMs ($P \leq 0.0005$). Such data deserve particular attention in emphasizing the peculiar features of midbrain reticular neurons compared to more caudally located reticular elements. Actually, some observations on pontine FTG cells in unrestrained animals (Siegel *et al.* 1977, Vertes 1977, *oper. cit.* in Borbély's, Jouvet's and Vanderwolf's commentaries), while denying the selectivity of those neurons during REMs of D sleep (Hobson *et al.* 1974 *op. cit.*), do *not* show that these elements are tonically active throughout the W state (as would be expected if they were responsible for the changes seen in thalamic and cortical cells) and rather indicate that pontine FTG neurons might be motor-related cells in both W and D sleep. ("Related" does not imply that such cells *generate* movements; see my remarks above.) Another point worth mentioning from our findings on mesencephalic reticular neurons is that the difference between W without movements and D without REMs is not significant ($P \leq 0.2$); the high rate of tonic discharge in both these activated states (median: 19.9/sec in motionless W, and 18.5/sec in D without REMs) does not depend upon phasic motor events. Furthermore, such equally-increased firing rates in W and D (compared to S) are uncommon for cerebral neurons, as most studies have reported much higher rates in D than in W (see Table 2 in Steriade & Hobson 1976 *op. cit.*). In other words, midbrain reticular neurons seem at present to be the best candidates for inducing and maintaining the *tonic* activation of cerebral neurons in both W and D states without phasic events. (2) Midbrain reticular cells increase firing rate from S sleep to W, *before* genuine EEG and behavioral signs of the W state, during a transitional period we conventionally term SW ($P \leq 0.007$). They are probably leading elements for activation processes in the S → W transition, analogous to neurons found by Hobson *et al.* (1974a *op. cit.*) in the pontine FTG during transition from S to D sleep. And, to complete the story, mesencephalic reticular cells decrease their firing rates during the transitional period from W to drowsiness, *in advance* of the complete induction of S sleep ($P \leq 0.0005$). Besides their histological location in the FTC area, some of the elements we investigated in the midbrain core could be physiologically identified as receiving multiple converging inputs from rostral or caudal areas (a qualification of brain-stem reticular neurons) and/or projecting to rostral structures (e.g. to the CM-Pf thalamic complex). As a conclusion to all my work during the past decade, and a perspective for my future research, I feel that this is the right place to pay a tribute to Moruzzi and Magoun (1949) as having introduced the concept of reticular arousal, by suggesting that "the presence of a steady background . . . within this cephalically-directed brain stem system . . . may be an important factor contributing to the maintenance of the waking state, and that absence of such activity in it may predispose to sleep" (p. 470).

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Call for papers (and topics, authors, nominations, and suggestions)

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We are also accepting informal nominations for (A) Corresponding Associate Commentators (who perform *Open Peer Commentary* on accepted articles) and for (B) members of the Board of Editorial Commentators (who referee submitted manuscripts in addition to performing *Open Peer Commentary*). A list of those who have thus far joined the Associateship hierarchy is available from the editorial office (and will appear in Volume I, Number 4 of this Journal). Qualified professionals in the behavioral and brain sciences who have either (1) been formally nominated by a current BBS Associate, (2) refereed for BBS, or (3) had a commentary or article accepted for publication can serve in capacity A or B.

To help in optimizing the service of *Open Peer Commentary* to the behavioral and brain science community, the editorial office would welcome suggestions from readers and Associates as to the optimal (1) length of a commentary, (2) number of commentaries per treatment, and (3) format for treatments.

Communications regarding these matters should be addressed to the Editorial Office, Behavioral and Brain Sciences, P.O. Box 777, Princeton, N.J. 08540.