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PUTATIVE NEUROTRANSMITTER EFFECTS DURING EPILEPTIFORM AFTERDISCHARGES IN THE CEREBRAL CORTEX

by

ERNIE ALLEN PUIL

A THESIS

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled PUTATIVE NEUROTRANSMITTER EFFECTS DURING EPILEPTIFORM AFTERDISCHARGES IN THE CEREBRAL CORTEX submitted by ERNIE ALLEN PUIL in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

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PUTATIVE NEUROTRANSMITTER EFFECTS

DURING EPILEPTIFORM AFTERDISCHARGES IN THE CEREBRAL CORTEX

by

ERNIE ALLEN PUIL

ABSTRACT

Subconvulsive electrical stimulation of the cortical surface in cerveau isolé preparations, evokes both excitation and inhibition. Although the inhibition phase appears to be more intense at higher stimulation rates, early excitation occupies a greater proportion of the inter-pulse interval as the effective stimulus frequency approaches an afterdischarge threshold. During threshold stimulation, the inhibition which usually follows single pulses of a stimulus train is interrupted by an intense repetitive firing with a rapid diminution in spike amplitude. The reduced spike amplitude persists into the ictal period and supports other evidence that seizure activity is produced and maintained by excessive, excitatory synaptic activation. The firing pattern of neocortical neurons subjected to iontophoretic application of L-glutamic acid and/or γ-aminobutyric acid (GABA) was examined during tonic and clonic phases of the electroseizure. Both glutamate and GABA exhibited at times, their well known respective excitatory and depressant effects during seizures. For the most part, however, the effects of glutamate and GABA could not be detected during the course of an afterdischarge. In addition, GABA was often found to increase the appearance of single spikes and to increase spike amplitude. Similarly, glutamate was

capable, on occasion, of causing occlusive effects and decreasing spike amplitude. These findings are interpreted as being a reflection of synaptic input during the afterdischarge. Thus the data suggest that a strong depolarization and inactivation of cells may occur during an afterdischarge and provide presumptive evidence that excessive excitatory synaptic drive may play a role in the maintenance of seizures. The termination of a seizure is accompanied by a decreased responsiveness to iontophoresed glutamate. This can be overcome with larger doses of glutamate and suggests a hyperpolarizing type of blockade. The afterdischarge can be prolonged for periods up to 30 minutes by epicortical stimulation of a low repetition rate. Either glutamate or GABA applied during this epileptiform sustaining response (ESR) depress the entrained activity of single units. These and other data suggest that a "state of facilitation" exists during an ESR which provides a basis for prolongation of seizure activity.

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A. GENERAL INTRODUCTION

In general, an afterdischarge refers to repetitive potential discharges of an excitable cell or a system of such cells. It may appear to be spontaneous or to follow a period during which electrical or chemical stimuli were applied to the cell or to the tissue. Within the spinal cord, the afterdischarge seems to be a physiological event. Sherrington in 1906, first used the term to describe the flexor reflex contraction which persists after the moment of cessation of the stimulus which initiated the spinal reflex (cf. Sherrington, 1947). Although the study of afterdischarges began with the spinal cord, the term especially refers to the cerebral cortex where afterdischarges may be recorded as gigantic electrocorticographic (ECG) potentials many orders of magnitude larger than any other electrical activity of the brain. Within the cortex, afterdischarges appear to be primarily pathological occurrences although normal sensory stimulation has been reported to evoke afterdischarges of cells in the cerebral cortex (Bremer and Bonnet, 1950). However, it has been known for a long time that strong artificial stimuli delivered to the surface of the cerebral cortex of the cat or rabbit may elicit a discharge which continues after cessation of the stimulus and strongly resembles the activity recorded from the skull of a patient undergoing a focal epileptic seizure (Adrian, 1936). Thus it has been demonstrated that stimulation of the retina in epileptics can evoke an afterdischarge. If this spreads sufficiently from sensory cortex to involve motor cortex it will develop into a seizure episode

of the grand mal type (Walter and Walter, 1949). This kind of after-discharge, named by Adrian as the "epileptiform afterdischarge" (EAD), can be produced by a plethora of stimuli in both epileptogenic and normal cortex. For example, cortical seizures may be induced by any of the following:

(1) generalized or local stimulation of the cortex at high frequencies

(2) audiogenic or photic sensory stimulation

(3) metabolic alterations such as those produced by anoxia, endocrine disturbances, water-electrolyte imbalance, etc.

(4) pharmacological agents such as the convulsant drugs administered systemically or applied topically to the cortex

(5) local irritation of the cortex such as by the application of alumina cream

(6) deafferentiation by undercutting a large cortical area (Sharpless and Halpern, 1962).

In addition, electrical stimulation of various subcortical nuclei can cause epileptiform discharges in the corresponding cortical projection (Goldring and O'Leary, 1951; Rakic, Buchwald and Wyers, 1962), and the literature abounds with evidence that subcortical structures do play a significant role in the clinical manifestations of the epileptic discharge. On the other hand, not all seizures caused by any of the above e.g., anoxia, are similar, in electroencephalographic terms at least, to naturally occurring seizures. It is also probable that any cellular abnormality existing in the neuropathology of epilepsy does

not exist in electrically-induced seizures in normal neocortex. Thus, it follows that the mechanism of epileptogenesis may be distinct from the mechanism of the afterdischarge but it does not preclude a common basic mechanism of initiation, maintenance and termination of cortical seizures induced by different stimuli.

Initially, the EAD is a local self-sustained discharge, but when sufficiently intense and repetitive, it can spread from the particular point stimulated to incrementally involve all neurons in a neurologically-isolated slab of cortical tissue (Burns, 1949; Kristiansen and Courtois, 1949). It is clearly evident, therefore, that the synaptic circuitry of the cortex itself is capable of providing the neural substrate for self-sustained, rhythmic neuronal discharge independent of the synaptic activation that it usually receives from afferent input.

The many theories postulated to account for the self-sustained repetitive firing of epileptic cortical cells are based on anatomical, biochemical or metabolic, and physiological alterations of the properties of cortical neurons. Although most of these theories are useful to explain focal cortical epilepsy with neuropathology, they do not necessarily suggest a cause for the electrically-induced EAD in normal cortex. On the other hand, they do have certain aspects which may be applicable in the latter case. The electroseizure appears to represent a distorted or bizarre utilization of normal excitatory, inhibitory, and control mechanisms of a large population of cortical cells. Thus it is conceivable that tetanic stimulation of the pial surface of the brain might cause a transient abnormality or disturbance in the external environment of a population of cortical cells; an example would be a

depletion of an inhibitory transmitter which precipitates (or is a predisposing factor to) a repetitive discharge of aggregates of cortical cells. Indeed, the recent observation that iontophoretic application of penicillin onto a cortical unit clearly increases the amplitude of a test excitatory postsynaptic potential [EPSP](Walsh, 1970) indicates that seizures may be generated by this unusual agent in synaptic areas in a manner somewhat analogous to the potentiation after tetanic stimuli (Franz and Esplin, 1965). For the most part, however, the large body of accumulated evidence of theories of repetitive firing induced by chemical agents, lesions, etc., remains thus far inapplicable to discussions of electroseizures. The material to be reviewed in the ensuing section suggests cortical afterdischarges may be generated in synaptic areas and provides some historical explanations of their mechanism of initiation, maintenance and termination.

B. HISTORICAL REVIEW

There are much experimental data correlating the various kinds of clinical epileptic patterns, i.e., muscle movements, with both chemically- and electrically-induced experimental afterdischarges. In addition, the behavior of many different cortical units encountered by microelectrode recording can be temporally related one to the other and be correlated even to the gross ECG itself (Enomoto and Ajmone Marsan, 1959). In 1939, Adrian and Moruzzi published the first records of discharges from single cortical cells during paroxysmal activity and since that time there are numerous examples of unit activity correlative with the gross surface discharges (for an analysis of the actual contributions of different cortical elements to surface potential, q.v., Creutzfeldt, 1969). Two mechanisms of epileptic discharges from single cortical cells have been suggested (Li, Chou and Howard, 1961):

(1) An intrinsic mechanism characterized by an instability of the resting membrane potential or rhythmically oscillating membrane potentials.

(2) An extrinsic mechanism by which the synaptic input may alter the discharge activity of a given cortical unit.

Adrian (1936) as well as Rosenblueth and Cannon (1942) maintained that the 'intrinsic ability' of cortical neurons to fire repetitively during excitation was sufficient to explain the afterdischarge. Jung (1951) further suggested that the pathological absence of a "braking mechanism" would permit the development of repetitive activity. Bremer (1958a) thought it necessary to postulate a mechanism by which "residual membrane

depolarizations constitute an endogenous stimulus for the self-sustained activity". The existence of regenerative processes suppressed under normal conditions but requiring massive synaptic impingement from the surrounding neuronal pool has thus been the target of experimental investigations.

1. Theories of the Epileptiform Afterdischarge

Some of the earliest observations on afterdischarges indicated that the repetitive firing of cortical cells during an EAD could be explained by the reverberating-neuron-chain theory of Forbes (1929) [see Lorente de Nó, 1933]. About twenty years later, Burns (1951) tested and subsequently rejected the self-reexcitation basis for the EAD. He found that it was not possible to interrupt an afterdischarge by an electrical stimulus presumably strong enough to simultaneously excite a large proportion of cells within an isolated cortical area. On the other hand, the surface-positive burst response which is regarded by Burns as a single epileptic paroxysm meets this important criterion for being due to a recirculation of neuronal activity in a network of interconnected neurons (Burns, 1958).

The early studies of Jasper and Erickson (1941) warrant brief mention since they found that electrical stimulation of the cortex produced vasodilation and an increased blood flow in the area where an afterdischarge was recorded. They concluded that such effects were secondary to the mechanism underlying the afterdischarge. Penfield and Jasper (1954) subsequently arrived at similar conclusions.

a) The Theory of Differential Repolarization

If the positive bursts are repeatedly evoked in one minute, there follows a period of up to an hour during which a series of positive bursts continue. An investigation of these "positive after-bursts" led Burns (1954) to formulate a theory of differential repolarization which has been more recently extended to explain the underlying mechanism of the EAD (Pinsky and Burns, 1962). Burns suggested that following a period of driven activity, the deeper or somatic part of each 'type-B' neuron repolarizes at a slower rate than does its superficial process which extends toward the pial surface of the brain. During recovery from a period of discharging activity, the potential gradient would, therefore, cause current to flow to the apical dendritic region from the relatively depolarized somatic region. If the current flow reaches a critical level, the dendritic region will discharge because of the catelectrotonus due to the positive current leaving the dendrite of the neuron. An analogous process in the cat is believed to underlie the generation of repetitive action potentials of a skeletal muscle fiber whose endplate has been poisoned with decamethonium (Burns and Paton, 1951). Thus repetitive activity is generated by differential repolarization and if the process is repeated following the end of each action potential, convulsive activity is also maintained. Although the idea received originally some endorsement by Bremer (1958b) in his review, the essential evidence upon which this theory is based is mostly indirect. Burns (1953) and others, notably Goldring and O'Leary (1951; 1957; Goldring, O'Leary and King, 1958; O'Leary and Goldring, 1960)

observed that the mean DC potential recorded transcortically from a focus in normal cortex became surface-positive while active. This finding suggested that during seizures, the deep portions of the radially arranged structures were depolarized. King, Schricker and O'Leary (1953) found that an increased surface-negative response to each stimulating pulse was a necessary preliminary for an afterdischarge to occur. Pinsky (1961; 1963) observed that this transcortical potential was a brief (1-2 sec) negative shift appearing maximum between 0.8 and 1.0 mm deep in the cortex. The amplitude of the "negative after-deflection" appeared to be critically related to the stimulus intensity and to the threshold. Similar conclusions have been reached by Gloor and his co-workers (Gloor, Vera, Sperti and Ray, 1961; Gloor, Sperti and Vera, 1962; Gloor, 1962; Gloor, Sperti and Vera, 1964a; 1964b). They found that a critical potential gradient between the apical dendrites and the soma must be generated for an EAD to occur. They suggest that somatic depolarization fires the cell repetitively and thereupon repolarizing sufficiently each time to permit the discharge to continue until cathodal block or synaptic inhibition stops the process. A similar DC "flip-flop" oscillation between pyramidal cells and dendritic layers of the hippocampus has been observed during epileptic paroxysms (Gloor, Sperti and Vera, 1963a; 1963b). In the latter case, the pyramidal cell layer became relatively negative during excessive cellular discharge while a potential shift in the reverse direction was concomitant with the cessation of discharge. Ferguson and Jasper (1971) [see also Jasper, 1969] have recently shown each paroxysmal rhythmic discharge activated by acetylcholine is associated with an abrupt negative shift

in DC potential of the cortical surface which extended down to the fifth layer in which large pyramidal cells lie. The work of Goldring and O'Leary (1951) and Gloor et al., (1961) implicated the theory of differential repolarization in the EAD since they found that artificial surface-positive polarization caused or enhanced afterdischarges while the opposite polarization inhibited seizures.

There are conflicting reports as to whether the dendrites repolarize more rapidly than the soma or vice versa. For example, Ward (1961) and others (Morrell, 1960; Esberard, 1961) have claimed that seizure initiation in cortex with penicillin or aluminum hydroxide lesions may be due to differential repolarization; the depolarized dendrites act as a current sink for the soma. That dendrites require about seven times longer to repolarize than the cell body has been reported to be the case for the giant Mauthner cells of the catfish (Tasaki, Hagiwara and Watanabe, 1954). Also dendritic responses in the cat's striate cortex have been found to be longer than those of the cell body or axon (Tasaki, Polley and Orrego, 1954). A similar phenomenon has been also observed to occur in the lateral geniculate (Tasaki, et al., 1954; Vastola, 1959a; 1959b) and in the cat's pyramidal cells where the apical dendrites repolarize at a slower rate following excitation than the soma (Clare and Bishop, 1955). Irrespective of the polarity of the potential gradient along the cell, it has not been proved if different repolarizing rates are due to differential properties of the somatic or dendritic membranes, or if the gradient is due to differences in synaptic input. Indeed, spike initiation in the central nervous system (CNS) is known to occur from the axon hillock region

(Phillips, 1956a; 1956b; Fuortes, Frank and Becker, 1957). This area is believed to repolarize at a faster rate than the soma or the dendrites in spinal motoneurons (Terzuolo and Araki, 1961) and also have a lower threshold of excitation in pyramidal cells of neocortex (Phillips, 1961; Li, 1961). Different rates of repolarization in cortical cells is of fundamental importance to the study of afterdischarges. For example, during propagated seizures in neocortex, Sypert, Oakley and Ward (1970) found the clonic phase corresponded to a slow rate of repolarization of neurons during which time, recovery of the A (initial segment) spike generator was followed by recovery of the B (soma-dendritic) spike generator.

b) Repetitive Discharge in Nerve Terminals

There is evidence that repetitive synaptic activation produces changes both presynaptically, and postsynaptically. Standaert (1963) has suggested by analogy to the formation of generator potentials of afferent nerves, that the failure of nerve terminals to repolarize induces a post-tetanic repetitive activity (PTR) in the terminals. PTR has been demonstrated to be a cause of post-tetanic potentiation [PTP](Standaert, 1964) which can also be induced by a high frequency stimulus in the neuromuscular junction, in spinal polysynaptic pathways and in various other pathways of the brain. On the grounds that anti-convulsant doses of diphenylhydantoin suppresses both PTR (Esplin, 1957; Paris and Raines, 1963; Raines and Standaert, 1966) and PTP (Franz and Esplin, 1965), it has been suggested that the primary site of repetitive activity in an epileptic seizure is the nerve terminals. Raines and

Standaert (1969) have stated what the mechanism of PTR is in the following way:

(1) At the nerve terminal, the axon loses its myelin and there is juxtaposition of a large diameter myelinated and a small diameter unmyelinated fiber. The parent myelinated fiber is assumed to respond to an adequate pre-tetanic stimulus with an action potential followed by brief negative and positive afterpotentials. The fine unmyelinated terminal responds similarly but with larger and longer lasting afterpotentials.

(2) After high frequency stimulation the axon is supposed to be more hyperpolarized than the terminal. The action potentials during the post-tetanic period are consequently augmented and have greatly enhanced afterpotentials.

(3) The negative afterpotential of unmyelinated nerves appears to be affected to a greater degree than that of myelinated nerves (Brown and Holmes, 1956) so that a potential difference will occur between axon and terminal. The axon would be expected to discharge because the "cathodal focus" in the nerve ending produces a flow of current exceeding the rheobase of the last node of Ranvier.

The above explanation is only one of several possible mechanisms for PTR and PTP. For example, post-tetanic hyperpolarization does not always accompany PTP. Thus Esplin and Zablocka (1969) emphasize a more basic membrane process to underlie PTP in which they have demonstrated transmitter release to be enhanced for several minutes following

presynaptic tetanization. An implicit assumption of this hypothesis is that PTP would progressively recruit more neurons from subliminal firing levels by temporal summation of the effects of enhanced quantities of transmitter and thus produce a spread of the seizure. Recently, Izquierdo and Nasello (1970) have presented pharmacological evidence, albeit indirect, that PTP and seizures in the hippocampus may be due to a common mechanism since both processes are similarly affected by drugs which alter extracellular potassium accumulation brought about by the firing of afferent fibers.

Weight (1968; see also Toman, 1969) has recently examined the high frequency repetitive activity which was formerly attributable to the Renshaw cells and suggests that it is essentially a locally stimulated repetitive firing of motor nerve terminals. It is of interest that the first spike in the Renshaw cell repetitive discharge appears to be unblockable by drugs like dihydro-β-erythyroidine and may be due to an antidromic potential reflecting a PTR. PTP has been demonstrated for cortical neurons (Krnjević, Randić and Straughan, 1966a) and other evidence from work unrelated to PTP has implicated the phenomenon in epileptogenic cortex. Using interval analysis and joint interval density technique, Calvin, Sypert and Ward (1968) found the interval between the first and second spikes of a burst during an EAD to be longer and more variable than the subsequent intervals. In addition, the remainder of spikes within a burst were "quite stereotyped and locked to the second spike". Ward and his co-workers (see Ward, 1969 for discussion) chose to interpret these data on the basis that it was obtained from an axonal extracellular recording site. They proposed

that the first spike in these instances is a spontaneous antidromic one which in some way, possibly through recurrent excitation, elicits the remainder of a burst. Furthermore, to explain the spread and maintenance of seizure activity it was suggested that both the antidromic spike and the subsequent burst propagate via axon collaterals to normal cells, and presumably by virtue of the "potent input" induce repetitive firing in cell populations away from the epileptogenic focus.

c) Persistence of Transmitter

A type of repetition due to an excess of persistence of transmitter has been demonstrated for skeletal muscle fibers in the presence of an anticholinesterase agent. If a frog skeletal muscle fiber is treated with physostigmine, the response of the fiber to a single nerve volley is a repetitive discharge. This has been held due to the persistence of the transmitter acetylcholine so that the endplate remains depolarized long enough to form the origin of a series of action potentials in the muscle fibers. Essentially, the depolarized endplate region of each muscle fiber becomes a current sink for the membrane potential of neighboring normal fiber. As repolarization of the fiber progresses an outward current which flows across the membrane adjacent to the endplate increases to the threshold point at which moment another action potential is generated. This type of mechanism has been used to explain the so-called "physiological afterdischarge" of the Renshaw interneurons in the spinal cord (Eccles, Fatt and Koketsu, 1954; see also Eccles, 1964) and has been held responsible for the antidromically-induced inhibition first described by Renshaw in 1941. Since anticholinesterase

agents increased the discharge of these cells while dihydro-β-erythroidine abolished their repetitive response, it has been suggested that the prolonged afterdischarge which follows a single antidromic volley is maintained by the persistence of transmitter. This type of afterdischarge bears strong resemblance to that caused by physostigmine at the neuromuscular junction since both begin with very short latency with the highest frequency of discharge occurring at the start of each burst. In this regard, the afterdischarge produced in frog skeletal muscle fibers with local application of veratrine sulfate (Burns, Frank and Salmoiraghi, 1955) affords a better comparison with cortical afterdischarge bursts because the highest frequency of discharge does not always occur at the start of each burst.

Eccles (1964) has suggested that the repetitive firing in central neurons is attributable to a prolonged action of transmitter produced by a single synchronous synaptic bombardment. However, the condition of prolonged transmitter action has not been found to be essential for multiple discharge of central neurons. In motoneurons, Kuno and Miyahara (1968) and Kuno (1969) showed that the presence of an "intense synaptic action" was necessary but not the only prerequisite for the occurrence of multiple discharges since the latter can be absent when large EPSPs are observed. That is, no multiple discharge occurred because of the limited firing rate at which motoneurons can discharge. On the other hand, dorsal spinocerebellar tract (DSCT) neurons are able to discharge at higher frequencies (< 1000/sec) so that their refractory period is short enough to allow a multiple generation of spikes when the transmitter action lasts about 2 msec. Thus the multiple

discharge of DSCT neurons to single afferent volleys was considered by Kuno to be a reflection of synaptic input.

2. Unit Behavior during Afterdischarge Activity

It is misleading to imply that any single theory discussed in the previous section explains adequately the mechanism of afterdischarge activity in normal cortex. The aforementioned theories save Forbes' hypothesis resemble one another in the sense that a critical potential gradient is required for repetitive firing to occur. Also, the theories of "persistence of transmitter" and "PTP" might even be considered a single mechanism. The enhanced transmitter release produced by PTP might cause a persistence of transmitter inducing a recruitment of neurons from previously non-responding members of the postsynaptic population. Both "differential repolarization" and "PTR" theories may depend upon gradients along the cell for a discharge which might be considered 'pacemakers' once initiated. The "follower neurons" of Aplysia, a marine mollusc, have been shown to have an endogenous rhythm due to a pacemaker property intrinsic to the neuron which persists in the absence of synaptic input (Alving, 1968; Frazier, Kandel, Kupfermann, Waziri, Coggeshall, 1967; Strumwasser, 1965). However, the complexities of cortical connectivity make it very difficult to prove experimenaally if a given cell is capable of an autonomous discharge, i.e., whether its rhythmic discharges during an EAD are occurring in the absence of all inputs from other elements. Clearly, extrinsic mechanisms underlying the EAD may evoke two types of unit behavior:

(1) where the firing frequency of a cortical unit is related to the time course of synaptic depolarization.

(2) where the synaptic input augments a state of facilitation or autonomous activity which keeps the cell firing after removal of the stimulus.

The evidence to be reviewed in the present section has been selected to provide attention to the aforementioned extrinsic mechanisms, particularly to the nature of synaptic input during afterdischarge activity.

a) Synaptic depolarization

To account for self-sustained repetitive firing of cortical units during an EAD, it has been maintained that either of two conditions must exist (Ajmone Marsan, 1961). Firstly, the cortical neuron might undergo an extremely slow repolarizing process when the cells were excessively depolarized, an idea obviously incompatible with known values for membrane time constants in general (Eccles, 1957) and cortical membrane time constants in particular (8.5 ± 2.2 msec, cf. Creutzfeldt, Lux and Nacimiento, 1964). The second, better possibility would be the establishment of a steady state depolarization, a condition believed to occur in peripheral nerve fibers, receptor organs, and in spinal motoneurones (Alvord and Fuortes, 1953; Barron and Matthews, 1938). It has been postulated (Ajmone Marsan, 1961) that excitatory and inhibitory connections in an "epileptic neuronal pool" and consequent "reciprocal impulse impingement" among its various elements would result in this steady state of depolarization. Thus, in the absence of an autonomously discharging process, a self-sustained repetitive activity may occur with

constant or cyclically-altered frequency which would vary according to the degree and uniformity of impulse input to the cell. When spinal motoneurons are subjected to intense synaptic action, no repetitive discharge occurs because of the limited frequency at which motoneurons can discharge (Kuno, 1969). Complete recovery of the resting polarization level of the membrane cannot occur with higher frequency stimulation since each subsequent stimulus establishes progressively lower values of membrane polarization. With sufficiently long stimulation, an excess of depolarization is reached, i.e., the firing level is exceeded and the cell becomes inactivated (Curtis and Watkins, 1959; Eyzaguirre and Kuffler, 1955; Fuortes, 1958; Granit and Phillips, 1956). It has also been shown that stimulation of the superficial radial nerve of the cat, i.e., a relatively physiological synaptic input, can depolarize a cell in somatosensory cortex to inactivation of the spike generator (Li et al., 1961). Thus it has been demonstrated that an excessive depolarization in the course of an epileptic seizure can inactivate the spike generator (Kandel and Spencer, 1961a; 1961b; Matsumoto and Ajmone Marsan, 1964; Sawa, Maruyama and Kaji, 1963; Sawa, Kaji and Usuki, 1965; Sawa, Maruyama, Kaji and Nakamura, 1966; Sypert et al., 1970).

b) Unit activity during an afterdischarge

The activity of single cortical neurons recorded during electrically induced seizures has been observed by extracellular methods (Ajmone Marsan, 1961; Creutzfeldt, 1957; Creutzfeldt, Lux and Watanabe, 1966; Fernandez-Guardiola, Manni, Wilson and Dow, 1962; Gerin, 1960; Sypert et al., 1970) and intracellular techniques (Sawa et al., 1963;

1965; Sawa, Nakamura and Naito, 1968; Sugawa, Goldring and O'Leary, 1964; Sypert et al., 1970). The results of these studies generally support evidence that the soma of a cell is excessively depolarized (see INTRODUCTION B-1). The onset of a propagated seizure in epileptogenic foci is characterized by the replacement of afterhyperpolarizations with depolarizing waves that become longer and more enlarged as the seizure approaches. Action potential size and shape remains quite uniform at this time but during repetitive stimulation and the subsequent tonic phase (i.e., when large populations of units discharge in a random pattern of single spikes after the tetanic stimulus) of a seizure, the same spikes undergo a rapid reduction in spike amplitude (Sypert et al., 1970). Also evident during the tonic phase is a progressive increase in the rate of occurrence of large depolarizing waves with high frequency bursts of decrementing spike amplitude. The transition of the tonic to the clonic phase of a propagated seizure is characterized by a depolarizing block of the spike-generating mechanism verified by the failure of antidromic invasion (Sypert et al., 1970). Repolarization allows some soma firing again and a progressive increase in spike amplitude occurs until the preictal spike size is again reached. During the clonic phase (i.e., when a more regimented pattern of unit firing is present and coincident with surface waves), the increasing repolarization of the membrane toward the original resting potential is accompanied by a gradual diminution in number of large depolarizing waves (Sawa et al., 1963; 1968; Sypert et al., 1970).

There are conflicting reports regarding the behavior of cortical cells at the end of a seizure. For example, the arrest of an afterdischarge

has been attributed to a long afterdepolarization (Matsumoto and Ajmone Marsan, 1964); an excess of depolarization (Grafstein, 1956; Marshall, 1959), spreading depression (Krnjević and Randić, 1965; Krnjević, Randić and Straughan, 1966b), and membrane hyperpolarization not due to prolonged inhibitory post-synaptic potentials [IPSPs](Ayala, Matsumoto and Gumnit, 1970; Sawa et al., 1963; Sypert et al., 1970) with a concomitant elimination of EPSPs (Sawa et al., 1965; 1968).

The aforementioned unit behavior during seizures is not invariably the case. Sypert et al. (1970) have classified, according to their behavior, three types of cells as types I and II (active) and III (passive). The active types exhibit the characteristics described above except that their patterns of firing differ in qualitative aspects such as an increase in firing frequency during the period of tetanization observable only in type I neurons. In the passive neurons, rhythmic depolarizations associated with high frequency bursts of spikes occurred throughout the tonic and clonic phases without inactivation of the spike-generating mechanism. The intracellular potential changes observed by Sawa et al. (1968) were similarly considered to reflect three states of cellular activity. Cells in the "propagated seizure state" would correspond to type I neurons of Sypert and his coworkers since a sustained membrane polarization was generated after the end of tetanization. According to Sawa et al. (1968) little or no sustained depolarization occurs in cells of the "projected seizure state" which may be correlated to the type III neurons of Sypert et al. (1970) or the "passive neurons" of Matsumoto and Ajmone Marsan (1964). Also, Kandel and Spencer (1961c) have described cells which have behaved similarly with predominant

inhibitory changes during hippocampal seizures. These cells appear capable of generating oscillatory transients with or without spikes even in the postictal phase. Cells in a "depressed seizure state" do not display normal membrane transients; the EPSP and IPSP responses to single stimuli are depressed and membrane oscillations and spontaneous spikes do not occur in these neurons. Sawa and his associates believed that a cell is in the "depressed seizure state" when it is under excessive synaptic bombardment originating at a remote epileptogenic region. When this state is overcome and IPSPs eliminated, the cell goes into a "projected seizure state". Where a resultant summation of EPSPs occurs and a sustained membrane depolarization is generated the cell is said to be in the "propagated seizure state" (see Sawa et al., 1968).

The suggestion that cell participation in seizures is by depolarization maintained by synaptic activity is in contrast to the concept that the soma is depolarized more than the dendrites as stated by Burns (1958). In epileptogenic cortex, Schmidt, Thomas and Ward (1959) found no attenuation of spikes during high frequency activity of bursts and suggested that spike initiation was not occurring on the axon hillock. They marshalled evidence that the dendrites are depolarized relative to the soma in epileptic foci. The capacity for sustained autonomous discharge is, according to these workers, a fundamental property of the "epileptic neuron". However, as pointed out above, some cells are evidently maintained in a partly depolarized state throughout most of the seizure, others hyperpolarize and do not fire during ECG afterdischarge activity, and still others, e.g., the type III neurons of Sypert's group, exhibit rhythmic depolarizations and bursts without spike attenuation.

Also, from the predominance of synaptic potentials that occur approximately concurrently with the EAD waves of the ECG, it is evident that synaptic activation plays a large role in the maintenance of seizures. While it can be proposed that massive synaptic impingement is the mechanism by which otherwise normal cells are driven into ictal activity, the question is whether it is due to an increased excitatory synaptic drive or to the loss of inhibitory mechanisms. Purpura, McMurtry, Leonard and Malliani (1966), for example, have observed that repetitive stimulation of fimbria or dorsal fornix caused epileptiform activity in hippocampal cells in the absence of sustained depolarization of their membrane. In addition, recent evidence suggests that cell behavior during an after-discharge is, to a large degree, determined by a balance between excitatory and inhibitory drives, i.e., synaptic input (Ayala et al. 1970).

C. STATEMENT OF THE PROBLEM

The coexistence of inhibition with excitation appears to be particularly critical in the cerebral cortex where large IPSPs are prominent in synaptic activity. From the evidence reviewed in the previous section, it is reasonable to suppose that an excessive excitatory input or the relative absence of such inhibitory potentials may precipitate the development and maintenance of the seizure discharge. On the other hand, the termination of an afterdischarge might reflect an absence of excitation or a regained inhibitory control mechanism. Depression of cell firing during an afterdischarge may result from:

(1) synaptically-mediated inhibition.

(2) an excessive depolarization and reduction of the firing threshold to a level of inactivation (see previous sections).

(3) disfacilitation.

Conversely, disinhibition may be due to a lack of inhibitory cell activation or of postsynaptic effectiveness of inhibitory transmitter. While intracellular techniques are ideally suited for exposing the neuron's ability to fire impulses, the conditions in the cerebral cortex are not favorable for prolonged intracellular observations, and limit the number of cells that can be studied. Also, the presence of an electrode inside the cell has been reported to profoundly affect synaptic potentials (Coombs, Eccles and Fatt, 1955). Moreover, intracellular measurements of membrane potentials are not simply correlated with changes in neuronal excitability since inhibition cannot always be

associated with a specific directional change of membrane potential, if any change at all (Kuffler, 1960).

The responsiveness of single cells can, however, be tested from outside the cell by the application of excitants and depressants released from an extracellular micropipette. L-glutamate, a general excitant of cortical neurons (see Krnjević, 1970), can be easily controlled by an iontophoretic current and has been used to provide a background of unit firing for the superimposition of inhibition evoked by surface electrical stimulation (Krnjević et al., 1966b). It is possible, therefore, to investigate the balance between excitation and inhibition against a background of glutamate-evoked firing as the seizure threshold is approached or during the entrainment of seizure discharges. If excessive excitatory synaptic activation is present during a seizure, glutamate would be expected to reduce the firing rate and spike amplitude or be unable to cause firing because the membrane potential would be near the inactivation level. Under these conditions, the application of a hyperpolarizing substance may increase spike amplitude or possibly initiate firing. The responsiveness of cortical units during an EAD can be tested by the iontophoretic application of γ-aminobutyric acid (GABA) particularly in cognizance of the strong possibility that this hyperpolarizing depressant is a natural inhibitory transmitter in the cerebral cortex (Krnjević and Schwartz, 1967; 1968). The hypothesis that the termination of a seizure is due to one of the mechanisms described in the previous section, (viz. exhaustion, hyperpolarization, spreading depression etc.) can likewise be investigated. Thus the responsiveness of single cortical cells to surface electrical stimulation,

glutamate and/or GABA can be examined during various phases of an after-discharge and be interpreted as being a reflection (i.e., an indirect observation) of synaptic input.

II. EXPERIMENTAL

II. EXPERIMENTAL

A. METHODS

1. Preparation

Experiments were performed on decerebrate adult cats of either sex weighing between 2.4 and 4.0 kg. The animals were anesthetized in a closed box (33 cm cube) containing a piece of cotton soaked with diethyl ether. The anesthetized cat was removed from the box, the trachea exposed while ether was administered by mask, and continued by tracheal cannula attached to a Trimar^{R} Vaporizer (Ohio). In most cases, the right femoral vein was also cannulated for the purposes of plasma volume replacement. The head of the cat was then fixed in a Czermak-type holder and a wad of saline-soaked cotton wool was placed in the animal's mouth around the tooth-bar to provide a grounding point for the animal. The scalp was incised in the midline to expose the temporal muscles which were reflected, clamped as near as possible to their insertion, and excised above the clamp. One trephine hole about 7 mm in diameter was made in the left temporal eminence of the skull. Bone was nibbled away with a rongeur, from the bony tentorium to the anterior border of the ectosylvian gyrus, and from the middle of the marginal gyrus to the inferior border of the ectosylvian gyrus. Bleeding from bone was controlled with bone wax and the entire operating field kept moist with warm (~37°C) physiological (0.9% NaCl) solution throughout the course of the surgery. The dura mater was incised and removed along the length of the exposed area, and connecting blood

vessels sealed with a high frequency cauterizer (Birtcher Corp.).

To eliminate possible interference by anesthetics, cerveau isolé preparations were then performed as in the original description by Bremer (1937), i.e., by a section of the brain stem along the plane of the bony tentorium. Several large blood vessels from the tentorium to the posterior part of the hemisphere had to be sealed by electrocautery before the introduction of the decerebration spatula. The brainstem was severed with a blunt, plastic or metal knife curved to facilitate using the edges of the tentorium cerebelli as a guide. In a few cases, 0.1 mg/kg methoxamine hydrochloride (Burroughs Wellcome) was administered intravenously to elevate blood pressure. A small surface area of cortex in the posterior horn of the suprasylvian gyrus was made bloodless by electrocautery. In this area, a hole about 2 mm in diameter, extending from the outside of the brain into the ipsilateral ventricle was made by suction. The purpose of this opening was to permit drainage or reduce swelling of the brain and to reduce the pulsatile movements of the brain. The animals were taken off ether after decerebration and allowed at least three hours to recover. During this time, a large piece of saline-soaked cotton wool was placed over the exposed cortex; this served to reduce swelling and edema. Successful operations usually resulted in the appearance of a characteristic decerebrate rigidity.

To prevent drying and to reduce cooling, the cortex was covered with film strips of thin, opaque polyethylene. The area within the suprasylvian gyrus left free for the insertion of the micropipette was irrigated by a continuous drip of Locke's solution (without glucose)

heated by an automatic thermistor control unit to 37 ± 0.5°C. Most of this area was covered by a transparent acrylic disk (1.2 cm diameter) pressing lightly on the cortical surface to reduce local pulsations; the disc had a small (~1 mm) central hole through which the multi-barrelled micropipette was inserted via a small rip in the pia into cortical tissue. At all times, the rectal temperature of the animal was maintained with a heating pad under the abdomen and automatically controlled at 37 ± 1°C by a thermostat with a thermistor probe (YSI Thermistemp). In cases where it was desired to maintain the preparation viable for periods longer than eight hours, 5% dextrose in saline was dripped intravenously. The contralateral or right hemisphere was then prepared by the aforementioned procedure for experimentation.

2. Experimental Arrangements

a) Stimulation

Surface electrical stimulation was delivered through bipolar platinum ball-tipped electrodes embedded 2 mm apart in an acrylic pressor foot. The ball-tips were filed flat and smooth on the under-surface of the pressor foot so that the stimulating surfaces (each 1 mm in diameter) lay flat on the pial surface of the brain. Function generators (Tekronix 160 series) were arranged to provide trains of 100 pulses at a desired rate (for convenience, stimulation rate will be referred to as stimulus "frequency"). The frequencies used were logarithmic increments from 1.6 to 100 per second. The stimulating pulses were either 0.1 or 1.0 msec square waves of 10 or 20 V amplitude which were delivered

through a photocoupled stimulus isolation unit.

b) Micropipettes

Five-barrelled micropipettes were pulled from borosilicate glass 'blanks' [6.5 mm outside diameter, 4.0 mm bore (Wesley Coe)] to overall diameters of less than a micron. All barrels were filled by boiling the electrodes in 50% methanol solution under reduced pressure. Most of the methanol but for that in the tip (about 1 to 2 cm) was evacuated with a syringe and the individual barrels filled with the appropriate solution. All solutions used including the storage bath for the electrodes were filtered through a 0.22μ pore grate (Millipore^{R}). The electrodes were then allowed at least 48 hours in distilled water at 4°C before the tips were broken under microscopic examination to outside diameters of 4 to 12μ. Electrodes stored in the above way were generally used up to a month.

The center and one side barrel of the micropipette was filled with 90% saturated NaCl (~4.6 M). Resistance of the center recording barrel was 1 to 4 MΩ. The side barrel containing NaCl was used to demonstrated current effects for control purposes; its resistance was 10 to 20 MΩ. Two surrounding barrels were filled with 1 M Na-L-glutamate (pH 6.5, Nutritional Biochemicals) and 1 M GABA (pH 4.2, Nutritional Biochemicals). The resistance of each of these barrels was 30 to 70 MΩ. In some cases, a fifth barrel was filled with a pharmacological agent; in this barrel, 10 mM ouabain octahydrate (Sigma Chemical Company) dissolved in 330 mM NaCl had a resistance of 25 to 50 MΩ. The drug-containing barrels were connected with platinum-tipped leads to a

constant current source. The spontaneous outflow of substances from the side barrels of the micropipette was prevented by small steady retaining currents of the appropriate polarity. The substances were administered iontophoretically with currents derived from high impedance (100 MΩ) sources. Glutamate was ejected as an anion, GABA as a cation while ouabain was released by an outward current (electrode tip positive). The intensity of the ejecting currents (in nanoamperes) was read directly on a series galvanometer (Fluke, Inc.) and the relative level displayed on the split beams of the lower trace on the dual beam oscilloscope.

c) Recording

The center barrel of the micropipette was connected with a platinum-tipped lead to the grid of a cathode follower (Model P6, Grass Instruments) and used for recording extracellular unit spikes. The output of the preamplified stage was AC coupled to the amplifier (Type 3A9 differential amplifier, Tektronix) of a dual beam, cathode ray oscilloscope (Type RM 565, Tektronix)[input capacitance 1 nF].

Electrocorticographic recording was obtained with 0.9% NaCl-agar wick electrodes made from 3 mm Pyrex glass tubing. The silk wick of one electrode was placed beside the entry site of the microelectrode into the cortex while the reference electrode wick was placed on a cauterized cortical area on bone. The surface potentials were led by silver chloride-tipped leads into a DC amplifier stage of a pen recorder (RP Dynagraph^{R}, Beckman Instruments). The frequency of extracellular unit activity was measured during the experiment with a rate-meter (Ferch Electronics) having a variable window discriminator input;

the integrated output was then traced out on a second channel of the pen recorder. The stimulating and recording arrangement is shown in Figure 1.

The appearance of extracellular potentials was monitored with an audio amplifier. A Schmitt trigger (Perch Electronics) was used to intensify the z-axis of extracellular potentials as they appeared over or below the noise level and displayed on the CRO for the purposes of photography. When required, photographic records were taken by superimposing three or four sweeps or by moving the film at 1 to 2 cm/sec with the beams stationary. The horizontal axis of both beams were driven externally by function generators to provide a time base identical to the reciprocal of the stimulus frequency. The synchronizing pulses provided by coupled but separate generators and the spikes with z-axis modulation were recorded on two channels of a four channel FM instumentation tape recorder (Precision Instrument). The spike input of the tape recorder was continuously monitored during an experiment on a second CRO (Tektronix 502A) in order to make certain that a faithful reproduction of unit activity was being made. The entire upper beam of the 565 oscilloscope was also recorded for possible later photography along with a verbal commentary of the experimental procedure. The experimental and data collection arrangements are shown in Figure 2.

3. Data Analysis

Two types of statistical analysis were employed: post-stimulus time histograms (PSTHs) where the events/sweep is set to some very large

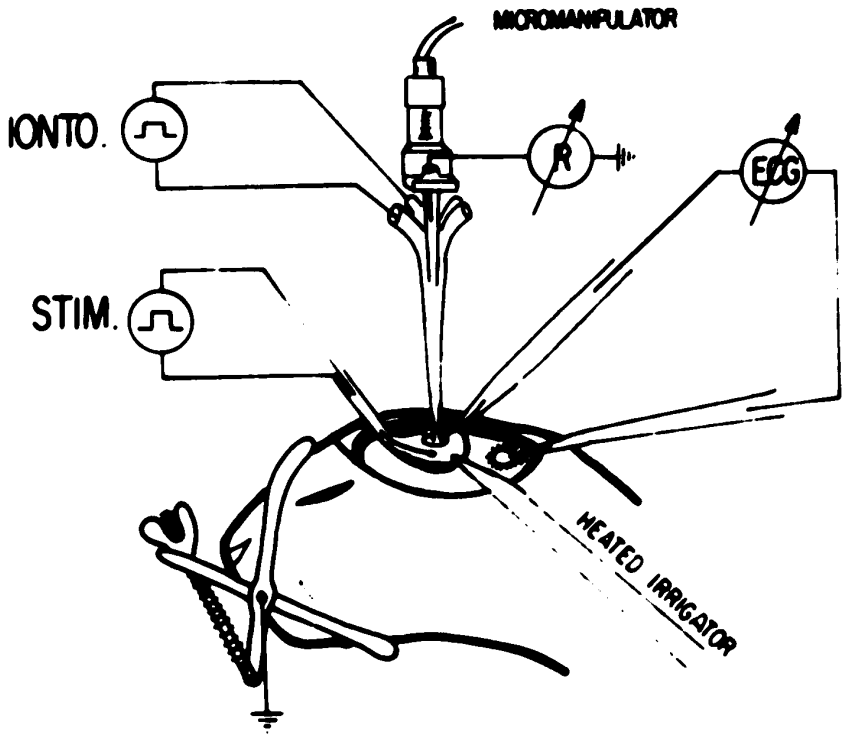

Figure 1. Stimulating and recording arrangement.

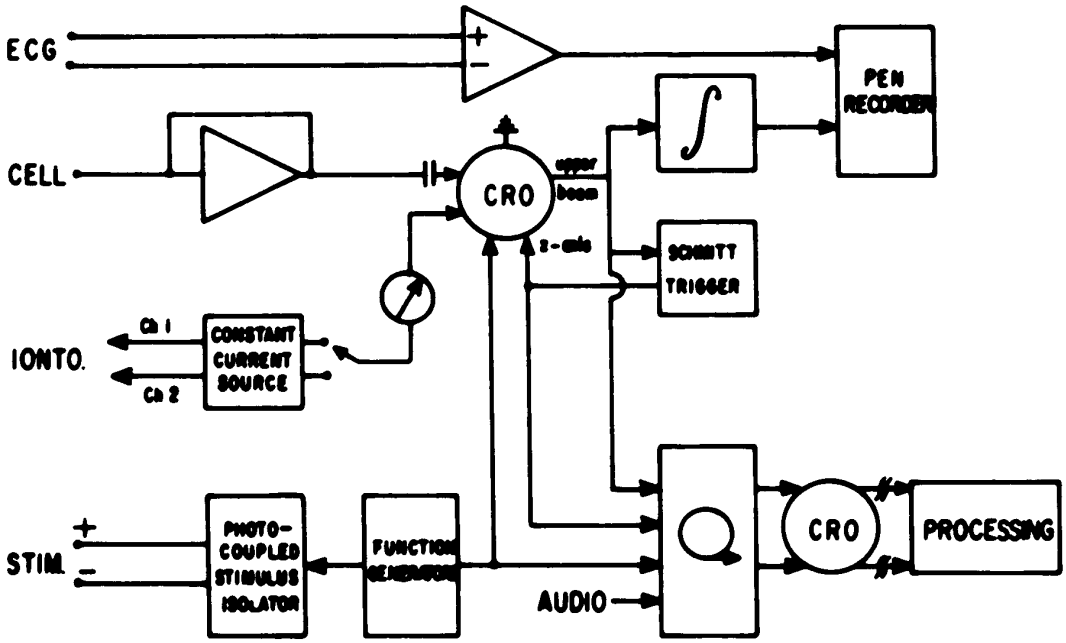

Figure 2. Block diagram of the experimental arrangement showing recording and stimulating apparatus.

number and interspike interval histograms where the events/sweep is 1. A post-stimulus time histogram is a plot of the number of nerve impulses occurring at different times after a stimulus to within the quantity δt (where δt = bin width in μsec). The interval histogram is a plot of the number of intervals in each duration category against the time interval. The interval distribution which is an estimate of the corresponding interval probability distribution function was determined by dividing the maximum number of intervals occurring in a particular bin by the total number n (where n = total number of intervals). The mean ($\hat{\mu}$) of the interspike interval distribution (or an estimate of the reciprocal of the mean firing rate) was calculated from the formula:

$$\mu = \frac{1}{n-1} \Sigma_{i=1}^{n} t_i$$

where t_i is the i'th interval

The probability ($\hat{p}$) of one spike following another was calculated for all interval distributions. $\hat{p}$ is defined by the assumption that within the observed period the probability of observing a spike is unity. Hence for any time bin, $\hat{p}$ is equivalent to the number of spikes within that bin width δt divided by the total number of spikes occurring during the interval "t". The programs used for the event/time histogramming in this study have been described in detail by French (1970). A flow chart illustrating the data analysis procedure is given in Figure 3. Tape recordings used for computer analysis were examined by oscilloscope display and the computer (LAB-8, Digital Equipment Corp.) started manually at the desired section of tape. The digital input buffers of

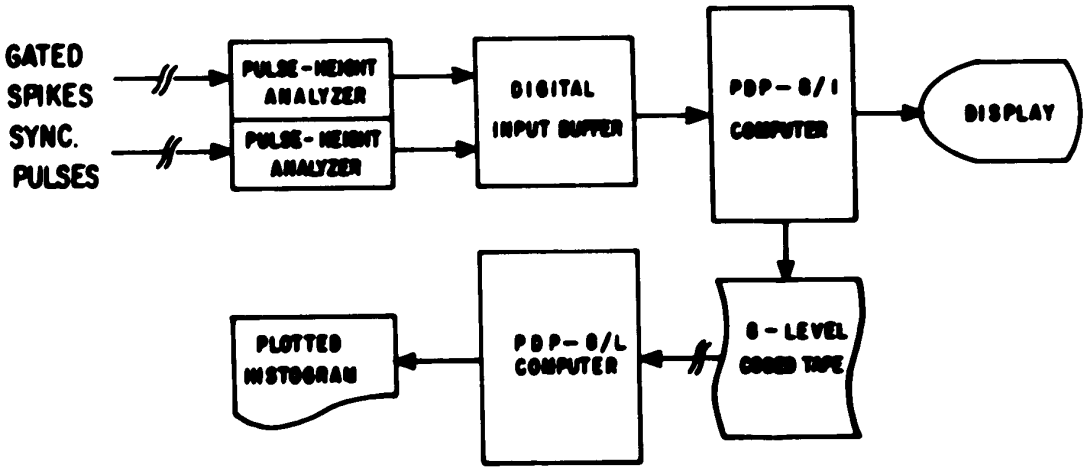

Figure 3. Flow diagram of data analysis procedure.

the computer were triggered with 450 μsec pulses from the output of the pulse height analyzers (Stein, 1968). The crystal clock of the computer limited the selection of bin widths to intervals of 100 μsec. In most cases, the bin width chosen was the sweep length divided by the maximum number of bins available i.e., 128. All PSTHs were compiled from 100 superimposed sweep lengths. The sweep length used for each series of histograms, i.e., for the pre- and post-stimulation control histograms and for the PSTH of the applied stimulus train, was the reciprocal of the stimulating frequency.

In some cases, the total number of spikes were calculated from the PSTH. In order to determine if the summed values were dependent on certain parameters of electrical stimulation (rate and pulse amplitude) correlation coefficients were computed for the two sets of data. This style of analysis was adopted to make maximum use of all cells' data, rather than using only cells for which more than one stimulation rate was used for "paired" analysis.

B. THE EFFECTS OF ELECTRICAL STIMULATION LEADING TO THE PRODUCTION OF AN EAD

1. Experimental Procedure

The experiments of the present section were designed to delineate the sequence of extracellular events leading to seizure initiation by electrical stimulation of normal neocortex. In particular, the question for which an answer was sought concerned the degree to which cells were inhibited as the seizure threshold was approached, i.e., does disinhibition contribute to electroseizure genesis? The results to be described are based on observations of electrical responses from single cortical neurons, excited by iontophoretically-released glutamate, to electrical stimulation of the pial surface of the brain. Because most cortical cells are excited by glutamate (Krnjević, 1970), a steady stream of this amino acid from the micropipette was used as a means of locating unit activity. Approximately 50 nA of glutamate was continously applied whilst the microelectrode was advanced with a micromanipulator into cortical tissue in steps of about 6μ. The identification of unit activity was twofold: (1) the appearance of diphasic or triphasic waveforms above the level of noise, and (2) a firing rate which varied with amount of glutamate applied. The position of the microelectrode was adjusted after encountering a unit to obtain a maximal spike amplitude in response to glutamate, and the depth recorded. The spontaneity of cellular discharge was checked for by switching off the glutamate current. It was also noted if glutamate accelerated its firing rate. In cases where the observed spikes were not of constant amplitude, the

position of the microelectrode was altered to intentionally discriminate against the smaller spikes. If this proved impossible or if it was apparent that more than one cell was responding to the excitant, further study was not attempted. Some cells exhibited high frequency burst activity with decrementing spike amplitude characteristic of cell injury and were also excluded from further study. Exception to the above were cases where two or more spikes of different amplitude were observed in close temporal relation to one another (i.e., a burst in which individual spikes were clearly discernable): if the ratio of spike amplitude between concomitantly-occurring spikes remained the same after moving the microelectrode away from the cell, the burst was considered to reflect the activity of a single cell. Subsequently, a minimal amount of glutamate was applied to cells which were not spontaneously firing to obtain a constant rate of firing of about 5 to 10/sec. The cell under observation was tested for its responsiveness to GABA and the magnitude of GABA current required for inhibition noted for future use during an afterdischarge. Only those cells responding to 40 nA, or less, of either glutamate or GABA were investigated. A period of five minutes usually elapsed after initial contact with a cell before subconvulsive electrical stimulation was commenced for 100 pulses at a frequency of 2/sec. The size and configuration of the electrical stimulus was chosen to provide a maximum afterdischarge duration (Pinsky and Burns, 1962); both voltage and pulse duration remained constant while the EAD threshold was determined. The frequency of electrical stimulation was increased logarithmically for succeeding periods of stimulation until a threshold for an afterdischarge was obtained [for

the purposes of this investigation the term "threshold for an after-discharge" will be implicit with the use of the word "threshold" unless otherwise stated]. Each period of electrical stimulation was preceded and followed by a control period of the same length (equivalent in time to the period of surface stimulation). All cells were observed for their responses during 2/sec stimulation but not necessarily during all other frequencies of stimulation.

2. Results

a) Cell Distribution

The iontophoretic application of L-glutamic acid into the close vicinity of cells of the suprasylvian gyrus causes the cortical units to discharge repetitively with short latency and rapid cessation upon termination of the application. These results are in agreement with a number of investigators, notably Krnjević and his co-workers (1966a; 1966b; 1966c). Also in agreement with the above workers is that no evidence was found in this investigation that cells excited by glutamate are damaged or desensitized in any way. A distribution of cells within the cerebral cortex which responded to minimal amounts of glutamate (2 to 40 nA) and upon which this dissertation is based, is illustrated in Figure 4. The skewed distribution indicates the preponderance of cells recorded from 65 cats in the range of 600 to 1000 microns beneath the pial surface of the brain corresponding to layers III and IV of typical visual cortex. The tip dimensions of the microelectrodes used there are relatively gross so that larger cells would be expected to

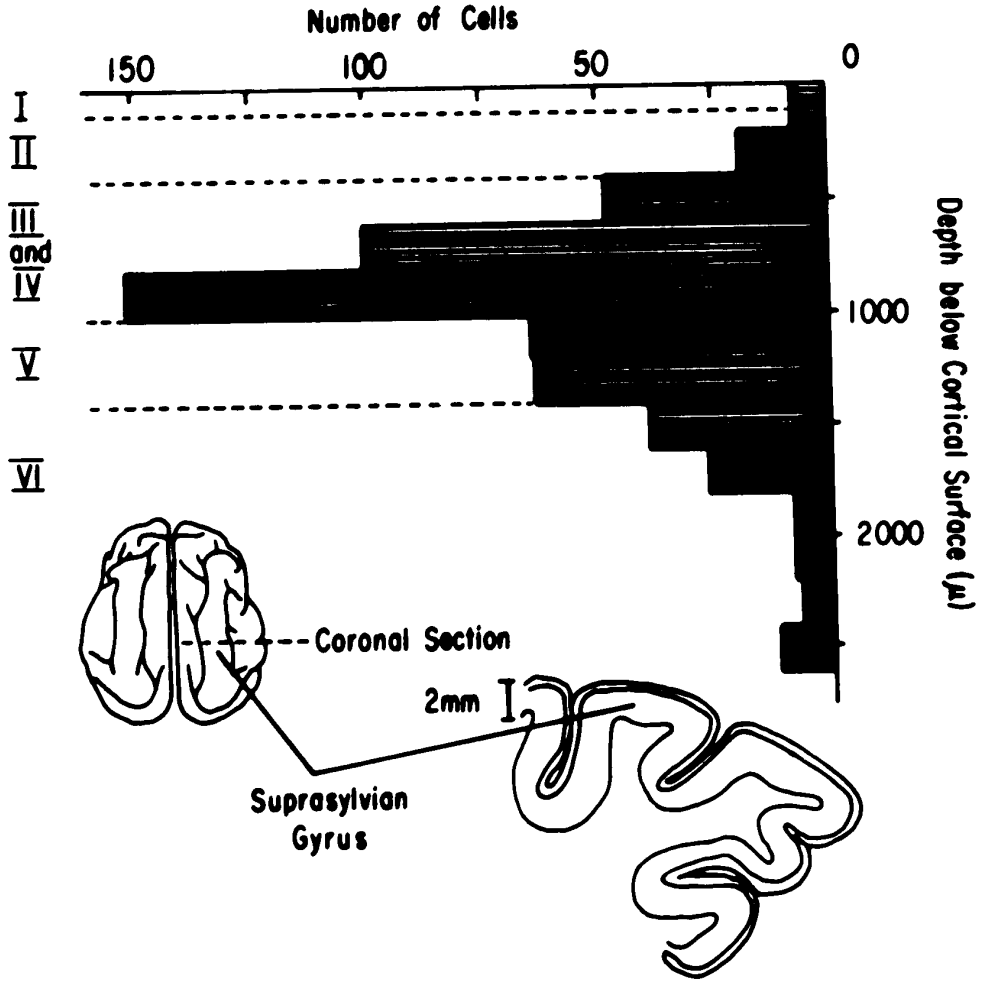

Figure 4. Location of cells studied in cerebral cortex. Lower diagrams are of cat brain as seen from above (anterior is up) and in coronal section (redrawn from Hubel and Wiesel, 1969). Upper plot illustrates the distribution of glutamate-sensitive cells from which responses were obtained and classified according to their depth. Roman numerals on left indicate, approximately, the layers of striate cortex which the depths correspond to.

be recorded more readily than smaller diameter cells (Towe and Harding, 1970). Since the large pyramidal cells of the cortex are located in the layer V, one might visualize the tip of the micropipette resting in layer IV while recording from the soma-dendritic portion of a layer V cell. Some variability, however, has been introduced here. For example, it was necessary to ensure under visual microscopic examination that the tip of the micropipette was inserted into cortical tissue and not just dimpling the pia. An estimate of this variability would be + 100 microns which would tend to bias the distribution of cells found in this study in favor of those in layer V. There have been no reports thus far on a preferential sensitivity of cells of different cortical layers to glutamate.

b) Subthreshold stimuli - qualitative data

The effect of subthreshold epicortical stimulation upon the glutamate-evoked repetitive discharges of a cortical unit can be seen from Figure 5. These data consist of a photograph of about five superimposed oscilloscope sweeps during three periods; prior to stimulation, during stimulation and after stimulation. As can be seen from the photographs during stimulation at 2/sec (Figure 5A) there follows after the stimulus artifact an enhanced firing for about 20 msec. This is particularly evident at 3/sec stimulation in Figure 5B. Typically, this period varies from 10 to 35 msec at 2/sec and 10 to 50 msec at 3/sec. At higher frequencies this early firing may not always be present. The period of enhanced firing is followed by a relative absence of discharge for an excess of 100 msec whereupon firing resumes. No difference

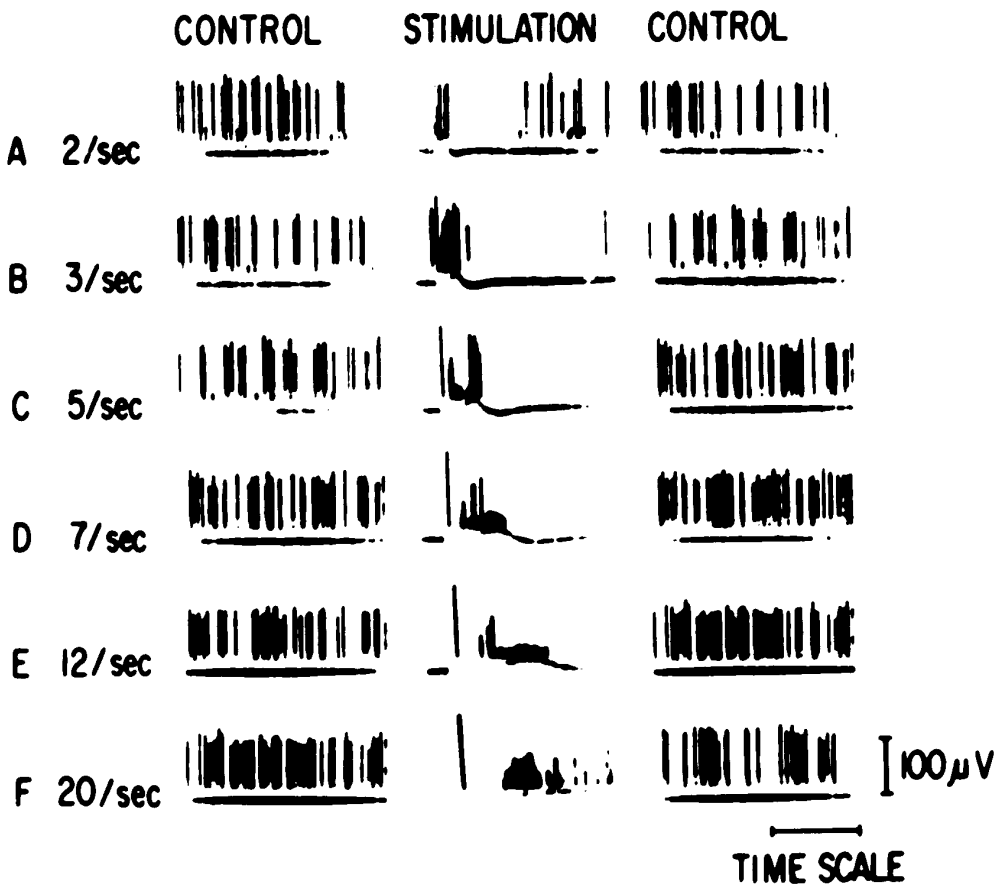

Figure 5. The effect of subthreshold electrical stimulation of the cortex on glutamate-evoked firing of cortical units. Each record is of at least five superimposed sweeps of discharges of cell at depth 781μ in response to 10 nA glutamate. Time scale (msec): in A, 150; in B, 99; in C, 60; in D, 39; in E, 24 and in F, 15.

is apparent between the depression of firing of a spontaneous unit or that of a unit responding to glutamate with a similar background firing rate. That this pattern can be demonstrated for a large number of units is evident from Table I. This tabulation represents the numbers of cells from which film records were obtained and visually examined for depression of glutamate-evoked firing at various subthreshold frequencies in 30 cats. Virtually every cell encountered exhibited this phenomenon save one qualification; in a few cases (< 6 cells) the depression of firing following the early appearance of spikes was visible (during recording) after alternate stimuli or after 2 or 3 stimuli, and not necessarily following every stimulus.

From Figures 5 A to F, it is evident that as the stimulus frequency is raised, there is a progressive diminution of responses to glutamate as indicated by the responses to glutamate before and after stimulation. On the other hand, the stimulus artifact can be seen to occupy a greater proportion of the entire sweep length as the stimulus frequency is raised. Thus at 20/sec in Figure 5F, the stimulus artifact has entered the gate and been brightened before the 'early firing' has taken place. The presence of an artifact did not hamper observations of unit activity which were visible above the level of the artifact itself (cf. Figures 5 C to E). This type of early firing was present at frequencies up to 30/sec; the stimulus artifact prevented definite observations concerning the short latency appearance of spikes at higher frequencies. Early firing was most intense during 3/s stimuli and although present at 5/sec, did not increase as the threshold was approached with higher frequencies of stimulation. In contrast, the depression of

TABLE I

NUMBER OF CELLS EXAMINED UNDER VARIOUS PARAMETERS OF ELECTRICAL STIMULATION[†]

FREQUENCY of STIMULUS (per sec)	10 V	20 V
	NUMBER of OBSERVATIONS[*]	
2	137[††]	33[††]
3	123	11
5	95	2
7	63	3
12	49	1
20	33	2
31	28	1
50	22	3
77	10	0
100	3	4

[*] each observation at a given frequency was made on a different cell.

[†] pulse duration 0.1 or 1.0 msec.

[††] value here represents number of cells stimulated at above pulse strength out of total of 170 cells, i.e., some cells were tested at more than one frequency but only once for any individual frequency. cells used at 20 V are different from those used at 10 V.

responses to glutamate following the stimulus artifact was distinguishable at frequencies above 20/sec since few large spikes can be seen. The periods of observed depression tended to increase with the stimulus frequency. Typically, these periods were of the order of 100 to 300 msec for 2 or 3/sec and usually over one half the time length at higher frequencies. Further analysis of photographic records of subthreshold phenomena was abandoned with the advent of computer analysis reported herein.

c) Subthreshold stimuli - quantitative data

The computer analysis records consisting of post-stimulus time histograms generally complement photographic records of the responses of cells to subthreshold stimuli. Figure 6 shows the responses of a spontaneously-firing cortical unit whose firing rate (~12/sec) is reduced to zero by 35 nA application of GABA and slightly accelerated by 40 nA application of glutamate. The striking feature of the center histrogram is the responses of short latency, i.e., after about 12 msec. [The stimulus artifact in this histogram and in subsequent histograms has been identified visually with an oscilloscope monitor while the computer compiled the histogram and deleted from the record.] The artifact usually was located within the first bin and always within the first two bins at low frequencies of stimulation [at higher frequencies, the stimulus rebound artifact shown in Figure 6 and described previously, has also been excluded from entering the gate level during the actual recording]. Compared with the pre-stimulation control, some depression of glutamate-evoked firing is discernible for an excess of 200 msec

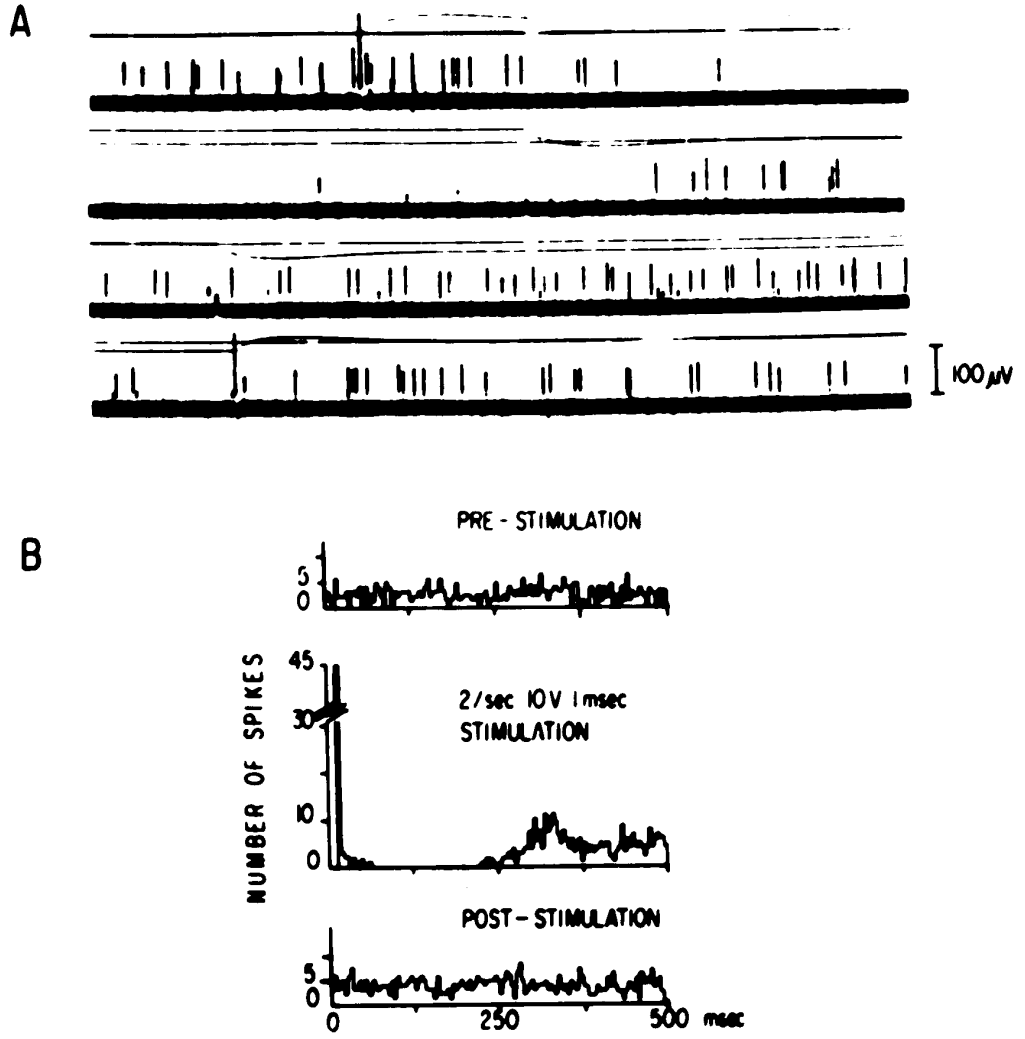

Figure 6. (A) Continuous extracellular record of spontaneously-active unit at depth 980μ. Lines above extracellular record indicate relative level of glutamate or GABA applied. In above and all subsequent records, movement of line in upward direction indicates application of GABA (35 nA in upper trace in A). Movement in downward direction indicates glutamate application (40 nA in 3rd trace). Time marks on iontophoresis reference line are 1 sec.

(B) Post-stimulus time histogram (middle) of same unit as in (A) and during epicortical stimulation at 2/sec. Spontaneously firing in all three histograms.

following the first two bins (7.8 msec). After a slight rebound, the number of spikes tapers off to a level which is above the level of background of glutamate-firing. Relatively few spontaneous cells have been encountered in this investigation and Figure 6 is a representative series of PSTHs taken from one of five spontaneous active units.

The effect of surface electrical stimulation is much more readily seen against the background of glutamate excitation as in Figure 7. The background frequency in this sequence visible in the pre-stimulation histogram was intentionally higher (>20/sec) for the purposes of illustration. Electrical stimulation at 2/sec allows firing of short latency (~25 msec peak) while depressing firing in response to glutamate for about 300 msec. Firing resumes toward the normal background frequency in the last 125 msec of the histogram. When one compares the histograms taken from the periods prior to, and after stimulation, a post-stimulatory depression of the response of the cell to applied glutamate is evident; the number of spikes occurring after the stimulation period is approximately 50% of the number occurring before stimulation commenced.

At 3/sec stimulation (Figure 8) only slight depression of glutamate firing appears after the short latency firing (~30 msec peak). The amount of depression during stimulation is clearly evident by comparison with its control histogram. The time course of depression during stimulation is similar to that observed for 2/sec. However, in contrast to the 2/sec series, no post-stimulatory depression is present, but rather the opposite occurs. That is, a type of 'rebound excitation' (cf. Andersen and Andersson, 1968) occurs which is apparent as a greater number of spikes occurring during the post-stimulatory compared with

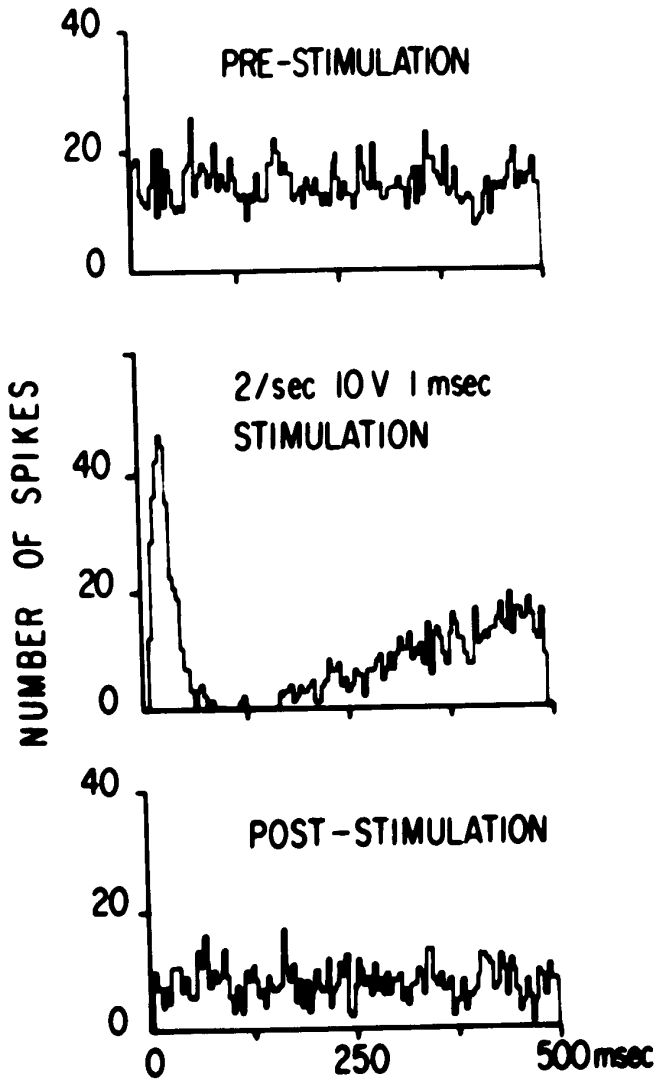

Figure 7. Post-stimulus time histogram (middle) of unit at depth 656μ during 2/sec epicortical stimulation. Glutamate 35 nA during three histogram periods.

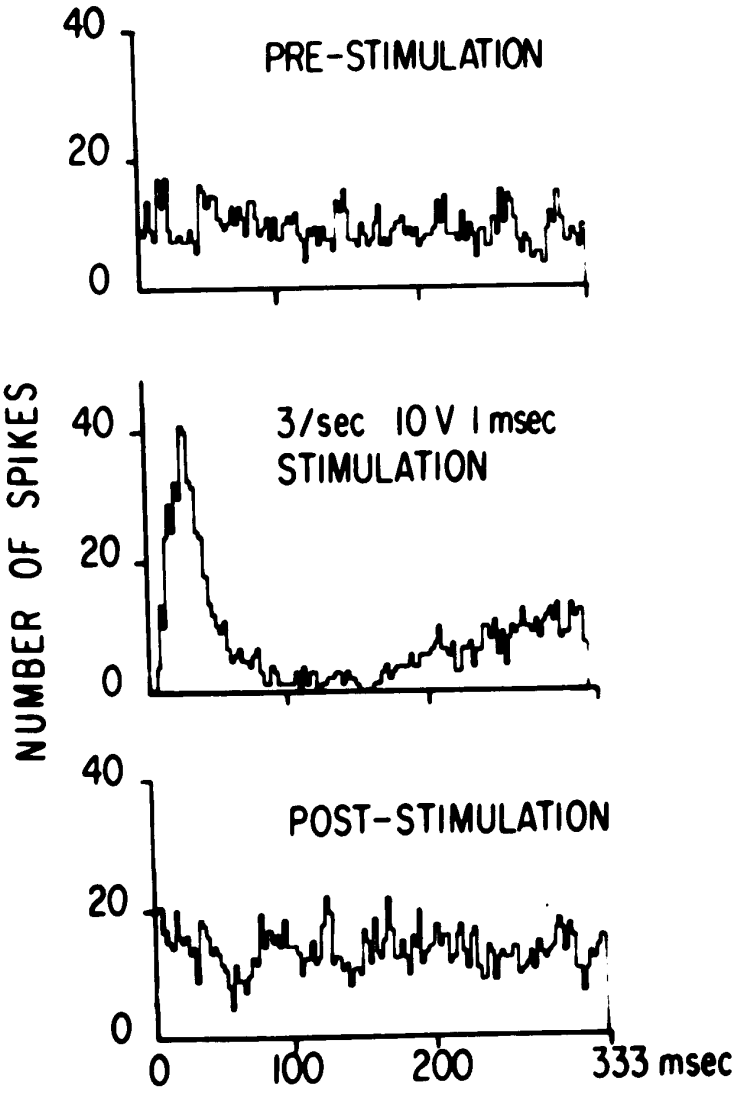

Figure 8. Post-stimulus time histogram (middle) of unit at depth 656μ during 3/sec epicortical stimulation. Glutamate 35 nA during three histogram periods.

the number occurring over an identical time and with the same amount of glutamate as before stimulation.

That this rebound does not always occur can be seen from Figure 9, where the stimulus frequency is 5/sec. The histograms taken before and after stimulation reveal little difference when the two are compared. Depression during stimulation is present but the early firing occupies a considerable proportion of the total time length. When the stimulus frequency is raised to 7.7/sec (Figure 10), the cell appears relatively depressed for some 70 to 80 msec in spite of the early appearance of spikes which are present up to 40 or 50 msec after the stimulus. In both cases where there is strong depression of glutamate-spikes during the stimulus (e.g., Figures 10 and 11), there appears to be a tendency for few spikes to occur in response to glutamate during the post-stimulatory period.

While the aforementioned histograms may be taken to be typical based on more than 300 PSTH series, a considerable range in the duration of excitation and depression occurs during epicortical stimulation of the glutamate-activated cell. Thus the peak of early excitation which is, in most cases, clearly above the level of the glutamate-activated spikes of the control histogram varies between 10 msec and 35 msec after the stimuli of the 2 to 30/sec range. The relative depression visible during stimulation at 2 or 3/sec is usually 65 to 300 msec in duration. PSTHs of the higher frequency stimuli used in this study are characterized by a total absence of firing following the early excitation period. There are a few exceptions to the above. For example, in PSTHs of 9 cells observed at 2 and 3/sec stimulation, the usual depression was not

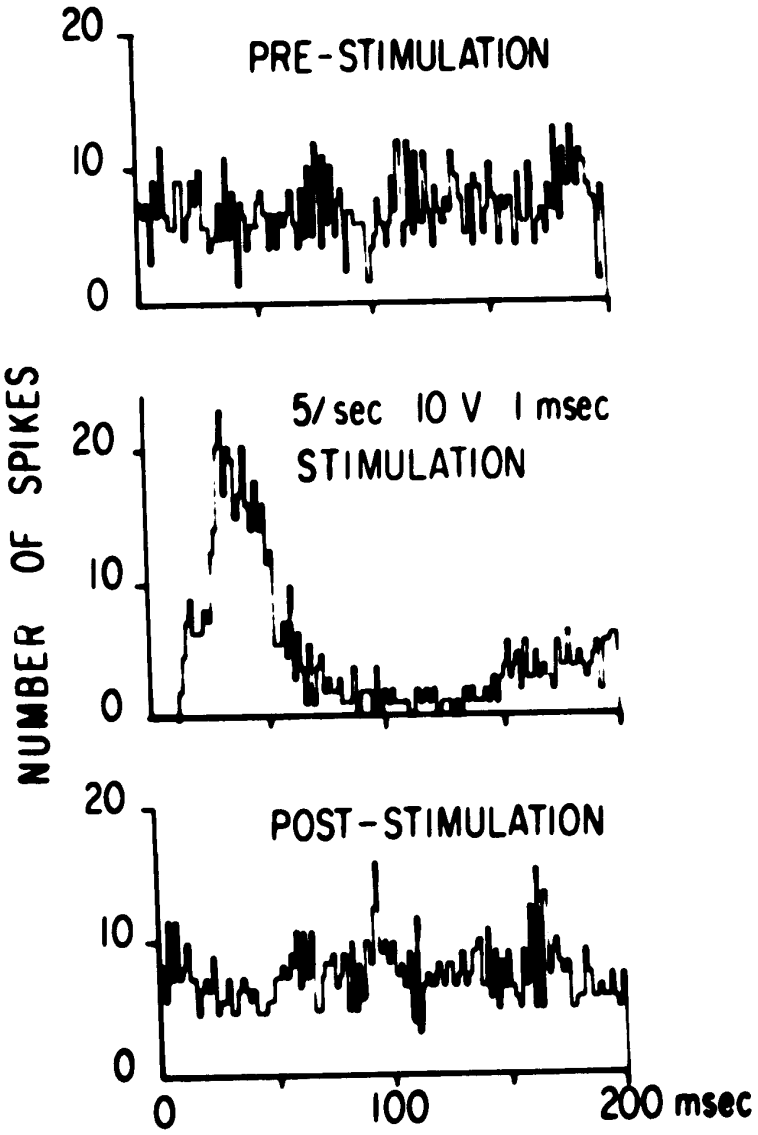

Figure 9. Post-stimulus time histogram (middle) of unit at depth 656μ during 5/sec epicortical stimulation. Glutamate 35 nA during three histogram periods.

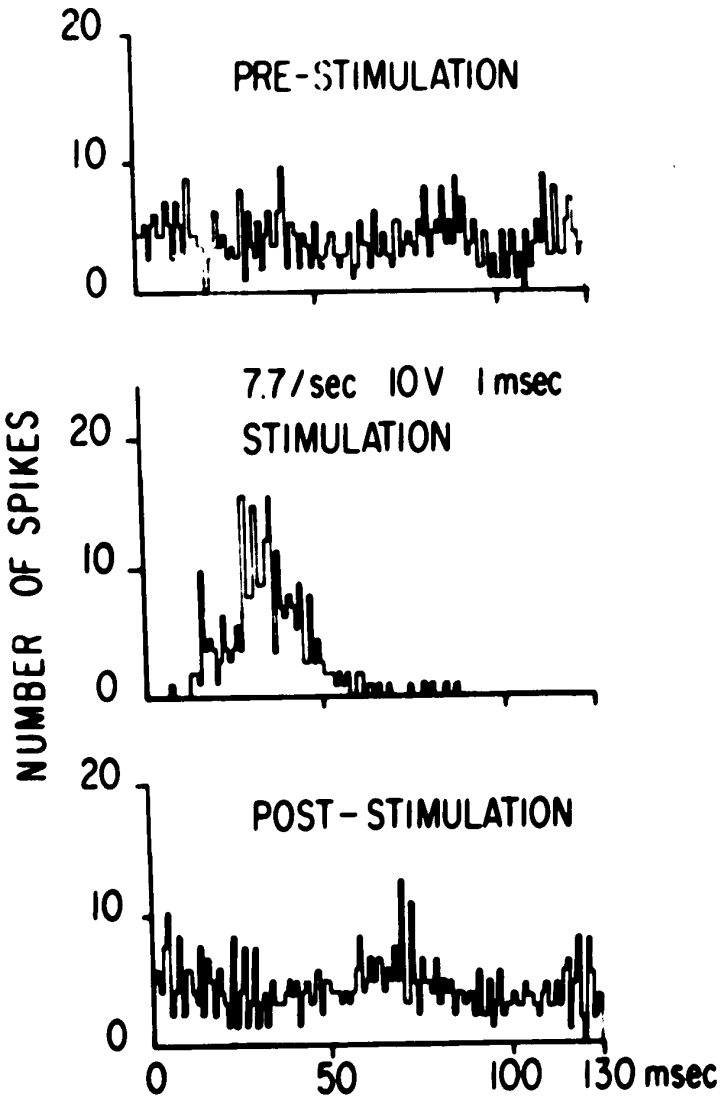

Figure 10. Post-stimulus time histogram (middle) of unit at depth 656μ during 7.7/sec epicortical stimulation. Glutamate 35 nA during three histogram periods.

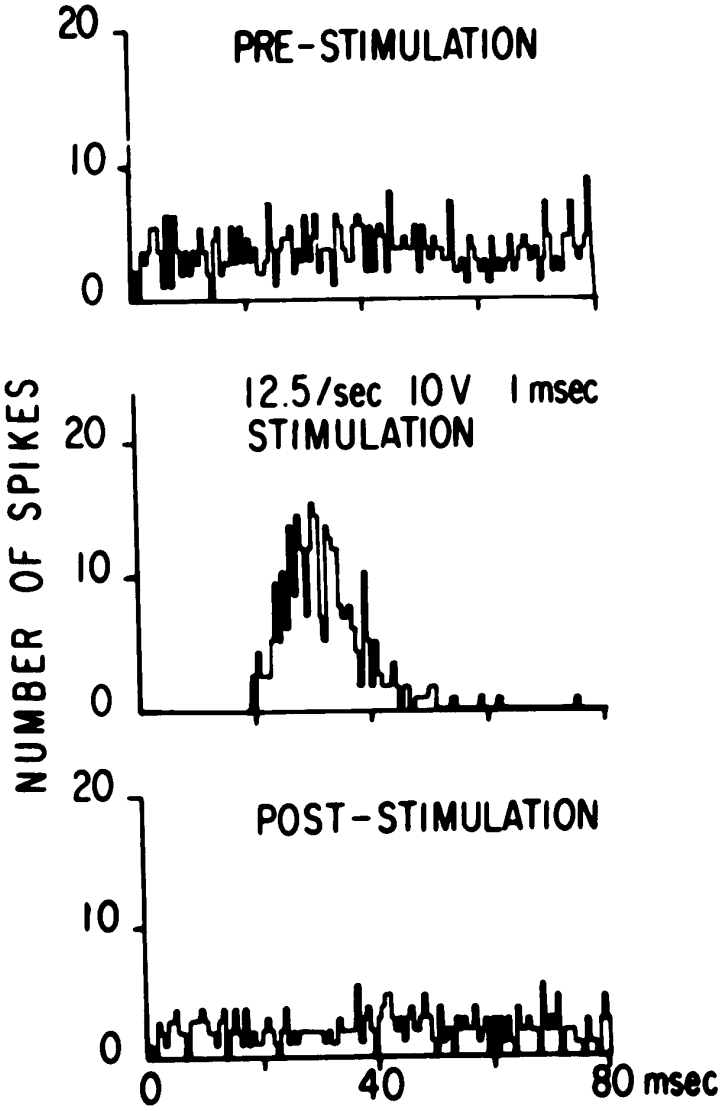

Figure 11. Post-stimulus time histogram (middle) of unit at depth 656μ during 12.5/sec epicortical stimulation. Glutamate 35 nA during three histogram periods.

present when the final histogram was compared with its control. However, extracellular activity monitored during compilation of the histograms indicated that a slightly different process was occurring during stimulation. In 3 of these 9 records, depression of firing was present from 0.3 to 1.0 sec following the stimulus in an alternating fashion. After alternate stimuli, glutamate appeared to cause its characteristic responses to the same extent as present before stimulation. The remainder of the anomalous records exhibited the usual depression pattern in response to low frequency stimulation for approximately one-third to one-half the duration of the stimulus train (25 sec at 2/sec and about 15 sec at 3/sec). Firing for the remainder of the histogram was mostly uninterrupted. There was no evidence that the cell was in any way damaged during this time. One explanation for this type of behavior is that the cortex was in the process of approaching an afterdischarge threshold and that excitatory input was "swamping" the inhibition produced by the individual stimuli.

In an effort to determine if a relationship exists between the stimulus frequency and the amount of excitation or depression, the total number of spikes occurring in each of the three identical time periods (pre-, during, and post-stimulation) were computed from their PSTHs for various frequencies of stimulation. In all cases reported here, the stimulus artifacts were measured on an oscilloscope monitor and their number (100) subtracted from the total. The entire procedure was abandoned for frequencies higher than 20/sec since the artifact's duration relative to the inter-stimulus interval leaves the credibility of the measurement of total spikes in question.

By this type of analysis two conditions have been realized: (1) where the number of spikes in the PSTH during stimulation is less than the number that occurred during the period preceding application of the electrical stimulus. [A time equivalent to the total duration of the applied stimulus train, i.e., 100 pulses at a particular frequency, was used for the control responses to glutamate. The end of this period preceded the initial stimulus pulse by no more than 3 seconds.] This condition will be hitherto referred to as "net depression" during stimulation. The term, (100 - net depression) is defined as "net excitation". (2) where the total number of spikes in the control histogram following the stimulation period is less than the number occurring during the equivalent time period preceding the stimulus train. [The spikes following the stimulation period commenced no later than 3 sec after the last pulse of the stimulus train.] This condition will be hitherto called a long-term "post-stimulatory depression". The term, (100 - post-stimulatory depression) is defined here as "post-stimulatory excitation" and will not be referred to as rebound excitation in order to take cognizance of the possibility of a different mechanism accounting for the latter type of excitation which occurs very shortly after the stimulus in the thalamus.

The incidence of cortical cells exhibiting a net depression during stimulation can be seen from Table II. At first glance, the absence of an increase in net depression as the stimulus frequency is elevated might appear a discrepancy. It must be remembered, however, that the period of early firing within the total time of the histogram does not decrease as the stimulus frequency is raised in a manner similar

TABLE II

INCIDENCE OF CELLS EXHIBITING NET DEPRESSION DURING EPICORTICAL STIMULATION†

SUBTHRESHOLD FREQUENCY of STIMULATION (per sec)	10V			20V		
	NUMBER TESTED	NUMBER DEPRESSED	%*	NUMBER TESTED	NUMBER DEPRESSED	%*
2	80††	27	33.8	70††	23	32.9
3	26	6	23.1	20	9	45.0
5	18	5	27.8	13	7	53.8
7.7	11	4	36.4	11	6	54.5
12.5	9	3	33.3	8	3	37.5
20	3	0	0	2	0	0
THRESHOLD and JUST SUPRATHRESHOLD FREQUENCIES	35	7	20.0	36	8	22.2

† pulse duration 1.0 msec.

†† value here is number of cells from total of 150 cells from 35 cats, i.e., some cells were tested at more than one subthreshold frequency but only once for any individual frequency. cells used at 20V are different from those used at 10V.

* correlation coefficient for relation between subthreshold percentages at 10 and 20V is: $r = 0.83$.

to the increase in the depression phase. Thus there is no apparent trend to an increase of net depression during subthreshold stimulation as one moves down the columns of the Table, at 10 V at least. Because fewer numbers of cells were tested at frequencies greater than 2/sec, a frequency dependence for the 20 V column is not different from a chance occurrence tested by the binomial theorem (5% level of significance). The high correlation coefficient ($r = 0.83$) between 10 and 20 V indicates an absence of a dependence of net depression during stimulation upon the voltage of stimulating pulses. The result of pooled data from separate cells stimulated at various threshold and suprathreshold frequencies suggests that less net depression occurs during threshold stimulation of the cell than during subthreshold conditions. The percentages of net depression during subthreshold stimuli given in Table II can be misleading; each of the 150 cells, for example, may have been stimulated at frequencies other than 2/sec, as well. However, pooled data from the different cells stimulated at a 2/sec subthreshold frequency (cf. Table II) also indicate that more net depression occurs during subthreshold stimulation than during threshold stimulation (i.e., about 33% at 10 or 20 V for subthreshold stimuli versus a mean of about 21% for threshold and suprathreshold stimuli, for all pulse strengths).

Table III is a tabulation of cells exhibiting a long-term depression of glutamate spikes that follows one or two seconds after the stimulus is discontinued. A trend is suggested toward greater long-term depression following high frequency stimuli. However, percentages of post-stimulatory depression from the relatively small sample of units tested at frequencies greater than 3/sec are not significant

TABLE III

INCIDENCE OF CELLS EXHIBITING LONG-TERM POST-STIMULATORY[†] DEPRESSION OF GLUTAMATE-EVOKED FIRING

SUBTHRESHOLD FREQUENCY of STIMULATION (per sec)	10V			20V		
	NUMBER TESTED	NUMBER DEPRESSED	%*	NUMBER TESTED	NUMBER DEPRESSED	%*
2	72[††]	30	41.7	62[††]	28	45.1
3	26	11	42.3	11	9	81.8
5	18	13	72.2	12	6	50.0
7.7	12	8	66.7	8	6	75.0
12.5	7	4	57.1	5	4	80.0

[†] stimulus pulse duration 1.0 msec.

[††] represents number of cells from total of 135 cells from 35 cats, i.e., some cells were tested at more than one frequency but only once for any individual frequency. cells tested at 10V are different than those used at 20V.

* correlation coefficient for relation between percentages at 10 and 20V is: r = -0.095.

at the 5% level when tested for being chance occurrences (binomial theorem). Thus a frequency dependence cannot be demonstrated. The correlation coefficient for the relation between percentage depression following 10 and 20 V stimuli is negative and close to zero ($r = -0.095$). This indicates that the two columns of percentages are not related and suggests that they are dependent on the respective voltage of the stimuli that they follow.

d) Correlations of responses

In addition to the dependence of long lasting post-stimulatory depression of firing on the strength of the stimulus of the preceding period, some correlation was noted between post-stimulatory depression and the behavior during the previous stimulation period. That is, cells excited during the stimulus tended to remain excited in the post-stimulus period and vice versa. Figure 12A histograms at 10 or 20 V show that the cells exhibiting post-stimulatory depression (from Table III) had a previous history of being depressed during stimulation. The net excitation displayed by the rest of the population of cells (from which data were tabulated in Table III) is represented by the total area of the histograms in Figure 12B. Many of those cells that exhibited post-stimulatory excitation (hatched area) had some previous history of net excitation during the preceding stimulation period. This correlation and that of post-stimulatory depression with net depression are more evident for 2/sec since larger numbers of cells were sampled compared with higher frequencies (cf. Figure 12). However, a smaller proportion of these cells continued the enhanced firing rate into the post-stimulus

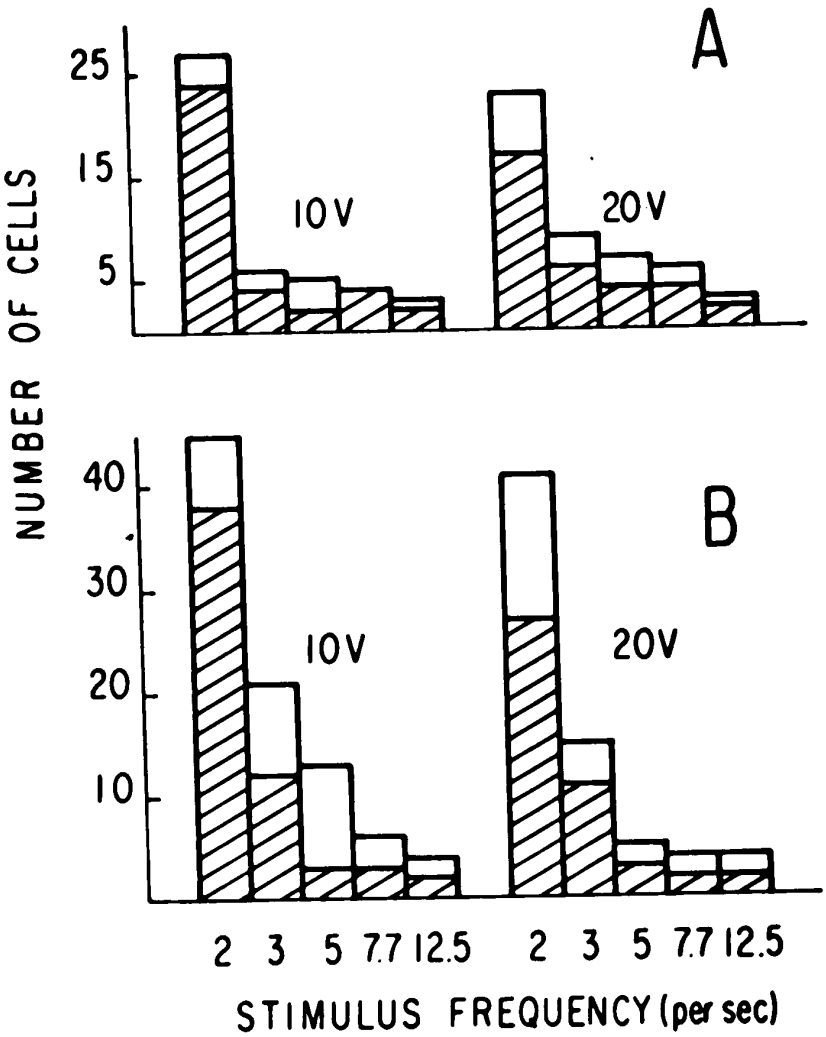

Figure 12. (A) Histogram sample of cells stimulated at 10 V or 20 V which exhibited net depression during stimulation (total area) and post-stimulatory depression (crossed area).

(B) Histogram sample of cells stimulated at 10 V or 20 V which exhibited net excitation during stimulation (total area) and post-stimulatory excitation (crossed area).

period, compared to the proportion of cells which continued an inhibited firing rate from the stimulus period into the post-stimulus period. Thus there is an overall tendency to post-stimulatory depression.

The question was considered whether the responses of net excitation and depression during the stimulation were restricted to particular layers of the cortex or characteristic of some other population of cells in terms of depth. Figures 13 and 14 are semi-logarithmic plots of the responses obtained from the 150 cells used in this study against their depth. A general scatter is evident in the plot of cells stimulated at 10 or 20 V although some "layering" of cells exhibiting net excitation during stimulation at 20 V might be construed. In general, however, cells giving a particular response (obtained during stimulation) did not occur at a preferential depth; the responses did not seem to be attributable to a specific group of neurons or layer within the cortex.

3. Summary and Discussion

The cells studied in this investigation were obtained from all cortical layers. However, the distribution of these cells according to their depth was skewed in the direction of layer IV. This finding suggests that the cells studied are the large pyramidal cells of layers IV and V. These results lend credence to the rule of Towe and Harding (1970) on extracellular sampling bias in the cortex. This is, of course, based on the assumption that most cortical cells are excited by glutamate

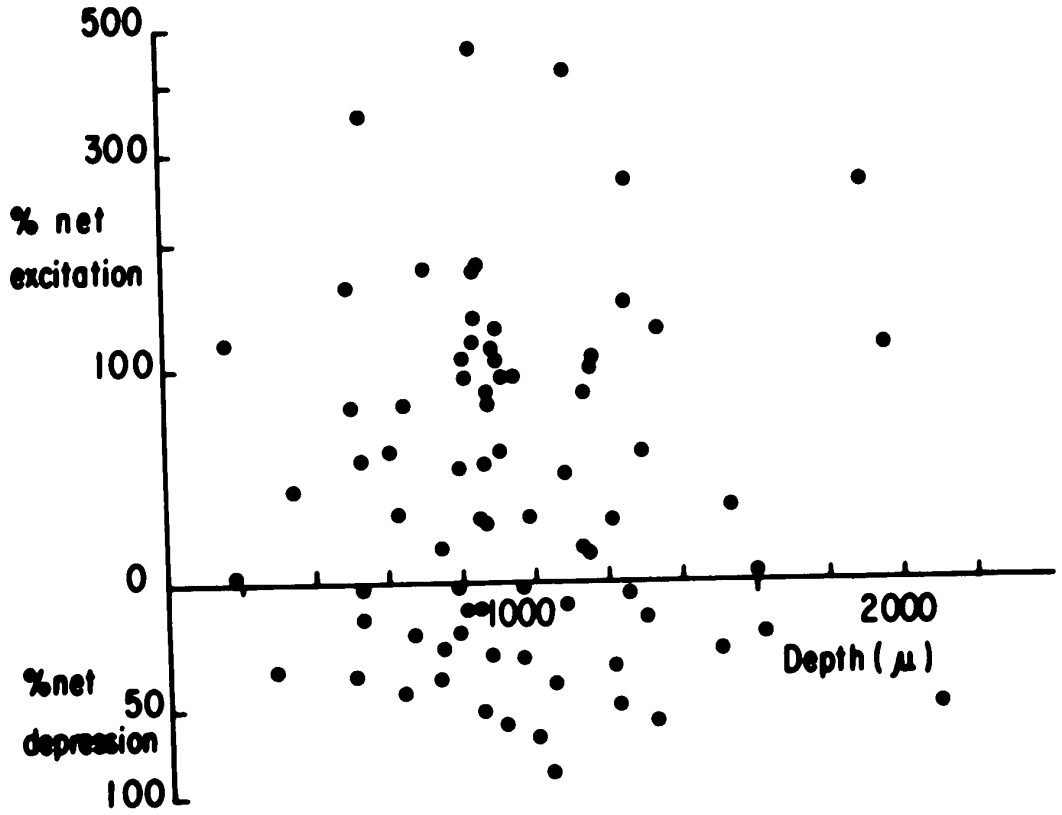

Figure 13. Graph shows relationship between the net excitation or net depression obtained during epicortical stimulation at 2/sec, 10 V, and depth of cell from which response was obtained.

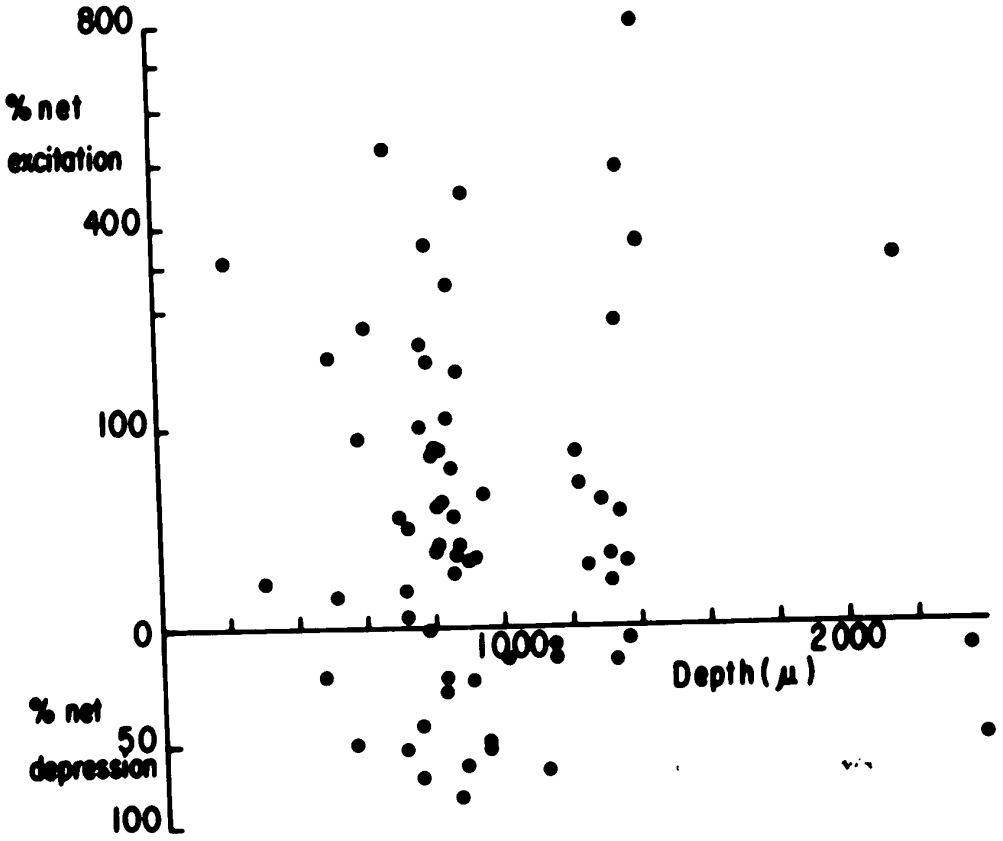

Figure 14. Graph shows relationship between net excitation or net depression obtained during epicortical stimulation at 2/sec, 20 V and depth of cell from which response was obtained.

(cf. Krnjević, 1970). The large pyramidal cells of these layers are known to possess well-developed recurrent inhibitory pathways (Phillips, 1956; Suzuki and Tukahara, 1963; Stefanis and Jasper, 1964; Brooks and Asanuma, 1965a; 1965b). The recurrent excitatory effects have been attributed to the small pyramidal tract cells (Takahashi, Kubota and Uno, 1967). However, the responses of this distribution of cells could not be categorized on the basis of their depths (cf. Figures 13 and 14).

Subthreshold electrical stimulation of the surface of the cerebral cortex elicited an inhibition of discharge of neurons in the presence of glutamate, following each stimulus pulse. This suppression was usually preceded by an early excitation of cells which lasted 10 to 35 msec. These effects have been studied in considerable detail by Krnjević and his associates (Krnjević et al., 1966a; 1966b; 1966c; Krnjević and Schwartz, 1967; 1968). Their studies have established that the depression which follows the period of early firing represents a "true inhibition" lasting 100 to 300 msec and in all probability is mediated through the action of GABA as a synaptic transmitter. The results of the present investigation confirm many of their observations and those of Creutzfeldt, Baumgartner and Schoen (1956) and Berlin (1964) on the time courses of these effects. Evidence has been presented that these events are mediated synaptically (see Li and Chou, 1962; Berlin, 1966). The pertinent points which remain for discussion are those which are relevant to the induction of an afterdischarge.

The simplest explanation for the observed early excitation and subsequent inhibition is that epicortical stimulation activates excitatory and inhibitory synapses. Several assumptions are crucial to this

hypothesis. Excitatory pathways appear to have a lower threshold to electrical stimulation but the stimulus current density used in this investigation is likely high enough to directly affect a large proportion of cells and nerve endings beneath the stimulating leads (see discussion by Krnjević et al., 1966b). Hence stimulation of the surface could cause synaptic activation directly or indirectly. Raising the current strength to 5 V has the effect of maximally shortening the latency of spike responses (Li and Chou, 1962) and early excitation was observed to have a fairly constant latency at either 10 or 20 V in the present experiments. The absence of a difference in responses of cells during stimulation at 10 V or 20 V (cf. Table II) suggests that the current density at 10 V is sufficient to cause direct activation of cell bodies and their impingements. The excitatory nerve endings are preferentially located on the basal and apical dendrites and the inhibitory nerve endings on the soma (Colonnier, 1968). The apical dendrites are believed to have a very high electrical threshold (Purpura and Grundfest, 1956) so that surface stimulation would presumably cause direct activation of excitatory and inhibitory nerve endings and the soma in that order. The durations of the two effects i.e., early excitation followed by inhibition, might then be accounted for by the respective time courses of the EPSPs and IPSPs. The EPSP reaches a maximum amplitude within 2 msec lasting less than 20 msec. The measured peaks of early excitation range from 10 to 35 msec which suggests that an additional mechanism must be postulated to explain this extent of excitation. One possibility is that excitation of the cell might evoke recurrent excitatory pathways, and inhibitory pathways. The onset of

an IPSP is slow (>50 msec) so that early excitation would be maximum during the minimal amplitude of the IPSP and minimum when the IPSP approached its maximum amplitude. This time course of an EPSP-ISSP sequence is reflected in the PSTH (q.v., Figure 5). It is not likely that recurrent inhibition contributes greatly to the observed inhibition period. This pathway degenerates in chronically isolated cortex (Rosenberg and Echlin, 1965) which still shows the same response pattern after stimulation of its surface (Creutzfeldt and Struck, 1962; Krnjević et al., 1966b; Krnjević, Reiffenstein and Silver, 1970).

Unitary responses after the period of early excitation are generally abolished at higher frequencies. This might be attributed to the idea that a greater part of the remaining inter-stimulus time would be covered by a greater degree of inhibition as the reciprocal of the stimulus frequency was decreased. Another explanation would be on the basis of a temporal summation of EPSPs and IPSPs caused by the higher stimulus frequency. This explanation is valid if EPSPs and IPSPs do not arrive in close spatial relation to one another since excitation would be expected to predominate from a non-linear summation of the effects of excitatory and inhibitory transmitters acting on a small area of the membrane (cf. Rall's dendritic model, Rall, 1964). Given this condition, the increase in inhibition following early excitation (with an increase in stimulus frequency) suggests that IPSPs may be temporally summating on the soma.

Net depression during stimulation has not been observed to increase when the stimulus frequency is raised (cf. Table II). This can be explained on the basis that the early excitation period occupies a

greater proportion of the inter-stimulus interval when the latter is decreased with a higher frequency stimulus (see section B-2). Although a relationship between net depression and subthreshold stimulus frequency could not be demonstrated quantitatively in the experiments described in the foregoing sections, threshold frequencies of stimulation produce a pronounced drop in net depression compared with subthreshold frequencies. These data indicate that the production of an afterdischarge depends on the amount of activation to which a population of cells are driven over a stimulation period. This is supported by the studies of Pinsky and Burns (1962) which clearly show that the production of afterdischarges bear an all-or-nothing relationship to the strength, duration and number of stimuli used to produce them. In addition, they demonstrated that the threshold stimulus strength is independent of the total area of cortex (over an eight-fold range) being stimulated. Thus their concept of a focus for an afterdischarge is that which is produced by a conditioning stimulus which causes a minimum density of neurones to be excited a critical number of times.

Of all the stimulus parameters, pulse frequency is the only one which does not bear an all-or-nothing relation to the afterdischarge (Pinsky, 1961; Pinsky and Burns, 1962; Reiffenstein, 1964). Thus Pinsky and Burns suggested that an afterdischarge is initiated during recovery from a "state of exhaustion" induced in fixed amounts with each driven discharge and wearing off between stimuli. That this state might persist after unsuccessful EAD induction is suggested in the present experiments by the presence of a post-stimulatory depression (cf. Table III). Less than one-half of the cells tested exhibited this phenomenon

which was found also to be dependent on the stimulating current strength. The experiments described herein support the reports of Krnjević et al. (1966a) who noted some dependency of the "silent" period at the end of repetitive stimulation upon the stimulus intensity. An increase of post-stimulatory depression with an increase in stimulus frequency was also noted (cf. Table III) but the dependence was not found to be statistically significant at the 5% level when tested for being a chance occurrence. Some correlation was obtained between the period of reduced firing following repetitive stimulation and whether the same cell had exhibited a net depression during stimulation (see Figure 12). Moreover, cells excited during stimulation tended to become depressed after the end of the stimulus train. This suggests that some effects of surface stimulation, i.e., the inhibition observed during stimulation, may be cumulative. A corollary of this would be that net depression during stimulation and post-stimulatory depression may be mechanistically related. On the other hand, no difference was observed in net depression whether the stimulating strength was 10 V or 20 V (cf. Table II). As noted earlier, post-stimulatory depression after 20 V stimulation is different from that observed after 10 V stimulation. Thus the types of depressions may not be entirely due to an identical mechanism. Since the reduction of amino acid evoked firing is somewhat greater after 20 V (see Table III), it is suggested that a cumulative exhaustion or fatigue may contribute to this state. This suggestion is not incongruous with the observations of Krnjević et al., 1966a that "inhibition was less complete" when a faster release of glutamate was employed following stimulation [suggesting a hyperpolarizing type of block] because both

mechanisms might be present during the post-stimulatory period.

In more than 50% of the cells studied, an increased responsiveness to glutamate was observed following the period of repetitive stimulation. As in the above case of depression, post-stimulatory excitation was associated with a history of net excitation during the stimulation period (see Figure 12). This effect appears analogous to the "post-anodal exaltation" in the thalamus explained by Andersen and Sears (1964) on the basis of phased excitatory influences from extra-thalamic areas. Thus post-stimulatory rebound in the cortex might be explained by the idea that surface stimulation activates corticothalamic afferents which induces repetitive activity in the thalamus [although it is not possible to state with certainty whether an antidromic impulse or a corticothalamic impulse is the triggering event (Andersen and Andersson, 1968)]. After a long delay, excitatory influences returning to the cortex via thalamocortical afferents might act in phase with other excitatory influences such as the iontophoresed glutamate to produce an apparent rebound. There is little doubt that subcortical areas are involved in this phenomenon since it cannot be found in acutely isolated cortex (see Creutzfeldt and Struck, 1962).

C. THE PRODUCTION OF AN EAD AND THE RESPONSIVENESS OF CORTICAL UNITS TO GLUTAMATE AND GABA DURING THE EAD

1. Experimental procedure

In order to avoid Leão's spreading depression, the threshold for an EAD was not determined each time a cell was encountered. Also, recording of electrical activity of many cells was lost before EAD threshold was obtained. In each cat, a new threshold level of stimulation was established if the site of microelectrode penetration was moved a considerable distance away (since the stimulus was applied close to the puncture) or if several hours had elapsed since the brain was stimulated. The purpose of these experiments was to compare the behaviour of cells during threshold stimulation with that during subthreshold stimulation.

The purpose of other experiments described in this section was to provide information about the responsiveness of single cortical cells to iontophoresed glutamate and GABA in an effort to determine the following: (1) whether a cortical unit retained its excitability to these extracellularly applied substances during the EAD and (2) to obtain in qualitative terms an estimate of the synaptic input of a cortical cell participating in the EAD, i.e., to try to modify the extracellular responses during an afterdischarge with glutamate or GABA to test for occlusion, inactivation, etc. (see STATEMENT OF PROBLEM). Upon evoking an afterdischarge, glutamate and/or GABA were applied with currents that were effective in producing spike excitation (up to 10/sec) or depression of firing respectively, prior to seizure initiation. The

iontophoretic currents remained on for periods of a few seconds to over a minute in the course of afterdischarge activity. If the threshold frequency of electrical stimulation produced an afterdischarge of comparatively short duration, e.g., a few seconds, the next logarithmic increment in stimulating frequency was used to evoke EADs for testing the responsiveness of neurons in that area of the cortex. Cells were also tested to various doses of glutamate, GABA and NaCl during this time. The responses are based on observations of 234 cells.

2. Results

a) Threshold stimulation

After the train of a subthreshold stimulus which is delivered at a high frequency (20 to 100/sec) there often follows a long period of depression or inhibition (Figure 15 B to D). The cell under observation does not respond to the application of a previously effective amount of glutamate. In Figure 15, A and D, the 80 μV unit under investigation, discharges to glutamate with small bursts. About a second after subthreshold stimulation (Figure 15B), the unit responds with a burst followed by about 10 seconds of no response to continuously applied glutamate (Figure 15 B to D). A smaller unit (or equivalently, a unit more distant from the recording electrode) firing with isolated spikes rather than bursts of spikes was also inadvertently recorded (see Figure 15) and exhibits a nearly identical response to an intense subthreshold stimulus. Figure 16, B and C are examples of unit behavior that is evident during epileptogenesis by electrical stimulation.

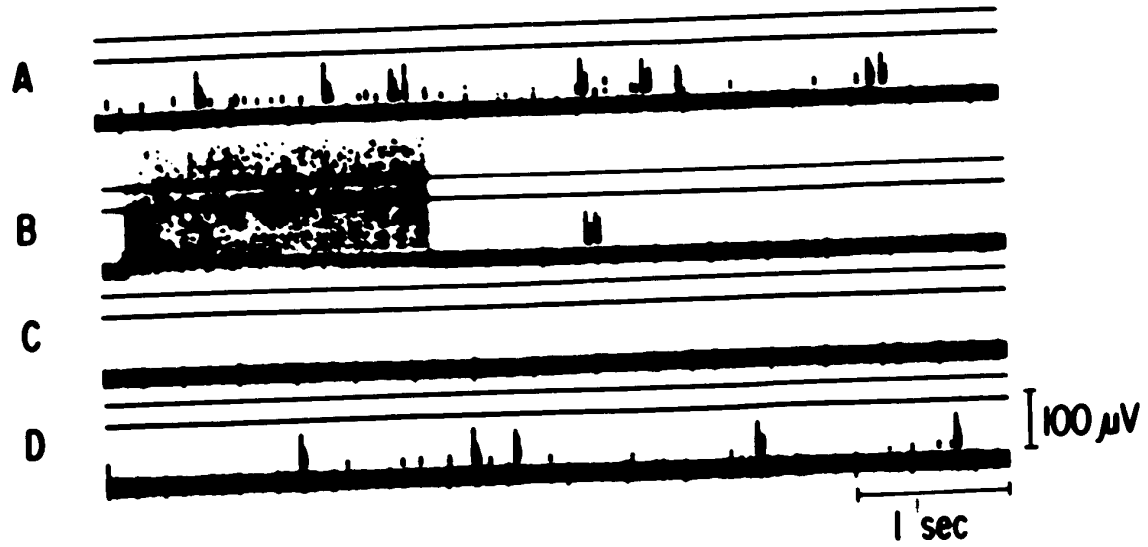

Figure 15. Continuous extracellular record of cell at depth 920μ during subthreshold epicortical stimulation at 77/sec, 20 V (in B). Glutamate 30 nA.

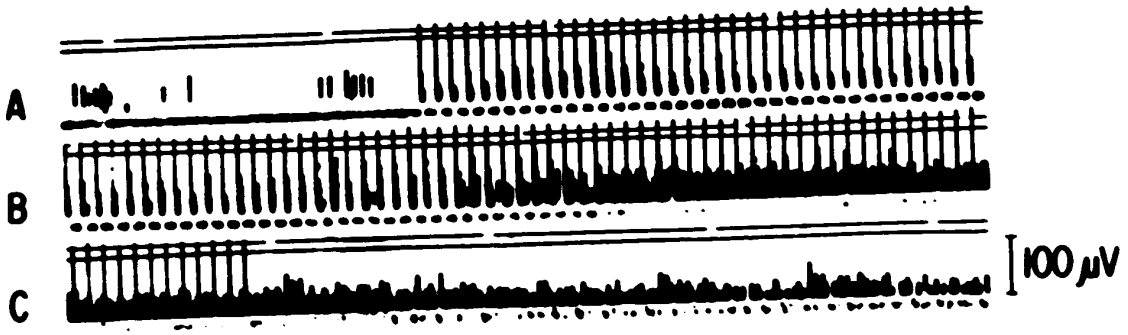

Figure 16. Extracellular record of cell at depth 512μ during epicortical stimulation at 12.5/sec, 10 V. Figure illustrates development of EAD during threshold stimulation. Stimulus artifacts are large vertical, equidistant lines. A, B and C are continuous records. Glutamate 5 nA throughout. Time marks on iontophoresis reference line are 1 sec.

Between the large, equidistant stimulus artifacts, early firing is visible at the beginning of the stimulus train. There is no apparent or gradual transition in behavior to where an intense discharge takes place towards the end of the stimulus train. However, examination of Figure 16B does reveal some tendency for the cell to respond with an increase in early firing for about 9 stimuli prior to the intense discharge (see also Figure 17D). The increase in early firing before the discharge of decrementing amplitude (see below) is a common feature whenever the latter is present. There does not appear to be a preferential time within the stimulus train where such high frequency discharges occur (see for example, Figure 17D). The presence of high frequency firing at the onset of a stimulus train (as in Figure 17D) was not always evoked with suprathreshold stimuli. Concomitant with the high discharge rate in both examples (Figures 16 and 17) is a progressive diminution of spike amplitude which persists after the last stimulus pulse. Spikes can be seen to regain their pre-stimulus amplitude in Figure 17H and are unaffected when glutamate application is stopped and when surface epileptiform activity is present. This typical response to threshold stimulation has also been seen in the absence of glutamate and at several different frequencies of threshold stimulation.

Figure 18 is a typical afterdischarge record from the surface of the brain and corresponds closely to the tonic-clonic type of seizure first analyzed by Rosenblueth and Cannon (1942). A tonic phase is a period at the beginning of an afterdischarge when fast frequency (about 20/sec) waves predominate. The tonic phase record has more large amplitude components than the spindling patterns of the ECG

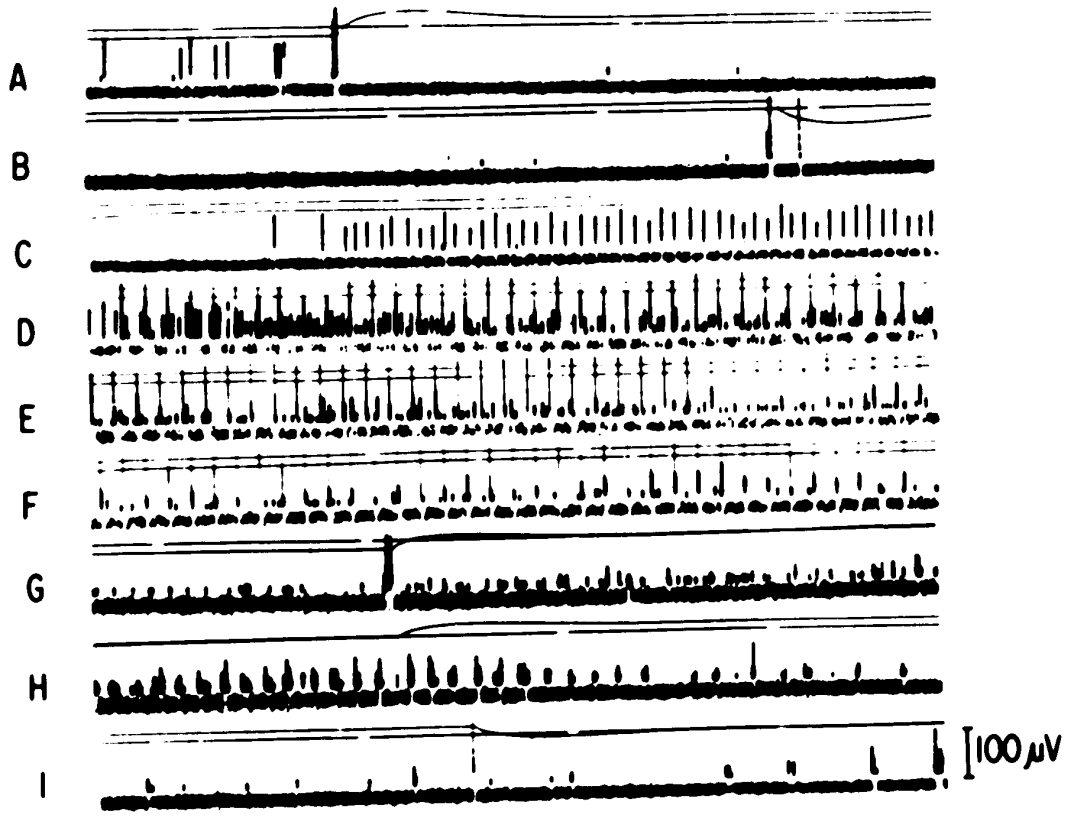

Figure 17. Induction of afterdischarge during (continuous) recording from unit at depth 752μ. A to C shows pre-stimulation record. Stimulation at 7.7/sec, 10 V starts in D, ends in F. Seizure develops during G to I. 10 nA GABA and 25 nA glutamate when on. Time marks or iontophoresis reference line are 1 sec. Seizure burst pattern as seen in H started to recover after termination of GABA in I. Full amplitude recovery is shown as last burst in I.

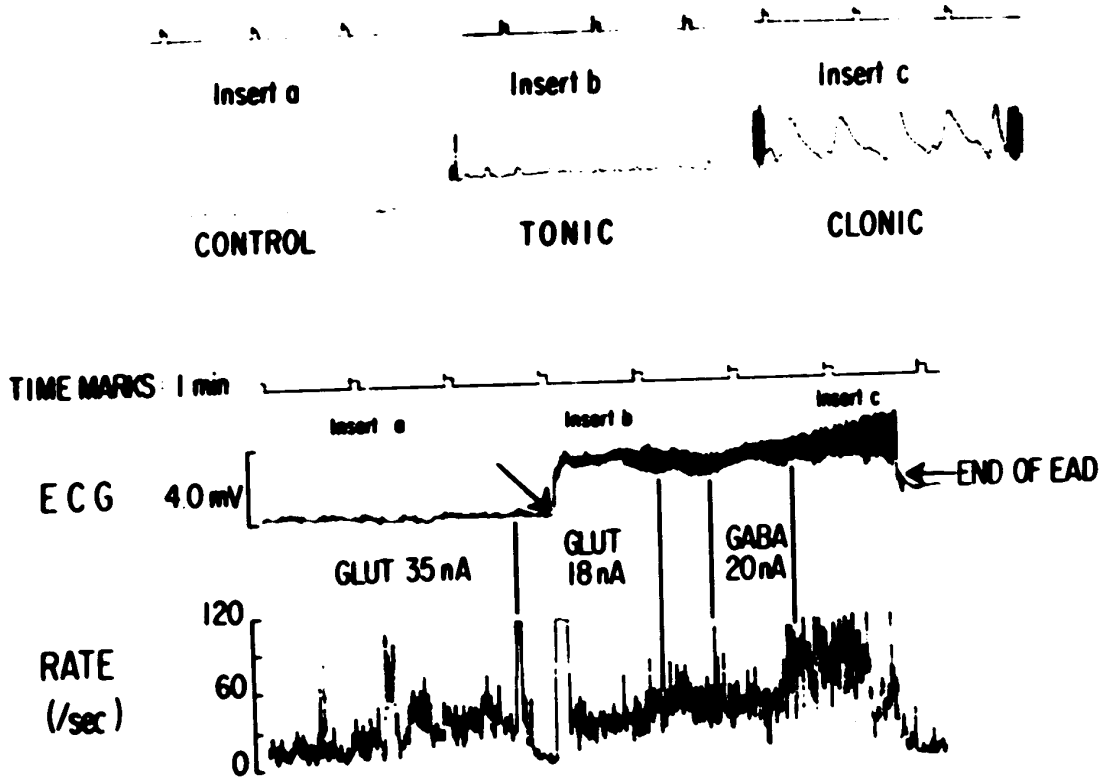

Figure 18. Surface record of electrocorticographic activity and firing rate of unit at depth 699μ illustrates afterdischarge activity. 30/s 10 V stimulation at arrow. Upper records are taken at higher paper speed during indicated periods in lower record. Time marks in upper record are 1 sec; same voltage calibration as in lower record. Glutamate and GABA iontophoresed during indicated periods.

(compare Insert a with Insert b of Figure 18).

The transition from tonic surface waves (see Insert b, Figure 18) to the slower large amplitude waves of a clonic phase (see Insert c, Figure 18) is characterized by a progressively more regimented pattern of surface waves. These slow to the large amplitude 2/sec waves indicative of a clonic phase (see Figure 18, Insert c).

The rate of cell firing during an afterdischarge is variable and not entirely predictable. For an afterdischarge which takes several seconds to develop, the rate of discharge usually increases with the development of the tonic phase. If an afterdischarge is well developed after cessation of the stimulus, then the rate of firing usually remains at a high level tending to decrease in the transition to the clonic pattern. There may be a slowing of the rate of cell firing or the surface record just before the end of an EAD. On occasions, however, the rate of cellular discharge increases slightly before the abrupt cessation of both the EAD surface waves and the cell firing. Iontophoresis of glutamate or GABA had no definite effect on the rate of firing of cell during the afterdischarge in Figure 18. These and other data suggest that the effects of glutamate and GABA are not easily detected from the rate records alone. The purpose of using the surface electrographic record in the present experiments was to be aware of when an afterdischarge was in process and approximately where in the EAD pattern the responsiveness of the cell was being tested.

b) <u>Responsiveness of units</u>

For the most part, GABA and glutamate applied iontophoretically

during the EAD do not produce the usual effects that one sees prior to seizure initiation. Few records in fact, provide clear evidence that glutamate can cause excitation [in spite of the range tested (10 to 100 nA)] of a cortical unit during an afterdischarge. On occasion, particularly if the discharges of a unit under observation are developing into a burst pattern in which several spikes are evident, glutamate can cause excitation. Relatively large amounts of glutamate can also cause excitation as the end of an EAD is approached. Two of the five records in which glutamate can be seen to evoke firing during an EAD are shown in Figure 19. Figure 19A shows the firing pattern developing just after 20/sec stimulation (not shown) while Figure 19B demonstrates that glutamate at the level of 30 nA is capable of causing more single-spike discharges from the unit. When glutamate application is stopped, the firing pattern (in Figure 19C) resumes as in Figure 19A. Figure 19 D to F (cell different from Figure 19 A to C) illustrates the extracellular record from the last 15 seconds of an EAD. Glutamate can be seen to provoke some firing after a two second latency during the late clonic phase (Figure 19E). Firing continues to nearly the end of the EAD (evident in extracellular record as the last burst in Figure 19F). Note that the bursts are still visible among the spikes evoked by glutamate (cf. Figure 19, E and F). Usually, single unit discharge remains unaffected by even large amounts of glutamate when tested following threshold stimulation or during the development of an EAD.

If glutamate is applied during an EAD (particularly during the clonic phase) with the same magnitude of current as before seizure genesis, there is often no perceptible change in the rate of cellular

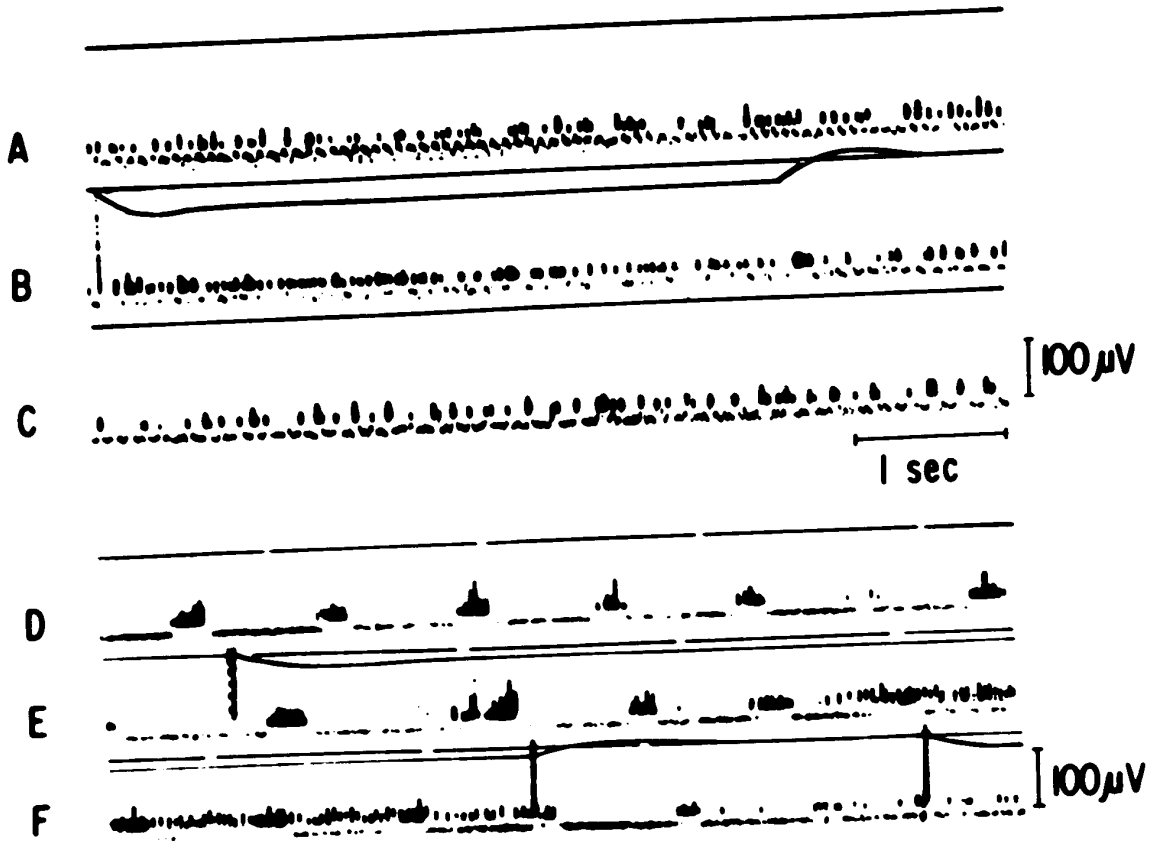

Figure 19. Extracellular records of two different cells during different after-discharges. A to C is unit activity at depth 1570µ. Glutamate 30 nA when on in B. D to F is also continuous record but of cell at depth 552µ. Glutamate 20 nA when on in E. Time marks in D to F on iontophoresis reference line are 1 sec.

discharge. 10% of these cases showed a reduction of spike amplitude during glutamate administration. An example of this phenomenon is given in Figure 20. The onset of the effect is nearly immediate while the termination of the effect requires one or two seconds before the original amplitude is regained. If one concedes that the small spikes of Figure 20B are discharges of the same cell whose firing appears in Figure 20A, then it can be said that glutamate decreases the amplitude of the potentials to one-quarter their control size. When current of opposite polarity to the ejecting current was applied to the glutamate barrel, no such effect has been observed. GABA or NaCl applied similarly with either polarity of current have not been noted to cause the above effect.

Glutamate has been observed to cause occlusion while the surface record shows a well developed clonic pattern (see Figure 21). In all seven records of this effect, glutamate was observed to decrease interburst firing without affecting either the pattern of bursts or the actual size of each burst. In many records other than the aforementioned, a similar reduction in the number of potentials during glutamate iontophoresis was evident. However, upon cessation of glutamate administration the firing rate of the unit remained indistinguishable from that observed during application of the amino acid. It was not possible in these cases to demonstrate that the effect was due to glutamate application since the reduction in interburst firing may have been due to a 'natural' progression of extracellular discharge during epileptiform activity.

When GABA is iontophoresed during the transition to the clonic phase, in addition to glutamate, a relatively common finding (~35% of 32

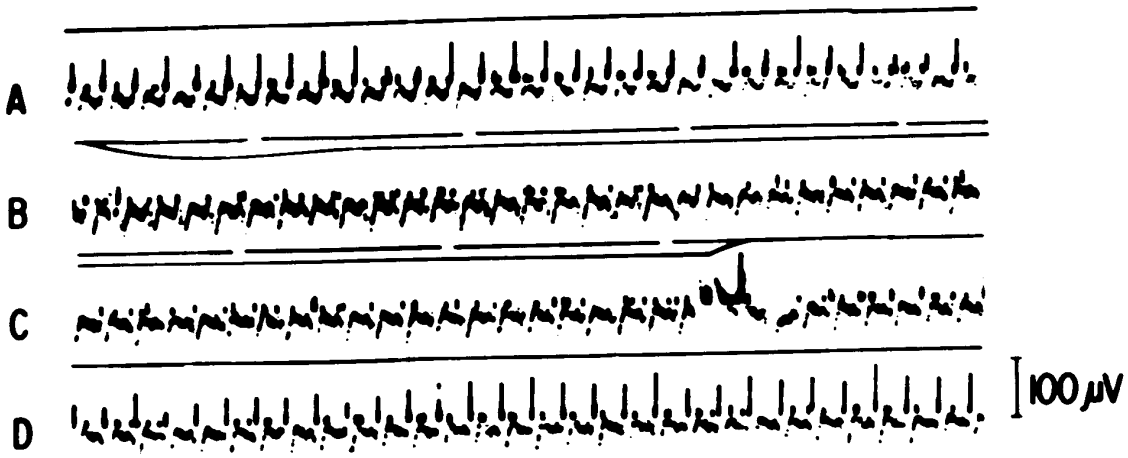

Figure 20. Continuous extracellular record during an EAD (provoked by 3/s, 10 V stimulation) of cell at depth 807µ. Glutamate 40 nA when on in B. Time marks on iontophoresis reference line are 1 sec.

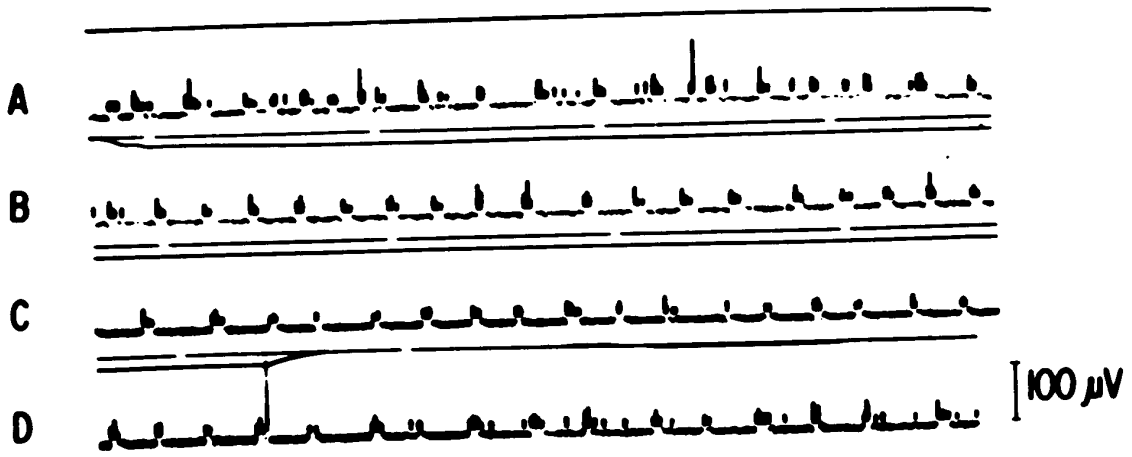

Figure 21. Continuous extracellular record during EAD (provoked by 30/s, 10 V stimulation about 1½ min before A) of unit at depth 541µ. Glutamate when on 35 nA as in B. Time marks on iontophoresis reference line are 1 sec.

tests) is a short (2 or 3 seconds) depression of glutamate-evoked firing followed by a pronounced excitation (see Figure 22). During the latter, a disruption of the organized burst pattern is present as in Figure 22B. In this case, the intense activity did not cease immediately when GABA application was subsequently stopped; the pronounced firing continued (Figure 22C) but later developed into a burst pattern once again (Figure 22D just before GABA added). When GABA was used to test the responsiveness of this unit after this unusual phenomenon it depressed all detectable activity of that unit after short latency (Figure 22, D and E) despite continual glutamate iontophoresis.

A typical example of another pattern of response (~12% of 205 tests) to amines is given in Figure 23. In Figure 23B, GABA administered alone during afterdischarge activity caused complete blockade of extracellular activity followed by a series of large spikes. This phenomenon was often observed five or ten seconds after the last stimulus pulse (during a slow development of an EAD or the tonic phase). The 50% increase in spike amplitude observed in Figures 23 A and B is not an invariable occurrence. However, GABA has often been observed to cause an increase in the size of potentials elicited by glutamate. Also, an increased amplitude has been seen as a rebound effect when GABA application (in the absence of glutamate) has been stopped during the tonic phase. This is not the case in Figure 23 C and D where spike amplitude returned to the same height as prior to GABA administration (cf. Figure 23, A to C); however, spike frequency was increased. Such pronounced increase in the number of extracellular potentials has been recorded from 20 units after the cessation of GABA current. The brief rebound increase in the

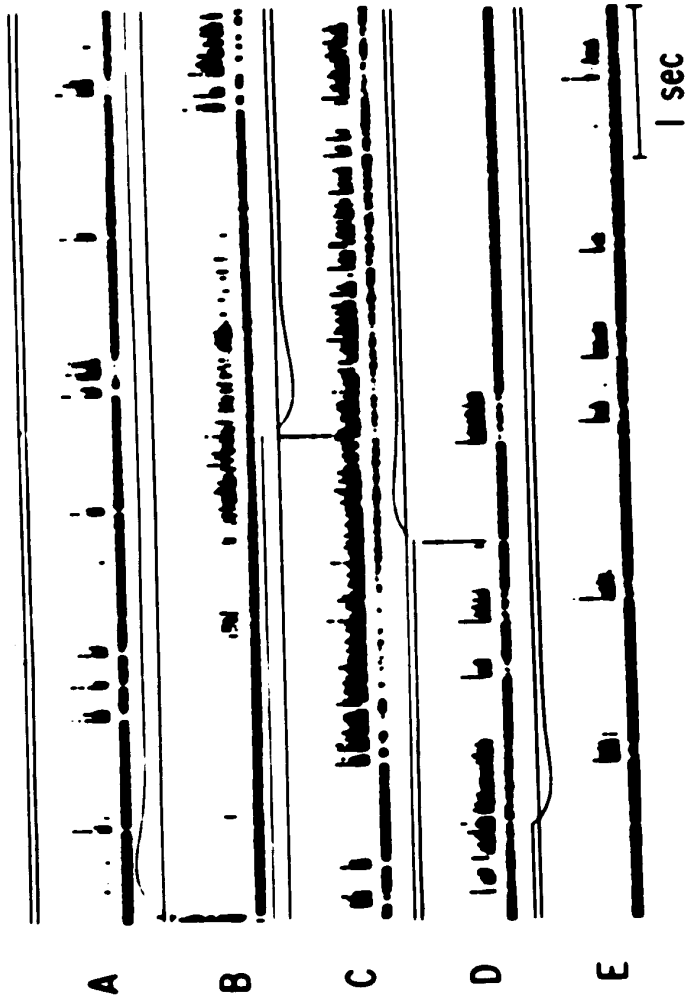

Figure 22. Continuous extracellular record during EAD (provoked by 50/sec, 10 V stimulation) of cell at depth 728µ. Glutamate 10 nA in A, C and E. GABA 10 nA plus glutamate 10 nA on in B and D as indicated by upward movement of iontophoresis line.

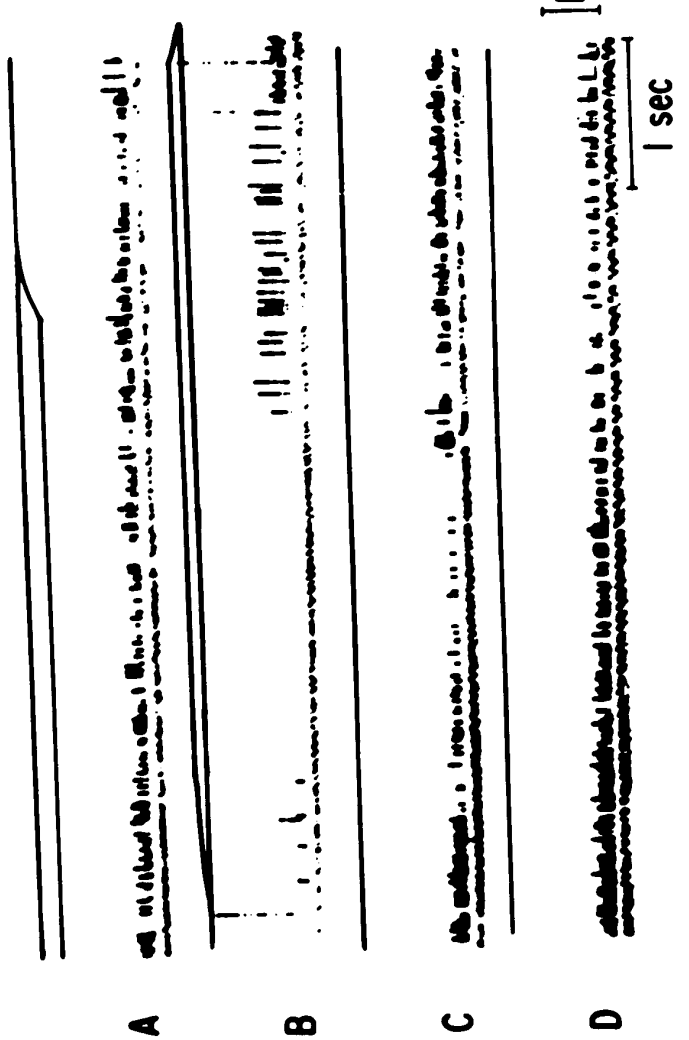

Figure 23. Continuous extracellular record during EAD (provoked by 20/s 20 V stimulation just prior to A) of unit at depth 1000µ. Glutamate 30 nA in A; GABA 30 nA in B.

firing rate after termination of GABA can be seen in the beginning of Figure 23C.

The depressant effect of GABA is not restricted to the early EAD; depression by GABA has been demonstrated late in the clonic phase. GABA can be seen to decrease the extracellular discharges in Figure 17 H and I which were recorded during the clonic phase. The above mentioned effects of GABA have not been observed when the polarity of current opposite to that normally used for GABA ejection is applied. Further this type of response has not been detected after glutamate or NaCl application with either polarity during the course of after-discharge activity. 9 out of 48 tests showed this GABA effect.

GABA was observed to increase interburst firing during an after-discharge. This response was never observed with the usually effective amounts of GABA required for total inhibition of pre-seizure firing. However, if about twice the above magnitude is applied during a developed afterdischarge pattern spikes become visible between the bursts (see Figure 24). A concomitant occurrence is the disruption of intraburst firing. This takes the appearance of two stages: (1) there is a reduction in the number of large amplitude spikes within the burst (or equivalently, a reduction of the amplitude of some spikes within the burst; compare Figures 24, A and B). (2) From Figure 24 C and D, a reduction in the duration of each burst is accompanied by an increase in number (and thus frequency) of single spikes between each burst. These spikes become indistinguishable from bursts in Figure 24E. This type of intense activity ceases in Figure 24F and reverts to the former pattern of repetitive activity when GABA iontophoresis is ended (Figure

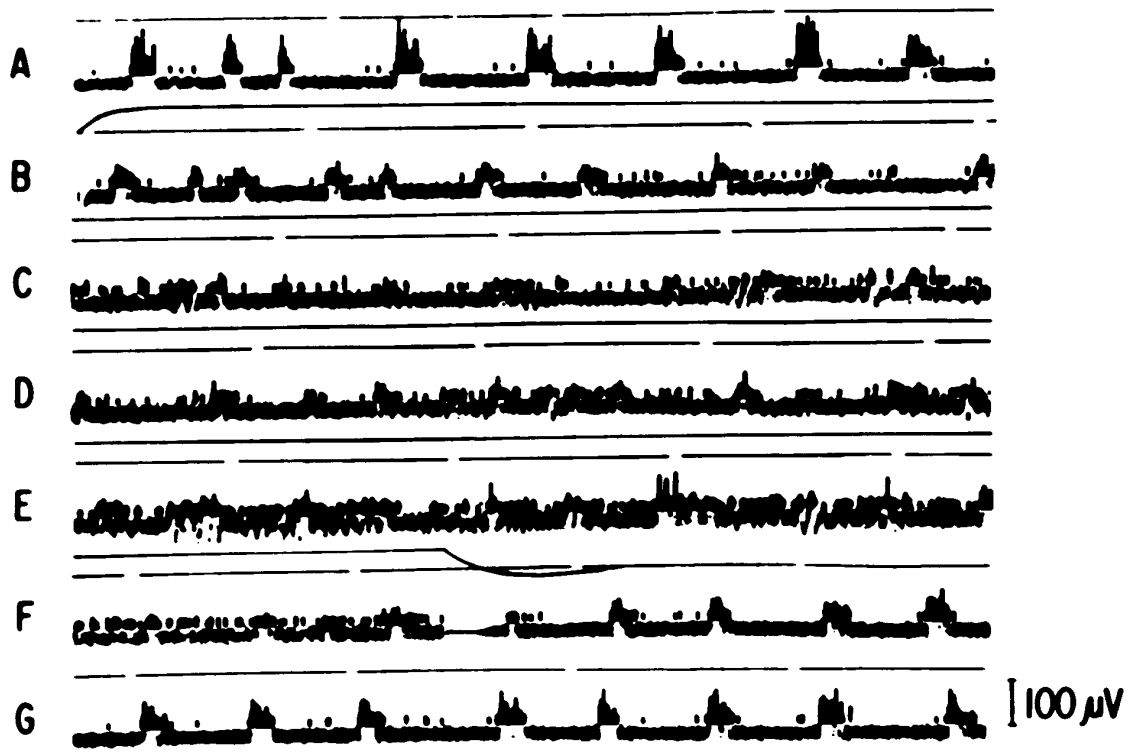

Figure 24. Continuous extracellular record during EAD (provoked by 12.5/sec 10 V stimulation 30 sec prior to A) of cell at depth 211µ. GABA 60 nA in B to F. Time marks on iontophoresis reference line are 1 sec.

24, F and G). The responses of (more than 25% of total) cells to GABA described above are unique in the sense that they have not been found to be caused by other substances in the examination of records from 234 afterdischarges.

c) Interval distributions

Interval histograms were done on a selection of cells representing previously described firing patterns. Examination of interval distribution of cell spikes revealed that firing patterns during EADs may be altered by GABA without changing the mean firing rate. An example of the sorts of changes which might produce such a result may be seen by comparing Figure 24, A and C.

Figure 25A shows the activity recorded extracellularly during the clonic pattern of the EAD where GABA decreased firing rate. The pattern of activity is given by the histograms in Figure 25B. The uppermost and lowest histograms are controls which preceded and followed the time of GABA iontophoresis (middle histogram) by no more than five seconds. The upper histogram of the discharge of the unit shows a trimodal distribution of interspike intervals. The very short interspike intervals (indicated by the smallest markers in Figure 25 A and B) are less than 20 msec in duration. These correspond to the intraburst intervals. About an equal number of intervals have a distribution peak at approximately 100 msec (intermediate sized markers). These intervals belong to the distribution of longer intervals between successive isolated spikes and bursts. These represent basically interburst intervals. The very long intervals (largest markers in Figure 25, A and B) represent

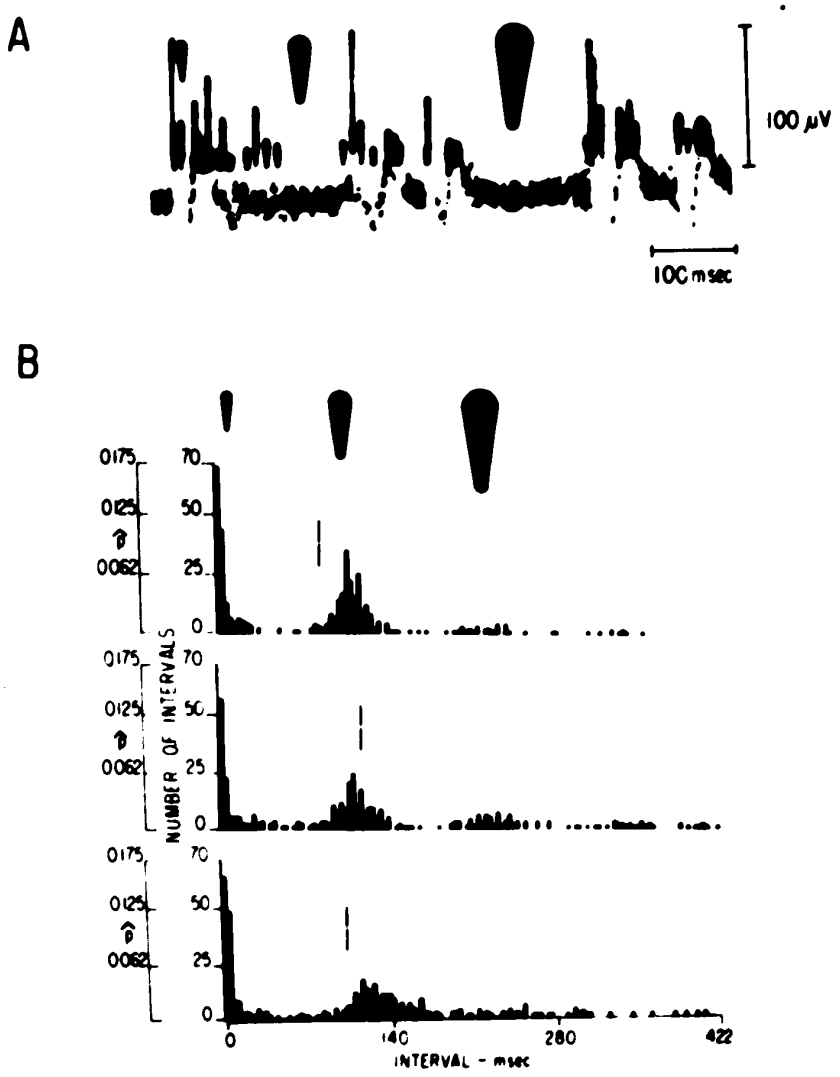

Figure 25. (A) Extracellular record of cell at depth 810μ during an afterdischarge and from same period as top histogram in B. Wedge markers indicate intervals which correspond with interval markers in B.

(B) Interspike interval histograms of same cell as in A before (top), during (middle) and after (bottom) 40 nA GABA iontophoresis. (Reciprocals of mean firing rates during analyzed period are indicated in above and subsequent interval histograms by vertical bars above histograms.) Each histogram contains measurements during EAD of 400 intervals, classified in 128 bins which are 3.3 msec long and represent intervals of up to 422 msec in duration (equal to total sweep length).

the long silent period (~200 msec) between individual bursts. The latter tend to be more dispersed than the other two distribution modes. The probability of one burst following another without interburst firing is lowest. On the other hand, the probability of one spike following another within the burst is very high ($\hat{p}$~0.175 where the intervals are of the order of 5 msec). This is reduced by GABA iontophoresis as indicated by the middle histogram ($\hat{p}$~0.0135). The trend of interval distribution as a result of GABA application appears to be a decrease in the number of intervals with rapid periodicity and a conspicuous increase in the number of very long intervals. There is also a filling in of the histogram between the short and intermediate intervals. These data suggest that (1) the firing during the bursts disperses slightly to give a lower intraburst frequency (2) a few of the later spikes in the intraburst firing drop out to reduce the number of intermediate intervals and increase the number of longer intervals (~200 msec). The time between bursts is, of course, controlled by the whole cell population. This shift of intra- and inter-burst firing is the cause of the increase in the mean interval estimate (see Figure 25B). Much of the distribution of intervals remains the same after GABA is discontinued (see lowest histogram in Figure 25B).

No change in the mean firing rate is evident from the effects of GABA on a different cell represented by the histograms of Figure 26. Figure 26 demonstrates the effect of GABA on the distribution of 500 consecutive interspike intervals of a cortical unit during a developed clonic phase and excludes interspike interval durations longer than 102 msec; thus the longer intervals are omitted, and intraburst

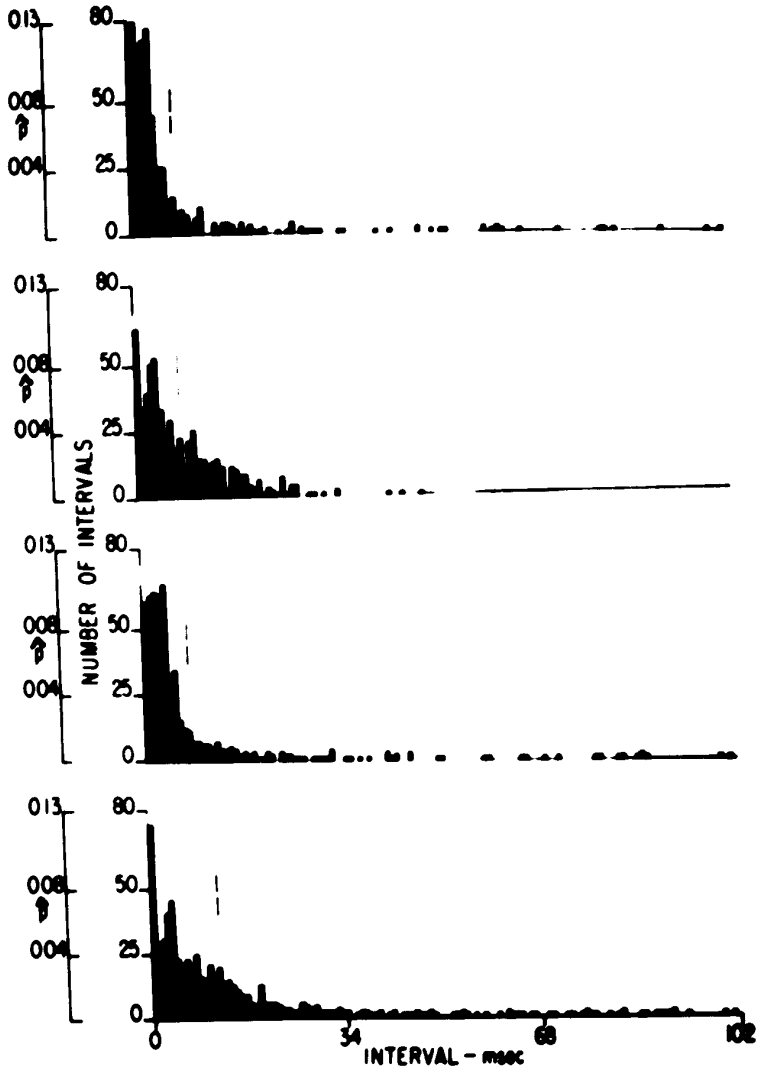

Figure 26. Interspike interval histograms of cell activity at depth 766μ during four consecutive analyzed periods of EAD. Second from top and last histograms were analyzed during 50 nA GABA iontophoresis. Each histogram contains measurements of 500 intervals, classified in 128 bins which are 800 μsec long and represent intervals of up to 102 msec in duration (equal to total sweep length).

intervals are emphasized compared to Figure 25B. The second and last histogram of this Figure are representative of the intervals during GABA administration and are not different. However, both lack the very short intervals (1 to 4 msec) which are due to intraburst spike discharges and are present in the control histograms. Only in the bottom histogram does GABA cause a reduction in the mean firing rate as indicated by the shift in the estimate of the mean interval to the right. This decrease in mean firing rate is caused by a reduction of very short intervals (~6 msec) and the occurrence of longer intervals. This is in some contrast to the second histogram where GABA decreased the very short intervals without increasing the very long ones. In both cases, however, the results indicate that GABA is capable of disrupting intraburst firing during the clonic phase of an EAD. In particular, the probability of one spike following another within the burst is considerably reduced by GABA (cf. Figure 26).

To investigate the shortest intervals between intraburst spikes, a large number of these short intervals was sampled (while disregarding the intermediate and long intervals). This fastest repetitive activity should be representative of the functional refractory period of the cell. Figure 27 is an example of such an analysis, where 1800 consecutive short intervals were sampled from a neuron discharging during the clonic phase. The uppermost and lowest histograms represent control periods of afterdischarge activity. These preceded and followed the period of GABA iontophoresis (middle histogram) by no more than five seconds. Only short (intraburst) intervals are examined. The overall distribution of interval durations within the burst is unimodal

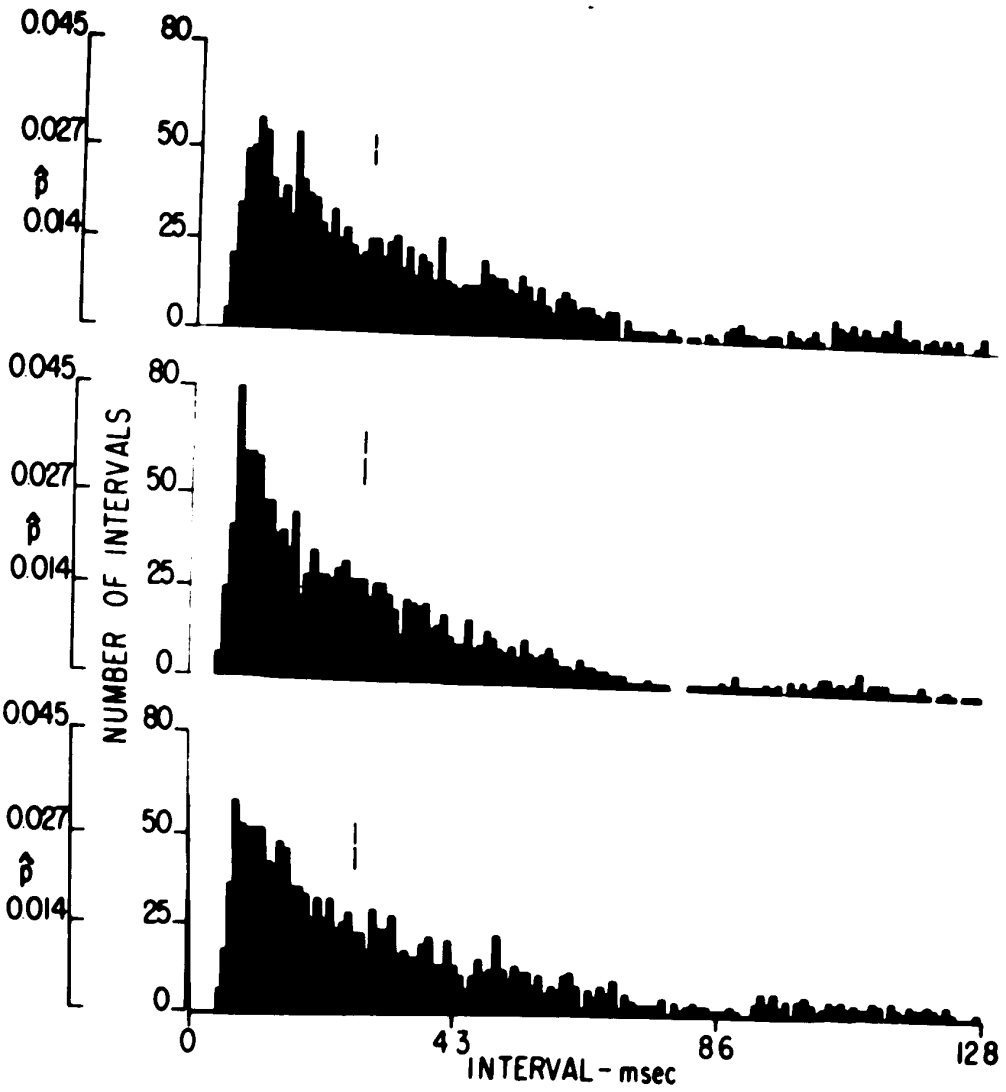

Figure 27. Interspike interval histograms of cellular activity at 810μ (same unit as Figure 25). The three consecutive analyzed periods from top are: control; 40 nA GABA; control. Each histogram contains measurement of 1800 intervals, classified in 128 bins which are 100 μsec long and represent intervals of up to 12.8 msec in duration (equal to the total sweep length).

and has been suggested to be a logarithmic normal distribution (Sherwin, 1970). The interval distribution starts at 500 μsec which might be considered a measure of the functional refractory period of the neuron [the response time of the pulse-height analyzer is of the order of 400 μsec]. The probability of one spike following another is highest when the intervals are about one millisecond ($p \sim 0.027$) and follows the exponential decrease in interval durations to where probability is lowest between 8.6 and 12.8 msec. GABA does not basically alter this distribution. The mean interval is not a true mean since intermediate and lont intervals are not sampled.

The functional refractory period was not altered by GABA. The transient nature of the afterdischarge itself (i.e., it is not a stationary process) precludes any definitive statement (using this type of analysis) about the effect of GABA iontophoresed for periods longer than a few seconds. It is also for this reason that the use of interval analysis during the afterdischarge is limited.

3. Summary and Discussion

There appears to be no gradual transition of responses of cortical units from a high frequency subthreshold stimulus to those that occur during a threshold stimulus. The long period of reduced firing to glutamate (see section B-2) following repetitive stimulation of the cortical surface often takes the form of a complete cessation of firing for many seconds after which the initial rate gradually resumes (see Figure 15). The duration during which there was an absence of cell

firing following a high frequency subthreshold stimulus was, in most cases, about 10 seconds. This confirms the report of McLennan (1970) but is considerably shorter in duration than the period of nearly a minute reported by Phillis and York (1968). Some of the apparent discrepancy can be accounted for since the latter investigators measured the time taken for the full initial rate to be restored and not just time for the return of sustained firing. Although Phillis and York (1967; 1968) reported that the so-called 'long-inhibition' could be abolished with strychnine, evidence for this has not been substantiated (McLennan, 1970). The failure of many pharmacological agents to alter post-stimulatory depression remains one of its characteristic features and suggests that some mechanism other than a synaptically-induced inhibition might be responsible for the lowered excitability of these cells during this time to excitatory amino acids. Since the studies of Krnjević et al. (1966b) suggest that the complete suppression of firing following an intense subthreshold stimulus is a hyperpolarizing type of block rather than a cathodal depression, an electrogenic sodium pump might be considered as appropriate mechanism. However, this cannot be documented.

As mentioned previously, the studies of Pinsky and Burns (1962) make it clear that an afterdischarge is the result of driven activity. They have described this factor as a state of fatigue, exhaustion or a persistent depolarization of neurons at the focus. The above theory is supported by the observations of this investigation on unit behavior during threshold stimulation. Although strong depression of unit firing was evident during induction of some seizures, a large percentage

(~50%) exhibited evidence of a persitent depolarization during repetitive threshold stimulation. As observed by Gerin (1960) extracellular spike amplitude and frequency undergo characteristic changes during this time (see Figures 16 and 17). However, the interpretation of changes in extracellular potential amplitudes is difficult because the cortex does not behave as a perfect ohmic resistor nor is it an isotropic medium. As a first approximation, however, these are not sources of grave distortion (Hubbard, Llinas, Quastel, 1969). If an electrode is near the soma, the biphasic negative-positive wave recorded can be considered a derivative of the intracellular spike which is in turn related to the rate of change of membrane transients (Freygang and Frank, 1959; Hubbard, Llinas and Quastel, 1969). The second assumption to be made is that the spike mechanism generates a peak voltage of an intracellular spike which is near the sodium equilibrium potential (Hodgkin and Katz, 1949). In the absence of a change in microelectrode tip potential, therefore, the rapid diminution of extracellular spike height [from a relatively stable spike amplitude] and the high frequency nature of the discharge (see Figures 16 and 17) can be taken as indirect evidence of an extensive depolarization of the cell. This supports the studies of Gerin (1960) and Ajmone Marsan (1961) that temporal summations of depolarizations are the basis of seizure genesis. This interpretation is supported by the intracellular studies of Sypert et al. (1970) who observed a strong depolarization with rapid spike attenuation during threshold stimulation (transhemispherically) by synaptic mechanisms.

Following the end of the stimulation period, spike amplitude often regains its initial height. Such data suggest that at the cellular

level, a seizure develops out of a period in which cells are strongly depolarized. During this period and near the end of an EAD, glutamate can excite the cell (Figure 19). This was not a common finding nor a striking one. Mostly, glutamate caused no perceptible change in unit behavior, or occasionally, caused a reduction of spike amplitude and occlusion (see Figures 20 and 21). Since glutamate is believed to act by depolarization (Krnjević, 1970) the latter effects might be explained on the speculation that the amino acid reduced the driving potential ($E_{Na} - E_r$; where E_{Na} is the sodium equilibrium potential and E_r is the resting membrane potential). In cases of occlusion, it is conceivable that glutamate could cause sufficient reduction in the driving potential to inactivate the cell. These data suggest that during most of the afterdischarge period, a large number of cells that are participating in the EAD are under intense synaptic excitatory influences to the extent that their membrane potentials are near the firing threshold level.

The micro-iontophoresis studies with GABA give more conclusive evidence for the periods of profound depolarization during the EAD. In contrast to the effects of glutamate, those of GABA during an EAD were more striking and frequently observed. In addition, the functional role as a possible inhibitory transmitter and its action (i.e., hyperpolarization) are better documented than that of its precursor amino acid (cf. Krnjević, 1970). Thus the observations that GABA increases spike amplitude, initiates discharges in the presence of glutamate, and causes the appearance of single spikes between bursts, during the EAD are more convincing evidence [than the results obtained with glutamate]

that cells are relatively depolarized and inactivated during this time. One of the major shortcomings of this investigation, however, is the inability to characterize precisely the tonic-clonic sequence according to the observed responses to GABA and glutamate. However, the effects of increased spike amplitude, and paroxysmal discharges when GABA was added in addition to glutamate, were not observed during the well-developed clonic pattern. Such effects might be ascribed to a hyperpolarizing action of GABA causing an increase in spike amplitude in cells where a small-driving potential exists. This condition would permit a greater effectiveness of ongoing excitatory mechanisms or iontophoresed glutamate to initiate spikes of larger amplitude and higher frequency. These data are consistent with the hypothesis that a population of cells participating in the afterdischarge activity are under excessive synaptic (excitatory) bombardment during the tonic phase of the EAD.

In some cells, a depressant effect of GABA was noted during the development period (i.e., the early tonic phase) but more often, depression appeared to be most effectively produced towards the end of an afterdischarge (i.e. clonic phase). During the tonic phase, this action of GABA is consistent with the behavior of Type II cortical cells (see Sypert et al. 1970) which are known to show an absence of, or a slight change in membrane potential during tetanization. In the post-stimulatory period, these cells rhythmically discharge to recurring depolarizing waves (Creutzfeldt, Watanabe and Lux, 1966; Sypert et al. 1970) and might therefore be expected to be inhibited by GABA. The action of GABA towards the end of an afterdischarge might be inferred from

some descriptions of the clonic phase, i.e., an increasing repolarization of the membrane toward the resting preictal resting potential occurs during the clonic phase (Matsumoto and Ajmone Marsan, 1964; Sawa et al., 1965). Spikes occurring during this phase would therefore be blocked by GABA while more spikes would be excited by glutamate (see Figure 19).

Earlier in the clonic phase GABA has been observed in the present experiments to reduce bursts (in amplitude and size) and cause the appearance of spikes during the interburst interval. In the distribution of intervals within the burst some short intervals are absent after GABA (cf. top and middle histograms in Figure 27) which indicates that high frequency firing within the burst is disrupted. Concomitant with a decrease in short intervals, is the appearance of longer intervals [without a reduction in the mean firing rate] which gives support, quantitatively, to conclusions derived from filmed records (see Figures 23 and 24) of unit behavior. That is, GABA causes a disruption of firing within the burst where the probability of one spike following another is high, and causes the appearance of single spikes during the interburst interval. A corollary of the above is that GABA decreases the tendency of a cell to fire repetitively within a burst while increasing its tendency to discharge at other times. A single mechanism can explain both of these observations. If one assumes that each burst during the clonic phase is a manifest discharge from depolarizations phasing with periods during which the membrane is not inactivated, GABA would inhibit the high frequency firing thereof. If during the subsequent period, the membrane of a participating cell was inactivated (i.e., depolarized to levels more positive than the firing threshold),

GABA would hyperpolarize the membrane to a level more negative than the firing threshold. Thus the cell would no longer be inactivated and in the presence of an excitatory input, spike discharge with more random interspike intervals would be apparent during the interburst interval. The above explanation is supported by numerous intracellular observations during the late tonic and early clonic phases of rhythmically-occurring oscillations in membrane potentials well before the end of a seizure (Creutzfeldt et al., 1966b; Sawa et al., 1963; 1965; 1968; Sypert et al., 1970). Another characteristic can be predicted from the above for periods during which the cell is not inactivated, i.e., when the oscillations of the membrane are in the hyperpolarizing direction relative to the threshold firing level. An irregular, decrementing spike amplitude is often seen in clonic bursts whether recorded intracellularly (e.g., Figure 5F of Sypert et al. 1970) or extracellularly (e.g., Figure 24A). This behavior is to be expected from depolarizations occurring at various points along the positive slope of an oscillating polarization curve. The above interpretation implies the existence of a long time course of repolarization which cannot be attributed to a membrane time constant (cf. Creutzfeldt et al. 1964). Gerin (1960) suggested that a "depolarizating after-effect following each spike" might cause the delay in repolarization but put more emphasis on excitatory synaptic input causing substantial slowing of the repolarization process. While the results described in this section suggest periods of inactivation of the driven neuron, they might also be considered equivalent to the hypothetical state of exhaustion suggested by Pinsky and Burns (1962). The latter could result, for example, from

inability of the sodium pumping mechanism to restore the normal intracellular concentration of sodium in a restricted portion of the neuron (Eccles, 1957; Shanes, 1958).

D. THE TERMINATION OF THE EAD AND THE EPILEPTIFORM SUSTAINING RESPONSE (ESR)

1. Experimental Procedure

At the end of each afterdischarge, the responsiveness of the cortical unit under observation was tested to glutamate or GABA. In a few cases, ouabain was continuously delivered electroosmotically during the afterdischarge and the postictal period. These substances were applied in an effort to determine whether inactivation, hyperpolarization, or spreading depression contribute to the arrest of an EAD. Cortical afterdischarges can be entrained by relatively weak stimuli applied during an EAD at low repetition rates (Rosenblueth and Cannon, 1942) or by appropriate peripheral stimulation once a focus in a particular area of the brain has been initiated (Rosenblueth, Bond and Cannon, 1942). This type of procedure was employed to determine what cellular responses could be obtained when surface EAD waves were entrained. A second question which was considered was the relative ease with which the cell responded to glutamate or GABA during this surface entrainment. A similar procedure to that employed in section II-B, was used, i.e., a type of experimental format suitable for post-stimulus time histogram analyses. Once entrainment was established, a control period of 100 stimulus pulses was analysed, followed by a similar period during iontophoresis of GABA or glutamate, and then another control.

2. Results

a) Termination of the EAD

As previously mentioned, there may be no slowing of the rate of cellular discharge as the end of surface afterdischarge activity is about to cease. Figure 28 illustrates an extracellular record during a brief but entire afterdischarge. After the last stimulus pulse in Figure 28C, spike amplitude rapidly gains in height until the pre-stimulus amplitude is attained. The last large amplitude spike in Figure 28D occurs at the moment of cessation of surface afterdischarge activity and is followed by a period of depression of all responses. When glutamate application is stopped in Figure 28E and GABA is iontophoresed, the blockade continues. In Figure 28F firing resumes when glutamate is on in the absence of GABA. Cells which were spontaneously firing prior to induction of seizure activity exhibit a similar long-lasting depression at the end of an EAD. This is illustrated in Figure 29 which is the extracellular record after the induction of epileptiform activity. In the absence of glutamate cell firing does not return for about 17 seconds. When prompted by a 3 second pulse of glutamate, spontaneous firing resumes. GABA never induced firing in any cell tested when given during the post-seizure depression.

Although depression of firing was witnessed at the end of most afterdischarges in this investigation it is not invariably the case. Figure 30 shows that in the presence of glutamate very little interburst firing is evident in the late clonic stage (Figure 30, C and D) but after the last burst the cell responds to glutamate with a firing

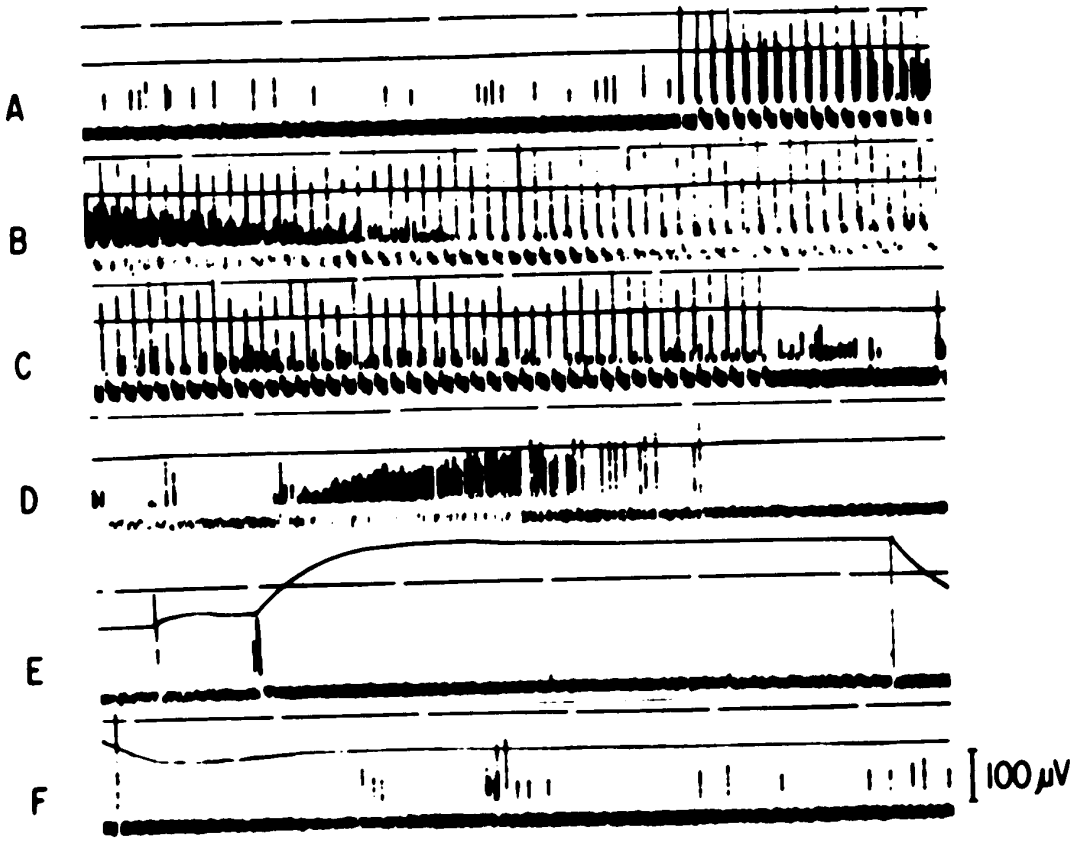

Figure 28. Continuous extracellular record of unit at depth 752μ and of entire afterdischarge. Glutamate 10 nA in A to D and F. Stimulation of 10/sec 20 V commences in A, continues to D. GABA 10 nA in E. Time marks on iontophoresis reference lines are 1 sec.

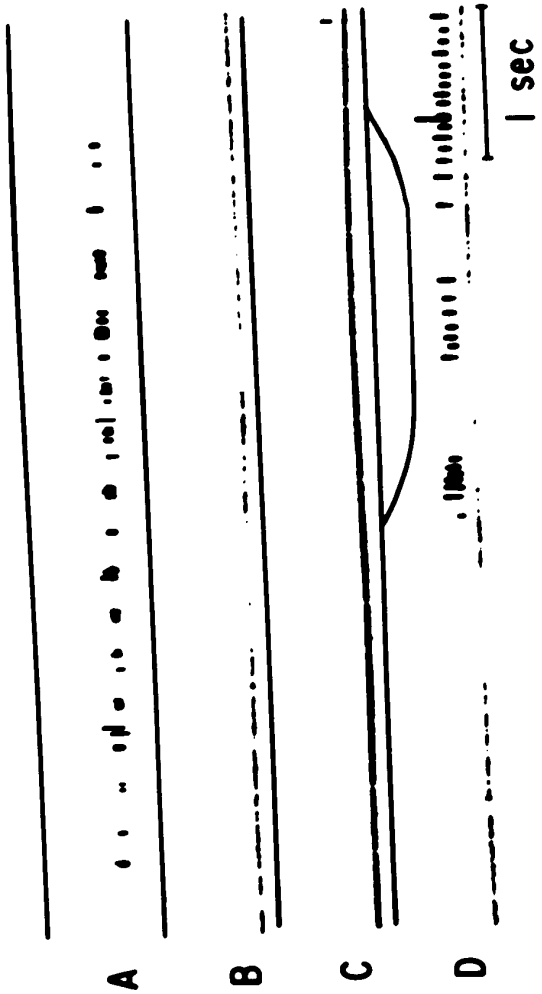

Figure 29. Continuous extracellular record of spontaneously-active unit at depth 781µ during late afterdischarge in A, end of EAD in B to D. 3 sec pulse of 10 nA glutamate in D.

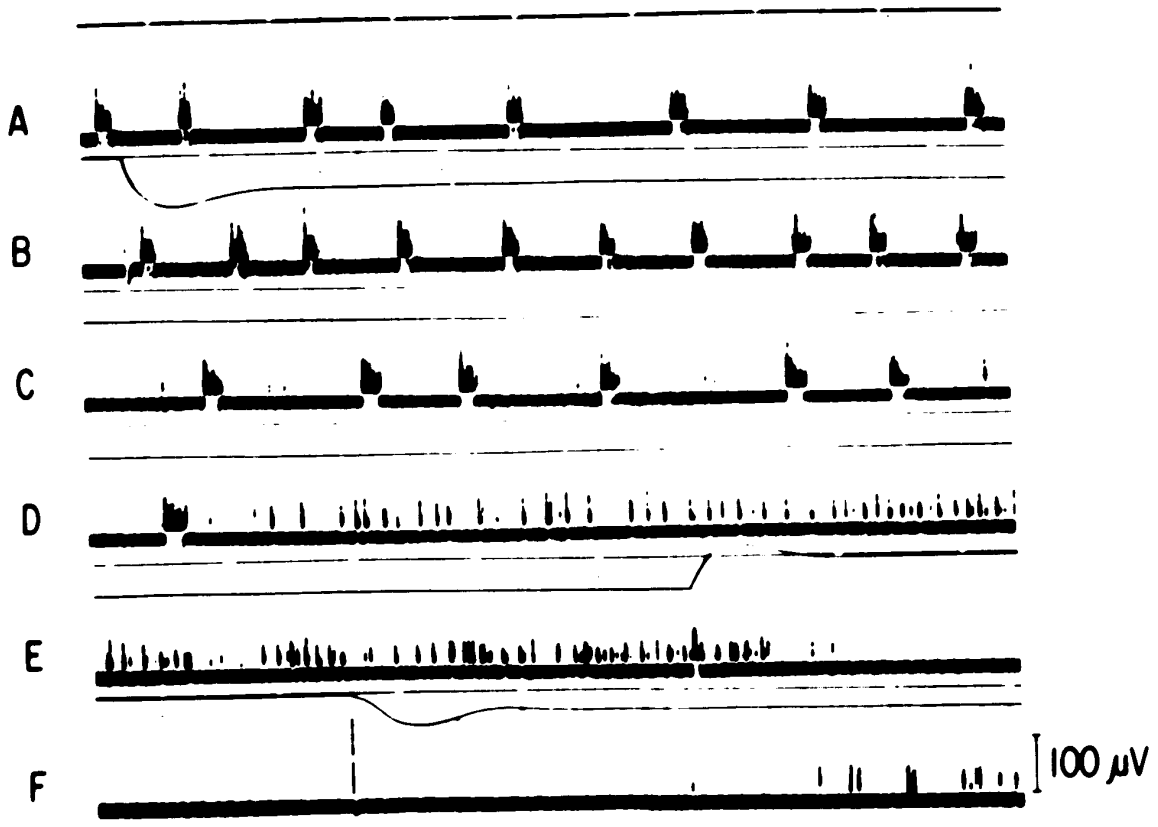

Figure 30. Continuous extracellular record of unit at depth 480μ during clonic phase of EAD. Glutamate 20 nA in B to E. Although relative level of iontophoresis is indicated as being less than B to F, three times more (60 nA) glutamate is being applied. Last burst in EAD can be seen in D. Time marks on iontophoresis reference line are 1 sec.

frequency which after a few seconds reaches 10/sec (the firing rate which was elicited by glutamate prior to convulsive stimulation). However, once glutamate was stopped and restarted, resumption of firing was sluggish, requiring about 5 sec to return. This contrasts with a less than one second onset in the pre-seizure state.

In the majority of cases, as noted above, there is a prolonged absence of firing for a few to 60 seconds following an EAD despite the presence of glutamate. If this amount of glutamate is tripled, a response, which is sluggish in onset, will usually occur. Three or four times the pre-seizure magnitude of glutamate is required to provoke a firing rate of about 10/sec, five to ten seconds after all surface epileptiform activity has ceased.

The possibility was considered that the depression of glutamate action at the end of an afterdischarge might represent a long hyperpolarizing response such as that held due to an electrogenic pump (Nishi and Koketsu, 1967; Pinsker and Kandel, 1969). Since ouabain is known to inhibit the electrogenic sodium pump mechanims (Skou, 1965), this agent was continuously applied electroosmotically to four different cells for periods not less than four minutes before the end of EAD's in two cats. In each case, ouabain (5 to 50 nA) abolished all extracellular activity within three minutes during which neither glutamate nor GABA (currents -50 nA) were able to activate any extracellular discharge. Also, no electrical activity could be picked up by the recording micropipette during the course of an afterdischarge when layers immediately above and below the cell were probed for extracellular activity. For these reasons, the experiments with ouabain were not pursued, and the

effect of ouabain was regarded as a "nonspecific poisoning" of the cells.

b) Epileptiform Sustaining Response (ESR)

One of the problems which has plagued tests of the responsiveness of cells during seizures is that is is difficult to control the duration of the afterdischarge. The ESR is believed to resemble an EAD during the period of regimented surface waves, i.e., the clonic phase (Sanders and Pinsky, 1967) and in this respect represents a convenient method for studying EAD activity. More than 100 ESRs have been studied in this investigation; nearly all of these were sucesssful for a period of 4 to 30 minutes. Epileptiform activity was not sustained if the "natural" frequency of the EAD bursts was very different (particularly, faster) from the frequency of the entraining pulses. Part of the longest ESR encountered is shown in Figure 31. This Figure also shows the induction of epileptiform activity in A to C. The development of a large burst pattern is rapid in Figure 31, D and E. In the latter trace, the bursts fire with a periodicity of about 1.6 per second. At the end of Figure 31E and beginning of F, this burst periodicity is roughly matched with a 1.6/sec continuous stimulus pulse. Almost all of the firing thereafter takes place within 200 msec after the stimulus pulse (entrainment). Activity of this type continued for 30 minutes after which the stimulus was shut off; the seizure waves and cell firing then abruptly stopped. The same cell can be seen to follow a subthreshold pulse of a similarly low frequency in the pre-seizure record (Figure 31G). The extent of early firing in the subthreshold

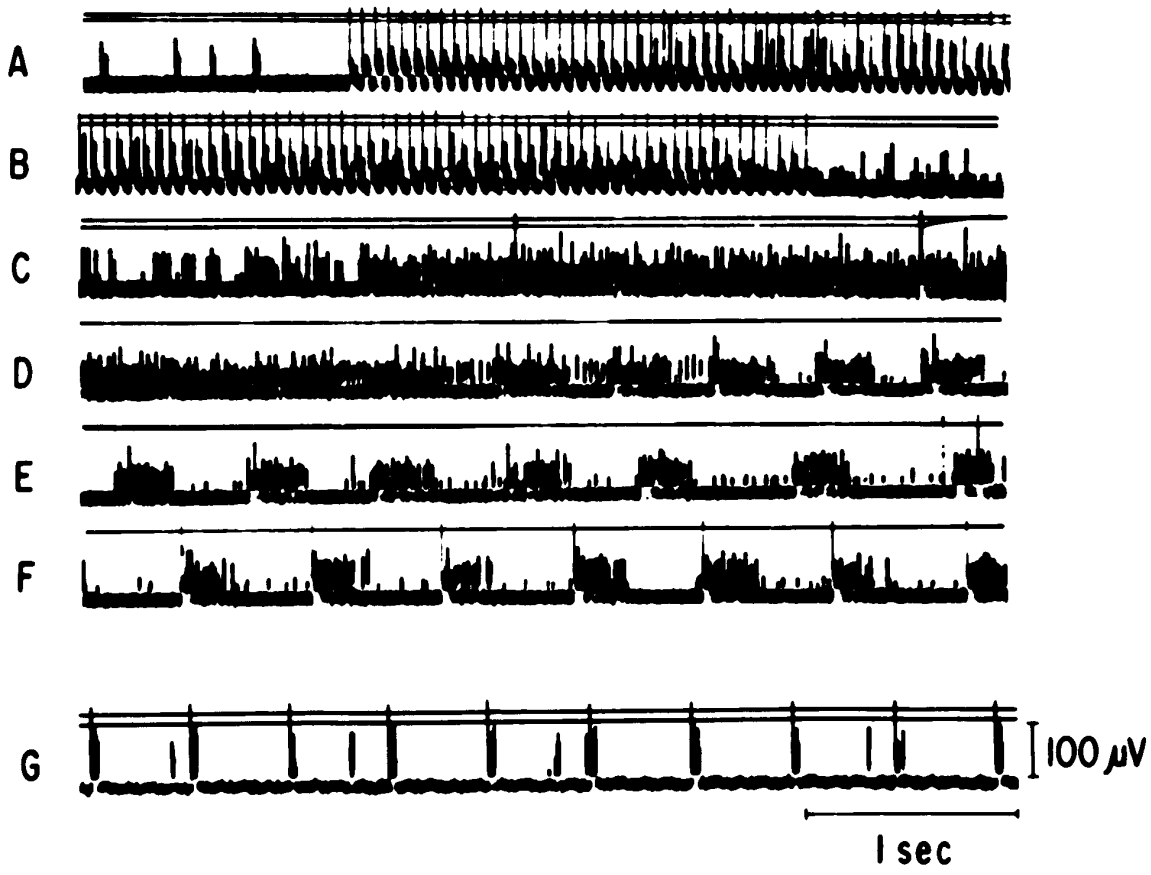

Figure 31. Continuous extracellular record during afterdischarge (A to F) and subconvulsive stimulation (G). Cell is at depth 845μ and glutamate 10 nA on in A to C, 15 nA in G. Induction of EAD in A is by 16/sec, 10 V stimulus. In F, prolongation of afterdischarge activity commences with 1.6/sec stimulus. In G, subthreshold stimulation rate is 2/sec.

record is approximately 10% of the burst duration following an entrainment pulse.

Part of the surface record and firing rate of the same cell as above is pictured in Figure 32 when the entraining stimulus frequency was increased to 2/sec. At the start of the ESR, the ECG record shows stereotyped pattern of 1 mV waves which increase in excursion to about 2 mV after 8 minutes. When GABA or glutamate is applied to the cell the rate of cell discharge during the indicated periods is decreased. 60 nA of glutamate decreased the rate of firing during the ESR to about 50% its previous value. More analysis of this record is provided by the PSTHs (Figure 33) taken from periods indicated by their respective letters in Figure 32.

GABA causes a generalized reduction of firing in the burst following the stimulus in Figure 33B in comparison with the two control histograms (Figure 33, A and C_1). Spikes appearing 150 to 300 msec after the stimulus are considerably reduced in number. The latter observation is the only indication of any disruption of burst activity such as that demonstrated for GABA during the EAD (viz. Figure 24). Another infrequent observation is the small number of spikes that have occurred between 300 and 500 msec during the application of GABA. This probably reflects interburst firing which was also caused by GABA during the EAD (cf. Figure 24).

The next series of PSTHs (Figure C_2, D and E_1) demonstrate that 40 nA of glutamate (twice the pre-seizure amount) causes occlusion during the ESR. [The pre-seizure dose also occluded firing (Figure 32 at B)]. The post-stimulus pattern in all three histograms has four

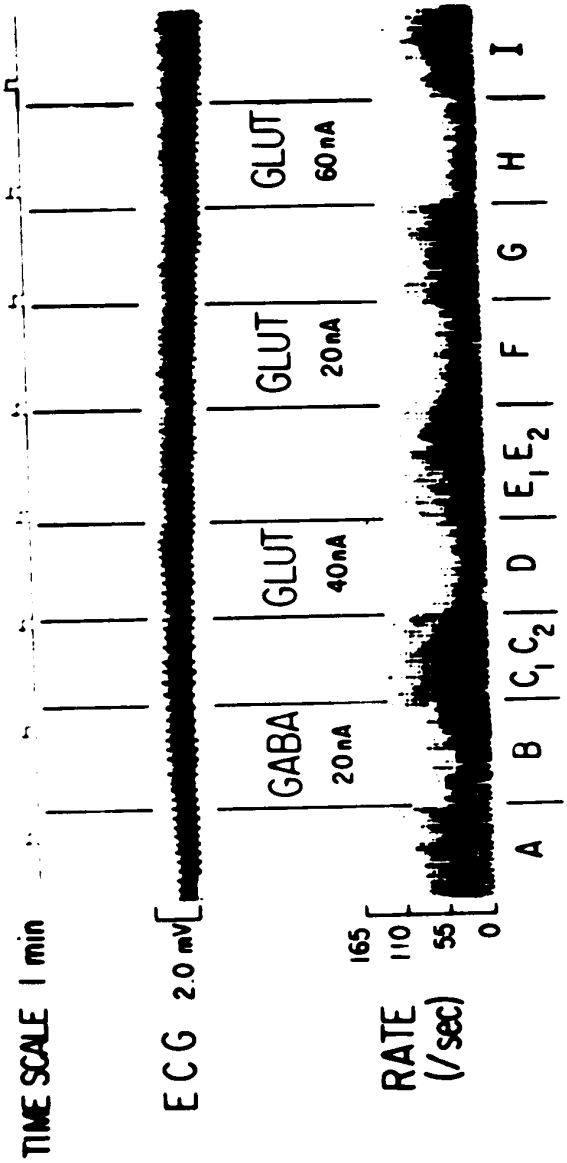

Figure 32. Surface electrographic and rate meter records (same unit as in Figure 31) during ESR. Letters at bottom indicate analyzed periods and A to G correspond to histograms similarly marked in Figure 33.

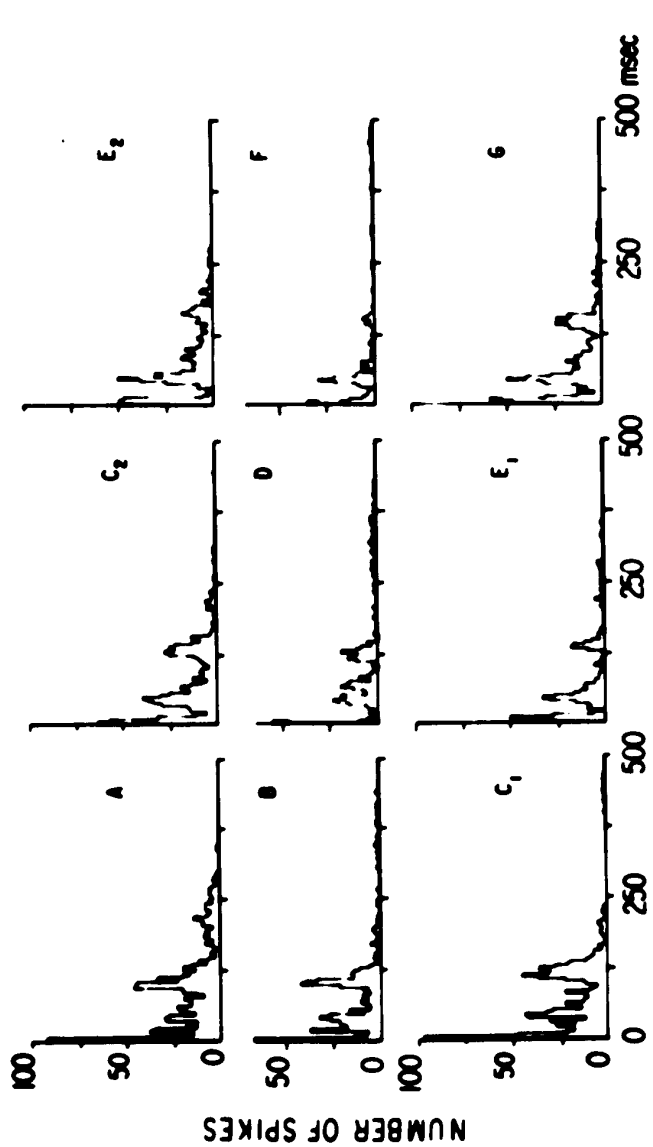

Figure 33. Post-stimulus time histograms of effects of GABA and glutamate during an ESR (same unit as in Figures 31 and 32). Periods analyzed are taken from respective letters of Figure 32. During B, GABA 20 nA; during D and F, glutamate 40 and 20 nA; remaining PSTHs are control periods, before and after iontophoresis (see Figure 32).

peaks up to 150 msec. These are reduced by glutamate. The interburst firing is increased with glutamate between 150 and 400 msec following the stimulus. These data suggest that 40 nA of glutamate is more effective than GABA in reducing intraburst firing and increasing interburst discharges during the ESR. Figure E_2, F and G show that 20 nA of GABA (the magnitude effective in producing total inhibition of firing elicited by 20 nA glutamate prior to the seizure) reduces intraburst firing while epileptiform activity is being prolonged. Both GABA and glutamate abolished most discharges that occurred towards the end of each burst, i.e., about 250 msec after the stimulus. Since no change in firing pattern up to 150 msec after each stimulus pulse was evident with glutamate, it cannot be stated that this agent causes a disruption of the burst pattern.

On the other hand, relatively large amounts of GABA cause burst disruption during the ESR. The series of histograms of Figure 34 from different recordings of the same cell as in Figure 32 show a pronounced abolition of organized firing. The top and bottom histograms are of control periods which preceded and followed, respectively, the 'high-dose' period of GABA application during the ESR. The tracing beside each histogram illustrates the firing pattern which the cell exhibited during the analyzed period. The histogram in A shows four large peaks at 8, 70, 350 and 400 msec after the stimulus. The latter three peaks are diminished during GABA administration (histogram B) although a remnant of the 70 msec peak is still visible (followed by about 150 msec of relative depression). The total number of spikes during GABA application is only 38% of the previous control period

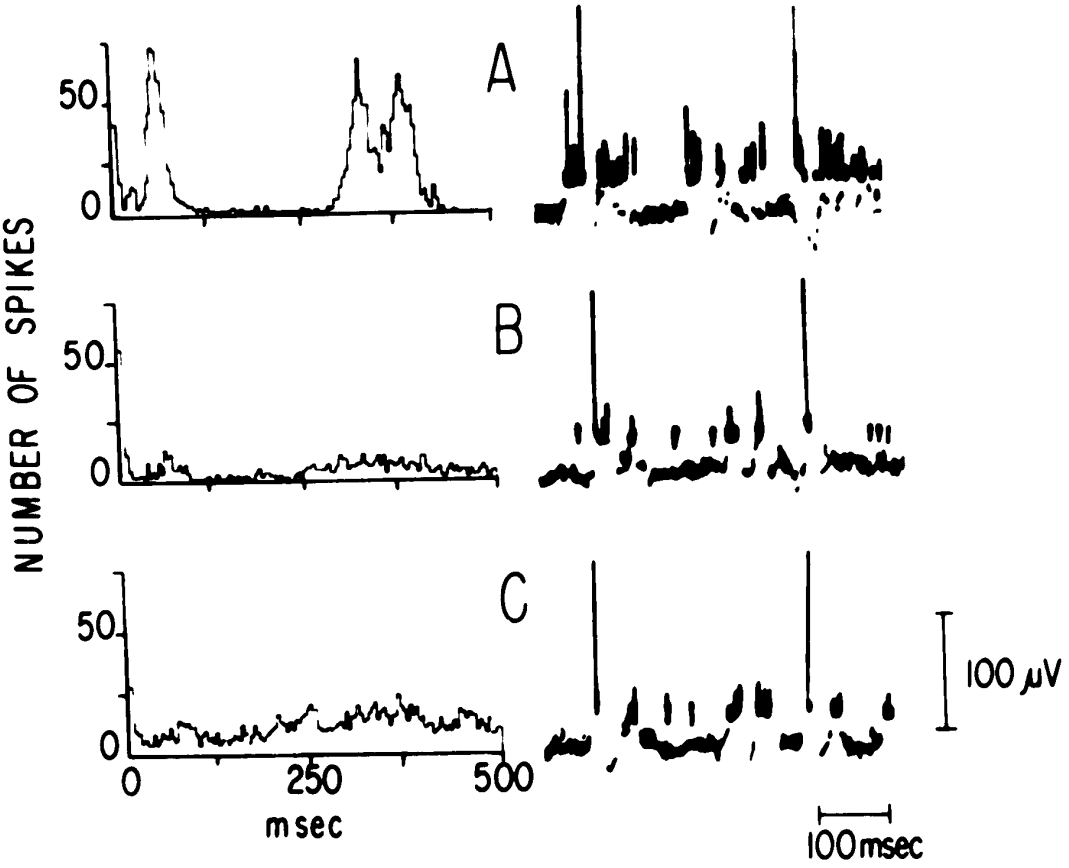

Figure 34. Post-stimulus time histograms and extracellular records before (top), during (middle) and after (bottom) 20 nA GABA iontophoresis during ESR. (Same cell as in previous three Figures). Extracellular records were taken from periods during which corresponding histogram was compiled. Presence of stimulus artifact can be seen in extracellular record as large amplitude vertical lines.

which suggests that a considerable depression of driven activity is produced by GABA. In the histogram C (the end of which is less than one minute after the end of histogram B) there is no indication that the cell has returned to its pre-GABA firing pattern. Cells exhibiting this behavior (<20% of total) eventually resumed their control firing rates and pattern. In this case the control pattern resumed within 2 minutes after the end of histogram B (end of GABA application).

The above results may be considered typical of this investigation since a depression of unit activity during the ESR by glutamate or GABA occurred in 90% of all ESRs studied. The cell responds to low frequency stimulation of the ESR with a similar pattern as cells stimulated at 2/sec when no EAD is present (see Figures 6B and 7). However, the effect of glutamate during subthreshold stimulation at 2/sec is in some contrast to the above findings. Glutamate increases early firing (compare A and C with B in Figure 35). GABA however, has been observed to decrease early firing associated with a subthreshold stimulus (Figure 35).

As mentioned previously, an ESR may end at a time when the burst following the stimulus pulse becomes progressively out of phase with the latter. This is not always the case. About 50% of the units studied have been observed to cease discharging after only a small decrease in the size of each burst when the ESR has ended without the ESR-stimulus being turned off. This is illustrated typically in Figure 36 A to D. Some depression of a response to glutamate has been seen after the termination of an ESR (Figure 36, E to G). The usual pre-seizure response to glutamate is obtained only 5 or 6 seconds after

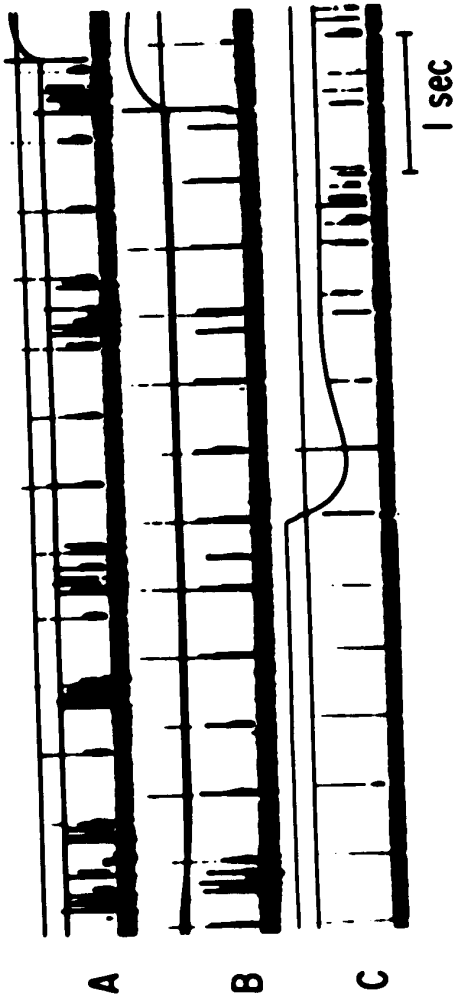

Figure 35. Continuous extracellular record of unit at depth 920μ during continuous subthreshold stimulation. 30 nA glutamate in A and part of C. 40 nA GABA on in B and off in C. Stimulus artifacts in most cases reach iontophoresis lines.

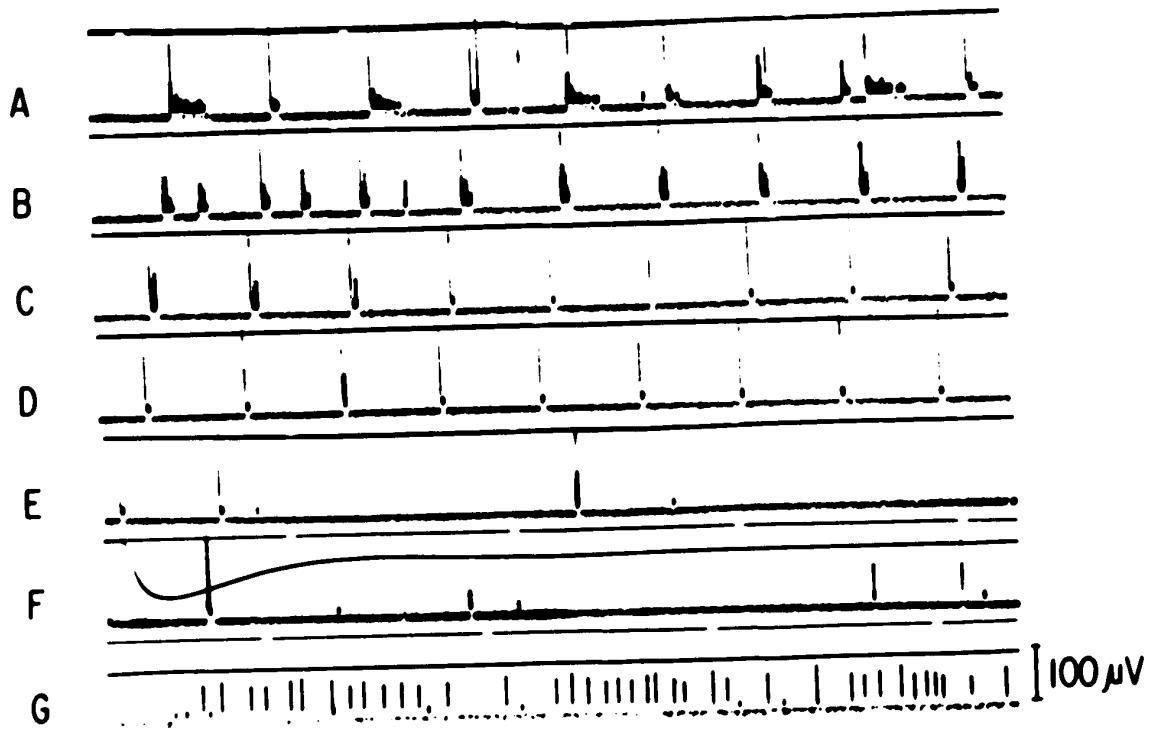

Figure 36. Continuous extracellular record of unit at depth 807μ during an ESR. Entraining pulse artifacts are equidistant and sometimes reach iontophoresis lines. Time marks on latter are 1 sec. Glutamate 30 nA in F, 50 nA in G. End of ESR is evident early in C.

glutamate iontophoresis has commenced. In this regard, the depression following termination of the ESR bears some resemblance to that following subthreshold stimulation or the EAD, where the cells sluggishly resumes firing, often only to greatly increased glutamate doses.

3. Summary and Discussion

As a rule, a depression of amino acid evoked firing or firing of spontaneously active cells is observed at the end of an afterdischarge whether the seizure ended spontaneously or was terminated by stopping the entraining stimuli. This depression bears some resemblance to the period of 'silence' which follows high frequency subthreshold stimuli. Both last for many seconds and the block can be surmounted by a larger dose of glutamate. During the end of a short seizure spike amplitude has been observed to undergo a progressive increase in height until its preictal size is reached (cf. Figure 28). The implication that an increase in repolarization of the membrane (or indeed, hyperpolarization) toward the preictal resting level occurs near the end of a seizure (Matsumoto and Ajmone Marsan, 1964; Sawa et al., 1965) is supported by the finding that glutamate can excite and GABA depress a units in the late clonic phase (see Results section C). Thus the data suggest a hyperpolarizing-type of block which is not in accordance with many of the mechanisms proposed for the termination of a seizure (see INTRODUCTION). In addition, surface electrical activity was usually very low at the end of an EAD and a negative spreading depression wave was not observed when unit inhibition was present. These data provide

additional presumptive evidence that the termination of a seizure is followed by a membrane hyperpolarization (Ayala et al,, 1970; Sawa et al., 1963; 1965; 1968; Sypert et al., 1970). All of the above-cited investigators agree that the long hyperpolarization is not due to prolonged IPSPs. One might speculate that this period of hyperpolarization might be due to an electrogenic sodium pump [as suggested for the depression following subthreshold stimuli]. Experiments were designed to test this hypothesis but ouabain abolished all cell activity before observations could be made.

The results of the present experiments also indicate that conditions which terminate the EAD can be attenuated or delayed by continuous low frequency stimulation of the cortical surface. Hence the original findings of Rosenblueth and Cannon (1942), and observations by Sanders and Pinsky (1967) who used neurologically-isolated cerebral cortex, are confirmed. Sanders and Pinsky (1967) have interpreted the ESR to mean that neurons participating in the EAD are in a "state of chronic facilitation". This was based on the fact that stimuli which had been previously ineffective in causing detectable surface responses, produced the large amplitude waves characteristic of an ESR when applied to any area of the cortex where an EAD could be recorded. Occlusion of unit discharge with a reduction of firing rate was seen when glutamate was applied during an ESR (see Figures 32 and 33). Relative susceptibility to occlusion would be an expected property of neurons with an excessive excitatory input, i.e., neurons with resting membrane potentials that oscillate or fluctuate near the threshold firing level. This type of activity has been postulated for epileptogenic cortex to explain the

large-amplitude surface responses to weak surface stimulation (Smith and Purpura, 1960). Surface and intracellular responses which have been studied in cortex made epileptogenic by a topical application of penicillin suggest a synaptically-activated mechanism. Matsumoto (1964) has demonstrated that the surface epileptiform bursts resulting from weak stimuli being applied to the cortex are synchronous with and dependent upon a critical level of the "paroxysmal depolarization shift (PDS)". Thus the ESR may involve the participation of synaptic mechanisms but this does not necessarily require the accompanyment of EPSPs (see Sanders and Pinsky, 1971). In the presence of a long-lasting depolarization level, glutamate would conceivably contribute to cause periods of inactivation. GABA might also be expected to prevent the production of 'all-or-nothing' spikes and thereby reduce the rate of firing (see Figures 33 and 34). The change in firing pattern induced by GABA (see Figure 34) is also predicted from the state of a reduced firing threshold. The disruption of the burst by GABA during the ESR is similar to that caused by GABA with or without concomitant application of glutamate during the EAD (cf. section C). It is suggested that in the presence of a recurring level of depolarization and inactivation, GABA might cause an apparent disruption of organized activity in the presence of an excitatory input. The reduced threshold condition suggested above is implied by other observations. First, there is a marked dependency of bursts to follow surface stimuli on the applied stimulus frequency, and on the 'spontaneous' firing pattern of the EAD (present observations and Sanders and Pinsky, 1967). Since the time course of activation of cell firing by low frequency subthreshold

stimuli differs from that observed at higher frequencies (see section B), occlusion might be produced if stimuli were applied at frequencies which did not approximate the "natural" frequency of the EAD bursts. Indeed, more inhibition or depression of firing is present following the early excitation after subthreshold stimuli than following cell firing in an ESR of the same stimulus frequency. This implies that a reduction of inhibition or state of facilitation may be present during epileptiform activity. Second, if one or two pulses are left out of the ESR stimulus train, the ESR ceases (present observations and Sanders and Pinsky, 1967). This suggests that the period of recurring facilitation is about one second and that each stimulus establishes a recurring low firing threshold which, in a population of neurons is critical if the ESR is to continue.

III. GENERAL DISCUSSION

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The data from this investigation provide some basis for suggestions concerning the underlying mechanisms of afterdischarge activity. However, much of the evidence obtained for existing theories of epileptiform activity is equivocal since more than one interpretation is possible. A possible exception in this regard is data obtained from studies on the ESR. The intention of this dissertation was to determine how excitatory and inhibitory mechanisms might be responsible for epileptiform activity. An imbalance of the two mechanisms rather than just an absence of inhibitory control is suggested by this investigation. Unit behavior as a result of this imbalance during seizures has led to a number of predictions:

> "The occurrence of high frequency discharges, oscillations of the membrane potential, and prolonged membrane depolarization leading to inactivation can all render the response to an externally applied test stimulus susceptible to the effects of occlusion, absolute and relative refractoriness, or membrane inactivation". (Berlin, 1966).

These effects have been demonstrated either directly or indirectly during seizures (see previous sections).

In order to understand how the epileptic state comes about, it is necessary to review some of the events during its development. Cortical cells respond to subthreshold stimulation with brief discharges followed by a period of depression (see section II-B). Other investigators (Li, 1959; Li and Chou, 1962; Sawa et al. 1963; Krnjević et al. 1966b) noted

that these events are associated with depolarizing potentials followed by long hyperpolarizing potentials and there is evidence to believe the latter represents "true inhibition" (Krnjevic et al. 1966b). Since there is no absence of depression of unit firing to glutamate as the seizure threshold is approached (see section II-B) it is suggested that the relative absence of inhibition is not the cause of electrically-induced afterdischarges in normal cortex. As previously mentioned, stimulus frequency is the only parameter of stimulation which can alter the afterdischarge duration and this, with other evidence, led Pinsky and Burns (1962) to suggest a "state of exhaustion" is induced by each stimulating pulse. They postulated that cells recover from this state during the inter-stimulus pulse interval so that a seizure develops out of the sum total of "exhaustion" of the stimulation period. Pinsky (1961) was explicit about the way in which this state could cause a seizure: a persistent depolarization of the soma of cells at the focus would initiate the process of diferential repolarization. The results of this investigation are basically consistent with this theory. The period of excitation following a stimulus to the cortex occupies a greater proportion of the inter-stimulus pulse interval as the effective stimulus frequency approaches an afterdischarge threshold (see section II-B). Moreover, the stimulus at higher rates produces effective early firing despite the presence of strong IPSPs still remaining from the previous stimulus pulse. Thus the excitatory input due to the stimulus must be very intense. Therefore, Pinsky and Burns theory might be interpreted in the following way: a focus for an afterdischarge may be induced by a conditioning stimulus which causes a minimum density of

neurons to be excited (driven) at a minimum net firing rate, a critical number of times. [The modification of their postulate has been underlined]. It appears therefore, that a focus for an afterdischarge can evolve only after a population of neurons has been activated at a particular rate over a number of stimulus pulse intervals. The total number of stimulating pulses does not seem to influence the afterdischarge duration (Pinsky and Burns, 1962) but this does not preclude some mechanism of seizure initiation, which is conceivably dependent on cell firing rate. Pinsky (1961) deliberately chose the hypothetical state to be in accord with the process of differential repolarization but excluded other mechanisms on grounds which have since become questionable. For example, an afterdischarge of the Renshaw cells in which the most rapid rate of repetitive discharge is found during its onset (Eccles et al., 1954), has been held due to a persisting transmitter (Burns, 1958). There is evidence that a rapid rate of repetitive firing occurs during stimulation (see Figures 16 and 17) and hence before the surface record indicates the start of an EAD. In addition, the hypothesis that the afterdischarges in the spinal cord are mediated by a persistent humoral transmitter is now in some controversy (see Weight, 1968; Curtis and Duggan, 1969, Eccles, 1969). Finally, a property expected for an EAD due to differential repolarization is that the latent period (as indicated by the surface record) between the end of the stimulus and the beginning of repetitive potential discharges is longer than the intervals between the first few potential discharges. While this implies that an EAD may develop out of an exhausted or depolarized state of a very large number of cortical cells, no cognizance

is taken of the subsequent occurrence of a mechanism other than differential repolarization.

Thus a condition of a sustained depolarization during and at the end of the stimulation period has been demonstrated both indirectly with extracellular recording techniques (Gerin, 1960; Sypert et al., 1970; see also section II-C) and directly with inteacellular recording techniques (Sawa et al., 1963; 1965; 1968; Sypert et al., 1970). However, there are suggestions that the genesis of seizure activity is not due to the intrinsic property of basal areas of neurons to repolarize at a slower rate than dendrites. Gloor et al. (1964a) have advanced the hypothesis that the negative DC-shift (observed during seizure genesis) in the basal layers of archicortex is the result of "summation of residual depolarizations in neuronal membranes subjected to afferent bombardment". They suggest that this condition facilitates the discharge of pyramidal cells within the layer since the activition of contiguous pyramidal cells over recurrent collaterals would constitute a positive feedback system. Hence the recovery between discharges would be prevented by a recurring, intense synaptic input. It is clearly evident, therefore, that seizure initiation need not be a process of differential repolarization and might even be a process of PTP in which an enhanced transmitter release takes place (see INTRODUCTION and also Gloor et al., 1964b).

Gloor et al. (1964a) were aware that the depolarized state with a positive feedback would inevitably result in a cathodal inactivation of pyramidal elements during the seizure. The observation that spike initiation occurs when GABA is applied iontophoretically during a slow

development of a seizure (see Figure 23) indirectly supports their contention. The above authors proposed a recurrent negative feedback system of hyperpolarizing potentials (IPSPs) which "resets" the membrane potential of inactivated cells to levels where excitatory synaptic input can "reactivate the seizure discharge" and maintain seizure activity. Similar reports of a restoration of a firing level of inactivated cells by hyperpolarizing potentials provide additional evidence for such a mechanism (Li, et al., 1961; Klee and Lux, 1962). The participation of synapses was suggested by Gloor and his associates because of observations that a positive (sustained depolarization with inactivation) or negative (restoration of a normal firing level) "resetting" could be obtained by stimulating one or other of the afferent paths to the hippocampus. This hypothesis is consistent with numerous observations of inactivation during the early and later stages of the seizure process in either archicortex (see Spencer and Kandel, 1969) or in neocortex (see INTRODUCTION). This investigation also supports these findings with indirect evidence of membrane inactivation (see section II-C).

Since the present studies also imply recurring conditions of excessive excitatory input, it is conceivable that a "state of facilitation" exists during maintenance of a seizure as suggested by Sanders and Pinsky (1967). However, the findings that either GABA or glutamate cause occlusion during an ESR (see section II-D) are not consistent with any contertion that differential repolarization mediates a regenerative activity during prolongation of an EAD. If anything, glutamate should prolong and GABA should reduce unitary repetitive discharges due to a process of differential repolarization by increasing or

decreasing, respectively, the proposed current sink of the soma (irrespective of the action of these substances upon nerve endings on the soma). In this reagard, the maintenance of an afterdischarge is implicit if an afterdischarge were due to a persistence of transmitter. Thus the continued application of weak stimuli at low repetition rates (during an EAD) is predicted by this theory to prolong an afterdischarge. The action of GABA is consistent with the above. The occlusive action of glutamate during an ESR could only be reconciled as further increasing the depolarization of an already depolarized cell to the point of inactivation. Direct evidence for this is lacking, since no intracellular studies during the ESR have been made.

As already discussed in the preceding sections, the termination of afterdischarge activity has been attributed to a number of different mechanisms including membrane inactivation. The observation that a cell can be excited by glutamate only at the end of the clonic phase (see section II-D) provides presumptive evidence that an increase in repolarization level from levels of strong depolarization and inactivation occurs during the clonic phase. These results are supported by direct observations of a decreased occurrence of large depolarizing waves during the clonic phase followed by membrane hyperpolarization at the end of the seizure (Sawa et al., 1963; 1965; Sypert et al., 1970). The absence of electrical activity at the end of the seizure period can be overcome with glutamate (see section II-D) and, therefore, concurs with the data for a hyperpolarized state. This condition does not occur in all cells because Gloor et al. (1964a) have observed an arrest of a seizure when an excessive negative DC-shift develops in basal layers

of the hippocampus. However, the arrest of seizure activity has not been well studied in neocortex (Levy and O'Leary, 1965) and the existence of cell populations with differing excitabilities at the end of seizures appears likely.

There appears little doubt that the large repetitive potentials recorded by surface electrodes during the clonic phase must result from a very large number of cortical neurons discharging synchronously (see section II-C). Various mechanisms have been proposed to account for the observed synchronous behavior of cortical units. Synchrony has been held due to synaptic transmission (Burns and Grafstein, 1952; Purpura and Grundfest, 1956), to interstitial current flow (Katz and Schmitt, 1940; Libet and Gerard, 1941) or to ephaptic transmission (von Baumgarten and Schaefer, 1957). Also, Minsky (1969) has recently presented a theoretical exposition of the principles of synchrony which predict that during afterdischarge activity, neuronal assemblies will take on a single repetitive rhythm of discharge and eventually arrest in occlusion. The present experiments, however, cannot provide evidence for or against any of the above mechanisms. The studies of Morrell (1969) have some bearing on the present finding that the EAD can be prolonged as in the ESR. He found that units in visual cortex can be made to burst at the same frequency as that of light flashed to the animal subjected to surface-positive transcortical polarization. When the animal was later (30 sec to 20 min) presented with 3/sec light flashes in the absence of a transcortical polarization visual units responded with 3/sec bursts. The polarization of cortical layers during an EAD is well documented (see INTRODUCTION) and its influence upon the neuronal

responses to subsequent low repetition stimuli (either endogenously-occurring or externally-applied) remains open as an area for speculation. Other concepts of "cooperative" behavior of organizations of neurons (Cragg and Temperley, 1954; Beurle, 1956) have some applicability to the maintenance of seizures and the ESR. Cragg and Temperley (1954) suggest that in a "co-operative analogy", nerve cells would discharge when its immediate environment became occupied by a domain of high excitation. The rate of firing of a single cell would be dependent on the excitation voltage of the domain while its synchrony would depend on whole patterns of domains. Beurle's theory is similar but is a quantitative treatment that assumes a mass of cells to have properties identical to a single cell. A cooperative state may appear as a wave which may be initiated in a mass of cells by individual stimuli. If some property of an individual cell (e.g., threshold) changes with repeated use, the mass of cells also displays a tendency to modify its response according to its previous experience. The size and geometry of the mass of cells and its maintenance in the cooperative state is predicted by Beurle's theory to be largely dependent upon the properties of the conditioning stimuli. Although the present studies cannot substantiate evidence for theories of cooperative processes, the results of this investivation (see section II-D) are consistent with them.

IV. SUMMARY AND CONCLUSIONS

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1. In cats maintained as cerveau isolé preparations, subconvulsive epicortical stimulation produced a pattern of early post-stimulus-pulse firing followed by relative depression of discharges of a cortical unit whether spontaneously-active or excited by iontophoretically-released L-glutamic acid. Maximum early firing occurred at 10 to 35 msec following a stimulus pulse and at stimulation rates of 3/sec. These results confirm the studies of Krnjević and his co-workers (1966a; 1966b; 1966c). An increase in the extent of early excitation was not detected by PSTH analysis as the stimulation rate approached the frequency effective for EAD initiation. However, early excitation occupies a greater proportion of the stimulus inter-pulse interval as the latter is decreased. The period of relative inhibition which followed excitation was about 65 to 300 msec in duration and appeared to increase in intensity as a seizure threshold was approached by increasing the stimulation rate. More net depression occurred during subconvulsive stimulation than during an induction of an EAD. An apparent 'rebound' excitation or depression followed a period of subconvulsive stimulation and appeared to be dependent upon the stimulus strength (voltage). None of the above responses were attributable to a specific group of neurons or layer within the cortex.

2. The above original findings suggest that the loss of inhibitory control mechanisms is not responsible for the induction process of an EAD [although GABA usually ceased to be effective once the seizure was underway (see below)]. The degree of activation of a population of cortical units over a number of time intervals appears to be an important factor for an afterdischarge to occur. An EAD may develop out of the "state of exhaustion" postulated by Pinsky and Burns (1962).

3. There is no apparent gradual transition in subthreshold events to a production of an EAD. The behavior of many cells during threshold stimulation exhibited indirect evidence of a paroxysmal, intense depolarization thus confirming the intracellular observations (see INTRODUCTION). The reduction of extracellular spike amplitude persists into the ictal period. For the most part, glutamate or GABA were ineffective in altering cellular discharge during an EAD. When iontophoretically applied in amounts that were effective in producing excitatory effects prior to seizure initiation, glutamate infrequently caused excitation when a burst pattern was developing or as the end of an EAD became evident. During the above periods, a reduction of spike amplitude and occlusion was occasionally produced by glutamate. When GABA was iontophoresed in addition to glutamate, particularly during the tonic phase, a blockade was produced followed several seconds later by a high frequency discharge. This phenomenon was observed [with or without disruption of burst activity] when GABA was applied alone early in the EAD. During the late clonic phase, GABA caused depression of afterdischarge activity.

GABA was not found to alter the functional refractory period as indicated by interspike interval analysis.

4. The above studies provide new evidence that excessive excitatory synaptic input is present throughout most of the afterdischarge in a large population of cortical cells. The relative refractoriness and occlusion of units observed during glutamate application especially during the tonic phase is interpreted as indirect evidence for membrane inactivation by depolarization. The paradoxical excitant effect of GABA is similarly reconciled.

5. The termination of an afterdischarge or an ESR is accompanied by an absence of cell firing whether spontaneous or glutamate-induced. Relatively large amounts of glutamate can excite cells during this period. As discussed previously (see INTRODUCTION) there are conflicting reports concerning the underlying mechanism of the termination of the EAD. These data however provide presumptive evidence that cells are strongly hyperpolarized during the late clonic phase and at the termination of EAD activity.

6. An EAD may be prolonged by low frequency continuous epicortical stimulation for periods up to 30 minutes and thus confirms the observations of Rosenblueth and Cannon (1942) and Sanders and Pinsky (1967). Both glutamate and GABA applied during the ESR produce depression of entrained activity. This is in contrast to the effects of glutamate during subconvulsive stimulation. These new data suggest that surface entrainment produces a "state of facilitation" similar to that described by Sanders and Pinsky (1967).

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