J. Physiol. (1986), **379**, *pp.* 429–449
With 11 *text-figures*
Printed in Great Britain

THALAMIC BURST PATTERNS IN THE NATURALLY SLEEPING CAT: A COMPARISON BETWEEN CORTICALLY PROJECTING AND RETICULARIS NEURONES

BY L. DOMICH, G. OAKSON AND M. STERIADE

From the Laboratoire de Neurophysiologie, Département de Physiologie, Faculté de Médecine, Université Laval, Québec G1K 7P4, Canada

(*Received 20 November 1985*)

SUMMARY

1. Unit discharges were extracellularly recorded from antidromically identified thalamocortical neurones of ventralis lateralis (v.l.) and centralis lateralis (c.l.) nuclei as well as from reticularis thalami (re.) neurones during wakefulness and electroencephalogram-synchronized sleep of the behaving cat. Various parameters of sleep-related discharge bursts were analysed.

2. Statistical analyses revealed striking similarities between motor relay (v.l.) and intralaminar (c.l.) neurones. More than 60 % of bursts consist of three to five spikes at 250–400 Hz. The defining feature of bursts in all cortically projecting neurones is a progressive increase in the duration of successive interspike intervals.

3. As in thalamocortical cells, all re. neurones change their tonic discharges in waking to bursting firing in sleep, regardless of the increased or decreased firing rates from wake to sleep in individual neurones. The bursts of re. neurones are essentially different from those of thalamocortical cells. In re. neurones, burst structure consists of an initial progressive decrease in duration of interspike intervals, followed by an increase in duration of successive intervals, eventually leading to a long-lasting tonic spike train at about 100 Hz. In contrast with bursts of thalamocortical neurones, only 6 % of re. bursts are shorter than 50 ms; the total duration of the burst extends between 50 ms and 1·5 s. Population periburst histograms show the beginning of a decline in firing probability about 1·5 s prior to burst onset and an increased firing probability persisting for 300–350 ms after burst onset.

4. The different electrophysiological properties underlying the burst structure of cat's thalamocortical and re. neurones are discussed, with emphasis on dissimilar aspects of re. bursts in unanaesthetized and barbituratized preparations. Various factors that may account for the transition from tonic mode in waking to bursting mode in sleep are envisaged.

INTRODUCTION

During electroencephalogram (e.e.g.)-synchronized sleep thalamic neurones fire spike bursts interspersed with long periods of silence in close time relation with sequences of spindle waves (7–14 Hz), as opposed to their single-spike sustained

activity during e.e.g.-desynchronized behavioural states. The high-frequency burst is characteristic of thalamic neurones during sleep: it appears in the lateral geniculate (l.g.) nucleus in spite of unaltered discharge patterns in the afferent optic fibres (Hubel, 1960); it is unchanged in ventrolateral (v.l.) neurones following lesions of their major afferent sources, the deep cerebellar nuclei (Steriade, Apostol & Oakson, 1971); and it disappears by advancing the micro-electrode beyond thalamic limits into other diencephalic structures (Glenn & Steriade, 1982). The bursts recorded from relay thalamic nuclei are defined by their short duration and high frequency of spikes.

In this paper we analyse quantitatively the burst parameters in antidromically identified thalamocortical neurones recorded from two nuclear groups (relay and intralaminar) and compare the burst patterns of cortically projecting neurones with those of reticularis thalami (re.) neurones. We first examined whether thalamocortical neurones of the intralaminar centralis lateralis (c.l.) nucleus, known to have widespread cortical projections and a peculiar propensity to spindle oscillations (Morison & Dempsey, 1942), exhibit a burst structure similar to that of thalamic relay neurones of specific nuclei. More importantly, we were concerned with the basic features that distinguish the bursts of re. cells from those of thalamocortical neurones. Immunohistochemical and electron-microscopic evidence suggest that the former elements exert inhibitory influences upon the latter: re. neurones are GABAergic (Houser, Vaughn, Barber & Roberts, 1980) and their axons terminate in the dorsal thalamus with flattened vesicles at symmetrical synaptic profiles (Ohara, Sefton & Lieberman, 1980; Montero & Scott, 1981). Negishi, Lu & Verzeano (1962) were first to mention that the duration of bursts of neurones recorded from the caudal, peri-l.g. part of re. nucleus is longer than that of l.g. relay cells. Recently, prolonged spike barrages of re. neurones were described during drowsiness and sleep as extending over a whole sequence of spindle waves, lasting for 1·5–2 s (Steriade, Domich & Oakson, 1986). It is known that, simultaneously, thalamocortical neurones exhibit long-lasting hyperpolarizations interrupted occasionally by brief bursts (see reviews by Andersen & Andersson, 1968; and Steriade & Deschênes, 1984). It seemed worthwhile to make a systematic evaluation of burst structure in re. neurones, as compared to bursts of thalamocortical neurones in v.l. and c.l. nuclei that are major targets of re. nucleus (Steriade, Parent & Hada, 1984). We have chosen the extracellular approach in the behaving animal. In such conditions the complicating factor of anaesthetics is avoided and, in addition, one can determine whether the burst patterns of re. cells are dependent upon the behavioural state of sleep with e.e.g. synchronization.

METHODS

Preparation, stimulation and recording

The data were obtained from chronic experiments on twenty-two adult cats of either sex. Surgery was performed under deep anaesthesia with sodium pentobarbitone (35–40 mg/kg). Recording leads consisted of cortical surface electrodes for e.e.g. rhythms, electrodes for neck electromyogram (e.m.g.), and silver-ball electrodes for ocular movements. Two arrays of bipolar stimulating electrodes (each consisting of four to six wires with tips bared 0·1–0·3 mm, about 1–1·5 mm apart) were inserted in deep layers or white matter underlying pre- or post-cruciate and anterior suprasylvian gyri. These electrodes served for antidromic identification of thalamocortical neurones

recorded from c.l. or v.l. nuclei, and for orthodromic activation of re. neurones. The calvarium overlying the thalamic target area (A8–13·5, L1–5) was removed and replaced with a plate. Four fixation cylinders were anchored to the bone with screws and dental cement; during recording sessions, bars were inserted into the cylinders to permit the head to be restrained rigidly in a stereotaxic position without pain. Recordings began a week after chronic implantation. The animals were not deprived of sleep between recording sessions.

Single neurones were extracellularly recorded by means of tungsten micro-electrodes (2–3 μm, 1–5 MΩ at 1 kHz) in c.l. and v.l. nuclei, and in the rostral pole, lateral and ventral parts of re. nucleus. Presumed fibres (exclusively positive discharges with durations shorter than 0·8 ms) were discarded. Cortical stimulation (0·05–0·2 ms pulses, 0·05–0·2 mA) was applied below the threshold for movements and did not disturb the normal wake–sleep cycle. Criteria for antidromic identification of thalamocortical neurones were fixed latency, collision with spontaneously occurring action potentials, and ability to follow high frequencies (> 250/s). The conduction velocities of thalamocortical neurones, as inferred from their antidromic response latencies, were in the range of 7–11 m/s for c.l. neurones and 10–16 m/s for v.l. neurones, as reported previously (see Steriade & Glenn, 1982; Steriade *et al.* 1986). The re. neurones were excited monosynaptically from pericruciate areas (Steriade & Wyzinski, 1972).

Unit discharges and focal slow waves were recorded simultaneously by the micro-electrode on direct (50–10000 Hz) and FM (1–700 Hz) channels of a tape recorder, along with physiological variables indicating the state of vigilance. Small lesions (10–20 μA, 20–30 s) were made at one or two sites along the micro-electrode tracks. At the end of the experiment, the animals were deeply anaesthetized with sodium pentobarbitone and perfused intracardially with formaldehyde. The stimulating electrodes and micro-electrode tracks were examined in frontal frozen sections (50 or 80 μm) stained with cresyl violet or thionine. The location of recorded neurones in c.l., v.l. and re. thalamic nuclei was possible by combining lesion sites along tracks with micrometer readings.

Data analysis

Electrographic criteria of behavioural states of vigilance analysed in this study were as follows. Quiet waking (w.) was accompanied by continuous e.e.g. desynchronization, that is, low-voltage and fast e.e.g. rhythms; it did not include periods (> 2 s) with overt movements since motor events may introduce uncontrolled factors in discharge patterns due to the proprioceptive drive. The state of sleep with e.e.g. synchronization (s.), characterized by high-amplitude spindles (7–14 Hz) and slow waves (0·5–4 Hz), started after the transitional period of drowsiness, when e.e.g. synchronization was no longer interrupted by desynchronization periods.

Stable episodes in s. that showed bursting activity were selected from polygraphic recordings of unit discharges, focal thalamic waves and surface-cortical e.e.g. waves, filtered to show amplitudes of slow waves and spindle waves. Graphic computer plots of unit firing rate, focal thalamic and cortical wave amplitudes were also used for epoch selection (see Fig. 2*C* in Steriade *et al.* 1986). Representative epochs in w. were selected for the same neurones. For all selected epochs interspike intervals were measured to a precision of 0·1 ms by a laboratory microcomputer and transferred to the central campus computer.

The criteria for automatic computer selection of bursts from the stored interval data bank were determined by trial and error testing, and by using several burst criteria on sample epochs from several cells. Computer burst listings of start times and interval lengths were compared with bursts determined visually from polygraphic records for the same epochs. The criteria which permitted detection of the largest number of visually identifiable bursts in the test epochs were retained.

Burst criteria for thalamocortical neurones in relay (v.l.) and intralaminar (c.l.) nuclei were: the burst began at least 50 ms after a preceding spike, the first interval in the burst was not greater than 5 ms, and the burst ended on the interval preceding a post-burst interval greater than 40 ms. The values taken for preburst and post-burst intervals are justified by intracellular studies indicating that the bursts of thalamic relay cells follow long-lasting hyperpolarizations, lasting usually for 70–150 ms (Andersen & Andersson, 1968), and that the absolute refractory period of a post-inhibitory rebound is around 40 ms, with a relative refractory period of up to 170–200 ms (Deschênes, Paradis, Roy & Steriade, 1984; Jahnsen & Llinás, 1984*a*). Structural characteristics of c.l. and v.l. bursts in s. were examined by classifying bursts from each neuronal group according to size (number of intervals) and computing the median interval length for each serial position in the burst for all bursts of the same size (see Fig. 1).

For re. neurones, the criteria for burst selection were: the preburst and post-burst intervals were greater than 100 ms and the sum of the first five intervals in the burst did not exceed 100 ms. During s., prolonged spike barrages of re. neurones are separated by exceedingly long periods of neuronal silence that explain the criteria of relatively long preburst and post-burst intervals. Other arguments for having chosen burst criteria for re. neurones different from those of thalamocortical cells are indicated in Results (see also Figs. 4–5). The bursts of re. cells were classified into two groups: those having at least twenty and not more than thirty intervals, and those having at least fifty intervals.

To verify any possible correlation between various burst parameters in our re. neuronal sample, the Spearman rank correlation coefficient was computed for each cell between preburst interval and the remaining burst parameters, as well as between burst duration and remaining parameters. Although some individual neurones exhibited significant correlations for some parameters, we decided to test for significance of the sign of the correlation coefficients over all cells. Since in the random case positively and negatively signed correlations are equally probable, the binomial test showed that at least seventeen of twenty-five like-signed coefficients were required to indicate a correlation in our sample of twenty-three re. cells ($P < 0{\cdot}04$, two-tailed test).

Periburst histograms were computed for re. bursts in s. to ascertain the expectation of spikes at times relative to the first spike in a burst (time zero, t_0). The beginning of each burst in the epoch was aligned with t_0 and spikes were counted in bins before and after this time. The result for one epoch was normalized as a percentage relative to the firing rate for the epoch and the periburst histogram for all epochs was determined by bin-by-bin averaging.

To test the relative frequency of re.-cell bursts in s. and w., the number of bursts per minute was computed for each cell in each state and the Wilcoxon paired-rank test was used for significance testing. Interspike interval histograms (i.s.i.h.s) were computed for each of twenty-three re. neurones in both s. and w. The i.s.i.h.s for each state were divided into two classes: cells that decreased and cells that increased their firing rates in s. when compared to w. A grouped i.s.i.h. for each class was computed by combining, with equal weighting, the i.s.i.h.s from a given class.

RESULTS

Thalamocortical neurones

It is known from previous studies on relay (McCarley, Benoit & Barrionuevo, 1983) and intralaminar (Glenn & Steriade, 1982) nuclei that the bursts of thalamic neurones characteristically occur during s. state, when they are associated with decreased firing rates, as compared to both e.e.g.-desynchronized states of w. and rapid-eye-movement (r.e.m.) sleep.

We analysed 2057 bursts from 16 c.l. neurones and 1255 bursts from 11 v.l. neurones, taken from s. epochs. All these neurones were thalamocortical, as identified by antidromic invasion. The top row in Fig. 1 depicts the activity of an intralaminar thalamocortical c.l. neurone, with single-spike discharges in w. and burst discharges in s. The higher incidence of bursts in s. was statistically significant for both c.l. and v.l. cortically projecting neurones ($P < 0{\cdot}001$, Wilcoxon paired-rank test).

Striking similarities were observed between the two cell populations recorded from an intralaminar and a relay (motor) nucleus. In both cases, the bursts of thalamocortical neurones consist of a few spikes at 250–400 Hz. Fig. 1 shows that about 63–65 % of bursts have three to five spikes (two to four intervals); 15 % of bursts have only two spikes and 13–14 % have six or seven spikes. The remaining (6–10 %) bursts have eight to ten spikes (see panel *D* in Figs. 2 and 3). There is a clear tendency to a progressive increase in the duration of successive interspike intervals within a burst; and the longer the burst duration, the shorter the first interval (Fig. 1). Similar aspects were described in unidentified neurones recorded from a specific sensory, l.g., nucleus (McCarley *et al.* 1983).

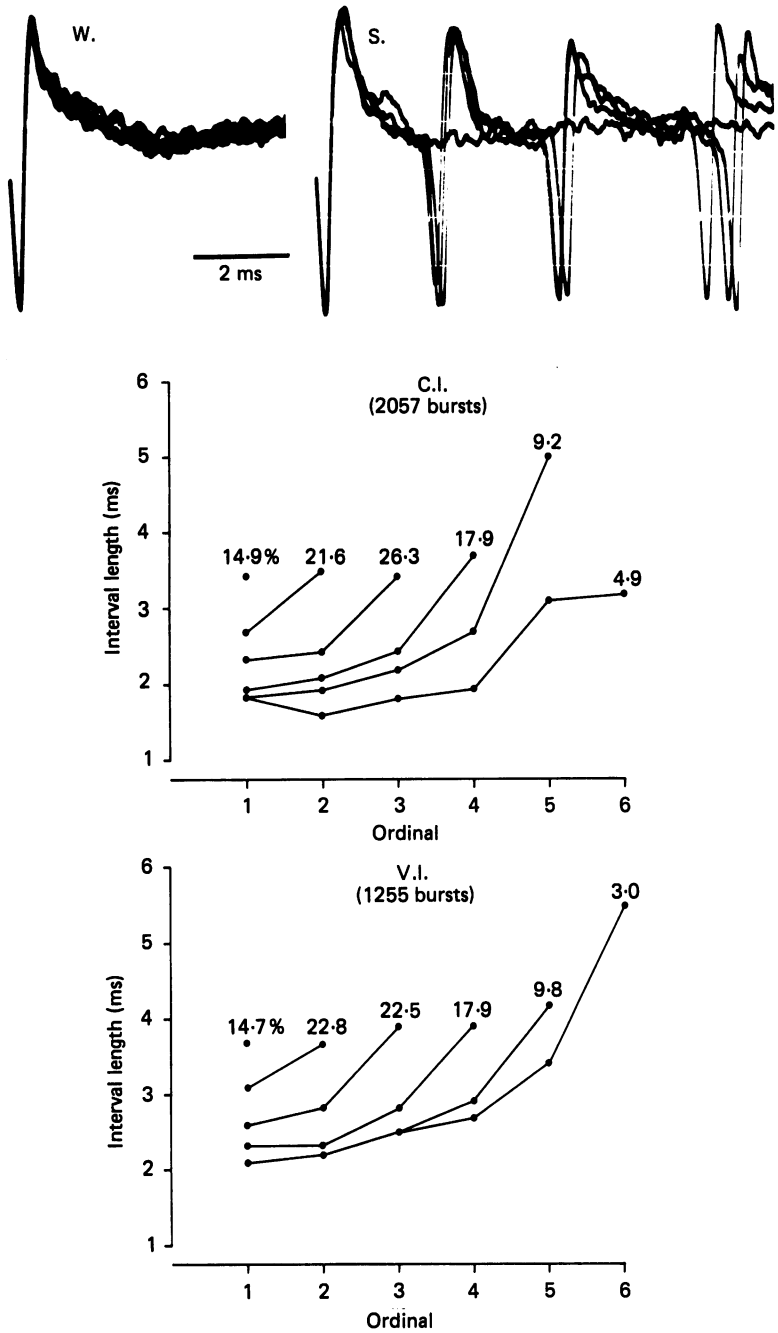

Fig. 1. Burst features of thalamocortical neurones. Single-spike discharges during w. and high-frequency bursts during s. are depicted in the top row for a thalamocortical c.l. neurone; sweeps triggered by spikes. In this and other similar Figures, positivity downward. Below, the interval duration (ordinate, ms) *versus* serial position (ordinal) in bursts of sixteen c.l. and eleven v.l. thalamocortical cells during s. Each curve depicts median interval length for each serial position for all cells (equal weighting) having number of intervals in burst as shown by highest ordinal in curve (single point denotes spike doublets). Percentage figures denote proportion of all bursts analysed having length indicated. In both cell groups curves show that longer bursts have an initial higher frequency than shorter bursts and all bursts show progressively lengthening intervals.

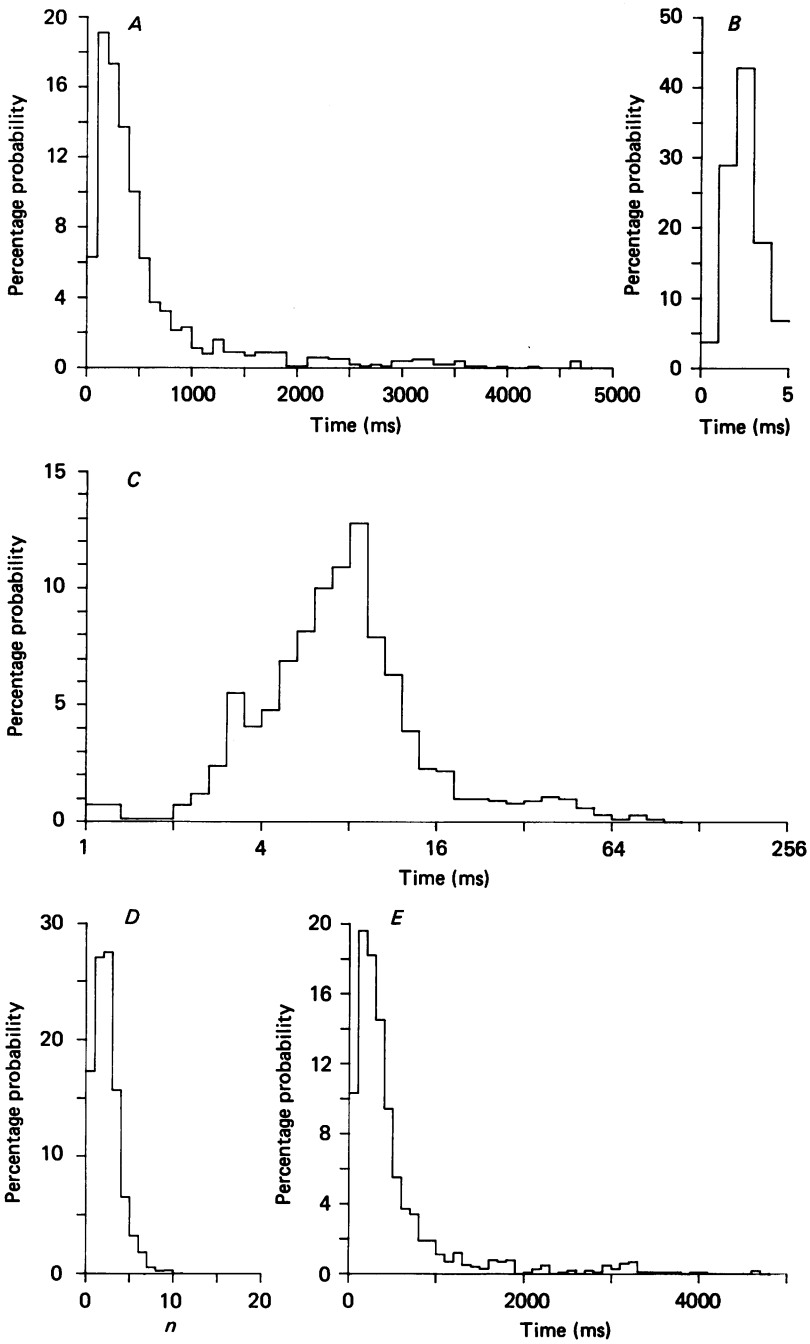

Fig. 2. Distribution of parameters in 2057 bursts of sixteen thalamocortical c.l. cells in s. sleep. Percentage probability on ordinate; in panels *A*, *B*, *C* and *E*, time (in ms) on abscissa. *A*, preburst interval; *B*, first burst interval; *C*, burst duration; *D*, number of intervals in burst; and *E*, post-burst interval.

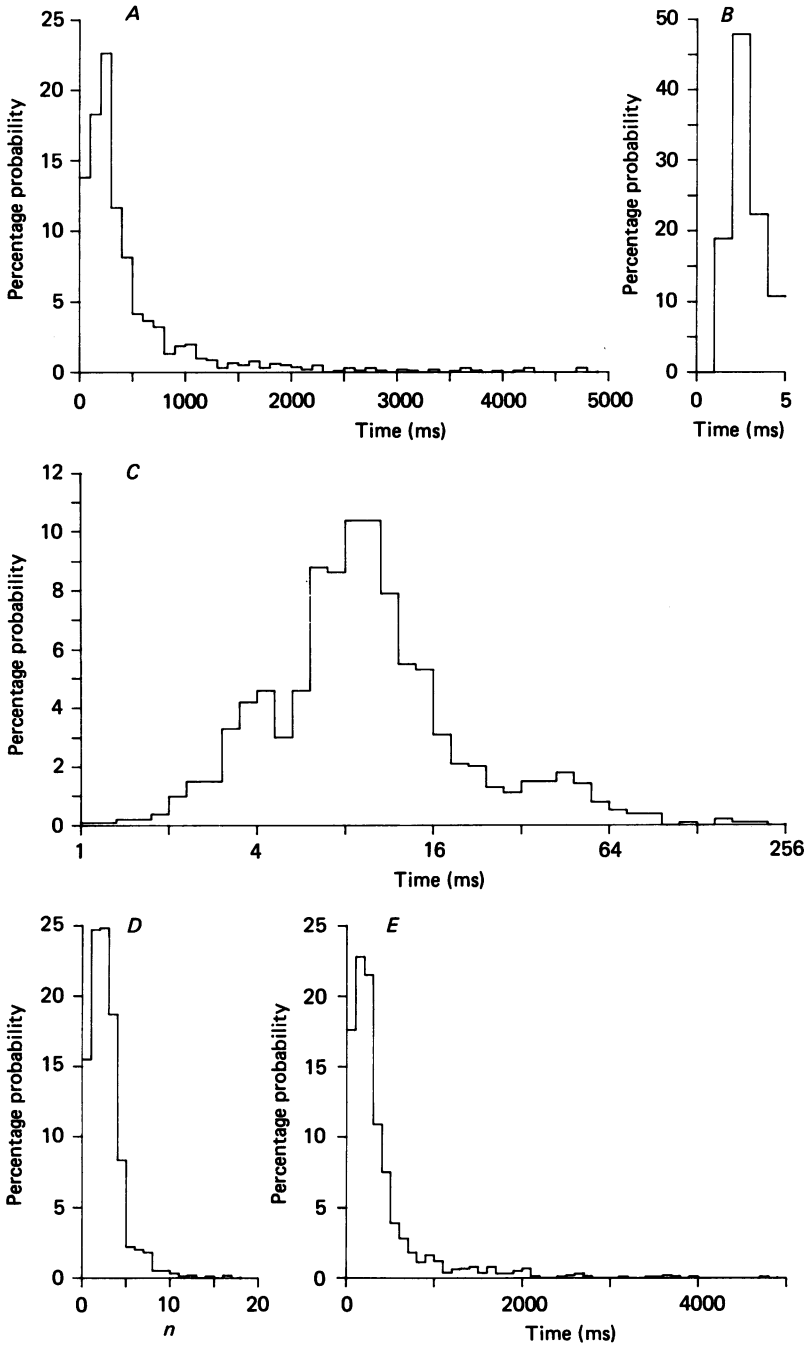

Fig. 3. Distribution of burst parameters in 1255 bursts of eleven thalamocortical v.l. cells in sleep. Percentage probability on ordinate; in panels *A*, *B*, *C* and *E*, time (in ms) on abscissa. *A*, preburst interval; *B*, first burst interval; *C*, burst duration; *D*, number of intervals in burst; and *E*, post-burst interval.

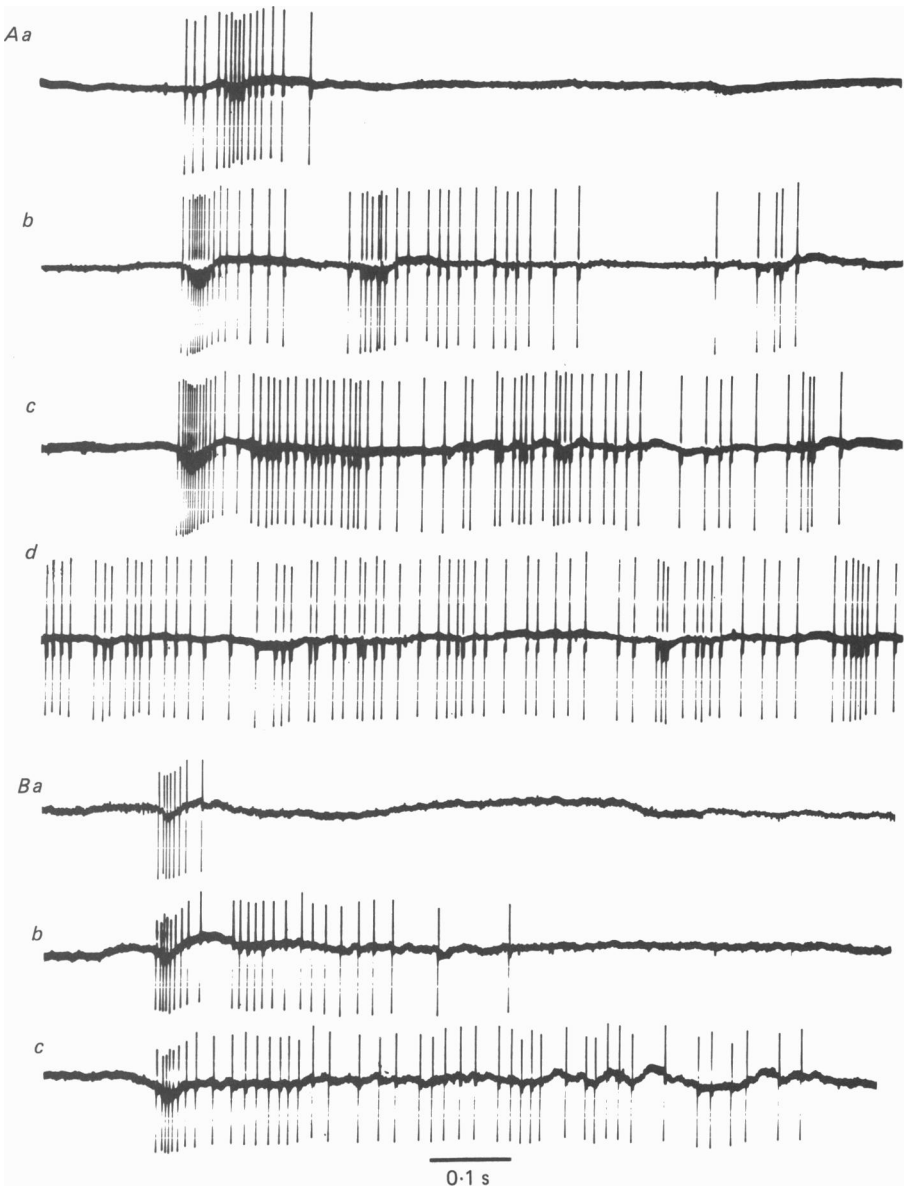

Fig. 4. Simple and complex burst patterns of re. neurones in sleep. *A* and *B*, two neurones recorded in the rostral pole of re. nucleus. In both cases, *a–c* depict successive bursts. Note the simple form of the high-frequency burst core (*a*) and complex bursts with a tonic tail (*b–c*). In *Ad*, tonic discharge pattern during w.

All burst parameters are almost identical in c.l. (Fig. 2) and v.l. (Fig. 3) neurones. More than 50 % of preburst and post-burst intervals (panels *A* and *E*) are between 200 and 400 ms, with peaks at 200–300 ms. (For the post-burst intervals, the above values fit in well with the refractory period of the spike burst, as revealed intracellularly (see Methods). Preburst intervals between 200 and 400 ms do not,

however, imply that this is the average duration of the preceding hyperpolarizations; as known, the duration of hyperpolarizations usually ranges between 70 and 150 ms. The explanation of this apparent discrepancy is that only one of two or three successive hyperpolarizations within a spindle sequence leads to fast repetitive action potentials that are detectable in extracellular recordings (Roy, Clercq, Steriade & Deschênes, 1984).) The duration of the first interval in the burst (panel *B*) peaks at 3 ms in more than 40 % of cases. The total burst duration (panel *C*) ranges between 6 and 16 ms in 50–60 % of cases.

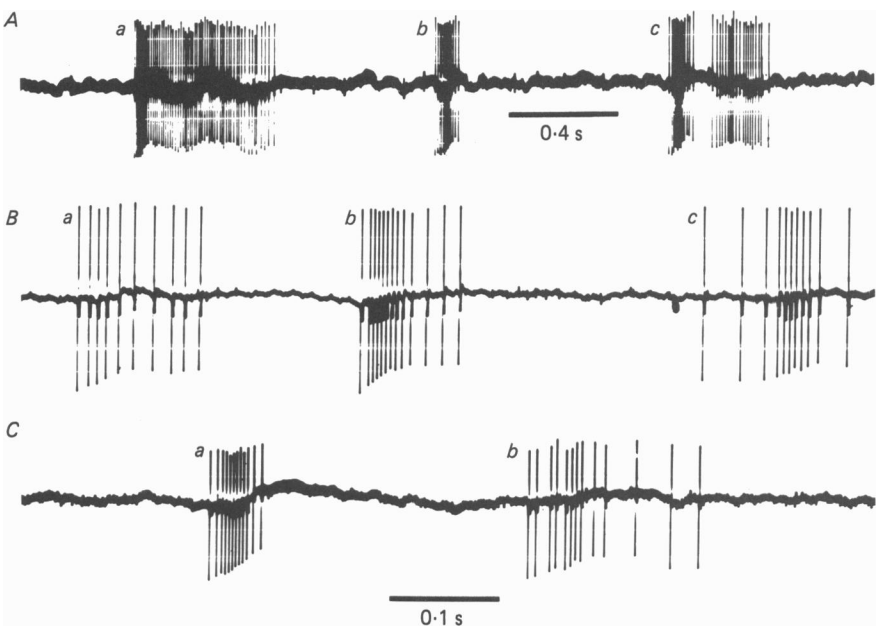

Fig. 5. Burst variability in re. neurones. Three different neurones recorded in the lateral part (*A* and *C*) and rostral pole (*B*) of re. nucleus. Time in *C* also valid for *B*. Note great differences in reaching the peak frequency in successive bursts (*b* and *c* in *B*; *a* and *b* in *C*).

Re. neurones

In the twenty-three-cell sample analysed during w. and s. states, the mean rate was 31·9/s during w. and 19·7/s during s. The higher discharge rate in w. is significant at $P < 0{\cdot}05$ (Wilcoxon paired-rank test).

During w., re. neurones are tonically active with single-spike discharges, similarly to thalamocortical neurones (see Fig. 4*Ad*). The tonic firing pattern appears during e.e.g.-desynchronized states of w. and r.e.m. sleep and was described in both the rostrolateral region (Steriade & Wyzinski, 1972) and caudal parts (Mukhametov, Rizzolatti & Tradardi, 1970; Barrionuevo, Benoit & Tempier, 1981) of re. nucleus.

During s. the spontaneous activity of re. neurones is characterized by prolonged spike barrages and long periods of silence. The re. bursts differ from the stereotyped bursts of thalamocortical neurones in their intrinsic structure, variability, and duration. The basic feature of re. bursts is their accelerando-ritardando structure, i.e.

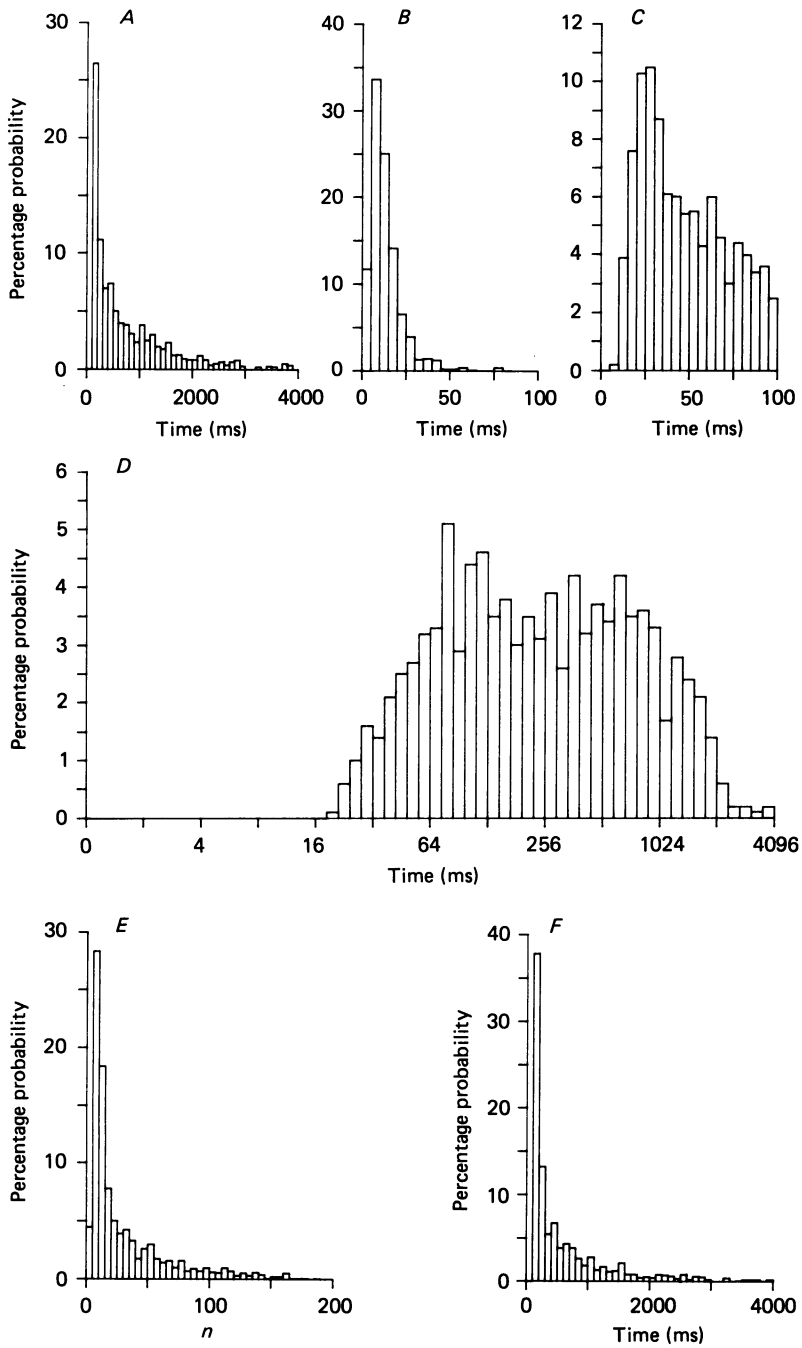

Fig. 6. Distribution of burst parameters in 1402 bursts of twenty-three re. cells in s. sleep. Percentage probability on ordinate; in panels *A*, *B*, *C*, *D* and *F*, time (in ms) on abscissa. *A*, preburst interval; *B*, first burst interval; *C*, duration of first five intervals in burst; *D*, burst duration; *E*, number of intervals; and *F*, post-burst interval.

an initial progressive decrease in duration of interspike intervals is followed by a progressively increasing duration of intervals. This is the core of the burst (Fig. 4 *Aa* and *Ba*) and it is often completed by a long-lasting tail of variable duration (Fig. 4 *Ab–c* and *Bb–c*). Both forms, the simple core and the more complex barrage, may occur in the same neurone as successive spike trains. This burst structure is characteristic for neurones recorded from all investigated zones of the re. nuclear complex: rostral pole, lateral parts adjacent to ventralis anterior (v.a.), v.l., lateralis posterior (l.p.) and ventrobasal (v.b.) nuclei, and ventral wing underlying v.b. nuclei. Fig. 4 shows that the core of the burst may appear in isolation (*Aa* and *Ba*) but may be followed in immediately successive bursts by a tonic barrage without a pause between the two components (*Ac* and *Bc*) or with a short pause (*Ab* and *Bb*).

Clearly, a long-lasting tonic spike train is a distinct component that ends many barrages of re. neurones without any pause after the initial burst. This final component would be lost in statistical analyses when a pause of > 40 ms separates it from the initial burst, if the criterion of the post-burst interval duration were similar to that chosen for thalamocortical neurones (40 ms). Moreover, such a criterion would be a misleading factor in the evaluation of burst structure in re. cells because the tonic tail, that is merely the end of a spike barrage, would be analysed among initial bursts.

The variability of re. bursts is also due to great differences in the time required to reach the peak frequency (200–300 Hz). In some bursts this frequency appears after five to six intervals, whereas in immediately subsequent ones the highest frequency may occur after only one to two intervals (Figs. 4 *Aa–b*, 5 *B–C*). These variable features explain the more encompassing criterion of 100 ms for the total duration of the first five intervals.

The values of various parameters in 1402 bursts recorded from twenty-three re. neurones during s. (Fig. 6) are as follows. About 50–60 % of preburst and post-burst intervals (panels *A* and *F*) are between 200 and 500 ms. The duration of the first interval (panel *B*) is between 5 and 15 ms in 58 % of bursts, with only 12 % having a first interval shorter than 5 ms. This stands in contrast with thalamocortical neurones in which 95–97 % of bursts have a first interval shorter than 5 ms. The total duration of the progressively shorter first five intervals peaks between 20 and 40 ms (panel *C*). The total duration of the burst (panel *D*) extends between 50 ms and 1·5 s. Only about 6 % of re. bursts are shorter than 50 ms; indeed, the initial component (what we termed core) is by itself longer than 50 ms (see again Figs. 4–5). There are only 3 % of re. bursts shorter than 30–35 ms, whereas virtually all bursts of thalamocortical neurones are below this value. About half of the bursts have 10–20 intervals, with the remaining half having up to 100–150 intervals (panel *E*).

The population periburst histograms confirmed the exceedingly long silent periods and long spike barrages of re. neurones during s. A decline in firing probability begins about 1·5 s prior to burst onset. The increased firing probability after burst onset persists for 300–350 ms at about the double of the average discharge rate. These values are similar in the periburst histogram from all twenty-three re. neurones (Fig. 7 *A*) and in the periburst histogram from nine re. neurones with discharge rates of at least 20/s during s. (Fig. 7 *B*).

Two statistically significant correlations were found between various burst para-

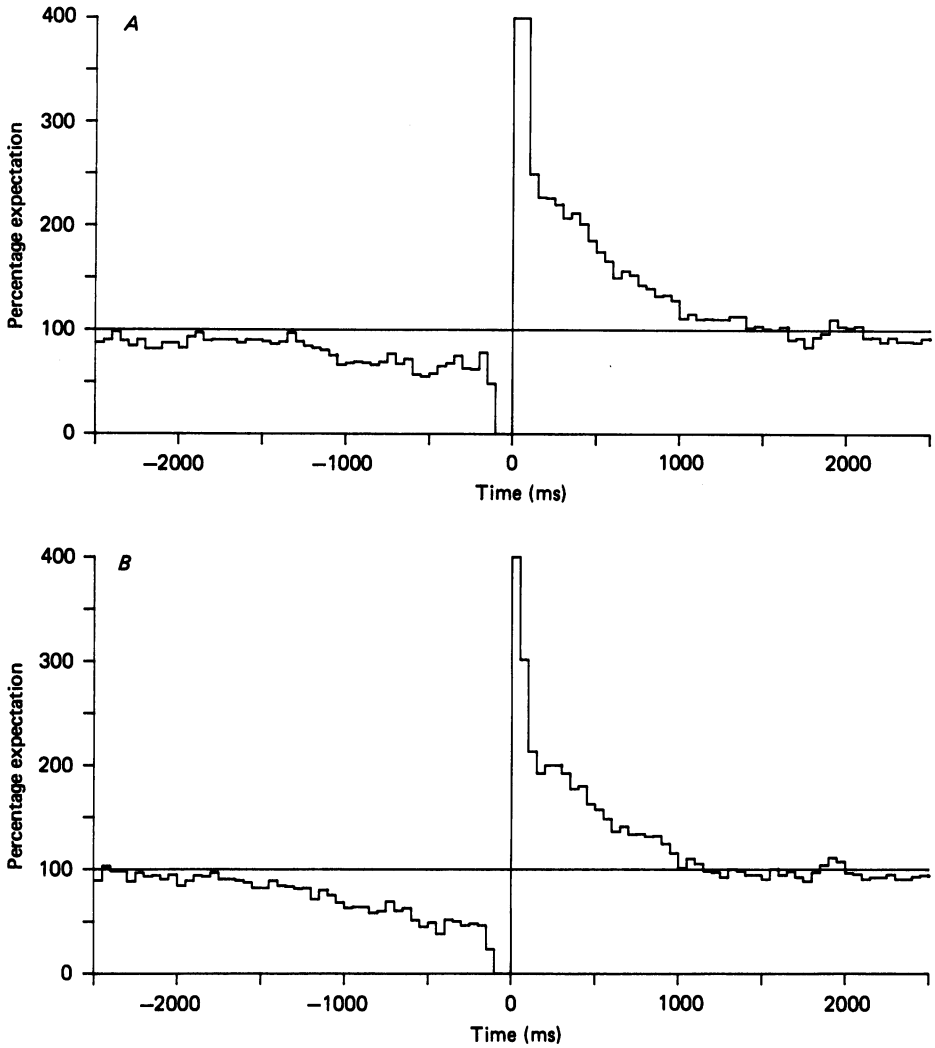

Fig. 7. Periburst histograms depicting percentage expectation of a spike firing relative to the first spike in a burst for re. cells in s. 100% denotes expectation for randomly distributed intervals of equivalent firing rate. Individual histograms were averaged bin-by-bin. *A*, plot for 1402 bursts from all twenty-three cells. *B*, plot for 522 bursts from nine cells having a firing rate in s. of at least 20/s.

meters over all cells (see Methods). The longer the preburst interval, the shorter the duration of the first five intervals in the burst. This inverse correlation was seen in twenty-two out of twenty-three re. neurones ($P < 0{\cdot}01$). And the duration of the burst was directly correlated with the number of intervals in all twenty-three neurones ($P < 0{\cdot}001$). (While this second correlation may seem a truism, neurones recorded from another (peri-l.g.) zone of re. nucleus were reported to display the same number of spikes within short-duration (median 90 ms) and long-duration (median 150 ms) bursts (Barrionuevo *et al.* (1981).)

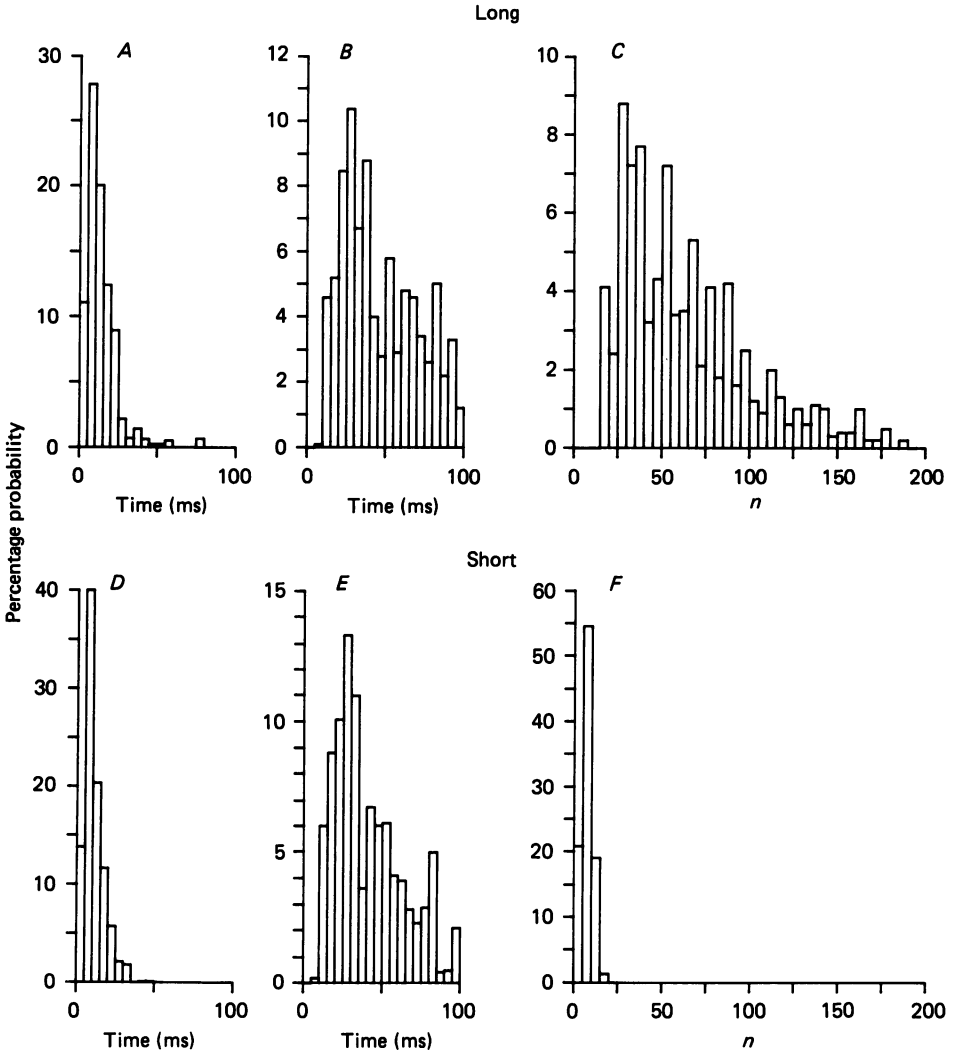

Fig. 8. Distribution of some burst parameters for long and short bursts of twenty-three re. neurones. Long bursts were defined as those lasting at least 500 ms while short bursts were those lasting 100 ms or less. *A* and *D*, first burst interval; *B* and *E*, duration of first five intervals; and *C* and *F*, number of intervals in burst.

The parameters of the high-frequency burst are similar whether or not this core of the burst is followed by a long-lasting tonic tail. This may be seen in Figs. 4*B* and 5*A* with original spikes and is further depicted in Fig. 8 with group analyses. The durations of the first interval and of the first five intervals are almost identically distributed in both long and short spike barrages. That the intrinsic structure of the core of the burst is very similar regardless of the presence or absence of the continuing tonic discharges is shown in Fig. 9. Progressive acceleration (i.e. decreasing duration of successive intervals) takes place within the burst until the fifth to sixth intervals.

It is followed by a few short intervals of 4–6 ms and, thereafter, by a progressive deceleration between the eighth to ninth and twelfth to fifteenth intervals. This biphasic pattern extends over 50–70 ms in both short and long spike barrages. The latter are prolonged by thirty to forty or more intervals, lasting around 300 ms. In most re. neurones this tail is tonic, but in six out of twenty-three re. neurones a rhythmic modulation at 7–10 Hz was observed within the long-lasting final component (Fig. 10).

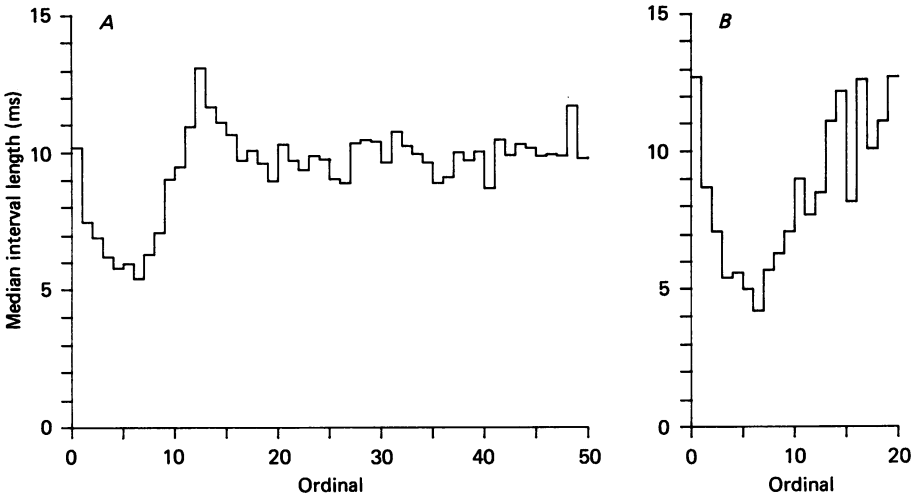

Fig. 9. Structure of bursts of re. neurones. Plots of median interval length (ordinate, in ms) *versus* serial or ordinal position of intervals in the burst (abscissa). Criteria for computer selection of bursts are: the pre- and post-burst intervals are greater than 100 ms and the sum of the first five intervals in the burst is not greater than 100 ms. In *A*, long bursts (10 cells, 189 bursts) defined as having at least 50 intervals. In *B*, short bursts (7 cells, 84 bursts) defined as having at least 20 and not more than 30 intervals. Note in both *A* and *B*, accelerando-ritardando feature at burst onset.

We examined the incidence of bursts during different behavioural states and the possibility that re.-cell bursting is specifically determined by a constellation of physiological events underlying s. sleep, independently of rate changes. All twenty-three re. neurones had a significantly higher incidence of bursts in s. compared to w. ($P < 0{\cdot}0001$, Wilcoxon paired-rank test). The increased burst occurrence in s. was not dependent on rate changes because all units of the twenty-three-cell sample had a higher incidence of bursts in s. whereas ten of them increased their median firing rates in s. (21·6/s) compared to w. (15·7/s) and the remaining thirteen neurones decreased discharge rates in s. (20·0/s) compared to w. (39·7/s). The median of burst occurrence in units increasing firing rates from w. to s. was 3·7/min in w. and 25·5/min in s.; for units decreasing firing rates from w. to s. it was 0 in w. and 23·3/min in s.

Since burst patterns are revealed in first-order analyses by a great proportion of short intervals, we computed grouped i.s.i.h.s in both cell samples, i.e. with

decreasing and increasing firing rates from s. to w. Fig. 11 shows that, regardless of rate changes, the probability of intervals shorter than 5 ms is eight to ten times greater in s. compared to w. Comparing the differences between the probabilities of intervals within the 0–5 ms bins and within the 0–10 ms bins during s. and w., we found that the greater probability of short intervals in s. is highly significant ($P < 0{\cdot}001$, Mann–Whitney U test) in both cell samples, with either increasing or decreasing firing rates from w. to s.

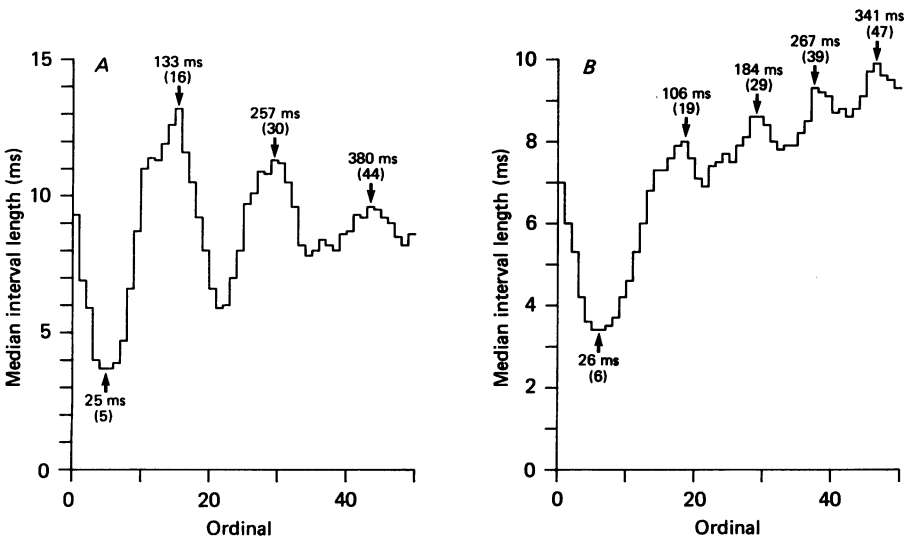

Fig. 10. Rhythmic (7–11 Hz) fluctuations during the long-lasting tail of re. bursts during s. sleep. Two neurones recorded in peri-v.b. part (*A*) and rostral pole (*B*) of re. nucleus. In *A* and *B*, respectively, seventeen bursts and twenty-six bursts were analysed. Curves depict median interval length (in ms, as shown on ordinate) *versus* serial position of intervals in bursts (ordinal on abscissa). The time elapsed from burst onset to a given interval position is shown (for instance, in *A*: 25 ms to the fifth interval, 133 ms to the sixteenth interval, etc.). Mean of differences between peaks is 135 ms in *A*, and 85 ms in *B*. These values indicate that rhythmic accelerations and decelerations within the long bursts are within the frequency range of spindles (7·4/s in *A*, 11·7/s in *B*). The rhythmic fluctuations depicted in these two neurones are lacking in group analyses shown in Fig. 9 because of two factors: some cells exhibit a tonic tail (such as in Fig. 4*Bc*) instead of phasic rhythms; and intraburst rhythmicity, when it exists, is not necessarily time locked with the beginning of the burst in all re. neurones.

Five of the twenty-three re. neurones investigated during w. and s. states were also recorded during r.e.m. or e.e.g.-desynchronized sleep. While such a small number does not allow statistical analyses, we note that all those neurones had a much lower incidence of bursts in r.e.m. sleep compared to s. sleep; their burst occurrence was closer to w. than to s. sleep.

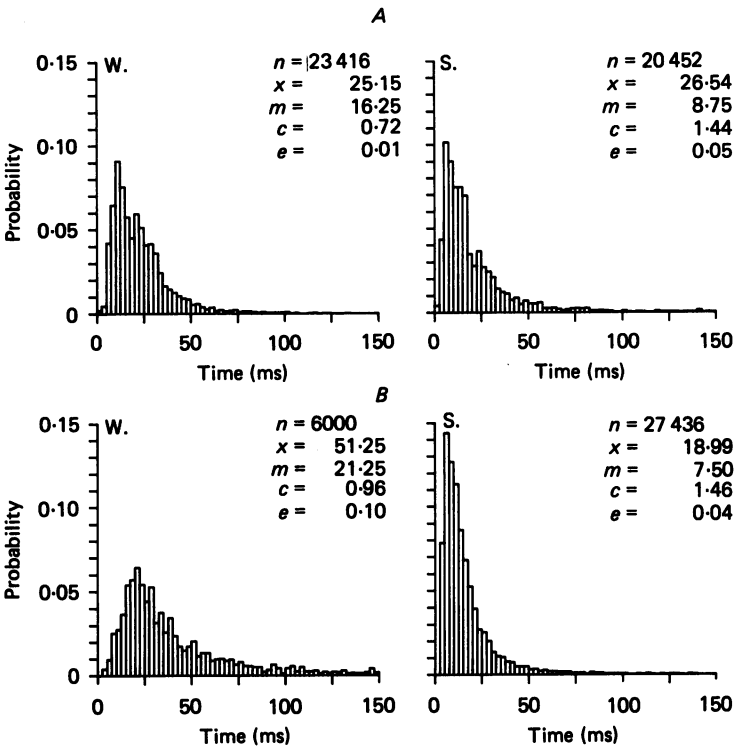

Fig. 11. Grouped interspike interval histograms (i.s.i.h.s) of re. neurones during w. and s. states. I.s.i.h.s with 2·5 ms bins. Symbols: n = number of intervals; x = mean interval; m = interval mode; c = coefficient of variation; e = intervals in excess of depicted time range. *A*, a thirteen-cell sample with decreasing firing rates from w. (39·7/s) to s. (20/s). *B*, a ten-cell sample with increasing firing rates from w. (15·7/s) to s. (21·6/s). Note great increase in probability of short intervals reflecting bursting patterns (first two or four bins) during s., regardless of rate changes from s. to w.

DISCUSSION

The present data show that the burst organization is identical for thalamocortical neurones in both relay and intralaminar nuclei, that the structure of bursts is essentially different in re. neurones compared to cortically projecting cells, and that re. bursting patterns specifically occur in s. sleep, independently of changes in firing rates.

Burst structure of thalamocortical neurones

Our analyses of a motor relay nucleus and an intralaminar nucleus are consistent with an investigation of a sensory relay nucleus (McCarley *et al.* 1983). These data afford a characterization of burst discharges during natural s. sleep that may be generalized to virtually all major types of cortically projecting neurones.

The burst of thalamocortical neurones can be defined as a stereotyped event, generally consisting of three to five spikes at 250–400 Hz, with progressively lengthening intervals. The increasing duration of successive intervals is an essential

feature that characterizes the bursts of thalamocortical neurones in a variety of experimental conditions: extracellular recordings during sleep of behaving animals as well as intracellular recordings in thalamic slices (Jahnsen & Llinás, 1984*a*) or *in vivo* under barbiturate anaesthesia (Roy *et al.* 1984). Long (200–300 ms) periods of neuronal silence before bursts (see panel *A* in Figs. 2 and 3) also suggest that the bursts occurring in natural sleep are generated by the same mechanism as recently disclosed in acute experiments, that is a low-threshold somatic slow spike de-inactivated by membrane hyperpolarization and underlying fast repetitive spikes (Deschênes *et al.* 1984; Jahnsen & Llinás, 1984*a*). Different ionic conductances (Ca^{2+} and Na^{+}) are at the basis of the slow spike and superimposed fast spikes (Llinás & Jahnsen, 1982). The increasing number of spikes in a burst, paralleled by shorter first intervals (see Fig. 1), is related to the increasing amplitude of hyperpolarization preceding burst discharges (Andersen & Andersson, 1968; Deschênes *et al.* 1984). The fact that burst discharges are state specific and depend upon the cell hyperpolarization fits in with measurements of resting membrane potential in behaving animals, showing that hyperpolarization of l.g. relay cells is specific for s. sleep (Hirsch, Fourment & Marc, 1983). Long-lasting hyperpolarizations and very brief rebounds imply an over-all depressed responsiveness of thalamocortical neurones during s., compared to both e.e.g.-desynchronized behavioural states of w. and r.e.m. sleep. This is indeed the case, as demonstrated by testing with antidromic and monosynaptic volleys (Steriade, 1984). The lower probability of single-spike discharges evoked by synaptic volleys during e.e.g.-synchronized sleep is associated with occasional burst responses during this state (Filion, Lamarre & Cordeau, 1971; Steriade *et al.* 1971; MacLeod & James, 1984).

While the burst structure of cortically projecting thalamic neurones is generated by intrinsic cell properties, the rhythmicity of spontaneous bursts is related to focal thalamic and cortical e.e.g. spindle oscillations and depends on synaptic interactions between re. and thalamocortical neurones. The disconnexion of thalamic relay and intralaminar nuclei from their afferents of re. origin abolishes rhythmic spindle waves as well as grouped, spindle-related bursts of thalamocortical neurones, but the structure of single bursts remains identical to that found in intact animals (Steriade, Deschênes, Domich & Mulle, 1985).

Burst structure of re. neurones

In the same manner that stereotyped high-frequency bursts are characteristic of thalamocortical neurones recorded from all cortically projecting nuclei, the structurally different bursts of re. neurones are found throughout the re. nuclear complex, from its rostral pole to its caudal part. Whereas the burst of thalamocortical neurones is a unique event consisting of a few short intervals, the burst of re. neurones is much longer in duration and exhibits at least two distinct components: an initial part with frequency acceleration (up to 200–250 Hz) and deceleration (that contrasts with the continuously lengthening intervals in bursts of thalamocortical neurones), followed by a long-lasting tonic barrage of spikes at about 100 Hz. The differences between the bursts of these two thalamic cell classes suggest that the electrophysiological features underlying bursts of cat's re. neurones are dissimilar to those generating bursts of thalamocortical neurones.

Intracellular recordings reveal that the core of re. bursts is a low-threshold slow spike de-inactivated by membrane hyperpolarization. In guinea-pig re. neurones, this event is similar to that found in thalamic relay-type cells (Jahnsen & Llinás, 1984*a*). In cat re. neurones, the low-threshold Ca^{2+} spike that underlies burst discharges is thought to be located in dendrites because it requires much larger somatic hyperpolarizations for its de-inactivation (Mulle, Madariaga & Deschênes, 1986) than in other thalamic cells where it is of somatic origin (see above). The hypothesized dendritic origin of re. bursts de-inactivated by membrane hyperpolarization fits in with the ultrastructural features of the feline re. nucleus that shows dendrodendritic inhibitory synapses (Deschênes, Madariaga-Domich & Steriade, 1985; Yen, Conley, Hendry & Jones, 1985). Such vesicle-containing presynaptic dendritic profiles are lacking in the rodent re. nucleus (Ohara & Lieberman, 1985). If dendritic, the low-threshold burst rebound of re. neurones is more similar to that of neurones recorded from pars compacta of substantia nigra (Llinás, Greenfield & Jahnsen, 1984) than to bursts of thalamocortical neurones.

The re. bursts described in the present experiments on naturally sleeping, unanaesthetized animals resemble those recorded in re. nucleus of barbiturated cats only to the extent that the first and last intraburst intervals are longer than the intervals in the middle of the burst. Under barbiturate anaesthesia, however, smooth acceleration and deceleration are lacking and the tonic tail is absent in spontaneously occurring bursts, thus reducing re. activity to phasic bursts whose total duration does not exceed 25–35 ms (Waszak, 1974; Mulle *et al.* 1986). Note that, in more than 90% of re. bursts recorded in the unanaesthetized condition, the initial component is longer than 50 ms and the tonic tail further extends from 0·2 s to more than 1 s (see Figs. 4, 5 and 9). The exceedingly long re. bursts that appear spontaneously during natural sleep could be mimicked under barbiturate anaesthesia by injecting a long-lasting depolarizing trapezoidal current pulse on a background of hyperpolarization, a manipulation that activated a presumed Na^+ current (Mulle *et al.* 1986), similar to the slowly inactivating Na^+ currents described in other C.N.S. neurones including thalamic ones (Jahnsen & Llinás, 1984*b*). It is likely that barbiturates cut off the prolonged tonic barrage of spikes that ends the spontaneous re. bursts in unanaesthetized animals either by a depressing effect on the persistent Na^+ current and/or by enhancing the K^+ current responsible for post-burst repolarization. As to the 7–11 Hz modulation of the prolonged tail of re. bursts (see Fig. 10), it may be ascribed to either intrinsic properties of re. neurones or spindle-related rhythmic bursts of thalamocortical neurones whose axons collateralize and excite re. neurones. At this point, *in vitro* studies of cat's re. neurones are needed to elucidate the ionic basis of the complex burst patterns as they appeared in the present experiments on unanaesthetized preparations.

Tonic and bursting discharge patterns of re. neurones during w. and s. states

We emphasize that, at variance with a prevailing view that the activity of re. neurones is invariably characterized by burst discharges, there are two distinct modes of re.-cell functioning in two behavioural states: burst firing during s. sleep and tonic firing during w. Of course, the latter mode can only be detected in unanaesthetized preparations. The direct relation between s. sleep and burst discharges was statistically

demonstrated in two subsamples of re. neurones and was found to be independent of rate changes. How do re. neurones change their discharge mode from one behavioural state to another?

The transition from arousal to sleep is associated with a slowing down of re.-cell discharges. Differences between the rates of tonic discharges during the first seconds of arousal from sleep and the lower firing rates during the last period of w., preceding drowsiness, are statistically significant at $P < 0{\cdot}001$; bursts occur later, during drowsiness and s., following this progressive decrease in discharge frequencies (Steriade *et al.* 1986). This evolution of firing rates parallels that of neurones recorded from the major input sources of re. neurones: neocortical areas and dorsal thalamic nuclei (see Hobson & Steriade, 1986, for a review) as well as rostral brain-stem reticular formation (Steriade, Oakson & Ropert, 1982). It is then plausible that the w.-to-s. transition would be accompanied by a progressive removal of excitatory afferent impulses to re. neurones. If the excitatory nature of afferent axons originating in neocortex and dorsal thalamus is commonly accepted, the influence of mid-brain reticular axons upon re. neurones has been controversial. During the 1970s, it was reported that the initial response of re. neurones to electrical stimulation of the mid-brain reticular core is a long-lasting suppression of discharges (Schlag & Waszak, 1971; Dingledine & Kelly, 1977). Since this result could be due to co-stimulation of passing fibres issuing from brain-stem monoaminergic cell aggregates that are known to exert hyperpolarizing effects at their targets, we stimulated mid-brain reticular neurones after chronic degeneration of passing fibres and were able to drive re. cells at short latencies (Steriade *et al.* 1986). This initially excitatory response may be direct or transmitted through axon collaterals of thalamocortical neurones excited by ascending mid-brain reticular projections. The reticular-elicited excitation of re. neurones in behaving animals was consistent in the same experiments with the effect of natural arousal on re. neurones (Steriade *et al.* 1986). Thus, the occurrence of burst patterns of re. neurones during s. sleep is ascribable, at least partially, to their hyperpolarization through disfacilitation at sleep onset. An additional factor would be the focal hyperpolarizations in pools of thalamocortical neurones that lead to burst discharges which may be effective in triggering the dendrodendritic re. apparatus, with the consequence of bursting and oscillations in re. neurones.

This work was supported by Grant MT-3689 from the Medical Research Council of Canada. L. Domich was a graduate student in this laboratory. Thanks are due to M. Cardinal, P. Giguère and D. Drolet for technical assistance.

REFERENCES

ANDERSEN, P. & ANDERSSON, S. A. (1968). *Physiological Basis of Alpha Rhythm.* New York: Appleton-Century-Crofts.

BARRIONUEVO, G., BENOIT, O. & TEMPIER, P. (1981). Evidence for two types of firing pattern during the sleep–waking cycle in the reticular thalamic nucleus of the cat. *Experimental Neurology* **72**, 486–501.

DESCHÊNES, M., MADARIAGA-DOMICH, A. & STERIADE, M. (1985). Dendrodendritic synapses in the cat reticularis thalami nucleus: a structural basis for thalamic spindle synchronization. *Brain Research* **334**, 165–168.

DESCHÊNES, M., PARADIS, M., ROY, J. P. & STERIADE, M. (1984). Electrophysiology of neurons of lateral thalamic nuclei in cat: resting properties and burst discharges. *Journal of Neurophysiology* **51**, 1196–1219.

DINGLEDINE, R. & KELLY, J. S. (1977). Brain stem stimulation and the acetylcholine-evoked inhibition of neurones in the feline nucleus reticularis thalami. *Journal of Physiology* **271**, 135–154.

FILION, M., LAMARRE, Y. & CORDEAU, J. P. (1971). Neuronal discharges of the ventrolateral nucleus of the thalamus during sleep and wakefulness. II. Evoked activity. *Experimental Brain Research* **12**, 499–508.

GLENN, L. L. & STERIADE, M. (1982). Discharge rate and excitability of cortically projecting intralaminar thalamic neurons during waking and sleep. *Journal of Neuroscience* **2**, 1387–1404.

HIRSCH, J. C., FOURMENT, A. & MARC, M. E. (1983). Sleep-related variations of membrane potential in the lateral geniculate body relay neurons of the cat. *Brain Research* **259**, 308–312.

HOBSON, J. A. & STERIADE, M. (1986). Neuronal basis of behavioral state control. In *Handbook of Physiology*, vol. IV, ed. MOUNTCASTLE, V. B. & BLOOM, F., pp. 701–823. Bethesda: American Physiological Society.

HOUSER, C. R., VAUGHN, J. E., BARBER, R. P. & ROBERTS, E. (1980). GABA neurons are the major cell type of the nucleus reticularis thalami. *Brain Research* **200**, 341–354.

HUBEL, D. H. (1960). Single unit activity in lateral geniculate body and optic tract of unrestrained cats. *Journal of Physiology* **150**, 91–104.

JAHNSEN, H. & LLINÁS, R. (1984*a*). Electrophysiological properties of guinea-pig thalamic neurones: an *in vitro* study. *Journal of Physiology* **349**, 205–226.

JAHNSEN, H. & LLINÁS, R. (1984*b*). Ionic basis for the electroresponsiveness and oscillatory properties of guinea-pig thalamic neurones *in vitro*. *Journal of Physiology* **349**, 227–247.

LLINÁS, R., GREENFIELD, S. A. & JAHNSEN, H. (1984). Electrophysiology of pars compacta cells in the *in vitro* substantia nigra – a possible mechanism for dendritic release. *Brain Research* **294**, 127–132.

LLINÁS, R & JAHNSEN, H. (1982). Electrophysiology of mammalian neurones *in vitro*. *Nature* **297**, 406–408.

MCCARLEY, R. W., BENOIT, O. & BARRIONUEVO, G. (1983). Lateral geniculate nucleus unitary discharge in sleep and waking: state and rate specific aspects. *Journal of Neurophysiology* **50**, 798–818.

MACLEOD, N. K. & JAMES, T. A. (1984). Regulation of cerebello-cortical transmission in the rat ventromedial thalamic nucleus. *Experimental Brain Research* **55**, 535–552.

MONTERO, V. M. & SCOTT, G. L. (1981). Synaptic terminals in the dorsal lateral geniculate from neurons of the thalamic reticular nucleus: a light and electron microscope autoradiographic study. *Neuroscience* **6**, 2561–2577.

MORISON, R. S. & DEMPSEY, E. W. (1942). A study of thalamo-cortical relations. *American Journal of Physiology* **135**, 281–292.

MUKHAMETOV, L. M., RIZZOLATTI, G. & TRADARDI, V. (1970). Spontaneous activity of neurones of nucleus reticularis thalami in freely moving cats. *Journal of Physiology* **210**, 651–667.

MULLE, C., MADARIAGA, A. & DESCHÊNES, M. (1986). Morphology and electrophysiological properties of reticularis thalami neurons in cat: in vivo study of a thalamic pacemaker. *Journal of Neuroscience* **6**, (in the Press).

NEGISHI, K., LU, E. S. & VERZEANO, M. (1962). Neuronal activity in the lateral geniculate body and the nucleus reticularis of the thalamus. *Vision Research* **1**, 343–353.

OHARA, P. T. & LIEBERMAN, A. R. (1985). The thalamic reticular nucleus of the adult rat: experimental anatomical studies. *Journal of Neurocytology* **14**, 365–411.

OHARA, P. T., SEFTON, A. J. & LIEBERMAN, A. R. (1980). Mode of termination of afferents from the thalamic reticular nucleus of the rat. *Brain Research* **197**, 503–506.

ROY, J. P., CLERCQ, M., STERIADE, M. & DESCHÊNES, M. (1984). Electrophysiology of neurons of the lateral thalamic nuclei in cat: mechanisms of long-lasting hyperpolarizations. *Journal of Neurophysiology* **51**, 1220–1235.

SCHLAG, J. & WASZAK, M. (1971). Electrophysiological properties of units of the thalamic reticular complex. *Experimental Neurology* **32**, 79–97.

STERIADE, M. (1984). The excitatory–inhibitory response sequence in thalamic and neocortical cells: state-related changes and regulatory systems. In *Dynamic Aspects of Neocortical Function*, ed. EDELMAN, G. M., GALL, W. E. & COWAN, W. M., pp. 107–157. New York: Wiley-Interscience.

STERIADE, M., APOSTOL, V. & OAKSON, G. (1971). Control of unitary activities in cerebellothalamic pathway during wakefulness and synchronized sleep. *Journal of Neurophysiology* **34**, 384–413.

STERIADE, M. & DESCHÊNES, M. (1984). The thalamus as a neuronal oscillator. *Brain Research Review* **8**, 1–63.

STERIADE, M., DESCHÊNES, M., DOMICH, L. & MULLE, C. (1985). Abolition of spindle oscillations in thalamic neurons disconnected from nucleus reticularis thalami. *Journal of Neurophysiology* **54**, 1473–1497.

STERIADE, M., DOMICH, L. & OAKSON, G. (1986). Reticularis thalami neurons revisited: activity changes during shifts in states of vigilance. *Journal of Neuroscience* **6**, 68–81.

STERIADE, M. & GLENN, L. L. (1982). The neocortical and caudate projections of intralaminar thalamic neurons and their synaptic excitation from the midbrain reticular core. *Journal of Neurophysiology* **8**, 352–371.

STERIADE, M., OAKSON, G. & ROPERT, N. (1982). Firing rates and patterns of midbrain reticular neurons during steady and transitional states of the sleep–waking cycle. *Experimental Brain Research* **46**, 37–54.

STERIADE, M., PARENT, A. & HADA, J. (1984). Thalamic projections of nucleus reticularis thalami of cat: A study using retrograde transport of horseradish peroxidase and fluorescent tracers. *Journal of Comparative Neurology* **229**, 531–547.

STERIADE, M. & WYZINSKI, P. (1972). Cortically elicited activities in thalamic reticularis neurons. *Brain Research* **42**, 514–520.

WASZAK, M. (1974). Effects of barbiturate anesthesia on discharge pattern in nucleus reticularis thalami. *Pharmacology, Biochemistry and Behaviour* **1**, 339–345.

YEN, C. T., CONLEY, M., HENDRY, S. H. C. & JONES, E. G. (1985). The morphology of physiologically identified GABAergic neurons in the somatic sensory part of the thalamic reticular nucleus in the cat. *Journal of Neuroscience* **5**, 2254–2268.