Inhibitory Mechanisms in the Cerebral Cortex

ABSTRACT

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A LINC-8 computer facilitated quantitative analysis of the magnitude and duration of conduction and potential changes during recurrent pyramidal and surface evoked IPSPs in pyramidal tract neurones of barbiturate anaesthetized cats. Potentiation of recurrent pyramidal IPSPs by surface evoked IPSPs and a similar IPSP reversal potential for both, coupled with evidence of occlusion following simultaneous activation of various inhibitory pathways suggested a common inhibitory pathway to corticospinal neurones. Exploration of the cortex with closely spaced multi-barreled micropipettes revealed that glutamate evoked firing of some nonpyramidal neurones was associated with a decreased firing frequency of neighbouring pyramidal and other nonpyramidal neurones, an inhibitory pattern in joint scatter diagrams and unidirectional negative cross-correlations at latencies sufficiently short to suggest a monosynaptic connection. Further studies on these putative inhibitory neurones evaluated their spontaneous activity patterns, responses to stimulation of inhibitory pathways and to iontophoretic applications of acetylcholine.

INHIBITORY MECHANISMS IN THE CEREBRAL CORTEX

by

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To Lillian, Francine and Barbara

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Mr. Kit McSweeney wrote computer programs to handle two trains of spikes and to perform auto- and cross-correlations.

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Chapter 1

INTRODUCTION

Inhibition is defined as anything decreasing the probability that a given neurone will discharge, with the proviso that there has been no previous excitation of that neurone. Synaptic inhibition implies release of an inhibitory chemical transmitter, from nerve endings of inhibitory neurones, which acts upon the membrane of the postsynaptic neurone in such a way so as to stabilize the membrane and to reduce the efficacy of any excitatory synaptic activity. This description applies primarily to post-synaptic inhibition which is the more common and better understood mechanism operating in the mammalian central nervous system

A. HISTORICAL BACKGROUND

From a historical viewpoint, it is only in very recent times that neurophysiologists have come to recognize inhibition as having an independent role in information transfer, possibly as important a role as that of excitation. In the early nineteenth century it was viewed mainly as a peripheral process. For example, in 1838 Volkmann first recognized and described a phenomenon which was one of the earlier clear manifestations of neural inhibition, namely cardiac standstill following vagal

stimulation. However, owing to the criticism he received from eminent contemporary physiologists, who rejected this "new" concept of inhibition as a basic principle of nervous operation, Volkmann finally conceded that his results were probably invalid and most likely the result of fatigue in sensitive motor nerves. Despite similar opposition, in 1845 the Weber brothers revived the concept of vagal inhibition of the heart, and extended the notion of nervous inhibition to central nervous system functions. They suggested that the increased spinal reflex activity which could be demonstrated following ablation of the brain resulted from the removal of a nervous mechanism which inhibited the spinal reflex machinery. In 1863 additional support for a central inhibitory mechanism came from Sechenov's demonstration on the frog that weak but prolonged stimulation of mesencephalic roof structures increased the latency of reflex movements elicited by stimuli to the skin (c.f. Meltzer 1899; Schäfer 1900).

Ablation experiments were followed by other experiments on the intact brain, which further suggested an inhibitory influence of the cerebral cortex on spinal centres. Siminoff (1866) elicited movements of contralateral extremities by stimulation of the frontal cortex, but he also noted that a stronger stimulus was required to produce the same effect if, in addition, simultaneous electrical stimulation was applied to the frontal portion of the forebrain. Bubnoff and Heidenhain (1881) demonstrated that stimulation of contralateral motor and

other cortical areas as well as midbrain structures produced inhibition of tonic contraction of the extensor digitorum communis longus in morphine-anaesthetized dogs. Signs of inhibition could also be seen in unanaesthetized preparations; for example, Libertini and Fano (1895) observed a decrease in magnitude and duration and an increased latency of the withdrawal response of the limbs following both contra- and ipsilateral cortical stimulation. By the turn of the century both excitation and inhibition were recognized as active and essential features of cortical function.

It was largely through the efforts of Sherrington and his associates that inhibition was elevated to a role equal in important to that of excitation, as was clearly emphasized in his treatise on the integrative function of the nervous system (Sherrington 1906). In 1893 Sherrington observed that cortical stimulation produced inhibition of antagonistic muscle groups in the contralateral limbs. Also noted was the enduring character of the central inhibitory process, which considerably outlasted the stimulus producing it. Furthermore, Graham Brown and Sherrington (1912) observed a mutual interference between the effects of simultaneous cortical stimulation at two different points, indicating that inhibition was present at both cortical and spinal levels.

The contributions of two Russian physiologists deserve particular mention. Sechenov's earlier experiments (1863, 1882) were followed by his description of the general importance of

inhibitory mechanisms in central integrative processes (Sechenov 1935). Pavlov (1927) fruitfully and extensively utilized the concept of cortical inhibition in his analysis of conditioned reflexes. He believed inhibition was an active neuronal function, not simply an exhaustion of cortical elements, and postulated that spinal or cortical centres were capable of inhibiting not only other areas but also themselves (disinhibition). Sleep was considered as an extension of the same internal inhibitory process and a means whereby fatigued neurones recovered their normal condition. He went on to postulate that some psychopathological disorders occurred because of a conflict between excitation and inhibition which could not be resolved by the cortex. The latter hypothesis was generally not acceptable to most neurophysiologists who gradually segregated themselves from psychological disciplines. Consequently for several years to follow there was a temporary neglect of further study of processes underlying cortical inhibition. Nevertheless, of more importance was the acceptance by this time that inhibition was an independent physiological process, even though the mechanisms by which inhibitory phenomena were produced were largely unsolved.

B. EARLIER THEORIES OF INHIBITION

As aptly stated by Gasser (1937) "... the large number and diversity of the theories about the nature of inhibition in the nervous system may be taken as a measure of the obscurity which has surrounded the subject". Fatigue of nerve fibres and vasoconstriction of nerve arteries appeared as explanations in

much of the earlier literature. Nerve excitation was proposed by Hering (1872) to be a catabolic process with inhibition acting as an anabolic activator to restore equilibrium. Gaskell (1882) felt that such a mechanism explained the rebound acceleration of heart rate which followed vagal stimulation. Hering's hypothesis was mentioned not infrequently in the writings of both Sherrington (1906) and Pavlov (1927). In 1905 MacDonald presented a rather interesting proposal. He suggested that during inhibition positive charges, presumably derived from presynaptic activity, induced colloidal changes in the neuronal membrane. This prevented excitation by impeding movements of certain ions, notably potassium, across the post-junctional membrane.

Schiff (1858) first described in frog nerve-skeletal muscle preparations the decreased muscle contractions which followed prolonged repetitive nerve stimulation. Wedensky (1885, 1903) demonstrated that such an event was not caused by nerve exhaustion but rather the result of neuromuscular junction blockage due to summation of subnormality when impulses arrived too frequently for recovery of the postsynaptic membrane. For the next half-century the mechanism of "Wedensky inhibition" was to be mentioned as one possible explanation for certain features of 'central inhibition' (Adrian 1924; Lucas 1917; Forbes, Battista and Chatfield 1949).

The introduction of more refined techniques to the study of neurophysiology permitted differentiation of two categories of the phenomenon described collectively as 'central inhibition'.

Provided that central neurones were activated prior to the appearance of inhibition, a prolonged period of subnormality of recovery (refractoriness), first described by Graham (1935), sufficed to explain so-called "indirect inhibition" (Eccles and Sherrington 1931; Gasser 1937). Conditions for "direct inhibition" required demonstration of inhibition without prior discharge of either the tested motoneurones or premotor interneurones (Gasser 1937). One such example was described by Renshaw (1941). He demonstrated that antidromic activation of some motoneurones depressed the response of neighboring uninvaded motoneurones to reflex activation for as long as 40-50 ms. As one possible explanation, which also took into account the short latency of the response, Renshaw postulated that the antidromic inhibitory action was mediated either directly (monosynaptically) to neighboring motoneurones or indirectly (disynaptically) through an interneurone over recurrent motoneurone axon collaterals described earlier by Kölliker (1891), Lenhossek (1893) and Cajal (1909).

There was much speculation concerning the mechanism of inhibition. The older theories of Wedensky were inadequate to account for the inhibitory phenomena, in part due to the enduring nature of 'central inhibition'. One view proposed that different synaptic knobs had varied effects depending upon where they impinged on the soma. By presupposing a constant flow of current between dendrites and axons, contacts on the soma and dendrites would tend to add/to the current flow and

therefore excite, while those on the axon hillock or further downstream would tend to substract from it and therefore inhibit the cell. Gerard (1932) first gave this theory clear expression in his comprehensive view of chemical and electrical mechanisms of nerve function. He postulated that there was a spatial separation of excitatory and inhibitory endings on opposite poles of a motoneurone. The same local change in one case caused an increase, in another a decrease in the polarization gradient along the cell, thereby influencing its state of activity.

Because many neurophysiologists at the time believed in the electrical nature of synaptic transmission, any explanations for excitatory or inhibitory events had to be formulated in terms of an electrical process. Grundfest (1940), Lloyd (1944) and Lorento de Nó (1947a) ascribed inhibition to polarization of adjacent neurones as a result of extrinsic currents generated by active neurones. Brooks and Eccles (1947) offered a theory of central inhibition which was based on the two neurone arc. They proposed that presynaptic activity of Golgi neurones caused an inward flow of current into the postsynaptic cell and through hyperpolarization prevented a spread and fusion of excitatory foci.

C. INHIBITORY PATHWAYS

The Webers (1845) through their studies on the autonomic nervous system were possibly the first to recognize that tissues could be innervated by two pathways, one excitatory (sympathetic), the other inhibitory (vagus). Subsequent studies in invertebrates revealed the presence of specific inhibitory (and excitatory)

nerve fibres to the adductor muscle of the bivalve Anodon (Pavlov 1885) and claw muscles of the crayfish (Biedermann 1887). However, in the peripheral nervous system of vertebrates no inhibitory efferent fibres could be detected which could provide one explanation for the mechanisms underlying reciprocal inhibition in the spinal cord (Sherrington 1906). One notable pioneer contribution came from the laboratories of the Japanese physiologist Kato (1934) which suggested the presence of a specific inhibitory class of primary afferent fibres in amphibia. Although such a finding would have added new insight into the mechanisms of spinal inhibition, for which Sherrington (1906, 1925) had provided good experimental evidence,it appears that Kato was mistaken in the details of the pathway since his results have not been reproduced by other physiologists.

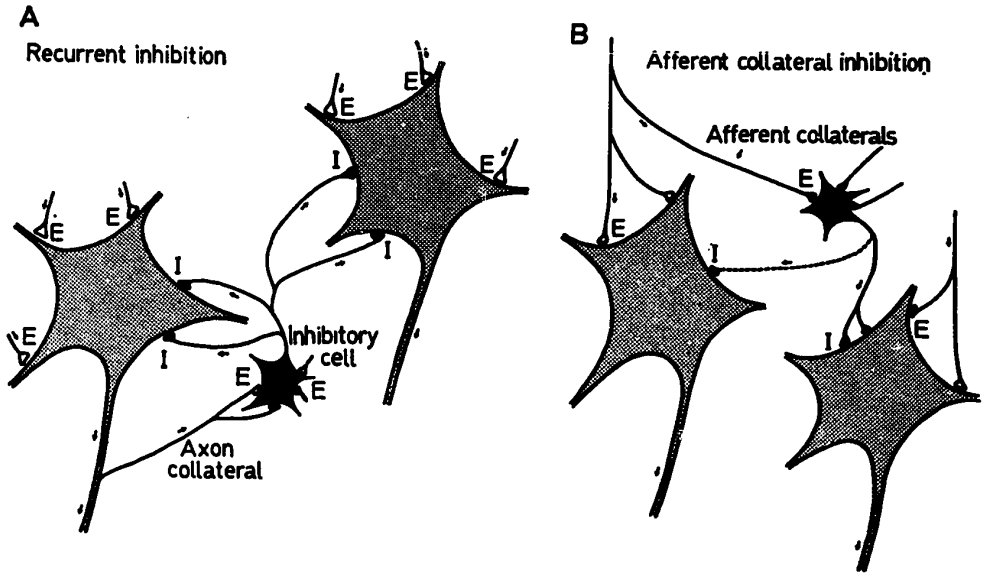
Sherrington's concept of central inhibition did not require the existence of separate inhibitory pathways or neurones. This is somewhat reminiscent of Cajal (1909, 1911) who, despite his magnificent achievements in morphology,never recognized the existence of discrete inhibitory pathways or neurones (nor for that matter the existence of inhibition). Sherrington simply envisaged that a given nerve exerted excitatory synaptic action at some of its branches (e.g. to flexor motoneurones) and inhibitory synaptic action at others (e.g. extensor motoneurones), without necessarily revealing a morphological synaptic differentiation.

For almost two more decades it was strongly believed that inhibition occurred as envisaged by Sherrington, with both

excitatory and inhibitory synapses originating from the same nerve fibre. Many ingenious solutions were offered (e.g. Eccles 1953) in order to accommodate Sherrington's concept of 'ambivalent' nerve fibres, having both excitatory and inhibitory effects, with the belief that a nerve fibre released the same transmitter substance at all its synapses (Dale 1935). Even with the advent of intracellular recording (Ling and Gerard 1949), the discrete hyperpolarizing inhibitory postsynaptic potentials (IPSPs) demonstrated in motoneurones following an afferent volley (Brock, Coombs and Eccles 1952) were not recognized as resulting from the activity of specific inhibitory pathways i.e. the axons of inhibitory interneurones.

Lloyd (1941) first described one of the better understood inhibitory pathways in the spinal cord i.e. that from Ia fibres to motoneurones of antagonistic muscles. Due to the brevity of the inhibitory response Lloyd (1946) was led to postulate that this was "direct (i.e. monosynaptic) inhibition". Intracellular recordings revealed that the inhibition of reflex discharge observed by Lloyd was associated with a motoneurone hyperpolarization (the IPSP) with a comparable time course (Brock, Coombs and Eccles 1952). The IPSP latency was almost a millisecond (i.e. one synaptic delay) longer than the latency of the monosynaptic EPSP (Eccles, Fatt and Landgren 1954, 1956). Further studies (Eccles, Fatt and Landgren 1956; Eccles, Eccles and Lundberg 1960) revealed that Ia impulses selectively produced monosynaptic excitation of interneurones in the intermediate nucleus, and suggested these were interpolated in the Ia inhibitory pathway. Confirmation was obtained by recording

Figure 1. Two Elementary Inhibitory Circuits. In A, the inhibitory circuit is brought into action through axon collaterals of active neurones, with an inhibitory neurone (in black) feeding back onto the same and and other neurones responsible for its activation, thereby producing recurrent inhibition. In B, the inhibitory effects are produced through excitation of inhibitory neurones by collaterals of afferent fibres, and therefore, the inhibitory circuit does not depend on prior excitation of neurones which they inhibit. (E) represents excitatory and (I) inhibitory synapses. Reproduced from Eccles 1969.


A
Recurrent inhibition
E
I
Inhibitory cell
Axon collateral
B
Afferent collateral inhibition
Afferent collaterals

short latency IPSPs in motoneurones following brief electrical stimuli in the intermediate nucleus (Eide, Lundberg and Voorhoeve 1961).

In 1954, Eccles, Fatt and Koketsu demonstrated that antidromic activation of motoneurone collaterals resulted in monosynaptic activation and repetitive discharge of small ventral horn "Renshaw" interneurones, called after the man who first described the effects of antidromic stimulation of ventral roots (Renshaw 1946). The same antidromic volley also produced inhibition of neighboring motoneurones (Renshaw 1941) and this was reflected intracellularly as an IPSP with a central latency sufficiently long to allow one synapse between the excitatory effects on Renshaw cells and the inhibitory effects on motoneurones. The evidence was clearly in favor of a disynaptic inhibitory pathway mediated by Renshaw inhibitory neurones.

Based on this evidence it appeared that there were but two classes of nerve cells in the central nervous system: those purely excitatory and those purely inhibitory (Eccles, Fatt and Landgren 1954, 1956; Eccles 1957). A further outcome was the recognition of two elementary inhibitory networks controlling neuronal activity: the recurrent and the afferent collateral pathways (Eccles 1965). In the former (Figure 1A) the inhibition acts as a negative feedback to already excited neurones; one example includes Renshaw cell inhibition of spinal motoneurones. As depicted in Figure 1B, inhibition can be

exerted in the forward direction relative to the excitatory lines of action, hence the term feed forward or afferent collateral inhibition. Included in this classification are the spinal inhibitory interneurones of Rexed's lamina VII responsible for inhibition of motoneurones to antagonistic muscles following annulospiral Ia afferent fibre activation.

Further systematic investigations have supported Eccles' earlier findings and provided electrophysiological evidence to support the existence of neurones whose sole function is inhibitory in the spinal cord (for review see Eccles 1969), cuneate nucleus (Andersen, Eccles, Schmidt and Yokota 1964), cerebellum(Eccles, Llinas and Sasaki 1966), thalamus (Andersen, Eccles and Sears 1964; Marco, Brown and Rouse 1967), hippocampus (Andersen, Eccles and Løyning 1963) and hypothalamus (Murphy and Renaud 1969). Many of these cells reveal features different from other CNS neurones. They usually are difficult to locate and have small spikes. They are active when other neurones show inhibition. Their axons are widely dispersed but mainly in the neighbourhood of cells which they inhibit. On the other hand, the cerebellar Purkinje cell is one obvious exception to this generalization.

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neurone interposed in the corticospinal inhibitory pathway. The meaning of their statements became clearer when Lundberg and his colleagues (1962 a,b) showed that the corticospinal pathway could facilitate the Ia inhibitory pathway to motoneurones and had excitatory synapses on the Ia inhibitory neurones. Similar results were noted following volleys in the rubrospinal tract (Hongo, Jankowska and Lundberg 1965). Therefore, it was clear that the long descending 'inhibitory' pathways were in fact excitatory and that supraspinal control of transmission was a complex operation mediated in part through spinal inhibitory neurones (c.f. Lundberg 1967). More recently monosynaptic IPSPs have been observed in some thoracic motoneurones, probably originating in the contralateral medial vestibular nucleus (Wilson, Yoshida and Schor 1970) implying that there may be other long direct inhibitory pathways elsewhere in the CNS (c.f. length of the Purkinje cell axons).

One other feature of inhibitory neurones is their ability to inhibit themselves (Figure 2). In attempting to explain the excitatory action that Renshaw cell activation had on some motoneurones (Renshaw 1941; Wilson 1959) Wilson and Burgess (1962a) postulated that some interneurones inhibitory to motoneurones were themselves subject to recurrent inhibition. The motoneurone facilitation was therefore brought about by a process of <u>disinhibition</u>. Many investigators have observed an inhibitory action of motor axon collaterals on spinal interneurones (Frank and Fuortes 1956; Hunt and Kuno 1959; Koizumi,

Ushiyama and Brooks 1959; Wilson and Burgess 1962b; Biscoe and Krnjević 1963; Smerdlov and Maksinova 1965; Willis and Willis 1966) without identifying the interneuronal excitatory afferent connections. A series of systematic investigations has now revealed that Renshaw cells exert an inhibitory action not only on other Renshaw cells in the neighbouring segments (Ryall 1970) but also on Ia inhibitory interneurones (Hultborn, Jankowska and Lindström 1968, 1971a,b; Ryall and Piercey 1971). Disinhibitory pathways can be found in other CNS areas, for example the inhibition of cerebellar Purkinje cells through parallel fibre activation of inhibitory stellate and basket cells (Eccles, Llinas and Sasaki 1966; Ito and Yoshida 1966).

D. SYNAPTIC TRANSMISSION: ELECTRICAL OR CHEMICAL?

After discovery of the Leyden jar had made the study of electricity possible, physiologists became keenly interested in this force as a possible explanation for nervous transmission. Throughout the latter half of the eighteenth century much effort was devoted to formulating an electrical theory of transmission. Varied explanations were offered by such prominent physiologists as du Bois Reymond (1877), Kühne (1888) and Biedermann (1895). The development of the neurone theory (c.f. Cajal 1954) emphasized the need for an explanation of transmission as occurring between contiguous rather than interpenetrating structures (see reviews by Grundfest 1957 and Brazier 1959). Claude Bernard's earlier discovery of transmission blockade by curare (1849) provided but one of many stumbling blocks to

the electrical theory of transmission. Different solutions to the problem were proposed by Lucas (1907) and Lapique (1926), with the last serious attempt at an electrical explanation being given by Eccles (1946).

Although many physiologists entertained doubts concerning the electrical transmission hypothesis, pharmacology provided the major impetus in formulating a chemical transmission hypothesis, dating from Elliott (1904, 1905) who first suggested that adrenalin might be the chemical stimulant released when impulses reach sympathetic nerve endings. In 1921 Otto Loewi began his series of experiments which were to establish the neurohumoral transmission concept, beginning with his demonstration of the inhibitory effect of "Vagusstoff" on the frequency of contractions of the frog heart. The subsequent work of Dale and his colleagues was complementary to that of Loewi particularly in establishing the role of acetylcholine as a chemical mediator (Dale 1937; c.f. bibliography by Schild 1971). Meanwhile, Cannon and his colleagues were obtaining experimental evidence in support of adrenergic transmission in the sympathetic nervous system (c.f. Cannon and Rosenblueth 1937).

The most convincing electrophysiological evidence for chemical transmission was obtained from the experiments of Fatt and Katz (1951, 1952) on the mechanisms underlying the formation of the end-plate potential, and the similar changes produced by both the neuromuscular transmitter and by acetylcholine (del Castillo and Katz 1954, 1955a,b; c.f. Kuffler 1943, 1949).

To these can be added studies on synaptic inhibition in the crayfish nerve-muscle preparation (e.g. Kuffler and Katz 1946; Fatt and Katz 1953). The results of these studies were no doubt influential in convincing proponents of the electrical theory (c.f. Eccles 1946) to abandon this hypothesis in favor of the chemical nature of junctional transmission (c.f. Eccles 1953).

Studies of inhibitory mechanisms in crustacea (e.g. Kuffler and Katz 1946; Fatt and Katz 1953; Kuffler and Eyzaguirre 1955) led to investigation on the actions of gamma aminobutyric acid (GABA) on the crayfish stretch receptor (Elliott and Florey 1956; Kuffler and Edwards 1958; see review by Ginsborg 1967). Similarities between the action of GABA and synaptic inhibition served to identify GABA as the inhibitory transmitter agent (see later section below) and provided an important model for the study of mechanisms of synaptic inhibition in other systems.

E. QUANTAL THEORY OF CHEMICAL SYNAPTIC TRANSMISSION AND THE PRESENCE OF SYNAPTIC VESICLES

In 1950 Fatt and Katz observed that in isolated resting muscle, the junctional region was the site of spontaneous discharges about 0.5 mV in amplitude and occurring at random moments. These were called miniature end-plate potentials (Fatt and Katz 1952), no doubt arising from localized impacts of small quantities of acetylcholine upon the postjunctional muscle membrane. The phenomena has been observed at all kinds

of vertebrate myoneural junctions, and similar spontaneous potentials have also been found to occur at neuronal synapses in the central nervous system (Katz and Miledi 1963) where the excitatory chemical transmitters remain to be precisely identified. At the myoneural junction statistical analysis revealed that the amplitude distribution of the miniature potentials fitted accurately a Poisson series (Boyd and Martin 1956) enabling one to state confidently that they were the result of quantal release of acetylcholine from motor nerve terminals in minimum packets of large multimolecular size. Coincidental with the above was the discovery of vesicles in presynaptic endings (de Robertis and Bennett 1954; Palade and Palay 1954) and the suggestion that the vesicles formed the structural units responsible for quantal release (del Castillo and Katz 1955a,b). The presence of vesicles at other synapses in the central nervous system (reviews by Palay 1958; de Robertis 1959; Whittaker and Gray 1962) has led to the generalization that these are also "chemically transmitting presynaptic terminals". However, as Katz (1966) has stated 'without more direct evidence this generalization would be difficult to defend because the presence of vesicles, though characteristic of such terminals, is certainly not restricted to them' . As will be described in a later section, the shape of the vesicles themselves has also been recently implied to be of functional significance.

F. ION MOVEMENTS DURING SYNAPTIC INHIBITION: EQUILIBRIUM POTENTIALS

The cell membrane potential is a diffusion potential generated by the marked differences in concentration of K^+ inside and outside the cell and the differences in membrane permeability (P) to K^+ and Na^+. The resting potential (V_m) may be quantitatively expressed by Goldman's constant field equation as adapted by Hodgkin and Katz (1949) so that

$$V_m = \frac{RT}{F} \log_e \frac{P_K (K_i) + P_{Na} (Na_i) + P_{Cl} (Cl_o)}{P_K (K_o) + P_{Na} (Na_o) + P_{Cl} (Cl_i)}$$

in which each ionic concentration is multiplied by a permeability term (P) and R, T and F have their usual electrochemical significance (RT/F at 20°C is approx. 25mV). The calculated value of V_m (approx. -70mV) agrees with the observed levels of resting membrane potentials in the nervous system. The unequal distribution of ions and maintenance of V_m in the steady state depends on the utilization of metabolic energy to drive a Na-K pump, and possibly also a chloride pump. This builds up the large ionic concentration gradient across the cell membrane, with maintenance of V_m occurring as a result of the much higher conductance for K^+ than for Na^+. The ionic conductances of the membrane can be represented by an electric circuit diagram with three separate conducting channels corresponding to the three prevalent inorganic ions (c.f. Katz 1966). Each channel has a characteristic electromotive force (emf), known as the equilibrium potential for the particular ion, and represents the potential difference across the membrane which would just

balance the tendency of the ion to diffuse in the direction of its concentration (chemical activity) gradient. The equilibrium potentials (E) are given by the Nernst equation so that E_K = -90mV; E_{Na} = +50mV; and E_{Cl} = -80mV. The resting membrane potential (i.e. -70mV) rests somewhere between the extreme values given by the equilibrium potentials for Na^+ on the one hand and K^+ (and Cl^-) on the other. If one of the ionic conductances predominates the membrane potential will move close to the emf of that particular channel.

It is convenient to account for ion movements in electrical terms (c.f. Ginsborg 1967). Fatt and Katz (1951, 1953) first used an electrical model to explain the action of excitatory and inhibitory transmitters. Their mode of action may be explained by supposing that they open additional channels through the membrane for one or several ions. If the ion pathways opened allow for the passage of only a single ion species, the transmitter equilibrium potential would be given by one of the values for the Nernst potentials. Therefore, knowledge of the equilibrium potentials is valuable in determining the nature of a transient permeability change such as occurs during synaptic excitation or inhibition. The channels opened by the transmitter may be represented by an equivalent circuit diagram (c.f. Ginsborg 1967, Fig. 6B) for the transmitter equilibrium potential "e" in terms of the Nernst potentials and the additional conductance (Δg) resulting from the interaction of transmitter and receptor, so that

$$e = \frac{\Delta g_K E_K + \Delta g_{Na} E_{Na} + \Delta g_{Cl} E_{Cl}}{\Delta g_K + \Delta g_{Na} + \Delta g_{Cl}}$$

This equation makes no assumptions about the resting state of the cell and the resting fluxes of ions, and follows the equations of Hodgkin and Huxley(1952).

During synaptic inhibition the values of "e" are usually more negative than the resting potential (e.g. Eccles 1964, 1966). Therefore, the additional conductance produced by inhibitory substances is greater for K^+ or Cl^-, or both, than for Na ions. In the vertebrate central nervous system investigations have revealed that the inhibitory transmitter causes an increase in membrane conductance for Cl ions (Coombs et al 1955; Eccles et al 1964; Kelly et al 1969; Dreifuss et al 1969) and possibly K^+(c.f. Eccles et al 1964). With the movements of Cl ions down their electrochemical gradient to the Cl^- equilibrium potential, excitation is prevented by making depolarization more difficult. The increased conductance shortens the space constant of the cell membrane, thereby reducing the efficacy of axo-dendritic excitatory synapses.

The existence of equilibrium potentials for the action of both the inhibitory transmitter and inhibitory substances applied artificially (perfusion or iontophoresis) has been established for a variety of cells (see Ginsborg 1967). The similar effects induced by the natural occurring inhibitory transmitter and those produced by iontophoretic applications of glycine to spinal neurones (Werman, Davidoff and Aprison

1968; Curtis, Hösli and Johnston 1968) and gamma-aminobutyric acid (GABA) to brain stem (Obata, Ito, Ochi and Sato 1967; Obata, Takeda and Shinozaki 1970; Ten Bruggencate and Engberg 1971) and cortical neurones (Krnjević and Schwartz 1967) has served as one of the major criteria by which to identify these amino acids as inhibitory CNS transmitter substances (Werman 1966).

G. VARIETIES OF INHIBITION

Postsynaptic Inhibition. From experimental studies on inhibition in crustacean neuromuscular junctions (Kuffler and Katz 1946; Fatt and Katz 1953 ; Boistel and Fatt 1958) and stretch receptor neurones (Kuffler and Eyzaguirre 1955; Kuffler 1958), heart muscle (Burgen and Terroux 1953), mammalian motoneurones and spinal interneurones (Eccles et al. 1954; Fuortes 1954; Coombs et al. 1955; Frank and Fuortes 1955; Kolmodin and Skoglund 1958; Haapenen et al. 1958) as well as cerebral (Li 1955, 1959; Phillips 1956, 1959; Branch and Martin 1958) and cerebellar cortical neurones (Granit and Phillips 1956), it was conclusively demonstrated that inhibition was accompanied by a stabilization of membrane polarization. This process prevented the depolarizing action of excitatory impulses and maintained the membrane at a potential close to that of the resting membrane potential. Since it involved the action of a specific chemical inhibitory transmitter which produced a selective increase in ionic permeability in the postsynaptic membrane, the process became known as postsynaptic inhibition (Coombs, Eccles and Fatt 1955). The ionic mechanisms described

earlier relate primarily to postsynaptic inhibition, which underlies one of the most commonly recognized forms of chemical inhibition in the mammalian central nervous system.

Presynaptic Inhibition. In the spinal cord Frank and Fuortes (1957) observed an inhibitory phenomenon characterized by (a) a reduction in the monosynaptically evoked EPSP without any evidence of altered membrane potential or conductance, either at resting potential or when altered by a background of depolarizing or hyperpolarizing current, (b) no change in the motoneurone excitability tested either by the intracellular application of current pulses (Frank and Fuortes 1956) or by the responses to antidromic invasion in the motor axon. It was concluded that diminution of the EPSPs was a result of decreased excitatory action of Ia presynaptic impulses and therefore they introduced the term presynaptic inhibition. However, Frank (1959) proposed an alternate explanation which attributed the EPSP depression to an action so far out on the dendrites of the motoneurones that no trace of the inhibitory influence itself was detectable by the microelectrode in the motoneuronal soma. Since both explanations located the inhibitory action at a site remote from the soma, Frank suggested that it was more appropriate to designate the phenomenon as "remote inhibition". Eccles et al. (1961) observed no detectable change in the time course of the depressed EPSPs, and in light of Frank and Fuortes (1957) original observations and the evidence for primary afferent fibre depolarization

(Eccles and Krnjević 1959; Eccles, Eccles and Magni 1960), they concluded this inhibitory phenomena was presynaptic. Eide et al. (1968) confirmed these earlier findings adding that they could detect no conductance change in the motoneuronal membrane during presynaptic inhibition. On the other hand, Granit (1968) Kellerth (1968) and others claim that so called presynaptic inhibition is often associated with definite postsynaptic effects and, therefore, have retained the possibility that this form of inhibition may be due to activity of relatively remote dendritic synapses. Schmidt (1971) has extensively reviewed the recent literature on presynaptic inhibition, and concludes that there is considerable evidence for its existence, certainly at the level of the spinal cord.

The first histological description of axo-axonic contacts in the mammalian spinal cord was given by Gray (1962) who suggested that these contacts formed the morphological basis for presynaptic inhibition, i.e. a chemically operated synapse located near the presynaptic terminals of primary afferent fibres (Eccles 1961). Since then this type of structure has been found in dorsal column nuclei (Walberg 1965; c.f. physiological observations of Andersen, Eccles, Schmidt and Yokota 1964), cranial nerve nuclei (Kerr 1966, 1970; c.f. Darian-Smith 1965) and thalamus (e.g. Szentagothai 1963; Pappas et al. 1966; Tömbol 1967; c.f. Andersen, Eccles and Sears 1964).

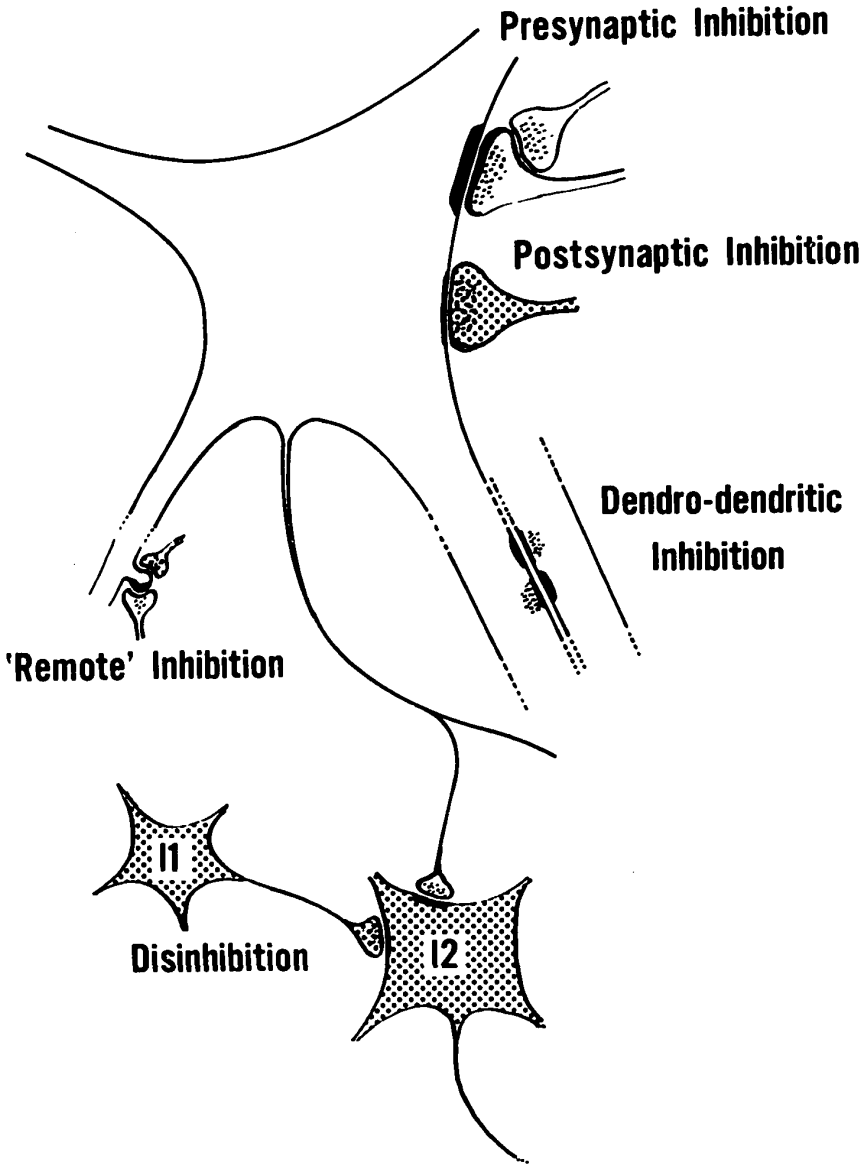
More recently electronmicroscopy has demonstrated axo-axonic synapses in which the presynaptic knobs have flattened

vesicle profiles synapsing onto knobs with round vesicle profiles (Walberg 1965; Conradi 1968; Westrum and Black 1971; Dyachkova et al. 1971). Axo-axonic profiles having the reverse arrangement i.e. profiles containing round vesicles presynaptic to those containing flattened vesicles are more usual in other areas (Guillery 1969; Lund 1969; Ralston and Herman 1969). Although histological results have fully validated the model for presynaptic inhibition in some cases, there are other instances where either the typical histological picture is totally absent in regions where physiological results would locate them, or the functional polarity of the synapse, as judged by presently accepted electronmicroscopical criteria, is the wrong way round (c.f. review by Schmidt 1971).

Recent studies have suggested the possibility that the same inhibitory transmitter (gamma-aminobutyric acid) may mediate presynaptic as well as post-synaptic inhibition in the mammalian cuneate nucleus (Galindo 1969; Davidson and Reisine 1971; Davidson and Southwick 1971; c.f. Kelly and Renaud 1971), and frog spinal cord (Davidoff 1972). In the cuneate nucleus, such evidence fits with the morphological picture of both axo-axonic and axo-dendritic synapses formed by the same presynaptic boutons (Walberg 1965).

At the present time neither axo-axonic contacts of the same nature nor physiological evidence of presynaptic inhibition have been described in the cortex suggesting that postsynaptic inhibition forms one of the major neural control

Figure 2. Diagram illustrating the various inhibitory synaptic connections referred to in the text. Presynaptic inhibition of the large neurone occurs through presynaptic depolarization of an excitatory axon terminal (illustrated as a Gray type I synapse) through an axo-axonic synapse. Postsynaptic inhibition is considered to be the result of interaction between a chemical inhibitory transmitter, liberated from endings of inhibitory neurones (dotted synaptic knob), and the postsynaptic membrane. Dendro-dendritic inhibition apparently involves some form of reciprocal synaptic action between adjacent dendrites through specialized dendro-dendritic junctions. A pair of synapses, one excitatory and the other inhibitory contacting a dendritic spine serves to mediate one type of "remote" inhibition. Disinhibition implies inhibition of inhibitory neurones themselves, shown as a synaptic connection between I1 and I2.


Presynaptic Inhibition
Postsynaptic Inhibition
Dendro-dendritic
Inhibition
'Remote' Inhibition
I1
Disinhibition
I2

mechanisms at that level.

'Remote' Inhibition. Recently Colonnier (1968) has described coupled synapses situated remotely on cortical pyramidal cell dendritic spines, one of which has the morphological features of an inhibitory synapse (Figure 2). Operation of inhibitory synapses so far removed from the cell soma may not necessarily produce any detectable change in potential, resistance or even excitability in the soma (Smith, Wuerker and Frank 1967; Rall 1970) and, therefore, could not be distinguished from a hypothetical presynaptic inhibitory mechanism.

Dendro-Dendritic Inhibition. Another type of local inhibitory mechanism involving reciprocal activity between dendrites and operating through dendro-dendritic synapses has been proposed for the granule cell inhibition of mitral cells in the olfactory bulb (Rall, Shepherd, Reese and Brightman 1966; Rall and Shepherd 1968; Rall 1970; Shepherd 1970). Conceivably a similar mechanism operates between retinal amacrine and bipolar cells (Werblin and Dowling 1969; Shepherd 1970). Dendro-dendritic junctions with similar morphological characteristics have been described recently in primates by different investigators (e.g. Wong 1970; Wong-Riley 1971) and their significance has been discussed in a current paper by Ralston (1971). Morphological or physiological evidence of such an organization in neocortex has yet to be demonstrated (but c.f. Sloper 1971).

Electrical Inhibitory Synapses and Other Mechanisms.

In 1963 Furukawa and Furshpan described a type of inhibition in Mauthner neurones of goldfish which could not be explained by either the increase in postsynaptic membrane conductance described above or the presynaptic inhibition described by Dudel and Kuffler (1961) or Eccles, Eccles and Magni (1961). All evidence pointed to an electrical type of inhibition. To date there has been little evidence of purely electrical transmission across synapses in the mammalian brain (c.f. Baker and Llinas 1971) and the predominant mechanisms of transmission appear to be mediated chemically.

After-hyperpolarization and delayed rectification (Nelson and Frank 1967; Baldissera and Gustafsson 1970), both involving changes in K^+ conductance, have been implied as limiting mechanisms to control neuronal discharge frequency, and are only mentioned for completeness, since by definition they are not true inhibitory mechanisms.

H. PHARMACOLOGY OF POSTSYNAPTIC INHIBITION

Knowledge of the ionic mechanisms and various pathways for postsynaptic inhibition antedated by almost a decade identification of the inhibitory transmitter substances themselves. The precepts of chemical transmission were largely derived from knowledge of cholinergic transmission. A substance suspected of functioning as a transmitter had to fulfill several requirements (Florey 1961; Werman 1966). Briefly these were (i) the presence of a synthesizing and (ii) inactivating enzyme system; (iii) presence and (iv) collectability of the transmitter;

(v) identity of action of the natural and postulated transmitter substance when applied to the postsynaptic membrane; (vi) agents which interact at postsynaptic structures with the natural transmitter should interact with the suspected transmitter in the same way.

Two significant developments, the introduction of the crayfish stretch receptor preparation (Wiersma, Furshpan and Florey 1953) and the development of the microiontophoretic technique applied to the study of neurophysiology (Nastuk 1953; del Castillo and Katz 1955; Curtis and Eccles 1958), led to considerable progress in the search for inhibitory transmitter substances. A large number of compounds were shown to have depressant effects on central neurones (Curtis and Watkins 1960; Krnjević and Phillis 1963). Of particular relevance were two amino acids, gamma-aminobutyric acid and glycine.

Gamma-aminobutyric acid (GABA). GABA was first identified as a biochemical substance in 1910 by Ackermann and Kutscher and later found to be present in considerable quantity in the grey matter of mammalian brain (Roberts and Frankel 1950; Awapara et al. 1950). Extracts of mammalian brain and spinal cord contained an agent called Factor I which had an inhibitory action on the crayfish stretch receptor organ (Florey 1953, 1954). With the help of an assay developed for Factor I (Elliott and Florey 1956) the active substance was identified and shown to be GABA (Bazemore, Elliott and Florey 1957). The similarities noted between the action of GABA and the natural inhibitory

transmitter (Elliott and Florey 1956; Boistel and Fatt 1958; Robbins and van der Kloot 1958; Kuffler 1960; Hagiwara, Kusano and Saito 1960; Florey and Hoyle 1961; Dudel and Kuffler 1961; Dudel 1965; Takeuchi and Takeuchi 1965, 1966a,b) served to substantiate GABA as the crustacean inhibitory transmitter. Experimental results strongly implied that GABA was also a promising transmitter agent in mammalian CNS except for two pieces of evidence militating against this possibility. Curtis and Watkins (1965) found that GABA produced depression of firing of motoneurones without producing the hyperpolarization expected if the conductance change was the same as that produced by the transmitter. Secondly, the depressant action of GABA on either spinal cord or cortical neurones was not blocked by strychnine (Curtis and Watkins 1965; Krnjević, Randić and Straughan 1966c; Curtis 1967), although strychnine effectively diminished spinal cord inhibition (Sherrington 1906; Bradley, Easton and Eccles 1953; Brooks, Curtis and Eccles 1957; Curtis 1962).

Further investigations demonstrated that cerebral and cerebellar cortical inhibition were in fact strychnine resistant and clearly pointed to GABA as the inhibitory transmitter. (a) Microiontophoretic applications of GABA produced hyperpolarization of neurones in both cerebral cortex (Krnjević and Schwartz 1967) and Deiter's nucleus (Obata et al. 1967). (b) In the cortex both GABA and the inhibitory transmitter increased the ionic permeability of cortical neurones for

anions, mainly Cl^- (Kelly, Krnjević, Morris and Yim 1969). (c) Picrotoxin (but not strychnine) antagonized both GABA induced and synaptic inhibition of neurones in Deiters (Obata et al. 1970; Ten Bruggengate and Engberg 1969, 1971), cuneate (Galindo 1969; Renaud and Kelly 1971) and oculomotor nuclei (Obata and Highstein 1970). More recent studies with bicuculline have shown that this alkaloid has a selective antagonistic action on both synaptic and GABA induced inhibition in several CNS regions (Curtis et al. 1971a,b). (d) GABA is present in high concentration in mammalian brain (Fahn and Cote 1968) particularly in the cerebral cortex (Awapara, Landua, Fuerst and Seale 1950; Roberts and Frankel 1950) and is found within the synaptic vesicle fraction (Whittaker 1965, 1968; Krnjević and Whittaker 1965; Salganicoff and de Robertis 1965; Kurijama, Roberts and Kakefuda 1968; Neal and Iversen 1969). (e) The CNS contains a system capable of synthesizing and degrading GABA (Elliott 1965, 1970; Roberts 1956, 1969). (f) A release of GABA is related to the state of cortical activation (Jasper, Khan and Elliott 1965; Jasper and Koyama 1969) and particularly to the activity of inhibitory synapses (Obata and Takeda 1969; Mitchell and Srinivasan 1969; Iversen, Mitchell and Srinivasan 1971). (g) In cortical neurones both the inhibitory response which follows epicortical stimulation and iontophoretically applied GABA are potentiated by agents which interfere with the removal of GABA from the synaptic cleft (Kelly and Renaud 1971; Gottesfeldt, Kelly and Renaud 1972).

Glycine. Glycine was one of the amino acids originally noted by Curtis and Watkins (1960) to depress the excitability of spinal neurones. It is among the most abundant of five amino acids present in the spinal cord (Aprison and Werman 1965) and is concentrated mainly within ventral horn interneurones (Davidoff et al. 1967; Graham et al. 1967). Applied by iontophoresis to spinal motoneurones and interneurones, the inhibitory action of glycine is identical with that of the natural inhibitory transmitter, and both types of inhibition are blocked by strychnine (Werman, Davidoff and Aprison 1968; Curtis et al. 1968a,b). Both glycine and GABA have significant depressant effects on neurones of the lateral vestibular (Obata et al. 1967) and cuneate nuclei (Galindo, Krnjević and Schwartz 1967). However, synaptic inhibition in these nuclei is not altered by strychnine, but rather is blocked by compounds which antagonize the action of GABA (Galindo 1969; Obata, Takeda and Shinozaki 1970; Ten Bruggencate and Engberg 1971; Kelly and Renaud 1971), suggesting that GABA occupies a dominant physiological role in these brain stem structures.

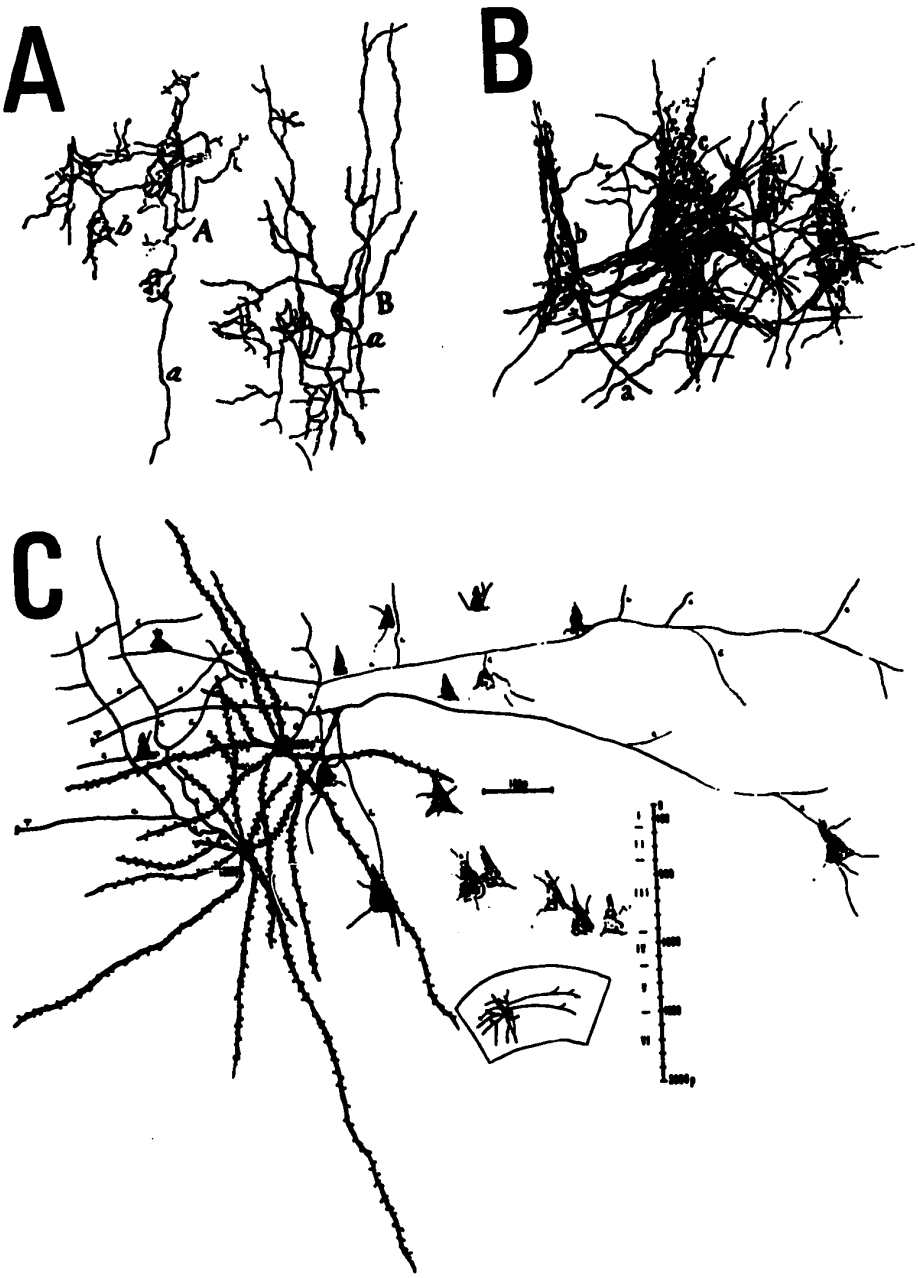
The cerebral cortex contains lower mean tissue glycine concentrations and is less able to concentrate glycine from an external medium compared with the spinal cord (Aprison et al. 1969; Neal 1971). Compared with the action of GABA and the natural inhibitory transmitter, glycine has a weak and poorly sustained inhibitory action on cortical neurones (Kelly and

Krnjević 1969). Therefore, it is unlikely to be of major significance in cortical inhibition.

I. MORPHOLOGICAL ASPECTS OF INHIBITION

Precise correlation of morphological characteristics with functional identity still remains a formidable barrier to the study of mammalian CNS inhibitory pathways. At present only two types of inhibitory interneurones have been so identified. One is the cerebellar Purkinje cell (Cajal 1911; Ito and Yoshida 1966; see also Eccles, Ito and Szentagothai 1967); the other is the spinal cord Renshaw cell (Eccles, Fatt and Koketsu 1954; Scheibel and Scheibel 1966; Jankowska and Lindström 1971). There are, however, many histologically described neurones in various CNS areas which have been tentatively identified as inhibitory interneurones. This includes interneurones situated in Rexed's spinal lamina VII which mediates Ia afferent inhibition (Scheibel and Scheibel 1966a; Hultborn, Jankowska and Lindström 1971; Ryall and Piercey 1971), triangular, multipolar or fusiform cells in the cuneate nucleus (Cajal 1911; Andersen, Eccles, Oshima and Schmidt 1964; Andersen, Eccles, Schmidt and Yokota 1964; Kuypers and Tuerk 1964), basket cells in the hippocampus (Cajal 1911; Lorento de Nó 1934; Andersen, Eccles and Løyning 1964a,b), basket, stellate and Golgi cells in the cerebellar cortex (Cajal 1911; Eccles, Llinas and Sasaki 1966; Hamori and Szentagothai 1965, 1966a,b), short axon non-relay ventrobasal thalamic neurones (Cajal 1911; Andersen, Eccles

Figure 3. Cortical stellate "Basket" neurones. A. Golgi profile of a stellate neurone 'B' and its axon 'a' breaking up into numerous axon branches 'b' which together with similar axonal arborizations from other neurones constitutes a "basket" around the soma and proximal dendrites of pyramidal neurones. Several of these are illustrated in B (from Cajal 1911). C. Two stellate basket cells from the motor cortex of a new-born infant demonstrating that the complete length of two horizontal axon fibres of one of the neurones measures approximately 1000 um and gives many collaterals which contribute to the formation of baskets around pyramidal neurones. Note that these long axons traverse the cortex perpendicular to the long axis of the gyrus, as shown in the insert (from Marin-Padilla 1969).


A
B
C
b
A
a
B
a
b
c
a

and Sears 1964; Marco, Brown and Rouse 1967; Szentagothai 1967b; Tömbol 1967), thalamic reticular neurones (Scheibel and Scheibel 1966b; Schlag and Waszak 1971) and bipolar cells in the hypothalamic ventromedial nucleus (Szentagothai, Flerko, Mess and Malasz 1968; Murphy and Renaud 1969).

Neocortical Stellate Basket Cells. In the neocortex, golgi studies (Cajal 1911; Poljakow 1958; Colonnier 1966; Marin-Padilla 1969) have revealed that axons of one type of stellate cell converge to form pericellular baskets around pyramidal cell somata (Figure 3). Axons from a single stellate cell may participate in the formation of a large number of these perisomatic networks (Poljakow 1958). Basket type stellate interneurones establish short range pericellular connections especially in the more superficial layers (Szentagothai 1969) but the axons of large basket interneurones in deeper layers may extend for 0.5-1.0 mm in the horizontal direction (Colonnier 1966; Szentagothai 1969; Ramon-Moliner personal communication).

In the hippocampus, basket cell axons form a dense network around the somata of pyramidal cells, which is precisely the region of maximum extracellular current flow generated by the inhibitory synapses on hippocampal pyramidal cells (Andersen, Eccles and Løyning 1963, 1964a). The pathway for hippocampal postsynaptic inhibition, therefore, is postulated to be through these basket cells (Andersen, Eccles and Løyning 1964b). The hippocampal pyramidal-basket cell morphology bears

Figure 4. Two main patterns of synaptic differentiation in cerebral cortex. (1) Electronmicrograph (x 32,500) illustrating two synaptic knobs, one of which (a) contains spheroidal vesicles. The asymmetrical membrane differentiation (arrows) resembles the type I synapse originally described by Gray (1959). Synaptic knob (b) contains flattened vesicles which are frequently associated with the symmetrical membrane differentiation shown in (2), and which bears similarity to the type II synapse of Gray. Arrow points to a dense core vesicle which is seen intermingled with flattened vesicle profiles (from Colonnier 1968).

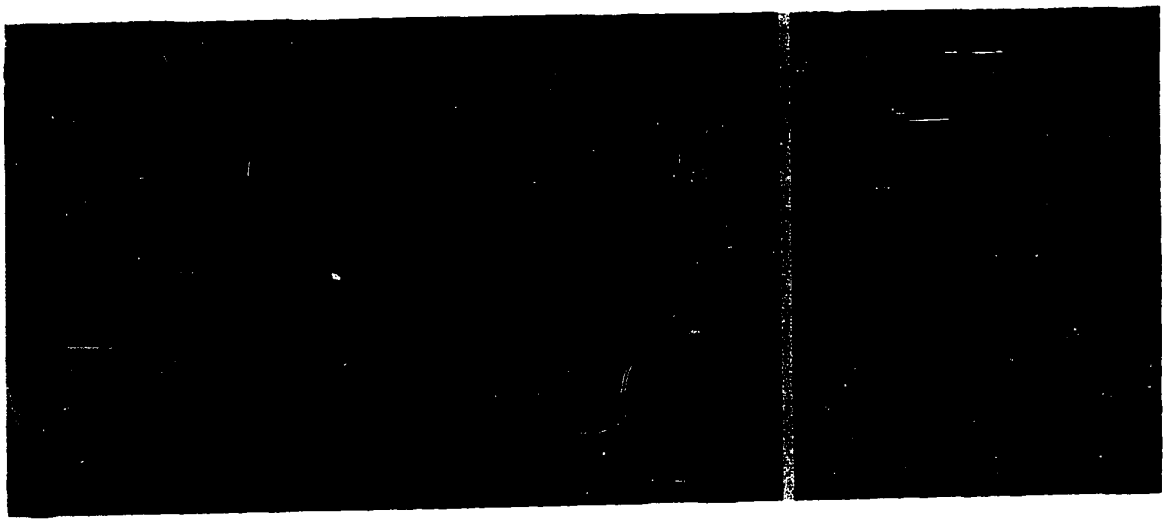
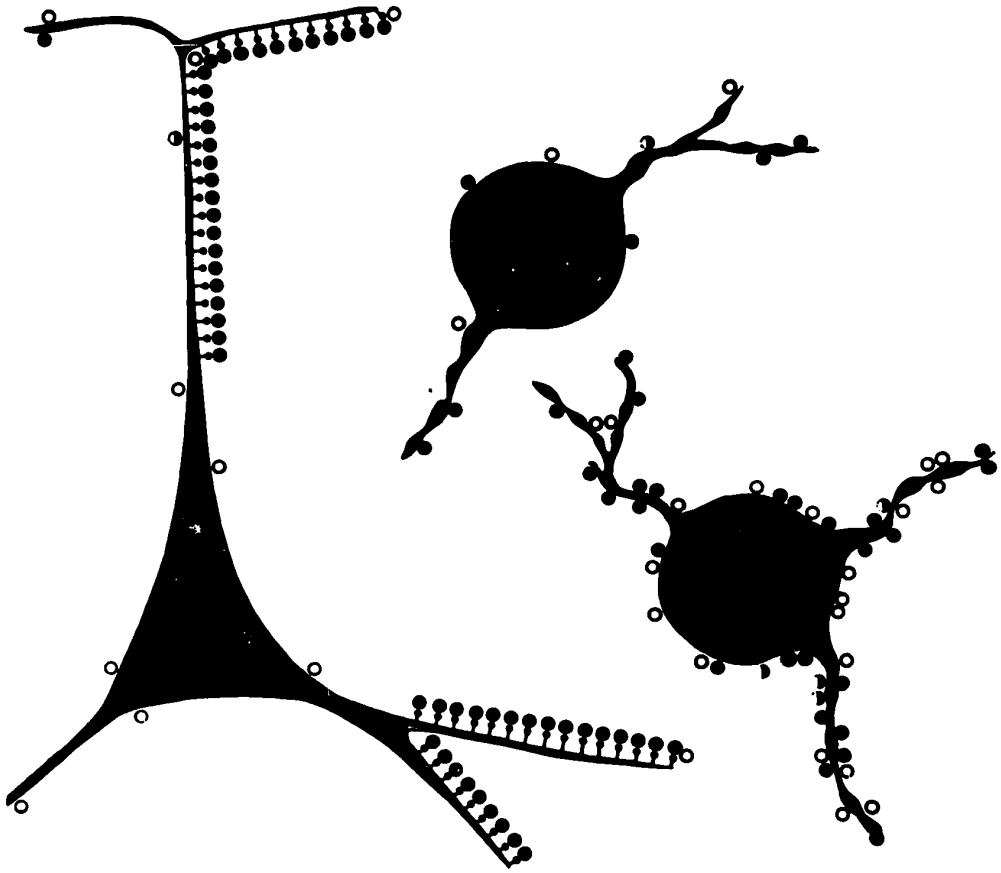


Figure 5. Distribution of different synaptic endings on stellate and pyramidal cells. Open circles represent symmetrical contacts with flattened vesicles (SF contacts) and closed circles represent asymmetrical contacts with spheroidal or round vesicles (AR contacts). Half-closed circles represent synapses difficult to classify as belonging to either type. Note the absence of AR contacts on the pyramidal neurone soma but predominance on the apical and basal dendritic spines. This regional distribution contrasts sharply with the more uniform pattern observed on stellate neurones. The occasional SF contact is found on dendritic spines of pyramidal neurones but is always coupled with an AR contact (from Colonnier 1968).



a striking resemblance to the neocortical pyramidal cell-basket cell arrangement. This analogy supports the argument that basket type stellate cells in neocortex probably also have an inhibitory function.

Ultrastructure of Inhibitory Synapses. Electron-microscopy has revealed two types of synaptic endings in neocortex. According to the synaptic membrane differentiation, Gray (1959) classified those endings with asymmetrical thickenings as type I and other endings with symmetric pre- and postsynaptic densities as Type II. Two varieties of synaptic vesicles have also been described i.e. round vesicles and flattened vesicles (Uchizono 1965; Bodian 1966). In the cerebral cortex (Colonnier 1968, 1969; Jones and Powell 1969) and in other CNS areas (e.g. Ralston 1968; Guillery 1969) round vesicles are usually associated with asymmetric membrane thickenings (AR synapses) and flattened vesicles with symmetric membrane differentiations (SF synapses). Examples of both types of synapses are illustrated in Figure 4. In the visual cortex different spatial arrangements exist for the two synaptic types (Colonnier 1968). As illustrated in Figure 5 they are distributed over both the cell soma and the dendrites of stellate neurones. However, pyramidal cell somata receive only SF contacts, their main dendritic shafts both types, and their dendritic spines mainly the AR variety with few SF synaptic contacts. Chronically isolated cortical slabs continue to

show evidence for axo-somatic termination, even though many of the contacts on the dendritic tree undergo degeneration (Szentagothai 1965; Colonnier 1966). This would imply that pyramidal cell somatic synapses are derived from axons of intracortical neurones, possibly from the basket-type stellate cells.

Correlative physiological and morphological studies have assigned a functional significance to the two types of synaptic contacts. In the cerebellum for example, SF contacts correspond to the location of inhibitory synapses from basket and stellate interneurones (Andersen, Eccles and Voorhoeve 1964; Uchizono 1965) and subsequent studies have supported the association of excitation with AR synapses and inhibition with SF synapses (Larramendi 1969; Gray 1969). In the neocortex SF contacts may be inhibitory whereas AR synapses are likely to be excitatory, and some of the axo-somatic SF (inhibitory) contacts on pyramidal neurones may be derived from axons of basket type stellate cells.

From a physiological standpoint, certain characteristics of pyramidal tract neurone IPSPs certainly suggest a close spatial approximation between the location of inhibitory synapses and the tip of an intracellular recording microelectrode (which is presumably situated in the cell soma). These features are the large IPSP conductance increase (Pollen and Lux 1966; Krnjević and Schwartz 1967; Dreifuss, Kelly and Krnjević 1969) and their easy reversibility either through anion or current injection

(Purpura and Schofer 1964; Kelly, Krnjević, Morris and Yim 1969). Since the pyramidal cell somata are contacted by less than 100 SF type (inhibitory) synapses, compared with literally thousands of the AR type (excitatory) synapses located on the dendritic tree (Colonnier and Rossignol 1969), the postulated inhibitory synapses occupy a very strategic position optimally situated to prevent neuronal discharge (Eccles 1964). Stellate cells on the other hand have a more random distribution of the two synaptic types (Figure 5) which must imply a considerable difference in their ability to process afferent information.

Axo-axonic synapses postulated to be the morphological correlates of presynaptic inhibition (Walberg 1965) have not been observed in neocortex, except on the initial axonal segments of some pyramidal cells (Westrum 1966, 1970; Peters, Proskauer and Kaiserman-Abramof 1968). However, Colonnier (1968) has described both AR type and SF type synapses contacting the same pyramidal dendritic spine which raises the possibility of a "remote" type of cortical inhibitory mechanism. Globus and Scheibel (1967a,b) have studied transsynaptic degeneration of dendritic spines of pyramidal cells in the visual cortex and have concluded that there is a regional distribution of cortical afferent fibre terminals on the pyramidal dendritic tree (see Chou and Leiman 1970). It has been proposed that these remotely located inhibitory synapses may operate so as to discriminate between different excitatory afferent sources of information and thereby prevent their depolarizing influences

from ever reaching the main dendritic trunks (Colonnier and Rossignol 1969). Experimentally, a microelectrode placed in the cell soma probably could not detect the presence of such a remote inhibition.

Functional Organization Within the Cerebral Cortex. Neurophysiological studies of the receptive properties of neurones in the primary sensory and motor cortex of the cat have revealed the presence of a functional organization of neurones into vertical columns perpendicular to the pial surface (Mountcastle, 1957; Welt et al. 1967). The cortical efferent system also contains discrete radially aligned colonies of corticospinal and other corticofugal neurones which when stimulated activate particular motor units and which in turn receive sensory information topographically related to the motor units (Asanuma and Sakata 1967; Asanuma, Stoney and Abzug 1968). Estimates of the dimensions of these functional columns vary from 0.3 to 1.3 mm and, therefore, would contain many hundreds of neurones.

Anatomically the evidence strongly supports the postulate of a vertical columnar organization. There are less long distance intracortical interconnections in the tangential direction compared, for example, with the predominantly vertical or columnar distribution of the thalamocortical afferents (Szentagothai 1965; Scheibel and Scheibel 1970) as well as certain intracortical elements themselves (Colonnier 1966). Located within these

dimensions are the tangential branches of stellate basket neurones (Colonnier 1966; Szentagothai 1969; Marin-Padilla 1969) which have been suggested to secure local inhibitory interconnections (see above).

J. INHIBITION IN CEREBRAL CORTEX

By the turn of the century neurophysiologists had been presented with substantial experimental evidence indicating that the cerebral cortex exerted both inhibitory and excitatory influences on proximal and distal spinal reflexes. In fact the intrinsic function of the cortex itself appeared to be controlled by inhibition. Dusser de Barenne and McCulloch (1937, 1939, 1941) introduced the terms suppression and extinction to describe two special types of cortical inhibitory processes. Suppression was a decreased responsiveness of one cortical region (e.g. area 4) observed to follow stimulation of specific inhibitory strips of cortical tissue (e.g. the adjacent area 4s). Extinction, as well as other types of cortical inhibitory phenomena such as spreading depression (Marshall 1959; Ochs 1962), were considered the result of previous or excessive activity of cortical neurones. Few considered inhibition as an independent neuronal function (e.g. Rosenblueth and Cannon 1942; Leão 1944). Although they had only indirect evidence, Rosenblueth and Cannon were of the impression that inhibition resulted from activity in a different population of neurones rather than the result of neuronal refractoriness or exhaustion.

With the introduction of techniques for recording from

single cortical neurones (Amassian 1953; Jung 1953) extracellular studies revealed that there was a temporary pause in their spontaneous discharges following stimulation of the epicortical surface (Creutzfeldt, Baumgartner and Schoen 1956), transcallosal pathways (Creutzfeldt et al. 1956; Asanuma and Okamoto 1959), thalamocortical fibres (Li 1956), caudate nucleus (Sphelmann, Creutzfeldt and Jung 1960), pyramidal tract (Suzuki and Tukahara 1963; Asanuma and Brooks 1965; Brooks and Asanuma 1965) or peripheral structures (Mountcastle 1957; Mountcastle, Davies and Berman 1957).

Intracellular recording provided direct proof that the 'silent period' or pause in activity was the result of an active inhibitory process. IPSPs were observed to follow direct cortical shocks (Phillips 1956b; Li and Chou 1962) and various other indirect forms of stimulation, for example to the pyramidal tracts (Phillips 1956a, 1959; Stefanis and Jasper 1964), thalamus (Branch and Martin 1958; Lux and Klee 1962; Li 1963; Purpura and Schofer 1964; Nacimiento, Lux/Creutzfeldt and 1964; Pollen 1964) and contralateral cortex (Creutzfeldt, Baumgartner and Schoen 1956).

IPSPs recorded from cortical neurones all demonstrate the following characteristics: (a) Typically they last well over 100 ms (Albe-Fessard and Buser 1953; Phillips 1956; Li and Chou 1962). (b) Intracellular current of Cl^- injection causes a reduction and eventual reversal of the membrane potential change induced by the natural inhibitory transmitter (Purpura

and Schofer 1964; Creutzfeldt, Lux and Watanabe 1966c). (c) IPSPs are accompanied by a marked fall in membrane resistance (Lux and Pollen 1966; Pollen and Lux 1966; Krnjević and Schwartz 1967; Dreifuss, Kelly and Krnjević 1969), the result of an increased membrane anion conductance, mainly for Cl^- (Kelly, Krnjević, Morris and Yim 1969). (d) The permeability change which occurs during synaptic inhibition is indistinguishable from that caused by iontophoretic applications of GABA (Krnjević and Schwartz 1967; Dreifuss, Kelly and Krnjević 1969). (e) Because of the long duration of IPSPs, inhibition is easily maintained by low frequency (5-7/s) stimulation. This latter point was best demonstrated by Krnjević and his colleagues (Krnjević, Randić and Straughan 1966a) in their studies on a particularly potent form of cortical inhibition i.e. that evoked by stimuli applied directly to the epicortical surface.

Krnjević et al. (1966a,b) found that inhibition was present throughout the neocortex and in all layers. They reasoned that the effect was produced by local inhibitory neurones, since the inhibitory effect was still present in isolated cortical slabs (c.f. Creutzfeldt and Struck 1962; Creutzfeldt et al. 1966a,c), and was possibly the result of activating a pool of cortical inhibitory neurones through a variety of pathways (e.g. transcallosal or thalamocortical).

Eccles, Fatt and Koketsu (1954) were the first to present evidence that inhibitory (Renshaw) neurones, interposed

in the recurrent pathway through motoneurone axon collaterals, were responsible for the antidromic inhibitory action of a ventral root volley. The central latency for Renshaw cell discharge was sufficiently short to be monosynaptic, whereas, the motoneurone IPSP latency was at least one synaptic delay longer, indicating a single interpolated neurone (the Renshaw cell). The high frequency repetitive discharge evoked from the Renshaw neurone was present throughout the duration of the IPSP. Analogous with these findings in spinal cord, presumed inhibitory interneurones discharging simultaneously with the appearance of inhibition in target neurones has also been observed in other CNS areas, for example the cuneate nucleus, cerebellum, hippocampus, thalamus (see Eccles 1969 review) and hypothalamus (Murphy and Renaud 1969).

Antidromic pyramidal tract stimulation also evokes IPSPs in cortical pyramidal neurones, with a latency sufficiently greater than the antidromic spike to suggest the presence of an interpolated inhibitory neurone (Phillips 1959; Stefanis and Jasper 1964a,b). Despite the long duration of cortical IPSPs, interneurone discharges lasting more than 20 ms are rarely (c.f. Stefanis 1966) seen to follow stimulation of cortical inhibitory pathways (Krnjević, Randić and Straughan 1966b). Nevertheless, the persistence of surface evoked inhibition and IPSPs in both acute and chronic isolated cortical slabs (Krnjević, Randić and Straughan 1966b; Krnjević, Reiffenstein and Silver 1969) attests to the intracortical localization of

the inhibitory generating mechanism (inhibitory interneurones). Therefore, assuming that cortical inhibitory interneurones are capable of spike generation, some of the interneurones initially excited by stimulation of known inhibitory pathways most likely function as inhibitory cells. On the other hand, the lack of prolonged repetitive high frequency discharges (similar to that of Renshaw cells for example) may not be required if cortical inhibition is produced by a chemical transmitter different from that in the spinal cord.

IPSPs can be generated in the same pyramidal tract neurone whether from direct stimulation of the cortical surface (Krnjević, Randić and Straughan 1966a,b) or indirectly through recurrent pyramidal (Phillips 1956a, 1959; Stefanis and Jasper 1964) thalamocortical (Branch and Martin 1958) or transcallosal pathways (Creutzfeldt, Baumgartner and Schoen 1956). Because of this apparent convergence of inhibition it has been suggested that these IPSPs are the result of activation of a similar pool of cortical inhibitory interneurones, in effect a common inhibitory pathway (Phillips 1956a, 1959; Krnjević, Randić and Straughan 1966b; Renaud, Kelly and Provini 1969). This proposal resembles the explanation offered for the inhibitory convergence seen on relay cells of the dorsal column nuclei (Gordon and Jukes 1964; Renaud and Kelly 1971). Verification of this hypothesis requires demonstration of convergence of excitation onto the same inhibitory neurone following activation of several inhibitory

pathways. Stefanis (1966) has illustrated this feature in some non-pyramidal cortical neurones but he lacked a reliable means by which to identify them as inhibitory interneurones. The ability to reliably identify cortical inhibitory neurones poses a major challenge to any investigation of cortical inhibitory pathways and organization, and constitutes the major purpose of the present experiments.

There is indirect evidence of possible intracellular recordings from cortical inhibitory interneurones. Andersson(1965) noted that dorsal columns or spinocerebellar tract stimulation evoked both EPSPs and IPSPs in neurones of somatosensory cortex. The latencies of the IPSPs were approximately 0.8 ms longer than for the EPSPs. Oscarsson and his colleagues (Oscarsson 1966; Oscarsson, Rosen, and Slug 1966) have also described both EPSPs and IPSPs in somatosensory cortex following group I muscle afferent stimulation. The EPSP latencies indicated that some were produced monosynaptically from thalamocortical fibres, while the IPSP latencies were on the average one synaptic delay later. These investigators suggested that many of the monosynaptically excited cells were inhibitory interneurones. Again the data, while being suggestive, lacks direct proof that such cells have an inhibitory function.

K. PURPOSE OF THE PRESENT STUDY

The general aim of this research was to obtain a better understanding of inhibitory mechanisms and pathways in the

cerebral cortex by attempting to identify and characterize the features of cortical inhibitory neurones. A LINC-8 computer, interfaced to the neurophysiological experiment, was used to analyze intracellular and extracellular data from identified pyramidal and non-pyramidal neurones in the pericruciate cortex of the cat under DIAL or nembutal anaesthesia.

More specifically, intracellular IPSPs were characterized both with respect to amplitude and conductance changes at various intervals along their time course. By conditioning of pyramidal recurrent IPSPs with surface evoked IPSPs it was hoped to test the hypothesis that a common inhibitory interneuronal pathway might mediate the inhibition observed in pyramidal tract neurones following activation of various inhibitory pathways.

Another phase of this research dealt with a search for the inhibitory interneurones themselves. A correlation analysis performed on spike trains recorded simultaneously from two different neurones was used to identify neurones with a presumed inhibitory function and thereby characterize the patterns of activity displayed by cortical inhibitory interneurones, particularly in response to stimulation of inhibitory pathways. By recording the activity of two neurones less than 700 μm from one another it was also hoped to obtain some insight into the mode of operation of neurones, both excitatory and inhibitory, within a functional cortical column.

Chapter 2

METHODS

A. EXPERIMENTAL PREPARATION

All experiments were performed on 3-4 Kg adult cats of both sexes.

For extracellular recording, initial anaesthesia consisted of a single 35 mg/kg intraperitoneal (I.P.) injection of pentobarbital sodium (Nembutal, Abbott) followed by supplemental intravenous (I.V.) doses of 5-10 mg every four to six hours. This regime produced a depth of anaesthesia which was adequate to prevent spontaneous movement and shivering without suppressing the limb withdrawal reflex to a noxious stimulus.

Intracellular recordings were obtained from animals anaesthetized either with Nembutal (35 mg/kg I.P. initially, with additional I.V. supplements of 5-10 mg at regular 4 hour intervals) or DIAL compound (CIBA, administered as a single 70 mg/kg I.P. injection). These cats were immobilized by the continuous I.V. infusion of succinylcholine (Anectine, Burroughs Wellcome) at a rate of 1-2 mg/min. and ventilated artificially. A wide bilateral pneumothorax helped to minimize respiratory movements and stabilize the recordings.

In all experiments the mean blood pressure was continuously monitored via an indwelling femoral arterial catheter.

Whenever necessary, blood pressure was maintained at an adequate level (>90 mm Hg) by I.V. administration of plasma substitute (Subtosan, Poulenc), sodium bicarbonate, methamphetamine or noradrenaline. The temperature was maintained at 37.5±0.5 C electronically through a feedback circuit from a rectal thermistor probe to a 12V heating pad inserted under the animal. Since the duration of the extracellular experiments varied from 15 to 22 hours these animals were given 10-20 cc of 5% glucose in saline by a slow intravenous infusion every six hours.

B. SURGICAL PROCEDURES

The trachea was opened just above the suprasternal notch and a cannula was inserted. The trachea and the esophagus were then cut at that point, the rostral parts retracted, and the deep anterior cervical muscles were removed. A burr hole 6-8 mm in diameter was made in the base of the occipital bone through which the dura was incised. This permitted exposure of the pyramids just caudal to the ponto-medullary junction. The opening was kept covered with thin polyethylene until later when, under visual control, stimulating electrodes were placed on the appropriate pyramidal tract.

The remaining surgical and experimental techniques were performed with the animal prone while held in a stereotaxic frame (David Kopf). Cisternal drainage was obtained by enlargement of the foramen magnum and removal of part of the occipital bone, thereby exposing the posterior third of the cerebellar vermis. This procedure also helped to diminish any remaining

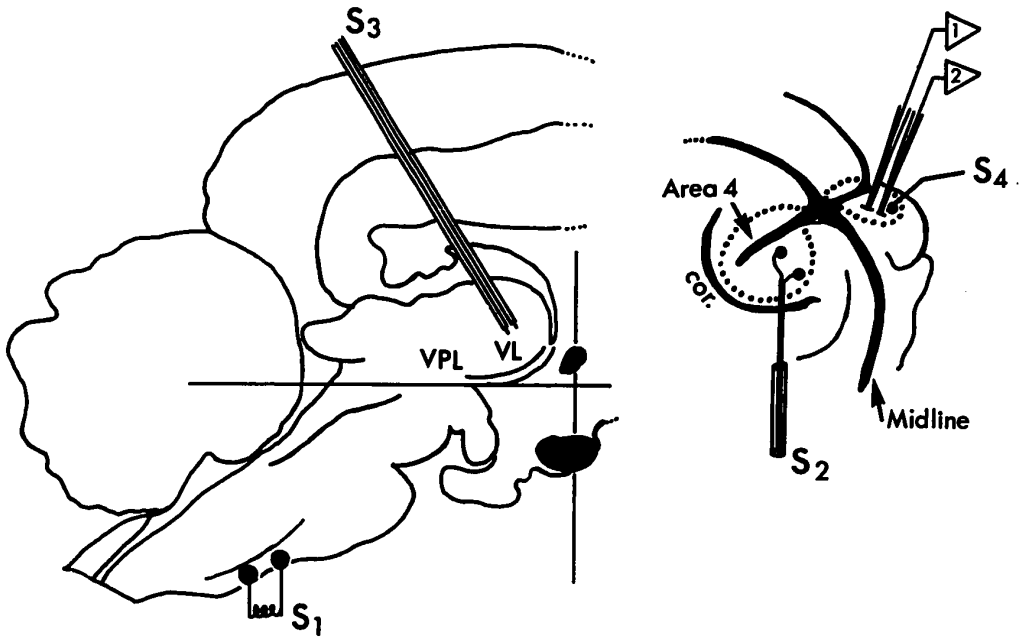
cardiovascular or respiratory pulsations. The pericruciate cortex was exposed bilaterally by removing the frontal sinus, the roof of the orbit and a large portion of the frontal bone back to the coronal suture. The dura mater was incised and reflected, and the exposed cortical surface was covered with thin strips of opaque polyethylene in order to prevent drying and reduce cooling. The recording sites were kept moist and warm by continuous irrigation with mammalian Ringer solution (Composition: NaCl 137 mM; KCl 2 mM; $NaH_2PO_4.H_2O$ 1 mM; $CaCl_2$ 2 mM; $MgCl_2$ 1 mM; $NaHCO_3$ 12 mM), maintained at a constant temperature of 38°C electronically through a feedback circuit from a thermocouple situated at the efflux end of a heated tube delivering the fluid.

In order to facilitate electrode penetration a small tear was made in the pia mater with the aid of jewelers' forceps (IREX) and a binocular dissecting microscope (Nihon-Kohden). A small perspex pressor plate, having a 1 mm hole through which the recording electrodes were inserted, was gently pressed against the pial surface to minimize pulsations but without impeding the cortical circulation which could be inspected periodically through the dissecting microscope.

C. STIMULATION

Isolated stimulation units (Devices, Mk III) controlled by a Digitimer (Devices) were used to deliver current pulses to three different types of stimulating electrodes placed to activate

Figure 6. Schematic diagram of the experimental preparation. On the left, a line drawing of the cat brain in sagittal section indicating the position of bipolar silver ball electrodes on the medullary pyramidal tract (S1), and two bipolar concentric electrodes positioned in the thalamic nuclei (VPL or VL) (S3). The two lines illustrate the axis of the stereotaxic plane in this section. On the right, a line drawing of the anterior and posterior sigmoid gyri, shown bilaterally. Area 4 of Hassler and Muhs-Clemente (1964) is outlined by the dotted line and is delimited laterally by the coronal sulcus (cor). Bipolar transcallosal electrodes (S2) were placed on the gyrus contralateral to the recording site (electrodes 1 and 2). Monopolar epicortical stimulation (S4) was obtained through a ball electrode placed on the ipsilateral cortical surface less than 2 mm from the recording site. The reference electrode was a silver plate embedded in the neck musculature.


S3
VPL
VL
S1
1
2
S4
Area 4
cor.
Midline
S2

inhibitory pathways (Figure 6).

(a) Bipolar stimulation of the medullary pyramids (S_1) with single current pulses (0.1 ms duration) of 0.2 - 10V was achieved through two silver ball (0.75 mm) electrodes positioned 3.0 mm apart in the sagittal plane on the ipsilateral pyramidal tract, with the rostral electrode acting as cathode. For transcallosal activation current pulses (0.1-0.2 ms duration) of 10-24V were delivered to similar electrodes positioned on the pial surface of the contralateral precruciate gyrus (S_2) at approximately the same site as the ipsilateral recording electrodes.

(b) Thalamocortical pathways were activated through stimuli of 5-15V (0.1 ms duration) applied between the tip and ring (distance of 1.0 mm) of bipolar concentric 23 gauge stainless steel electrodes. To facilitate selection of the thalamic site yielding the best responses, two electrodes were placed 1.5-2.0 mm apart in the coronal plane and positioned stereotaxically according to the atlas of Jasper and Ajmone-Marsan (1960). Neurones in the precruciate gyrus were tested with stimuli (S_3) delivered to nucleus ventralis lateralis (VL) at stereotaxic coordinates of F+11.0, L 4.5 and 6.0, H+2.0, since VL projects largely to area 4 (Macchi 1958; Yoshida, Yajima and Uno 1966). In one preparation the responses of postcruciate neurones were determined while stimulating nucleus ventralis posterolateralis (VPL) at stereotaxic coordinates F+9.5, L 5.5 and 7.0, and H+0.5.

(c) A fine 0.5 mm ball-shaped platinum electrode used for epicortical surface stimulation (S_4) was embedded into the pressor plate at the edge of the opening, within 1.5 mm of the recording microelectrodes. Monopolar cathodal current pulses 0.1ms in duration and 2-7V intensity were most commonly employed, the indifferent electrode consisting of a chlorided silver plate embedded in the muscles of the neck.

D. RECORDING MICROPIPETTES

Single or multibarrel micropipettes were prepared from pyrex glass tubing (Kimax) having an outside diameter of 0.9-1.1 mm.

(a) Extracellular studies were performed with double or triple barrelled micropipettes. Prior to filling by boiling under reduced atmospheric pressure, the electrodes were bent approximately 30-35° some 4-6 mm from the tip. This allowed the two electrodes to be positioned as close as 50 µm from each other. One channel, filled with 2.7 M sodium chloride was used for recording; another contained an excitatory amino acid, Na L-Glutamate (Matheson, Colemann and Bell 1.0 M, pH 7.0), and an additional channel if available contained acetylcholine (ACh) chloride (British Drug Houses 1.0 M, pH 4.0-4.5). Useful electrodes had a total outside tip diameter of 3-5 µm and impedances of 2-5 Megohms for the recording barrel and 20-70 Megohms for the other barrels, as measured by a 60 Hz source at 0.5V.

(b) Intracellular potentials were recorded with

single micropipettes filled with 0.5 M potassium citrate (pH 7.0) or 1 M potassium formate (pH 6.0) and having tip diameters under 0.5 um with impedances of 15-20 Megohms measured under similar conditions.

E. IONTOPHORESIS

Iontophoretic release of an excitant amino acid, L-Glutamate, has proved to be a useful and reliable means of obtaining a controlled increase in the rate of cell discharge because of glutamate's sharp and quickly reversible action and an apparent lack of serious desensitization following prolonged applications (Krnjević and Phillis 1963a,b; Krnjević, Randić and Straughan 1966a). The excitatory action of glutamate is predominantly due to its effect on the postsynaptic membrane. Consequently a pause in the glutamate evoked spike discharge which follows any of several stimuli used in these experiments can be attributed with a reasonable degree of certainty to a postsynaptic inhibitory process (Galindo, Krnjević and Schwartz 1967; Kelly and Renaud 1971).

Iontophoresis was useful in several different ways:

(a) Glutamate controlled neuronal discharge provided a suitable background of activity upon which to compare the effective inhibition converging on a particular neurone over different pathways. This was quantified by computation of post-stimulus latency histograms which specifically showed the

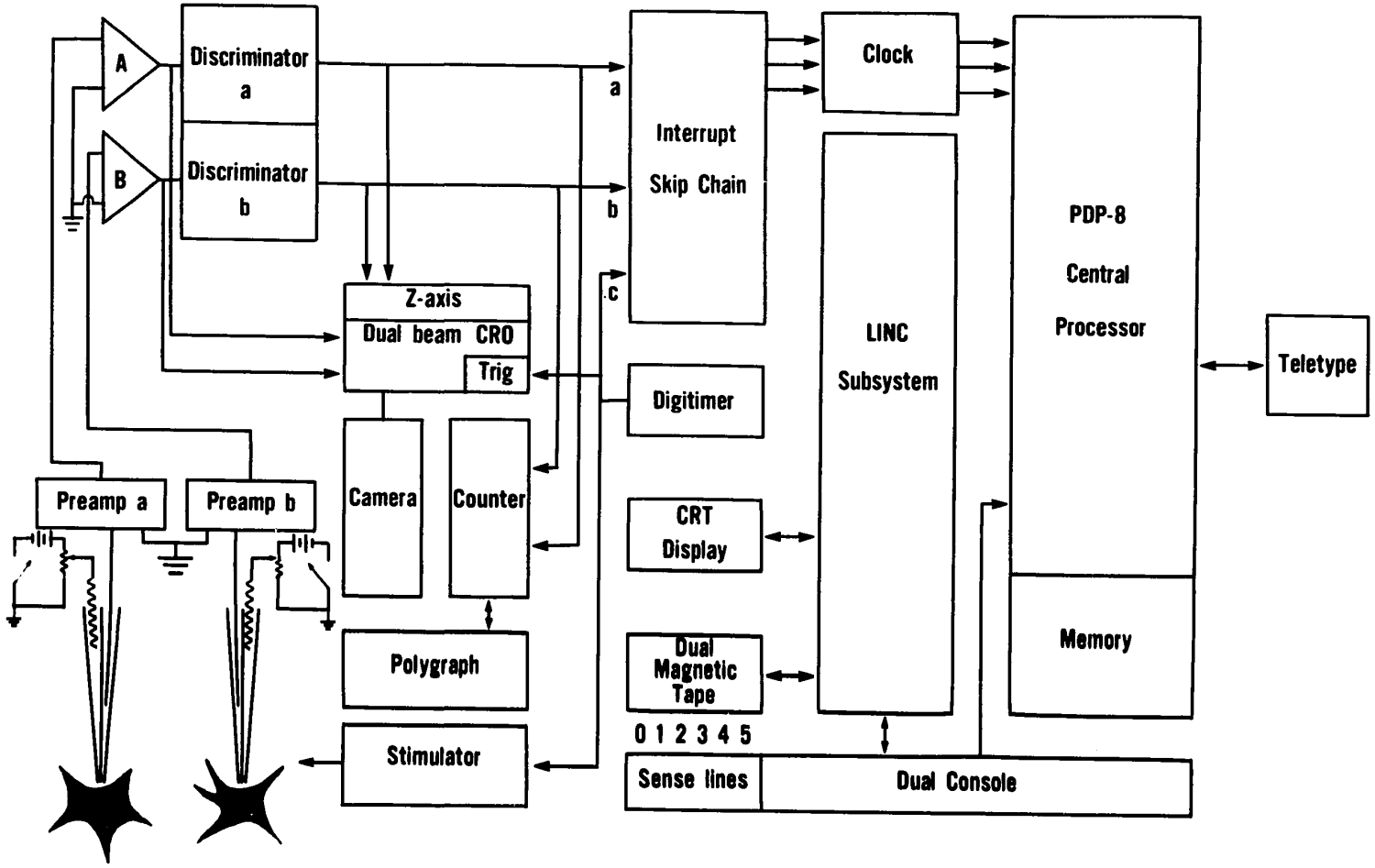
probability of cell firing as a function of time after the stimulus (Gerstein and Kiang 1960). The validity of this method rested on the maintenance of a relatively constant background frequency of neuronal discharge during the testing of any particular neurone.

(b) The interaction between two simultaneously recorded neurones, and in particular the search for inhibitory interneurones, was facilitated by iontophoretic applications of glutamate to one neurone while observing the effects of this augmented neuronal discharge on the background activity of a neighbouring neurone. A very localized form of intracortical microstimulation confined to the region of one recording electrode was, therefore, attained by careful iontophoretic control over the rate of glutamate ejection.

(c) The two cell data acquisition system used in these experiments had inherent limitations (see below) which required that both neurones be moderately active. Continuous release of a small amount of glutamate provided a method whereby slowly discharging or silent neurones, which otherwise would have been ignored, were useful for analysis.

(d) In some experiments iontophoretic application of ACh was used to test and extend previous observations (Krnjević, Pumain and Renaud 1971) on the effects of ACh on different types of cortical neurones.

Figure 7. Block diagram of the LINC-8 computer and data acquisition system for two trains of spikes. The activities of two neurones (in black) were recorded from one of two channels of micropipettes which contained an excitatory amino acid applied iontophoretically from the second channel. The occurence of action potentials from either neurone was detected by amplitude discriminators connected to the PDP-8 interrupt bus. Additional pulses of variable duration were useful for CRO Z-axis enhancement. The output of the counter which determined the number of spike occurrences over a defined period was displayed on a polygraph. A Digitimer controlled the computer data cycle periods (c), the CRO trigger and stimulators. The data was displayed on-line on the CRT as a joint scatter diagram (Figure 8). The program was controlled either via the teletype or through the left-hand console switches of the LINC-8 computer.


A
B
Discriminator a
Discriminator b
a
b
c
Interrupt Skip Chain
Clock
PDP-8 Central Processor
Teletype
Z-axis
Dual beam CRO
Trig
Digitimer
LINC Subsystem
Preamp a
Preamp b
Camera
Counter
CRT Display
Memory
Polygraph
Dual Magnetic Tape
0 1 2 3 4 5
Stimulator
Sense lines
Dual Console

F. EXTRACELLULAR RECORDING

The extracellular recording set-up is illustrated schematically in Figure 7. Two micropipettes (double or triple barrelled) mounted on independent micromanipulators (Narishige) were aligned visually so that their tips were parallel to one another and penetrated in a direction perpendicular to the cortical surface. The recording barrels were connected through chlorided silver wires to high impedance input (10^8ohms) pre-amplifiers having capacitance neutralization (Transidyne Model MPA-5). Action potentials,led into single ended differential amplifiers with a bandpass of 100 Hz to 10 KHz (Tektronix 3A9) were displayed on a dual beam oscilloscope (Tektronix 565). The amplified action potentials, converted by DC coupled adjustable voltage gates (discriminators) into standard pulses which were monitored by an audio-amplifier, were used to brighten selected spikes with respect to the background activity by modulating the oscilloscope Z-axis intensification. This technique offered two advantages: spike intensification enhanced the contrast for photography, and provided the experimenter with a continuous visual monitor of the data being selected for further analysis.

G. LINC-8 COMPUTER

The LINC-8 (Digital), a laboratory oriented small general purpose digital computer, is comprised of two subsystems:

a standard Programmed Data Processor -8 (PDP-8) and the LINC subsystem consisting of a central processing portion, a display scope and a dual tape transport. Both systems are connected by a special interface section which mediates the interchange of data and control, and both share a dual console. The LINC-8 is designed to operate in one of two modes. In one mode, it operates as a standard PDP-8 computer system, and is programmed using the basic PAL III twopass assembler. The LINC-8 is set up for operation in the LINC mode using the interpretive PDP-8 program PROGOFOP, and has its own class of instructions, assembled with the LAP6-3L system. In the LINC mode, the PDP-8 operates primarily as a supporting system, supplying special subroutines for some of the more complex LINC instructions.

Each mode of operation offers distinct advantages for individual applications. Consequently programs were designed and written for either PDP-8 or LINC mode depending upon the nature of the task at hand. The programs are specified as such in the following descriptions.

One of the limitations of using machine language for numerical problems and data reduction programs is its inefficiency in program coding. This can be overcome through use of the on-line interpretive language FOCAL (Digital, 1969 version). In order to circumvent some of the restrictions of this software language (limited speed and inability to handle large volumes of data), a number of changes and additions were made in FOCAL function calls (Covington 1969; Covington and Kelly 1969). This

permitted handling of data stored on magnetic tape and was particularly useful for off-line analysis of changes in neuronal discharge resulting from stimulation.

H. EXTRACELLULAR DATA ACQUISITION

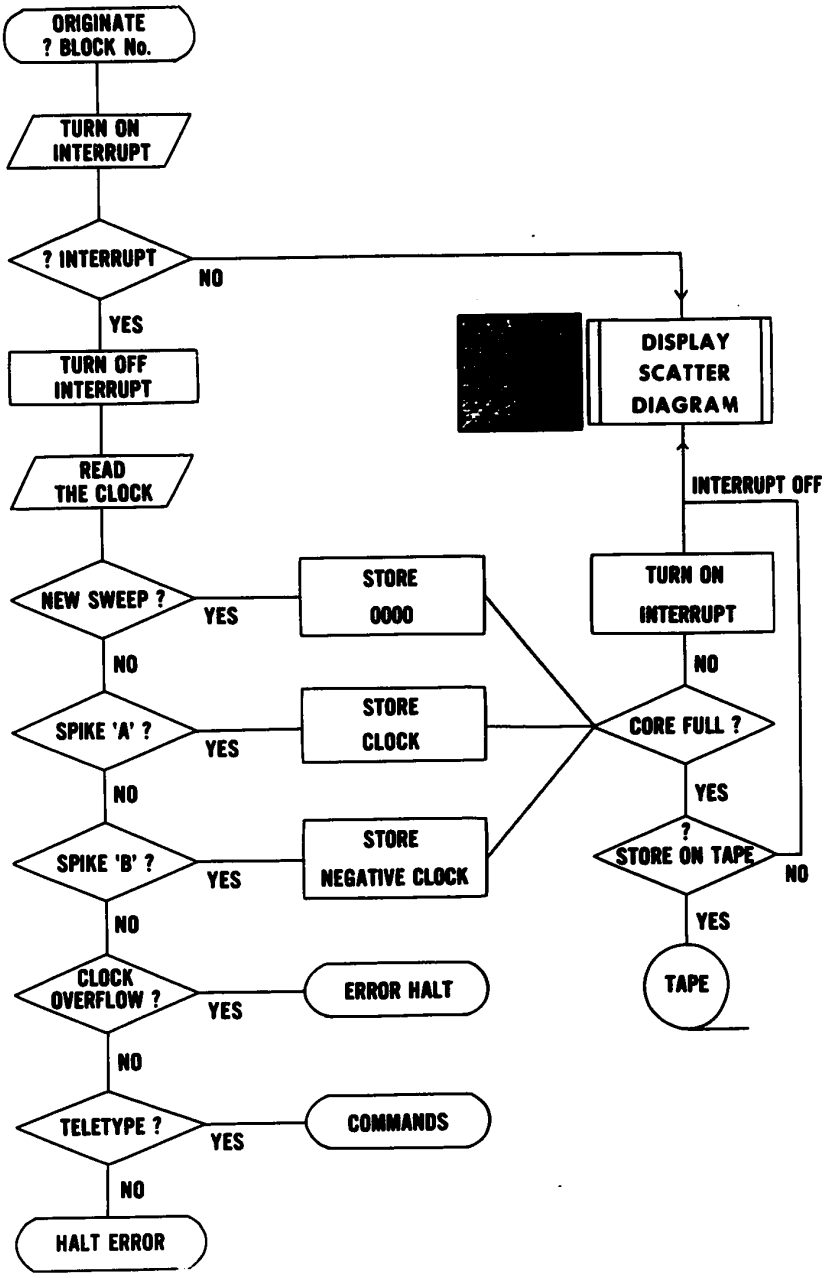
Two sets of data handling and analysis programs were utilized depending upon whether the data consisted of one or two trains of spikes.

Single trains of spikes. In order to measure a change in neuronal excitability following a given stimulus the computer was programmed to display on-line post-stimulus latency histograms (PSLHs) and store the data on magnetic tape.

Every second, synchronized with the start of each oscilloscope sweep, a pulse from the Digitimer started a new data cycle by resetting a simple crystal clock (12 bit, frequency 2 KHz) to zero. The presence of an action potential, signaled by a pulse from the gate unit to one Schmitt trigger input, interrupted the display and caused the contents of one of 2000 memory registers, determined by the current reading on the clock, to be incremented. The number of clock resets, and therefore the number of one-second periods available for data sampling, was usually limited to 128. Upon reaching this number, the contents of the 2000 memory registers were stored on consecutive 20 block segments of magnetic tape from which the data could be recalled for further off-line analysis.

Two trains of spikes. In order (a) to detect the

Figure 8. Flow diagram of the PDP-8 computer program used for data acquisition from two trains of spikes. The program initially requested the block number for output of data onto magnetic tape. Amplitude detectors connected to the PDP-8 interrupt bus signalled the presence of a spike in either train A or B. Either one of these events, or a pulse from the Digitimer, signaling the onset of a new data cycle (NEW SWEEP?), interrupted the display routine. If the interrupt originated from the Digitimer, an integer (0000) was stored in the next location in the memory table. If the interrupt was due to the presence of a spike, this caused a simple 2 KHz crystal clock to be read. Depending on whether the spike originated in train A or B, either the current clock reading or its complemented value was stored in the next location in the memory table. The 12 bit clock was never allowed to exceed 3777 (octal), i.e. to have a negative reading. Once the area assigned in the computer memory for data was filled, the data were written on 30 blocks of magnetic tape beginning at the block number specified at the onset.

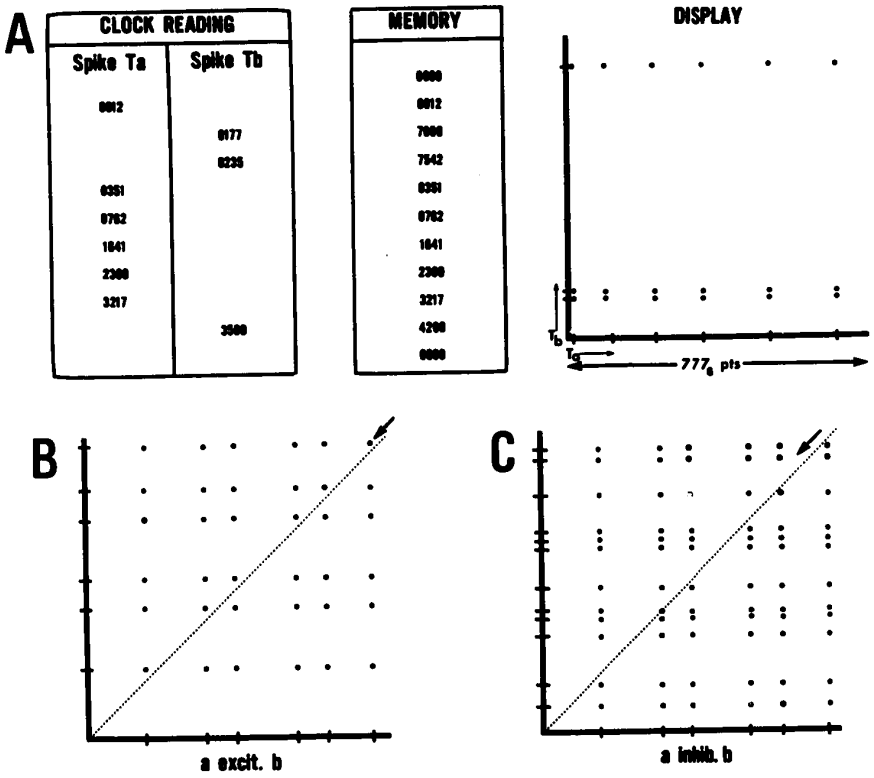

ORIGINATE
? BLOCK No.
TURN ON INTERRUPT
? INTERRUPT
NO
YES
TURN OFF INTERRUPT
READ THE CLOCK
DISPLAY SCATTER DIAGRAM
INTERRUPT OFF
NEW SWEEP ?
YES
STORE 0000
TURN ON INTERRUPT
NO
SPIKE 'A' ?
YES
STORE CLOCK
CORE FULL ?
NO
YES
SPIKE 'B' ?
YES
STORE NEGATIVE CLOCK
? STORE ON TAPE
NO
YES
NO
CLOCK OVERFLOW ?
YES
ERROR HALT
TAPE
NO
TELETYPE ?
YES
COMMANDS
NO
HALT ERROR

presence and type of functional interactions between closely situated neurones, and possibly characterize them as excitatory or inhibitory cells and (b) to compare the potency of various inhibitory pathways on both pyramidal and non-pyramidal neurones, the activities of two neurones were recorded simultaneously. A rapid qualitative answer could be obtained through the on-line construction of a joint scatter diagram (JSD) display (Gerstein and Perkel 1969; c.f. Kristan 1971). More quantative derivations (auto- and cross-correlograms) were obtained off-line from data stored on magnetic tape.

Although the JSD may not be the most sensitive detector of the interactions between two cells, it had the advantage in the on-line situation that the program necessary to generate the display (Figure 8) was relatively short, occupying the first 1K of the computer's memory addresses, leaving the remaining 3K memory addresses for data storage. Once the data area was filled, the data were transferred onto 30 blocks of magnetic tape and the memory area cleared for new data.

The basic data consisted of three events: a Digitimer pulse signaling the start of each data cycle, and the two trains of action potentials. Each event was detected by a Schmitt trigger input, and caused an interruption in the display program. An interrupt initiated by the start of a new data cycle (usually every 500 or 1000 ms) caused the integer 0000 to be stored in a memory address (c.f. figure 9A) and reset a simple crystal clock (12 bit, frequency 2KHz) to zero. Interrupts from action potentials (neural

Figure 9. Computer generation of the scatter diagram display. A. On the left are illustrated clock readings of one hypothetical data cycle as explained in the text. Clock readings for the 6 spikes from cell a remain as positive numbers while those for cell b are first complemented and then stored in individual memory addresses (centre). The beginning of each data cycle is indicated by a separate number (0000) in the memory table. The CRT display for this cycle is presented on a square matrix 777 octal points on a side. Positive numbers in the memory address table determine the X coordinates (Ta) while negative numbers, signifying spike train b occurences, are again complemented (i.e. now positive numbers) to provide the Y coordinates (Tb). Values are scaled to 11 bits and the points determined by the X and Y coordinates representing spikes a and b are displayed on the screen. B. Hypothetical scatter diagram in which a spike in train b is made to occur just after a spike in train a (i.e. a excites b). A consistent clustering of points above the 45° diagonal (arrow) appears in the scatter diagram plot. C. Hypothetical example in which a spike in cell a causes transient inhibition of spike production in cell b. This situation results in an absence of points immediately above the 45° diagonal (arrow) of the scatter diagram.


A
CLOCK READING
Spike Ta
Spike Tb
0012
0177
0235
0351
0762
1641
2300
3217
3500
MEMORY
0000
0012
7000
7542
0351
0762
1641
2300
3217
4200
0000
DISPLAY
T_b
T_a
777 pts
B
a excit. b
C
a inhib. b

trains a and b) caused the clock to be read. In the event of a neurone a spike, the current clock reading was deposited in the next address in the memory data table, while for neurone b spikes the complemented clock reading was stored (c.f. Figure 9A, Ta and Tb). Since clock values were prevented from exceeding 11 bits (i.e. 3777, octal numbers) the two spike trains could be distinguished by their sign (positive or negative).

The data were displayed on-line whenever the computer was not occupied in the servicing of interruptions from the Digitimer or the two spike trains. Points were displayed on the oscilloscope the coordinates of which were derived from the nine most significant bits of the contents of memory addresses (i.e. clock readings) for each data cycle. Figure 9A illustrates the principle diagramatically. The position of each point in the JSD display (a square of 512 decimal or 777 octal points to a side) was determined as follows: the positive (Ta) numbers provided the X coordinates, while the negative (Tb) numbers (complemented) provided the Y coordinates. The total number of points displayed from each data cycle was the product of the number of positive and negative integers (e.g. 18 points in Fig. 9A).

Interpretation of the joint scatter diagram. The JSD provides two kinds of information: one pertains to the interactions between two neurones, the other pertains to the responses of the two neurones to a periodic stimulus.

Figures 9B,C and 10E illustrate the three basic patterns

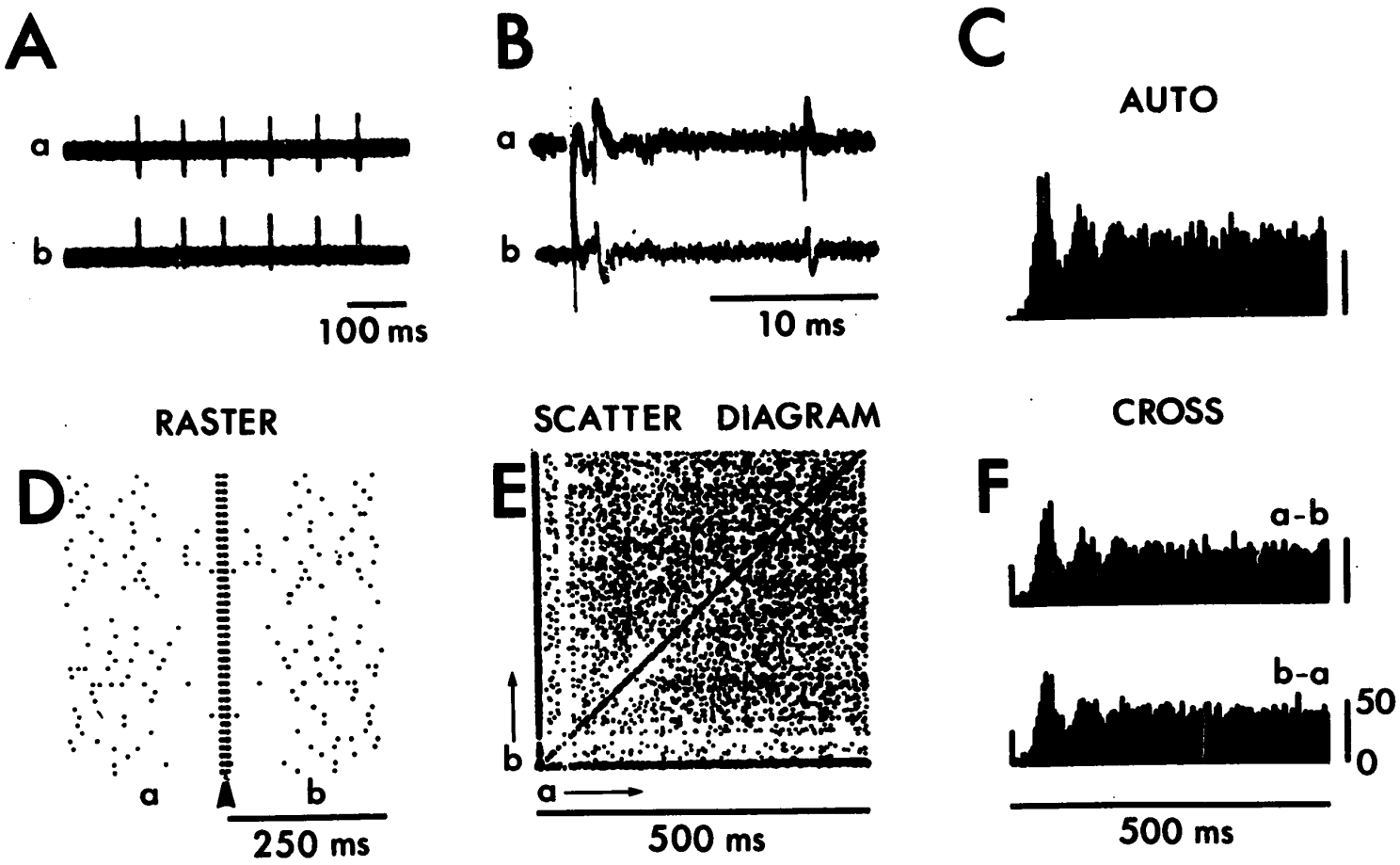
which relate to interactions between two spike trains. In the schematic diagram in Figure 9B for a single data cycle the spike from neurone a is followed a short time later by a spike from neurone b (excitation), while in Figure 9C the spike from cell a is followed by a decreased probability of cell b discharge (inhibition). This results in a increase or decrease respectively in the density of points which would appear above the 45°diagonal if many data cycles were superimposed. On the other hand, if the two neurones tended to discharge synchronously, for example due to the activity of a common excitatory source, there will appear a high density of points along the 45°diagonal. This is illustrated in the extreme case of Figure 10E in which the spike discharges in each train originated from the same PT neurone (Fig. 10A, B). In the extreme opposite situation where no interaction occurs between spike discharges, the JSD would show a uniform distribution of points.

Excitatory or inhibitory neuronal responses appear as a high or low density of points parrallel to the X or Y axis. For example, recurrent inhibition of the PT neurone by volleys to the pyramidal tract resulted in the scanty distribution of points along the major axes in the JSD in Figure 10E.

I. EXTRACELLULAR DATA ANALYSIS

Single trains of spikes. Off-line, a FOCAL program was used to calculate the average discharge rate in the 250 ms period immediately preceeding the stimulus. The decrease in the number

Figure 10. Types of analyses performed on data from 2 neurones. A. Two oscilloscope traces illustrating the simultaneous presence of spikes from both electrodes a and b which were positioned next to the same PT neurone. B. The antidromic spike appears in both channels (upper channel inverted) following a pyramidal tract shock 2 ms after the onset of the sweep. C. The auto-correlogram computed for one channel, over 500 ms of time, in this case was identical to that computed for the other channel. D. A raster diagram of the pyramidal stimulus data shown in B. Each spike occurence appears as a dot, and each row corresponds to a new sweep on a data cycle. The arrow represents the onset of each oscilloscope trace which then courses to the left (train a) or right (train b). Note the symmetry in the distribution of dots on either side of the arrow, and the paucity of dots at the onset of each sweep representing recurrent pyramidal inhibition. E. Scatter diagram of the same data as shown in B and D. The increase in the density of points on the 45° diagonal results from the simultaneous appearance of spikes in both train a and b, and the less dense distribution of points parallel to the major X or Y axes is the result of recurrent pyramidal inhibition. F. Cross-correlograms of spontaneous data shown in A, computed in two portions. The upper histogram represents the influence of spikes in train a on train b spikes, while the lower histogram represents this relationship with the spike trains reversed. In this case the data originates from the same neurone; therefore, the auto-correlograms and cross-correlograms will appear similar since the cross-correlogram becomes simply an auto-correlogram.


A
a
b
100 ms
B
a
b
10 ms
C
AUTO
RASTER
D
a
b
250 ms
SCATTER DIAGRAM
E
b
a
500 ms
CROSS
F
a-b
b-a
50
0
500 ms

of spikes observed during the same interval of time, but immediately following the stimulus, was expressed as a percentage of the average discharge rate (% inhibition) and served as a measure of inhibition (c.f. Figure 26 and 27 in Chapter 3).

Another program recalled the data from tape and displayed a post-stimulus latency histogram containing 128 bins, each with a duration of 8 ms. The contents of each bin were normalized as a percentage of the average frequency of neuronal discharges computed from the FOCAL program described above.

Two trains of spikes. Off-line, several programs were available to perform various functions (c.f. Figure 10):

1. The data could be displayed either as joint scatter diagrams or raster diagrams. In the latter, action potential occurrences are represented as dots, and the individual oscilloscope sweeps for each data cycle arranged so as to course to the left (for train a) or right (for train b) from the centre of the diagram. A program option permitted expansion of the X axis of the display up to five-fold by sampling a potentiometer connected to one of the input channels of the multiplexed A-D conversion system.

2. Cross-correlation. The presence and the nature of any dependency between two spike trains can be quantatively derived through computation of the cross-correlogram (Moore et al. 1970). This is a standard method for detection of statistical dependence and measures the probability that the occurrence of an action potential from one unit changes the firing pattern of another unit (Moore, Perkel and Segundo 1966; Perkel, Gerstein and Moore 1967).

The aim primarily centered on defining short latency correlations; therefore, the correlation period was usually below 1000 ms. Correlograms were displayed above one another, with the upper representing the correlation from spike trains a to b and the lower vice versa (c.f. Figure 10F).

3. Auto-correlation. Periodicities in the discharge pattern of pre- and postsynaptic neurones influence the shape of the cross-correlogram (Moore et al. 1970). Therefore, interpretation of the cross-correlograms require visualization of the auto-correlograms for each individual cell. As applied to the analysis of spike trains, the probability of encountering an action potential as a function of time after a given action potential is the auto-correlogram, also called expectation density (Poggio and Viernstein 1964), post firing interval distribution (Lamarre and Raynauld 1965) and renewal density function (Moore, Perkel and Segundo 1966). Auto-correlation was studied over the same time period as the cross-correlation (c.f. Figure 10C).

J. INTRACELLULAR DATA ACQUISITION

The aim of intracellular recording was to characterize and compare the IPSPs evoked by two forms of inhibitory stimuli i.e. surface and pyramidal recurrent IPSPs. Since the IPSP amplitude alone poorly reflects the magnitude of the membrane conductance change resulting from synaptic inhibition (Dreifuss, Kelly and Krnjević 1969) it was necessary to measure both membrane resistance and potential, not only at the peak of the IPSP but also

Figure 11. Operational amplifier circuit used to interface the LINC-8 computer with the neurophysiological experiment. The computer digital to analog (D-A) output consists of a 0 to -10V pulse which was converted to +10V to -10V by operational amplifier number 4. The output of this amplifier was applied directly to the bridge network, and also to an analog channel (SAM 10) after reducing the pulse to +1V to -1V maximum with operational amplifier number 5. The latter provided the current sample for voltage-current plots. The voltage sample, obtained through a high input impedance preamplifier with unity gain (Pico-metric) was first amplified and displayed (amplifiers 1 and 2), then rendered as a positive signal by adding 0.51 V (amplifier number 3) and connected to a second analog channel (SAM 11). Bridge balance was maintained through a 7.2 Kohms potentiometer.

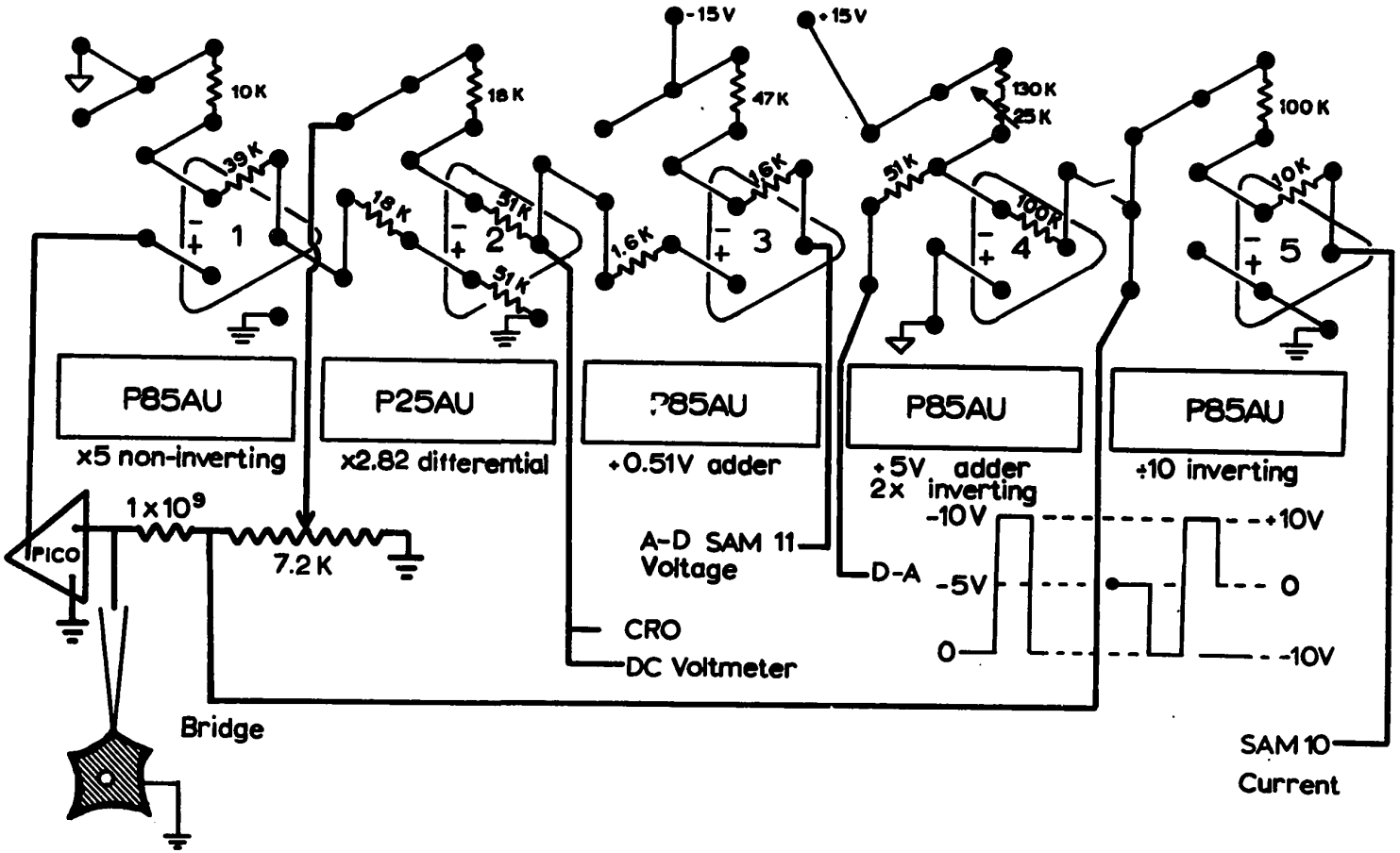
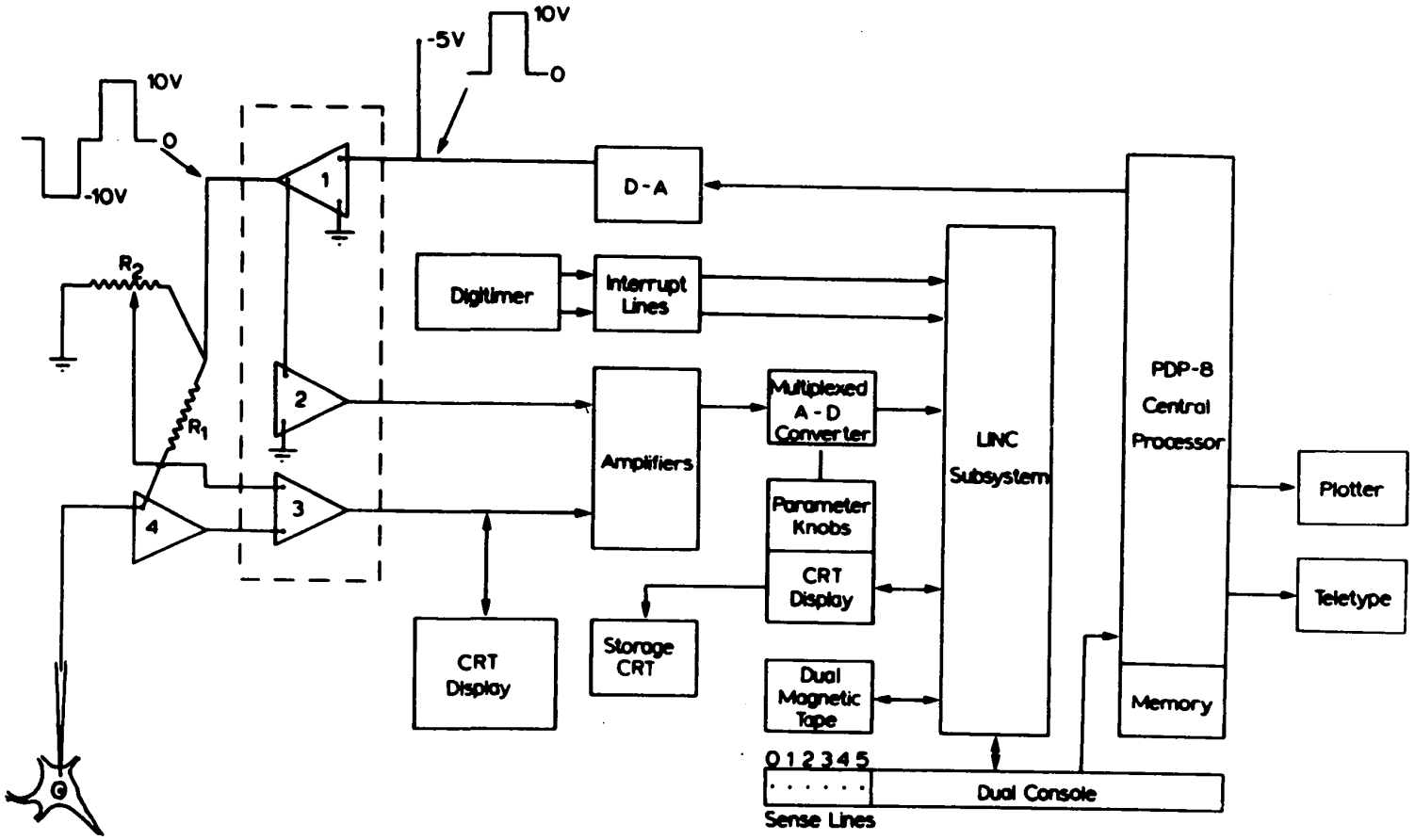

-15V
+15V
10K
18K
47K
130K
25K
100K
39K
18K
51K
51K
1.6K
1.6K
51K
100K
10K
1
2
3
4
5
P85AU
P25AU
P85AU
P85AU
P85AU
x5 non-inverting
x2.82 differential
+0.51V adder
+5V adder
2x inverting
+10 inverting
1x10⁹
IPICO
7.2K
A-D SAM 11
Voltage
D-A
-10V
+10V
-5V
0
0
-10V
CRO
DC Voltmeter
Bridge
SAM 10
Current

Figure 12. Block diagram of the LINC-8 computer and data acquisition system for intracellular experimental analyses. The recording electrode, together with R_1 and R_2 formed components of a balanced bridge network. Current pulses (0 to -10V generated by the LINC-8 computer through a D-A converter and changed to +10V to -10V through an operational amplifier circuit shown in Figure 11) were applied to the bridge and recording microelectrode. The computer sampled the membrane voltage (amplifier 3) and the applied current (amplifier 2) ten times per second by means of the computer's multiplexed analog to digital converter (A-D). The values served as coordinates for voltage-current plots displayed on-line and subsequently stored on magnetic tape. Pulses from a Digitimer and its associated gated oscillator connected to the computer interrupt line were used to initiate each train of current pulses, and determine the pulse frequency within each train. The Digitimer was also used to trigger the oscilloscope and stimulators. Parameter knobs connected to the analog inputs of the computer determined (i) the duration and number of current pulses (1-10) to be delivered per data cycle, (ii) the maxima and minima of the pulse amplitudes and (iii) which set of voltage-current plots were to be displayed. The program was controlled either through the teletype or sense switches.

On-line, the computer was restricted to displaying voltage-current plots and storing data on magnetic tape. The data were later reduced to printed tables on the teletype or graphs on the X-Y plotter.


10V
0
-10V
-5V
10V
0
R2
R1
1
2
3
4
G
D-A
Digitimer
Interrupt Lines
Amplifiers
Multiplexed A-D Converter
Parameter Knobs
CRT Display
Storage CRT
CRT Display
Dual Magnetic Tape
LINC Subsystem
PDP-8 Central Processor
Memory
Plotter
Teletype
0 1 2 3 4 5
Dual Console
Sense Lines

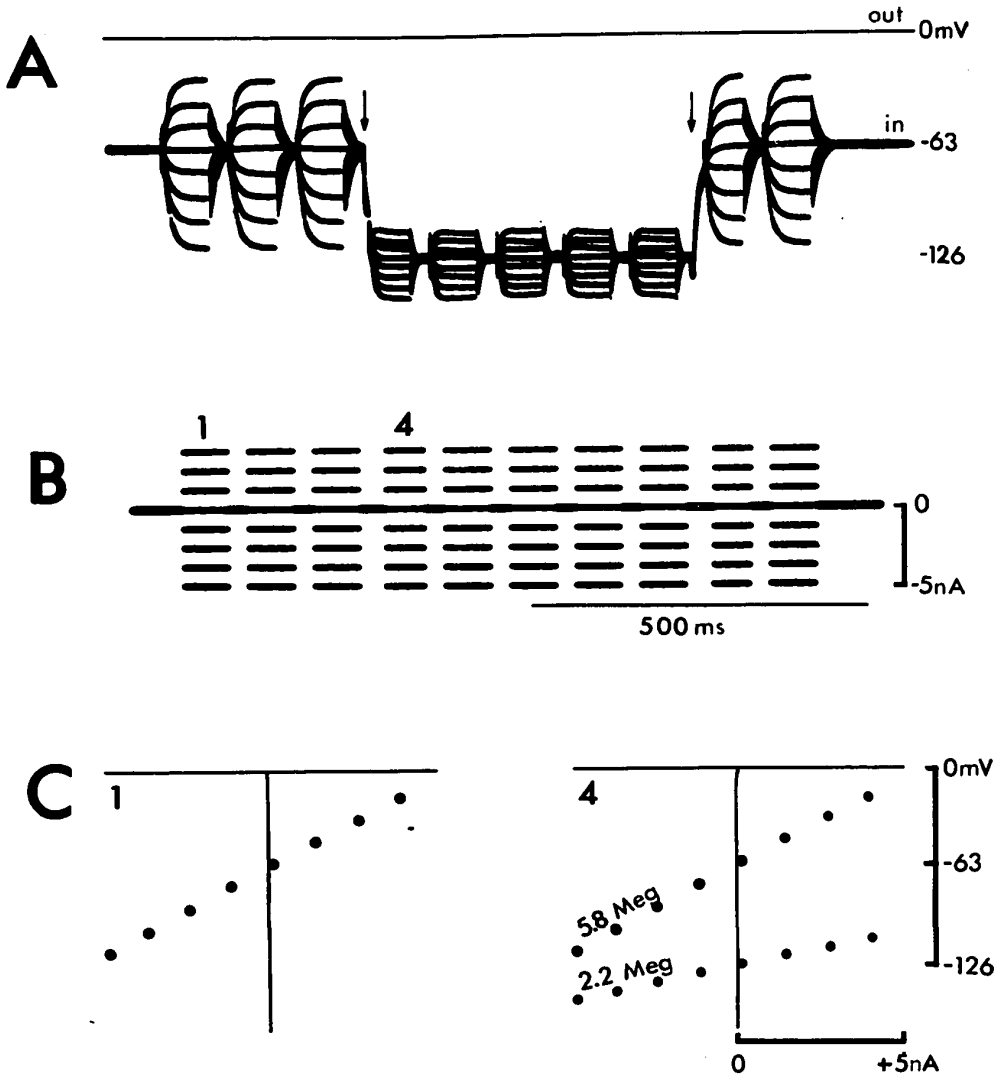
at various intervals along its time course. To facilitate these measures it was necessary to interface a LINC-8 computer with the neurophysiological experiment in such a way so as to provide an on-line display of voltage-current plots, with storage of the data points on magnetic tape for subsequent analysis off-line.

The recording micropipette was linked through saline and a chlorided silver lead to a preamplifier with a high input impedance (greater than 10^{12} ohms), low grid current (less than 10^{-12} A) and compensation for high frequency loss at the input (Pico-metric, Instrumentation Laboratories Inc.). The input circuit was designed so that the electrode and preparation were part of a resistive bridge which could be balanced by adjusting a potentiometer. The principle was similar to the method utilized by Krnjević and Schwartz (1967) except that a high resistance (10^9 ohms) was used in the high resistance arm of the bridge (Figure 11).

Current pulses, 20-30 ms in duration, were applied across the bridge to test for bridge imbalance. Since the resistance for useful electrodes was less than 50 Megohms (measured in situ) the current through the electrode could be assumed to be equal to the applied voltage (10V) divided by 10^9 , without the error exceeding 5%.

The degree of bridge imbalance observed on penetrating a neurone was assumed to represent the effective resistance of the cell membrane. The maximum of the recorded voltage pulse divided by the applied current was taken as the resistance. The margin of error was small as long as the electrode resistance

Figure 13. Simulation of an intracellular experiment using an artificial cell. A,B. Photographic records from an experiment in which the membrane potential and resistance, the IPSP and accompanying decrease in membrane resistance were simulated by two circuits, each of which consisted of a battery and resistance in series. (A) illustrates the simulated membrane potential (63 mV) and the IPSP (63 mV) between arrows. Eight consecutive sweeps were examined, with each sweep containing a train of ten equal current pulses whose amplitude was incremented after each train (B). The downward deflection of potential and the decreased amplitude of the current pulses (numbers 4-8) mimic the IPSP. In (C) are illustrated voltage-current lines displayed by the LINC-8 computer (voltage on the Y axis, negative and positive current on the X axis) from the values of the bridge current and voltage sampled and stored immediately before termination of each current pulse. On the left, the plot derived for the first family of pulses (number 1). On the right, points derived from the first and fourth pulses illustrating the change in membrane resistance (slope) and potential (intersect with the Y axis) during the simulated IPSP.


A
out
0mV
in
-63
-126
B
1
4
0
-5nA
500 ms
C
1
4
0mV
-63
-126
5.8 Meg
2.2 Meg
0
+5nA

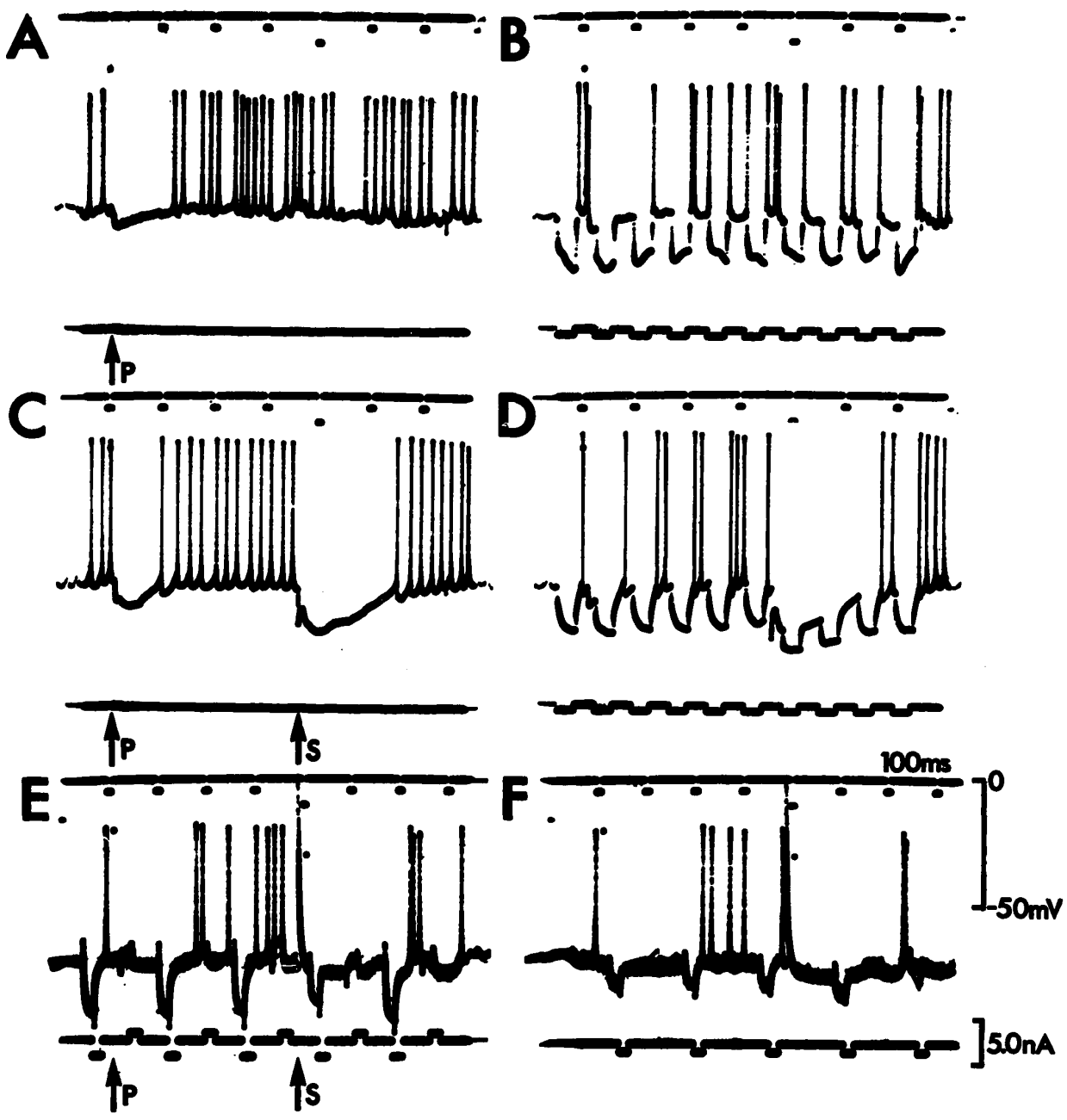
was small in comparison with the fixed resistance in the high resistance arm of the bridge.

Changes in resistance or conductance during the surface or pyramidal IPSPs were determined by measuring changes in the amplitude of the recorded pulses applied ten times per second at 80 ms intervals along the time course of the IPSPs (c.f. Figures 14B,D and 15). The first pulse, occurring prior to the stimulus, gave an estimate of the resting resistance. The next pulse, or first test pulse during the IPSP, was usually applied 40 ms after the stimulus in order to minimize interference by early EPSPs. Sufficient data for comprehensive voltage-current plots (providing both reasonably accurate resistance and IPSP reversal potential estimates) were obtained by varying the intensity of the current pulses once every second for eight consecutive seconds (c.f. Figure 15).

Facilities for automating most of these tasks were available with the LINC-8 computer. The computer was programmed to deliver through a D-A converter, a train of ten equal voltage pulses, with the pulse amplitudes incrementing by equal amounts once after each train and the sequence being repeated eight times (Figure 13). A Digitimer initiated each train of pulses once every second and triggered the stimulators, oscilloscope sweep and a gated oscillator which determined the pulse frequency (one every 80 ms) within each train. Potentiometers connected to the input channels of the multiplexed A-D conversion system controlled both the duration as well as the maximal and minimal values of the voltage pulses.

In order to obtain positive and negative values from the unidirectional D-A voltage output (0 to -10V maximum) an

Figure 14. Intracellular recordings from PT neurones illustrating pyramidal and surface evoked IPSPs and changes in membrane resistance. In each series of oscilloscope traces the upper line is the time scale, the central line the voltage recorded inside the neurone and the lower line the applied current pulses. A,C: A pyramidal evoked IPSP (P) alone, or followed by a surface evoked IPSP (S). B,D: Voltage changes produced by ten negative current pulses applied to the intracellular microelectrode. Note the decreased amplitude of the voltage output at the peak of the IPSPs, particularly during surface evoked IPSPs, representing a marked decrease in the membrane resistance. E: Similar sequence with two IPSPs but with ten current pulses alternating in polarity. Only five negative pulses are shown in (F).


A
B
P
C
D
P
S
E
F
100ms
0
-50mV
5.0nA
P
S

adder-inverter operational amplifier circuit (Figure 11, No.4) converted the voltage signals to ±10V maximum. These pulses, applied to the bridge network through the 10^9 ohm resistor, allowed for current pulses with maximal intensities of ±5 nA to be delivered to the recording microelectrode. The current pulses, reduced to analog pulse signals of ±1V (maximum) by another operational amplifier circuit (Figure 11, No. 5) were monitored on the lowest oscilloscope trace and led to one of the computer's analog inputs (SAM 10 in Figure 11).

In some of the earlier experiments, such as the example illustrated in Figure 14E (lowest trace), the computer was programmed so that consecutive current pulses in each train were of opposite polarity. In later experiments all pulses within a train were programmed to have the same polarity (Figure 14B,D). With suitably chosen electrodes, either approach proved satisfactory and no systematic differences were apparent.

The voltage output signal from the microelectrode was amplified through operational amplifier circuits (Figure 11, No. 1, No. 2), displayed on the middle trace of the oscilloscope and monitored on a DC voltmeter. After addition of 0.51 V, in order to bring the signal within range of the A-D converter (Figure 11, No.3) the signal was led to another of the computer's analog input channels (SAM 11 in Figure 11).

The computer tested for bridge imbalance by sampling the maxima of both the applied current (SAM 10; lower traces in

Figure 15. Complete data cycle obtained during intracellular recording. Intracellular records from a PT neurone in the precruciate cortex. A. Photographic records of eight oscilloscope sweeps illustrating in the middle trace the voltage changes recorded during application of ten current pulses (lower traces) which were incremented between successive sweeps. B. From left to right are ten separate voltage-current plots showing the membrane resistance at each of the ten intervals at which the current pulses were applied. The eight points were obtained after each series of eight oscilloscope sweeps. Note the change in slope of the voltage-current lines following the pyramidal (P) and surface (S) evoked IPSPs. Maximal changes occur for the second and seventh pulses applied at the peaks of the IPSPs. The horizontal line represents zero voltage and the vertical bars represent zero current.

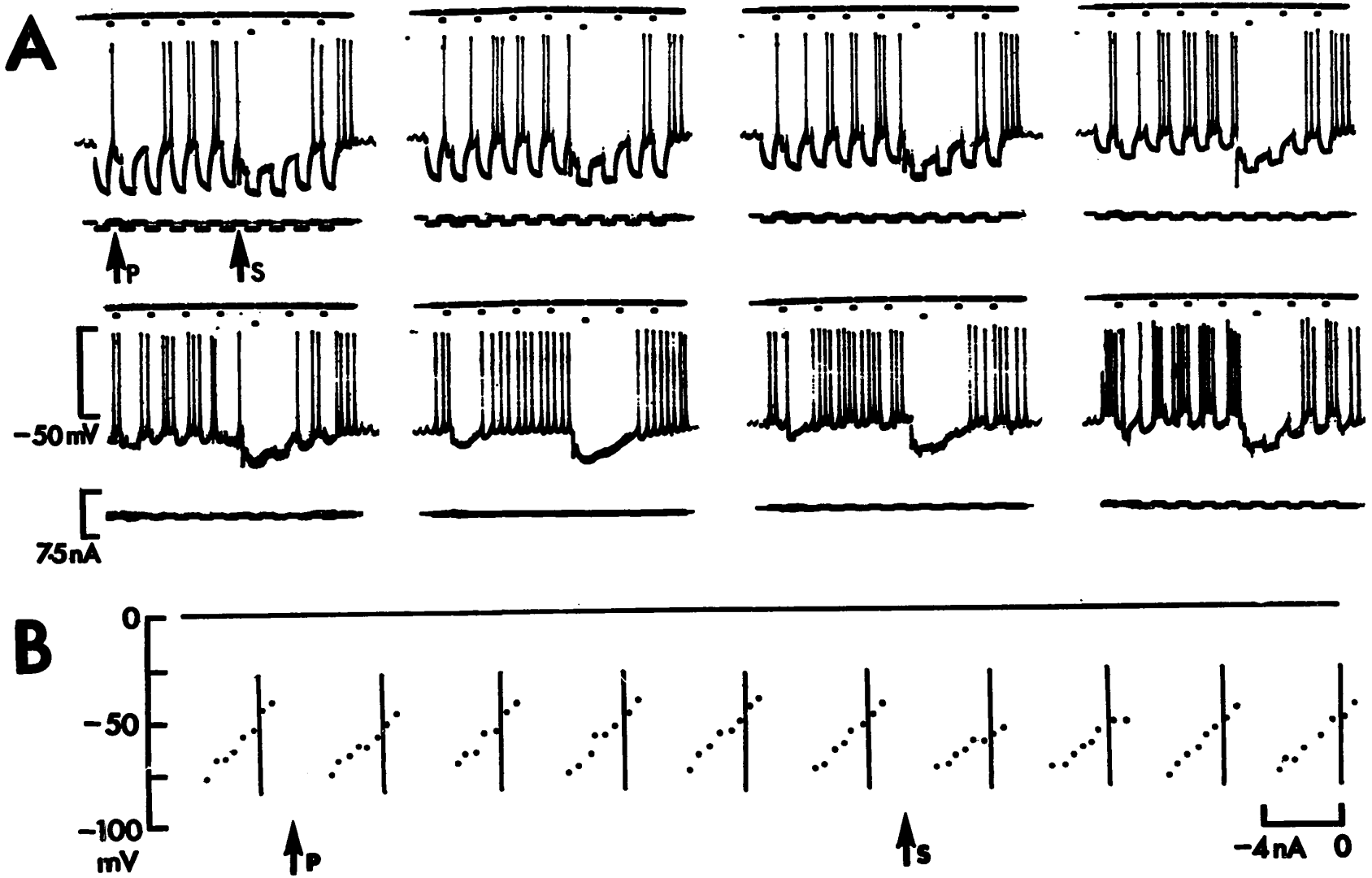
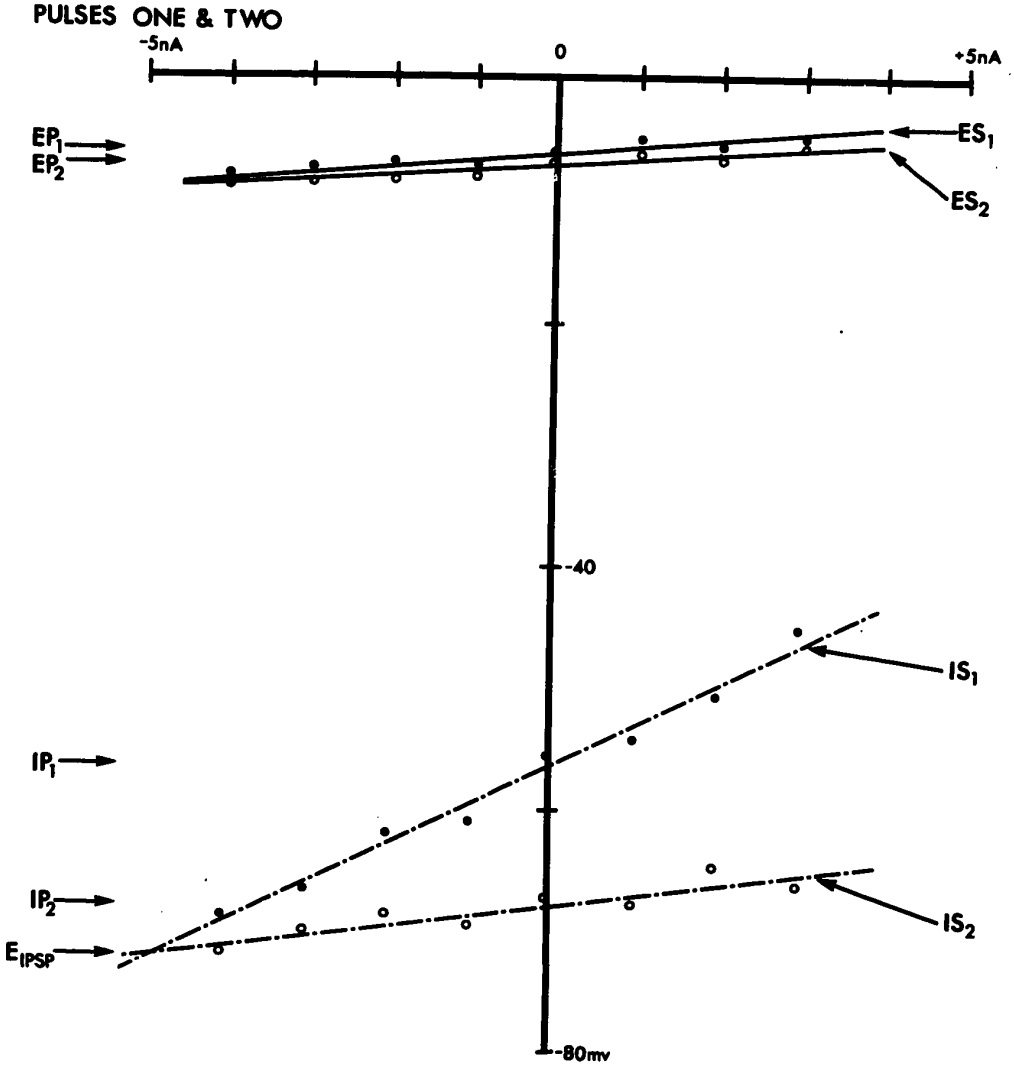

A
P
S
-50 mV
7.5 nA
B
0
-50
-100
mV
P
S
-4 nA
0

Figure 15A) and the voltage output (SAM 11; middle trace in Figure 15A). Voltage-current plots were drawn up after each cycle of eight traces and these were displayed individually on the LINC-8 cathode ray tube for each of the time intervals during which a pulse was applied to the recording microelectrode (Figure 15B). In the example shown (Figure 15B) reduction in the slopes of the second and especially the seventh voltage-current plots, when compared with the first control plot preceding the pyramidal stimulus, reflected the increased conductance occurring at the peak of the pyramidal (P) and surface (S) evoked IPSPs respectively.

In order to compare the influence of surface evoked IPSPs on the recurrent pyramidal IPSPs, the surface stimulator connected to one of the computer relays, was disengaged for each alternative data cycle (i.e. eight consecutive Digitimer cycles). X (current) and Y (voltage) coordinates of the data points were stored on consecutive blocks of magnetic tape, even numbered blocks containing information of pyramidal IPSPs alone and odd numbered blocks containing information relating to pyramidal IPSPs conditioned by surface evoked IPSPs.

Once a complete series of data had been obtained from any one neurone, the electrode was withdrawn from the cell and the full sequence repeated, without changing any of the applied current parameters. This data, also stored on magnetic tape, served as the extracellular control (c.f. Figure 16) for (a) any degree of bridge imbalance which was subtracted from the intracellular values, and (b) the reference voltage levels used to

Figure 16. Schematic illustration of voltage-current lines as generated with the LINC-8 computer. The ordinate represents the peak amplitude of the positive or negative applied current pulses (in nA) while the abscissa represents the absolute potential changes simultaneously sampled. Voltage-current lines were derived from each family of eight voltage-current points. The solid points were obtained before, and the open points obtained during the peak of the IPSPs. The resting membrane resistance was obtained by subtracting the slope of the intracellular voltage-current line (IS_1) from the corresponding extracellular control (ES_1). The difference between this value and that similarly obtained at the peak of the IPSP (IS_2-ES_2) was representative of the change in membrane resistance or conductance evoked by an inhibitory stimulus. Potential values were obtained at the intersects of the voltage-current lines with zero on the current axis. The resting membrane potential derived from the first pulse series before the IPSP was the difference between the intracellular (IP_1) and extracellular (EP_1) potentials. The maximum membrane displacement at the peak of the IPSP was obtained by subtracting EP_2 from IP_2. The change between resting potential and the IPSP potential was therefore IP_2-IP_1. The reversal or equilibrium potential (E_{IPSP}) was derived from the intersect of the first and second voltage current lines, corrected for the extracellular control potentials similarly obtained.


PULSES ONE & TWO
-5nA
0
+5nA
EP1
EP2
ES1
ES2
-40
IS1
IP1
IP2
IS2
EIPSP
-80mv

calculate the IPSPs, membrane and reversal potentials, since only the absolute voltage levels with reference to zero potential on the A-D and not the true intracellular potentials, determined the Y coordinates of the on-line voltage-current plot.

K. INTRACELLULAR DATA ANALYSIS

From the data stored on magnetic tape, a FOCAL-69 program was used to calculate the intracellular resistance and potential values. From all sets of voltage-current plots, the computer derived voltage-current lines of best fit by the method of least squares (dotted lines in Figure 16). Each of the 10 pulses had extracellular control lines and intracellular data lines.

Resistance measurements. The membrane resistance at each of the 10 points along the oscilloscope traces was calculated by subtraction of the calculated extracellular slope (ES_x) from the calculated intracellular control slope (IS_x), where (x) refers to the particular pulse (1, 2, 3...10). Values for the first pulse determined the resting membrane resistance, the second pulse determined values for the membrane resistance 80 ms later i.e. at the peak of the first (pyramidal) IPSP, and so on. The change (decrease) in membrane resistance during the course of the IPSPs was the difference between that obtained from the first and any subsequent voltage-current lines, and was usually expressed as a percentage conductance increase over resting conductance.

Potential measurements. The membrane potential calculated

at each 80 ms interval was the difference between the Y axis intercept of the calculated intracellular voltage-current line (IY_x) and the extracellular voltage-current line (EY_x), as illustrated diagramatically in Figure 16. The resting potential values were derived from the first voltage-current lines while the other voltage-current lines were used to derive membrane potentials during the IPSPs. The IPSP amplitude was the difference between the resting and IPSP membrane potentials. The reversal potentials (E_{IPSP}) were derived from the intercepts of the resting and IPSP voltage-current lines, subtracted from the intercepts of the corresponding extracellular control voltage-current lines. In practice, the reversal potentials calculated at the peaks of the IPSPs (second and seventh pulses) provided the most reliable and stable measurements.

Prior to each experiment, trial tests with known resistance and potential values were used to derive conversion factors so that the calculated values could be converted to millivolts and megohms directly. At the end of the experiment, by inserting these factors, a complete teletype listing of the desired values could be obtained.

Two additional programs calculated the means and standard errors for values selected from several data cycles, and utilizing the LINC-8 graphics package, plotted histograms prepared from the same data on a CALCOMP plotter; one example is illustrated in Figure 19.

L. IDENTIFICATION OF UNITS

Action potentials were considered to be recorded from somata of pyramidal tract (PT) neurones provided that an all-or-none mono- or biphasic spike followed at constant latency a single suprathreshold stimulus to the ipsilateral medullary pyramid. Additional criteria included the ability to follow stimuli up to 200 Hz and the presence of collisions of orthodromic with antidromic spikes. All cells not having a demonstrable corticospinal projection by this technique were classified as non-pyramidal tract (NPT) neurones, and these were further classified according to differences in their functional properties.

M. NEURONAL LOCALIZATION

The majority of neurones were encountered during microelectrode penetrations in the lateral half of the precruciate gyrus. In only one experiment were penetrations made in the lateral half of the postcruciate gyrus, anterior to the postcruciate dimple. According to the cytoarchitectonic classification of Hassler and Muhs-Clemente (1964) this would place all neurones within the limits of their area (4).

The intracortical depth of the cells was determined approximately from micrometer readings on the micromanipulators, with reference to the point at which the electrode tips barely touched the pial surface. Under the high power of the operating microscope, the precision of this technique was approximately ± 50 μm for each of the electrodes. On three occasions in which both

electrodes were positioned sufficiently close so as to record the activity of the same pyramidal cell, the micromanipulator readings differed by less than 50 μm indicating a reasonably accurate alignment at the pial surface. Several attempts were made to mark the depth of the electrode penetrations by iontophoretic extrusion of a 5% solution of Alcian blue (Croma-Gesellschaft, Schmid and Co.) prepared according to the method of Lee et al. (1969). However, the technique failed, either because the electrodes became blocked (indicated by the inability to pass more than 10 nA of current) or the dye spots were too small and/or dissolved during the thionin staining procedure to which the 20 micra frozen sections were subjected.

The separation of the electrode tips was measured by aligning them with a graduated 1 mm scale etched in the upper surface of the perspex pressor plate at the edge of the opening through which the electrodes were inserted. Both the tips and the scale were visible under the microscope. The margin of error in this measurement was also considered to be approximately ±50 μm. In order to keep the electrodes within the confines of one and two functional motor cortical columns (Welt et al. 1967) the tip separations were usually under 400 μm.

N. HISTOLOGICAL CONTROL

Before completing an experiment, the animals were given an intravenous overdose of Nembutal (60 mg/kg). With the chest open

and the thoracic aorta clamped, a transcardiac perfusion of the head and upper extremities with 200 cc of physiological saline was followed by 200 cc of 10% formalin in saline. The brains were removed and after further fixation in 10% formalin for 2-7 days, 20 micron frozen sections of the precruciate region and thalamus in the sagittal plane were stained with thionin and examined for precise localization of the electrode placements.

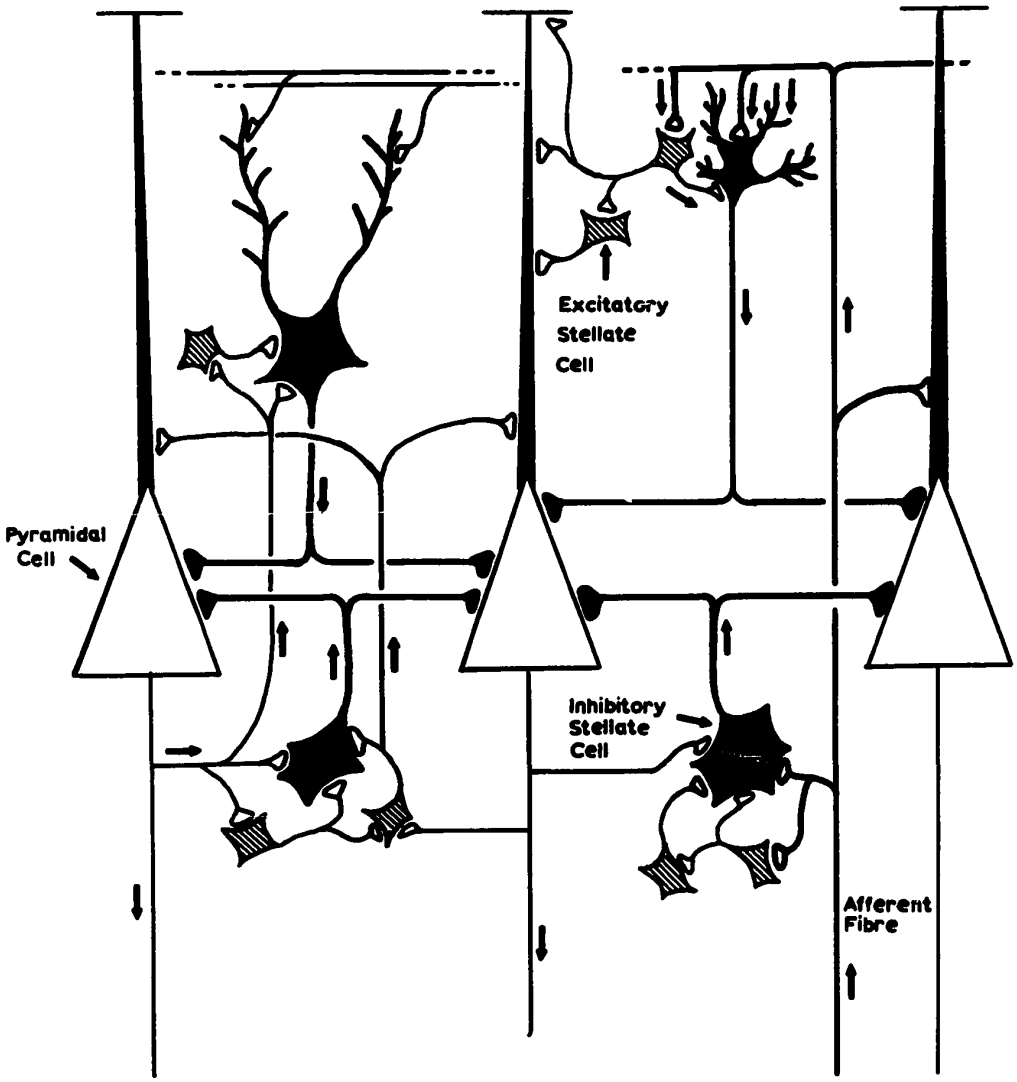
Chapter 3

RESULTS

CORTICAL INHIBITORY POSTSYNAPTIC POTENTIALS: POTENTIATION OF INHIBITION

Pyramidal tract neurones are important physiologically identifiable elements of the motor and sensory cortex (Patton and Amassian 1960). Their axons form the corticospinal (pyramidal) pathway and provide the most direct route through which the cortex can exert its influence on spinal neuronal activity. Prolonged hyperpolarizing potentials (IPSPs) are readily elicited in pyramidal neurones directly by epicortical stimulation (Phillips 1956b; Li and Chou 1962; Krnjević, Randić and Straughan 1966b) and indirectly by stimulation of recurrent pyramidal (Phillips 1956a, 1959; Stefanis and Jasper 1964a,b) and afferent transcallosal or thalamocortical pathways (Creutzfeldt, Baumgartner and Schoen 1956; Branch and Martin 1958; Lux and Klee 1962; Li 1963; Purpura and Schofer 1964; Nacimiento, Lux and Creutzfeldt 1964; Pollen 1964). The profound decrease in neuronal excitability observed during both intracellular and extracellular recordings is the direct consequence of a marked increase in neuronal membrane conductance for anions, mainly Cl^- (Kelly, Krnjević, Morris and Yim 1969). This has a powerful clamping effect on the membrane at a voltage appreciably negative to the resting potential for cortical neurones, which is the IPSP reversal or equilibrium

Figure 17. Circuit diagram illustrating postulated inhibitory pathways to pyramidal tract neurones. The arrows indicate the direction of impulse propagation. Inhibitory neurones, shown in black, may be activated through mono- or polysynaptic pathways either from axon collaterals of pyramidal neurones (feedback or recurrent inhibition) or afferent fibres (feed forward or afferent collateral inhibition). The recurrent inhibitory pathway is polysynaptic, a disynaptic path being activated with the briefest latency by an antidromic stimulus (c.f. Eccles 1969). Individual inhibitory neurones may be activated by more than one pathway, in effect forming a common inhibitory mechanism. Stimuli applied to the cortical surface may excite inhibitory neurones directly or synaptically. PT neurones can be inhibited without necessarily discharging an impulse and engaging a recurrent inhibitory mechanism. Excitatory connections are shown in white, inhibitory in black.


Excitatory
Stellate
Cell
Pyramidal
Cell
Inhibitory
Stellate
Cell
Afferent
Fibre

potential (E_{IPSP}).

The results presented in this section are from experiments designed to test the hypothesis that IPSPs evoked either directly or indirectly in pyramidal tract neurones are the consequence of a release of inhibitory transmitter from identical nerve endings, implying that they are generated through activation of a common inhibitory interneuronal pathway (c.f. Figure 17).

Surface evoked inhibition is apparently more effective than other forms of inhibitory stimuli (Krnjević, Randić and Straughan 1966a) which may be a result of indiscriminate activation of a large number and perhaps variety of cortical inhibitory interneurones. During slow repetitive epicortical stimulation (5-7/sec) there is a striking facilitation of cortical inhibition. Assuming that this is the result of the activity of inhibitory neurones common to other inhibitory pathways, it should also be possible with proper conditioning experiments to demonstrate a facilitatory action of surface evoked IPSPs on other inhibitory test IPSPs (for example the recurrent pyramidal IPSP) through careful measurement of either the IPSPs themselves or their accompanying excitability changes.

In cortical neurones, as in other cells (Kuffler 1960) the IPSP amplitude may not reflect the magnitude of the change in membrane conductance resulting from synaptic activity (Dreifuss, Kelly and Krnjević 1969). A more accurate estimate of both the magnitude and duration of inhibition can be obtained from measure-

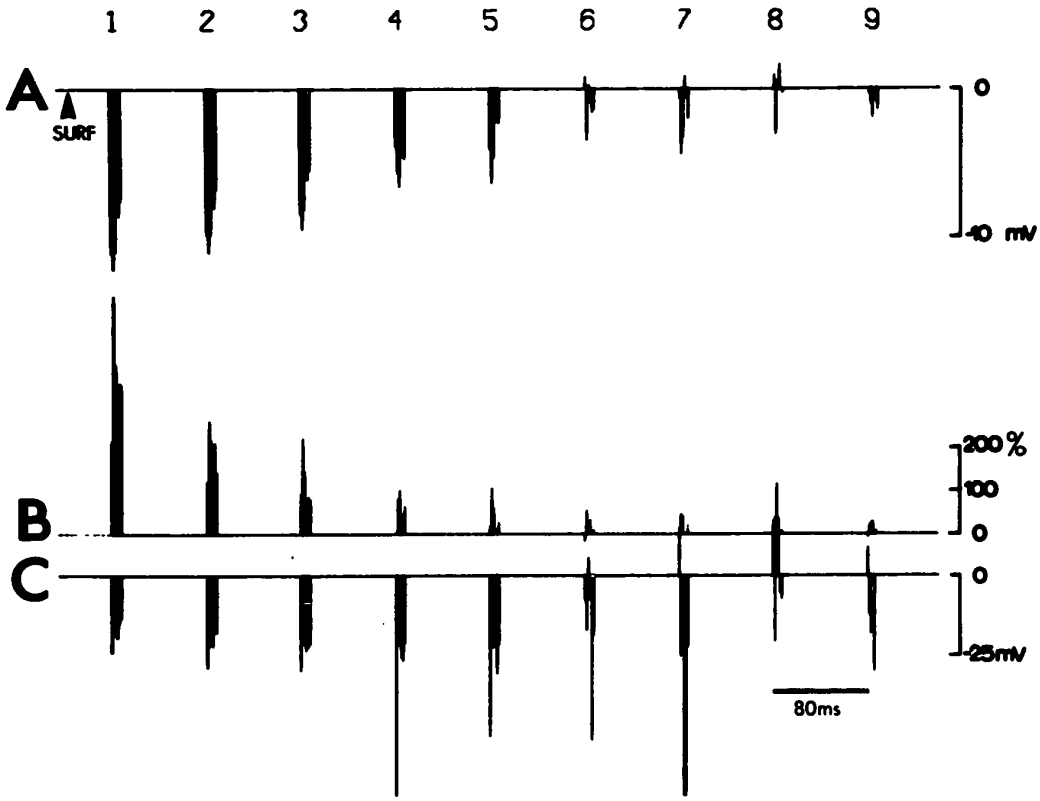
ments of the change in membrane resistance not only at the peak of the IPSPs, but also at various intervals along its time course. A technique for the measurement of inhibitory postsynaptic potential and conductance changes has been developed on a LINC-8 computer as described in METHODS, and some of the results presented here have been reported briefly (Renaud, Kelly and Provini 1969; Kelly 1970).

A. SURFACE EVOKED IPSPs

In accordance with previous studies (Creutzfeldt et al. 1966b,c; Krnjević, Randić and Straughan 1966b) single cathodal shocks (0.1 ms in duration) delivered once per second to the epicortical surface evoked hyperpolarizing inhibitory postsynaptic potentials with durations of 200 ms or longer and equilibrium or reversal potentials approximately 20mV more negative than the resting potential. During the peak of the IPSP there was a marked increase in membrane conductance of more than 200% over the resting membrane conductance (c.f. Pollen and Lux 1966; Krnjević and Schwartz 1967; Dreifuss, Kelly and Krnjević 1969).

The histograms in Figure 18 were prepared from data obtained during intracellular recordings from one pyramidal neurone located 1.2 mm below the precruciate cortex. Voltage-current plots were constructed at ten intervals during one-second oscilloscope sweeps. The surface stimulus was delivered between the first and second intervals (c.f. Figure 14B in METHODS). Relative to resting membrane values, the histograms reveal the changes in IPSP amplitude, conductance and reversal potentials

Figure 18. Potential, conductance and reversal potentials during surface evoked IPSPs. Histograms, derived from intracellular recordings of a PT neurone during 1/S surface stimulation (arrowhead), compare the changes in potential (A), conductance (B) and reversal potentials (C) at nine 80 ms intervals during the surface evoked IPSP. The first column of data was obtained 40 ms after the stimulus. The individual bars in each column illustrate the variability in the data collected when the cycle of eight sweeps was repeated six times. Potentials are expressed in mV, relative to the resting membrane potential, and conductances as a percentage increase over resting membrane conductance; both resting values were derived from the voltage-current lines obtained from the first of the ten pulse sequence applied before the stimulus. Note the stability of the reversal potentials (C), and the approximate linearity of the potential (A) and conductance change (B) for the second to the fifth columns.


1
2
3
4
5
6
7
8
9
A
SURF
0
-10 mV
200%
100
0
B
C
0
-25mV
80ms

at nine 80 ms intervals. The first column represents data obtained 40 ms after the onset of the surface evoked IPSP, and therefore show the maximum changes. Clearly detectable potential and conductance increases lasting up to and beyond 400 ms (Figure 18, fifth column) are characteristic for most surface evoked IPSPs following single monopolar shocks of 3-5V applied to the cortical surface. At stimulus repetition rates greater than 2/sec. these long duration changes tend to have a cumulative effect which at least partially explains the maintained inhibition observed in extracellular records by repetitive stimulation at frequencies of 5-7/sec. (c.f. Krnjević, Randić and Straughan 1966a).

During the later portion of the surface evoked IPSP (Figure 18, columns two to five) the changes in potential (A) and conductance (B) show a relationship which is for the most part linear, while the IPSP reversal potential during the same part of the IPSP is fixed at a level approximately 20 mV more negative than the resting potential. However the difference between the potential changes (A) in columns one and two is much less than would be predicted from a comparison of the conductance changes shown in (B) measured during the same period. The conductance change is perhaps therefore a much more reliable indicator of the magnitude of the transmitter-receptor interaction (c.f. Dreifuss et. al. 1969) since the potential change is always attenuated when the conductance change is large. This attenuation is in part a consequence of Ohm's law since the voltage

change evoked per unit of current flow across the membrane will drop as the conductance increases, and in part a result of the gradual approximation of the voltage levels of the IPSP reversal potential and membrane potential at the peak of the IPSP. A similar phenomenon at the myoneural junction was measured by Martin (1955), and resulted from non-linear summation of the unit potentials which make up the end plate potential when the amplitude of these potentials were large. On many occasions this attenuation of the voltage was accentuated by an increase in the membrane potential itself, perhaps as a result of a rise in the intracellular chloride concentration during intense inhibitory transmitter action.

B. PYRAMIDAL RECURRENT IPSPs

Inhibitory postsynaptic potentials evoked in pyramidal tract neurones by a single shock (0.1 ms duration) to the medullary pyramidal tract were illustrated earlier in Figure 14. In general, the duration of both the IPSP and the conductance change was shorter for pyramidal recurrent IPSPs (e.g. 100-150 ms) than for surface evoked IPSPs (e.g. greater than 200 ms). The histograms (D) in Figures 19 and 20 illustrate respectively the IPSP and conductance changes obtained during ten data cycles (c.f. Figure 15 in METHODS) derived from one precruciate pyramidal tract neurone, during pyramidal recurrent IPSPs. The mean and standard errors of these individual values are illustrated graphically in histograms (C) in Figures 19 and 20. Single oscilloscope sweeps of the activity of this pyramidal tract neurone, which

Figure 19. Changes in membrane potential during pyramidal evoked IPSPs with and without conditioning by surface evoked IPSPs. Histograms (A) and (D) were prepared from data collected during alternate cycles of the computer program, and illustrate the full range of variability of ten data cycles. Data were taken at 80 ms intervals beginning 40 ms after the pyramidal stimulus (arrowhead on the left), and each column contains the values expressed with reference to the resting membrane potential (downwards represents increasing negativity) at that point in time during one-second oscilloscope sweeps. A. Data collected during surface IPSP conditioning (arrowhead between the 5th and 6th columns) of pyramidal recurrent IPSPs. B. The mean potential changes of the data from ten cycles shown in (A) are plotted below the horizontal line, while the standard errors are plotted above the line, for each 80 ms interval. C, D. Similar to (A) and (B) with the exception that the data was obtained during unconditioned pyramidal IPSPs. Note the increased duration of the potential change for the surface conditioned pyramidal IPSP in histogram (B) compared with the unconditioned pyramidal IPSP in histogram (C). Same PT neurone as illustrated in Figure 14E,F.

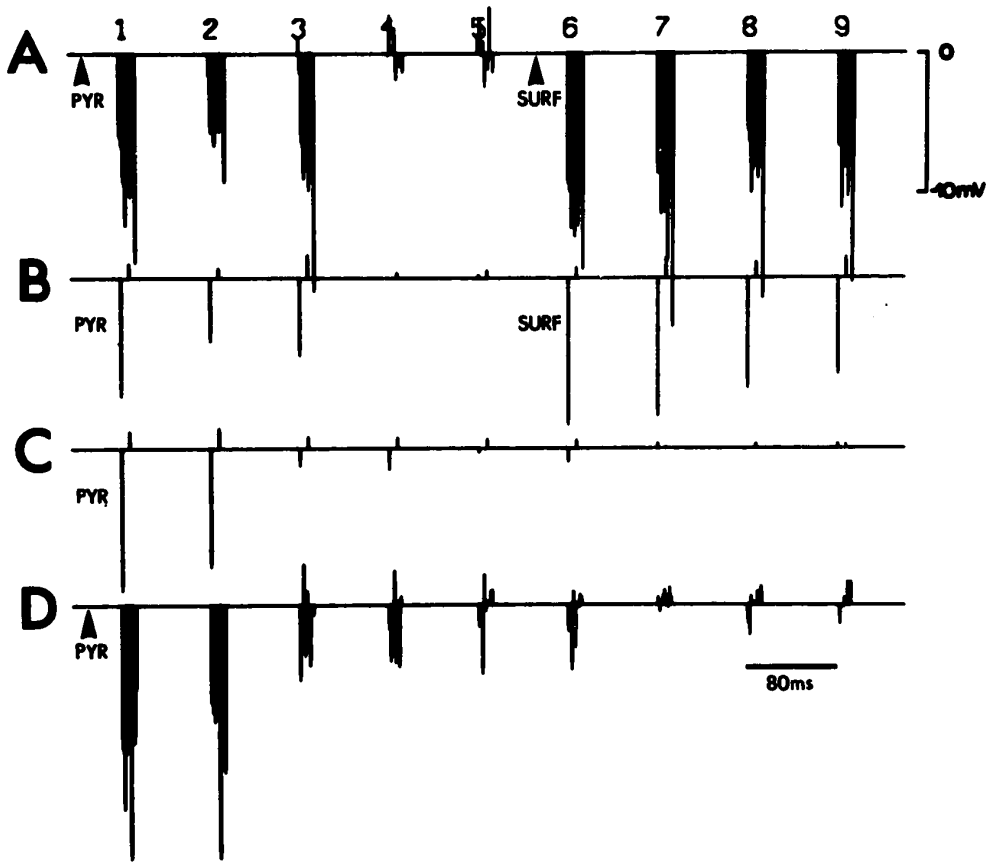

A
1
2
3
4
5
6
7
8
9
PYR
SURF
0
-10mV
B
PYR
SURF
C
PYR
D
PYR
80ms

Figure 20. Changes in membrane conductance during pyramidal evoked IPSPs with and without conditioning by surface evoked IPSPs. These histograms are complementary to those in the previous figure for potential changes. Histogram (A) illustrates the variability in the conductance increase over resting membrane conductance for ten data cycles, with the means and standard errors of this data appearing in histogram (B). Histograms (C) and (D) illustrate the mean and standard errors and the actual data respectively from ten cycles in which only the pyramidal tract was stimulated. Note the enhancement in both the magnitude and the duration of the pyramidal IPSP conductance change when conditioned by surface stimulation.

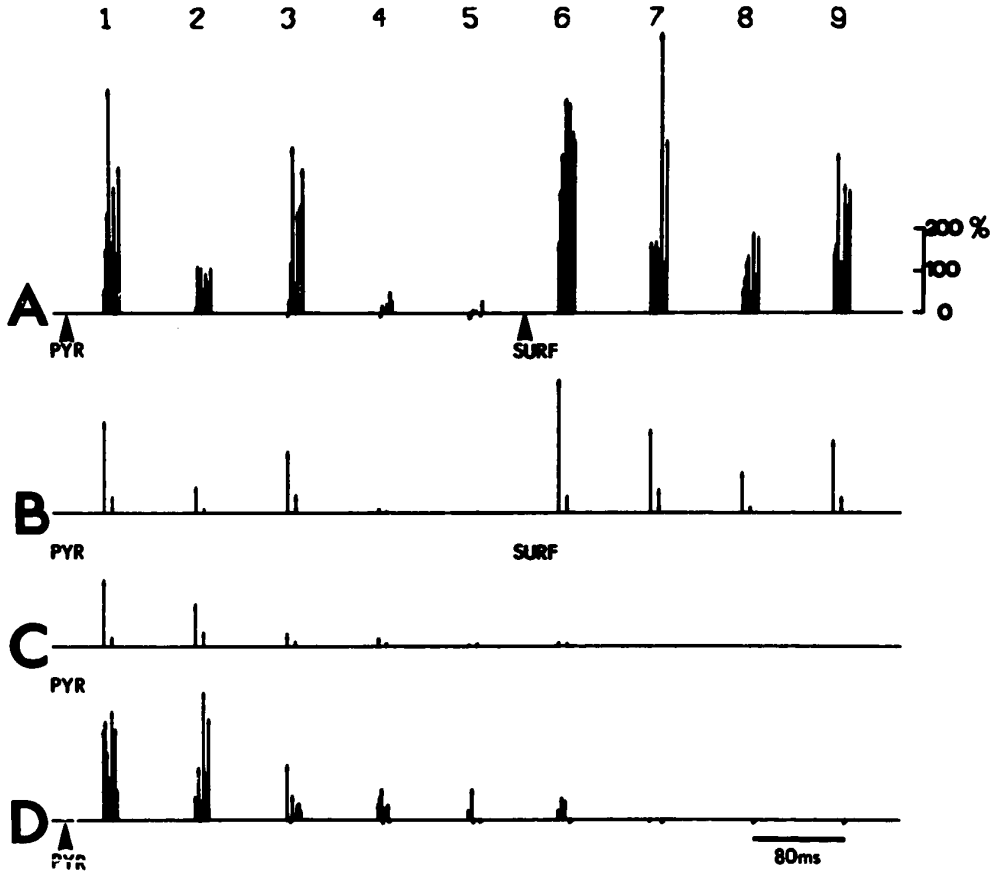

1
2
3
4
5
6
7
8
9
200 %
100
0
A
PYR
SURF
B
PYR
SURF
C
PYR
D
PYR
80ms

Figure 21. Potential, conductance and reversal potential changes in pyramidal evoked IPSPs conditioned by surface IPSPs. Composite illustration containing histograms obtained from ten alternate data cycles during surface conditioning of pyramidal IPSPs. Histograms (A) and (B) are the same as those seen in Figure 19A and 20A respectively representing potential and conductance changes with reference to resting membrane values. Histogram (C) contains the reversal potentials at nine 80 ms intervals, beginning 40 ms after the pyramidal stimulus and relative to the resting membrane potential. Note the consistent and similar values for reversal potentials obtained for the first and sixth columns, representing the stabilization of membrane potential and similarities in reversal potentials at the peak of pyramidal and surface evoked IPSPs.

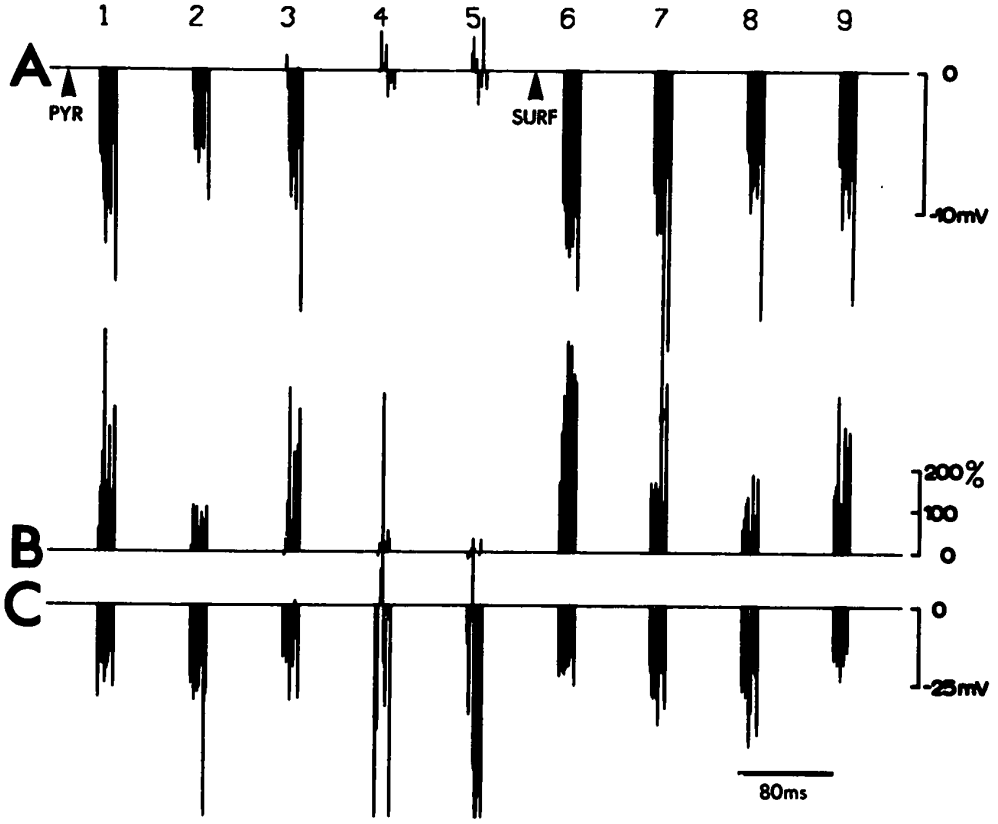

1
2
3
4
5
6
7
8
9
A
PYR
SURF
0
-10mV
200%
100
0
B
C
0
-25mV
80ms

Table 1

Experimental values computed from intracellular recording of the PT neurone illustrated in Figures 19, 20 and 21. The individual columns represent measurements (means ± SE) obtained at 40, 120, 200 and 280 ms after the stimulus applied once per second to the epicortical surface (SURF) and the pyramidal tract either alone (PYR) or conditioned 600 ms earlier by a surface shock (PYR + S). These values were corrected for any pre-existing bridge imbalance by subtracting the intracellular measurements from those obtained immediately outside the neurone (see Fig 16). Conductance values represent the increase in membrane conductance relative to the resting membrane conductance derived from the voltage-current plots obtained from the first pulse applied prior to the stimulus.

Time (msec) after IPSP onset		40	120	200	280
IPSP (mV)	SURF	-50.7 ±1.0	-49.8 ±1.7	-47.6 ±1.4	-46.4 ±1.8
	PYR	-45.2 ±1.4	-43.1 ±1.6	-35.1 ±1.0	–
	PYR(+S)	-48.2 ±1.3	-43.7 ±1.0	-44.8 ±2.0	–
COND increase %	SURF	234 ±14	191 ±25	147 ±21	181 ±27
	PYR	174 ±10	146 ±16	116 ±6	98 ±3
	PYR(+S)	201 ±19	132 ±13	170 ±30	106 ±8
REVERSAL POTENTIAL (mV)	SURF	-60.1 ±1.0	-66.8 ±2.1	-69.3 ±2.9	–
	PYR	-59.7 ±2.1	-67.1 ±5.4	-57.1 ±3.3	–
	PYR(+S)	-62.3 ±1.7	-69.4 ±8.1	-58.3 ±8.0	–

Figure 22. Changes in membrane potential during pyramidal evoked IPSPs with and without conditioning by surface evoked IPSPs; Results from ten PT cells. Format is similar to that in Figure 19, except that (A) and (B) were prepared from data collected during 28 data cycles, while (C) and (D) were derived from thirteen data cycles only. Note the prolongation in the duration and amplitude of pyramidal IPSPs in the presence of surface evoked IPSPs.

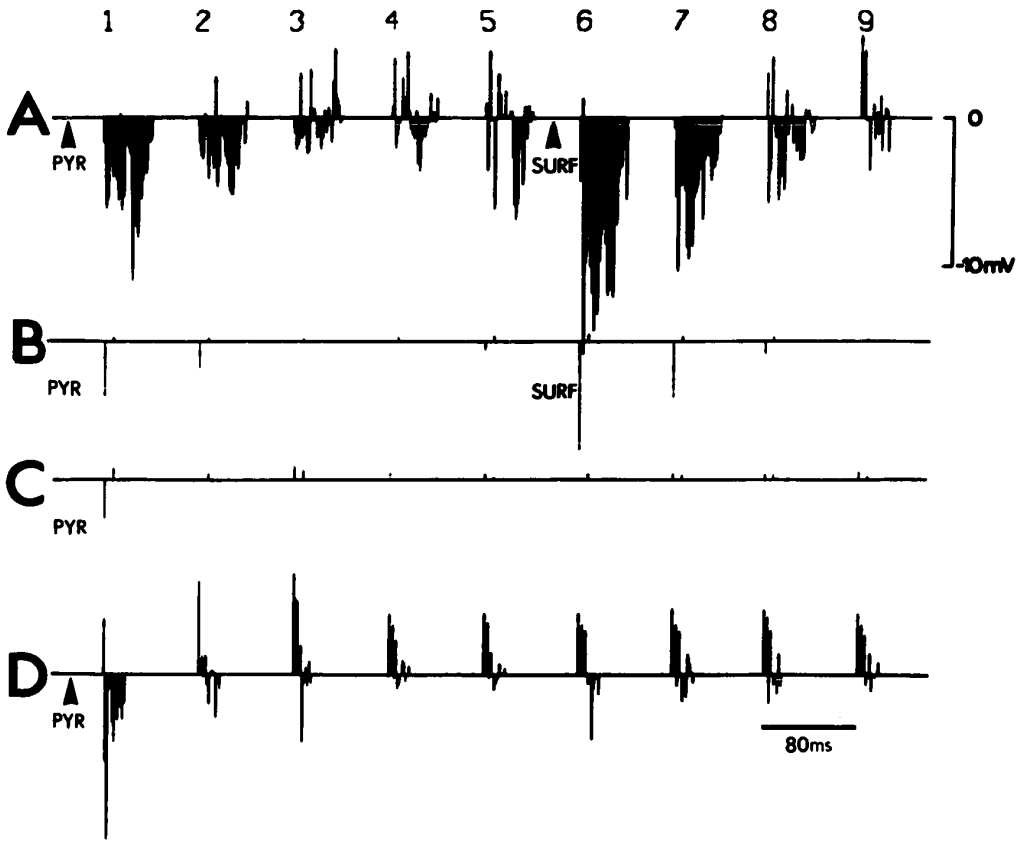

1
2
3
4
5
6
7
8
9
A
PYR
SURF
0
-10mV
B
PYR
SURF
C
PYR
D
PYR
80ms

Figure 23. Changes in membrane conductance during pyramidal evoked IPSPs with and without conditioning by surface evoked IPSPs; Results from ten PT cells. Format similar to that in Figure 20, except that (A) and (B) were prepared from data collected during 28 cycles, while (C) and (D) were derived from thirteen data cycles only. Note the prolongation in both the duration and magnitude of the membrane conductance change during surface conditioned pyramidal IPSPs.

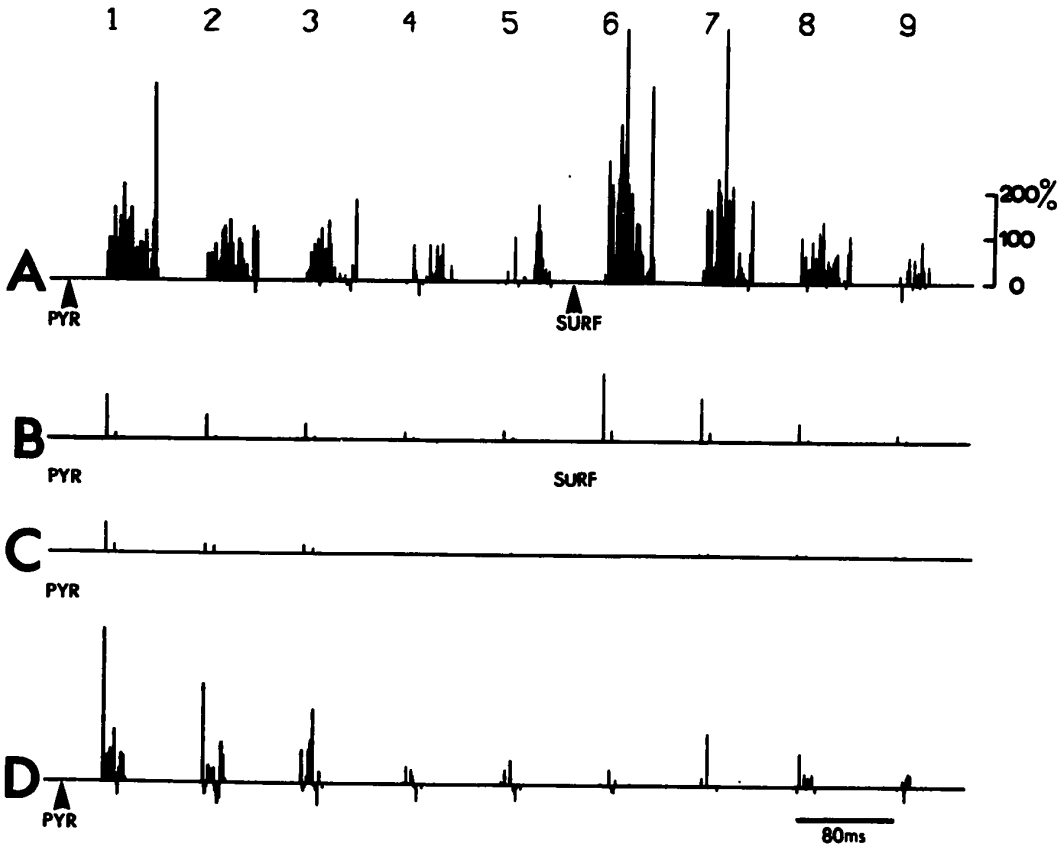

1
2
3
4
5
6
7
8
9
200%
100
0
A
PYR
SURF
B
PYR
SURF
C
PYR
D
PYR
80ms

Table 2

Means and standard errors of the IPSP and conductance changes computed for unconditioned and conditioned pyramidal recurrent IPSPs during individual intracellular recordings from ten other pyramidal tract neurones. In this table note that it is only the change in both the membrane potential and the membrane conductance which are listed. These data are illustrated graphically in Figures 22 and 23.

Time (msec) after IPSP onset		40	120	200
IPSP (mV) ($IPSP_{max}$ - RP)	PYR	-13.4 ±4.4	–	–
	PYR(+S)	-18.7 ±2.3	-10.0 ±1.7	-0.7 ±1.6
COND increase %	PYR	37 ±12	13 ±10	13 ±9
	PYR(+S)	50 ±8	29 ±4	18 ±5

had a resting membrane potential remaining greater than 40 mV for more than thirty minutes, are illustrated in Figure 14E,F. The numerical values (mean ± S.E.) of the membrane potentials during the IPSPs, and the corresponding conductance increases during these ten data cycles are contained in Table 1. These results indicate that not only the magnitude but also the duration of the changes observed during the peaks of pyramidal IPSPs were significantly less than those observed during surface evoked IPSPs. This feature was consistently present in useful recordings obtained for other neurones. For example, histograms (D) in Figures 22 and 23 illustrate the IPSP and conductance changes respectively for thirteen data cycles collected from ten pyramidal tract neurones during pyramidal evoked IPSPs. The means and standard errors of these values, illustrated in histograms (C) in Figures 22 and 23, are presented numerically in Table 2. Of note is the short duration and small magnitude of the change during pyramidal inhibition, which contrasts with the changes described above for certain individual surface evoked IPSP values.

C. FACILITATION OF PYRAMIDAL IPSPs BY SURFACE EVOKED IPSPs.

During intracellular recordings it was common to observe that single shocks delivered to the cortical surface at frequencies of 3-5/sec. had a stabilizing influence on the spontaneously occurring fluctuations in membrane potential. Under these circumstances the mean resting membrane potential was usually closer to the reversal potential than was the mean resting membrane potential obtained in the absence of stimulation. This

cumulative effect indicates a persistance of the inhibitory process, possibly the result of either prolonged release or prolonged action of inhibitory transmitter.

Surface evoked IPSPs also had a facilitatory influence on pyramidal recurrent IPSPs. Figures 19 and 20 graphically illustrate this effect in the results from an experiment on a precruciate pyramidal neurone. As described under METHODS, pyramidal IPSPs were conditioned 600 ms earlier by a surface evoked IPSP. The surface stimulation was disengaged for each alternate data cycle (i.e. eight consecutive oscilloscope sweeps). The histograms (A, B) in Figures 19 and 20, obtained from ten odd-numbered cycles, illustrate a prolongation in the duration of the conditioned pyramidal IPSP potential and conductance changes respectively compared with those obtained for the unconditioned pyramidal IPSPs shown in histograms (C, D) in Figures 19 and 20. Table 1, containing the mean values and standard errors for these ten measurements, detail the increase in both the detectable duration and magnitude of the potential and conductance changes during conditioned pyramidal IPSPs (PYR+S).

Similar changes were detected in recordings obtained from ten additional pyramidal neurones. The first five columns of histograms (A, B) in Figures 22 and 23 represent twenty-eight measurements obtained during surface conditioned pyramidal IPSPs. These reveal a significant prolongation and increase in magnitude of the potential and conductance change compared with thirteen measurements shown in the histograms (B, C) for the unconditioned

pyramidal IPSP. Table 2 contains the mean values for these graphs. These results compliment those shown earlier in Table 1 taken from a single pyramidal neurone.

It should be pointed out that the experiments were arranged so as to minimize the possibility that surface evoked IPSPs might influence pyramidal IPSPs through a cumulative effect, as described earlier. This was accomplished by utilizing a conditioning testing interval of 600 ms, at which time most of the control data from surface evoked IPSPs alone no longer revealed significant changes in potential or conductance (see Figure 18 and Table 1). There remained a possibility that residual changes in the cell membrane (i.e. hyperpolarization) resulting from surface inhibition were still present at the onset of each sweep (i.e. beyond 600 ms) and would therefore interfere with the voltage-current plots derived for the first of the ten pulses (and used to derive the control or resting membrane values). If so, then the slope of the voltage-current plots for the resting membrane would be less than expected. Consequently, all subsequent calculations of potential and conductance changes during the conditioned pyramidal IPSP would be less than the actual changes, which would mean that the changes detected under the present experimental conditions are even more significant.

D. IPSP EQUILIBRIUM OR REVERSAL POTENTIALS

Hyperpolarization observed in neocortical neurones following direct (epicortical) or indirect (pyramidal, thalamic

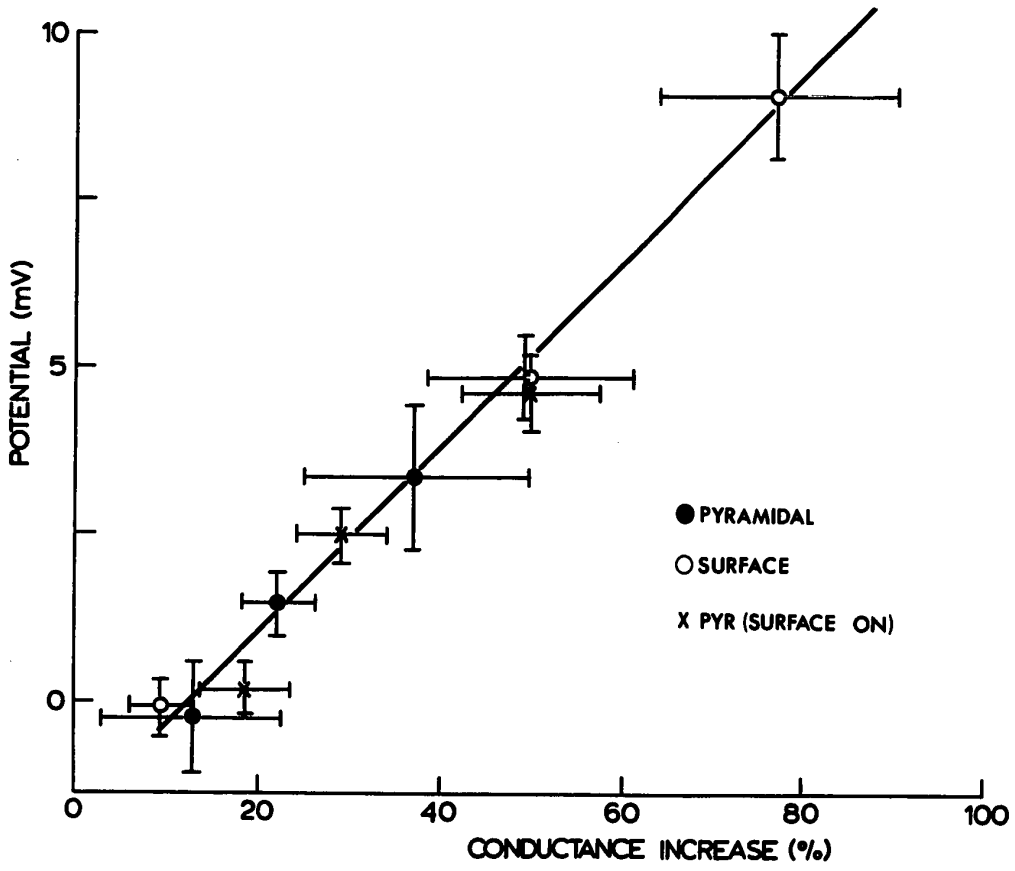
or transcallosal) activation are generated by ionic mechanisms similar to those responsible for IPSPs in other cells (Eccles 1961, 1964). Thus, at levels of membrane polarization greater than the equilibrium potential for the ionic movement generating the IPSP, the synaptically evoked hyperpolarizations will be reversed (Eccles 1957, 1961, 1964; Grundfest 1959; Ginsborg 1967). In the case of neocortical neurones attempts with ion or current injection techniques in order to define membrane potential levels at which synaptically induced hyperpolarizations were reversed have met with limited success (e.g. Purpura and Schofer 1964; Stefanis and Jasper 1964a). Alternatively, IPSP reversal levels can also be estimated from the intersects of voltage-current lines derived for the resting state and during the peaks of the IPSPs (c.f. Krnjević and Schwartz 1967; Dreifuss et al. 1969), and with the aid of a LINC-8 computer this technique has been used in the present analysis (see METHODS).

In neocortex, the IPSPs elicited indirectly by electrical stimulation of afferent (for example thalamocortical) or recurrent (for example pyramidal tract) pathways and by direct epicortical stimulation are comparable in shape as well as in duration (Phillips 1959; Li and Chou 1962; Stefanis and Jasper 1964; Creutzfeldt et al. 1966a). However, the shape of the IPSPs alone is not a sufficient criterion upon which to decide that the same synaptic endings are activated through various inhibitory stimuli. A more reliable indicator of

similar synaptic loci is the IPSP reversal potential (Rall 1967; Calvin 1969). For example, previous studies on motoneurones have detected similarities in reversal potentials of group Ia and recurrent IPSPs (Coombs et al. 1955) although on closer examination (Burke et al. 1971) it appears that the two pathways probably have a different spatial synaptic termination.

Based on the hypothesis that there is a common inhibitory mechanism, and therefore common inhibitory interneurones mediating cortical inhibition, the IPSPs generated by various types of stimuli ought to have similar reversal potentials. Only surface and pyramidal recurrent IPSPs were examined during recording of eleven pyramidal neurones. The mean values and standard errors derived from ten measurements on one pyramidal neurone are shown in Table 1 but are representative of the data obtained from the remaining ten neurones. Intersects of the voltage-current lines derived from the membrane at rest and during the peak of the IPSP revealed that there was no significant difference between the reversal potential for the surface IPSP as compared with the conditioned or unconditioned pyramidal IPSP. The individual reversal potential values (relative to the resting membrane potential) for the conditioned pyramidal and surface IPSPs are graphically illustrated in Figure 21C (compare the first and sixth columns obtained during the peaks of the IPSPs). Therefore, the data are in support of a similar spatial arrangement for the endings of inhibitory synapses producing both surface evoked and recurrent IPSPs (c.f.

Figure 24. Relationship between the change in conductance and the change in membrane potential during cortical IPSPs. Plots (means ± S.E.) of the conductance increase over resting membrane conductance versus the change in potential measured at three different time intervals during the course of surface evoked IPSPs (O), unconditioned pyramidal (●) and conditioned pyramidal IPSPs (X), indicating the approximate linearity in the relationship from data obtained with intracellular recordings of 10 pyramidal tract neurones.


10
5
0
POTENTIAL (mV)
0
20
40
60
80
100
CONDUCTANCE INCREASE (%)
PYRAMIDAL
SURFACE
X PYR (SURFACE ON)

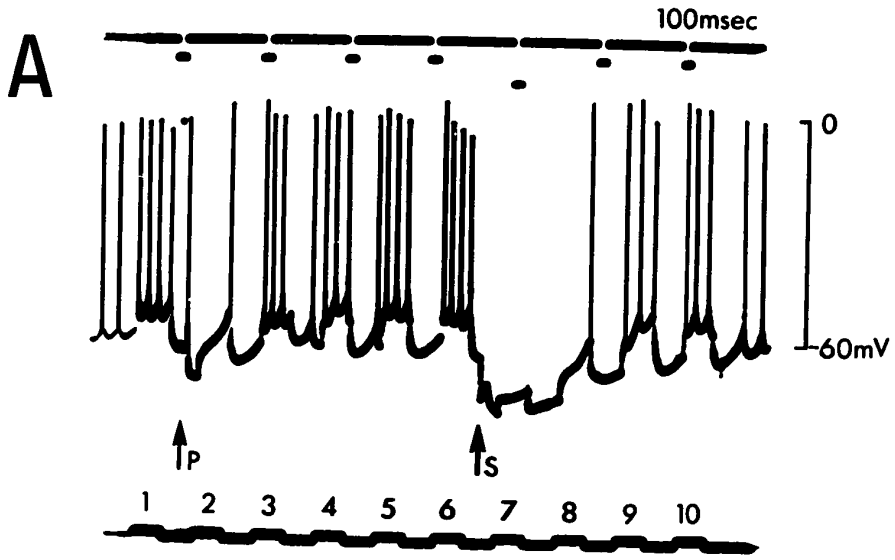
Rall 1964, 1967).

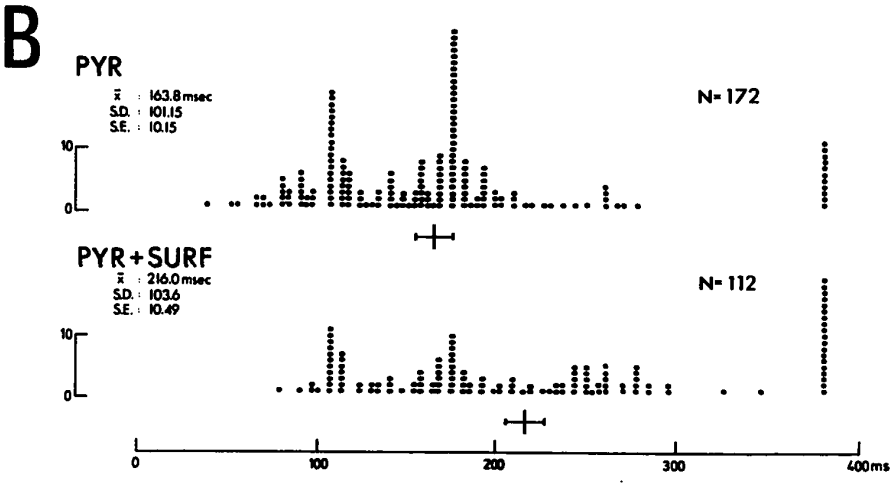
E. RELATIONSHIP BETWEEN THE POTENTIAL AND CONDUCTANCE CHANGES

Although the magnitude and duration of the conductance change during unconditioned and conditioned pyramidal IPSPs were sometimes quite marked (Table 1) most of the ten remaining pyramidal neurones tested did not reveal such a marked change. This is apparent from the lower mean values illustrated in Table 2. Nevertheless the pooled data still reveal a significant conductance increase for more than 120 ms following the pyramidal stimulus. On the other hand, changes in membrane potential during pyramidal IPSPs, generally less pronounced than those illustrated in Table 1, indicate that by 120 ms after the onset of an unconditioned pyramidal IPSP the mean potential values were not significantly different from the resting membrane potential and therefore not entered in Table 2. This is in accord with the earlier findings of Dreifuss et al. (1969) that the potential changes may not necessarily reflect the magnitude of the change in membrane conductance observed during intracellular recording of cortical IPSPs.

In Figure 24, the conductance increases measured at 40, 120 and 200 ms following unconditioned (●) and conditioned (X) pyramidal IPSPs and surface evoked IPSPs (O) are plotted against the corresponding potential changes measured simultaneously for seven pyramidal neurones. Points furthest from the origin were obtained during the peaks of the IPSPs and therefore show the greatest changes. The graph reveals an approximate linearity in

Figure 25. Changes in neuronal excitability during inhibition. A. The middle trace is an intracellular record of a PT neurone illustrating two IPSPs evoked by pyramidal tract (P) and epicortical surface (S) stimulation. Ten pulses of positive current (lowest trace) were applied to the recording microelectrode through a bridge network. Under resting membrane conditions each pulse usually evoked 3-4 spike discharges (e.g. first, fifth and sixth pulses). During the IPSPs there was a reduced neuronal excitability most pronounced with surface evoked IPSPs, with a time course approximating that of the potential and conductance changes described in the text. Spikes retouched where necessary. B. Histograms indicating the time from onset of the stimulus (time 0) to the occurrence of the first spike following the IPSP measured from photographic film taken during intracellular recording of the PT neurone. Each dot represents data from a photograph of single oscilloscope sweeps. In the upper histograms 172 measurements during the unconditioned pyramidal IPSP yielded a mean stimulus-to-first-spike interval of 163.8 ms. In eleven other measurements (column of dots on the right) the interval exceeded 380 ms. The enhanced duration of surface conditioned pyramidal IPSPs is reflected below by a longer mean stimulus-to-first-spike interval of 216 ms in 112 measurements with a larger number (19) exceeding 380 ms.


A
100msec
0
-60mV
P
S
1 2 3 4 5 6 7 8 9 10


B
PYR
x̄ : 163.8 msec
S.D. : 101.15
S.E. : 10.15
N= 172
10
0
PYR+SURF
x̄ : 216.0 msec
S.D. : 103.6
S.E. : 10.49
N= 112
10
0
0
100
200
300
400ms

the potential-conductance relationship, at least for smaller changes in membrane potential. Together with the similarity of the reversal potentials described earlier the data support the postulate that there is a similar spatial arrangement presumably on the cell soma for the origin of the inhibitory synaptic currents generated by transmitter release following pyramidal tract and epicortical volleys.

F. CHANGES IN EXCITABILITY OF PYRAMIDAL NEURONES DURING IPSPs

The long duration of the IPSP conductance change was also reflected as a decrease in the excitability of cortical neurones. For example, during the IPSPs evoked in the pyramidal neurones illustrated in Figures 14 and 15A, there was a silencing of any spontaneous spike discharges. Twenty millisecond intracellular pulses of positive current applied during the resting period generated regular bursts of 3-4 spikes at 150-200 cycles/second (Figure 25A; first, fifth and sixth pulses). Each burst was followed by a negative deflection, most likely an after-hyperpolarization rather than an autogenous recurrent IPSP since similar potentials in other neurones could not be reversed by intracellular injections of small anions (Dreifuss et al. 1969). These evoked discharges were fewer in number during pyramidal evoked IPSPs and totally abolished during surface evoked IPSPs, with a time course approximating the observed membrane conductance increases.

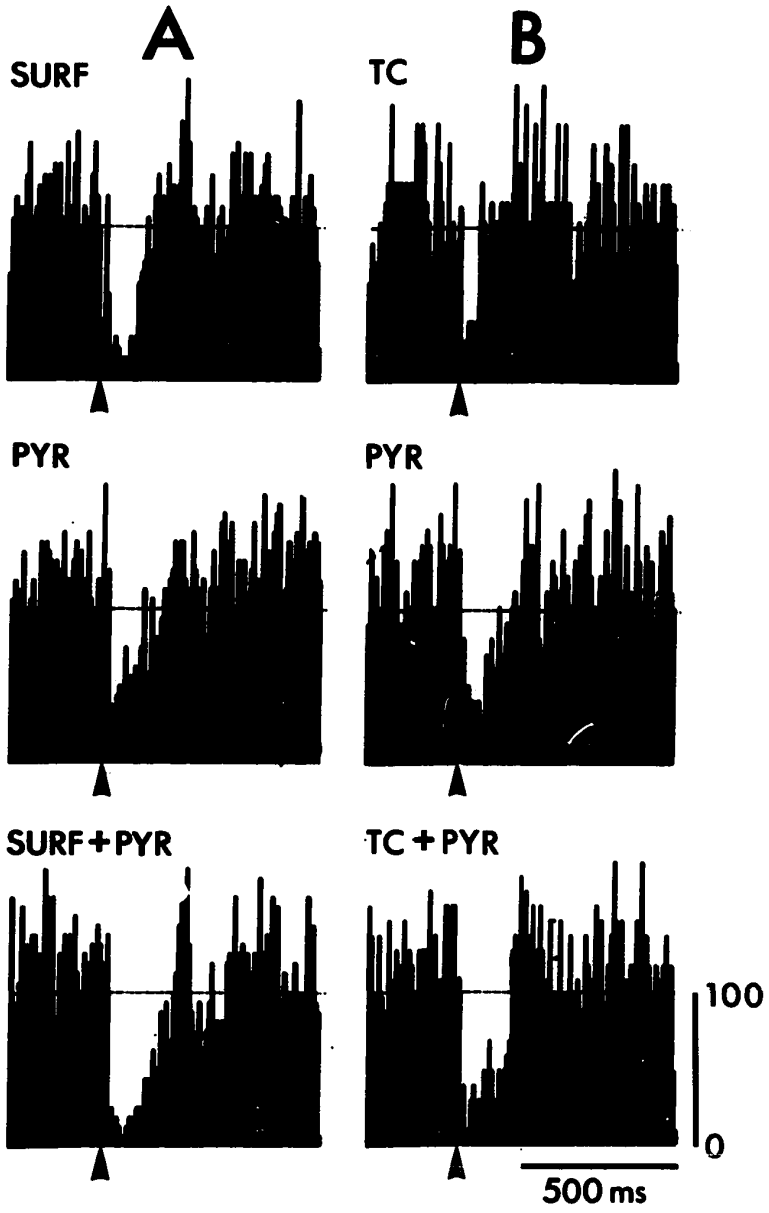
The enhanced increase in conductance during a surface conditioned pyramidal IPSP (Table 1 and 2) was also reflected

as an increase in the duration of reduced neuronal excitability. For example, from control data on one cell (Figure 25B) the mean time from the stimulus artifact due to the pyramidal tract shock to the appearance of the first spike after the onset of the recurrent IPSP was 163 ms (S.E. 10.15, n 172). This time interval increased to a mean value of 216 ms (S.E. 10.49, n 112) for measurements obtained for pyramidal IPSPs conditioned by surface stimulation.

These preliminary observations suggested that further tests of excitability measured quantitatively from post-stimulus latency histograms with extracellular recordings might be useful to extend the intracellular studies on the enhancement of recurrent pyramidal inhibition by various inhibitory stimuli. Similar studies were conducted on nine pyramidal neurones, tested against a steady background of glutamate evoked or spontaneous neuronal discharges. In four cells, conditioning with surface stimulation, similar to the approach used during intracellular recordings, accentuated a reduction in the probability of spike discharge following control sub-maximal pyramidal tract volleys. These data were therefore in support of temporal facilitation of inhibition. Results from five other pyramidal neurones were not conclusive, apparently because of a marked postinhibition excitation following epicortical stimulation (see Chapter 4).

Further studies were conducted in which the two inhibitory stimuli were presented simultaneously. The intensity was adjusted so that control stimuli evoked less than 100%

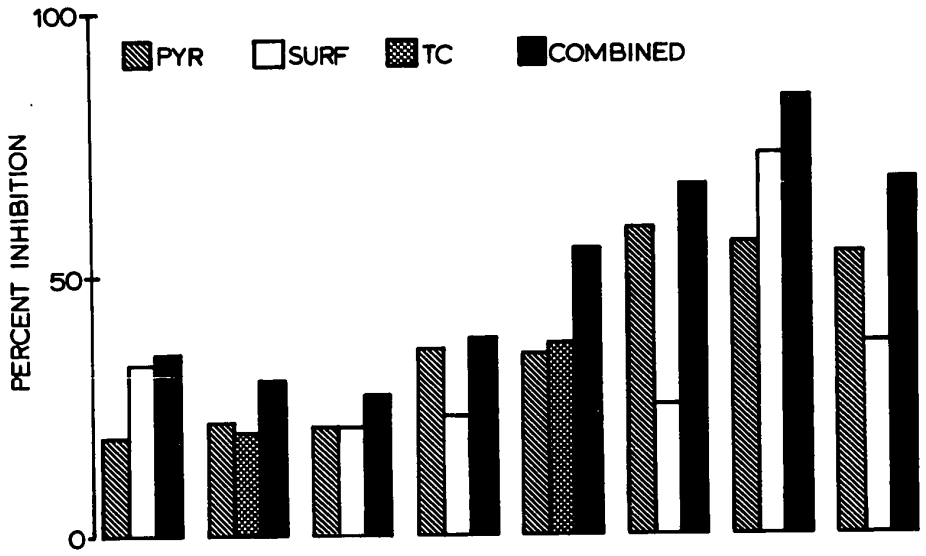
Figure 26. Post-stimulus latency histograms illustrating interaction on inhibitory pathways to PT neurones. Data obtained from extracellular recordings of two different PT neurones. The two upper histograms in columns A and B represent the post-stimulus decrease in the probability of spike discharge following individual stimuli sub-maximal for complete spike suppression presented to the epicortical surface (SURF), pyramidal tract (PYR) or transcallosal (TC) pathways. In the lowest histogram the two individual stimuli, shown above in each column and presented 300 ms after the onset of each of the 128 sweeps, were applied simultaneously. This produced a greater decrease in post-stimulus discharge probability. A separate LINC-8 program was used to quantitatively assess the reduction in spike discharge probability in each example and express the results as "percentage inhibition" relative to the discharge frequency of the neurone in the 250 ms immediately preceeding the stimulus (see METHODS). The values were used to construct the next figure.


A
B
SURF
TC
PYR
PYR
SURF + PYR
TC + PYR
100
0
500 ms

(preferably 50% or less) reduction in the probability of spike discharge in the immediate post-stimulus period. Examples obtained from two pyramidal neurones are illustrated in Figure 26. In A, both surface and pyramidal stimuli evoked a partial reduction in the probability of spike discharge from a neurone whose activity was maintained at a constant rate by iontophoretic application of glutamate. In a similar example, from another pyramidal neurone (Figure 26B) a transcallosal shock was tested with a pyramidal stimulus. The lowest histograms in each column reveal that the outcome of presenting the two stimuli together was further reduction in the probability of neuronal discharge.

In order to derive quantitative estimates from these and similar studies, the number of spike discharges occurring within the 250 ms period following the stimulus was subtracted from the number obtained in the 250 ms period immediately preceeding the stimulus, and the value expressed as a percentage inhibition of the prestimulus "spontaneous" spike discharge frequency (see METHODS). The results of eight experiments conducted on five pyramidal neurones testing pyramidal stimulation against surface and transcallosal stimuli are illustrated graphically in Figure 27. Each test confirmed that although there was a greater reduction in the probability of spike discharge (percentage inhibition) when the two stimuli were presented simultaneously, the amount of the increase was usually not much greater than the largest reduction obtained with an individual stimulus. This was observed even when the individual stimuli

Figure 27. Occlusion on the inhibitory pathways. Bar histograms representing the computed results of eight experiments of the type illustrated in the previous figure and performed on five PT neurones. Stimuli, sub-maximal for total spike suppression, were presented individually and together. In each situation the reduction in spike discharge probability for the 250 ms following the stimulus was expressed as a percentage of the probability of spike discharge under non-stimulated conditions and expressed as percentage inhibition. In each sample, with various combinations of stimuli, simultaneous presentations of these stimuli resulted in only a small increase in the percentage of inhibition.


100
50
0
PERCENT INHIBITION
PYR
SURF
TC
COMBINED

were adjusted so as to result in a less than 25% reduction in excitability (Figure 27, columns 2,3).

If inhibition of PT neurones following the different types of stimuli were mediated over separate pathways, one might have expected to see a greater summation of inhibitory effects particularly with lower intensities of stimulation. This is evidence which suggests occlusion within the inhibitory pathways. Although this study was not pursued systematically, employing different strengths of stimuli in each experiment and allowing for differences in conduction over longer (for example trans-callosal) pathways, the data support the previous intracellular observations in suggesting that there is some overlap of inhibitory pathways to individual neurones, possibly implying a common inhibitory interneuronal network.

In summary, a LINC-8 computer was utilized to obtain intracellular measurements of both membrane potential and resistance changes at various intervals during pyramidal and surface evoked IPSPs, in order to estimate more accurately the magnitude of the conductance increase evoked in pyramidal tract neurones during cortical inhibition. Temporal facilitation of pyramidal evoked IPSPs was observed to follow a conditioning surface evoked IPSP, applied at conditioning testing intervals beyond which any changes in membrane potential or conductance could be detected in control tests of surface evoked IPSPs. Despite the longer duration of surface evoked IPSPs, the reversal potentials did not differ significantly from pyramidal recurrent IPSPs. Combined with an approximate linear relationship between conductance and potential

changes, particularly during short range changes in potential, these data support the hypothesis that IPSPs evoked directly or indirectly in pyramidal cells are generated through similar inhibitory mechanisms and that at least some of the cortical inhibitory interneurones involved in the generation of both recurrent pyramidal and surface IPSPs are activated by both stimuli. Some of the extracellular findings, which also relate to other inhibitory pathways, are in agreement with this postulate.

Chapter 4

RESULTS

RESPONSES OF CEREBRAL CORTICAL NEURONES TO STIMULATION OF INHIBITORY PATHWAYS

Inhibitory postsynaptic potentials and periods of decreased neuronal excitability lasting more than 150 ms have been observed in cortical pyramidal and some non-pyramidal neurones following stimulation of several inhibitory pathways (see INTRODUCTION). Here, as in other areas of the central nervous system, considerable effort has been devoted to the identification of neurones which function as mediators of these inhibitory potentials.

In the spinal cord there is electrophysiological and neuropharmacological evidence that ventral horn interneurones, originally described by Renshaw (1941, 1946a) and bearing his name, discharge repetitively in response to acetylcholine released from motoneurone axon collaterals. These Renshaw cells monosynaptically generate the recurrent IPSPs observed during intracellular recording from motoneurones under the same experimental conditions (Eccles, Fatt and Koketsu 1954). Repetitive spike discharges from interneurones have been noted in other CNS areas in response to stimuli which evoke IPSPs and decrease

the excitability of neighbouring neurones and this type of activity has been considered as one prototype for the identification of inhibitory interneurones in general (see Eccles' 1969 review). However, similar repetitive discharges have seldom been observed during recording from cerebral cortical neurones (Stefanis 1966). The usual duration of any interneuronal excitatory response in the cortex has been in the order of 20 ms, which is a very short time by comparison with the duration of cortical IPSPs (Krnjević, Randić and Straughan 1966b).

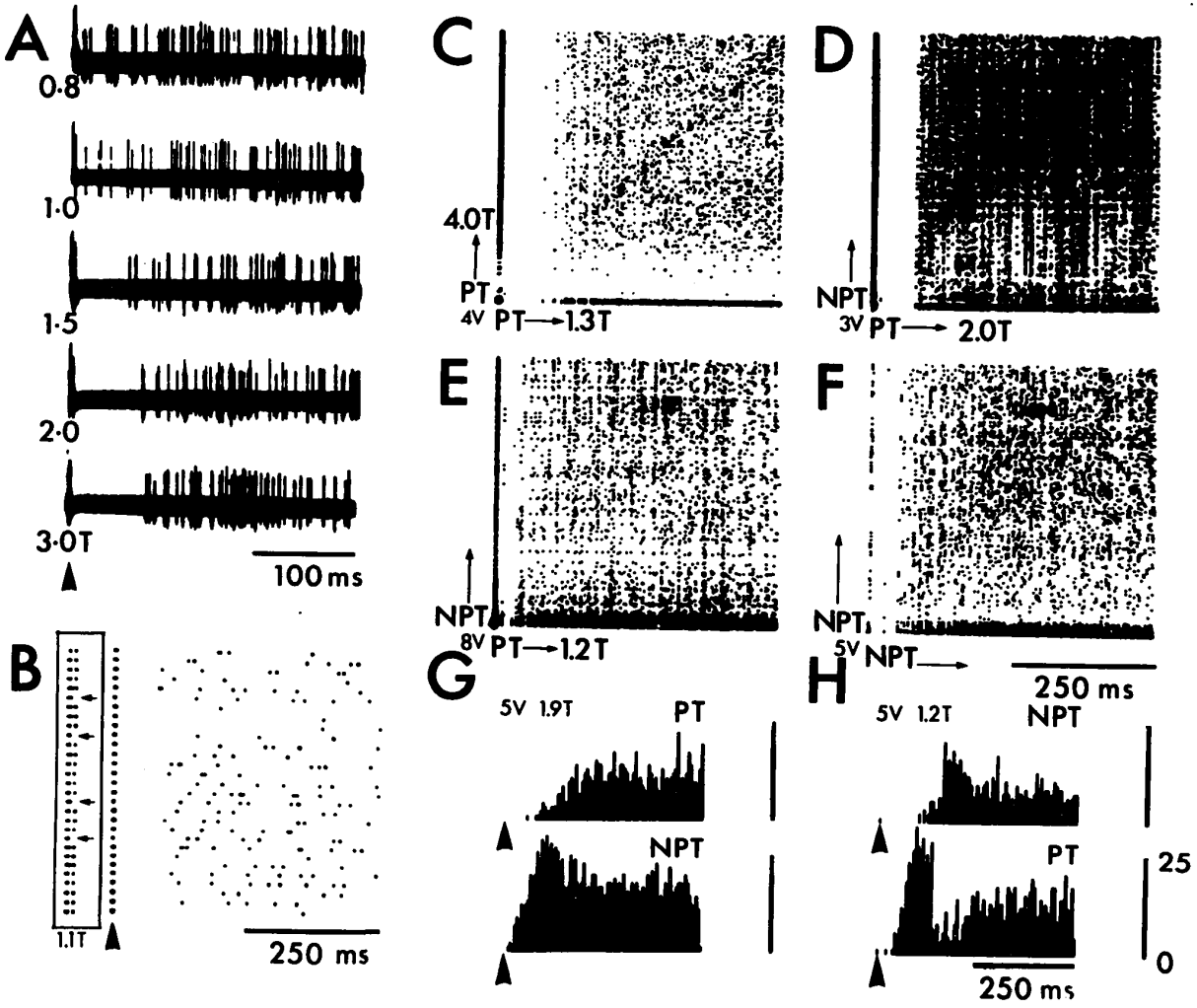
Apart from the problem of identification of cortical inhibitory neurones, there have been few experimental studies in which a comparison has been made between the responses of identified pyramidal and non-pyramidal tract neurones following stimulation of direct (epicortical) or indirect (recurrent, transcallosal, thalamocortical) inhibitory pathways, information which is necessary for a clearer understanding of cortical inhibitory mechanisms. As part of an investigation of the connections between two cortical neurones in search of short latency negative cross-correlation patterns (see Chapter 6), the responses of 110 pairs of cells consisting of two simultaneously recorded and identified PT or NPT neurones were studied. Whenever possible the cells were tested with as many forms of inhibitory stimuli as were available. This provided information concerning both the response characteristics of cortical neurones to individual types of stimuli, and also permitted comparison of the relative potencies of the various types of stimuli in evoking inhibitory and/or excitatory responses seen in individual neurones.

A. PYRAMIDAL TRACT STIMULATION

Both the excitatory and inhibitory synaptic events observed among cortical pyramidal neurones following stimulation of the pyramidal tract are presumably the consequence of activation of local interneurones over recurrent pyramidal axon collateral pathways (Chang 1955; Phillips 1956, 1959a,b; Li 1958; Suzuki and Tukahara 1963; Stefanis and Jasper 1964a,b; Armstrong 1965; Brooks and Asanuma 1965; Kubota et al. 1965; Brooks et al. 1968). Short latency excitatory responses have been observed in PT cells following pyramidal tract stimuli (Phillips 1959; Stefanis and Jasper 1964a,b; Kubota and Takahashi 1965). Takahashi and colleagues (1965) presented evidence that recurrent collaterals of slowly conducting PT neurones were themselves monosynaptically excitatory onto other PT neurones with faster axonal conducting velocities. The latencies of PT cell IPSPs generated by similar stimuli are most probably mediated through di- or polysynaptic pathways involving inhibitory interneurones (Stefanis and Jasper 1964a,b). Non-pyramidal neurones are also subject to recurrent inhibition (Krnjević, Randić and Straughan 1966a,b). This suggests that recurrent inhibition may function as a rate limiting control mechanism (Granit 1963) not only for corticospinal neurones and perhaps cortical projection neurones in general but may also be an active mechanism in the process of integration.

PT neurones. Eighty-three pericruciate neurones were identified as PT neurones according to the criteria outlined in

Figure 28. Responses of PT and NPT neurones to pyramidal tract stimulation. A. Six superimposed oscilloscope tracings indicating from above downwards progressive decrease in excitability of a PT neurone following pyramidal tract stimulation (arrow). Numbers indicate stimulus strengths relative to threshold (T) for antidromic soma invasion. Note the insignificant change in the duration of the silent period between 2.0 T and 3.0 T. B. Raster diagram indicating a similar duration in the post-stimulus silent period to the right of the arrow head, despite absence of the antidromic spike during the four sweeps indicated by the horizontal arrows in the encased section, which illustrates the peristimulus details seen at the arrowhead but on an expanded time sweep. C. Joint scatter diagrams showing the response of two PT neurones to a pyramidal stimulus of 4V (1.3 T for PT cell a and 4.0 T for PT cell b; see METHODS for explanation). Note the increased density of points along the X and Y axes representing stimulus artifact and antidromic spikes, and the absence of points parallel to each major axis representing recurrent inhibition. D. E. F. Scatter diagrams from other pairs of PT and NPT cells indicating their response to pyramidal stimuli at stimulus strengths indicated (threshold values for PT cells are included). Note the different response patterns for individual NPT cells: weak inhibition (D), excitation (E) and an excitation-inhibition sequence (F). G.H. Post-stimulus latency histograms illustrating similarities and differences in excitability for PT and NPT cells in response to pyramidal stimuli (arrows). Vertical bar represents number of spike occurrences for each of the 128 bins composing the histograms.


A
0·8
1·0
1·5
2·0
3·0T
100 ms
B
1.1T
250 ms
C
4.0T
PT
4V PT→1.3T
D
NPT
3V PT→ 2.0T
E
NPT
8V PT→1.2T
F
NPT
5V NPT→
250 ms
G
5V 1.9T
PT
NPT
H
5V 1.2T
NPT
PT
25
0
250 ms

METHODS. A pyramidal tract shock of less than 2.0 V was adequate to produce antidromic invasion in more than 80% of cells (range 0.2 to 10.0V). Graded stimuli resulted in a characteristic response pattern of which one example is illustrated in Figure 28A. At threshold (50% probability of activation) a weak but detectable decrease in spike discharge probability was observed to follow the antidromic spike (or shock artifact) in many PT neurones (c.f. Dreifuss and Kelly 1972). With suprathreshold intensities, this decrease in excitability which followed the antidromic spike was manifested as a clear silent period the duration which reached a maximum of 80-120 ms with stimulus strengths of 3 to 5 times threshold. The intracellular experiments described earlier (Chapter 3) revealed a similar time course for the IPSP and particularly for the membrane conductance changes observed in PT neurones following the pyramidal stimulus.

Following this inhibitory pause, a gradual smooth return of neuronal firing to prestimulus levels was characteristic of the activity of the majority of PT cells (c.f. upper histogram in Figure 28G). On three occasions however the inhibitory response was terminated rather abruptly by a period of enhanced excitability. In one example (the lower histogram in Figure 28H) the peak resulting from this enhanced excitability was followed by a second period of reduced excitability shorter in duration and less marked than the first and having a smoother return to control excitability levels.

Previous investigators (Phillips 1961; Stefanis and Jasper 1964a,b) have noted that multiple stimuli subthreshold for antidromic invasion also were effective in reducing PT neuronal excitability. The raster diagram in Figure 28B contains 4 sweeps (small arrows) in which a single shock stimulus of strength 1.2 times threshold for the PT cell under study failed to produce antidromic invasion (see the expanded sweeps in the left-hand enclosure). These may possibly be due to collision of antidromic with orthodromic spikes or to inadequate stimulus strength. In each instance there was still a silent period following the stimulus artifact (right side at slower time base). Similar observations from neocortical (Stefanis and Jasper 1964a) and hippocampal neurones (Dichter and Spencer 1969) indicate that the inhibitory pause is independent of antidromic soma invasion and is, therefore, an orthodromic synaptic process presumably generated through recurrent collaterals of other PT neurones whose axons were excited by the medullary pyramidal stimulus.

Although there are several reports in the literature describing pyramidal recurrent facilitation (Phillips 1959; Stefanis and Jasper 1964; Armstrong 1965; Kubota and Takahashi 1965) no clear evidence could be discerned for its presence here, and may have been overlooked due to the manner in which neurones were tested (see DISCUSSION). Only occasionally, when high intensity stimuli (10V or more) were necessary to produce antidromic invasion were short latency (2-5 ms) spike discharges

observed; these displayed features characteristic of orthodromic responses (variable latencies, inability to follow stimuli at 200 Hz). Since current spread to adjacent lemniscal structures could not be ruled out under such circumstances, these findings were excluded from the present observations.

On the other hand, evidence for recurrent inhibition was present for all PT neurones encountered. In the case of higher threshold neurones, there was usually a clear depression of spike discharge activity even at stimulus strengths subthreshold for antidromic invasion and was probably related to activation of other pyramidal axons with lower thresholds for axonal activation. These differences in threshold were probably a reflection of axonal size and proximity of the axon to the stimulating electrodes.

In nine instances the microelectrodes were positioned to monitor the activity of two PT cells simultaneously. Despite obvious differences in the antidromic invasion thresholds of any two cells, there was usually a remarkable similarity in the duration of the inhibitory pause following the antidromic spike (c.f. joint peristimulus scatter diagram in Figure 28C). Although not systematically investigated, the magnitude of any recurrent inhibitory event was apparently unrelated to the axon conduction velocity.

NPT neurones. A total of 88 NPT neurones, situated between 0.10 and 1.90 mm below the pial surface, were examined for changes in excitability following a single pyramidal tract stimulus with intensities of 1-10V.

The activity of 20% of NPT neurones (18 cells), distributed at depths ranging from 0.17 to 1.36 mm (mean 0.72 mm), was either unmodified to any significant extent or there was only a weak decrease in excitability noted at stimulation strengths sufficient to profoundly alter the activity of nearby PT cells. One example is illustrated in the joint scatter diagram in Figure 28D.

In 40% of NPT neurones (35 cells) the stimulus was found to produce a short latency orthodromic excitation. These neurones were also located 0.30 to 1.72 mm (mean 0.72 mm) below the pial surface. Usually the excitation appeared as a single spike with variable latency (Figure 34C, first column). Less frequently cells responded with a burst of spike activity (Figure 34D, first column) although seldom (3 cells) did the duration of any high frequency discharges last more than 50 ms and was always shorter than the duration of inhibitory events observed from other nearby PT or NPT neurones (Figure 28E,F). Increasing intensities of stimulation had two effects on the excitability of this neuronal population: first the initial excitation became more closely synchronized with the stimulus, and secondly a period of decreased neuronal excitability was observed to follow the initial excitation (Figure 28F, vertical arrow).

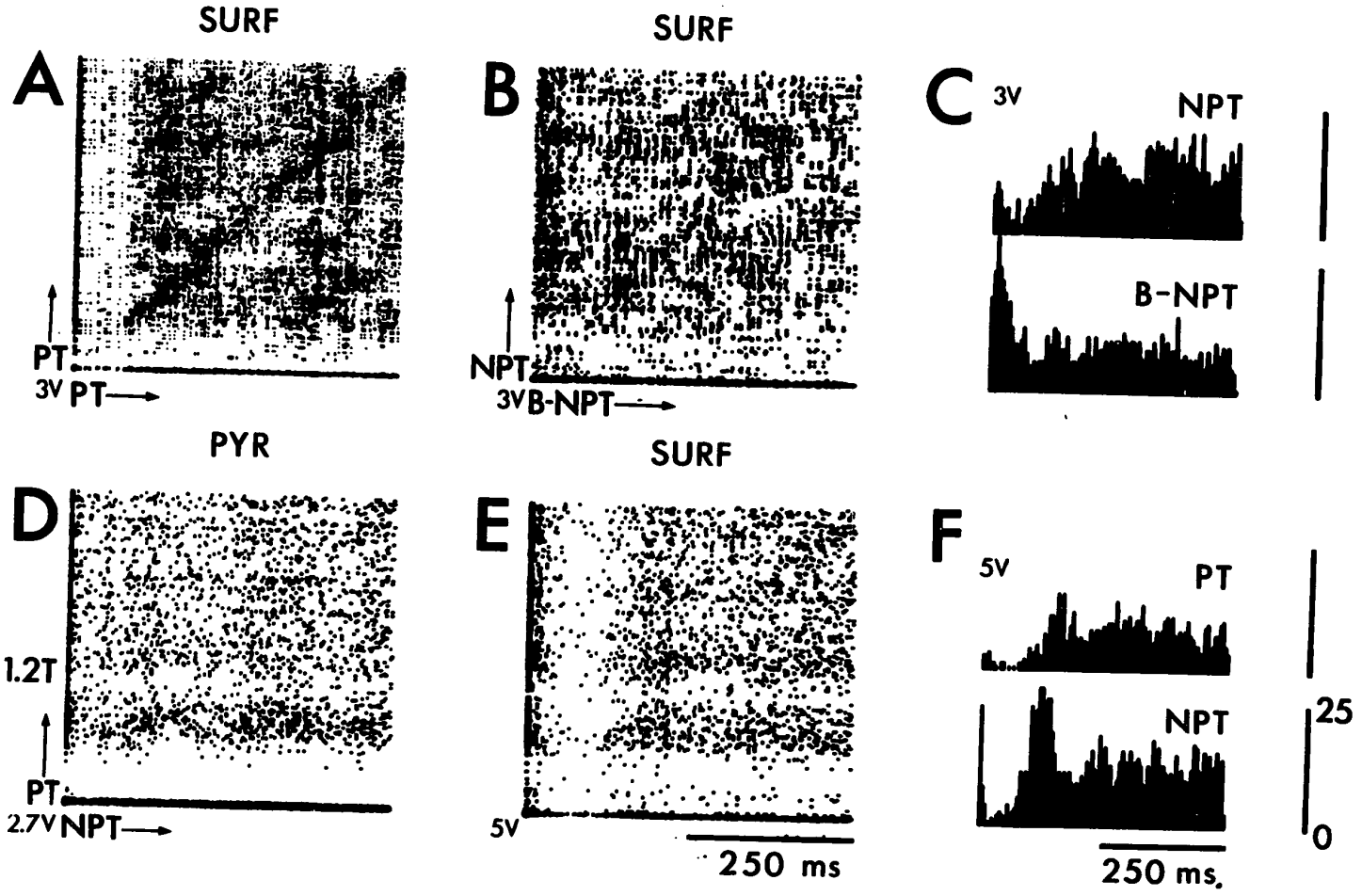
For the remaining 40% of NPT neurones (35 cells), for which there was no clear evidence of initial excitation, the pyramidal stimulus was followed by a period of decreased cell excitability having a duration comparable to that observed for

most PT cells (Figure 28F, horizontal arrow; c.f. the NPT post-stimulus latency histograms in Figure 28G,H; Figure 34A, first column). In comparison with the gradual return of excitability to prestimulus levels observed with most PT neurones, NPT neurones usually manifested a more or less temporary enhancement of neuronal excitability after the initial pause (NPT post-stimulus latency histograms in Figure 28G, H).

For the 80% of NPT neurones which exhibited a pause in their spike discharge pattern (with or without preceding excitation), in the majority (greater than 65%) of examples pyramidal stimulation was less effective in inducing a decrease in their neuronal excitability as compared with its effects on PT neurones. This statement was based on assessment of both the duration and the intensity of the pyramidal stimulus induced changes obtained from simultaneous recordings of the activity of both types of neurones(Figure 28D; Figure 35A,D,G).

In summary, pyramidal tract stimuli suprathreshold for antidromic soma invasion evoked a decrease in neuronal excitability, independent of antidromic invasion, in all PT neurones tested. A decreased neuronal excitability, with or without preceding excitation was observed in the majority (80%) of NPT cells. Comparable stimuli produced no change in the activity of 20% of NPT cells. Both the magnitude and the duration of NPT cell response was usually less intense than that observed for PT neurones.

Figure 29. Response patterns of pairs of neurones to epicortical stimulation. A. Scatter diagrams of two PT cells illustrating similar inhibitory responses to surface stimulation of 3V applied every 500 ms at the onset of each sweep. B. Reciprocal pattern of response of two NPT cells (one with bursting spontaneous discharges, labeled B-NPT), one excited, the other inhibited by a surface stimulus. C. Post-stimulus latency histograms of the data in (B). D. A pyramidal stimulus of 2.7V evokes only weak excitation of a NPT cell despite clear inhibition of a neighbouring PT cell. E. Surface stimulation, adjusted to produce approximately the equivalent amount of PT cell inhibition as the pyramidal stimulus in D evokes a clear excitation-inhibition response from the NPT cell. F. Post-stimulus latency histograms of two other simultaneously recorded neurones: (above) characteristic inhibitory response of a PT cell with a gradual return of excitability and minimal post-inhibitory excitation; (below) a NPT neurone with an initial excitation-inhibition response demonstrating a marked secondary peak or postinhibition excitation following the same stimulus (a 5V shock to the cortical surface once every 500 ms).


SURF
A
PT
3V PT
SURF
B
NPT
3V B-NPT
C
3V
NPT
B-NPT
PYR
D
1.2T
PT
2.7V NPT
SURF
E
5V
250 ms
F
5V
PT
NPT
25
0
250 ms.

B. STIMULATION OF THE CORTICAL SURFACE

An IPSP associated with a marked increase in ionic conductance of up to 300% over resting membrane conductance and lasting up to 500 ms following the stimulus (Dreifuss, Kelly and Krnjević 1969) underlies the powerful inhibition seen in most cortical neurones following a single epicortical shock (Creutzfeldt, Baumgartner and Schoen 1956; Phillips 1956b; Li and Chou 1962; Krnjević, Randić and Straughan 1966a,b).

PT neurones. A single monopolar cathodal shock of 3-5V (0.1 ms duration) delivered to the cortical surface evoked a transient decrease in excitability of all 20 PT neurones tested with durations of the pause or inhibitory "silent period" reaching a maximum of 200 ms. Excitation of the PT cell under observation was not a prerequisite for surface evoked inhibition, since only four PT cells exhibited a short latency (less than 4 ms) orthodromic spike preceding the onset of the inhibition (Figure 34C, arrow in third column). With the majority of PT cells the profound initial decrease in excitability was followed by a gradual return to prestimulus firing levels. On a few occasions (Figure 29F) post-stimulus latency histograms detected a weak enhancement of excitability following the inhibitory events, but seldom as marked as that observed for the majority of NPT neurones to be described below.

NPT neurones. Thirty-one NPT neurones, situated 0.10 to 1.20 mm (mean 0.77 mm) below the pial surface were tested with a single epicortical stimulus. In 19 neurones a pause in the

spontaneous or glutamate evoked spike discharge lasting more than 200 ms was observed to follow the stimulus. Ten additional neurones exhibited a short latency excitation preceding the pause, usually consisting of a single spike discharge (Figure 34C, third column). Approximately one-half of the NPT cells tested demonstrated an enhanced excitability following the inhibitory pause (Figure 29F, lower post-stimulus latency histogram). Only two NPT cells responded with an initial increase in excitability lasting up to 60 ms. In the example illustrated in Figure 29B and C, the same surface stimulus induced a reciprocal response in the activity of the two neurones: a decreased excitability of one neurone and an enhanced excitability of the other. The two NPT neurones exhibiting an enhanced excitability of this type were also noted to have spontaneous discharges consisting of bursts of several spikes at higher frequencies (see Chapter 7) and have therefore been labeled as B-NPT neurones.

Surface Evoked Inhibition versus Recurrent Inhibition. Whenever possible the same neurones were tested with more than one type of stimulus. In order to compare the ability of different stimuli to evoke PT and NPT cell inhibition, the intensities of both surface and pyramidal stimuli were adjusted so as to induce silent intervals of similar magnitude and duration in a PT neurone recorded with one microelectrode. Without changing parameters in stimulation, the inhibitory responses of several NPT or PT neurones less than 400 microns away and recorded from the other electrode were compared with the initial PT cell response.

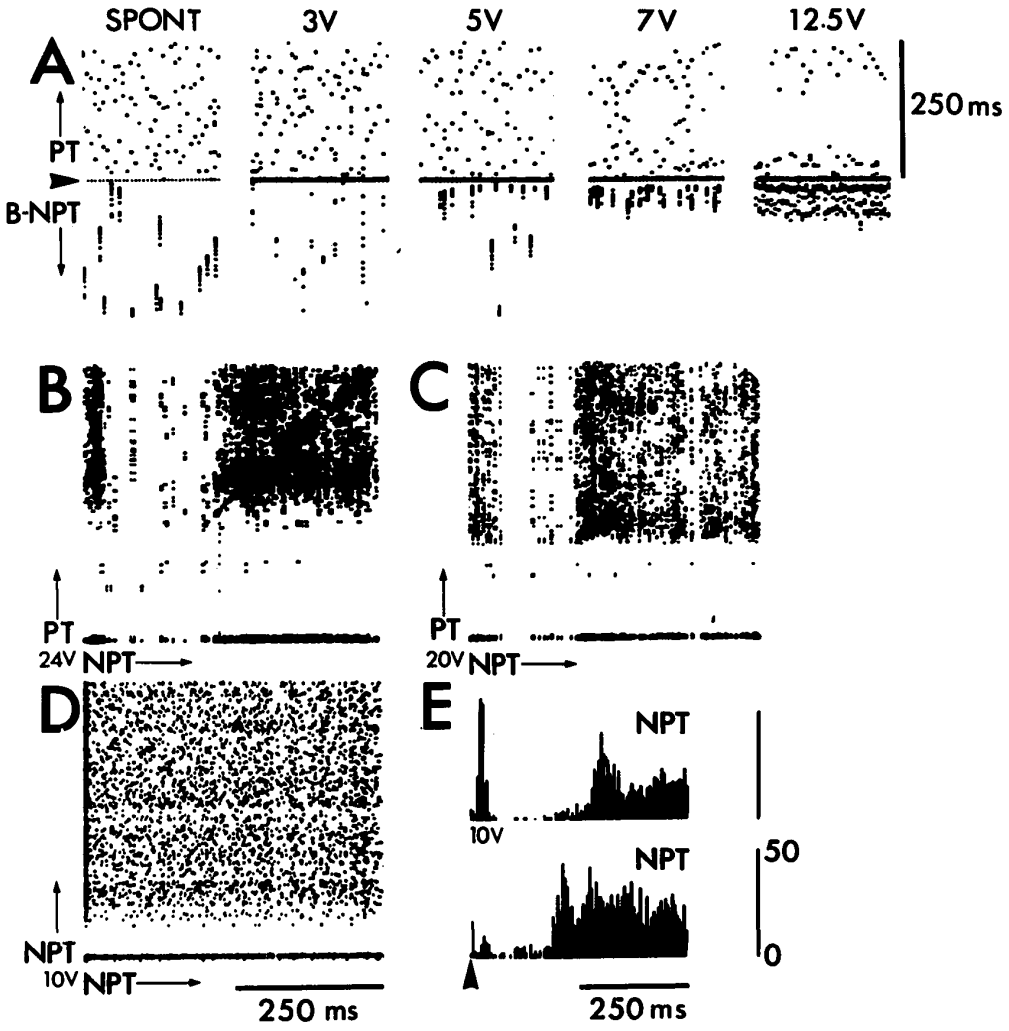
In the majority of instances where pairs of PT neurones were examined, both cells displayed similar responses to pyramidal and surface stimuli (Figures 28C and 29A). This pattern was in contrast to that observed when combinations of PT and NPT cells were recorded. NPT cell inhibition from the pyramidal shock was usually either weak or nonexistent despite clear PT cell inhibition, while surface stimulation was almost equally effective in inducing a decrease in the excitability of both neuronal types. Examples are illustrated in Figure 29 (compare D and E) and in Figure 35 (compare A with C, D with F, G with I).

Therefore, in terms of the NPT cell population these observations support the earlier findings of Krnjević et al. (1966b) on the greater potency of surface stimulation to evoke inhibition compared with that observed to follow stimulation of the recurrent pyramidal pathway. The results also point out the remarkable constancy with which recurrent pyramidal inhibition engages control of the pyramidal cell population compared with the cortical neuronal population in general.

C. ACTIVATION OF TRANSCALLOSAL FIBRES

Stimulation of homologous areas of the contralateral cerebral cortex has been shown to introduce both excitatory and inhibitory responses from ipsilateral cortical neurones (Creutzfeldt, Baumgartner and Schoen 1956; Asanuma and Okamoto 1959; Latimer and Kennedy 1961; Asanuma and Okuda 1962; Ajmone-Marsan and Morillo 1963). The results described in the previous sections following pyramidal

Figure 30. Responses of pairs of precruciate neurones to transcallosal stimulation. A. Raster diagram sequence illustrating the effect of increasing intensities of transcallosal (TC) shocks on the activity of a PT (upper) and B-NPT neurone (lower). The arrowhead refers to the onset of each sweep and stimulus artifact; the arrows point in the direction of each sweep. Note that excitation of the B-NPT cell appears at a lower stimulus strength than does the silent interval in the PT neurone discharge pattern (e.g. 5V vs 7V). B. Joint peristimulus scatter diagram (JSD) pattern characteristic of the response of the majority of PT and NPT cells, i.e. a narrow and a wide increased density of points along the X and Y axes respectively, in each case followed by a point deficit. C. JSD of another cell pair illustrating the usual PT response, and the presence of NPT cell long latency inhibition without preceeding excitation. D. Short latency onset of inhibition of one NPT cell at a strength of stimulation which was sub-threshold for influencing the activity of a neighbouring NPT neurone. E. Post-stimulus latency histograms illustrating two types of NPT responses seen in the JSDs in (B) and (D). Note the peaks of enhanced excitability following the long inhibitory pauses in spike discharges.


SPONT
3V
5V
7V
12.5V
A
PT
B-NPT
250 ms
B
PT
24V
NPT
C
PT
20V
NPT
D
NPT
10V
NPT
250 ms
E
NPT
10V
NPT
50
0
250 ms

tract activation and surface stimulation have pointed to differences between responses of pericruciate PT neurones and those of the majority of NPT neurones. Experimental evidence on the inhibitory effects of transcallosal activation is scanty and most of the recent information has been derived from PT cells (Asanuma and Okamoto 1962; Naito et al. 1970). The results to be presented here were also obtained from pairs of PT and NPT neurones recorded simultaneously thereby providing information concerning in particular the inhibitory responses of the two cell types.

PT neurones. Single stimuli with intensities ranging from 10-24V (0.1 to 0.2 ms duration) were delivered to the surface of the contralateral anterior sigmoid gyrus at approximately the same position on the gyrus as the ipsilateral recording microelectrodes. The initial response of twelve of fifteen PT neurones tested (80%) usually but not necessarily consisted of a single (more rarely double) orthodromic spike discharge at a variable latency of 6-12 ms. In all twelve neurones, whether or not an initial spike was present, a silent period followed, lasting up to 200 ms. Examples are illustrated in Figures 33B and 34C (arrow in centre column). The silent period was consistently observed from both spontaneous and glutamate-evoked spike discharges, therefore, indicating that the pause is most likely the result of postsynaptic inhibition (c.f. Galindo, Krnjević and Schwartz 1967; Kelly and Renaud 1971). The threshold was slightly lower for excitation than for inhibition.

Since the initial excitation for PT cells tended to be

brief and consisting usually of a single spike discharge, on scatter diagrams PT cell activity appeared as a narrow band containing a high density of points along one of the major axes. Parallel to this line was a zone containing a decreased number of points, representing the inhibitory effects (Figure 30B,C).

Three other PT cells were also seen to be inhibited by a transcallosal shock but these neurones did not demonstrate any clear initial excitation (Figure 35E), and the latency to peak of the inhibitory response was longer and the duration somewhat shorter than for other PT neurones studied. These neurones were located more medial than the usual site for microelectrode penetrations and may have been in the forelimb region of the cortex, which apparently does not receive a direct transcallosal projection (Curtis 1940; Krieg 1963; Ebner and Myers 1965; Jones and Powell 1968).

NPT neurones. The responses of 37 NPT neurones, distributed 0.10 to 1.90 mm below the pial surface, were studied following a transcallosal stimulus.

Twenty-three NPT cells (62.2%) responded with excitation followed by inhibition. In the majority of these neurones (19 cells) the initial excitatory response consisted of a burst of two or more spikes, examples of which are shown in the centre column in Figure 34. The latency of the initial spike was variable, but seldom greater than 10 ms. Some burst responses lasted for more than 40 ms, depending on the intensity of the stimulus applied. Cells of this type produced a wide band containing a high density

of points along the major axis of the joint scatter diagram, and contrasted with the narrow band resulting from initial excitation of PT cells (Figure 30B). Among NPT cells, those showing the most pronounced initial excitation were cells which tended to discharge spontaneously with bursts of high frequency spikes (see Chapter 7) and called B-NPT neurones. One example of the response of this type of NPT neurone to increasing stimulus intensities is shown in the lower half of the raster diagram in Figure 30A. As with PT cells, threshold was lower for excitation than for inhibition.

Four NPT neurones (10.8%) revealed patterns which were identical to those of most PT neurones, except that these cells were clearly not invaded antidromically either from the pyramids or the opposite cerebral cortex. The response of one such cell is illustrated in the scatter diagram in Figure 30D and the lower post-stimulus latency histogram in Figure 30E. This latter figure also illustrates the differences in the histogram patterns corresponding to the two types of NPT neurones described above. The spontaneous discharge patterns of these four NPT cells were not unlike those for PT neurones generally in that they were relatively free from high frequency bursts of activity.

The remaining 10 NPT neurones (27.0%) also demonstrated inhibition lasting up to 200 ms following a transcallosal shock, but without any apparent excitation, the pause appearing after a variable latency of up to 50 ms (Figure 30C). At lower intensities of stimulation these cells were usually uninfluenced by

transcallosal stimuli despite the fact that other neighbouring NPT cells presented clear evidence of inhibition (Figure 30D).

Similarities and differences between PT and NPT neurone responses to transcallosal stimuli. In general the characteristics of the responses i.e. a sequence of excitation-inhibition or only inhibition, were similar for both PT and NPT neurones. According to the extracellular spike discharges, the initial excitation of PT neurones appeared to be sharper and usually consisted of a single cell spike as compared to a longer lasting excitation often with the occurence of bursts of spikes for the majority of NPT neurones (compare Figure 33B with Figure 34, centre column).

Comparison of the responses of the two cell types recorded simultaneously revealed that in almost one-half of the examples a well developed NPT cell excitation or excitation-inhibition pattern was present at stimulus strengths below that necessary to produce PT cell excitation and/or inhibition. For example, the raster diagram sequence in Figure 30A illustrates a clear excitation of the NPT cell after a 7V transcallosal shock whereas a clear PT cell excitatory response only appears after a 12.5V transcallosal stimulus. Other examples are found in Figure 35B and E. It is tempting to speculate that some of these NPT neurones may be inhibitory interneurones which mediate the inhibitory responses observed after a transcallosal volley.

Following inhibition, post-stimulus time latency histograms from PT neurones generally showed a gradual return to

prestimulus excitability levels. Almost all NPT cells on the other hand revealed a postinhibitory increase in excitability (Figure 30E). In two of these (B-NPT cells) the pattern was quite remarkable resembling that observed in response to thalamic stimulation (see the following section).

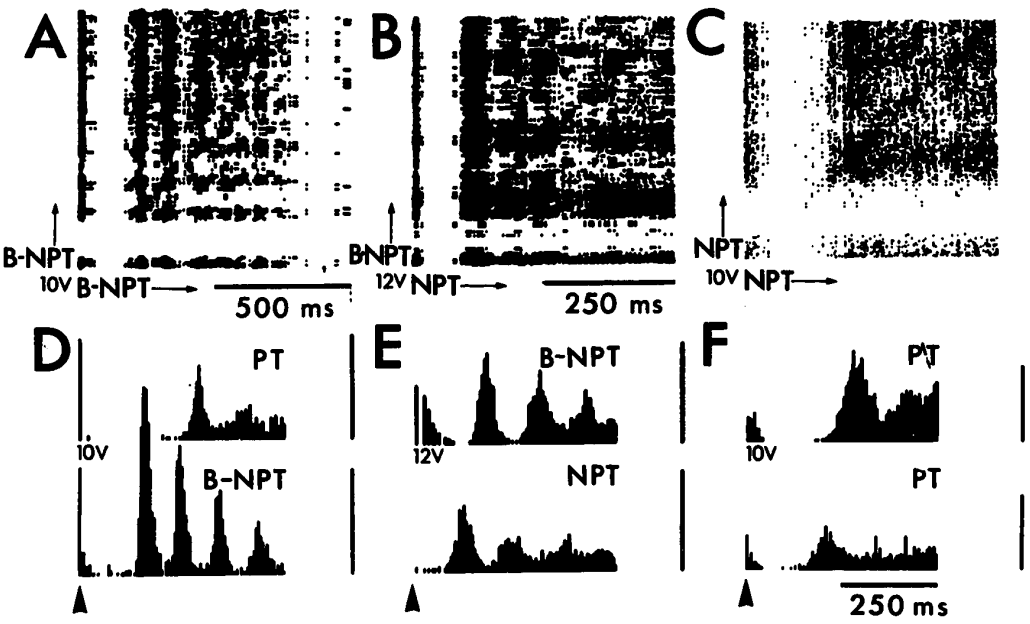
In summary. All PT and NPT neurones displayed an inhibitory pause following a transcallosal volley. In many cells inhibition was preceeded by excitation, which for some NPT cells appeared as bursts of two or more spikes lasting for more than 40 ms. This contrasted sharply with the brief single spike excitation displayed by most PT cells. Few PT neurones but almost all NPT neurones exhibited some degree of postinhibition excitation.

D. ACTIVATION OF "SPECIFIC" THALAMOCORTICAL PATHWAYS

Both excitation and inhibition have previously been shown to characterize the response of pericruciate PT and NPT neurones following stimulation of "specific" thalamic relay nuclei (Li 1956; Branch and Martin 1958; Lux and Klee 1962; Nacimiento et al. 1964; Schlag and Balvin 1964; Purpura et al. 1964; Klee 1966; Krnjević et al. 1966a). The present investigation concerned similarities and differences in the responses of the two populations of neurones following stimulation of nucleus ventralis lateralis (VL) or nucleus ventralis posterolateralis (VPL).

PT neurones. Activation of thalamocortical pathways produced inhibition of the spontaneous or glutamate evoked spike discharges recorded from all eleven PT cells studied. Included

Figure 31. Response of pericruciate neurones to stimulation of 'specific' thalamic relay nuclei. All records obtained from precruciate neurones in response to VL stimuli except those in (E). A. JSD pattern of alternating dense and less dense distribution of points obtained from the activity of two superficial (0.30 mm) B-NPT neurones. B. Similar but less pronounced JSD pattern obtained from a pair of neurones, one of which was a B-NPT neurone, the other a NPT neurone, also located superficially. Time scale is 500 ms in (A) only. C. Delayed inhibitory pause observed for two other deep (1.2 mm) NPT neurones. D, E, F. Post-stimulus latency histograms of pairs of simultaneously recorded neurones of the type indicated. PT cells were less likely to show more than one post-inhibition excitation peak (F), contrasting sharply with the multiple peaks observed for many NPT cells, especially B-NPT cells (D,E). Post-stimulus latency histograms in (E), were obtained from postcruciate NPT neurones in response to a VPL stimulus (arrowhead) illustrating the marked differences in phase of the excitatory peaks observed, even among cells situated less than 150 μm apart.


A
B-NPT
10V B-NPT
500 ms
B
B-NPT
12V NPT
250 ms
C
NPT
10V NPT
D
PT
10V
B-NPT
E
B-NPT
12V
NPT
F
PT
10V
PT
250 ms

in this group were eight precruciate neurones tested with a VL shock and three postcruciate neurones tested with a VPL stimulus. Excitation, appearing as 1-3 spike discharges superimposed on the initial negative component of the thalamic evoked cortical potential (Figure 33C), preceded the inhibitory response in seven of the eleven PT cells. The remaining four PT cells exhibited only inhibitory responses to thalamic stimulation, with latencies slightly greater than those for the excitatory responses described above. Following the inhibitory pause, which had a duration at times in excess of 150 ms, most PT neurones demonstrated a second peak of enhanced excitability best reflected in post-stimulus latency histograms (Figure 31D, F).

NPT neurones. Twenty-five cells, located 0.10 to 1.90 mm (mean 0.82, S.D. 0.16) below the pial surface all demonstrated evidence of inhibition following a single thalamic shock. Eight of these cells were postcruciate neurones tested with a stimulus to VPL, the remaining seventeen were precruciate neurones tested with a VL stimulus.

Ten of these NPT cells, located more superficially at a mean depth of 0.49 mm (S.D. 0.35), displayed an initial excitation to thalamic stimulation. This was usually manifested as a burst of 3-4 spikes at intermediate frequencies of 50-80/sec. and lasting up to 35 ms following the stimulus (Figure 34A, third column). Four such neurones, classified as B-NPT neurones (see Chapter 7) exhibited a more marked burst response of 3-10 spikes with intra-burst frequencies approaching 600/sec. Within the bursts it was

common to observe a progressive decline in the amplitude of consecutive spikes (Figure 34B and D, third column). In all neurones, initial excitation was followed by a silent period of 100 ms or more. The four B-NPT neurones exhibited a remarkable postinhibition pattern lasting up to 500 ms, consisting of rhythmic fluctuations in excitability. This was best illustrated in the scatter diagram in Figure 31A obtained from two such B-NPT neurones, and the post-stimulus latency histograms in Figure 31D and E (the B-NPT portion). The other NPT cells in this group of ten also exhibited similar but less dramatic patterns.

In fifteen other neurones the response to VL or VPL stimulation was characterized usually by a short latency inhibition. However on some occasions inhibition appeared only after a longer and variable latency reaching its maximum effect approximately 80 ms after the stimulus. These cells tended to be more deeply situated (mean 1.03 mm, S.D. 0.39) than others showing initial excitation. In the postinhibition period excitability fluctuations were also present (lower post-stimulus latency histogram in Figure 31E) but never were they as marked as for some other NPT neurones, particularly the B-NPT cells described earlier.

Similarities and differences in PT and NPT neuronal responses to VL or VPL stimuli. For those cortical neurones exhibiting excitation following VL or VPL stimuli, the previous sections have illustrated that PT cell excitation usually consisted of only 1-3 spike discharges appearing within a short time interval. NPT excitation tended to be more variable, with a longer time

course and not infrequently consisting of a burst of intermediate or high frequency spikes.

Compared with the duration of inhibition generated by VL or VPL stimuli on PT neurones (mean 190 ms, S.D. 24, n 11), stimuli of comparable strengths evoked shorter periods of inhibition of NPT neurones (mean 132 ms, S.D. 38, n 24). These values were obtained from measurements of post-stimulus latency histograms and were based on the time interval between the stimulus and the first bin following inhibition in which the probability of firing reached control level.

Following the inhibition, post-stimulus latency histograms of the activity of PT cells were seldom observed to contain more than one peak of enhanced excitability. While similar patterns were observed for some NPT neurones, the latter more frequently displayed several peaks of enhanced excitability with the most dramatic responses being derived from the superficial B-NPT neurones.

Apart from the general characteristics of postinhibition excitability for the two populations of neurones (i.e. PT and NPT) the patterns obtained from any two neurones were almost always different. This is illustrated best in the histograms in Figure 31D,E and F, obtained from three different pairs of cells. For example in D and E, the postinhibition peaks of enhanced excitability are almost 180° out of phase with each other. The two NPT cells in Figure 31E were located 1.27 mm (the upper B-NPT cell) and 1.10 mm (the lower NPT cell) below the cortical surface of the postcruciate gyrus and less than 150 μm from each

Figure 32. Postinhibition excitation of postcruciate neurones. A. Records obtained from the activity of two neighbouring NPT neurones located 1.25 mm below the surface of the postcruciate gyrus. Pyramidal tract (left column) and thalamic stimulation (right column) evoked rhythmic sequences of increased and decreased excitability in one member of the pair (NPT 2) indicating that this response pattern was specific only of the activity of NPT 2. B. Four pairs of superimposed oscilloscope sweeps from a postcruciate PT (upper trace) and the same NPT 2 neurone (lower trace) illustrated in (A). The cells were located less than 200 μm apart. Note the tendency for clustered firing of the NPT cell, particularly following the VPL evoked silent period, as compared with the more regular discharge pattern of the PT cell.

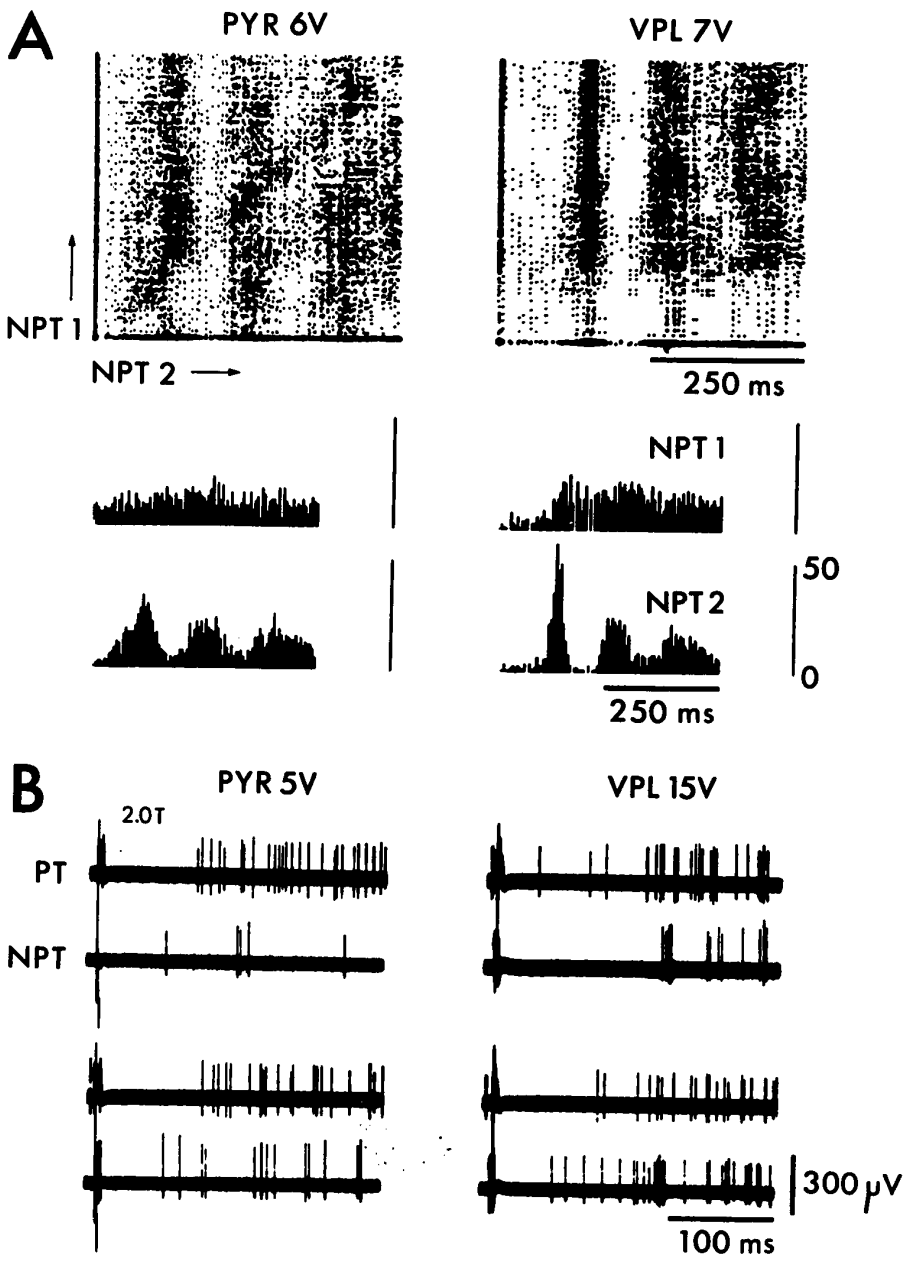

A
PYR 6V
VPL 7V
NPT 1
NPT 2
250 ms
NPT 1
NPT 2
50
0
250 ms
B
PYR 5V
VPL 15V
2.0T
PT
NPT
300 μV
100 ms

Figure 33. Responses of a precruciate pyramidal neurone to stimulation of various inhibitory pathways. PT neurone located 1.1 mm below the cortical surface. A. A single 2V shock to the medullary pyramid (arrowhead) evokes an antidromic spike at a latency of 1.1 ms. Not illustrated was a silent period lasting for approximately 120 ms following the antidromic spike. B. A single 15V transcallosal shock usually (but not necessarily) evoked a short latency orthodromic spike followed by a silent period lasting up to 200 ms. The lower trace (5 superimpositions) displays a clustering of spikes at 220 ms, the postinhibition excitation phenomenon referred to in the text. C. Single stimuli of 15V to the thalamic VL nucleus evoked one or two short latency spikes during the initial negative field potential (upper two traces) followed by a silent period of 180 ms (lowest trace containing five superimposed sweeps). Post-inhibition excitation was also noted to follow thalamic stimulation.

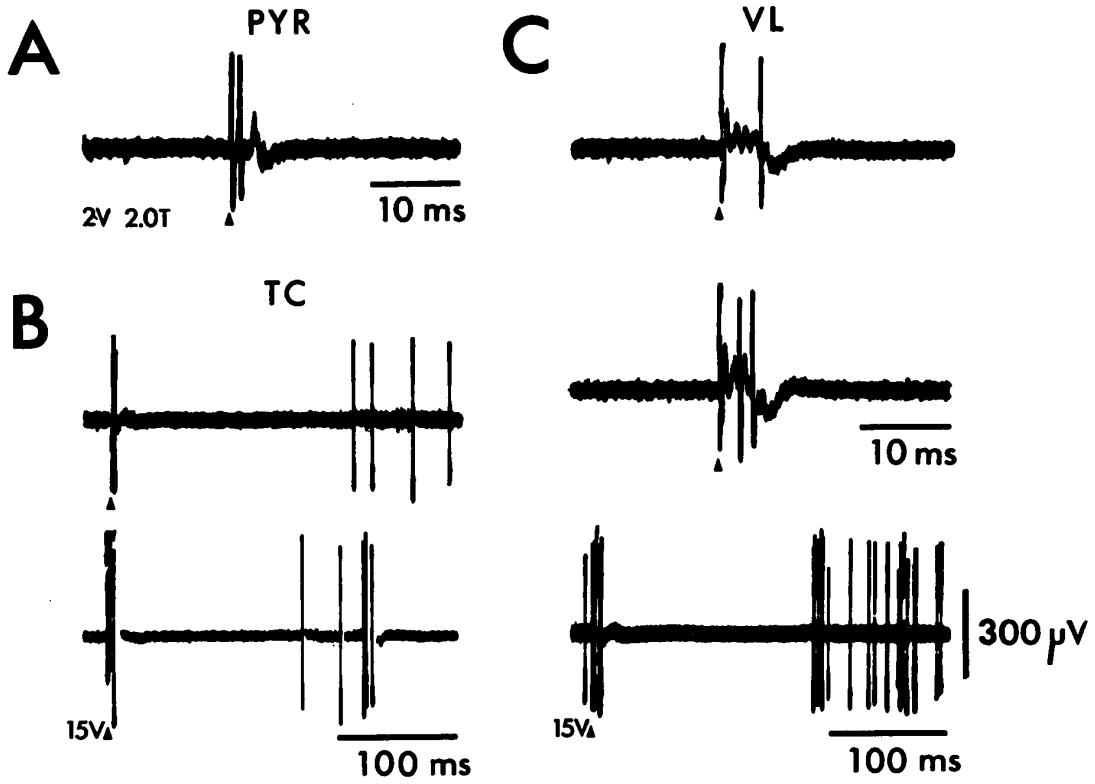
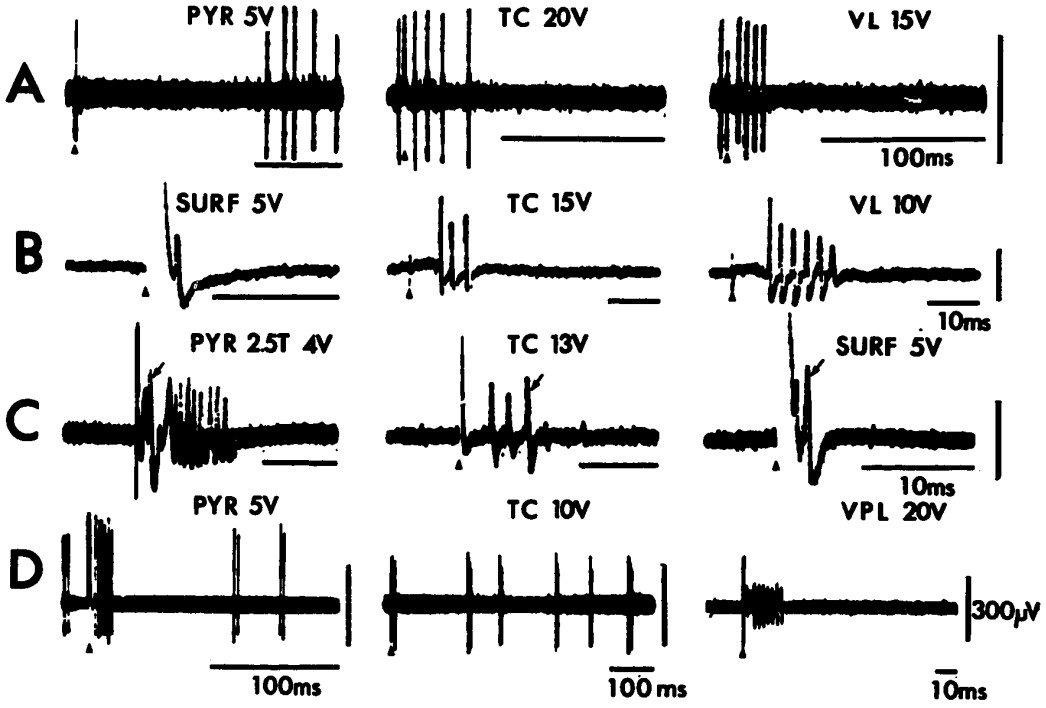

A
PYR
2V 2.0T
10 ms
B
TC
15V
100 ms
C
VL
10 ms
300 μV
15V
100 ms

Figure 34. NPT neurone multiple spike responses to various types of stimulation. A. Pyramidal tract stimulation evoked a 'silent' period while TC and VL stimuli evoke low frequency multiple spike responses followed by a 'silent' period in the discharge pattern of one precruciate NPT neurone (depth 0.08 mm). B. Another precruciate neurone (depth 0.30 mm) responded to all three stimuli with spike discharges, followed by a silent period (not seen in full at this sweep speed). Note the more pronounced excitation following a VL stimulus. C. The same electrode recorded the activity of a precruciate PT (arrow) and a NPT neurone. Five superimposed sweeps during pyramidal stimulation illustrate the variable latency of the NPT cell response. D. Responses of 3 different B-NPT cells. In the first column, pyramidal tract stimulation evoked a very pronounced high frequency burst of activity followed by a pause in the centre column, on a slower time base, in response to a TC stimulus this B-NPT cell demonstrated a pause followed by rhythmic bursts of activity. On the right, in response to a VPL stimulus, a post-cruciate B-NPT neurone discharged repetitively after a rather long latency and was then silent for more than 100 ms. Time bar represents 100 ms in (A), 10 ms in (B) and (C).


A
PYR 5V
TC 20V
VL 15V
100ms
B
SURF 5V
TC 15V
VL 10V
10ms
C
PYR 2.5T 4V
TC 13V
SURF 5V
10ms
D
PYR 5V
TC 10V
VPL 20V
300μV
100ms
100 ms
10ms

other horizontally, indicating that even neighbouring cells in the same cortical layers have their own response pattern.

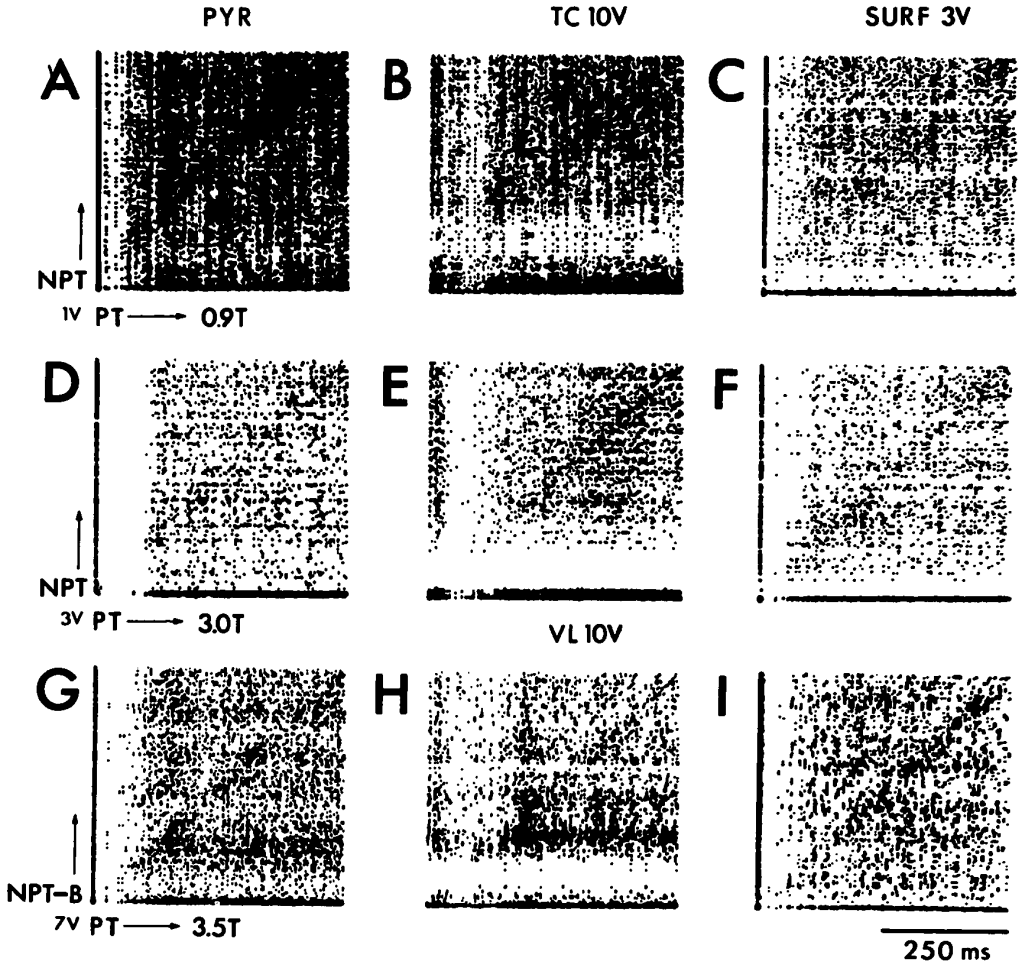
The phenomenon of postinhibition excitation appeared to be a characteristic feature of the neurone itself, and was reflected in the cell's response to more than one type of stimulus. This is illustrated for example in Figure 32A, where both pyramidal and thalamic stimuli evoked rhythmic responses in only one of two neighbouring NPT neurones. A nearby PT cell also failed to show this pattern of response even though the same NPT cell continued to show the rhythmic postinhibition excitability fluctuations (Figure 32 B).

In summary. Both PT and NPT neurones displayed inhibition following thalamocortical stimulation. This was not infrequently preceeded by excitation, most marked in certain NPT neurones which responded with a high frequency burst of spikes at shorter but variable latencies. Those cells having such a marked initial excitation often exhibited a marked and usually periodic postinhibition excitation pattern, which was seldom the same even among closely situated neurones.

E. RESPONSE PATTERNS OF CORTICAL NEURONES TO STIMULATION OF VARIOUS INHIBITORY PATHWAYS.

On many occasions it was possible to study the activity of a pair of neurones in response to several forms of stimulation. Several general features characteristic of most PT and NPT cell responses are exemplified by the three pairs of neurones illustrated in Figure 35, recorded in three different preparations. First,

Figure 35. Scatter diagram patterns of 3 different PT-NPT cell pairs in response to various stimuli. A. Pyramidal tract stimulation (PYR) just subthreshold for a PT cell (depth 0.66 mm) evoked only inhibition in the PT cell. B. Transcallosal (TC) stimulation evoked weak excitation and inhibition of the same PT cell but marked excitation and inhibition of the NPT neurone (depth 0.75 mm). C. Surface stimulation (SURF) evoked inhibition of both neurones. D, E. Same PT cell as in A. A different NPT cell, located at a depth of 1.03 mm, revealed no clear inhibition by PYR stimulation but much sharper excitation and inhibition following TC stimulation. F. SURF effectively inhibited both cells. G. Pyramidal stimulation weakly inhibited the activity of a B-NPT precruciate neurone, (depth 0.70 mm) in comparison with the clear inhibitory response evoked by VL stimulation (H). The other neurone is a PT neurone (depth 1.5 mm). I. Surface stimulation inhibited the activity of both cells, the other being a PT neurone (depth 1.5 mm) which demonstrated inhibition following both pyramidal tract and thalamic stimulation.


PYR
TC 10V
SURF 3V
A
B
C
NPT
1V PT ⟶ 0.9T
D
E
F
NPT
3V PT ⟶ 3.0T
VL 10V
G
H
I
NPT-B
7V PT ⟶ 3.5T
250 ms

the PT neurones exhibited recurrent inhibition more readily than did NPT neurones following a pyramidal tract stimulus (first column). Second, surface inhibition was more readily evoked in all types of cortical neurones, irrespective of their depths. Third, excitatory responses from NPT neurones tended to be more marked following activation of one particular pathway compared with any other. For example, the NPT cells in the first two rows in Figure 35B,E illustrate an excitation-inhibition sequence only to transcallosal activation.

Chapter 5

RESULTS

IN SEARCH OF INHIBITORY INTERNEURONES: INTRACORTICAL CHEMICAL MICROSTIMULATION

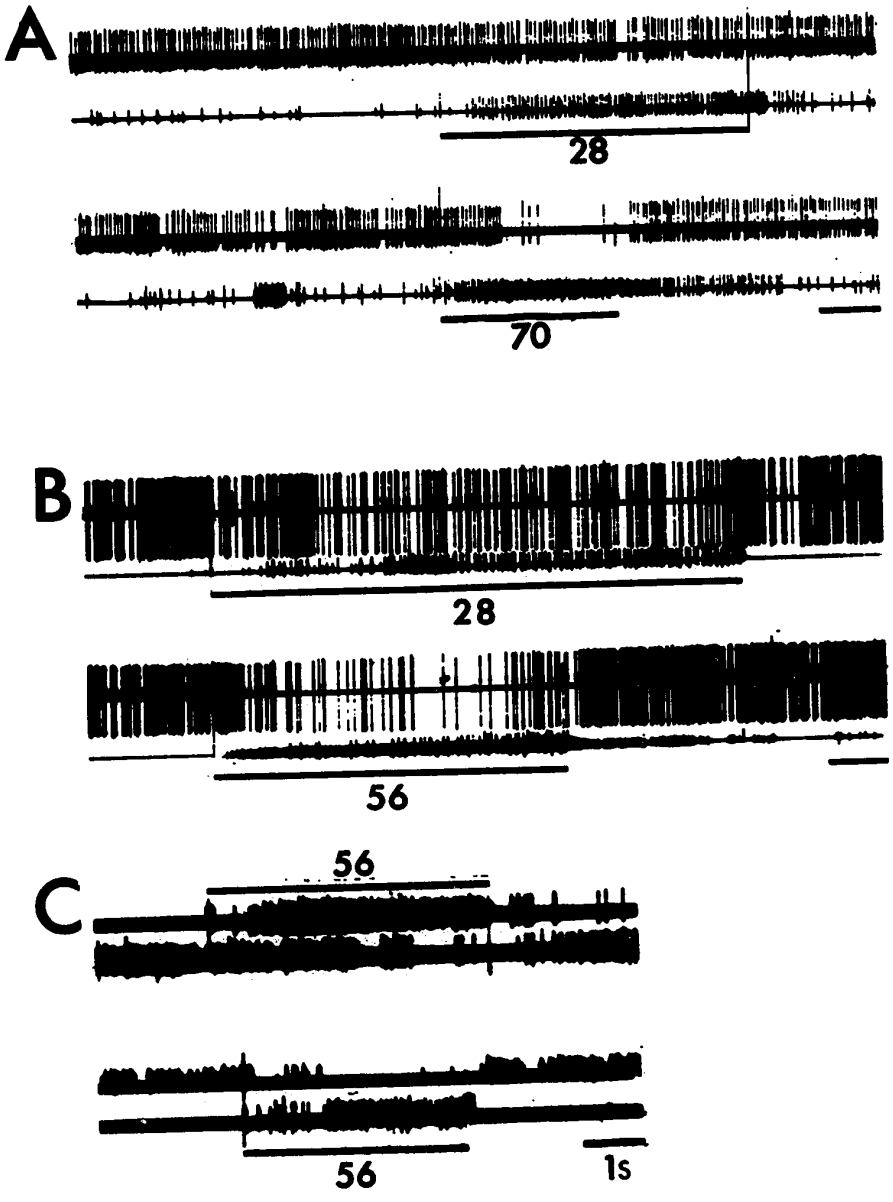
In Chapter 3 intra- and extracellular analysis of the response of the postsynaptic neurone to inhibitory stimuli provided indirect information pertaining to the inhibitory pathways (i.e. axon terminals of inhibitory interneurones) afferent to pyramidal tract neurones. A more direct approach to the investigation of cortical inhibitory mechanisms would be the study of the inhibitory interneurones themselves. One of the major barriers to this approach, however, is the lack of a reliable and accurate means of identifying these neurones. Cortical neurones which show multiple spike discharges in response to known inhibitory stimuli, and which are active during inhibition of other nearby cells, have been tentatively identified as inhibitory neurones (c.f. Stefanis 1966; Sukhov 1969). However, it is for the most part conjectural to attribute a functional role to any individual neurone based on information derived solely from single cell recordings. The function of any neurone, particularly an inhibitory function,

can be defined with certainty only if there is some direct means of testing the action which such a neurone exerts on other neurones.

Multiple unit recordings have been introduced in the study of simpler invertebrate neural networks, for example in Aplysia (Kandel et al. 1969; Kristan 1971) in order to clarify the functional role of individual neurones within the intact network. In the mammalian nervous system where the complexity of connections is increased tremendously and the anatomical connections cannot be visualized, there are few reports which describe the search for inhibitory neurones utilizing dual recording microelectrodes (Biscoe and Curtis 1967; Curtis et al. 1971), where neurones were functionally classified as either inhibitory or excitatory depending upon whether their activity was related to a decrease or increase in the probability of spike discharges of other neurones located at the tip of a neighbouring electrode.

In the study to be reported here two independently mobile multibarreled micropipettes, in parallel alignment but separated by 80-800 μm, were used to study the activity of 344 pairs of pericruciate neurones in a total of 18 experiments. Cell discharges of one neurone were initiated by iontophoretic release of an excitatory amino acid (L-glutamate). A search was made for any influence which this localized evoked activity might have on spontaneous or glutamate-evoked neuronal activity of nearby neurones.

Figure 36. Local and remote patterns of activity evoked by glutamate action on "I type" NPT neurones. A. Continuous oscilloscope traces of the activity of a PT neurone (upper trace, depth 1.09 mm) and a NPT neurone (lower trace, depth 1.29 mm), located approximately 300 µm apart in the precruciate cortex. Glutamate-evoked activity of the NPT neurone with a current of 28 nA resulted in a detectable slowing of PT cell activity; application of glutamate with a current of 70 nA evoked a brisk response with partial spike inactivation of the NPT cell, coupled with a transient arrest of PT cell spike discharges. B. Interactions observed between two superficial NPT neurones located approximately 0.15 mm below the cortical surface. A two-fold increase (from 28 to 56 nA) in the amount of iontophoretic current applied to the glutamate channel considerably enhanced the suppression of activity of the NPT cell in the upper trace. These cells were located less than 200 µm apart in the horizontal plane. C. The only example obtained in which glutamate-evoked activity of either of two closely apposed NPT neurones (less than 150 µm apart and located approximately 0.30 mm below the surface) was associated with suppression of the activity of the other neurone. Time calibration 1 second.


A
28
70
B
28
56
C
56
56
1s

A. NEURONAL ACTIVITY RELATED TO DEPRESSED FIRING OF NEIGHBOURING NEURONES

A decrease in the frequency of spike discharges from one neurone was observed to follow a burst of glutamate induced activity of another neurone during recordings taken from 45 (13.1%) pairs of cells. On twenty occasions it was the activity of a pyramidal tract (PT) neurone which was decreased by spike discharges evoked in a neighbouring non-pyramidal (NPT) neurone. Cells which appeared to evoke a decrease in the excitability of other neurones were classified as "I type" neurones. In one example, illustrated in Figure 36A, a PT neurone (upper trace) and two NPT neurones (lower trace), separated by approximately 200 μm, were recorded at cortical depths of 1.1 and 1.3 mm respectively. Glutamate released in the vicinity of the NPT neurones with a current of 28nA increased the discharge frequency of both NPT cells and was associated with a transient minimal decrease in the activity of the PT cell. Repeated short applications with progressively larger iontophoretic currents resulted in an increasing suppression of the activity of the PT cell. Eventually, 70nA of glutamate evoked an intense discharge of the NPT neurone with larger amplitude spikes, which was associated with an arrest of activity of the PT cell lasting almost two seconds.

"I type" neurones also appeared capable of suppressing spike discharges from other NPT neurones. Figure 36B

taken from one of twenty-five similar pairs of neurones illustrates the interaction between two NPT neurones, located approximately 0.2 mm below the cortical surface, in which larger applications of glutamate to one neurone (lower traces) resulted not only in more intense firing of this cell but also greater suppression of activity of a neighbouring cell (upper traces) situated approximately 250 μm away.

The pair of NPT cells illustrated in Figure 36C, situated less than 0.3 mm below the cortical surface and less than 150 μm from each other, was the only example in which either cell was capable of suppressing the activity of the other. In all other examples the effect was only present in one direction.

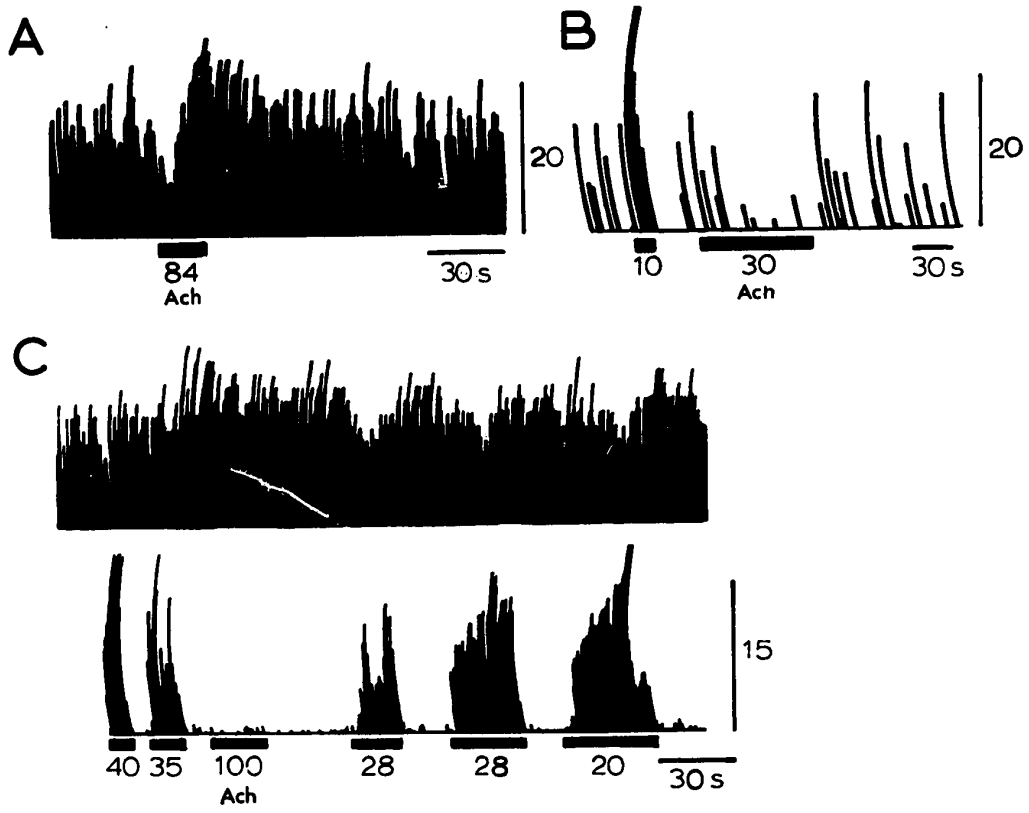
"I type" neurones. Only NPT neurones demonstrated the features described for "I type" neurones. Glutamate evoked spike discharges of PT neurones were never noted to be associated with a detectable decrease in the frequency of discharges recorded from neighbouring PT or NPT neurones.

NPT "I type" neuronal spikes were usually very small (less than 100-150 μV) in amplitude and difficult to gate properly. This became even more of a problem when larger doses of glutamate were applied, since these neurones appeared to undergo excessive depolarization with decrease in amplitude of the extracellular spikes so much so that they became lost in the baseline noise level. Such glutamate-evoked excessive depolarization was on several occasions associated with an inability to maintain maximal depression of the spike discharge activity from neighbouring

neurones. This would suggest that some inhibitory neurones do produce spike discharges and that their ability to inhibit other neurones is related to their ability to produce propagated action potentials.

On the other hand, there were also examples in which "I type" neuronal spikes were no longer visible on the tracings, or were not proportional to the amount of inhibitory effects simultaneously observed from nearby neurones. Figure 41 C and D are examples of this effect, and raise two possibilities. One is that conducted action potentials may not always be necessary for inhibitory transmitter release from axon terminals. Thus if the soma-dendrite membrane is maintained in a state of depolarization and the space constant λ (the distance at which the change in transmembrane voltage has fallen to 1/e) is in the order of a few hundred microns ($\lambda = \sqrt{r_m/r_i}$ where r_m is the specific membrane resistance and r_i is the axoplasmic resistance of a 1 cm length of axon; c.f. Gordon and Woodbury 1965) it may be that the amount of depolarization at the axon terminals of inhibitory neurones is itself sufficient to induce release of transmitter; therefore one would not be able to observe action potentials, but would be able to detect the result of transmitter release (i.e. inhibition). The other possibility is that glutamate is discharging interneurones too distant for the spikes to be recorded by the microelectrode. The data presented here are insufficiently clear to permit a definite conclusion on whether or not "I type" neurones do require the presence of

Figure 37. Acetylcholine (ACh) sensitivity of PT and some "I type" NPT neurones. A. Polygraph recording of the firing frequency of an ACh sensitive neurone (PT cell) illustrating the typical response to ACh which consisted of an initial depression of activity followed by an enhanced activity that considerably outlasted the iontophoretic application. Numbers below the heavy bars refer to the iontophoretic current in nA. Unlabelled bars refer to glutamate applications. Vertical bar represents counts per bin. B. A superficial B-NPT neurone produces an erratic polygraph pattern due to its sporadic bursting type of activity. A short iontophoretic glutamate application (10nA) evoked excitation while 30nA of ACh appeared to depress cellular activity, possibly an artifact due to positive current. C. Simultaneous polygraph recordings from a PT (upper) and a NPT (lower) neurone. Glutamate-evoked activity of the NPT neurone was usually associated with a temporary depression of the PT cell spike discharge frequency. The poor correspondence between the amount of current used to eject glutamate and the response shown here from the NPT cell was related partly to the difficulty of gating small amplitude spikes frequently recorded from these "I type" neurones. Note the latter's absence of response to even a large (100nA) ACh application.

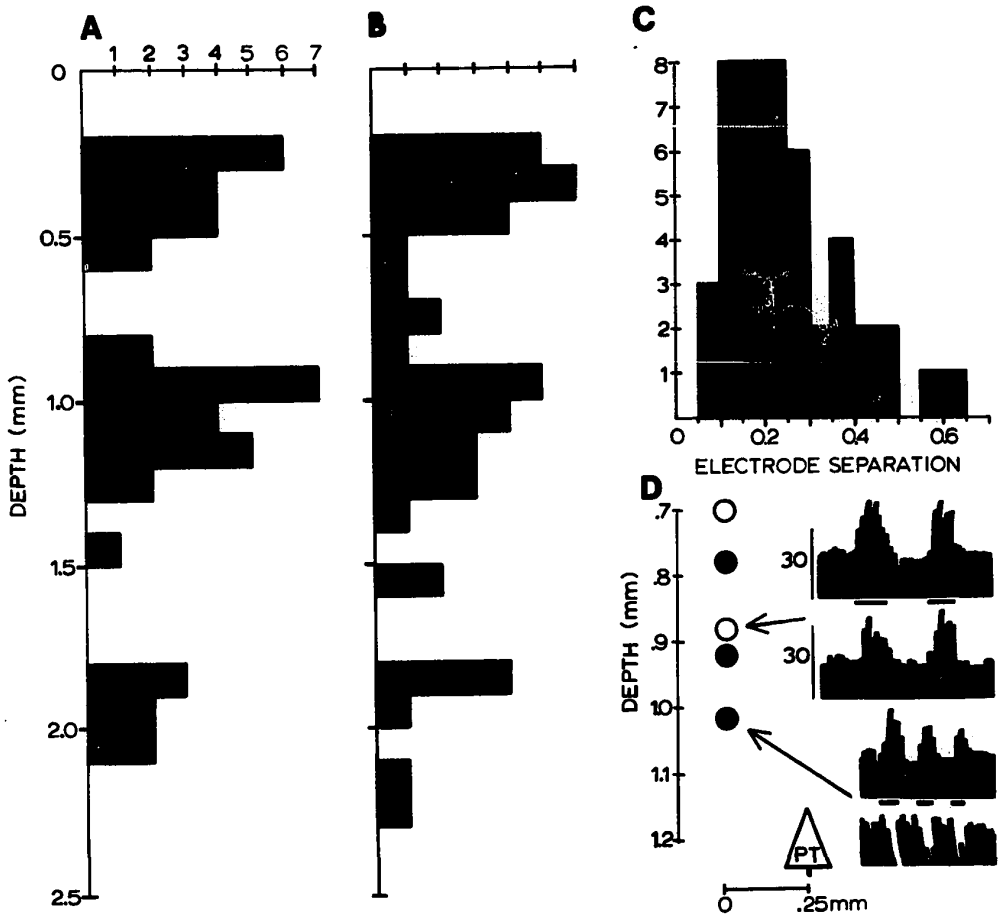

A
20
84
Ach
30 s
B
20
10
30
Ach
30 s
C
15
40 35 100
Ach
28
28
20
30 s

somatic action potentials in order to produce transmitter release. It may be that both possibilities are in existence.

Acetylcholine sensitivity. In some experiments the response to iontophoretic acetylcholine was investigated further. Cortical neurones excited by ACh frequently were spontaneously active and revealed a characteristic response (Figure 37A). The onset of firing was usually delayed by a period of 5-10 seconds, even when there was a brisk spontaneous discharge, indicating a relatively high level of excitability. During this initial phase the ongoing activity was often sharply reduced for some seconds, this initial depression being more pronounced the more intense the spontaneous firing. This was followed by an after-discharge which was the most prominent feature of the response, often outlasting the initial application by several tens of seconds (c.f. Krnjević and Phillis 1963 b,c; Krnjević, Pumain and Renaud 1971). This type of response was observed from 80% of neurones, including all PT neurones, located deeper than 0.8 mm from the cortical surface. Neurones more superficial to that point either showed no response or appeared to be depressed by ACh (c.f. Figure 37B).

Of five "I type" neurones tested all were unresponsive to ACh. In one example (Figure 37C), even 100 nA of ACh produced no significant excitation of the NPT cell shown in the lower polygraph tracing, although glutamate evoked the usual excitatory response which was associated with decreases in

Figure 38. Frequency distribution of inhibitory responses. A. Depth distribution of 45 neurones whose activity was suppressed following glutamate-evoked activity of other neurones which were located at the depth indicated in the histogram in (B). Included in this profile are 5 pairs of cells studied in the suprasylvian gyrus, and 18 pairs from the postcruciate gyrus; all others were from the precruciate gyrus. Note the tendency for both groups of cells to cluster at three different depths. C. Frequency distribution of the distance (in mm) separating the microelectrode tips during these recordings. Most penetrations were made with inter-tip distances of 0.2 to 0.3 mm. D. An example from one experiment illustrating that several neurones within a single penetration can influence the activity of the same PT cell located 1.2 mm below the cortical surface 0.25 mm in the horizontal direction from the NPT neurones. Open circles refer to NPT neurones evoking excitatory PT responses, filled circles refer to neurones evoking suppression of PT activity. The lower of each pair of polygraph records on the right illustrates the activity of the PT cell during glutamate-evoked activity of the NPT neurones (horizontal bar; longest application 12 seconds) illustrated in the upper of each pair of polygraph records, and taken from the NPT neurones indicated by the arrows.

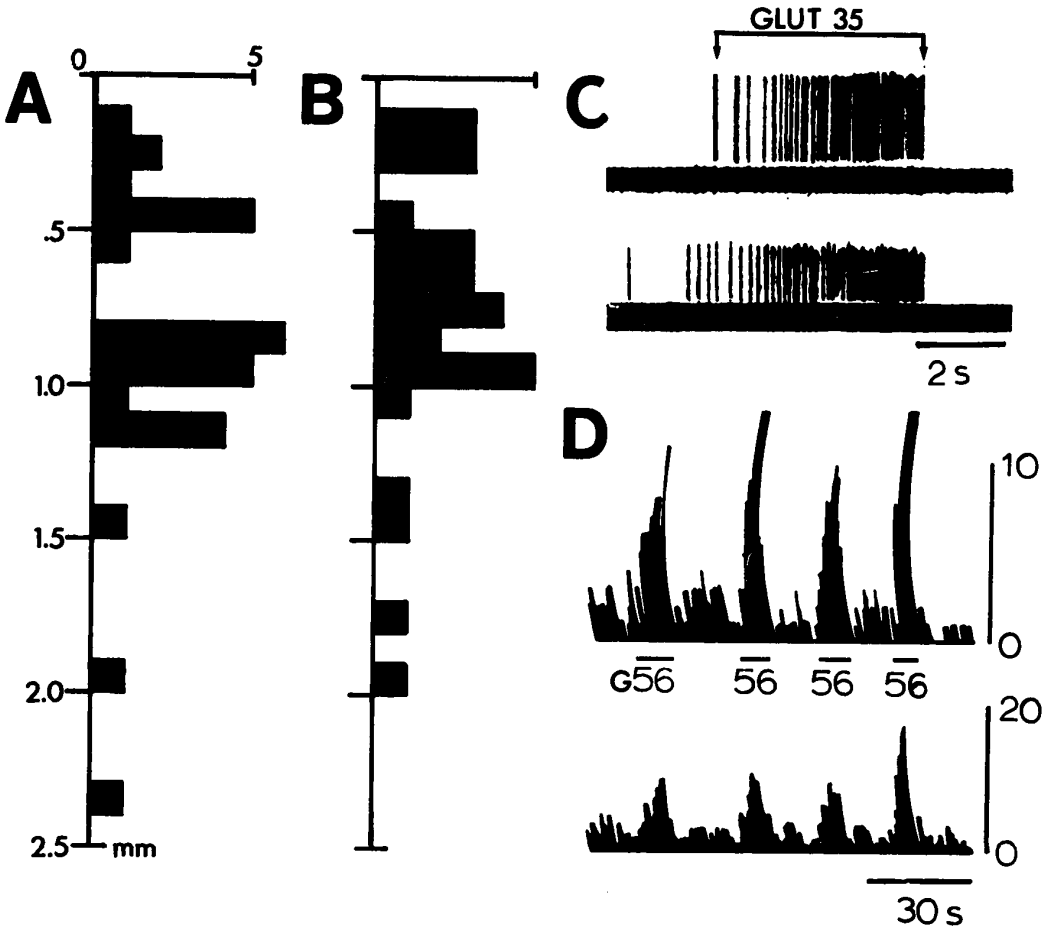

A
B
C
D
1 2 3 4 5 6 7
0
0.5
1.0
1.5
2.0
2.5
DEPTH (mm)
8
7
6
5
4
3
2
1
0 0.2 0.4 0.6
ELECTRODE SEPARATION
.7
.8
.9
1.0
1.1
1.2
30
30
PT
0 .25mm

discharge frequency of the PT neurone (upper trace). In one other example (not illustrated) a PT and a NPT were recorded from one electrode. Glutamate evoked activity from both neurones was associated with decrease in the discharge frequency of a neighbouring PT neurone recorded from the second electrode. ACh also evoked excitation, but only of the PT neurone; under these conditions, no change was observed in the firing rate of the neighbouring PT neurone.

B. INTRACORTICAL LOCALIZATION OF "INHIBITORY" EVENTS

Figure 38 gives the distribution by depth from the cortical surface of neurones whose activity was decreased (A) by iontophoretic release of glutamate in the vicinity of other neurones (B). It was observed that the inhibited cells tended to be located at three different depths, possibly related to different cortical layers although confirmation would require more precise histological controls. Cells producing the "inhibitory" effect also showed a tendency for a similar distribution. This was in agreement with experimental observations that frequently the cell pairs were located at similar depths below the surface. There were however exceptions, one example being illustrated in Figure 38D where three different neurones (in black) were able to evoke a clear suppression of firing of the same PT neurone located somewhat deeper in the cortex.

Figure 39. Intracortical glutamate microstimulation producing remote excitation. A. Depth distribution of 28 cortical neurones whose activity was increased in association with glutamate-evoked increases in spike discharge frequency of other neurones whose distribution is plotted in histogram (B). C. Oscilloscope traces illustrating one example of short latency increase in the activity of one precruciate neurone (lower trace) following a 35nA application of glutamate to the neurone in the upper trace, located 300 µm away (spikes retouched). D. Polygraph records from two other neurones illustrating a consistent excitatory response of both neurones following glutamate-evoked activity (56nA) of the neurone in the upper tracings. Vertical bar refers to number of spikes per bin.


A
0
5
.5
1.0
1.5
2.0
2.5
mm
B
C
GLUT 35
2 s
D
10
0
G56
56
56
56
20
0
30 s

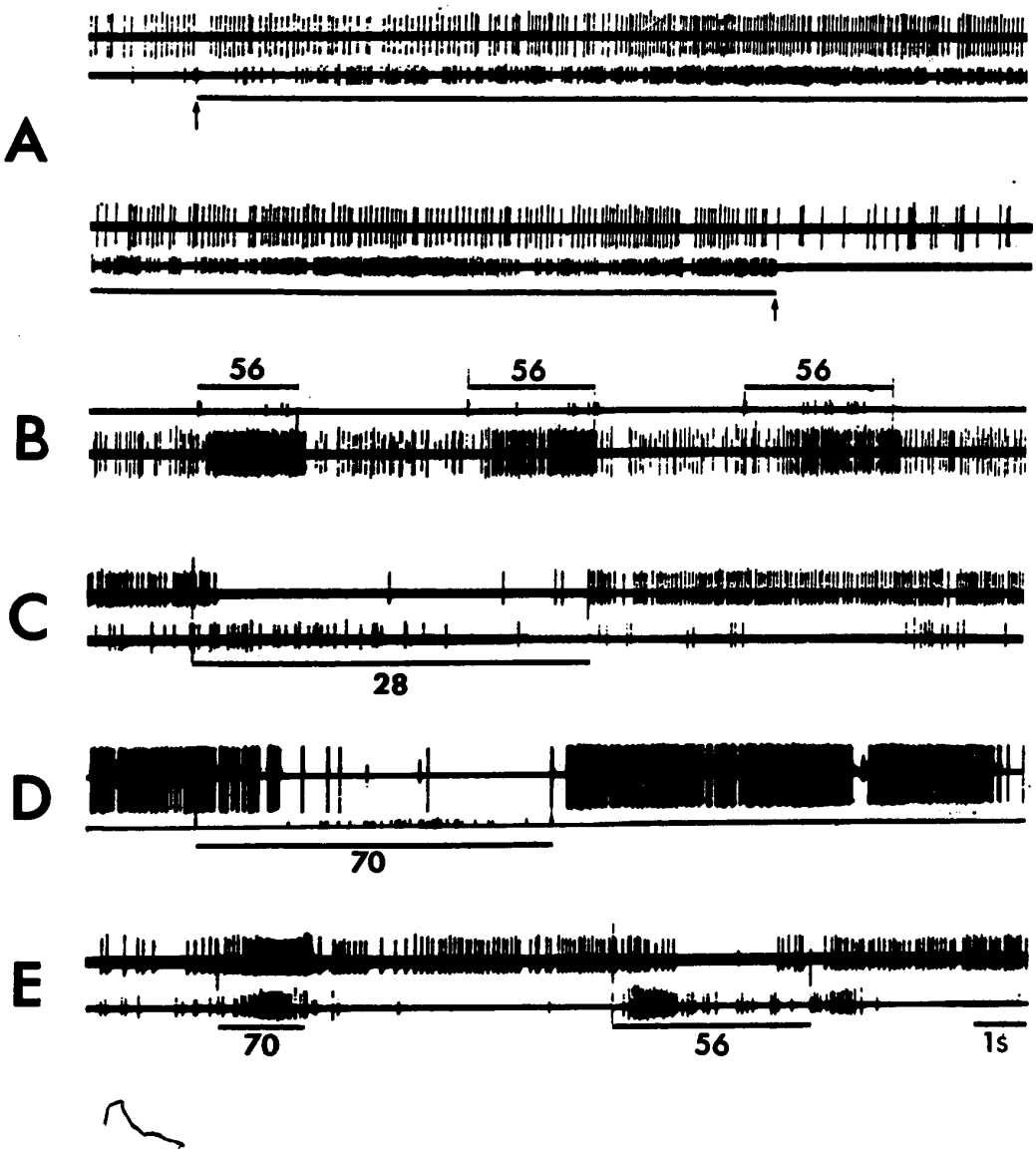
On several occasions it was possible to find several "I type" neurones which affected the activity of the same PT cell. One of these examples is shown in Figure 38D, and indicates a convergence of "inhibitory" influences on the same PT neurone. Figure 38D also illustrates one of several examples in which "I type" neurones were separated from one another by other NPT neurones (open circles) which, when activated with glutamate, appeared to evoke an increase in the activity of the same PT cell ("E type" cells; see below).

The bar graph in Figure 38C is a plot of estimated microelectrode tip separation (in mm) used during these experiments. Most penetrations were made with the tips 150-300 μm apart, but "inhibitory" events were observed (although rarely) even with tips separated by more than 600 μm.

C. NEURONAL ACTIVITY RELATED TO INCREASED ACTIVITY OF NEIGHBOURING NEURONES: "E TYPE" NEURONES

Aside from the "inhibitory" events, thirty-five pairs of cells were found in which glutamate evoked spike discharges of one neurone were associated with an increase in firing of the other neurone. Eight of these pairs of cells were excluded because of suspicion (vide infra) that direct spread of glutamate rather than true interneuronal interaction was producing the response. The remaining twenty-seven pairs (7.9%) exhibited the features illustrated in Figure 39C and D in which a short latency increase in discharge rate of the second neurone was observed to

Figure 40. Difficulties encountered during intracortical glutamate microstimulation. A. Continuous oscilloscope tracings of the activity of a PT (upper) and NPT neurone (lower) less than 50 µm apart. A gradual increase in the NPT neurone activity through controlled release of glutamate (between arrows) increased the activity of both neurones. However, subsequent examination revealed a negative correlation (NPT to PT) in their spike discharges (refer to Figure 46 in Chapter 6). B. Several short (2-3 seconds) glutamate applications evoked little local activity (upper trace) but caused a marked increase in the activity of a large amplitude neurone (lower trace) located 150 µm away. C. Local glutamate release (lower trace) evoked little change in the activity of the neurone shown in the lower trace despite clear depression of the activity of a neurone 200 µm away, illustrated in the upper trace. D. A 70nA application of glutamate resulted in the appearance of low amplitude spikes (lower trace) but which displayed a poor temporal correlation with the pause in spike activity (upper trace) noted from a NPT neurone approximately 200 µm away. E. The larger of two glutamate applications (70nA) to the lower trace neurone evoked a pronounced "delayed" excitation of the upper trace neurone 100 µm away, possibly due to glutamate spread and a direct action on the membrane of the remotely situated neurone since a lower dose of glutamate (56nA) appeared to evoke depression of the activity of this same cell. Time scale is 1 second.


A
B
56
56
56
C
28
D
70
E
70
56
1s

follow glutamate evoked activity of the first cell. The graphs in Figure 39A represent the depths of cells which could be excited by glutamate induced activation of other neurones whose distribution is shown in Figure 39B. During most penetrations the microelectrodes had inter-tip separations of 150-300 μm.

Excitation mediated synaptically or the result of glutamate diffusion? An increased neuronal activity of the remotely situated neurone, particularly a short latency response, observed to follow a localized release of the excitatory amino acid (glutamate) was assumed to be mediated over neural pathways. Delayed excitatory responses from a remote neurone were viewed with suspicion since such patterns could well have resulted from glutamate diffusion directly onto sensitive areas of its neuronal membrane (c.f. Herz et al. 1969). The dendritic expansion of cortical neurones is quite large, extending in some cases for hundreds of microns (Cajal 1911; Lorento de Nó 1949; Ramon-Moliner 1961; Marin-Padilla 1969). If glutamate were acting directly on dendritic surfaces one might expect to encounter situations in which glutamate acting on the remote neurone's dendrites would also result in short latency distant excitatory patterns and lead to the erroneous interpretation that the results were synaptically mediated. However, the results of Herz and his colleagues (1969) would tend to suggest that this is not the case since they found that 20 second applications of glutamate with currents of 200 nA took several seconds to evoke neuronal activity from cells located 150-300 microns away.

Presumably iontophoretically applied glutamate induces excitation by acting on that portion of the neuronal membrane forming the soma and proximal dendrites.

In all of the examples of excitatory neuronal interaction included here glutamate applications rarely exceeded 56 nA for longer than 5-10 seconds and the results were considered to represent true synaptically mediated effects rather than direct effects of glutamate itself.

In contrast to "I type" neurones "E type" cells could be classified as both PT (20%) and NPT (90%). All five of the PT neurones as well as six of twenty-two NPT neurones were excited by ACh applied iontophoretically (c.f. Figure 37A).

D. SOME LIMITATIONS OF INTRACORTICAL GLUTAMATE MICROSTIMULATION

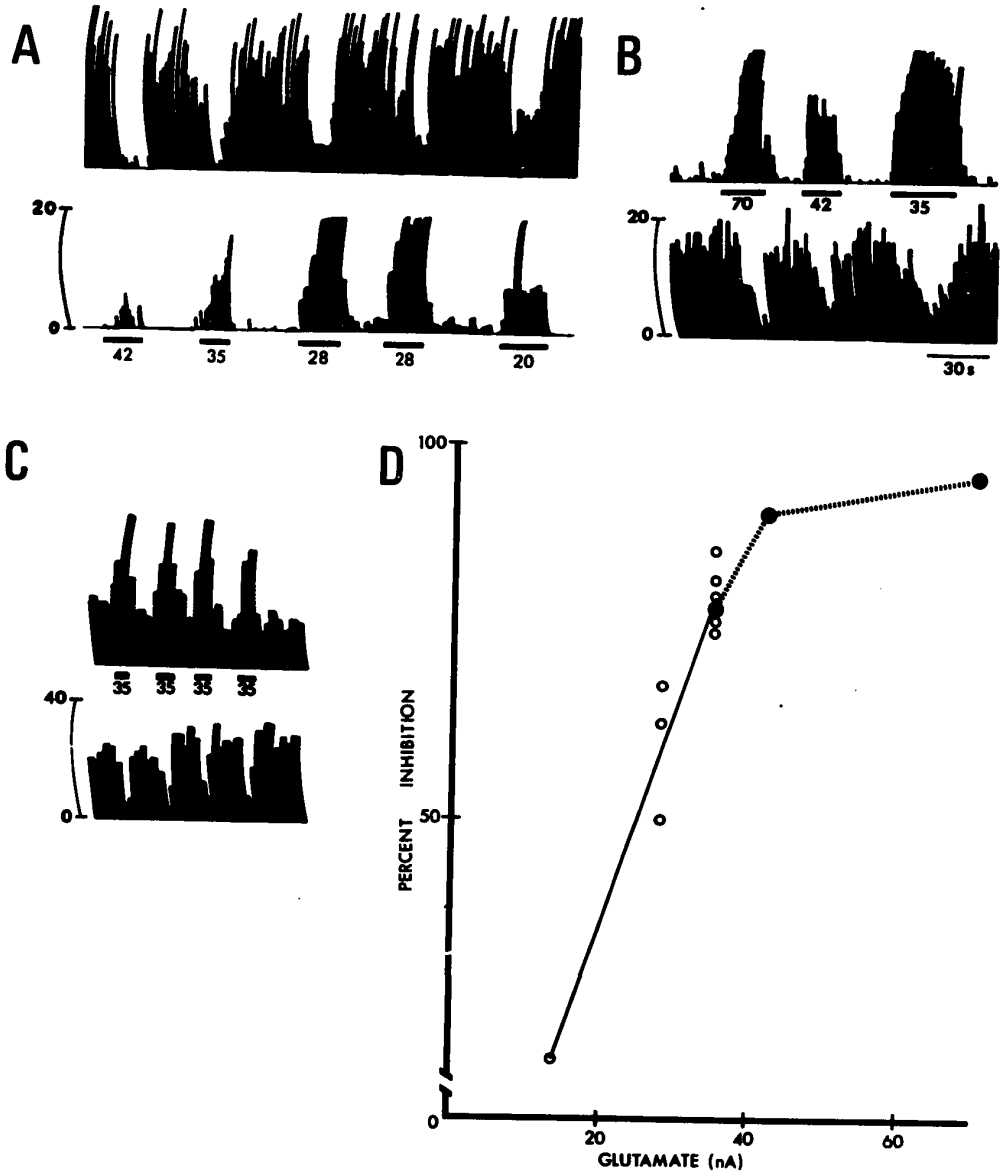
One of the troublesome technical faults with the method of dual micropipette recording is that one is often limited to recordings from neurones in the upper layers of the cortex. This arises from the tendency for the surface of the brain to 'dimple' as two electrodes in close apposition to each other are inserted into the depths of the cortical tissue. Since this undoubtedly results in added distortion, beyond that normally induced by the presence of the microelectrodes themselves, the appearance of dimpling was an indication to move the electrodes to a new recording site. This biased the recordings in favor of neurones in the upper cortical layers.

Quite apart from this difficulty, two types of peculiarities

were encountered relating to the recordings themselves. One, already mentioned earlier, is that of glutamate diffusion from the point of release to the region of the neighbouring neurones. The oscilloscope tracings in Figure 40A are one example in which glutamate applied in progressively greater amounts to a NPT neurone (lower trace) was associated with an increase in frequency of neuronal discharge of both this NPT cell and a PT neurone (upper trace). These neurones were situated less than 50 microns from each other. Cross-correlation analysis of spontaneous spike discharges (see Chapter 6, Figure 46B) clearly revealed that the NPT neurone's spikes were in fact followed by a decreased probability of PT neuronal spikes. In other words the visual impression derived from Figure 40A gives the erroneous impression that the NPT neurone had an excitatory influence on the PT cell. The most probable explanation is that glutamate, through diffusion to the PT cell membrane, evoked an increase in its discharge frequency while the increase in NPT cell activity through synaptically evoked inhibition tended to decrease the PT cell activity. The end result was that the NPT cell activity increased by a factor of two, a response which disappeared almost immediately upon terminating the glutamate application.

One other peculiarity related to glutamate release from one electrode was the paradoxical situation in which an apparent synaptically mediated response appearing from the remotely located neurone was unrelated to any events observed at the site of the

Figure 41. Relationship between glutamate dose and spike suppression. A. Glutamate released with various iontophoretic currents to one NPT neurone evoked suppression of activity of nearby NPT neurones 250 μm away, the response appearing to be dose-related. Note the poor correlation of glutamate-evoked spike counts in the lower polygraph record, largely due to the difficulty of gating small amplitude spikes. B. The degree of suppression evoked in the lower trace from a PT neurone was related to the dose of glutamate released on a NPT neurone (upper trace). C. A fairly consistent depression of activity of a PT neurone shown in the lower polygraph record followed several 35 nA glutamate applications to a NPT neurone whose activity appears in the upper tracing. D. Data obtained from two separate neuronal pairs (open and filled circles) indicating the relationship between "inhibition" or suppression of spike discharges of two PT neurones (ordinate) and varying amounts of glutamate released remotely onto two NPT neurones (abscissa). Time scale in (B) applies to all polygraph records. Vertical bar represents counts per bin.


A
20
0
42
35
28
28
20
B
70
42
35
20
0
30s
C
35
35
35
35
40
0
D
100
50
0
PERCENT INHIBITION
20
40
60
GLUTAMATE (nA)

glutamate application. In Figure 40B for example, 56 nA of glutamate barely yielded any local response although a marked short latency increase in spike discharge frequency appeared from a PT cell located at a distance of 150 microns. This effect was infrequent,however, and may have resulted from a crack some distance from the tip of the glutamate containing channel.

In an earlier section a comment was made concerning reasons for the inability to record spikes from "I type" neurones. The oscilloscope tracings in Figure 40C,D and the polygraph records in Figure 41 A,B further illustrate these discrepancies between the degree of "inhibition" observed from distant neurones and the spike activity recorded from "I type" neurones.

The traces in Figure 40E reveal two types of effects: in the first trial, 70 nA of glutamate applied to the neurones in the lower trace (a PT and a NPT cell) evoked a delayed increase in the activity at the tip of both electrodes. The long latency of the intense firing generated in the upper trace neurone (a PT cell) might well be the result of glutamate diffusion over the 100 microns separating the two neurones. The second trial using a lower iontophoretic current eventually yielded a suppression of PT cell activity but this was associated with a very erratic response pattern from the cells in the lower trace.

Although no systematic investigation was undertaken to relate the "inhibitory" response to the amount of glutamate released on "I type" neurones, for any one dose of glutamate applied iontophoretically a fairly consistent response was obtained with

repeated applications (Figure 41C). On several occasions repeated iontophoretic glutamate applications in different intensities evoked different degrees of neuronal "inhibition" and the results of two of these experiments have been plotted in the graph in Figure 41D.

E. SUMMARY AND COMMENT

Using dual multibarreled micropipettes and iontophoresis of an excitatory amino acid (Na L-glutamate) as a very localized form of intracortical stimulation, the relationships between individual members of 344 pairs of pericruciate neurones were investigated.

In 13.1% of pairs, glutamate evoked firing of certain NPT "I type" neurones was associated with a suppression of the spike discharges recorded from neighbouring PT and NPT neurones. Of the latter group 80% of those located in deeper cortical layers were excited by ACh; none of the "I type" NPT cells tested showed any clear response to ACh. "I type" neurones were often located at approximately the same depth as cells whose activity they appeared to suppress, although some examples were found in which glutamate evoked firing of several "I type" neurones at different depths was associated with suppression of the activity of the same PT cell. This type of response could be obtained with electrodes separated by inter-tip distances up to 650 microns.

In 7.9% of the neuronal pairs, glutamate evoked spike discharges of PT and NPT "E type" neurones, 80% of which were NPT

and some excited by ACh, were associated with an increase in the activity of more remote PT and NPT neurones. On some occasions more than one "E type" neurone was found to influence the activity of the same PT cell, and these cells were usually intermingled with "I type" neurones.

In conclusion, while the evidence suggests a possible role of the "I type" cells as inhibitory neurones, this can be confirmed only by demonstrating that they exert their effect monosynaptically. The next chapter contains the results of such a study.

Chapter 6

RESULTS

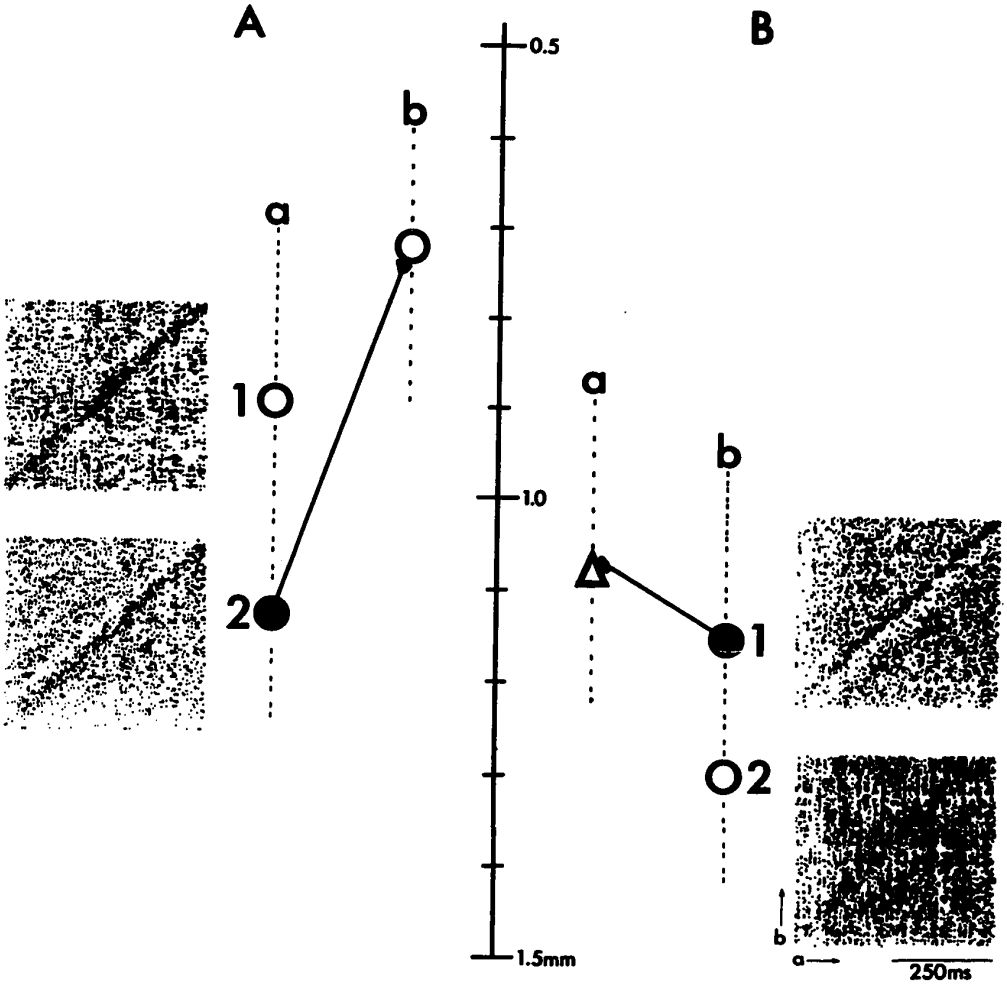
NEGATIVELY CORRELATED PAIRS OF CORTICAL NEURONES AND THEIR RESPONSE TO INHIBITORY STIMULI

In Chapter 5, experiments were described in which local chemical excitation of certain NPT cortical neurones was associated with depression of the activity of nearby PT and NPT neurones. Although these features are suggestive of the activity of inhibitory neurones, verification requires demonstration of a monosynaptic link between the "I type" neurones and those whose activity is depressed. Assuming that cortical inhibitory interneurones produce extracellularly recordable action potentials it should be possible to obtain this information by the demonstration of very short latency negative correlation between spike discharges of two cortical neurones (c.f. Moore et al. 1970).

A. "I TYPE" NPT NEURONES

From 110 neurone pairs studied, spike discharges in ten of them produced a scatter diagram pattern similar to the theoretical pattern of inhibition of the activity of one cell by another cell described in the METHODS (Figure 9C). This appeared as a decrease in point density along a line parallel to the 45° diagonal. As illustrated in Figure 42

Figure 42. Discrete joint scatter diagram patterns from adjacent pairs of neurones. Centre scale illustrates the depth below the cortical surface at which recordings were obtained from NPT (circles) and PT (triangle) neurones. Black circles and lines refer to postulated inhibitory monosynaptic neuronal connections between cells recorded during microelectrodes tracks a and b (less than 400 μm apart) in two separate experiments. A. The upper joint scatter diagram reveals an increase in point density along the 45° diagonal indicating a tendency for spike discharges from two NPT neurones (a1 and b) to occur synchronously. The discharges from a second NPT neurone (a2), located after a 220 μm advancement of microelectrode a, when correlated with the activity of the same NPT neurone in b results in a joint scatter diagram with a decrease in the density of points above the 45° diagonal (suggesting an inhibitory action of spikes from a2 neurone on the activity of neurone b), and an increase in the density of points on and below the 45° diagonal (suggesting an excitatory function of neurone b on the activity of neurone a2, or a common excitatory source impinging on the two neurones, or both). B. In a different experiment, a similar joint scatter diagram pattern, but with reversed polarity, results from correlating the activity of a NPT neurone (b1) and a PT neurone (a), suggesting an inhibitory function for the NPT neurone. Following advancement of microelectrode b, the activity of a new NPT neurone (b2) does not appear to bear any clear relationship with the activity of the PT neurone a, therefore, the more uniform distribution of points in the joint scatter diagram (lower right).


A
B
0.5
1.0
1.5mm
a
b
1
2
250ms

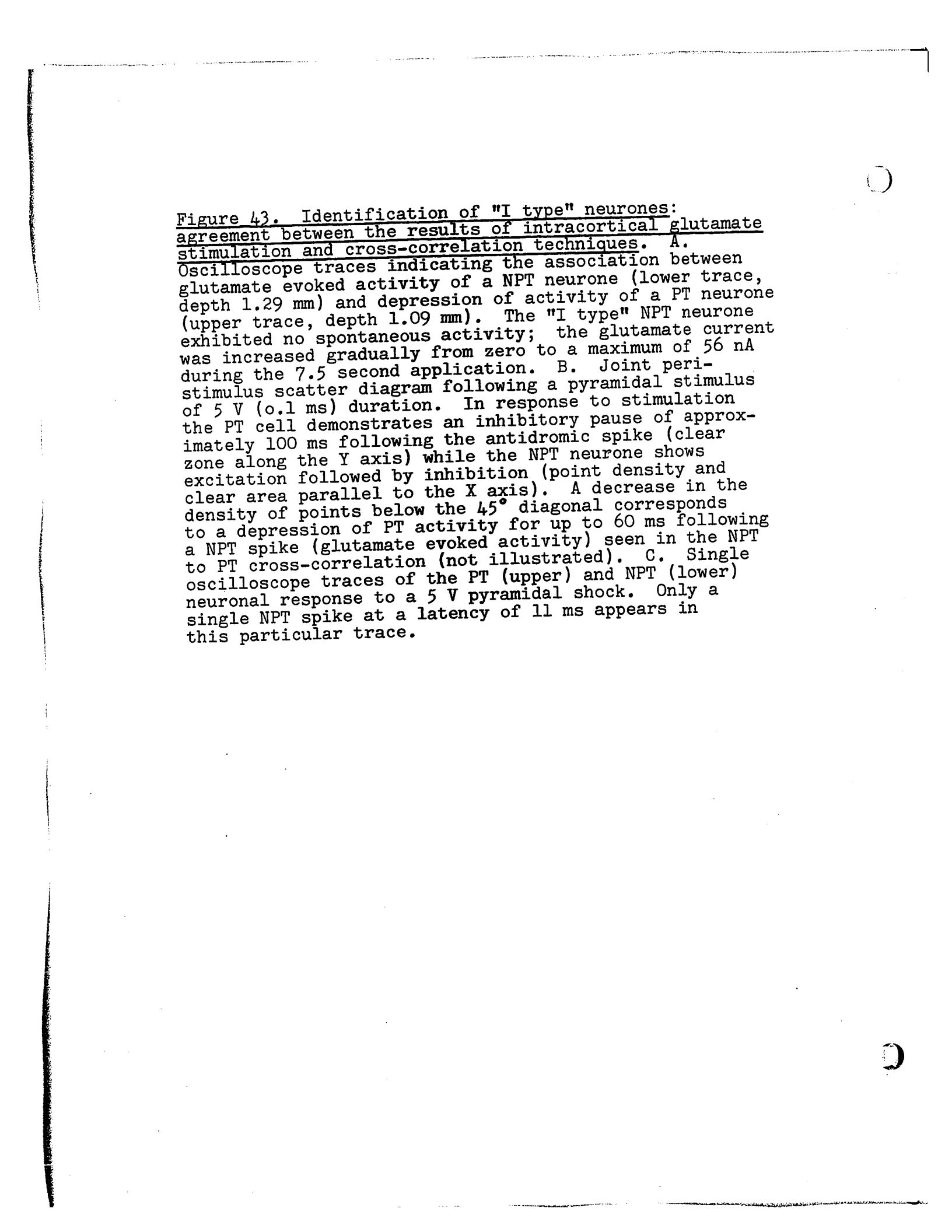
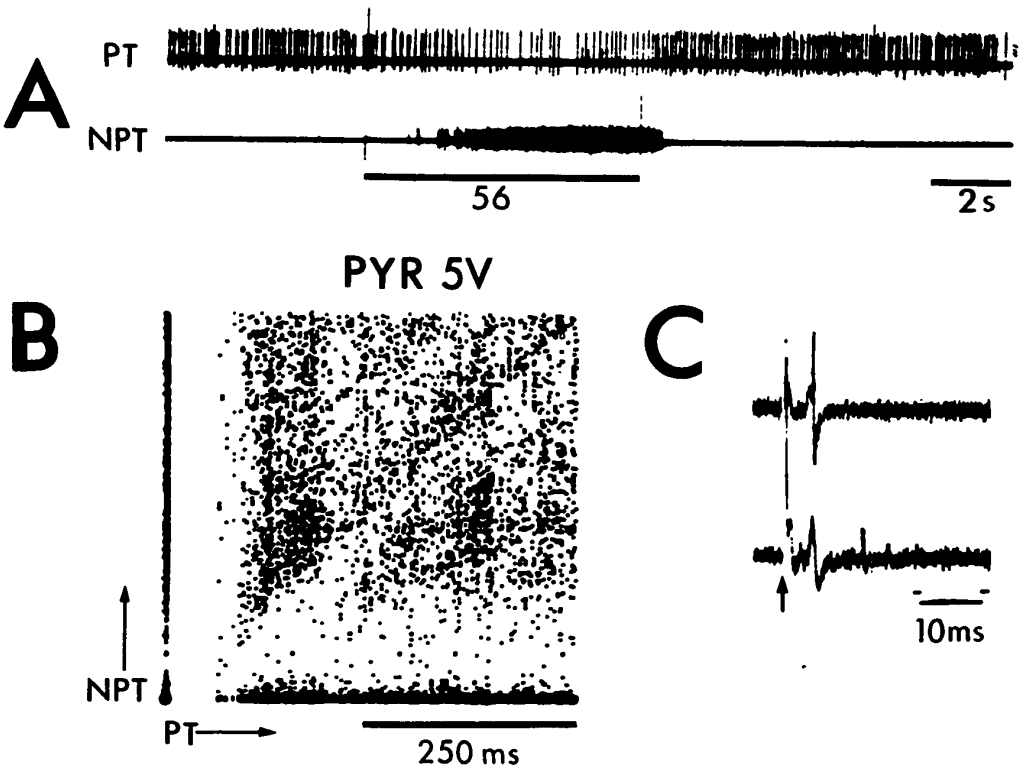


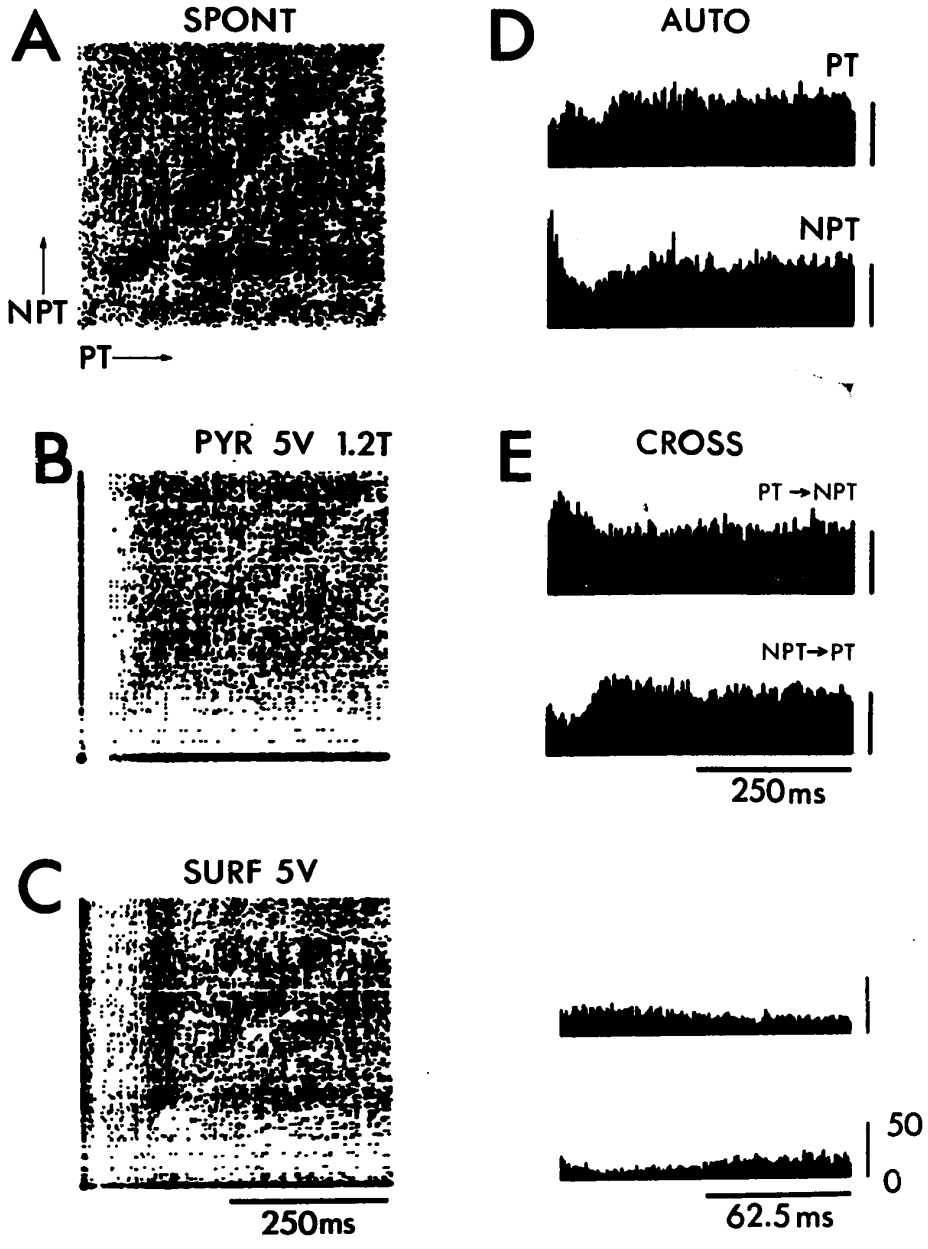
Figure 43. Identification of "I type" neurones: agreement between the results of intracortical glutamate stimulation and cross-correlation techniques. A. Oscilloscope traces indicating the association between glutamate evoked activity of a NPT neurone (lower trace, depth 1.29 mm) and depression of activity of a PT neurone (upper trace, depth 1.09 mm). The "I type" NPT neurone exhibited no spontaneous activity; the glutamate current was increased gradually from zero to a maximum of 56 nA during the 7.5 second application. B. Joint peri-stimulus scatter diagram following a pyramidal stimulus of 5 V (o.1 ms) duration. In response to stimulation the PT cell demonstrates an inhibitory pause of approximately 100 ms following the antidromic spike (clear zone along the Y axis) while the NPT neurone shows excitation followed by inhibition (point density and clear area parallel to the X axis). A decrease in the density of points below the 45° diagonal corresponds to a depression of PT activity for up to 60 ms following a NPT spike (glutamate evoked activity) seen in the NPT to PT cross-correlation (not illustrated). C. Single oscilloscope traces of the PT (upper) and NPT (lower) neuronal response to a 5 V pyramidal shock. Only a single NPT spike at a latency of 11 ms appears in this particular trace.


A
PT
NPT
56
2s
PYR 5V
B
NPT
PT
250 ms
C
10ms

the pattern was found either above (A) or below (B) the diagonal depending on which train of spikes was causing the inhibitory effect. The pattern was also specific for a certain pair of cells, disappearing if either electrode was advanced to record from a new cell. Note in these two examples that the area with fewer points on one side of the diagonal was accompanied by a narrow band of increased point density of the opposite side of the diagonal (vide infra).

Figure 43 illustrates one example of the experimental evidence which led to the retention of the term "I type" for the neurones which produced the scatter diagram pattern very suggestive of inhibition. The upper neurone in the continuous traces in Figure 43A was identified as a PT cell (Figure 43C). Glutamate evoked activity of the lower trace NPT cell was associated with clear depression of the activity of the PT neurone. The scatter diagram pattern resembled that in Figure 42B (c.f. Figure 9C) suggesting that spike discharges from the NPT neurone resulted in a transient decrease in probability of spike discharges appearing from the PT cell. Pyramidal tract stimulation was also applied once per second at the onset of each sweep. PT cell recurrent inhibition appeared as an absence of points parallel to the Y axis. The NPT cell responded with a short period of excitation (orthodromic) followed also by a silent period, and appeared as a dense distribution of points immediately above the X axis followed by a wider zone containing very few points.

Figure 44. Short latency negative correlation. A. Spontaneous activities of a PT and a NPT neurone (located at depths of 0.99 and 0.74 mm respectively) resulted in a joint scatter diagram pattern which indicated negative correlation (NPT to PT). B. In response to a pyramidal stimulus of 5 V, both cells showed evidence of inhibitory responses. C. Surface stimulation of 5 V evoked PT excitation followed by inhibition, while the NPT neurone showed a similar but less pronounced response. D. Auto-correlograms during spontaneous activity of these two neurones. E. Cross-correlograms computed with two different bin widths (3.9 ms per bin in the upper series, 0.9 ms in the lower). Note the negative short latency correlation (NPT toPT) which is coupled with a positive correlation (PT to NPT) having approximately the same time course. The onset of the negative correlation is already present in the second bin of the lower series, computed with a bin width of 0.9 ms.

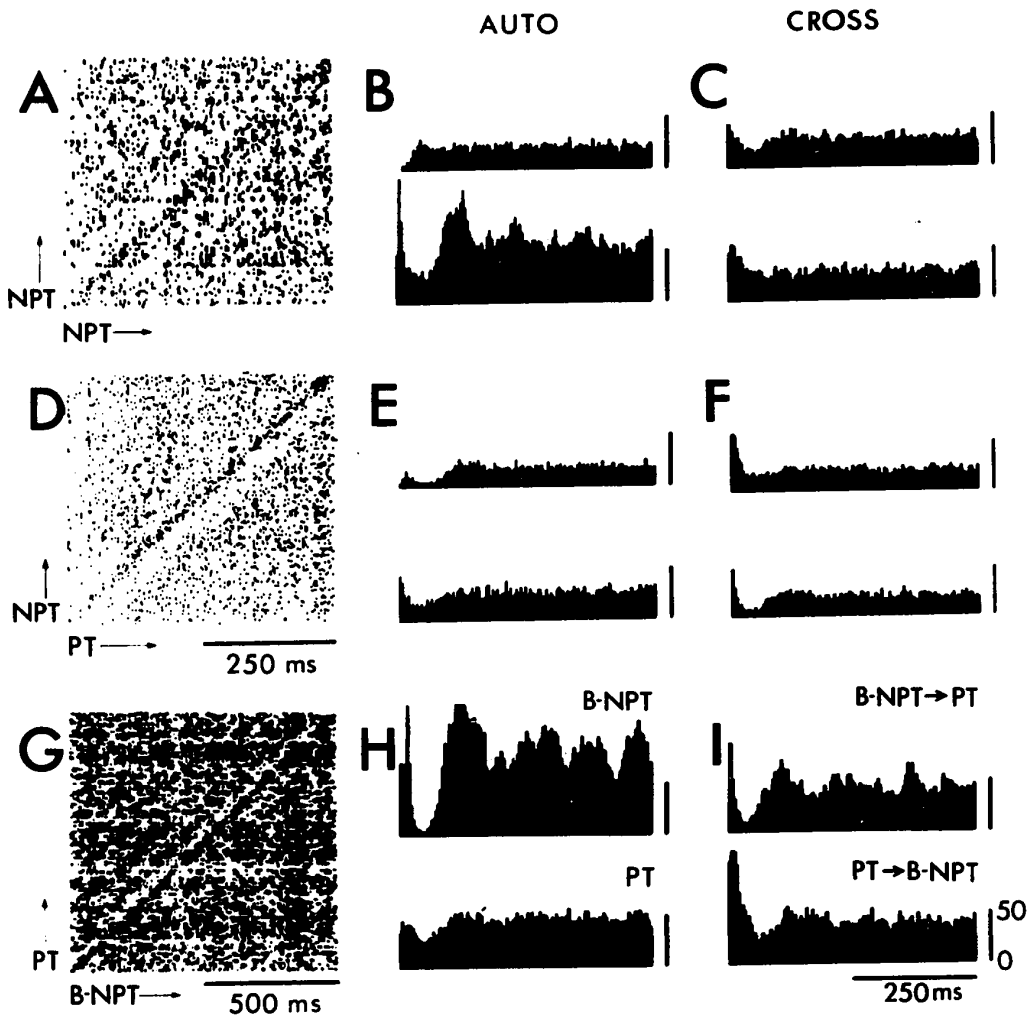

A
SPONT
NPT
PT
B
PYR 5V 1.2T
C
SURF 5V
250ms
D
AUTO
PT
NPT
E
CROSS
PT →NPT
NPT→PT
250ms
50
0
62.5 ms

B. SHORT LATENCY NEGATIVE CORRELATION

Cross-correlations of the spike trains from the 10 pairs of neurones exhibiting the above described scatter diagram pattern yielded negative correlations. In all pairs (see Table 3) examined it was a spike discharge from a NPT neurone which was followed by a temporary decrease in the probability of spike discharges of other PT (8 examples) or NPT neurones (2 examples). The latency of onset of the negative correlation was an important determinant for monosynaptic connections and probably was the single most important extracellular means of verifying that one of the two neurones in fact was inhibitory in function.

A short latency negative correlation for one PT-NPT pair is illustrated in Figure 44. A PT cell was located 0.9 mm below the cortical surface and had an antidromic latency of 4.3 ms (therefore, slow conducting). The NPT cell was estimated to lie 400 μm away in the horizontal plane and situated at a depth of 0.74 mm. The scatter diagram of spontaneous activity (A) showed a less dense region below the 45° diagonal indicating that NPT cell activity temporarily decreased PT cell activity. Cross-correlations were computed at two different bin widths (3.88 ms and 0.97 ms in E and F respectively) on two different 80 second segments of data, during which each neurone discharged more than 1400 impulses. From these the latency of onset of the initial depression (NPT to PT) could be observed to lie between 1.94 and 2.91 ms which, allowing for one synapse (0.80 ms) and an

Figure 45. Other examples of cell pairs exhibiting negative correlation. Three different types of "I type" NPT cells are illustrated. The labels in the bottom series indicate the order of each histogram. A-C: Two postcruciate neurones. The spontaneous activity of one NPT neurone (depth 1.48 mm) closely resembled that of PT neurones (see Chapter 7) and was negatively correlated with the activity of a B-NPT neurone located considerably more superficially (depth 0.55 mm). D.-F. Two precruciate neurones. The spike discharges of one NPT neurone (depth 1.12 mm) were negatively correlated with those of a PT neurone (depth 1.09 mm). G.-I. Two precruciate neurones. The spike discharges of a B-NPT neurone (depth 0.30 mm) appear to be negatively correlated with these of a PT neurone (depth 0.62 mm). The cross-correlation pattern is complex, consisting of a symmetric central peak with asymmetric positive and negative correlations, as well as "secondary" peaks which probably reflect the NPT cell auto-correlation. Note the change in time scale in the scatter diagram. As described in the text, note also that there is a tendency for the neurones in each pair to discharge synchronously. This is reflected as a peak at the onset of each cross-correlogram, and obscures the onset of the negative correlation.


AUTO
CROSS
A
B
C
NPT
NPT
D
E
F
NPT
PT
250 ms
G
H
I
B-NPT
B-NPT→PT
PT
PT→B-NPT
PT
B-NPT
500 ms
250ms
50
0

axonal length of 600 μm or more, would imply a conduction velocity of 0.6 to 1.0 metres per second. Note that the peak depression was not reached until several milliseconds later. The total duration of this initial depression of PT cell excitability lasted just under 80 ms and was followed by a slight enhancement of PT cell activity having a similar time course (Figure 44E). Coincident with the decrease in PT cell excitability was an initial enhancement of NPT cell excitability (PT to NPT) the time course of which paralleled the PT spike probability depression. This suggested some form of loop in which the PT cell discharge resulted in NPT excitation which in turn led to the PT cell depression.

Figure 45 illustrates scatter diagrams and correlograms from three other pairs of cells which also presented negative cross-correlograms in one direction coupled with positive correlation peaks in the other direction. However, in these examples the latency of onset of the effect in either direction was obscured by a symmetrical (central) peak. Similar symmetrical central peaks were detected in cross-correlograms obtained from 45.9% of all neurone pairs (see Chapter 8). This pattern most likely represents a common excitatory influence affecting both neurones and tending to synchronize their discharges. In view of the overall frequency of this pattern, one might expect to find a similar pattern superimposed on asymmetrical positive or negative correlograms. The duration of the troughs in these and the remaining negative correlograms measured from the centre bin of the cross-

TABLE 3

On the left, details of cell type and depth below the cortical surface for neuronal pairs demonstrating negative correlations. All "I type" neurones were the NPT variety, including three whose activity consisted of high frequency bursts of spikes (B_NPT). The activity of both PT and NPT neurones appeared to be decreased ("D" cells) as a result of "I type" neuronal activity. The antidromic spike latency (ASL) of PT neurones is included. Horizontal separation refers to the estimated distance between the tips of the electrodes at the time of recording. On the right are listed the response patterns (when tested) of "I type" neurones following stimulation of four inhibitory pathways; (E) for excitation, (I) for inhibition and (E-I) for an excitation-inhibition sequence.

NEURONE PAIRS EXHIBITING NEGATIVE CORRELATION

Pair	'I' cells		'D' cells			Horizontal	'I' cell response to stimulation			
	Type	Depth(mm)	Type	ASL	Depth(mm)	separation(μm)	PYR	SURF	TC	THAL
C1-8 Pre	NPT	0.66	PT	1.2	0.62	50	E	–	–	–
C1-5 Pre	B-NPT	0.30	PT	2.2	0.62	200	I	–	–	–
C1-15 Pre	NPT	0.68	PT	2.5	0.70	150	I	–	–	–
C5-5 Post	NPT	0.71	NPT	–	1.12	250	I	I	–	E-I
C6-12 Pre	NPT	0.74	PT	4.3	0.99	400	I	E-I	–	–
C6-15 Pre	NPT	1.29	PT	4.0	1.09	400	E-I	I	–	–
C6-17 Pre	NPT	1.12	PT	4.0	1.09	400	E-I	I	–	–
C10-15 Pre	B-NPT	0.49	PT	4.0	0.91	700	I	I	E-I	–
C12-2 Post	NPT	0.46	NPT	–	0.83	150	I	–	–	I
C12-10 Post	B-NPT	0.50	PT	3.0	1.40	200	I	I	–	E-I

correlogram was similar to that obtained from the data in Figure 44, having a mean value of 75 ms (range 55-80 ms). The duration of the asymmetric portion of the positive correlation was usually less than that illustrated in Figure 44 (for example compare the two portions of the correlogram in Figure 45 F and I).

As described in Chapter 5 of the RESULTS, with intracortical microstimulation it was not unusual within a single penetration to find several "I type" neurones converging onto the same PT neurone. Similarly, two neighbouring NPT neurones were found on one occasion to be negatively correlated with the activity of the same PT cell. Thus the two methods have yielded complementary results indicating convergence of the projections of the different "I type" neurones onto the same neurone.

C. CHARACTERISTICS OF "I TYPE" NEURONES

Table 3 contains information pertaining to the neuronal pairs exhibiting these negative correlations. The left-most column identifies the neurones according to the experiment and the cell pairs studied on that occasion as well as their location in the pre- or postcruciate gyrus. The next column lists the "I type" neurones and the cells which exhibited a transient decrease (D type) in the spike discharge probability following "I type" cell spikes. The ASL column refers to antidromic spike latency (in ms) of PT neurones. The Table also contains the estimated separation of the microelectrode tips and responses observed from the "I type"

cells following stimulation of different inhibitory pathways (E, for excitation, I, for inhibition).

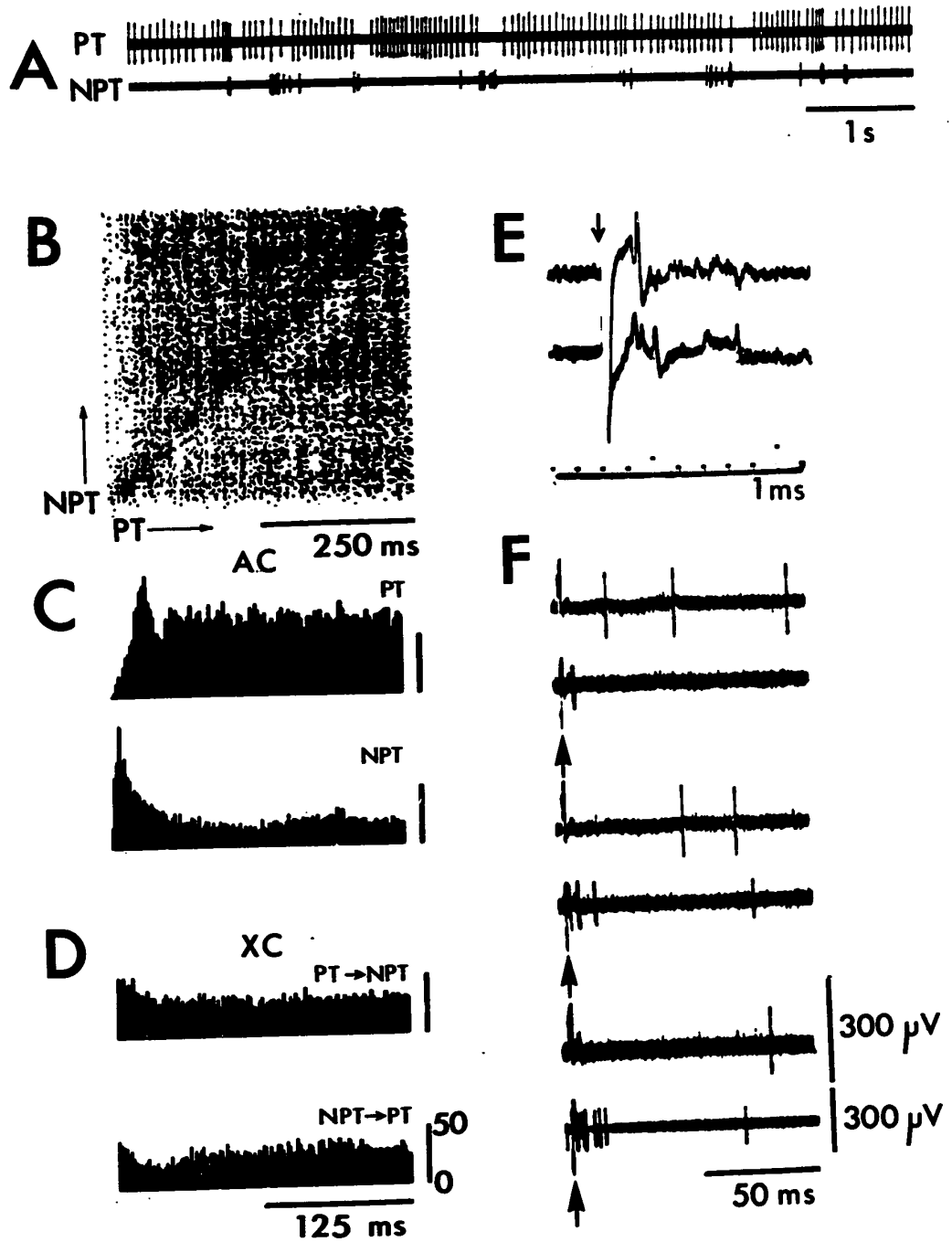
Observations on the activity of the ten "I type" neurones indicated that three were B-NPT cells, displaying irregular spontaneous or glutamate-evoked spike discharges frequently occurring in high frequency bursts of two or more spikes. Two of these are illustrated in Figure 45 (A to C and G to I). The pattern of activity exhibited by the other NPT "I type" neurones resembled the irregular type of activity characterized by most NPT neurones in general (see Chapter 7).

"I type" neurones were usually located near or slightly superficial to the "D type" neurones. Horizontal separations varied from 50 μm to more than 700 μm. Since relatively few penetrations were made with tip separations greater than this, it was impossible to give any estimation of the horizontal spread of the axons of "I type" neurones based on the data available.

D. RESPONSE OF "I TYPE" NEURONES TO STIMULATION OF INHIBITORY PATHWAYS

Despite the long duration of cortical IPSPs it was extremely rare to obtain recordings from cortical neurones which discharged repetitively for more than 20 ms in response to an inhibitory stimulus (see Chapters 3 and 4). Therefore, assuming that some of the cells observed were inhibitory neurones the inhibitory effect is unlikely to be mediated by a mechanism involving prolonged repetitive presynaptic activity of inhibitory interneurones, in contrast to the mode of inhibition generated by Renshaw cells on anterior horn motoneurones and interneurones (Eccles, Fatt and Koketsu 1954; Ryall 1970). Also the inhibitory

Figure 46. Short latency excitation of an "I type" neurone following antidromic pyramidal tract stimulation. A. Continuous oscilloscope tracings of the activity of a PT neurone (upper trace) and a NPT neurone (lower trace) situated less than 50 μm from each other. Note at times the tendency for NPT firing to coincide with interruptions in PT activity. B. Joint scatter diagram of their spontaneous activity illustrating a decrease in the density of points below and an increase in the density of points above the 45° diagonal. C. Auto-correlograms for each cell. D. The correlogram (NPT to PT) reveals a negative correlation lasting approximately 55 ms. E. A single oscilloscope tracing following pyramidal stimulation (arrow). The upper trace contains the PT cell antidromic spike. The NPT neurone response consists of a series of spikes, the latency of the first spike always being longer by at least 0.7 ms than the PT antidromic spike latency. So close are the two recording electrodes that the spikes of each neurone are visible in both traces. F. Three traces at slower sweep speeds with progressively increasing stimulus strength of pyramidal tract stimulation from above downwards (0.2V per frame). With each increase there occurs a more pronounced burst of activity from the NPT neurone (lower trace) associated with an increase in silent period in PT cell activity following the antidromic spike.


A
PT
NPT
1 s
B
NPT
PT
250 ms
AC
C
PT
NPT
D
XC
PT→NPT
NPT→PT
50
0
125 ms
E
1 ms
F
300 μV
300 μV
50 ms

effect is too long to be accounted for by the time constant of cortical neurones (Nacimiento, Lux and Creutzfeldt 1964; Creutzfeldt, Lux and Watanabe 1966; Klee 1966). The most likely explanation is that there is a prolonged interaction between the inhibitory transmitter and the postsynaptic membrane resulting from either delayed inactivation, uptake or diffusion processes (Krnjević, Randić and Straughan 1966b; Iversen and Neal 1968). Electrophysiological observations adding indirect support to this theory were obtained during the present experiments. For example, Figure 46A illustrates the spontaneous activity of two cortical neurones which at times appeared to be reciprocally related. Quite frequently bursts of activity from a NPT neurone (lower trace) located less than 50 μm away from a PT neurone (upper trace) were associated with definite silent periods in the PT cell discharge pattern.

Figure 46B indicates that this was more than just a casual relationship, since scatter diagrams revealed a decreased density of points below the diagonal and on the cross-correlogram a slight but definite trough (Figure 46D, lower correlogram) for NPT to PT with a duration of 55 ms. As with certain other examples previously described, the onset of this decreased probability of PT cell discharge was obscured by a low amplitude central peak in the cross-correlogram.

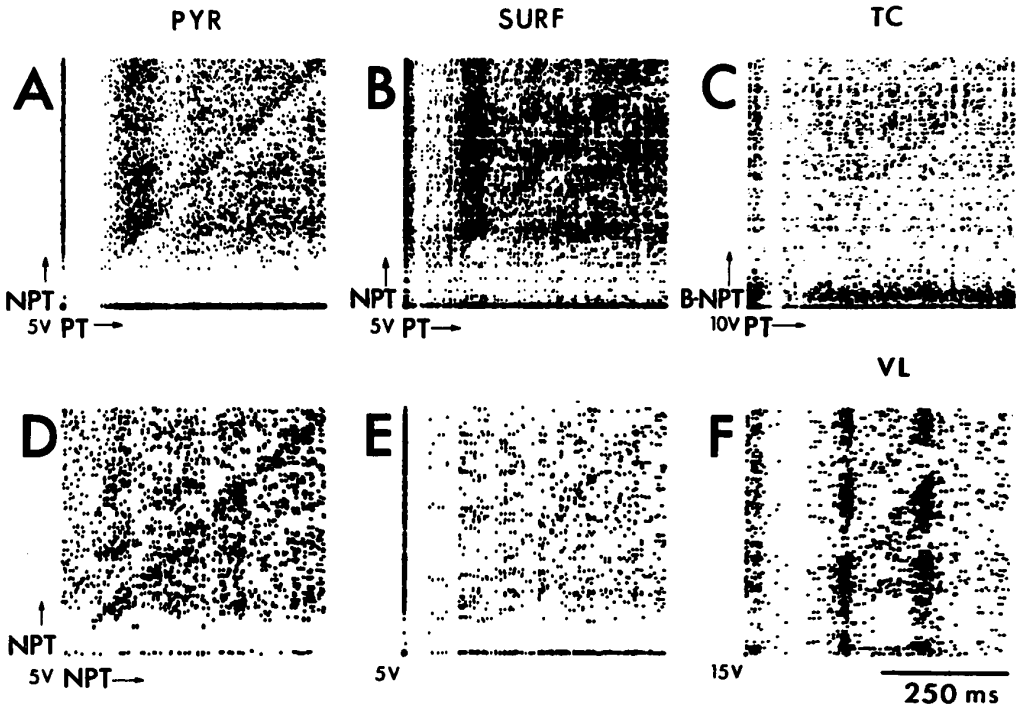
In response to stimulation of the medullary pyramids, the single sweep oscilloscope traces in Figure 46E reveal that the NPT cell responded with a burst of spikes (lower traces) in

comparison with the single antidromic PT spike. Although the latency of the first NPT cell spike was variable, even from sweep to sweep despite any change in the stimulus intensity, it always occurred later than the PT cell antidromic spike (latency 1.2 ms). The shortest time interval between the two spikes was 0.7 ms, sufficient for little more than one synaptic delay (Eccles 1964) and, therefore, consistent with monosynaptic excitation of the NPT cell over the pyramidal recurrent axon collateral pathway.

In the 3 lower examples in Figure 46F photographed at slower sweep speeds, step-wise increases of 0.2V in the pyramidal stimulus strength (from above downwards) induced (i) a progressive increase in duration of the pause in the PT cell spike discharge following the antidromic spike and (ii) an increase in the number of NPT neurone spike discharges per response, as well as the more consistent appearance of NPT initial spikes at the shortest latency (1.9 ms).

In summary the reciprocal activity patterns exhibited by these neighbouring PT and NPT neurones during spontaneous and evoked activity coupled with evidence of a short latency negative correlation (NPT to PT) would favour the identification of the NPT neurone as an inhibitory neurone in the recurrent pyramidal axon collateral pathway. Moreover, a short burst of spikes at high frequency would appear to characterize the activity of at least one type of inhibitory cortical neurone.

Figure 47. Response of "I type" neurones to various inhibitory stimuli. Joint scatter diagrams illustrating the direction of negative correlation and the response of both neurones to stimulation of inhibitory pathways. A, B. Data from neurone pair (C6-12 Pre in Table 3). Both PYR and SURF stimulation evoke a short latency inhibition of the NPT "I type" neurone. Surface evoked inhibition is preceeded occasionally by excitation. C. Neurone pair (C1-8 Pre) in Table 3. Only TC stimulation evokes longer latency excitation of this B-NPT neurone, followed by a decreased excitability. D. E. F. Cell pair (C5-5 Post). The NPT "I type" neurone corresponding to the horizontal arrow shows weak inhibition from PYR but clear inhibition following SURF. VL stimulation evokes a weak but prolonged excitation followed by inhibition and two post-inhibition excitation peaks (dark vertical lines in F). Note that the area of decreased point density above the diagonal is accentuated by these postinhibition excitation peaks.

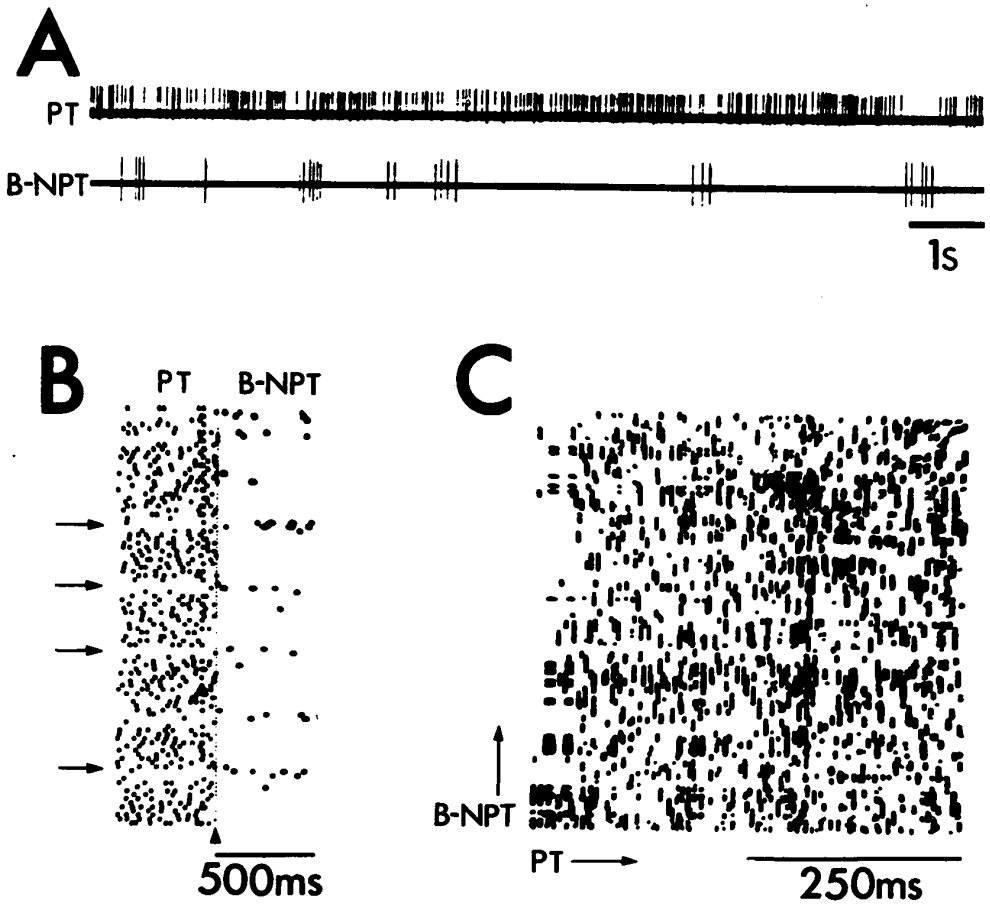

PYR
SURF
TC
A
B
C
NPT
5V PT
NPT
5V PT
B-NPT
10V PT
VL
D
E
F
NPT
5V NPT
5V
15V
250 ms

E. STIMULATION RESPONSE OF OTHER "I TYPE" NEURONES

Although excitation of any particular NPT "I type" neurone followed activation of one inhibitory pathway, a glance at Table 3 will indicate that this did not necessarily imply a similar response from stimulation of other inhibitory pathways. Results from actual experiments are illustrated in Figure 47. Scatter diagrams in A or B obtained from the same pair of neurones (C6-12 pre) exhibit short latency inhibition of the "I type" NPT cell both from pyramidal and surface stimulation. In C, from another pair of neurones (C10-15 pre) only transcallosal stimulation evoked longer latency excitation in this B-NPT "I type" neurone while the pattern with pyramidal stimulation was not unlike that shown in A. D to F were obtained from the same cell pair (C5-5 post). The NPT "I type" neurone activity is plotted in the horizontal direction and illustrates weak excitation followed by inhibition only in response to thalamocortical stimulation (F), whereas pyramidal stimulation (D) evokes only a weak inhibitory response and surface stimulation (E) evokes a prominent inhibitory response. Note that in F during the post-inhibitory excitatory sequences (dark vertical bars) there is enhancement and sharpening of the margin along which the decreased point density above the diagonal becomes apparent.

According to these results it appears that most "I type" neurones are excited preferentially by only particular forms of inhibitory stimuli. In most cases this initial excitation is followed by inhibition. Other inhibitory stimuli usually evoked only inhibition of spike discharges.

Figure 48. Reciprocal activity without negative correlation. A. Pauses in spontaneous activity of a PT neurone (upper) appears to be reciprocally related to the activity of a NPT neurone (lower trace). B. Raster diagram of the same neurones indicating that these pauses in PT activity are also visible (arrows) and correspond with NPT cell activity. C. Despite the visual impression that there may be a negative correlation on the continuous film traces (A), the joint scatter diagram reveals an irregular non-specific pattern.


A
PT
B-NPT
1s
B
PT
B-NPT
500ms
C
B-NPT
PT
250ms

F. RECIPROCAL ACTIVITY WITHOUT NEGATIVE CORRELATION

Occasionally during recording of the activity of pairs of cortical neurones one encountered cells whose activity appeared reciprocally related, but which did not produce negative correlograms. Figure 48 is one example in which a spontaneous discharge of a PT cell (A, upper trace) was interrupted now and again, and these interruptions were temporally associated with increases in activity of an NPT neurone (lower trace). Raster diagrams illustrated the same (arrows in B) although no distinct pattern was seen on the scatter diagram (C) and the cross-correlogram (not illustrated) was flat indicating no correlation.

Chapter 7

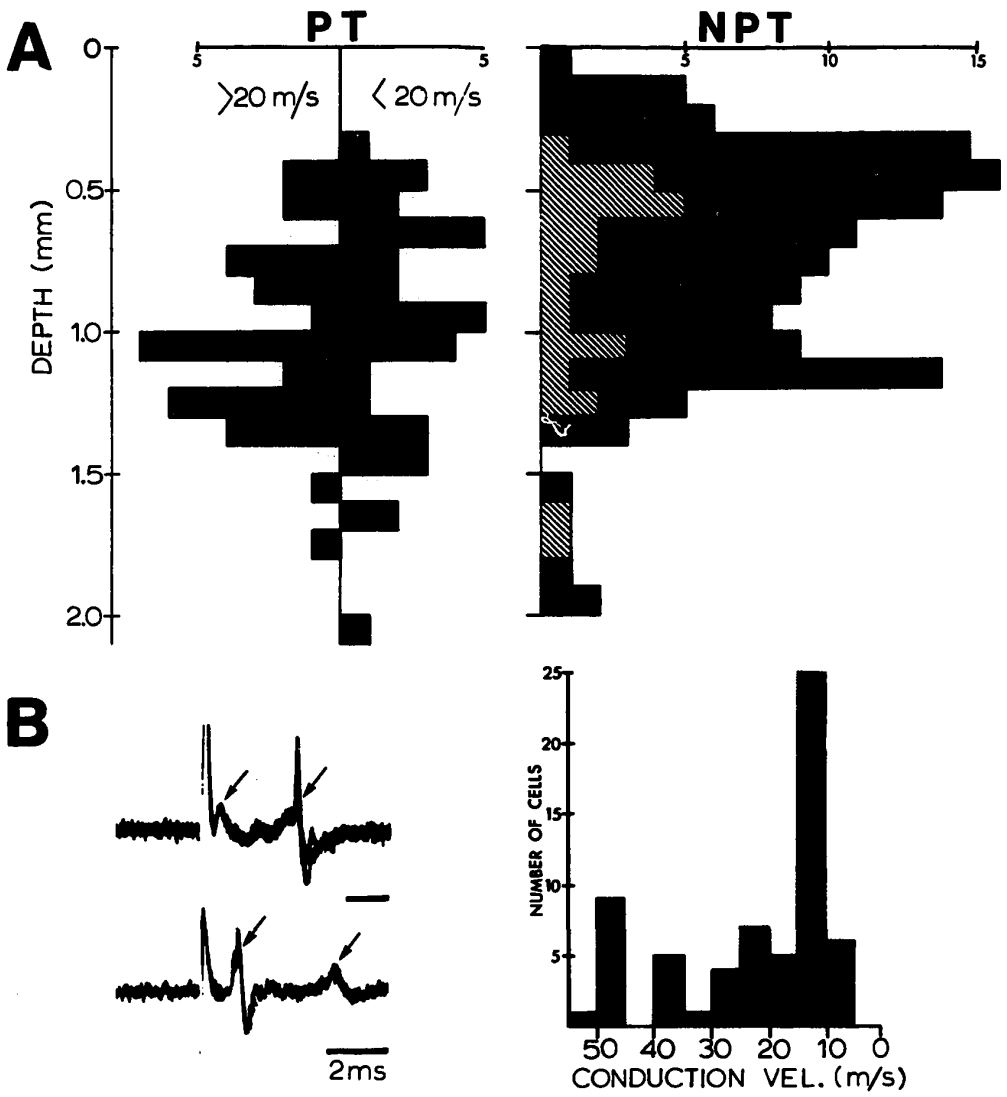
RESULTS

CHARACTERISTICS OF PERICRUCIATE NEURONES

A. PT NEURONES: DEPTH DISTRIBUTION AND CONDUCTION VELOCITIES

Seventy-eight pericruciate neurones fulfilled the criteria defined under METHODS for identification of PT neurones. Ninety-four percent of these were located in the lateral precruciate gyrus (area 4 of Hassler and Muhs-Clemente 1964). Figure 49A illustrates their depth distribution in mm below the cortical surface. Following a pyramidal tract stimulus, their latencies for antidromic invasion varied from 0.7 ms to 5.0 ms, which would allow for conduction velocities ranging from 66 metres per second for the fastest to 8 metres per second for the slowest PT axons, measured over the 40 mm distance (range 38 to 42 mm, measured in five brains) separating the recording and stimulating electrodes. Figure 49 also gives the PT neurone frequency distribution based on depth (A, left side) and conduction velocity (B, right side) for fast (greater than 20 ms^{-1}) or slow (less than 20 ms^{-1}) PT fibres. From this population it appears that neither type of distribution yields to a clear separation between fast and slow PT neurones. Therefore, in contradiction to earlier suggestions (c.f. Towe, Patton and Kennedy 1963), it would appear that the depth below the

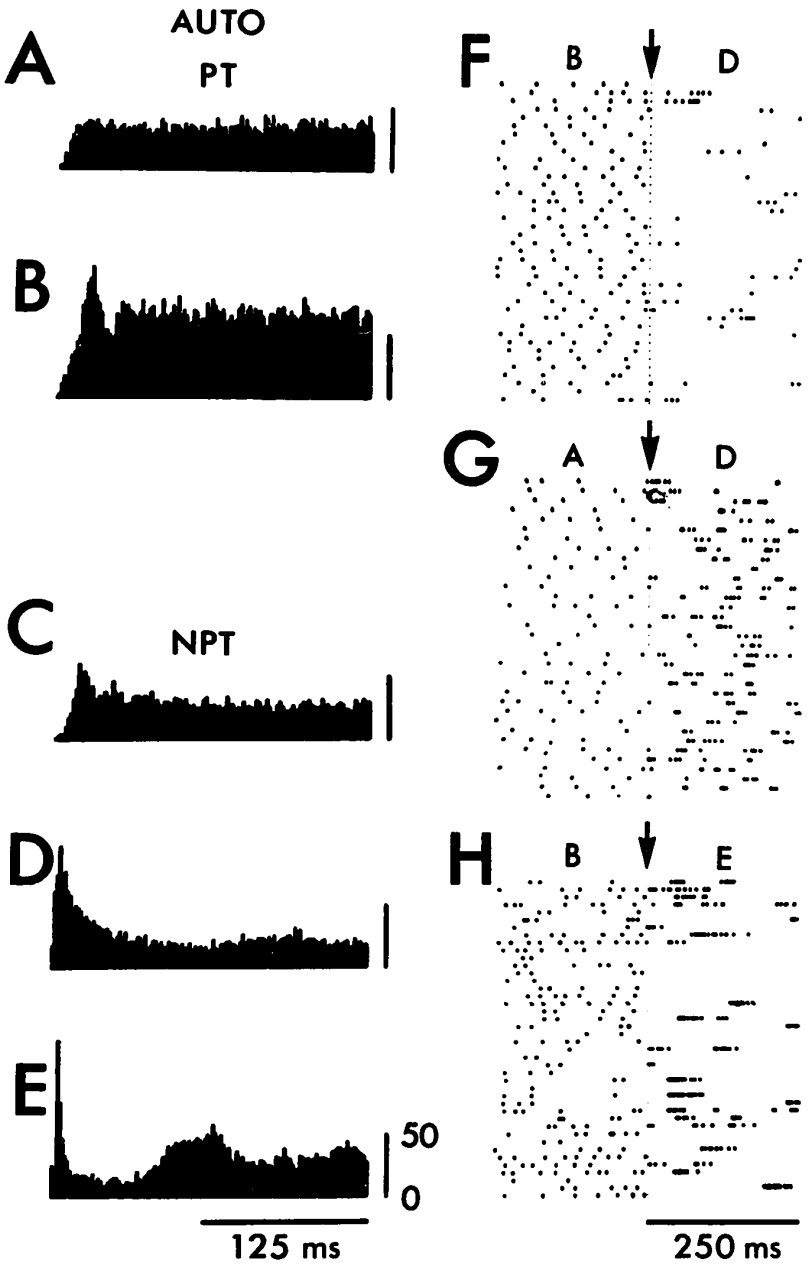
Figure 49. Frequency distribution histograms of cortical neurones. A. Depth distribution of PT and NPT neurones below the cortical surface. PT neurones (on the left) were plotted according to whether their axons conducted faster or slower than 20 meters per second. The histogram revealed no clear difference in the distribution of 'fast' and 'slow' elements. NPT neurones (on the right) were found in all cortical layers, but mainly above 1.4 mm. The small number of deeper neurones reflects the inability to reach these layers with two closely apposed electrodes (see METHODS). Diagonal hatching indicates those neurones classified as B-NPT type. B. On the left, oscilloscope traces illustrating antidromic action potentials of slow (upper) and fast (lower) conducting PT neurones. Arrows point to the early and late negative field potentials observed to follow pyramidal tract stimulation. On the right, the frequency distribution of PT neurones according to the conduction velocity of their axons (in meters per second).


A
PT
NPT
5
5
5
10
15
>20 m/s
< 20 m/s
DEPTH (mm)
0
0.5
1.0
1.5
2.0
B
2ms
NUMBER OF CELLS
25
20
15
10
5
50 40 30 20 10 0
CONDUCTION VEL. (m/s)

cortical surface is not a valid criterion to distinguish whether PT neurones may be of the fast or slow conducting type (c.f. Naito et al. 1969). Latency histograms for antidromically fired PT cells also fail to show any distinct peaks. The population of PT neurones sampled is probably too small to visualize a clear separation between fast and slow conducting cells (but c.f. Towe et al. 1963; Humphrey 1968b).

However, the intracortical evoked response to a pyramidal tract shock does reveal two distinct components (Figure 49B, left side, arrows) having peak latencies of approximately 1.0 and 4.8 ms, sometimes with superimposed PT antidromic spike potentials. Two components in the cortical evoked responses have been noted in earlier studies (Jabbur and Towe 1965; Kubota et al. 1965; Takahashi 1965; Humphrey 1968). When recorded with microelectrodes at a depth in the cortex where PT cells were penetrated, Takahashi (1965) observed that stimulation of the pyramidal evoked a field potential which consisted of two negative deflections (his N_1 and N_2). The times to the peaks of these waves were very similar to those found in the present study. Takahashi concluded that these were field potentials of the two groups (fast and slow) of antidromically invaded PT cells. Other authors have concluded that the evoked potentials are a reflection of more complex events, and are formed by contributions from post-synaptic potentials as well as antidromic spike potentials (Gorman and Silfvenius 1967; Humphrey 1968a). Therefore, it would be unwise for the present to relate any particular unitary

Figure 50. Spike discharge patterns of cortical neurones. The left hand column illustrates auto-correlograms computed for PT (A,B) and various types of NPT neurones (C,E,F). The right hand column (F,G,H) contains raster diagrams of PT-NPT cell pairs. The left half of each diagram illustrates the pattern of activity of different PT neurones, the right half the activity pattern of NPT neurones. The letters above each raster refer to the auto-correlogram pattern shown in the left hand column which corresponds to that particular type of neuronal activity. Note the more uniform pattern for PT neurones as compared to the generally irregular pattern for NPT neurones.


A
AUTO
PT
B
C
NPT
D
E
50
0
125 ms
F
B
D
G
A
D
H
B
E
250 ms

event (spikes, postsynaptic potentials) to the evoked potentials.

B. NPT NEURONES: DISTRIBUTION

All non-pyramidal cortical neurones examined were considered as a single group. Presumably this included neurones whose axons remained purely intracortical (e.g. stellate neurones) as well as corticofugal elements projecting to areas of the contralateral or association cortex, subcortical nuclei, etc. Neurones comprising this group were found in all cortical layers but most abundantly between 0.5 and 1.3 mm (Figure 49A, histogram on the right), and were further classified according to the discharge patterns (see below).

C. PATTERNS OF NEURONAL DISCHARGE

PT neurones. In the present series of experiments, performed on cats under light nembutal anesthesia, it was frequently observed that the spike discharges recorded from PT neurones appeared more randomly distributed and were relatively free of very short or very long interspike intervals when compared with the spike discharges recorded from NPT neurones. Auto-correlograms for most PT neurones revealed a pattern consistent with random discharges (Figure 50A, and left half of the raster diagram in G), remaining stable during recording periods on occasion exceeding 60 minutes.

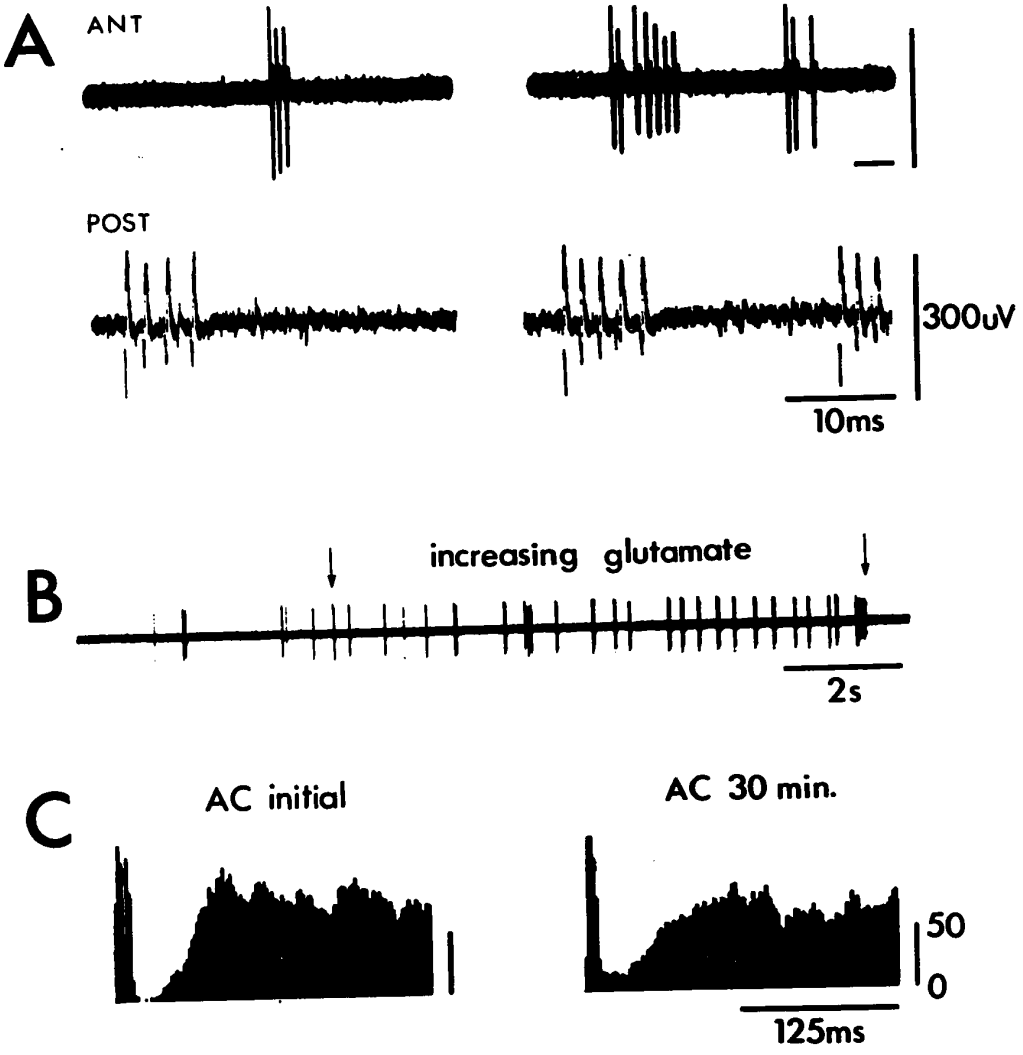
Less commonly, some PT neurones displayed interspike intervals which were quite regular, interrupted by sequences of apparently random discharges (Figures 46A; 50F,H). This activity

was reflected in auto-correlograms as an initial peak (Figures 46C; 50B). Occasionally, PT neurones which did not appear to present discharge patterns different from any other PT neurones on visual inspection, yielded auto-correlograms with an initial trough, such as the example seen in Figure 45E in chapter 6. The significance of this transient altered probability in the appearance of consecutive spikes is obscure, since it was seen so infrequently. In view of the time course of this trough, one possible explanation might be that certain PT neurones activate local inhibitory neurones monosynaptically over recurrent axon collaterals, and these in turn feed-back to the same PT cell, causing a transient decrease in membrane excitability. The contour in the auto-correlogram pattern (initially rising, then forming a temporary trough) may be caused by the time taken to activate the inhibitory mechanism.

NPT neurones. The spontaneous activity and auto-correlograms of 16 NPT neurones (12.1%) appeared to be very similar to those of the majority of PT neurones. Some of these could have been PT neurones whose axons were not activated by pyramidal tract stimulation, although only 6 of the 16 responded to a PYR shock with a short latency silent period suggestive of PT neurone responses (Chapter 4, Figure 28A).

Eighty-three NPT neurones (62.9%) were noted to have a more irregular pattern of discharge consisting of single spikes

Figure 51. Spontaneous high frequency burst activity from some B-NPT neurones. A. Spontaneous action potentials appearing as bursts of spikes at high frequency recorded from pericruciate B-NPT neurones located anterior or posterior to the cruciate sulcus. Note the different sweep speeds. B. Gradually increasing the amount of glutamate applied iontophoretically caused this B-NPT neurone to discharge more frequently, but always in an irregular burst fashion, sometimes with an increase in the number of spikes per burst (for example the last burst before the second arrow). C. Auto-correlograms obtained initially and 30 minutes later, indicating little change in the pattern of glutamate-evoked activity of the B-NPT neurone shown in (B).


A
ANT
POST
300uV
10ms
B
increasing glutamate
2s
C
AC initial
AC 30 min.
50
0
125ms

intermingled with discharges at varying frequencies. Figure 50C and D illustrate examples of their auto-correlograms, while the right hand raster diagrams in Figure 50F and G illustrate their discharge patterns.

The activity of 33 other NPT neurones (25.0%) was characterized by irregularly appearing bursts of 2-10 spikes at frequencies approaching 600 per second (Figure 51A). Almost without exception the first spike in a burst was of larger amplitude than subsequent spikes. Auto-correlograms characteristic of the activity patterns of these neurones are seen in Figures 50E and 51C, with the corresponding raster diagrams shown on the right side in Figure 50H. These "burst type" (B-NPT) neurones were observed in both superficial and deeper cortical layers (Figure 49A, diagonal hatching). B-NPT neurones were generally less active than PT cells and other NPT neurones, and could easily have gone unnoticed on several occasions if glutamate had not been applied iontophoretically in small doses as the microelectrodes were advanced into the cortex. Their response to glutamate varied from neurone to neurone but was usually quite typical: they were slower to respond to a glutamate application, often requiring higher iontophoretic currents than most other cortical neurones (no quantitative data available); once active these B-NPT cells were more susceptible to glutamate induced excessive depolarization and loss of recordable action potentials.

Their spontaneous or glutamate-evoked spike discharges continued to exhibit a bursting character, despite long periods of recording (Figure 51 B,C), and advancement of the recording microelectrode. Impalement resulted in injury discharges not unlike those recorded from other CNS neurones. Due to these irregular 'bursts', polygraph recordings of their activity displayed a rather bizarre appearance (Figure 37B in Chapter 5) which made them less suitable for pharmacological studies compared with more regularly discharging neurones. Nine of these neurones were tested for their response to ACh; none were excited and in fact most appeared to be depressed by ACh applications of 30-100nA (c.f. Figure 37B in Chapter 5).

The response of some B-NPT neurones to stimulation of inhibitory pathways has been described earlier (Chapter 4). In general their response consisted of bursts of spikes rather than single action potentials, and their excitatory responses tended to be more prolonged in comparison to other NPT neurones.

Chapter 8

RESULTS

CORRELATIONS BETWEEN DISCHARGES RECORDED FROM CORTICAL PYRAMIDAL AND NON-PYRAMIDAL NEURONES

The introduction of techniques for recording the activity of individual neurones in the cerebral cortex (Amassian 1953; Jung 1953) has permitted a more detailed examination of the relationship between neurones themselves, and particularly how they function as part of a neural network. Attempts to understand the formation of the EEG have revived earlier theories that Berger's rythm arises as a summation of extracellular currents from many individual neurones, located in the various layers of the cortex (Purpura 1959; Amassian 1961). Theories relating the EEG to 'synchronized' activity of many neurones have been supported to some extent by extracellular recordings of unitary action potentials (Li and Jasper 1953; Bremer 1958; Verzeano and Negishi 1961; Creutzfeldt 1963), but a clearer relationship can be established if intracellular postsynaptic potentials, instead of action potentials, are compared with the slower potentials either at the cortical surface or within its depths (Sugaya et al. 1964; Klee et al. 1965; Jasper and Stefanis 1965; Creutzfeldt et al. 1966a,b; Frost 1968).

Table 4

Results of cross-correlation analysis performed on the activity of pairs of closely apposed cortical neurones. Data compiled from 110 pairs of neurones in both the pre- and postcruciate cortex under Nembutal anaesthesia. See text for further explanations.

Correlation Patterns - All Cell Pairs				
Pair type	NPT-NPT	PT-NPT	PT-PT	Total
NO CORRELATION	11	27	4	42 (38.2%)
+ve CORRELATION				
symmetric	19	27	4	50 (45.5%)
asymmetric	3	4	1	8 (7.2%)
−ve CORRELATION				
symmetric	0	0	0	−
asymmetric	3	7	0	10 (9.1%)

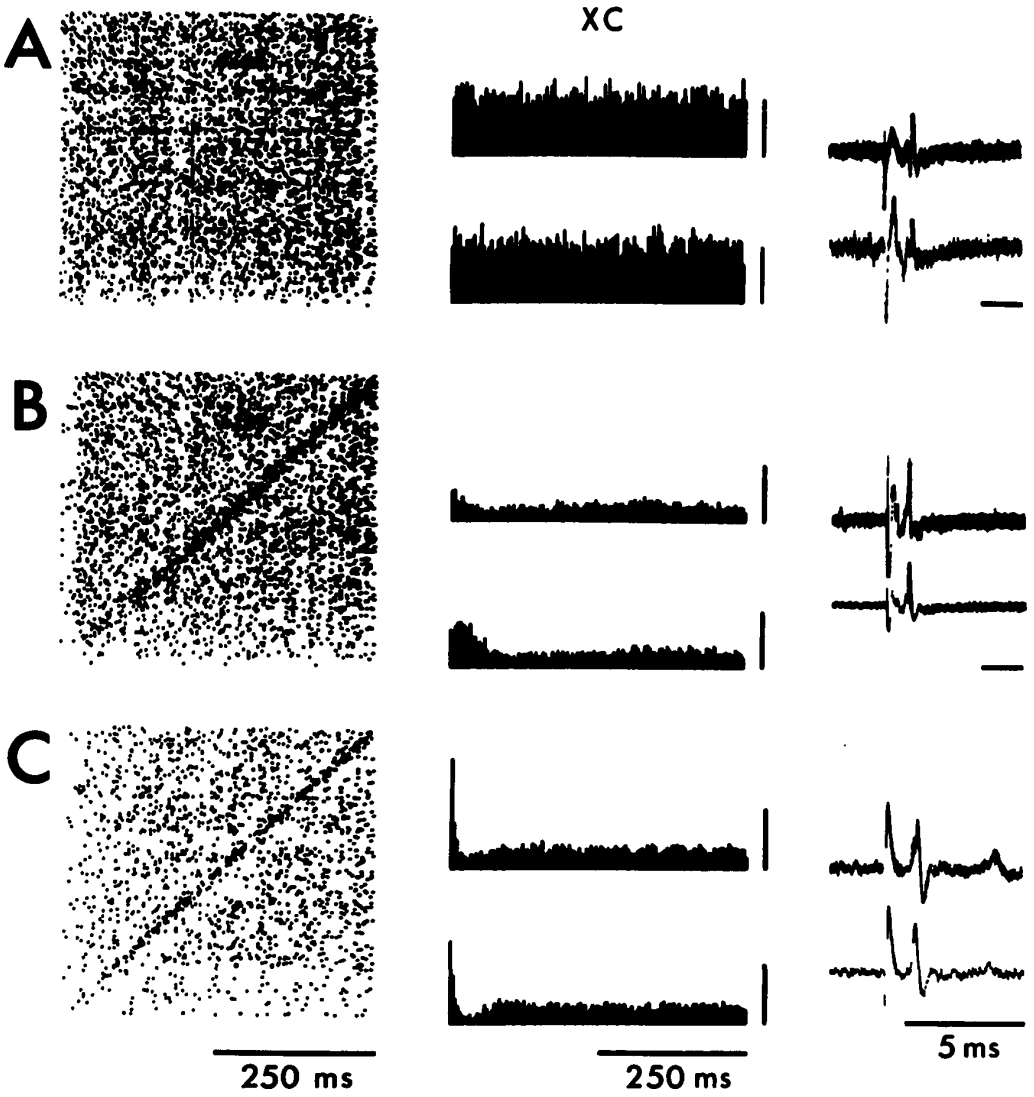
Although there have been estimates of the percentage of neurones which must display synchronized neuronal activity in order to produce a surface potential (Elul 1967, 1968), there has been little experimental data concerning the degree of synchronization actually present between two or more cortical neurones recorded simultaneously. Some recent results point out that the degree of correlation which exists between spontaneous activities of pairs of neurones displays a certain plasticity, changing with different behavioral states (Noda et al. 1969, 1970) or anaesthetic conditions (Holmes and Houchin 1966).

This chapter relates to the correlation patterns observed for neurone pairs with short interneuronal distances and under conditions of light Nembutal anaesthesia. Some of the findings have already been described in Chapter 6. The results are summarized in Table 4.

A. NO CORRELATION

Spike discharges from 38.2% of neuronal pairs formed joint scatter diagrams with a uniform distribution of points and cross-correlograms with a flat contour, indicating that discharges from the neuronal pair were independent of each other (Perkel et al. 1967; Gerstein and Perkel 1969). One example of this pattern is seen in Figure 52A. The locations of the neurones forming this group, and the estimated distances between the cells themselves were not different (significantly) from the same measurements made on neurone pairs which were

Figure 52. Scatter diagram and cross-correlogram patterns observed for pairs of PT neurones. A. Independent activity of two PT neurones results in a scatter diagram with a uniform distribution of points and a flat cross-correlogram. B. One example of an asymmetrical positive cross-correlation. A high density of points appears on the 45° diagonal of the scatter diagram. The cross-correlogram clearly reveals the asymmetry of this distribution. C. Highly correlated activity of another pair of PT neurones results in a high density of points on the 45° diagonal of the scatter diagram, and a sharp initial peak in each cross-correlogram. Column at the right illustrates the response of these PT neurones to a single pyramidal tract shock. Time bar represents 5 ms in each row. Vertical bar to the right of each correlogram represents 50 counts per bin.


A
B
C
XC
250 ms
250 ms
5 ms

positively correlated. In other words, some neurones 100-150 μm apart were found to have totally independent activity patterns. This statement deserves some qualification: it is possible that cross-correlograms with larger values of "T" (the usual value used here was 500 ms) might have revealed that the cells' activities were not totally independent (c.f. Holmes and Houchin 1966, where values of "T" up to 5 seconds were used). If there was in fact such a low-grade force tending to synchronize their activities, it was not seen with the present method.

B. POSITIVE CORRELATION

52.7% of neuronal pairs displayed an increase in the density of points along the 45° diagonal on the joint scatter diagram and a peak in the cross-correlogram.

Symmetric Correlograms. Most of the positive correlograms (45.5%) were more or less symmetrical about the origin. Examples are found in Figures 52C and 56A. Not all cross-correlogram peaks were as narrow and as marked as in these figures (c.f. Figure 53B). Nevertheless, the significance of this finding was that 45.5% of neurone pairs did exhibit a tendency to have spike discharges which appeared synchronously, and these were intermingled with another population previously described which appeared to be independent of each other. Of course, it must be recalled that only two of a large number of neurones were being examined at once, and that any individual neurone is related perhaps to hundreds of other neurones

(through axons and axon collaterals, similar afferent connections, etc) which are not being sampled. Reasons for synchronized discharges among cortical neurones will be considered in the DISCUSSION.

Asymmetric Correlograms. A separate category was created for those neurone pairs which exhibited a positive correlogram but which was asymmetric about the origin. One example appears in Figure 52B. Such a pattern implies that a spike discharge from one unit facilitates the appearance of a spike from the second unit (i.e. excitation) and resembles the converse pattern (negative correlogram) described in Chapter 6, which frequently was coupled with an asymmetric positive correlogram. The onset of the asymmetric peak in the correlograms implied that the excitatory effect was of a short latency, which could be produced by both PT and NPT neurones onto other neurones of a similar kind. Some of the asymmetric peaks seemed to be superimposed on a symmetrical central peak in the correlograms and might be interpreted in another way i.e. that both cells are subject to a common excitatory source but with a conduction delay (axon or synapse) before this influence reaches one of the neurones.

C. CORRELATION BETWEEN PAIRS OF PT NEURONES

Nine pairs of PT neurones were examined for correlation. Values for their depths below the cortical surface, and the latency of their antidromic action potential following a medullary pyramidal tract shock are listed in Table 5. Cross-correlation

analysis resulted in three patterns: four pairs of cells showed a strong tendency for spike discharges to appear synchronously (Figure 52C); in four other pairs the analysis revealed their spike discharges to be independent (Figure 52A); there was only one pair which displayed an asymmetric positive correlogram (Figure 52B). Negative correlograms were not observed after spike discharges from PT neurones. The one exception is shown in Figure 52C where the marked central peak in each correlogram is followed by a trough, most marked in the lower (PTb-PTa) of the two histograms. After artificially increasing the spike discharges of the PTb neurone (Figure 56B) the symmetric central peak remained but the trough was no longer evident. If there had been a monosynaptic inhibitory influence of PTb on PTa activity, one might have expected an enhancement of the trough under these circumstances. An alternative explanation would have PTb (and possibly PTa also) activating a recurrent inhibitory mechanism which feeds back to both neurones (disynaptically) but is more effective (in the spontaneously firing condition) on PTa. However, glutamate activation of PTb (Figure 56B) would be expected to enhance the inhibition, while in reality the 'apparent inhibition' is no longer seen. Another possible explanation would be a common excitatory source also activating an inhibitory pathway to PTa. Since many of these are very speculative it is best to simply concentrate on the central event for the present time.

The cross-correlogram pattern in Figure 52B is of interest since it implies that one PT neurone can facilitate spike discharges from another neighbouring PT cell. EPSPs have

Table 5

Data related to pairs of PT neurones whose spikes were subjected to correlation analysis. Both the depths of the neurones and antidromic latencies are tabulated. The right hand column contains the results of the correlation. The activity of the upper four pairs of PT neurones, which tended to have faster axonal conduction velocities, were positively correlated. The lower four pairs of PT neurones consisted of cells with slow axonal conduction velocities, and the spike activity of all pairs was apparently independent since the cross-correlogram was flat. Only one pair of PT neurones revealed an asymmetrical, positive correlogram. Examples of each of the three correlation patterns are illustrated in Figure 52.

PT-PT CROSS- CORRELATION				
PTa		PTb		CORRELATION
DEPTH(mm)	A.LATENCY(ms)	DEPTH (mm)	A.LATENCY(ms)	
1.17	1.2	1.26	1.3	+
1.40	2.7	1.26	1.3	+
0.90	–	0.92	–	+
1.37	1.1	1.30	0.8	+
0.86	2.8	1.38	3.0	+ b→a
1.37	2.8	1.31	3.0	0
1.10	3.2	1.15	3.0	0
1.10	3.2	1.18	4.0	0
0.77	3.0	0.80	4.0	0

been observed in PT neurones following pyramidal tract stimulation (e.g. Stefanis and Jasper 1964a; Armstrong 1965) and Takahashi et al. (1967) have presented evidence to suggest that slower conducting PT neurones facilitate faster conducting PT neurones. Therefore, it is not an unexpected finding that some correlograms should reflect these features.

A glance at Table 5 suggests that positive correlations were found between pairs of fast conducting PT cells while no correlation exists between slowly conducting PT neurones. Although of interest from the standpoint of the intercortical functional relationships between individual corticospinal neurones, the small sample size precludes any definitive statements.

D. VARIABILITY IN CORRELATION PATTERNS FOR DIFFERENT PAIRS OF NEURONES

An advantage to the use of two separately mobile micromanipulators was the ability to position one electrode near a neurone and move the neighbouring electrode over distances of several hundred micra, thereby, recording from a number of neurones and relating their activity to the one neurone recorded with the other electrode. In some instances the correlation pattern changed dramatically either from one which showed no correlation to one with some positive features (Figure 53A,B) or from a condition in which cells tended to discharge synchronously to one where there appeared to be no relationship between the spike trains (Figure 54).

Figure 53. Change in the correlation pattern following advancement of one microelectrode. A. The scatter diagram and cross-correlograms obtained from the spike discharges of two NPT neurones located at depths of 1.20 and 1.27 mm reveal no significant interaction between their spike discharges. Note the periodicity at approximately 10/S in the upper auto-correlogram (cell a). B. Following a 0.09 mm advancement of electrode b, the activity of a new NPT neurone was found to be positively correlated with the activity of the same NPT neurone a. However, the peak of the positive correlation is broader than that seen in the previous figure. In this case both cells show a periodicity in their auto-correlogram.

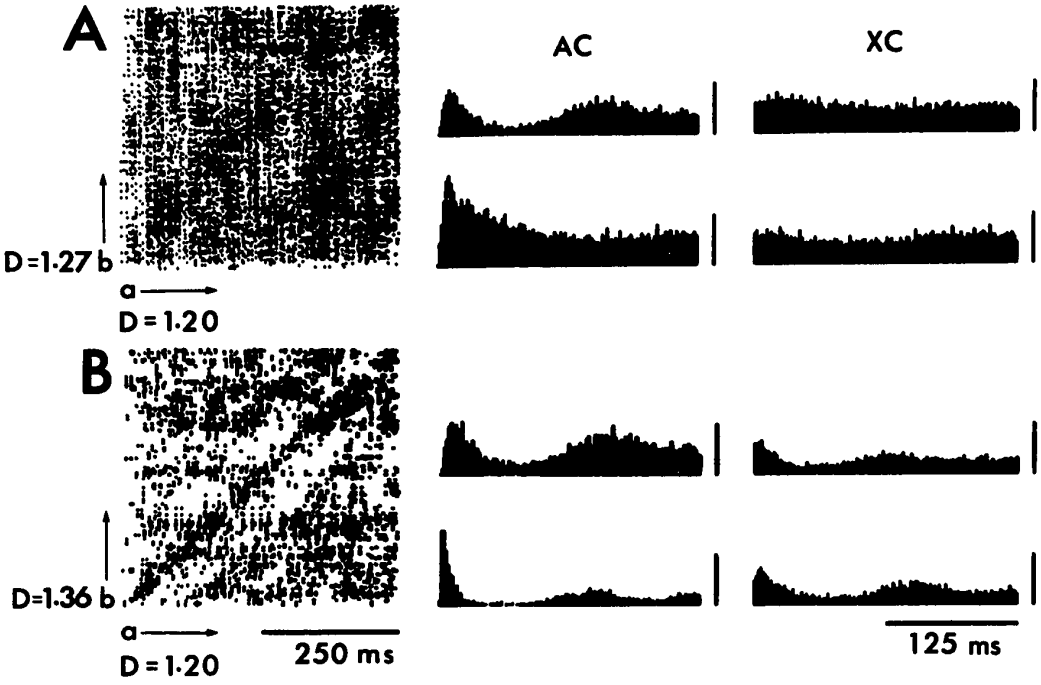

A
AC
XC
D=1·27 b
a
D=1·20
B
D=1·36 b
a
D=1·20
250 ms
125 ms

Figure 54. Change in the cross-correlogram pattern with movement of one electrode to record the activity of different NPT neurones. Scale on the left represents the depth in mm below the cortical surface. Electrode a continued to record the activity of one NPT neurone while electrode b was advanced sequentially to record the activity of different NPT neurones (1,2 and 3). There was little change in the auto-correlogram of cell a while the auto-correlograms derived from the activity of the three neurones recorded with electrode b showed some variability. Even more variable were the patterns in the scatter diagram and cross-correlogram showing a marked positive correlation in the first row but no correlation in the bottom row. The two microelectrodes were separated by approximately 250 mm in the precruciate gyms.

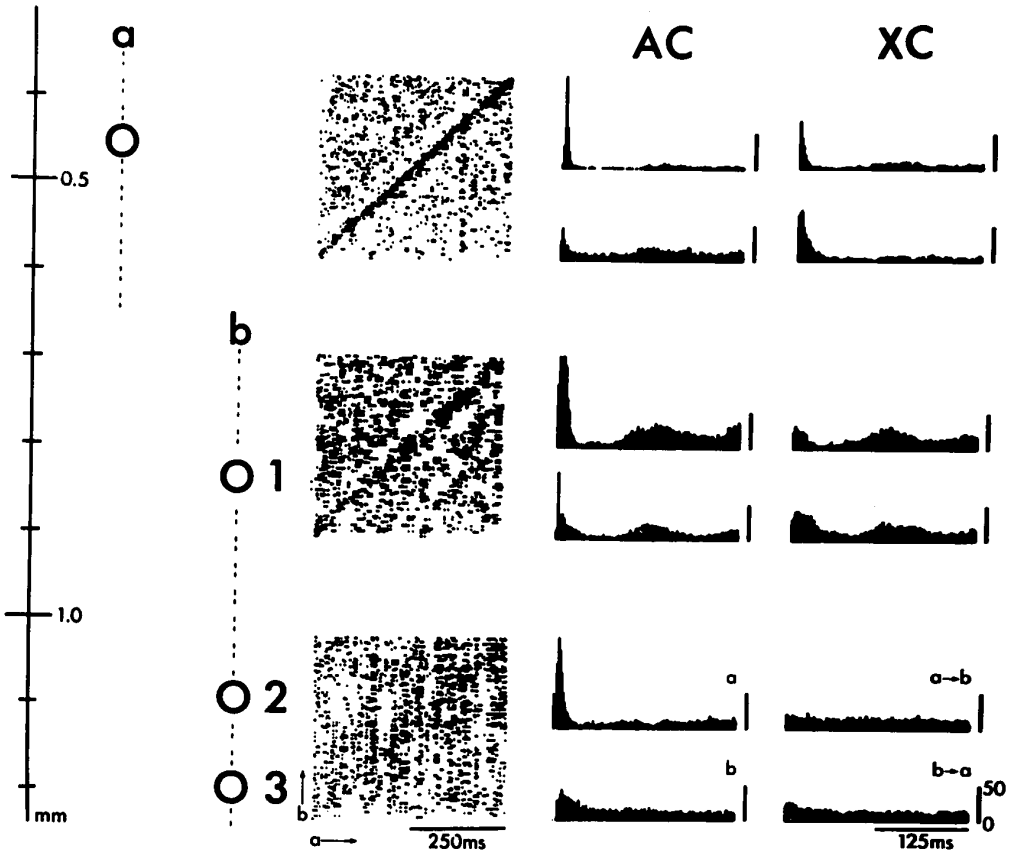
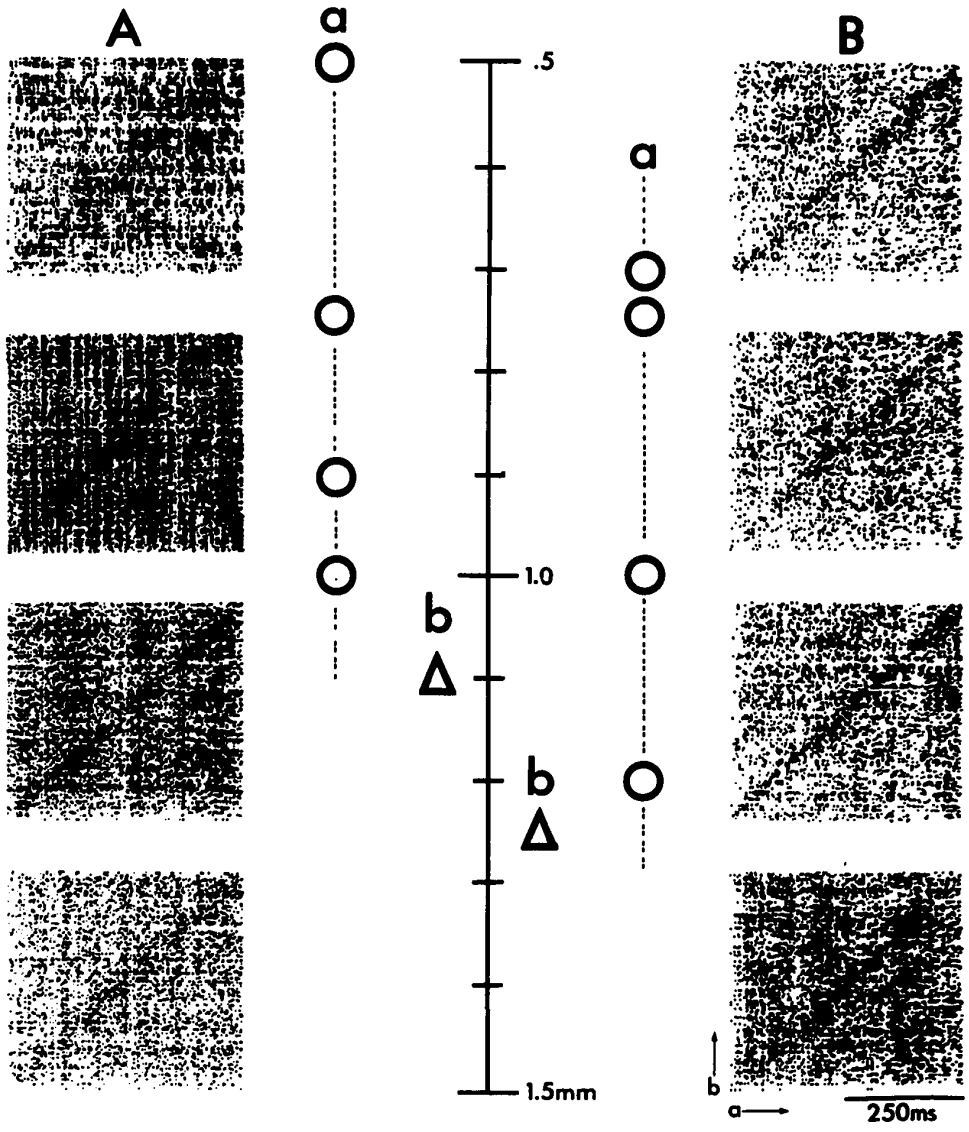

a
0.5
1.0
mm
b
1
2
3
AC
XC
a
b
a→b
b→a
50
0
250ms
125ms

Figure 55. Similar correlation patterns between some PT neurones and several neighbouring NPT neurones. A and B represent four scatter diagrams from two different experiments in which the electrodes b recording the activity of PT neurones (triangles) were not moved, but the other electrodes a were advanced to record the activity of four separate NPT neurones. Scatter diagrams computed at each point reveal that the activity of all NPT neurones within a microelectrode tract less than 200 mm away was positively correlated with the spike discharges of the PT neurones in each experiment.

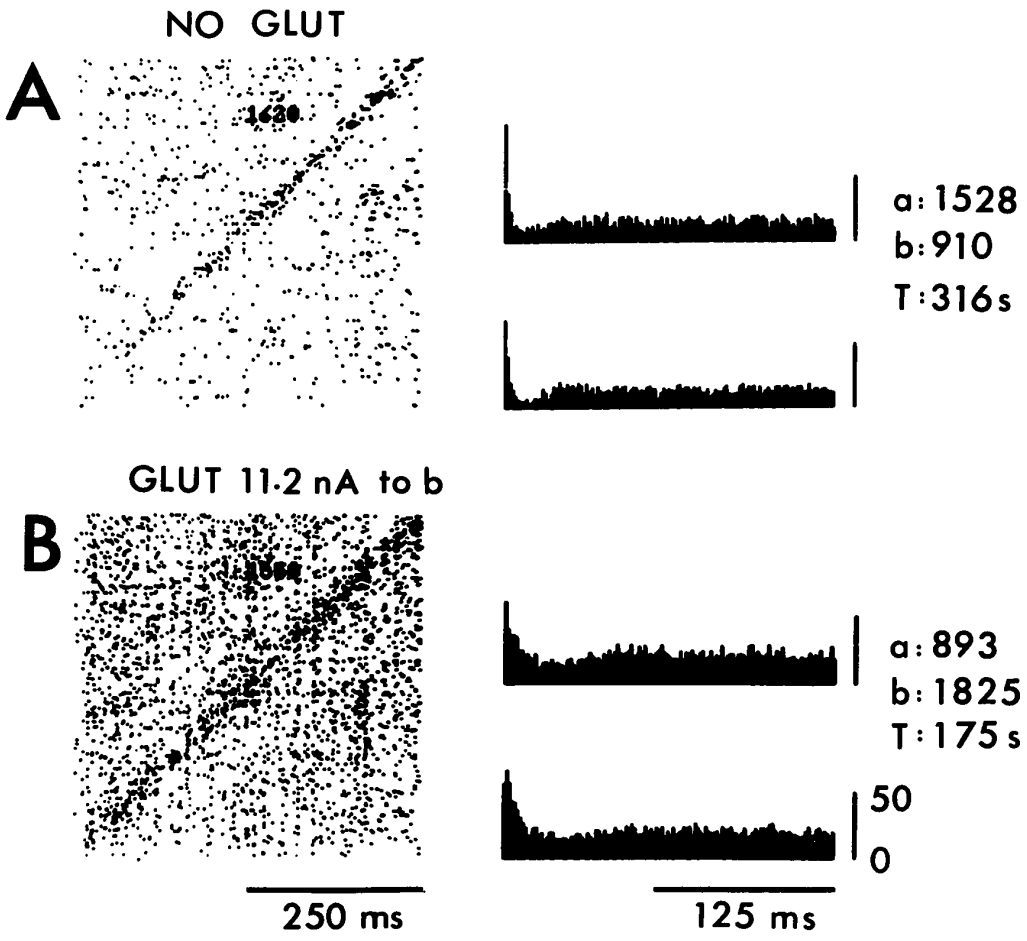

A
a
.5
B
a
1.0
b
b
1.5mm
b
a
250ms

One of the features often noted was that single PT neurones had a similar relationship to a group of NPT neurones in the vicinity. Figure 55 illustrates two such experiments in which the activity of a PT neurone (b) was correlated with that of four different NPT neurones (a) located during a microelectrode penetration. The usual results were joint scatter diagram patterns with an increased density of points along the 45° diagonal, suggesting that some force common to each pair of neurones examined was influencing their activity in such a way so as to synchronize their firing patterns. As the interelectrode distance was increased beyond 500 μm the chances of observing this effect decreased, and it was more common to observe joint scatter diagrams with a uniform point distribution (i.e. independent spike trains). Possible causes of these phenomena will be considered later in the DISCUSSION.

E. INFLUENCE OF IONTOPHORETICALLY APPLIED GLUTAMATE ON THE CORRELATION PATTERNS

It was often a distinct advantage to be able to increase the firing rate of slow discharging neurones or to study otherwise silent cells by the iontophoretic ejection of L-glutamate from an extra channel of the recording microelectrode. One question asked was whether or not glutamate could be, in part at least, responsible for synchronized behavior between two closely situated neurones. Assuming that glutamate does not spread to directly involve other neurones relaying excitatory or inhibitory information directly to those neurones which are

Figure 56. Influence of glutamate evoked activity on correlation patterns of two PT neurones. A. The spontaneous spike discharges of two PT neurones with antidromic spike latencies of 1.2 and 1.3 ms exhibit a marked positive correlation. On the right are the number of spikes from PT cell a and b, and the time (in seconds) during which these data were obtained. B. Glutamate applied to PT cell b evokes an increase in its spike discharge. This resulted in a decreased number of spikes available from PT cell b . For correlation because of the shorter time interval required to fill the data table in the computer's memory. Despite a reversed proportion of spikes from each neurone available for correlation, the positive correlation is still clearly evident although not quite so marked in amplitude and somewhat broader in appearance. In A, note the slight depression in the cross-correlogram after each peak, as referred to in the text.


NO GLUT
A
1620
a: 1528
b: 910
T: 316 s
GLUT 11.2 nA to b
B
a: 893
b: 1825
T: 175 s
50
0
250 ms
125 ms

being recorded, the pattern seen on the cross-correlation with or without glutamate should appear unchanged. Such an experiment is seen in Figure 56. In A, two PT neurones exhibited a clear tendency to synchronized behavior in their spontaneous activity (a dense line along the 45° diagonal of the joint scatter diagram, and a central peak in the cross-correlogram). On the right are the number of spike discharges from each neurone as well as the time in seconds over which the recordings were obtained. In B, 11.2 nA of L-glutamate were released iontophoretically from a second channel of the electrode b resulting in a reversal of the relative number of spikes and a shortening of the time interval over which data was collected (see METHODS). The pattern of the cross-correlation remains essentially unchanged except for an increase in the background contour. Similar studies of other neurones excluded the possibility that glutamate was influencing in some way the tendency for synchronized behavior among neurones. In each case where synchronized firing was evident from the joint scatter diagram, the pattern remained unchanged despite any artificial increase or decrease in background activity of one or other neurone.

Chapter 9

DISCUSSION

Recent investigations of the morphology and pharmacology of inhibitory synapses in the cerebral cortex have significantly advanced our knowledge of cortical inhibitory processes. For example, it appears that inhibitory synapses can be identified at the ultrastructural level (Colonnier 1968; Colonnier and Rossignol 1969) and these most likely represent the axon terminals of stellate basket neurones situated in cortical layers II to V (Marin-Padilla 1969, 1970b). Gamma-aminobutyric acid probably functions as the inhibitory transmitter agent which is released from these terminals during synaptic inhibition (Krnjević and Schwartz 1967; Dreifuss et al. 1969; Krnjević 1970; Mitchell and Srinivasan 1969; Iversen et al. 1971). However two main difficulties have been encountered in any further attempts to understand the mechanisms and pathways involved in inhibition at the cortical level; first, there is no precise method of measuring and comparing IPSPs evoked by various direct or indirect inhibitory stimuli; second, it has not been possible to identify with any degree of certainty the inhibitory interneurones themselves. The present study, including the on-line use of a digital computer interfaced to the neurophysiological experiment, was undertaken in order to overcome some of these difficulties.

A. QUANTITATIVE STUDIES OF CORTICAL IPSPs

The amplitude of IPSPs in cortical neurones, as in other cells (Kuffler 1960), is a poor indication of the magnitude of the change in membrane conductance resulting from activation of inhibitory synapses (c.f. Dreifuss et al. 1969). This was illustrated earlier in Chapter 3,in Figure 18, where the potential change at the peak of a surface evoked IPSP was much less than the observed change in membrane conductance. This attenuation of potential was readily apparent during large changes in conductance.

The results in Chapter 3 illustrate that direct stimulation of the cortical surface is much more efficient in engaging the activity of inhibitory synapses than is a stimulus to the pyramidal tract. This probably arises from the direct (or synaptic) excitation of a greater population of inhibitory neurones by the surface shock. The long duration of the conductance change during surface-evoked IPSPs (i.e. greater than 400 ms) provides an explanation for the maintained suppression of spontaneous activity of cortical neurones by repetitive surface stimulation at lower frequencies (3-7/s), probably owing to a 'cumulative' effect of inhibitory transmitter-receptor interaction at the postsynaptic membrane.

B. POTENTIATION AT INHIBITORY SYNAPSES

This cumulative effect is however different from longer term effects which have been observed at chemical inhibitory synaptic junctions. For example, in Crustacea (Dudel and Kuffler

1961) and in the abdominal ganglion of Aplysia (Waziri et al. 1969) potentiation of junctional potentials produced by inhibitory pathways has been observed to greatly outlast the conditioning stimulus. In a recent study, Spencer (1966) has reported similar changes in vertebrate inhibitory pathways. When a recurrent pyramidal IPSP is conditioned by a surface evoked IPSP at a conditioning-testing interval greater than 400 ms, i.e. beyond which the change in membrane conductance or potential induced by the surface shock is no longer detectable, potentiation is also apparent in the neocortex. Not only was there a significant increase in membrane conductance at the peak of the conditioned pyramidal recurrent IPSP, but also the duration of the change was prolonged. These findings could suggest that there has been an alteration in either the release of the chemical transmitter substance or in the responsiveness of the postsynaptic receptor to the inhibitory transmitter, and consequently that neocortical chemical inhibitory pathways show synaptic plasticity. Similar changes may also occur at excitatory synapses on the inhibitory neurones themselves. Consequently, it cannot be excluded that the observed potentiation occurs as a result of changes occurring in interneurones which have been activated by the surface shock. Possibly related to the phenomenon of synaptic plasticity is is the observation of Zimmerman and Kreisman (1970) that a single sensory stimulus evokes long term changes (up to 2000 ms) in cortical neurones, which would imply that a single stimulus sets up a sequence of events which considerably outlasts the

stimulus.

C. COMMON INHIBITORY PATHWAYS TO PYRAMIDAL TRACT NEURONES

Antidromic pyramidal tract volleys, as well as stimulation of afferent pathways (thalamocortical, transcallosal) and direct epicortical stimuli evoke IPSPs in pyramidal tract neurones (see Chapters 3 and 4 for references), with amplitudes and durations that are quite similar despite the pathway stimulated (c.f. Creutzfeldt, Lux and Watanabe 1966c). Two of these, the surface and pyramidal-evoked IPSPs were examined in greater detail in Chapter 3. Although there were differences in the intensities and duration of the conductance changes evoked by each stimulus, surface stimulation did potentiate pyramidal IPSPs, suggesting that at least some of the synaptic endings activated by both stimuli were identical. Also in favour of this hypothesis was the linear relationship between the conductance and potential changes, at least for small values of potential change, and the similarity of the IPSP reversal or equilibrium potentials for both surface and pyramidal IPSPs.

IPSPs evoked by both types of stimuli may be 'contaminated' by EPSPs, for example through direct excitation of excitatory neurones or their axons in the case of surface stimulation, or through recurrent excitatory connections in the case of pyramidal tract stimuli. Such 'composite' postsynaptic potentials may be rather poor indicators of synaptic loci (c.f. Rall 1964, 1967; Calvin 1969). However in pyramidal tract (and probably other pyramidal shaped) neurones, the major

inhibitory synaptic density appears to be concentrated in the region of the cell soma. Only synapses with a symmetrical membrane differentiation and flattened vesicles, presumed to be a morphological correlate of inhibitory synaptic activity (Uchizono 1965; Larramendi 1969; Gray 1969), make contact with the soma (Colonnier 1968; Lund and Lund 1970). Consequently any EPSP contributions presumably arise from the dendritic tree, remote from the recording electrode and the site of maximum subsynaptic current generated by inhibitory synapses. The magnitude of the EPSP potential change propagated electrotonically along the cable-like dendrites would have declined considerably at the soma (Rall 1964, 1967). In addition, the shunting and voltage-clamping effect of a large increase in chloride conductance during the IPSP shortens the space constant and further reduces the efficacy of axo-dendritic synaptic activity. Positioning intracellular current pulses 40 ms after the shock artifact also helps to avoid any early EPSPs which might interfere in the derivation of the reversal potential. Therefore it can be assumed that the reversal potentials for cortical IPSPs, at least in pyramidal tract neurones, as calculated here do reflect with a fair degree of accuracy the true inhibitory subsynaptic currents and are reasonable approximate indicators of synaptic loci.

The changes in excitability of cortical neurones during synaptic inhibition closely parallel the changes in membrane conductance (c.f. Dreifuss et al. 1969). They also reflect the enhancement of pyramidal IPSPs by a conditioning

surface evoked IPSP (see Figure 25 in Chapter 3). Extracellular studies in which the probability of occurrence of spontaneous or glutamate-evoked spike discharges were measured following a pyramidal tract shock conditioned by surface stimulation were also partly in support of the intracellular findings. On the other hand, they also provided evidence for occlusion (Figures 26 and 27 in Chapter 3) in studies which were extended to include the transcallosal inhibitory pathway. It could be concluded that at least some of the inhibitory neurones producing IPSPs in pyramidal tract neurones could be activated by surface, pyramidal tract and transcallosal stimuli.

D. IDENTIFICATION OF CORTICAL INHIBITORY INTERNEURONES

Although they support the hypothesis of a common inhibitory pathway, the previous data do not clarify the identity of the inhibitory neurones themselves. There is little evidence to suggest that inhibitory neurones in the cortex, like those found elsewhere in the CNS, give long bursts of high frequency discharges lasting throughout the duration of the IPSPs observed in nearby cells (c.f. Eccles, Fatt and Koketsu 1954, in the spinal cord; Andersen et al. 1964, in the cuneate nucleus; Andersen, Eccles and Sears 1964, in the thalamus; Eccles, Llinas and Sasaki 1966, in the cerebellum; Murphy and Renaud 1969, in the hypothalamus). As described in Chapter 4 and illustrated in Figure 34, some NPT neurones were found which responded with a brief high frequency burst to stimuli which coincidentally produced an inhibitory pause

in the activity of neighbouring cortical neurones. But neocortical neurones displaying this type of response have been observed only infrequently. For example Stefanis (1966) and Sukhov (1969) inferred that such cells were inhibitory neurones, but they lacked a means by which to directly test the action of these neurones on other cells. The results of the intracortical microstimulation experiments (Chapter 5) provided convincing evidence that a considerable number of NPT neurones, when activated by iontophoretically applied glutamate, appeared to cause 'inhibition' of neighbouring PT and NPT neurones (c.f. Biscoe and Curtis 1967). Cross-correlations performed on the action potentials of pairs of neurones exhibiting these features revealed an asymmetric negative correlation, with a latency that was sufficiently short to consider that the interaction was mediated through a monosynaptic pathway. This approach exemplifies one way in which cross-correlation analysis can be applied to the detection and interpretation of functional associations between cortical neurones (c.f. Gerstein 1970) and provides one of the more direct approaches presently available for the identification of cortical inhibitory cells (c.f. Asanuma and Rosén 1972).

E. CHARACTERISTICS OF SOME CORTICAL INHIBITORY NEURONES

One of the characteristics of all 'I Type' cells was their lack of antidromic invasion following a pyramidal shock (c.f. Table 3 in Chapter 6), suggesting that they could be local circuit neurones. These cells were located at various depths, ranging

from 0.25 to 2.3 mm below the cortical surface, and were situated between 50 µm and 700 µm from the neurones which appeared to be inhibited by their activity (Figures 38 and 57; see also Table 3). The spontaneous activity of 'I type' cells was always somewhat irregular, and some neurones displayed spontaneous or glutamate-evoked activity consisting of high frequency bursts of spikes (B-NPT cells).

ACh Sensitivity. These 'I type' neurones were not excited by ACh applied iontophoretically. This is a marked difference from the behaviour of Renshaw neurones in the spinal cord, which are excited by ACh (eg. Curtis and Ryall 1966). Although the limited number of tests made in the present series of experiments does not permit any definitive conclusions to be drawn, the data suggest that some cortical inhibitory neurones are not sensitive to (or may be depressed by) ACh, in comparison with PT and other possibly excitatory cortical interneurones which are excited by ACh (c.f. Phillis and York 1968).

F. RESPONSE TO STIMULATION OF INHIBITORY PATHWAYS

Excitation. A short latency excitation, manifested as a single spike or burst of spikes which seldom exceeded 40 ms in duration, was observed for a number of NPT neurones following stimuli delivered to the pyramidal tract, thalamo-cortical and transcallosal pathways. Perhaps many of these neurones were 'I type' NPT cells which could not be identified through cross-correlation since their axons did not directly

influence the activity of the particular cell recorded from the neighbouring microelectrode. Those that were identified as 'I type' cells gave clear excitatory responses, but only to stimulation of one of several pathways. In one example, illustrated in Figure 46F, pyramidal tract stimulation of increasing strength evoked from the NPT cell a progressively greater number of spikes at shorter latencies, coinciding with an increase in the silent period following the antidromic spike of the neighbouring PT cell. However the duration of the excitatory response from the NPT cell seldom exceeded 20-40 ms (c.f. Krnjević, Randić and Straughan 1966b) which was much shorter than the 100-120 ms pause in the activity of this PT cell, or the 100-200 ms duration of cortical IPSPs in general.

Inhibition and Disinhibition. If excitation was the initial response of some 'I type' NPT neurones, this was followed in all instances by a pause in activity lasting at least 50-70 ms (Figure 46F; c.f. Figure 47C). One of the possible reasons for this pause could be post-activation depression (c.f. Eccles 1969c). However, it is important to note that similar pauses in the activity of spinal Renshaw cells (Curtis and Ryall 1966) have been shown to occur as the result of an inhibitory action of Renshaw neurones on other Renshaw neurones, i.e. disinhibition (Wilson and Burgess 1962a; Ryall 1970). As mentioned above, another feature of putative inhibitory neurones was their failure to show excitation in response to stimulation of more than one inhibitory pathway. In fact it appeared as if

there was only one specific pathway which excited any one 'I type' neurone (c.f. Table 3), while stimulation of other pathways apparently resulted in its' inhibition (e.g. Figure 47A) or no clear effect (e.g. Figure 47D).

More recent studies on the spinal cord have yielded convincing evidence that Renshaw cells in addition to their inhibitory action on other Renshaw neurones also have a post-synaptic inhibitory action on inhibitory neurones located in other inhibitory pathways, i.e. the Ia inhibitory pathway (Hultborn et al. 1968, 1971a,b; Ryall and Piercey 1971). Some of these mechanisms observed to operate in spinal cord inhibitory pathways may also be common to inhibitory networks at higher levels of the neural axis, so that cortical inhibitory neurones may be capable of inhibiting other inhibitory neurones located in the same or separate inhibitory pathways.

The present evidence does throw some doubt on the initial hypothesis of a common inhibitory pathway and suggests that, although it may be partly correct, further experimentation will result in considerable modification of such a simple hypothesis.

G. TIME COURSE OF CORTICAL IPSPs: A PROLONGED TRANSMITTER-RECEPTOR INTERACTION?

Earlier it was mentioned that putative inhibitory neurones seldom were active for longer than 40 ms following an inhibitory shock, despite the long duration of cortical inhibition. This discrepancy is even more apparent during surface stimulation which is seldom followed by more than one or two

spike discharges, although generating a very potent form of cortical inhibitory action on virtually all neurones in all cortical layers (Chapter 4; c.f. Krnjević et al. 1966a,b). With surface shocks, it is possible that there occurs a direct activation of inhibitory fibers, thereby obviating the need for soma activation. While this may be so for surface stimulation, it obviously does not occur with remote (eg. transcallosal) stimulation. Therefore, this discrepancy requires some other explanation.

The time course of a cortical IPSP is too long to be explained by the probable membrane time constant of cortical neurones (Nacimiento et al. 1964). In view of the results presented here, a prolonged repetitive discharge of inhibitory neurones also seems rather unlikely (but c.f. Stefanis 1966; Sukhov 1969). A prolonged release of inhibitory transmitter not associated with repetitive presynaptic firing, or a prolonged transmitter-receptor interaction are more likely possibilities. Therefore, from the electrophysiological viewpoint, the prolonged changes in membrane conductance during cortical IPSPs may require only a short burst of activity (or even a single spike discharge) from a cortical inhibitory neurone. With the electron microscope, only 50-100 'inhibitory type' synaptic contacts have been found on the soma of individual pyramidal neurones (Colonnier and Rossignol 1969). From Golgi studies it would appear that a single axon may form several such contacts (Marin-Padilla 1969, Figure 4) on any one pyramidal

cell soma. This can be interpreted to mean that one impulse from an inhibitory neurone causes a release of inhibitory transmitter at several points on the pyramidal cell soma, and that perhaps 10% or more of the total inhibitory synapses on any one neurone are thereby activated by a single impulse from one inhibitory neurone.

Clearly these are suggestions which at the moment are highly speculative. However, they may serve to explain two physiological observations: first, that the effects of a single spike discharge from a putative inhibitory neurone can be detected statistically by monitoring the activity of a recipient neurone and, second, that the duration of the inhibitory response observed extracellularly is in the order of 50-70 ms.

H. 'UNITARY' OR 'SPONTANEOUS' CORTICAL IPSPs

In the intact cortex, spontaneously occurring IPSPs are 50-100 ms in duration, with rise times of 7-8 ms and decay time constants of 29.2 ± 4.9 ms (Creutzfeldt et al. 1966c). The duration of these IPSPs is very similar to the time course of negative correlation described in Chapter 6, and suggests that this is the extracellular manifestation of the 'unitary' or 'spontaneous' IPSPs recorded by Creutzfeldt and colleagues. If so, this would imply that a single inhibitory neuronal discharge is capable of decreasing the excitability of other cortical neurones for periods of 50 ms or longer.

The cross-correlation analysis as performed here

does not take into account bursts of spikes from postulated inhibitory neurones. A more interesting analysis would be to compare the time course of two types of correlation: one in which only single spike discharges of "I type" neurones were selected, with the time course of negative correlation in which only burst discharges of "I type" neurones were selected, ignoring the second and subsequent spikes of a burst and treating each burst as a single event. The latter analysis might be expected to yield a longer time course for negative correlations since presumably each burst would be associated with the release of a greater quantity of inhibitory transmitter.

Hexobarbital has been shown to prolong the recurrent IPSP in motoneurones (Larson and Major 1970). Both the present observations and those of Creutzfeldt and colleagues (1966b,c) were from Nembutal anaesthetized cats and raise the possibility that the duration of 'unitary' or 'spontaneous' IPSPs described here are longer than would be found in the absence of barbiturates. In addition, thalamocortical synchronizing activity during barbiturate spindles has been described to induce burst discharges from cortical neurones (Andersen et al. 1967) which, if influencing the activity of inhibitory neurones, may also promote the presence of longer periods of 'spontaneous' inhibition.

I. THE CORTICAL INHIBITORY INTERNEURONAL SYSTEM: ANATOMICAL-PHYSIOLOGICAL CORRELATIONS

There is now clear evidence that the IPSPs observed

in cortical neurones are due to activation of an intracortical system of inhibitory neurones: (a) Both intracellular IPSPs and extracellular silent periods have been observed to follow epicortical stimuli in isolated cortical slabs (Creutzfeldt and Struck 1962; Krnjević et al. 1966b; Watanabe and Creutzfeldt 1966; Krnjević et al. 1969) and (b) very localized activation of certain cortical neurones, either by electrical (Asanuma and Rosen 1972) or chemical means (Biscoe and Curtis 1967; see also Chapter 5 of RESULTS) evokes respectively monosynaptic IPSPs or short latency decreases in the activity of neighbouring neurones. The problem remains to relate the physiological observations with the morphological features found in the cortex.

One of the anatomical characteristics of isolated cortical slabs is the persistence of axo-somatic contacts (Szentagothai 1965; Colonnier 1966). Ultrastructurally, these axo-somatic synapses are composed of symmetrical pre- and post-junctional membrane differentiations and contain elongated presynaptic vesicles (Colonnier 1968, 1969; Szentagothai 1969), profiles which have been identified with an inhibitory synaptic function in other CNS areas (Uchizono 1966; Larramendi 1969; Gray 1969). The stellate basket neurone has been singled out as the most likely neurone to be involved in the formation of these contacts (Colonnier 1966; Szentagothai 1969; Marin-Padilla 1969, 1970b).

The question may be asked 'how does the morphological

picture of the basket-pyramidal system correlate with some of these physiological findings described here?'. Several points deserve mention. (a) Stellate basket cells are found in layers II to V (Marin-Padilla 1969) similar to the cortical depth distribution of 'I type' neurones shown in Figure 38B. (b) The axons of stellate basket neurones are related principally to pyramidal neurones in layers II, III, and V; they form baskets around pyramidal neurone somata located at the same cortical depth as well as with some pyramidal neurones in more superficial and deeper layers (Marin-Padilla 1970b). This may explain the tendency for inhibited neurones to be located at three different cortical depths as seen in Figure 38A. (c) Axons of basket cells contribute to the formation of many perisomatic baskets. The corollary is that several basket cells can influence the activity of single pyramidal neurones. Evidence to this effect was observed not only with the technique of intracortical glutamate microstimulation (Figure 38D) but also in the observation that spike trains from two separate NPT neurones were negatively correlated with the activity of an individual neurone (Chapter 6). (d) During the course of cortical ontogenesis, pericellular baskets derived from these cortical interneurones are formed progressively around the somata of all pyramidal neurones (Marin-Padilla 1970a). A significant portion of NPT (as well as PT) neurones appeared to be inhibited by NPT neurones (see Chapters 5 and 6). However, many of these NPT cells may have been pyramidal in shape, but without axons projecting into the

pyramidal tract. A clear distinction must be retained between the term 'PT neurone' and 'pyramidal neurone', as the latter term merely describes a morphological feature of cortical cyto-architecture. (e) In the human motor cortex, the axonal distribution and pericellular endings of basket cells form a rectangle no thicker than 0.2 mm and no longer than 1-2 mm extending from layers II to V and oriented perpendicular to the long axis of the precentral gyrus (Marin-Padilla 1970b). In the experiments described in Chapters 5 and 6, the two electrodes were usually orientated in the sagittal plane, and therefore perpendicular to the long axis of the pre- and postcruciate gyri. This was technically easier than recording with electrodes in any other plane. However in addition, such an arrangement was also noted to increase the chances of finding the desired type of neuronal interactions, even with separations of electrodes by as much as 700 um. In other experiments, with electrodes separated by 200-300 um in the coronal plane (therefore in the long axis of the gyrus) 'inhibitory' interactions were also noted, but less frequently.

The Basket-Pyramidal Cell System: One Model of Cortical Inhibition. Structural similarities exist between the basket-pyramidal cell system of the motor cortex, the basket-Purkinje cell system of the cerebellum and the basket-pyramidal cell system of the hippocampus. The latter two are inhibitory in nature (c.f. Andersen, Eccles and Løyning 1963, 1964a,b; Eccles, Llinas and Sasaki 1966) suggesting a similar type of

function for the former. Also the neurochemistry would seem to be identical since GABA is apparently the inhibitory transmitter in all three regions (Krnjević and Schwartz 1967; Dreifuss et al. 1969; Curtis, Felix and McLennan 1970; Curtis et al. 1971; Bisti et al. 1971).

However certain questions remain unanswered. There is as yet no anatomical model to explain the phenomenon of cortical disinhibition (see Chapter 6; also c.f. Hull et al. 1967; Steriade et al.1971). Not to be excluded are other types of inhibitory neurones besides basket cells, which for example would be involved in 'remote' inhibition on dendritic spines (Colonnier 1968) and the presumed inhibitory synapses on the soma and dendrites of stellate neurones (Colonnier 1968; Lund and Lund 1970; Peters 1971). Also to be explained are the axo-axonic synapses on the axon hillocks and initial segments of pyramidal cells (Jones and Powell 1969; Lund and Lund 1970).

J. SYNAPTIC ORGANIZATION OF PT NEURONES

Recurrent Collateral Pathway. As suggested by earlier extracellular studies (Jabbur and Towe 1961; Towe et al. 1963) and confirmed by intracellular recordings, two groups of PT neurones can be distinguished not only by their axonal conduction velocities (Takahashi 1965) but also by differences in the biophysical properties of their cell membrane (Takahashi 1965; Koike et al. 1968a,b; Oshima 1969).

These two varieties of PT cells have different synaptic

organizations. The monosynaptic EPSPs recorded in fast PT neurones following an antidromic pyramidal stimulus (Armstrong 1965; Kubota and Takahashi 1965) presumably occur through activation of collateral branches of slow PT cell axons (Takahashi et al. 1967). The correlation pattern in Figure 52B suggests that slow PT cell axons are also facilitatory to other slow PT neurones.

On the other hand, there is general agreement that IPSPs are evoked in both fast and slow PT neurones by pyramidal tract shocks (Phillips 1959; Stefanis and Jasper 1964a,b; Armstrong 1965; Kubota et al. 1965; Humphrey 1968a,b; see also RESULTS of Chapter 4), presumably through the action of impulses in collaterals of PT fibers. Two components have been identified in the antidromic IPSPs in PT neurones (Endo and Araki 1972), data which suggest that fast PT neurones receive IPSPs disynaptically through activity in collaterals of both fast and slow PT neurones, while slow PT neurones receive recurrent inhibitory impulses only from other slow PT neurones.

One of the more striking morphological features of recurrent pyramidal axon collaterals is their wide distribution. Axons of both large and small pyramidal neurones give off 3-10 axon collaterals prior to entering the white matter. These course for three mm or more either horizontally, or obliquely ascending towards the surface (Cajal 1911; Ramon-Moliner 1961; Scheibel and Scheibel 1970). Such fibres are therefore capable of contacting many hundreds of cortical neurones, some of which

must be the recurrent inhibitory neurones. Although not described explicitly in the literature, the direction of these recurrent collateral fibres may be in the long axis of the gyri. This would be important to verify because these, rather than the axons of basket neurones (which apparently are perpendicular to the long axis of the gyri c.f. Marin-Padilla 1970b) may be involved in the tangential spread of inhibition in the long axis of the gyrus as noted in earlier electrophysiological studies (Krnjević et al. 1966b).

Afferent Connections. Stimulation of thalamocortical pathways gives rise to both excitatory and inhibitory influences on PT neurones (e.g. Li 1963; Nacimiento et al. 1964). Fast PT cells were observed to have two types of EPSPs: one short latency i.e. monosynaptic (Yoshida et al. 1966; Amassian 1970) and another longer latency EPSP. Slow PT cells display only longer latency EPSPs. With single shock stimulation at low frequencies (1-2/s) both types of thalamic evoked EPSPs are followed by IPSPs with durations of 100 ms or longer, which in turn are often followed by rhythmically appearing summated EPSPs (Creutzfeldt et al. 1966c; c.f. RESULTS, Chapter 4).

Transcallosal evoked EPSPs in fast PT neurones appear to be transmitted monosynaptically, while EPSPs evoked in slow PT neurones are mediated by one or two synapses (Naito et al. 1970). The synapses generating transcallosal EPSPs are probably located on the dendritic membrane surfaces, while those generating transcallosal IPSPs are probably on the somatic

membrane surface (Nakamura et al. 1971). Transcallosal IPSPs are most likely di- or polysynaptically mediated, possibly by the same inhibitory neurones which mediate the IPSPs observed following stimulation of other pathways. Relevant to this point is the lack of degenerating boutons found on the somata of pyramidal cells following disruption of transcallosal fibres, as compared with the prevalence of degenerating axo-dendritic synapses (Lund and Lund 1970) suggesting that all transcallosal fibres are excitatory.

K. POSSIBLE EXPLANATIONS FOR THE DIFFERENT DISCHARGE PATTERNS OBSERVED FOR PT AND NPT NEURONES

There have been few experimental studies reported in which mention has been made of any differences in activity patterns for PT compared with NPT cells. Phillips (1959) observed that the activity of non-invaded cells (i.e. NPT) was more sporadic, with a greater prevalence of higher frequency discharges than the activity observed from Betz cells. Evarts (1964) noted that NPT neuronal discharges frequently exhibited a greater number of short interspike intervals, tending to occur in bursts, and were often very inactive in comparison with the activity of PT neurones. Stefanis (1966) pointed out that some non-pyramidal neurones displayed very high frequency (800 Hz) activity occurring in bursts and commented on this feature as one criterion by which to distinguish interneurones from PT neurones.

As described in Chapter 7, during the present experiments PT neurones were noted to have patterns of activity which

were relatively free from very long or very short interspike intervals, compared with most NPT neurones. This type of PT activity closely resembles that described by Evarts (1964) in the awake monkey, and would suggest that the level of barbiturate anaesthesia used in the present experiments was generally light. During changes in behavioural states, such as from waking to slow wave sleep, both PT neurones and thalamocortical relay neurones in VL nucleus show similar changes in their patterns of activity (c.f. Evarts 1964; Lamarre et al. 1971). Since thalamic relay neurones are known to influence PT activity (Schlag and Villablanca 1967; LeBlanc and Cordeau 1969) and to have direct projections to PT neurones (c.f. Jones and Powell 1970), the question arises as to whether the nearly random activity pattern displayed by PT neurones does not reflect a fairly random bombardment of afferent impulses. However, it can be argued that NPT cells also receive direct connections from similar areas (see Jones and Powell 1970), so why should they not also display similar activity patterns? As mentioned in Chapter 7, 12% of NPT cells do show this feature, but the majority of them do not. One other fact to be considered is the mode of termination of afferent synapses on the two categories of neurones. PT cells receive corticopetal connections primarily through axo-dendritic contacts, whereas NPT neurones receive afferent connections both on their dendrites and somata (Jones and Powell 1970). Axo-somatic contacts are in a more favourable position to influence the behaviour of any neurone compared with those remotely located on the dendritic tree

(Smith et al. 1967; Calvin 1969; Rall 1970). Consequently, NPT neurones may be more responsive to an afferent stimulus than PT neurones and therefore will exhibit greater fluctuations in activity patterns.

Inhibitory connections may also have a role in controlling activity. Phillips (1959) introduced the notion that recurrent collateral pathways exerted a regulatory function on Betz cell activity. Granit (1961, 1963) has commented on the role that recurrent inhibitory mechanisms may have in regulating motoneurone discharge. In the sensory-motor cortex recurrent collaterals of PT cells have both an inhibitory and facilitatory influence on near-by corticorubral neurones (Tsukahara et al. 1968). In addition, recurrent inhibition may serve to heighten the contrast between field focus and surround at cortical levels (Brooks and Asanuma 1965; Brooks et al. 1968). In Chapter 6, it was observed that recurrent cortical inhibition was more effective in arresting PT discharges as compared with most NPT neurones. This feature must be related in some way to the anatomical observations that synapses presumed to have an inhibitory function are distributed primarily around the soma and axon hillock of pyramidal neurones (Colonnier 1968; Jones and Powell 1969), whereas they have a wider distribution on both the soma and the dendrites of stellate neurones (Colonnier 1968; Peters 1971). It would seem therefore that PT cells in general are more likely to be subject to a stronger inhibitory control through the PT recurrent inhibitory pathway than NPT

neurones, particularly if their spike generating mechanism is similar to that of motoneurones and located somewhere near the axon hillock-initial segment region (c.f. Coombs et al. 1957; Fuortes et al. 1957). On the other hand, as mentioned earlier, the inhibitory endings on NPT cells may have a quite different origin. NPT neurones may also be subjected to strong inhibition, but from somewhere else.

Multiple Spike Discharges. Eccles (1964) has postulated that multiple discharges in central neurones result from a prolonged action of an excitatory transmitter, possibly arising from temporal dispersion of presynaptic volleys through polysynaptic pathways. While such an explanation may be valid for stimulus induced events, it can hardly be considered the cause of the 'bursting' activity recorded from some NPT neurones. Kuno and Miyahara (1968) have shown that identical time courses of transmitter actions evoke different patterns of activity depending on the type of central neurone tested. For example, in addition to the multiple discharges of dorsal spinocerebellar tract neurones, the doublet or burst firing of hair and touch cells in the cuneate nucleus is also clearly an intrinsic property of these cells (Galindo et al. 1968). Although the presence of an intense synaptic action and large EPSPs are apparent prerequisites for multiple discharge, Kuno (1969) has concluded that these differences also depend on (a) the maximum frequency at which neurones can fire i.e. the duration of the action potential and the brevity of the refractory period, (b) the presence or absence

of recurrent inhibition and (c) cell membrane adaptability and after-hyperpolarization. It is tempting to speculate that the membrane biophysical properties of cortical neurones displaying multiple discharges will be considerably different from those now known for PT neurones (e.g. Oshima 1969).

Post-Inhibitory Excitation. The marked increase in excitability of some cortical neurones following inhibition appears to depend largely on the integrity of the thalamocortical circuits. For example,neurones studied in undercut cortex do not show post-inhibition excitation (Krnjević et al. 1966a).

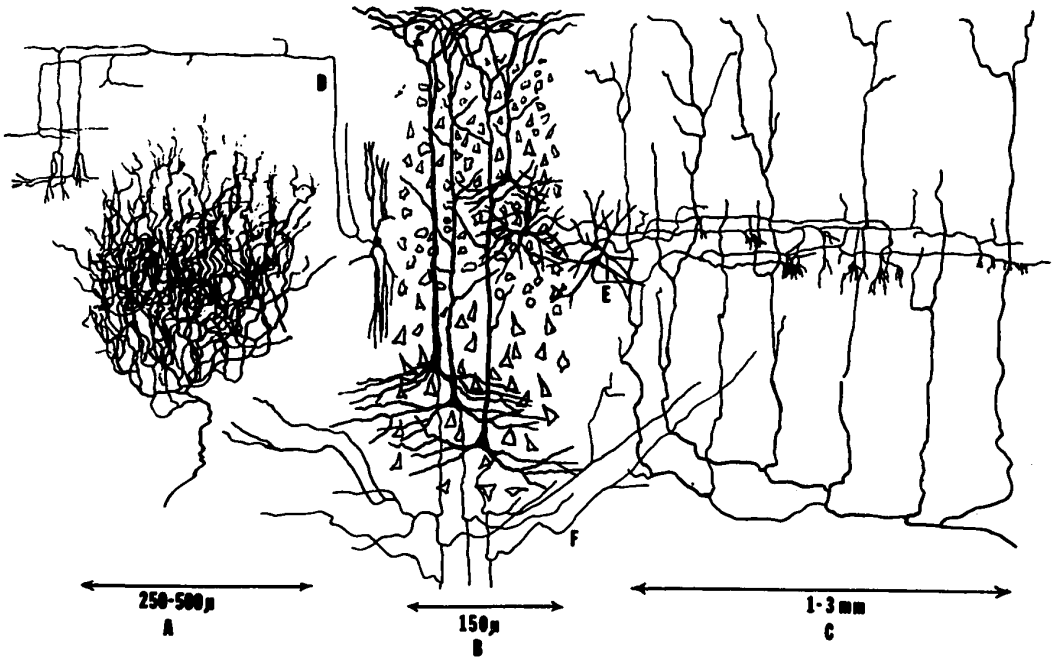
From their intracellular studies of thalamic neurones, Maekawa and Purpura (1967) concluded that this delayed activity was only found in depolarized (and therefore injured) neurones. This explanation is hardly adequate, since extracellular recordings of apparently intact cells show a similar event. A possible explanation of the depolarization associated with a burst of spikes observed in thalamic neurones after an IPSP is the phenomenon of postanodal rebound or exaltation (Andersen and Eccles 1962; Andersen and Sears 1964; Eccles 1964; Andersen and Andersson 1968). If such a rebound is a widespread phenomena, it is surprising that the most potent cortical inhibitory stimulus i.e. that to the cortical surface, was not followed more frequently by post-inhibition excitation. Nor has it been an obvious finding when synaptic inhibition is mimicked by application of short (less than 100 ms) pulses of GABA (c.f. Figures 6 and 7 in Gottesfeld et al. 1972), although there is often some

rebound of firing after a longer (five to ten seconds) iontophoretic application of GABA (Kelly and Renaud 1972, in preparation). Since the peaks of this enhanced excitability were frequently quite different even for neighbouring neurones (e.g. Figure 31E in Chapter 4) it most likely results from a complex synaptic interaction involving both thalamocortical pathways and local neuronal interactions, including disinhibition.

L. CORRELATED FIRING OF UNITS IN THE CEREBRAL CORTEX

In an effort to understand the complexities of interneuronal behaviour, there has been a growing interest in the correlation patterns found to exist between unit discharges recorded simultaneously in the vertebrate CNS. Some have studied action potentials recorded from two or more neurones using a single microelectrode (Mountcastle 1957; Baumgarten and Schaefer 1957; Mountcastle and Powell 1959; Hubel and Wiesel 1962; Griffith and Horn 1963; Evarts 1965; Rodieck 1967; Noda et al. 1969). In those experiments individual neuronal spikes were distinguished either by their amplitude (Griffith and Horn 1963; Noda et al. 1969; Noda and Adey 1970) or shape (Gerstein and Clark 1964). Other investigators have utilized independent recording microelectrodes separated by various distances (Li 1959; Amassian et al. 1961; Oikawa et al 1965; Holmes and Houchin 1966; Bell and Grim 1969). Two main factors appeared to determine the incidence of positive correlations: the distance between the two neurones, and the functional or behavioural state of the experimental preparation. There was a greater percentage of positively correlated spike discharges

Figure 57. Afferent fibre terminations in neocortex. A fragment of the cortical neuronal matrix is illustrated in B. Two of the afferent elements which impinge upon it are seen in A and C. In A, terminal axonal plexus generated by a specific afferent sensory fibre delimits a cortical column or module of 250-500 μm in diameter. In C, the larger terminal domaine established by a non-specific afferent fibre delimits a cortical module in the order of 1-3 mm. D and E represent the inter-cortical horizontal projections of local circuit Golgi type II neurones which together may form a most significant excitatory and/or inhibitory modulators reaching across the columnar modules. (F) indicates a group of recurrent collaterals from a small core of pyramidal neurones (From Scheibel and Scheibel 1970).


D
E
F
250-500μ
A
150μ
B
1-3 mm
C

when interneuronal distances were short (Baumgarten and Schaefer 1957; Li 1959; Griffith and Horn 1963; Noda et al. 1969) than when they were large (Amassian et al. 1961; Oikawa et al. 1965). Neurones whose discharges were positively correlated during spindling and slow wave sleep became independently active during arousal or REM sleep (Noda and Adey 1970). Under deep anaesthesia, unit firing also exhibited positive correlations which were absent under local or light general anaesthesia (Li 1959; Holmes and Houchin 1966).

Synchronized Neuronal Discharges. Statistical evidence of synchronized neuronal activity was given by 45.5% of cell pairs examined. This interpretation is based on the demonstration of a symmetrical peak at the onset of each cross-correlogram, which in the terminology of Moore et al. (1970) constitutes a primary effect. There are several possible explanations for this observation:

(i) symmetrical cross-correlation peaks may arise if both neurones receive a common excitatory input. A common inhibitory drive may have a similar effect, although broader primary peaks in the cross-correlogram might be expected under these circumstances (c.f. Moore et al. 1970). Golgi studies on the cerebral cortex have revealed structural details which are consistent with both of these effects. Figure 57A illustrates a terminal axonal plexus of a specific afferent fibre, which delineates a cortical column or module some 250-500 μm in diameter. Neurones encased in this plexus would therefore be subject to almost

synchronous excitation by an arriving impulse. Similar but more widespread and less intense activation could result from impulses arriving in non-specific afferent fibres, shown in Figure 57C. Impulses in axons of intracortical excitatory or inhibitory stellate neurones, especially basket neurones, may reach many cells virtually simultaneously at different cortical levels.

(ii) direct excitatory connections between the two neurones under observation also will result in nearly synchronous activity. Axons forming repeated parallel contacts along the dendrites of pyramidal neurones have been described by Szentagothai (1969) and are somewhat analogous to cerebellar climbing fibres. While such an axonal distribution would readily allow one neurone to influence the excitability of another, the frequency with which this arrangement occurs in the cerebral cortex requires further study.

(iii) electrotonic coupling between mammalian neurones has only recently been described (Baker and Llinas 1971). This type of coupling has been correlated with the 'gap' junctions seen with the electron microscope (Revel and Karnovsky 1967; Brightman and Reese 1969; Asada and Bennett 1971; Pappas et al 1971). Sotelo and Llinas (1972) have described similar morphological features in the cerebellum and speculated on their physiological significance. To date, there is little electrophysiological evidence of electrotonic coupling between cerebral neocortical neurones.

M. SIGNIFICANCE OF THE PRESENT STUDY

Almost two decades ago, extracellular studies from single cortical neurones revealed a temporary pause in their spontaneous discharges following an epicortical stimulus (Amassian 1953; Jung 1953). Although intracellular studies have confirmed that this phenomenon is a result of an inhibitory process which also can be evoked by other forms of stimulation, there is very little information about the identity of the inhibitory neurones and no precise knowledge of their connections.

One of the main difficulties retarding further neurophysiological studies has been the lack of a precise method by which to identify cortical inhibitory neurones. The present study has attempted to correlate statistical evidence of synaptic interactions between two simultaneously recorded cortical neurones with data obtained following stimulation of inhibitory pathways. This approach provides one of the most direct methods yet available for identification and study of the properties of inhibitory neurones. Furthermore, intracellular experiments have confirmed the need for more precise quantitative studies of IPSPs, and support the hypothesis that the excitability of PT neurones is controlled through a common inhibitory pathway.

Simultaneous recordings of the activity of two neurones from within an area having the approximate dimensions of a functional cortical column (Mountcastle 1957; Welt et al. 1967) has revealed that almost one-half of the cells display a positive correlation. When correlated with the morphological details, this finding supports the concept that the motor cortex is organized

into overlapping units or columns less than 1 mm in diameter, and suggests that this approach coupled with more recent refined physiological stimuli may be of value in the understanding of cortical integrative processes.

Chapter 10

SUMMARY AND CLAIMS TO ORIGINALITY

The results presented in this thesis are original. Except where mentioned specifically in the text, there have been no similar experimental results presented elsewhere. Features relevant to the original contributions of this work are summarized below.

Chapter 2. One of the unique characteristics of these experiments was the fact that they were interfaced to a LINC-8 computer, thereby providing the experimenter with direct on-line acquisition and storage of both intracellular and extracellular data. The intracellular data acquisition program was designed by Dr. J.S. Kelly. Mr. Kit McSweeney wrote the two-spike-train data acquisition program according to specifications. This chapter described some of the features entailed in the computer-experiment interface and the manner in which data were sampled and analyzed using the computer.

Chapter 3. The computer facilitated a quantitative study of cortical IPSPs in pyramidal tract neurones by sampling both membrane potential and conductance at various intervals along the time course of the IPSPs. Surface evoked IPSPs had a 'cumulative' effect at stimulation rates greater than 2/s owing to their prolonged conductance change (greater than 400 ms).

Recurrent pyramidal IPSPs had a shorter time course, but their IPSP reversal potential was similar to surface evoked IPSPs and could be potentiated by a conditioning surface shock. An approximately linear relationship was noted in the IPSP potential and conductance changes for both the unconditioned and conditioned pyramidal IPSP and the surface evoked IPSP. These data support the hypothesis of a common inhibitory pathway mediating both surface evoked and recurrent pyramidal inhibition. Based on excitability studies, occlusion was demonstrated between surface, pyramidal and transcallosal inhibition, suggesting that at least some of the inhibitory neurones which mediate these forms of inhibition are activated by more than one form of stimulation.

Chapter 4. Simultaneous extracellular recordings of the response of two neurones, identified as either pyramidal or non-pyramidal tract neurones, were compared following stimulation of the pyramidal tract, epicortical surface or transcallosal and thalamocortical pathways. Pyramidal tract recurrent inhibition was much more effective in PT neurones than in NPT neurones, whereas surface evoked inhibition was evident in both types of cells. Some NPT cells responded with a burst of spikes at high frequency, but seldom lasting more than 40 ms, while nearby neurones simultaneously were seen to have a 'silent' period in their extracellular spike discharge. This initial burst of NPT cell excitation was also followed by a 'silent' period. Some PT cells and many NPT neurones displayed a postinhibition excitation, the

peak of which was quite specific for each individual neurone.

Chapter 5. Parallel independently mobile multichannel micropipettes, positioned less than 800 µm apart, were used to search for neurones whose activation by iontophoretically applied L-glutamate was associated with a decrease in the firing frequency of neighbouring neurones. In 13.1% of neurone pairs increase in the spike discharges from a NPT "I type" neurone was accompanied by a decrease in the frequency of spikes recorded from nearby PT or other NPT neurones. Activity of "I type" neurones was either uninfluenced or depressed by iontophoretic acetylcholine, compared to the excitation which followed ACh application to 80% of the remaining PT and NPT neurones located more than 0.8 mm below the cortical surface. In 7.9% of neurone pairs artificially increasing the spike frequency of some PT and NPT neurones was associated with an increased activity of neighbouring cells; the former were classified as "E type" neurones, many of which were excited by ACh. On some occasions several "I type" and "E type" neurones were noted to have an influence on the activity of the same PT cell, indicating converging effects. The evidence suggested that "I type" neurones may be inhibitory interneurones, but confirmation required the demonstration that they exert their effect monosynaptically.

Chapter 6. In order to obtain information concerning possible monosynaptic connections between "I type" neurones and neighbouring cells, their spike discharges were subjected to cross-correlation analysis. Efforts to locate such pairs of cells

were greatly facilitated through the on-line construction of a joint scatter diagram. Ten of 110 cell pairs were found to display a short latency negative correlation in their spike discharge activity. The time of onset of the trough in the negative correlogram was sufficiently brief to consider a monosynaptic pathway, while the mean duration of the trough was 75ms. Only spikes from "I type" NPT neurones were associated with a transient decrease in the probability of discharge of other PT or NPT neurones, and therefore the former were tentatively identified as inhibitory neurones. "I type" neurones were located in various cortical layers and up to 600 μm from cells which they appeared to inhibit. One occasion of convergence of two "I type" neurones onto the same PT neurone was observed. In response to stimulation of inhibitory pathways, a burst of spikes rarely lasting more than 50 ms was observed from these neurones, but only following stimulation of one particular pathway. Inhibitory pauses were the rule following activation of other inhibitory pathways. The evidence, therefore, favors separate inhibitory pathways with inhibitory cells in turn inhibiting other inhibitory neurones (disinhibition).

Chapter 7. The firing patterns of pericruciate PT neurones were noted to be more random and free from a large number of very long or very short interspike intervals, compared with that of the majority of NPT neurones examined. Some NPT neurones, located at various depths in the cortex, displayed

spontaneous or glutamate-evoked activity consisting of short bursts of spikes at high frequencies (B-NPT cells); this pattern was constant during recording periods lasting more than thirty minutes and was not significantly altered by increasing the amount of iontophoretic glutamate applied to the neurone.

Chapter 8. Cross-correlation analysis performed on the activity of pairs of PT and/or NPT neurones revealed that out of 110 pairs of neurones no correlation was present among 38.2%. The activity of another 45.5% of neurones was synchronized to a greater or lesser extent, resulting in a symmetrical peak at the onset of each correlogram. 7.2% of cell pairs presented evidence of a short latency excitatory connection from one cell to another. 9.1% of cell pairs presented evidence of a short latency inhibitory connection (described in Chapter 6).

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