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Effect of Barbiturate Anesthesia on Discharge Pattern in Nucleus Reticularis Thalami[1]

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WASZAK, M. *Effect of barbiturate anesthesia on discharge pattern in nucleus reticularis thalami.* PHARMAC. BIOCHEM. BEHAV. 2(3) 339–345, 1974. – In unanesthetized preparations spontaneous EEG spindles are accompanied by a tonic increase in neuronal firing in the rostral pole of nucleus reticularis thalami. The effect of Brevital on this relationship was investigated in encephàle isolé cats in the present report. Small doses of the drug consistently decreased the firing frequency inbetween the appearance of cortical spindles, whereas the effect on the intraspindle discharge rate as well as the averaged overall firing frequency varied from cell to cell. The ratio of intra- vs. interspindle discharge frequency was enhanced during light and moderate anesthesia, and the tonic spindle-related firing was broken up into bursts which were in phase with individual EEG waves. An increase in dosage to a level producing persistent slow waves in the EEG was followed by continuous irregular reticularis firing with no apparent relationship to the slow EEG waves. Massive doses of Brevital resulting in a flat cortical EEG arrested spontaneous firing completely. The results are viewed as supporting the hypothesis that neurons in the rostral part of nucleus reticularis exert a tonic inhibitory influence on dorsal thalamic cells.

Thalamus Nucleus reticularis thalami Inhibitory interneurons EEG spindles Barbiturate anesthesia Sleep

IT HAS long been recognized that under certain conditions the presence of synchronized waves in the cortical EEG is related to particular states of behavior such as drowsiness, satiety, and suppression of movements [4, 9, 22]. As a consequence, knowledge about the physiological mechanisms responsible for different types of synchronized EEG activity is important for an understanding of the neuronal mechanisms involved in these behavioral states.

Synchronous activity of 8–12 Hz occurs periodically in the form of spindles in the cortical EEG of unanesthetized encephàle isolé cats as well as in animals under barbiturate anesthesia. However, at the level of the thalamus – which has been considered the pacemaker of synchronous electrocortical activity [2,7] – the membrane potential changes accompanying spindles in these two types of preparations do not appear to be identical. In unanesthetized animals, hyperpolarizing membrane potential shifts, sustained throughout the entire duration of the spindle although interrupted by small depolarizing wavelets, occur in conjunction with cortical spindles in neurons of nucleus ventralis anterior (VA) and nucleus ventralis lateralis (VL) [19] as well as in some ventrobasal cells [10], whereas during barbiturate narcosis, cortical spindles have been found to be accompanied in different thalamic nuclei by a rhythmic sequence of augmenting and summating inhibitory postsynaptic potentials (IPSPs) which are separated by short depolarizing phases frequently giving rise to burst discharges [1].

It has recently been shown in unanesthetized encephàle isolé cats that neurons in the rostral pole of nucleus reticularis thalami exhibit a strong tonic increase in firing rate in conjunction with cortical spindles [21], a result which has been viewed as lending support to the Scheibels' hypothesis [13] that rostral reticularis neurons, which project their axons throughout the thalamus [11,12], are the inhibitory interneurons which are responsible, at least in part, for the tonic hyperpolarizing potentials observed in dorsal thalamic neurons during spindles. Given the differences in membrane potential configuration in dorsal thalamic neurons during barbiturate anesthesia as compared with unanesthetized prepara-

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tions, the Scheibels' hypothesis would seem to require that rostral reticularis neurons continue to increase their discharge rate during barbiturate spindles, but that they do so in phasic firing bursts, interrupted by cellular silence, rather than tonically as in the absence of anesthesia. This prediction was tested in the present report by comparing the firing patterns of rostral reticularis neurons before and after the administration of a short-acting barbiturate. In addition, this study afforded an opportunity to investigate the firing pattern of reticularis neurons during another form of electrocortical synchrony, that is the continuous slow waves which are seen during deeper stages of barbiturate anesthesia.

METHOD

Cats were surgically prepared under ether and Brevital anesthesia. Following tracheotomy the animals were given artificial respiration, the neuraxis was completely transected by suction at the spinomedullary junction and the cerebral cortex was bilaterally exposed and covered with mineral oil continuously warmed to 38°C, while the body temperature was maintained by a heating pad. All sites of incision and the pressure points of the stereotaxic headholder were liberally infiltrated with 1% lidocaine. General anesthesia was terminated at least two hours prior to recording when Flaxedil was injected. The observation that spindle waves were periodically occurring in the cortical EEG and that the pupils remained fissurated throughout the experiments was considered evidence that the animals did not experience any discomfort.

The cortical EEG was monitored throughout the experiment from the surface of the ipsilateral anterior sigmoid gyrus with silver ball electrodes and displayed on one channel of an oscilloscope. Extracellular recordings were obtained with glass micropipettes filled with 2M potassium citrate by the method of Tasaki *et al.* [17], the potentials being led through a cathode follower into the second oscilloscope channel from where they were photographed.

The animals were killed with an overdose of pentobarbital. The depth below entry of the recording electrode into the cerebral cortex was recorded for each unit encountered and this information was transposed onto the individual micropipette tracts in the histological slides, which were 50–100 μ sections stained by thionin or cresyl violet. In addition, neurons in nucleus reticularis thalami were easily recognized during recording by their prolonged burst responses to repetitive intralaminar stimulation [16].

RESULTS

The relationship between cortical EEG and neuronal firing in a VL and a rostral reticularis neuron in unanesthetized animals is illustrated in Fig. 1. The development of the cortical spindle is shown to be accompanied by a hyperpolarizing shift in membrane potential in the VL unit (Fig. 1A). The shift was sustained throughout the duration of the period of EEG synchrony, reaching its highest value in excess of 10 mV in the middle of the spindle when the EEG waves were largest in amplitude. Depolarizing wavelets can be seen to arise out of the hyperpolarizing shift, especially in its early phase, but the sustained nature of the membrane hyperpolarization becomes easily visible in the middle and towards the end of the spindle. Neurons in the rostral pole of nucleus reticularis, in contrast, were found to enhance their dis-

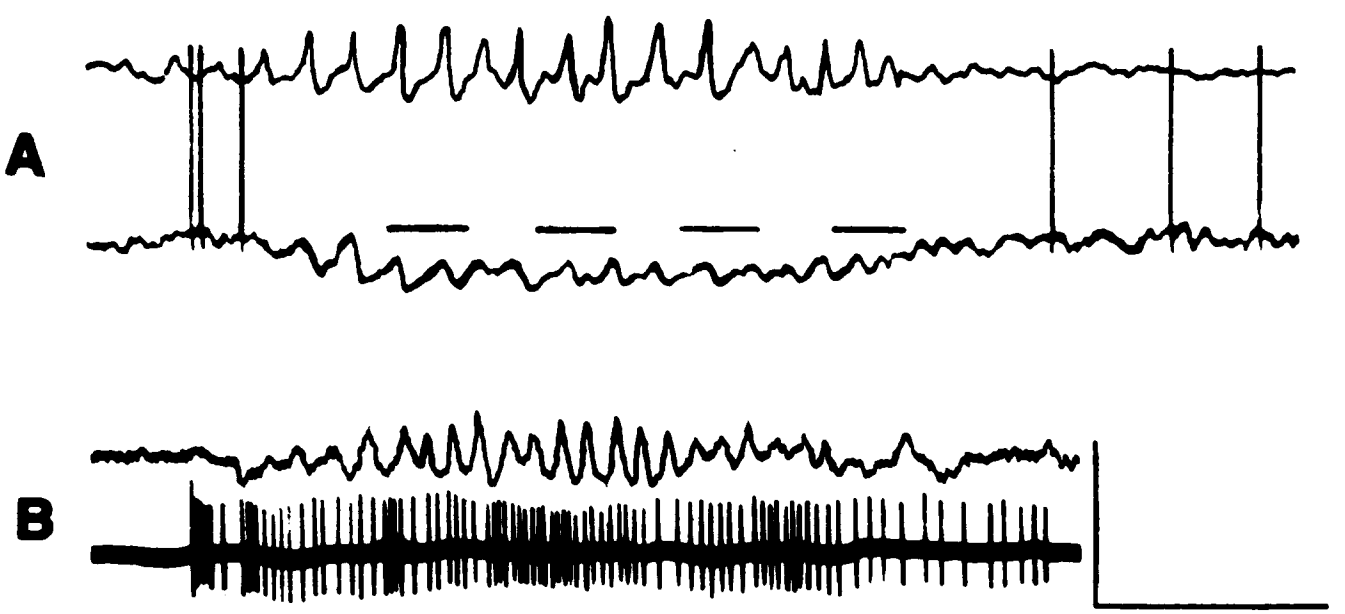

FIG. 1. Comparison of neuronal firing in VL and the rostral pole of n. reticularis thalami during cortical EEG spindles in unanesthetized animals. (A) Intracellular record from a physiologically identified VL unit. Dashed lines indicate membrane potential during EEG desynchronization. Note the sustained hyperpolarizing shift of the membrane potential in conjunction with the spontaneous spindle. (B) Extracellular record from a neuron in the rostral pole of n. reticularis thalami. Note the tonically sustained increase in discharge rate accompanying the spindle. In this and subsequent figures upper traces are surface recordings from the ipsilateral anterior sigmoid gyrus; voltage calibrations refer to cellular recordings. Time calibration: 0.8 sec in A, 1 sec in B; voltage bar: 50 mV in A, 10 mV in B.

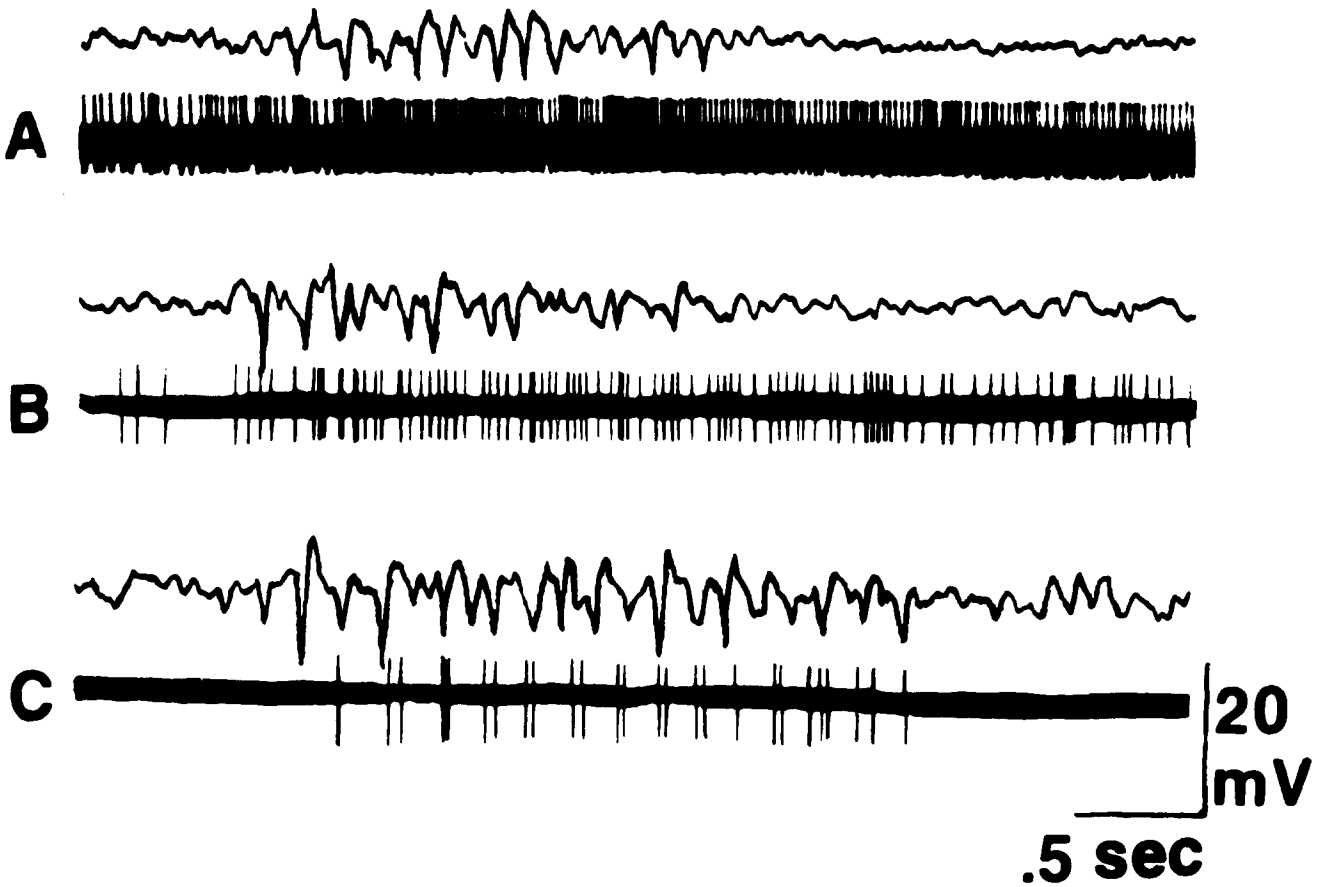

FIG. 2. Effect of intravenous injection of Brevital on spindle-related firing in a rostral reticularis neuron. (A) Unanesthetized animal; spontaneous spindle accompanied by 50% rise in discharge frequency. (B) Following injection of 0.7 mg/kg of Brevital; firing decreased both during and between EEG spindles. (C) Additional administration of 1.0 mg/kg; interspindle firing reduced to 0/sec, spindle-related discharges occurring in phasic bursts.

charge rate in conjunction with cortical spindles in unanesthetized preparations, as illustrated in Fig. 1B. The increase was tonically sustained throughout the duration of the spindle, and no phasic bursts at EEG wave frequency, separated by cellular silence, were encountered.

The effect of intravenous injections of the short-acting barbiturate Brevital on the spontaneous discharge pattern of rostral reticularis neurons was assessed in 30 cells. Fifteen of these were recorded while the drug was given for the first time during an experiment or after sufficient time had elapsed from a previous injection so that the cortical EEG and neuronal firing appeared as they did prior to Brevital administration.

Small doses of less than 1.0 mg/kg were found to exert their most pronounced and consistent effect on the interspindle firing rate of rostral reticularis neurons, reducing the frequency in all but one cell, where it was enhanced. The discharge rate during the spindle, in contrast, could be as high, higher or lower than was the case in the unanesthetized preparation. As a consequence, the overall firing rate, averaged over spindle and interspindle periods, remained either the same, increased or decreased depending on the extent to which the reduction in interspindle firing rate was compensated for by the increasing occurrence of EEG spindles and the associated enhancement of the neuronal discharge rate in the rostral sector of nucleus reticularis.

The degree of the firing increase during spindles as compared with interspindle intervals was obviously potentiated by Brevital in those neurons whose spindle-related discharge rates either increased or remained at preanesthesia levels, due to the drop in their interspindle firing frequency. However, the same tendency toward an enhancement of the ratio between intra- and interspindle firing was also observed in those neurons whose intraspindle discharge rate was decreased during light anesthesia, since this reduction tended to be less pronounced than that occurring in the interspindle intervals.

Increasing the dosage of Brevital in small increments to levels above 1.5 mg/kg caused a gradual decrease in the intraspindle discharge rate, as well as a much more pronounced reduction in interspindle firing to less than 1/sec. In addition, with these dosages the pattern of the intraspindle unit activity was changed in all cells from tonic firing to phasic bursts at the frequency of the individual EEG waves, as shown in Figs. 2C, 4, and 5C. In the absence of anesthesia, the unit illustrated in Fig. 2 showed an increase in firing rate during the spindle which was

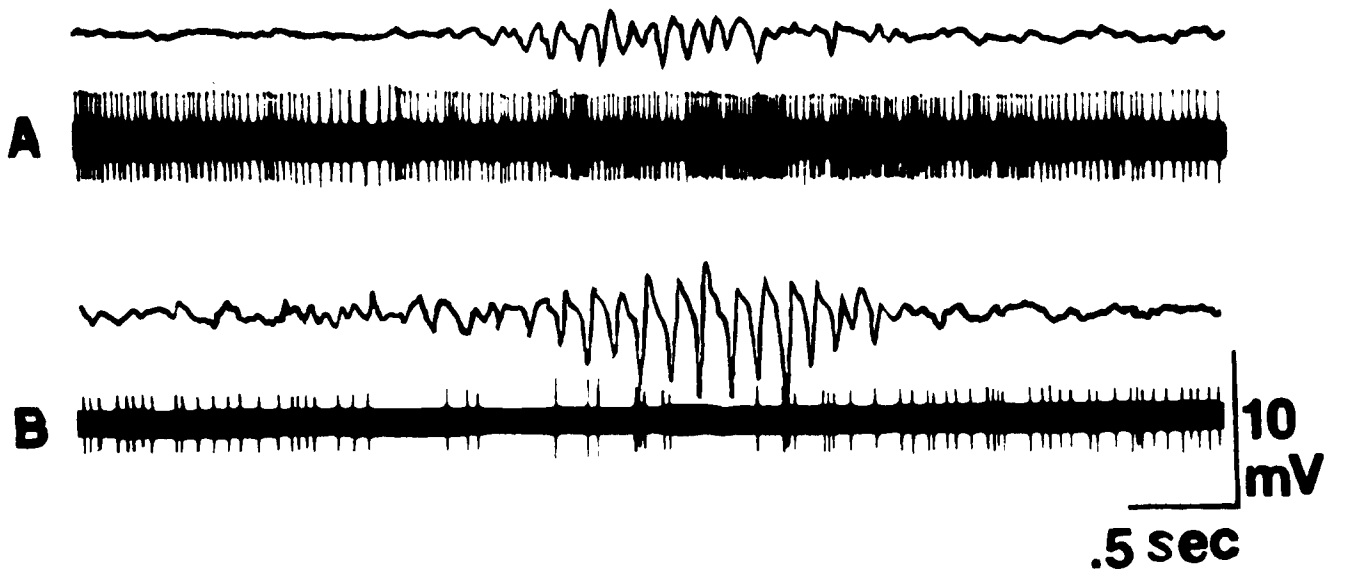

FIG. 3. Decrease in discharge rate of reticularis unit during EEG spindle after Brevital. (A) Unanesthetized animal; spontaneous spindle accompanied by increased firing (36 to 54/sec). (B) Following injection of 2.0 mg/kg Brevital; discharge rate is decreased.

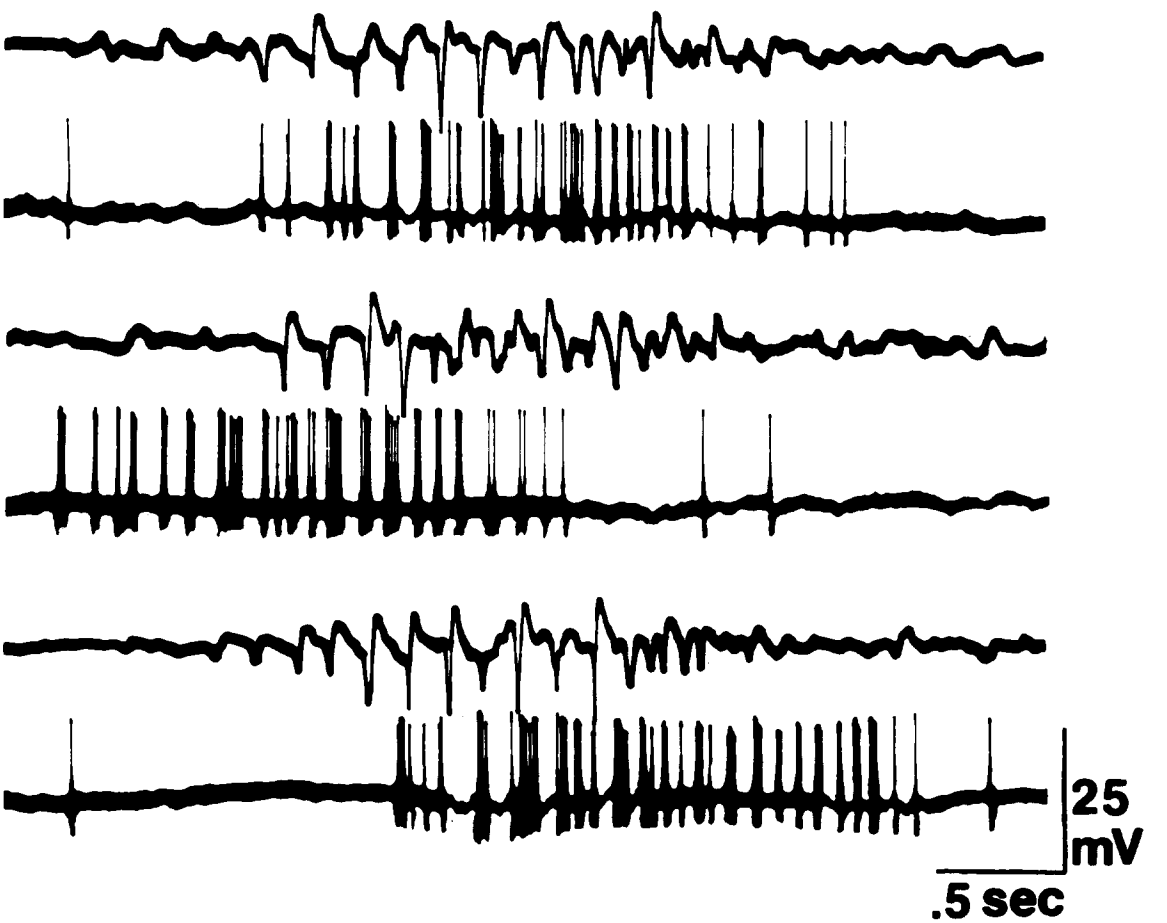

FIG. 4. Varying relationships between onset of EEG spindles and burst responses of rostral reticularis neuron after Brevital. Three spindles recorded within 30 sec; cellular bursts beginning and ending together with (top), before (middle), and after (bottom) the cortical spindle. Following 3.3 mg/kg Brevital injected over a period of 25 min. Interspindle firing frequency below 1.0/sec.

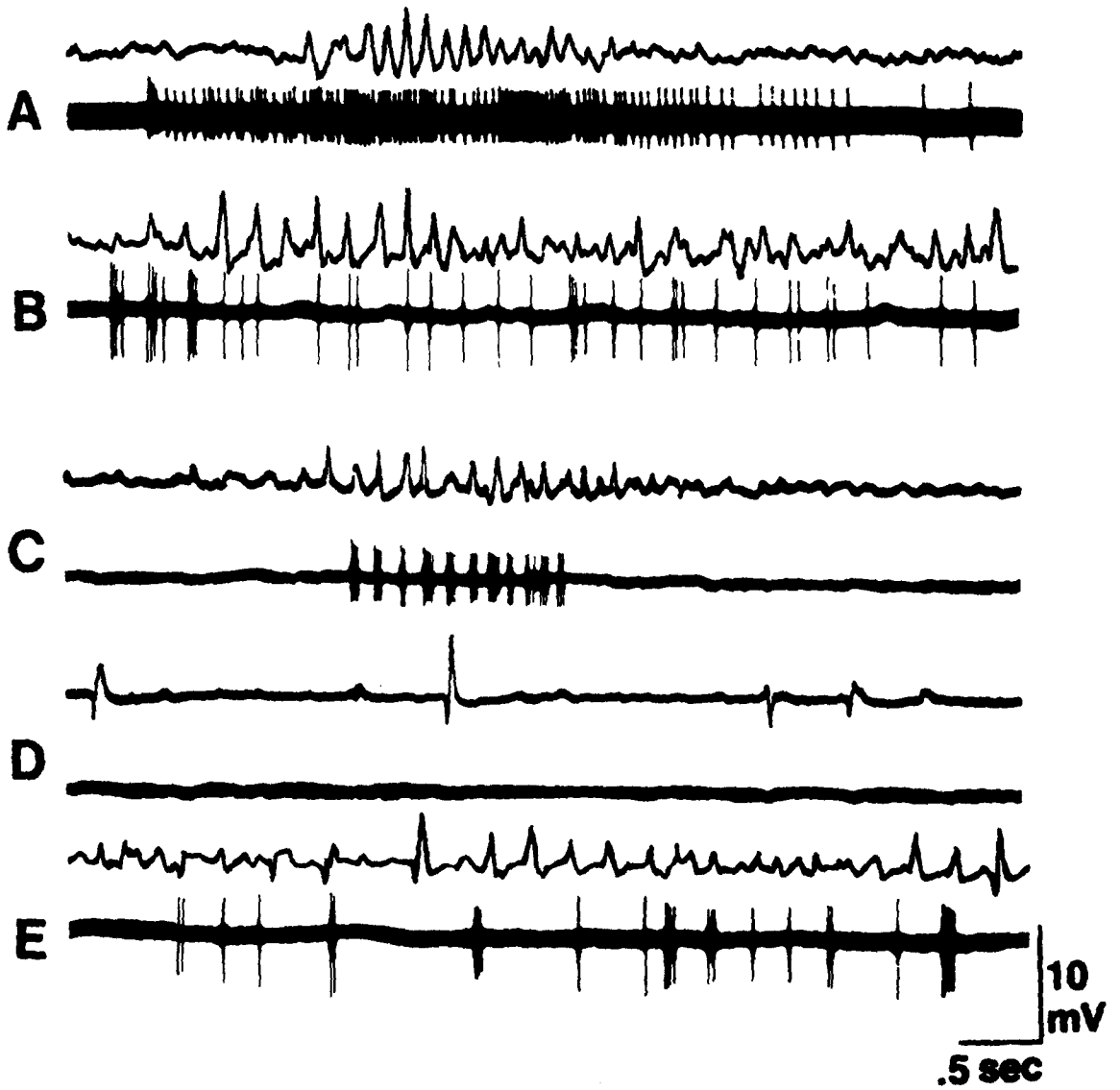

FIG. 5. Effect of large doses of Brevital on firing pattern of reticularis neurons. (A) Unanesthetized animal; EEG spindles accompanied by prolonged firing increase. (B) Thirty-five min later, following administration of 5 mg/kg of Brevital; EEG continuously synchronized, unit firing irregularly. (C) Different cell, same animal, 225 min after B, following injection of 1.4 mg/kg of Brevital; cell silent except for phasic bursts occurring during EEG spindles. (D) Sixteen min later; stepwise increase in dosage to 7.7 mg/kg resulted in spikes in otherwise flat EEG and total cellular silence. (E) Forty-five min after D; EEG continuously synchronous and unit firing irregularly again.

barely discernible upon visual analysis, but which nevertheless amounted to a 50% enhancement from an interspindle level of 36/sec to a rate of 57/sec in conjunction with the spindle depicted in Fig. 2A. Following 0.7 mg/kg of Brevital both intra- and interspindle firing was strongly reduced (Fig. 2B), and the addition of 1.0 mg/kg led to complete cellular silence except for the spindle-related phasic bursts which occurred at an average discharge rate of 12/sec (Fig. 2C).

Additional effects of moderate Brevital anesthesia (1.5 to 3.5 mg/kg) are shown in Figs. 3 and 4. Figure 3 illustrates that at this dose level a decrease in firing during the spindle as compared with interspindle intervals was occasionally observed, a finding which was never obtained in unanesthetized preparations [21]. In the absence of anesthesia this unit increased its firing rate from 36/sec between spindles to 54/sec concomitantly with the spindle depicted in Fig. 3A; the administration of 2.0 mg/kg of Brevital led to a decrease of the interspindle discharge rate and to a drastic reduction in firing during the appearance of the cortical spindle (Fig. 3B). Another result frequently observed at these dose levels is depicted in Fig. 4, which reveals that the relation between the onset and termination of the spindle and of the reticularis firing bursts became variable within the same neuron. After 3.3 mg/kg of Brevital given earlier the unit was firing at a frequency of less than 1/sec between spindles, while the beginning of EEG synchrony was either accompanied, preceded or followed by phasically occurring discharge bursts at average rates of 36/sec.

Dosages of Brevital in excess of 5 mg/kg caused a background EEG of almost continuous synchrony, with spindle trains observed only rarely, as shown in Fig. 5. In the absence of anesthesia, the unit illustrated in Fig. 5A, B showed a strong tonic enhancement in firing rate beginning prior to the period of cortical EEG synchrony, and sustained throughout its duration (Fig. 5A). Following 5 mg/kg of the drug (Fig. 5B), the EEG was characterized by the continuous presence of slow waves, while the activity of the reticularis units was similarly changed to continuous irregular firing, similar to that seen during EEG desynchronization in unanesthetized animals, except that the spikes frequently occurred in clusters of 2–4 and that the firing rate was strongly reduced. No clear relationship between individual EEG slow waves and reticularis activity could be discerned by visual analysis.

Further enhancement of the anesthesia to levels where the cortical EEG became isoelectric except for occasional spikes resulted in complete cessation of firing as illustrated in Fig. 5D. Following 1.4 mg/kg the cell depicted in Fig. 5C-E was completely silent except for phasic bursts occurring in conjunction with the spindles (Fig. 5C). A stepwise increase in dosage to 7.7 mg/kg resulted in the total absence of spontaneous discharges (5D), although activity could still be evoked by low-frequency repetitive intralaminar stimulation. Fig. 5E was recorded 45 min after 5D and shows the beginning of recovery both in the EEG and in the activity of the reticularis neuron.

DISCUSSION

The Scheibels have proposed the hypothesis that neurons in n. reticularis thalami exert an inhibitory influence on other thalamic cells [13]. This view was based on anatomical findings that the nucleus – which is a thin shell of neurons interposed between the internal capsule and the external medullary lamina – has its dendritic fields traversed by most of the thalamocortical and corticothalamic projections, with synaptic contacts frequently being made, and that the vast majority of reticularis axons project widely throughout different thalamic nuclei [11,12]; as a consequence of this arrangement the cells in this nucleus are strategically located to monitor all ongoing thalamocortical activity and to influence the discharge pattern of widely dispersed thalamic neurons. Subsequent electrophysiological [6, 8, 15, 16, 20] and behavioral [3] studies of this nucleus have been consistent with the Scheibels' hypothesis. In addition, the inference has been drawn that in unanesthetized preparations the postulated inhibitory influence is most likely of a tonic nature [8, 16, 21].

In encephàle isolé cats cortical EEG spindles are accompanied by a sustained increase in neuronal activity in the rostral pole, but not the ventral part, of nucleus reticularis [21], in contradistinction to the tonic hyperpolarizing shift observed at this time in VL [19] and some ventrobasal [10] units. It was noted in the introduction that in contrast to these prolonged hyperpolarizing drifts, EEG spindles in barbiturate anesthetized animals are paralleled by phasically recurring IPSPs – separated by strong depolarizing waves frequently giving rise to burst discharges – in the ventrobasal complex and intralaminar nuclei [1]. Consequently, if rostral reticularis neurons are indeed responsible for the tonic inhibition observed in dorsal thalamic units during spindles in unanesthetized preparations, the hypothesis would require that during barbiturate anesthesia the reticularis neurons still enhance their firing rate during spindles, but that they do so in phasic bursts, separated by cellular silence, rather than tonically.

The present results demonstrate that in animals under light to moderate Brevital anesthesia cortical EEG spindles were also accompanied by increased rostral reticularis firing; indeed, as a consequence of the pronounced drop in interspindle discharge rate following the administration of the drug, the degree of the increase in firing during spindles in relation with interspindle intervals was enhanced in comparison with unanesthetized preparations. In addition, the tonically sustained firing increase typically observed in unanesthetized animals [21] was replaced by phasic burst discharges occurring at the frequency of individual EEG spindle waves. The responses of the rostral reticularis neurons to Brevital anesthesia were thus exactly as would be expected if they were inhibitory interneurons which are responsible, at least in part, for the different patterns of hyperpolarizing potentials seen during spindles in dorsal thalamic neurons in unanesthetized animals as well as in preparations under light barbiturate anesthesia.

A primary role for reticularis neurons in the thalamic events leading to the continuous slow-wave synchronization in the cortical EEG during deeper stages of anesthesia is made unlikely by the results obtained in this report. No relationship between the neuronal firing pattern in nucleus reticularis and the EEG could be detected, and in addition the average firing rate was much reduced in these instances from that recorded during EEG desynchronization in unanesthetized preparations. A progressive decrease in discharge rates following increasing doses of barbiturates has also been observed for neurons in other thalamic nuclei [18], indicating that cells in the rostral pole of nucleus reticularis and in the dorsal thalamus may be subjected to a common disfacilitatory or inhibitory influence during deeper stages of barbiturate anesthesia, possibly originating from structures situated caudally to the diencephalon. The complete cellular silence observed in the rostral sector of nucleus reticularis concomitantly with an isoelectric EEG, finally, is paralleled by an absence of spontaneous firing under these conditions in other thalamic nuclei [14], the mesencephalon [14] and the cortex [5].

The findings obtained in this report indicate that the administration of small amounts of Brevital already exerts a pronounced effect on the firing pattern of rostral reticularis neurons during spindles, changing it from a tonic increase in discharge rate to phasically recurring spike bursts. Similar differences in the configuration of hyperpolarizing membrane potentials during spindles in dorsal thalamic cells in unanesthetized preparations [10,19] and in cats under light barbiturate anesthesia [1] seem to indicate that although the EEG spindles appearing on the cortex in these two physiological states look similar, they are accompanied by neuronal processes in the diencephalon which are in fact unequivalent. In this context it must be recalled that when Scheibel and Scheibel [13] proposed the hypothesis that neurons in nucleus reticularis thalami inhibit other thalamic cells they noted that they might do so "either tonically or phasically." The results of this and a previous study [21] can be interpreted as indicating that in unanesthetized animals rostral reticularis neurons inhibit cells in the dorsal thalamus tonically, concomitantly with the development of EEG spindles, while under light to moderate barbiturate anesthesia the postulated inhibitory influence is brought about in phasically recurring rhythmic waves.

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