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Review Article

FIRING PATTERNS OF EPILEPTIC AND NORMAL NEURONS IN THE CHRONIC ALUMINA FOCUS IN UNDRUGGED MONKEYS DURING DIFFERENT BEHAVIORAL STATES

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SUMMARY

This communication summarizes data from a series of experiments on the activity of single units in chronic epileptogenic alumina foci in precentral cortex of undrugged monkeys. The foci contained a mixture of normal and epileptic cells, which differed consistently in their spontaneous firing patterns under various behavioral conditions, and in their responses to electrical stimulation: (1) during restful waking the spontaneous activity of normal precentral cells rarely exhibited intervals less than 10 msec, whereas the activity of epileptic cells included high frequency bursts with intervals less than 5 msec. The percentage of total activity in bursts was defined as the 'burst index'; (2) responses evoked antidromically by pyramidal tract stimulation and orthodromically by stimulation of center median of thalamus consisted of single action potentials in normal cells and bursts in epileptic cells; the probability of evoking a burst in epileptic cells was proportional to the burst index; (3) bidirectional operant conditioning of firing rates was most readily successful in normal cells and appeared to be increasingly difficult in epileptic cells in proportion to their burst index and (4) during sleep, epileptic cells fired in longer and higher frequency bursts than normal cells.

To the extent that both types of cells receive similar inputs, these observations suggest that many epileptic cells in the alumina focus are intrinsically hyperexcitable, *viz.* they respond abnormally to normal inputs rather than responding normally to abnormally intense inputs. These hyperexcitable neurons may drive other cells in the focus, but activity of both may be operantly controlled.

INTRODUCTION

Experimental models of epileptic processes are essential for scientific investiga-

tion of neural mechanisms underlying epileptogenesis and their possible control. Such models fall into two broad classes, usually termed 'acute' and 'chronic'. Acute models of epilepsy generate patterns of neural hyperactivity, resembling interictal and/or ictal episodes, relatively quickly after application of a drug (penicillin, strychnine, Metrazol, etc.) or a stimulus (electrical, freezing, etc.), and typically complete their natural histories within minutes or hours. The experimental popularity of acute models can be attributed in part to the convenience of rapid onset, control of dosage, and intensity of neural response. Although such models may mimic some naturally occurring, acute epileptic phenomena, the degree to which acute convulsants accurately represent naturally occurring chronic human epilepsy remains debatable; moreover, concordance with the human disease is often further compromised by the use of anesthetic and/or paralyzing agents[14], and acute surgical procedures which preclude observation under normal behavioral conditions. The electrophysiological properties of various acute models have recently been reviewed elsewhere[2,4] and are not the subject of this review.

The second class of epileptic models, namely chronic, involves application of agents which results in a more gradual evolution of an epileptogenic focus which generates interictal and ictal patterns for longer times. Of these, the model generally acknowledged to most closely resemble chronic focal epilepsy in humans is that produced by application of aluminum hydroxide to neocortex of the rhesus monkey[15, 25,26]. Monkey alumina and human foci are remarkably similar with respect to ictal and interictal EEG patterns, natural history of ictal episodes, and patterns of single unit activity[7,12,15,25,26]. Therefore, the electrophysiological properties of cells in alumina focus would be of considerable relevance to understanding natural epileptogenic mechanisms. Furthermore, the behavioral control of neuronal activity in chronic foci can be investigated in trained animals; besides providing important data on modification of epileptic activity, such studies would have important implications for the learned control of human epilepsy.

Activity of single cells in chronic alumina foci was first recorded extracellularly in anesthetized monkeys by Schmidt *et al.*[21,27] and from undrugged monkeys by Sypert and Ward[24]; both groups found many cells in the focus which fired in high-frequency bursts during interictal periods and became tonically active or inactive during propagated seizures. A remarkably stereotyped burst pattern, the 'long-first-interval' burst, which characterized some cells in the alumina focus was first documented by Calvin, Sypert and Ward[8]. Since this stereotyped burst pattern is not readily explained in terms of normal synaptic or circuit mechanisms, and is never seen in normal cortex, it would seem to provide important clues to the nature of hyperactivity in these cells. Intracellular recordings have been obtained from alumina foci, despite technical difficulties presented by the gliotic scar[3,13,19,20]; these studies have confirmed the presence of unstructured and structured burst patterns, (often coincident with augmented depolarization shifts[19]) but have failed to elucidate the mechanism of the long-first-interval burst.

In a recent series of studies we have documented the firing patterns of cells in the alumina focus under a variety of natural behavioral conditions and have

investigated the degree to which monkeys could learn to control the activity of these cells. This review summarizes these separately reported observations on the interictal behavior of normal and epileptic neurons in the alumina focus during waking, sleep, operant conditioning of unit activity[11,28–33] and in response to antidromic and orthodromic stimulation[30,32]. This report is not intended to review data obtained from acute foci nor extensively review non-behavioral acute experiments on alumina foci, since such data does not address the present issue of this report, namely the activity of single cells recorded from chronic epileptic foci in alert, undrugged monkeys under different natural behavioral conditions.

METHODS

Eight *Macaca mulatta* monkeys were rendered epileptic by subpial alumina gel injections in sensorimotor cortex using the modified Kopeloff method[16] recently reviewed by Ward[26]. All monkeys developed EEG correlates of focal epilepsy and 7 who underwent 24-h seizure monitoring developed documented focal motor and/or generalized seizures.

Between 3 and 9 months following the development of seizures, all monkeys were implanted with a chronic recording mount and a bipolar pyramidal tract stimulating electrode. In addition, 5 monkeys had silver ball epidural EEG electrodes permanently placed peripheral to the focus, and two of these had bipolar stimulating electrodes in the ipsilateral nucleus center median (CM) of thalamus. These procedures have been described in more detail in previous reports[10,11,28–33]. The animals were then trained to bidirectionally control the activity of normal single precentral units by differentially reinforcing high or low firing rates[11,29]. Except for the anesthesia of surgery, these monkeys received no other medication.

Terminology

Because similar terms are used in slightly different contexts in the literature, the terms used in this report will be defined as follows:

(1) *Bursts* are considered the hallmark of abnormal (pathologic) single unit activity and consist of consecutive action potentials (AP) with interspike intervals shorter than 5 msec. Normal precentral units may exhibit high frequency firing under certain conditions, *e.g.* sleep, active movements or operant conditioning of high rates, but intervals rarely become shorter than 5 msec. As discussed elsewhere[24] bursts produced by electrode injury are distinguished from epileptic bursts by being more variable in duration, being a function of electrode position and being less stable over prolonged periods. Epileptic bursts encountered in the alumina focus can be further classified as follows:

(a) *Stereotyped bursts* have a repeatable sequence of short interspike intervals; thus, if successive stereotyped bursts are aligned along their first AP in a dot raster, the remaining APs of each burst are also aligned with little variance.
(b) *Structured bursts* have a repeatable timing sequence of successive interspike intervals, including a relatively long interval. The most frequently encountered

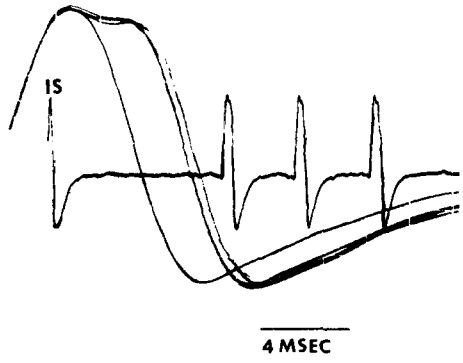

Fig. 1. Spontaneous long-first-interval burst recorded in alumina focus. Superimposed fast (1 msec) sweeps of individual action potentials comprising the long-first-interval bursts of the slower sweep. All action potentials occurring in the afterbursts had a 'compound' waveform whereas initial spikes and single spikes during normal activity had 'simple' action potentials. The second portion of the compound AP's corresponded to the wave form of simple AP's. The early and late portions of the compound AP varied independently as a function of electrode depth[30]. Peak-to-peak AP's were 1.5 mV (negativity upwards). The time bar at the bottom calibrates the slower sweep.

example is the *long-first-interval (LFI) burst*, which is initiated by a single AP called the initial spike followed by a relatively long interspike interval (4–12 msec), in turn followed by a higher frequency stereotyped burst, called the *afterburst*. (Fig. 1.)

(c) *Unstructured bursts* have no repeatable timing sequence from one burst to the next.

(2) *Burst index*. For an isolated unit the burst index is the per cent ratio of APs occurring in bursts to total APs within a 15-sec epoch. The burst index was determined by an on-line PDP8/e computer programmed to identify a burst on the basis of consecutive interspike intervals less than 5 msec. Thus, a normal precentral unit with all interspike intervals greater than 5 msec would have a burst index of 0.0, whereas a unit exhibiting only high frequency bursts would have a burst index of 100.

(3) *Pyramidal tract (PT)* neuron: a neuron which responds to each of 3 pyramidal tract stimuli at 500 pulses/sec with an invariant latency (usually less than 1.2 msec).

(4) Several types of *behavioral schedules* were used during operant conditioning sessions: *DRH*, differential reinforcement of high rates of unit activity; *DRO*, differential reinforcement of zero unit activity; *DRR*, differential reinforcement of regular activity (low burst index) and *DRB*, differential reinforcement of bursting activity (high burst index); S^{Δ}, an extinction or 'time out' period with no reinforcement. *Bidirectional conditioning* means that increases *and* decreases in a response were successively conditioned in the same session.

RESULTS

This report comprises observations on more than 300 distinctly isolated neurons of which over 200 were subjected to bidirectional operant conditioning; more than

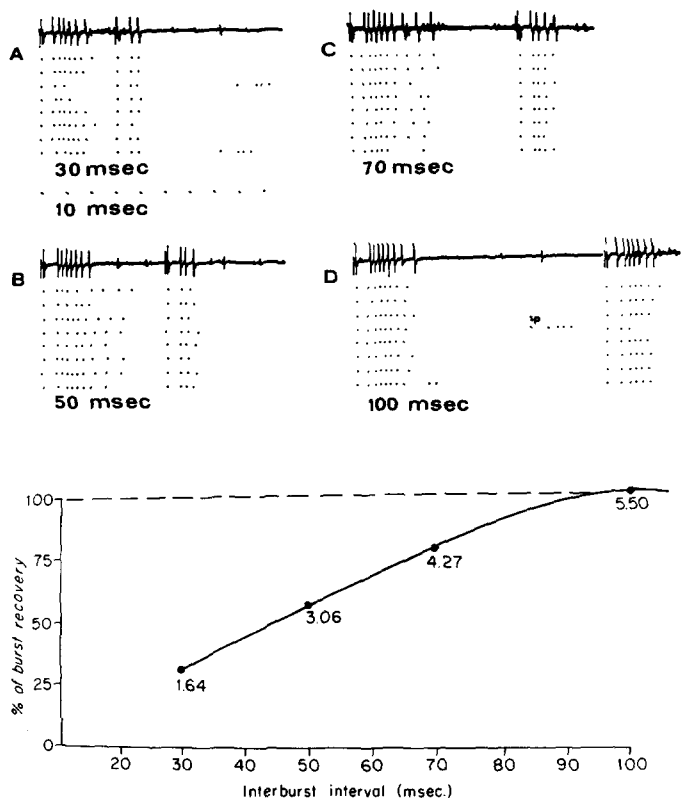

Fig. 2. Attenuation of antidromically evoked bursts as inverse function of preceding interburst interval. Top: long-first-interval bursts evoked by pairs of pyramidal tract shocks separated by specified intervals. A: 30 msec; B: 50 msec; C: 70 msec and D: 100 msec. (sp = spontaneous burst occurring between evoked bursts.) Bottom: mean duration of second burst as per cent of maximum, plotted as function of interburst interval. (Fom ref. 30.)

half of all the units were pyramidal tract neurons. Neurons were characterized as normal or epileptic if their burst index was lower or greater than 10 respectively. This criterion was quite reliable because normal precentral neurons rarely had interspike intervals shorter than 5 msec, even under conditions which generated shortest interspike intervals, such as DRH periods (Figs. 3 and 4) or slow wave sleep (SWS) (Fig. 6). Thus, all neurons with burst indices greater than 10 could reliably be considered to be epileptic. Epileptic neurons were further subdivided into two groups: group 1 epileptic neurons had a high burst index (generally greater than 60) whose variance during quiet wakefulness was low (typically less than $\pm$ 10). This variance was documented during periods when the animal was not moving, but alert by behavioral and EEG criteria. Group 2 epileptic neurons had lower and more variable burst indices (variance greater than $\pm$ 10); although the burst index of a group 2 neuron could temporarily exceed that of a group 1 neuron, this was never sustained. Of the total population of recorded cells, slightly less than 50% were epileptic as judged by these criteria.

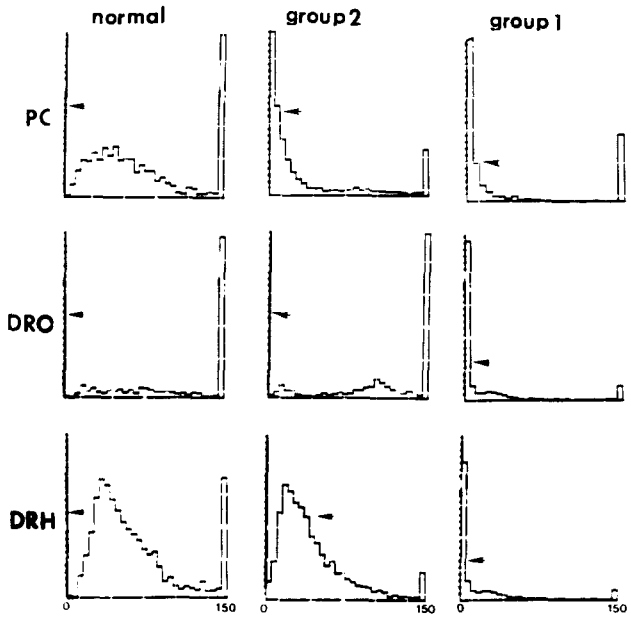

Fig. 3. Representative interspike interval histograms of activity from normal, group 1 and group 2 neurons recorded in the cortical focus of one monkey. Samples were taken during preconditioning period (PC), differential reinforcement of high rates (DRH), and differential reinforcement of zero activity (DRO). Examples of activity from which histograms were compiled are shown in Fig. 4. Bin width is 5 msec; the last bin contains all interspike intervals greater than 145 msec. Arrows indicate 1000 counts. (From ref. 29.)

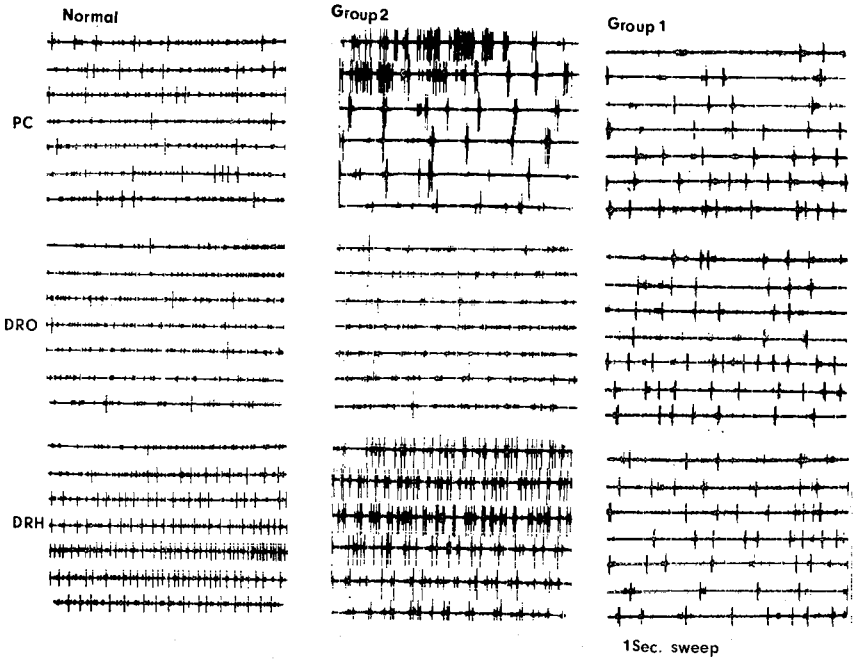

Fig. 4. Representative examples of unit activity from normal, group 1 and group 2 neurons from the same epileptic focus. Continuous sample of activity is shown in consecutive sweeps; samples were taken in different behavioral periods of operant conditioning sessions illustrated in Fig. 5. Because of slow sweep (1 sec), the single AP's comprising the high-frequency epileptic bursts of the group 1 and group 2 neurons are not resolved. (From ref. 29.)

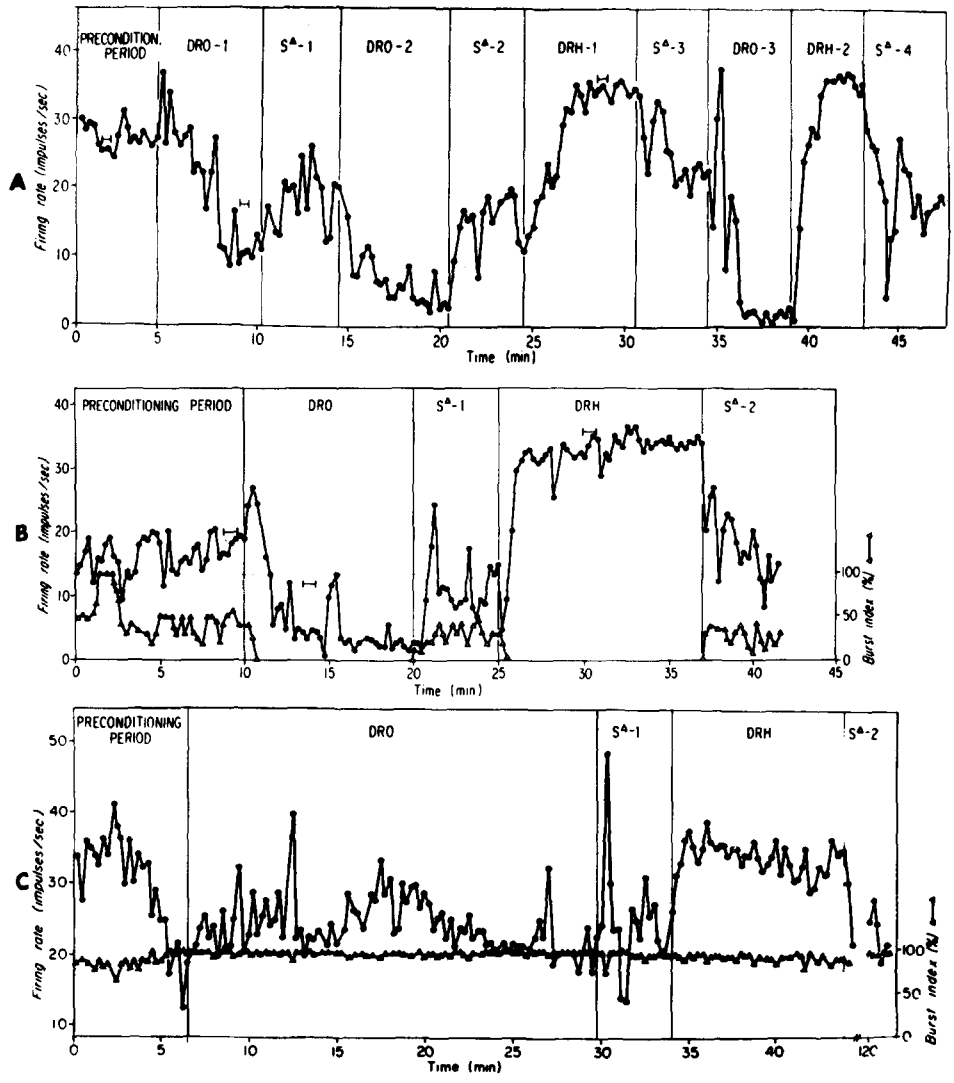

Fig. 5. Operant conditioning sessions with normal, group 1 and group 2 epileptic neurons in alumina focus of same monkey. Graphs plot average firing rate (scale at left) and burst index (scale at right) in successive 15-sec intervals. Firing rates of a normal neuron (A) were bidirectionally controlled, most convincingly in DRO-3 and DRH-2. Firing rates of a group 2 epileptic neuron (B) were also bidirectionally controlled, and burst index went to zero during both behavioral periods. Firing rates of a group 1 epileptic neuron (C) were not convincingly modified and burst index remained high and constant under all conditions. (From ref. 29.)

All cells which demonstrated the long-first-interval burst pattern were pyramidal tract (PT) neurons, and conversely long-first-interval bursts were never recorded from non-PT neurons. Since this structured burst pattern appears to be restricted to only pyramidal tract neurons, the following discussion will distinguish two main classifications of epileptic neurons: PT and non-PT neurons.

Before discussing the behavior of epileptic neurons within the alumina focus, we will describe the behavior of normal neurons recorded from the same cortical substrate.

1. Normal neurons in epileptic cortex

Cells exhibiting normal firing patterns were usually recorded in the same electrode tracks in which abnormal, bursting units were also encountered. These normal cells fired in regular patterns characteristic of neurons recorded from homologous regions of normal cortex. The majority responded to some form of peripheral stimulation, usually passive movement of contralateral joints. Normal units were often observed to fire single action potentials synchronously with bursts of a simultaneously-monitored neighboring epileptic unit, particularly when the monkey was either inattentive or asleep. When the monkey was alerted, or actively moving, such synchronous unit firing was often dissociated[29]. Interspike intervals were typically greater than 10 msec and these cells often fired in relation to spontaneous movements. During EEG events such as spindles, spikes, and K complexes, such units fired in high-frequency clusters of APs, but the firing frequency attained during such clusters was generally lower than that characteristic of epileptic cells (Figs. 6 and 7) [9,18,22,23,28]. In contrast to epileptic neurons, normal PT units responded with a single AP to a pyramidal tract stimulus. Normal neurons (both PT and non-PT) also re-

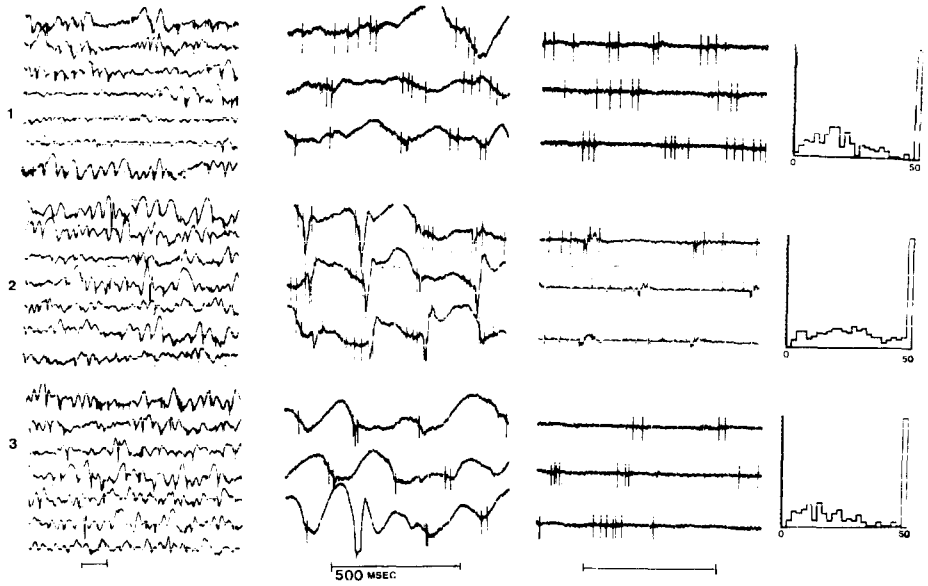

Fig. 6. Simultaneous EEG and unit activity of a normal non-PT neuron recorded in an epileptic focus during stages 1–3 sleep (rows 1–3 respectively). Left column taken at slow sweep, whereas the next column is at faster sweep. (Time bars calibrate 500 msec.) The third column shows the unit activity, with the high pass filter at 300 Hz. The right column shows interspike interval histograms of the unit activity. (From ref. 28.)

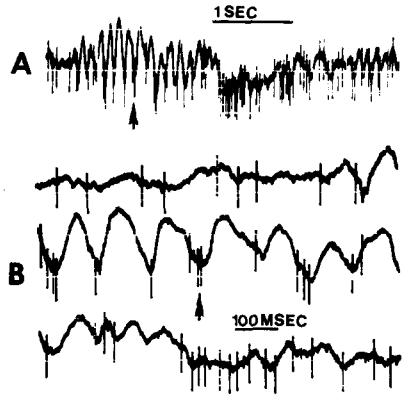

Fig. 7. Unit activity of a normal PT neuron recorded in epileptic cortex during a sleep spindle. Sweep A shows same activity as in 3 consecutive faster sweeps in B (negativity downwards). Arrows identify the same point in sweeps A and B. Note that the high-frequency unit activity during the peaks of the spindles does not approach the firing frequency or structure of epileptic bursts. (From ref. 28.)

sponded to a single center median (CM) stimulus with a single AP[32]. Repetitive stimuli to center median at frequencies from 1–10 hz evoked EEG recruiting responses with concomitant single unit activity consisting of variable latency, waxing–waning clusters of APs, but interspike intervals in such clustered APs were never less than 5 msec (ref. 32). After these monkeys demonstrated proficiency at operant control of normal cells, we found no normal pyramidal tract neurons that were not easily bidirectionally controlled (for example see Fig. 5); this fact allowed us to use normal PT cells as controls for behavioral variables in subsequent operant conditioning sessions of epileptic cells. Of the normal non-PT cells 98% were easily bidirectionally conditioned.

In summary, normal PT and non-PT cells recorded from the alumina foci responded to pyramidal tract stimulation, center median stimulation, slow wave sleep, and bidirectional operant conditioning like neurons recorded from homologous regions of normal cortex.

II. Highly epileptic cells (Group 1)

Cells in the alumina focus were designated as highly epileptic, or 'Group 1' if they fired predominantly in high-frequency bursts (mean burst index greater than 60), and exhibited relatively little variance in burst index during quiet wakefulness. Since PT and non-PT group 1 cells differed consistently in burst structure and other characteristics these two types of cells are best discussed separately.

A. Pyramidal tract cells

Group 1 PT neurons fired predominantly in structured long-first-interval (LFI) bursts during periods of *quiet wakefulness*. For a given cell, these bursts recurred with remarkably repeatable sequence of spikes, with the mean duration of the long-first-

interval often being close to an interger multiple of the first afterburst interval[11,30]. Some units exhibited first intervals with a bimodal distribution and a few showed an exceedingly invariant first interval[8,11,30]. In many cells the initial spike of the LFI burst had a 'simple' waveform, identical to single APs which occurred between bursts during regular firing; in contrast, the afterburst APs were typically larger and longer, often clearly compounded of two portions, suggesting multiple sites of spike initiation (Fig. 1 and ref. 30). PT stimulation evoked a simple antidromic AP, which usually formed the initial spike of a complete LFI burst, which was structurally similar to spontaneous LFI bursts. The fact that juxtathreshold intensity PT shocks evoked either a complete LFI burst or no response at all suggests that the afterbursts are generated within the cell consequent to the initial spike and do not depend on synchronous activation of neighboring cells. A second antidromic spike could also be timed to invade the cell during the long-first-intervals, indicating that the cell was not in cathodal block during this interval[30] (this conclusion has recently been substantiated by intracellular recordings from LFI bursting neurons in monkey cortex reported by Reynolds *et al.*[20]). The number of spikes per burst was relatively constant for a given cell, except when the preceding interburst interval was less than 100 msec, in which case the afterburst was shortened. This graded recovery of the burst generating mechanism was observed for both spontaneous and antidromically evoked LFI bursts (Fig. 2)[11,30,32].

Stimulation of center median nucleus of the thalamus orthodromically evoked a burst similar in timing to the afterburst of the LFI burst (Fig. 8); in two cells the CM stimulus occasionally evoked a complete LFI burst.

In *operant conditioning sessions*, the firing rates of group 1 PT cells could, in general, be more readily increased during DRH periods than decreased during DRO periods. Usually the mean burst index remained steady during conditioned

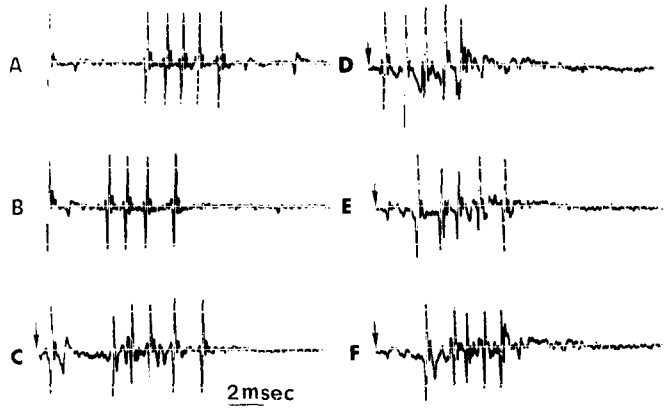

Fig. 8. Long-first-interval bursts of a group 1 PT cell, spontaneously occurring in A and B, and antidromically evoked in C. (Arrows indicate stimuli.) Sweeps D–F show variable latency response to thalamic stimuli; these orthodromically evoked bursts are similar in timing to the afterbursts of spontaneous and antidromically evoked afterbursts. Action potential amplitudes were 1 mV. (From ref. 32.)

rate changes; that is, mean firing rates changed without altering the relative proportion of bursts. However, during transient increases in firing rate, bursts often recurred rapidly enough to be attenuated. We also noted that after transient suppression of unit rates on DRO, the initial activity following the pause was invariably a burst. This suggests an inverse relation between firing rate and the probability of burst activity. When the monkey was reinforced for suppressing the burst index, on a schedule which differentially reinforced regular activity (DRR), he could change the firing patterns from bursting modes to regular firing patterns[11]. However, the drop in burst index was associated with a rise in total firing rate such that a decreasing interburst interval ultimately resulted in complete attenuation of the afterburst. This would suggest that the change in pattern was mediated by a net increase in synaptic drive[11].

During *sleep*, the structure of the burst changed such that afterburst interspike intervals approached their minimal duration of less than 2 msec, while the first interval became considerably shorter and extremely variable (Fig. 9). Occasionally the sleep bursts also were characterized by long second or long third intervals (Fig. 10)[28]. Although the total duration of the burst did not increase, the number of APs per burst increased by 100% or more. In all instances, the burst structure reverted to its original form when the monkey was awakened. During the transition into sleep, the mean interburst intervals tended to become slightly longer and less variable than during wakefulness (Fig. 10).

In summary, group 1 epileptic PT neurons fired abnormally with a high proportion of LFI bursts during all behavioral conditions; their response to pyramidal tract and center median stimulation was usually a burst, in contrast to single AP

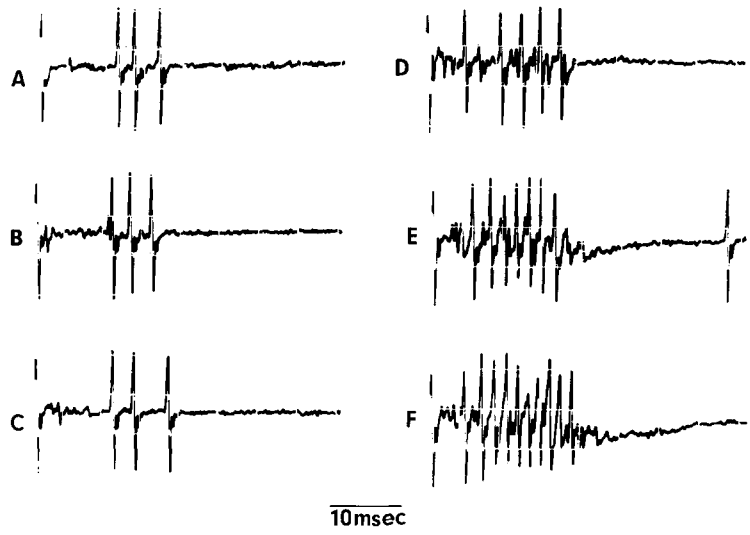

Fig. 9. Comparison of burst structure of an epileptic PT neuron occurring during quiet wakefulness (A–C) and stage 2 sleep (D–F). Although the total burst duration did not increase, the number of AP's per burst approximately doubled during sleep. (From ref. 28.)

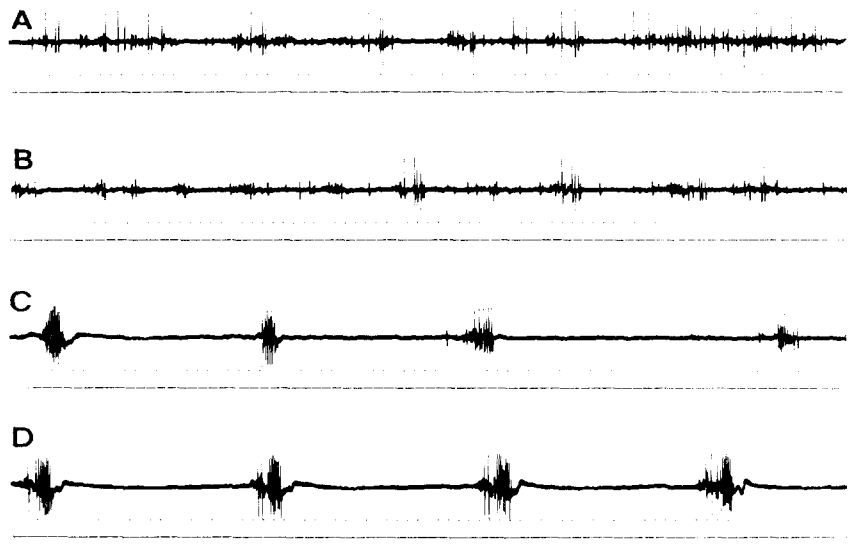

Fig. 10. Activity of a group 1 PT neuron during 4 behavioral states: A: during active wakefulness, with initiation of movement of the contralateral arm at end of sweep. B: during quiet wakefulness, in absence of overt movements. C: during early stage 2 sleep. D: during sleep spindle. Action potential was 0.9 mV, and timing markers calibrate 10 msec intervals. During sleep, the background units fired synchronously with the epileptic unit. (From ref. 28.)

evoked in normal PT cells from the same sites. During operant conditioning sessions, monkeys were able to successfully increase firing rates, but did not consistently succeed in suppressing activity, especially burst activity. (Fig. 5.) During sleep the burst activity was intensified and the burst index increased.

B. Non-pyramidal tract neurons

Group 1 non-PT epileptic neurons fired primarily with a mixture of stereo typed and unstructured bursts. Cells with higher burst indices tended to have a greater proportion of stereotyped bursts. By definition, PT stimulation did not elicit a short invariant latency antidromic response; however, several units responded to pyramidal tract stimulation with a burst structurally similar to spontaneously occurring bursts (Fig. 11). The brief but variable latencies of some of these responses (1.4 msec), suggests that these bursts were evoked orthodromically, via axon collaterals of neighboring pyramidal tract neurons. Single center median stimuli also evoked bursts similar in structure to spontaneous bursts (Fig. 11).

In *operant conditioning* sessions, the group 1 non-PT neurons were not successfully bidirectionally conditioned; in contrast to PT neurons, their rates were not significantly increased by reinforcement of high rates of activity. Although some cells demonstrated appropriate changes in the occurrence of interburst unit activity, such activity was such a small proportion of total activity that significant firing rate changes were not achieved[29].

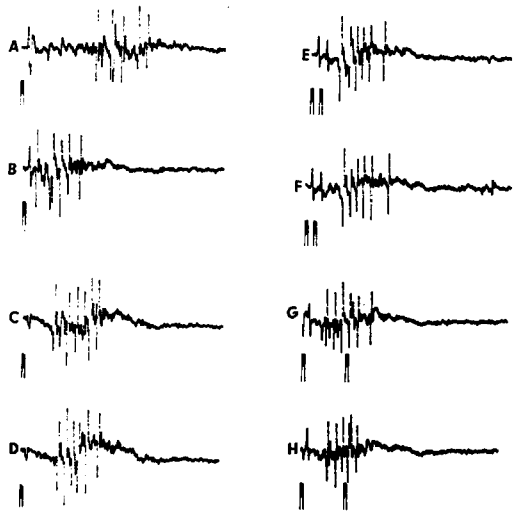

Fig. 11. Response of a group 1 non-PT neuron to thalamic and pyramidal tract stimulation. All sweeps triggered from stimulus marker. Sweeps A and B demonstrate variable latency response to thalamic stimulation. Sweeps C and D show the same cell's response to pyramidal tract stimulation. Response to paired thalamic stimuli separated by 2 msec in E and F and 10 msec in G and H. Action potentials were 600 μV. (From ref. 32.)

During *sleep*, the total burst duration increased (Fig. 12), but interspike intervals within the burst did not decrease significantly since, during wakefulness, they already approximated maximal firing rates of 500 per sec. During all periods of synchronized sleep, the interburst intervals were approximately 250 msec, only slightly longer than the average interburst interval seen during wakefulness. Moreover, these bursts recurred regularly, independently of EEG events such as spindles, K complexes or sharp waves.

In summary, group 1 non-PT neurons fired predominantly in structured and unstructured bursts, both spontaneously and in response to orthodromic stimuli (center median); they appeared more difficult to condition than other types of cells and exhibited prolonged bursts during all stages of sleep.

III. Moderately epileptic (Group 2) cells

The alumina focus also contained a large proportion of cells whose firing patterns were intermediate between normal and highly epileptic cells. These 'moderately epileptic' or 'Group 2' cells were characterized by a highly variable burst index, whose mean value was usually below 60. The bursts of most PT and all non-PT group 2 cells were unstructured bursts, with the exception of a few PT cells which exhibited LFI bursts. With this distinction in mind, the following observations applied to both PT and non-PT group 2 cells.

During *quiet wakefulness* firing patterns of group 2 cells by definition contained a smaller and more variable proportion of bursts than group 1 cells. The nature of

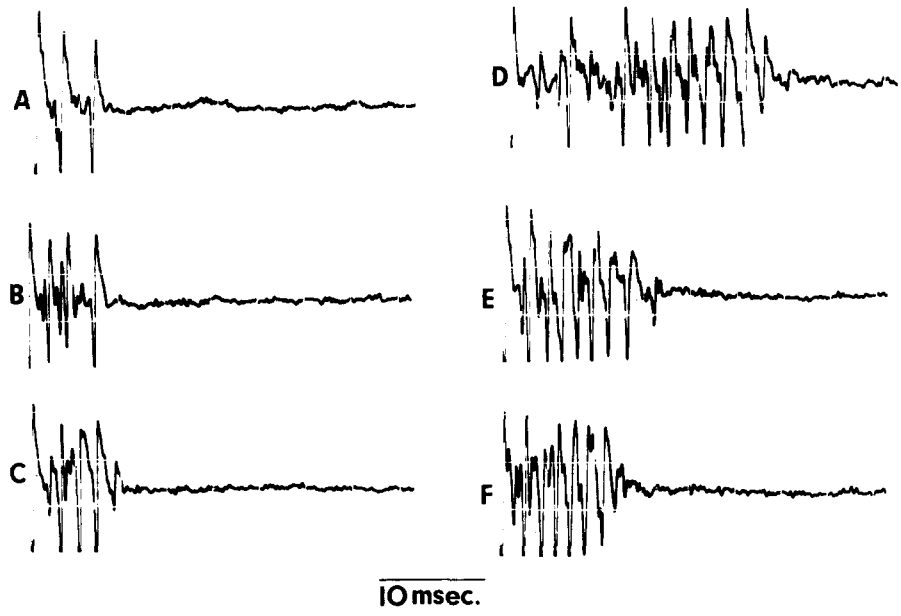

Fig. 12. Comparison of burst structure of a group 1 non-PT neuron during wakefulness and stage 2 sleep. Sweeps A–C show 3 spontaneous bursts during wakefulness; sweeps D–F show bursts recorded during stage 2 sleep. Peak-to-peak AP amplitude was 0.5 mV. (From ref. 28.)

the response evoked by electrical stimulation — either orthodromically from CM or antidromically from PT — tended to be similar to the spontaneous firing pattern at the time of stimulation. Thus, when the cell was firing in bursts, the evoked response tended to be a burst; when the cell was firing regularly, the evoked response tended to be a single AP. The probability of evoking a burst was proportional to the burst index at the time of stimulation and the duration of cell inactivity which immediately preceded stimulation: *i.e.*, the longer the duration between preceding activity and the stimulus, the higher the probability that the evoked activity (antidromic or orthodromic) would be a burst. Thus, the fluctuating propensity of group 2 cells to fire in bursts applied to both spontaneous and electrically evoked activity.

During *operant conditioning* sessions, experienced monkeys were successful in bidirectionally controlling firing rates of virtually all group 2 cells[29]; that is, they could increase firing rates on DRH and decrease them on DRO. Some group 2 cells with extremely variable burst indices occasionally showed complete suppression of burst activity during conditioning periods, regardless of whether the schedule reinforced high or low firing rates (Fig. 5B). This suggests that increased arousal or attention may reduce the conditions for bursting in group 2 cells.

During *sleep*, the burst index of all group 2 cells increased markedly[28]. Cells with higher waking burst index often fired exclusively in high-frequency bursts during sleep; those with lower waking burst index also increased their burst index, but not always to 100%. During sleep the bursts typically contained decreased interspike intervals, sometimes doubling the number of action potentials. Sleep bursts of group 2

cells were significantly higher in frequency than sleep-related clusters of APs from normal cells and often became indistinguishable from those of group 1 cells. Waking the monkey immediately reduced the burst index of group 2 cells.

In summary, group 2 cells fired with a moderate and highly variable proportion of unstructured bursts during quiet waking; responded to electrical stimulation with bursts or single spikes, depending on the spontaneous pattern at the time of stimulation; were readily bidirectionally conditioned and during sleep exhibited bursts of higher frequency firing than normal cells.

Effects of recording and conditioning on cell types and seizures

The relative proportion of normal and group 1 and 2 epileptic cells varied as a function of both location relative to the site of alumina injection and over time. Several millimeters from the injection site most cells were normal and the proportion of epileptic cells was highest near the focus defined by EEG spiking. A systematic mapping of cell types with respect to the injection site remains to be done to quantify this relation. The proportion of cell types also showed some variation for electrode tracks made at contiguous sites on separate days. However, such proportions became more repeatable when pooled over 5 day periods. In several animals the relative number of epileptic cells encountered was found to decrease systematically over several weeks of recording and conditioning[33]; these animals also experienced fewer seizures during recording and conditioning days and were generally successful in controlling firing rates of cells in operant conditioning sessions. In contrast, other monkeys who were exposed to similar recording and conditioning procedures had a constant and high proportion of epileptic cells; these animals did not succeed in controlling firing rates of units and experienced no reduction in seizures. As discussed elsewhere[33], a number of variables may be involved in these differences, including differences in the severity of the focus.

DISCUSSION

These observations suggest a number of conclusions concerning the nature of the pathological processes in the chronic epileptic focus and their possible behavioral manipulation. The precentral alumina focus clearly contains a spectrum of cell types, from normal cells, whose spontaneous and evoked responses resemble those observed in normal cortex, to highly epileptic cells whose predominant firing pattern under the same conditions consists of high frequency bursts.

Data pooled from several animals would indicate that for those regions of cortex in the immediate periphery (1–2 mm) of the injection sites the relative percentage of neurons is approximately: group 1, 10%; group 2, 40%; and normal neurons 50%. These approximations need clearer quantification since we do not know what variables in the preparation of the animal might influence these data.

For epileptic cells the proportion of cell activity occurring in bursts (burst index) ranges from low and variable (group 2 epileptic cells) to high and invariant

(group 1 epileptic cells). The propensity of epileptic cells to respond with a burst to conditions which produced single action potentials in normal cells was consistently observed over a wide range of behavioral situations. Thus, the responses evoked antidromically by pyramidal tract stimulation and orthodromically by thalamic stimulation tended to be bursts for epileptic cells and single spikes for normal cells. The degree to which burst responses were evoked by stimulation was directly proportional to the cell's burst index at the time of stimulation. Thus, the higher the burst index, the more consistently the cell exhibited a burst response to antidromic or orthodromic stimulation. Furthermore, during sleep, the same differences between normal and epileptic neurons were maintained; events such as sleep spindles associated with a doublet or triplet firing in normal neurons were correlated with exaggerated bursts in epileptic neurons.

These data bear on the classic debate of whether bursting neurons in epileptogenic cortex are intrinsically hyperexcitable or are simply normal cells responding to an abnormally intense synaptic input. Thus, there are two contrasting views of the epileptic focus: (1) the focus is maintained by a group of intrinsically bursting 'epileptic' or 'pacemaker' neurons[19,25,27] or (2) the focus is perpetuated by a group interaction within an 'epileptic aggregate'[1,4,5,17] of individually normal cells, synchronized perhaps by subcortical centers like thalamus. A common argument against the concept of intrinsically hyperexcitable neurons is the lack of evidence that such neurons respond in a hyperexcitable fashion to stimulation which would evoke normal responses in a normal neuron. Assuming that the normal and epileptic cells recorded at the same cortical sites in our studies received similar synaptic input, the fact that the latter responded with bursts would suggest an intrinsic hyperexcitability. Although burst responses to stimulation of pyramidal tract or thalamus could have been due to activation of a population of neurons converging on the epileptic cell, the same should apply to adjacent normal neurons. If both types of cells receive similar input, the fact that normal neurons responded with single spikes suggests that burst responses of epileptic cells reflect intrinsic hyperexcitability.

The highly structured long-first-interval burst pattern found in many group 1 cells further supports the concept of intrinsic hyperexcitability. It is difficult to account for the length and invariance of the long interval on the basis of reverberating circuits, which would involve two synapses. The fact that LFI bursts were antidromically evoked in all-or-none fashion by juxtathreshold PT stimuli strongly implicates intrinsic pathology. Moreover such structured burst patterns have been evoked from single cells in alumina gel foci by slight local mechanical and metabolic cellular injury[31]. It should be noted that LFI bursts have never been seen as part of injury patterns in normal cortex. In any case this data gives supportive evidence that such extremely structured burst patterns may be produced by damage to single cells in the alumina focus and probably does not involve extensive neuronal circuits. Calvin[6] has hypothesized that a normal neuron may be driven into bursting firing mode by biasing 2% of its synaptic input towards membrane excitation. One could argue that the epileptic units we observed were bursting in response to massive synchronized synaptic input. Although this may explain the labile, unstructured bursts of the very

weak group 2 epileptic neurons it is an unlikely explanation for the sustained, stereotyped bursts of group 1 units.

Although the above data clearly demonstrate that within the experimental alumina epileptic focus one may find pyramidal tract neurons whose spontaneous and evoked activity is pathologic, this data is not as conclusive for those cells classified as non-PT neurons. This latter group of neurons may comprise a heterogeneous population, some of which could be considered 'interneurons'. Steriade and coworkers[22,23] have recently reported data on a group of precentral neurons which they consider interneurons. Such cells had the following characteristics in normal cortex: (1) their 'normal' firing patterns were high frequency bursts, which by our criteria would be epileptic; (2) the majority responded orthodromically (monosynaptically) with a short latency burst to pyramidal tract stimulation; (3) such cells also responded orthodromically (slightly longer latencies) to VL stimulation with a burst; (4) duration of spontaneous bursts increased during sleep (5) their average firing rates changed with alerting and (6) during wakefulness the ability to evoke firing from these cells by pyramidal tract or VL stimulation decreased, but when evoked, their latencies were somewhat shorter. The above data is quite compatable with some of the group 1 non-PT neurons we considered 'epileptic' in that a few such cells had burst responses orthodromically evoked by pyramidal tract and CM stimulation, lengthened their bursts during sleep, stabilized their firing rates during alerting (see Fig. 4 in ref. 29), and had, on the average, smaller amplitude APs than the PT cells. If some of our group 1 non-PT cells were in fact interneurons, this might account for the inability to operantly control their firing patterns and the stability of their 'burst indices'. Although the above may exclude a few group 1 non-PT cells as being 'epileptic' it does not adequately explain the behavior of all the non-PT cells which demonstrated burst firing. Moreover, it now places more importance on the elucidation of mechanisms responsible for pathologic behavior of those cells clearly identified as pyramidal tract neurons: cells whose firing patterns during undrugged, behavioral conditions in normal cortex are clearly defined.

The hypothesis that normal cells may be synaptically driven to high frequency firing may be particularly relevant to understanding the rapid spread of epileptiform activity during propagated seizures, as well as explaining the continued high-frequency bursts characteristic of certain neurons during interictal periods. When epileptic neurons produce an ictal event, they presumably recruit surrounding neuronal activity into synchronous firing until a 'critical mass' is reached, at which point clinical manifestations of the propagation of the pathological cellular activity become apparent. We postulate that the group 1 epileptic neurons are relatively autonomous and act as 'pacemakers' to the focus, and that the group 2 epileptic neurons may represent the potential 'critical mass' available for rapid enlargement of the focus. Once this 'critical mass' has been activated, additional normal neurons may be recruited to produce the ictal event.

Ultimately, extracellular recordings cannot unequivocally resolve the issue of whether the primary pathology responsible for hyperexcitable single cell behavior is due to a hyperexcitable postsynaptic membrane or a hyperintense synaptic input,

although the above evidence suggests the former alternative for group 1 cells. The intracellular records of Prince and Futamachi[19] in alumina focus showed mixtures of normal and augmented synaptic potentials, and patterns of single spikes and bursts similar to those characteristic of our group 2 cells. Prince and Futamachi concluded that their observations were consistent with an abnormal synaptic input to cells that otherwise appeared normal. A direct test of intrinsic membrane hyperexcitability by intracellular current injection remains to be done for cells in the chronic alumina focus; such tests in the acute penicillin focus have failed to reveal any membrane pathology.

Although the mechanism by which the epileptic cells become hyperexcitable remains to be elucidated it appears that the bursts of group 1 PT neurons (LFI cells) are probably autonomously sustained, and thereby qualifies such cells as intrinsically hyperexcitable neurons. The concept of 'epileptic' or 'pacemaker' neurons[25] does not necessarily imply complete autonomy, nor does it exclude the possibility of synchronizing influences in a population of cells. Indeed the fact that the epileptic cells could be orthodromically activated and their firing rates modified during operant conditioning sessions confirms the existence of some synaptic input. The ease with which firing rates of cells could be operantly conditioned appeared to be inversely related to their burst index. Thus, an experienced monkey could be trained relatively quickly to bidirectionally control the firing rate of most normal and group 2 cells. In contrast, firing rates of group 1 cells appeared more difficult to operantly condition; rate increases in LFI cells could be more readily produced than decreases[11,29]. However, these findings should be interpreted with caution, since successful operant conditioning depends critically on numerous behavioral variables, particularly the amount of training. Thus, the fact that most group 1 cells were encountered in initial operant conditioning sessions[33] could perhaps partially account for the greater difficulty in conditioning them. The fact that firing rates of some group 1 PT cells with burst indices of 80–95% could be conditioned and the burst index decreased[11] suggests a degree of synaptic control over even highly epileptic cells. Whether this control over 'pacemaker' as well as recruited cells can become therapeutically significant remains an intriguing clinical challenge.

It should be reiterated that these results and hypotheses may only be applicable to alumina gel foci, and are not necessarily related to mechanisms operative in acute foci. Since all the patterns of burst firing (including long-first-interval bursts) recorded from alumina foci in monkey have also been documented in human foci[7], the data reported here may well be relevant to understanding mechanisms operating in human foci.

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