ABSTRACT

Ph.D. PHYSIOLOGY

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MECHANISMS OF ANAESTHESIA
AND SOME OBSERVATIONS ON SYNAPTIC INHIBITION

An experimental preparation was developed in decerebrated cats for the study of synaptic transmission through the cuneate nucleus. Anaesthetics, and other relevant substances, were given by microiontophoresis, by superfusion of the nucleus, or by systemic administration. Procaine readily depressed the excitability of all cuneate cells, as well as the excitability of presynaptic nerve fibres. Sodium pentobarbitone (Nembutal) preferentially depressed the post-synaptic excitation of all types of cells, but higher doses were required to block conduction in nerve fibres. Halothane had no direct effect on the post-synaptic elements, or on the nerve fibres. However, it depressed the synaptic excitation of cuneothalamic units (but not that of "interneurones"). It also potentiated synaptic inhibition, as well as the depressant effects of GABA and glycine. These observations indicate that anaesthetics may depress synaptic transmission by at least three different modes of action; a) non-specific depression of excitation (procaine), b) specific depression of post-synaptic excitation (Nembutal) and c) facilitation of inhibition (halothane). Picrotoxin antagonizes post-synaptic inhibition and the action of GABA. Post-synaptic inhibition in the cuneate is therefore likely to be mediated by GABA. In addition, GABA may have a presynaptic inhibitory action comparable to that observed at the crustacean myoneural junction.

MECHANISMS OF ANAESTHESIA
AND SOME OBSERVATIONS ON SYNAPTIC INHIBITION

by

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TO

Georgina and the children

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A - GENERAL INTRODUCTION

General anaesthesia is a well defined clinical term manifested by a depression of the central nervous system. Several theories have been proposed to explain its basic mechanism; they are mostly based on a single, non-specific mode of action covering all anaesthetic agents. However, with the advances in scientific knowledge, these theories have become specific, suggesting the possibility of not one but several mechanisms.

Claude Bernard proposed in 1875 that anaesthesia could be mediated by a "reversible semicoagulation of cell proteins". Linus Pauling, in 1961, explained the anaesthesia produced by inert gases as a depression of "brain oscillations" by clathrates obstructing transmembrane ionic movements.

Moruzzi and Magoun (1949) localized the preferential site of action of anaesthetic agents in the brain stem reticular formation. Destruction of this area suppressed behavioural and electrical arousal (Lindsley, Bowden and Magoun, 1949). Later studies by Larrabee and Posternak (1952) on sympathetic ganglia demonstrated that some anaesthetic drugs depress synaptic transmission more readily than the process by which action potentials are propagated.

According to modern ideas, the action of anaesthetics could be explained by a synaptic depression that preferentially affects complex neuronal networks, such as those in the ascending reticular system, suspected of being involved in behavioural arousal (Arduini and Arduini, 1954; French, Verzeano and Magoun, 1953a). This simple picture became less apparent as further studies of the brain stem reticular formation were made. On the one hand the reticular system is formed by multiple functional units organized as an integrating circuit - the input of this system can be controlled at different levels -; on the other hand some experimental observations demonstrate a dissociation between behavioural and electrical arousal (Funderburk and Case, 1951; Wikler, 1952; Bradley and Elkes, 1957).

An alternative to this idea is to relate the conscious state to a diffuse multisynaptic cholinergic system involving the brain stem, striatum, and deeper cortical layers (Krnjević, 1965; Krnjević, 1967; Phillis, 1968) with functional rather than a anatomical localization of arousal and consciousness. This system would be preferentially affected by anaesthetic agents (Matthews and Quilliam, 1964).

The synaptic function is a complex process having several morpholigical correlates (Eccles, 1964; De Robertis,

(1966). Block of transmission can occur at several stages, for instance, at the presynaptic terminal, at the synaptic cleft or at the post-synaptic membrane. This blockade in turn can affect the excitable process through various mechanisms: by depolarization or hyperpolarization of the terminals or the post-synaptic membrane, or by non-specific decrease in membrane excitability. Furthermore, synapses can be classified as excitatory or inhibitory.

Centripetal conduction from peripheral receptors is transmitted through specific (e.g. dorsal columns) and non-specific channels (e.g. spino-thalamic tract). The latter have more peripheral and more complex relay structures, where the different modalities of sensation seem to compete for the same pathway (Wall, 1964). Input to higher centres can be modulated at these relay stations by synaptic inhibition, or "by gating the input" as it has been called in a new theory on pain mechanisms proposed by Melzak and Wall (1965). The selective input control appears to operate from the spinal cord to the cerebral cortex in the form of pre- and post-synaptic inhibition (Eccles, 1964). This synaptic function is probably mediated through the activity of small interneurones.

More basically, the nerve impulse is generated by the movement of ions across the cell membrane as demonstrated in the squid giant axon by Hodgkin and Huxley

(1952a, b). Interference with this movement can explain changes in cell excitability. It is precisely on this basis that Pauling (1961) and Miller (1961) proposed a theory of the mode of action of non hydrogen-bonding anaesthetics.

One may assume that the depression of the central nervous system produced by anaesthetics can be explained through several mechanisms: a) non-specific depression of all neuronal elements, b) selective depression of the excitatory process and c) facilitation of inhibition. On the other hand it is reasonable to suggest that the site of action determines the pattern of functional disruption, and that in turn the preference for this site is dependent upon synaptic organization, specificity of neurotransmitters and the characteristics of the synaptic membranes.

The neurophysiological study on the mechanisms of anaesthesia presented in this thesis, was limited to the effect of anaesthetic drugs on the synaptic structures of the cuneate nucleus. The results were evaluated with respect to the action of these drugs on the excitatory and inhibitory processes.

B - THEORIES OF ANAESTHESIA

Anaesthesia is the loss of pain sensation obtained by blocking afferent stimuli from reaching the central nervous system. This blockade may occur at the periphery - local or regional anaesthesia -, or it may occur in the C.N.S. - general anaesthesia. These terms define clinical states characterized by various signs. In the case of general anaesthesia, these signs are grouped in stages depending on the degree and pattern of neuronal depression (Gillespie, 1943; Laycock, 1953; Guedel, 1951; Woodbridge, 1957; Galla, Rocco and Vandam, 1958 and Dornette, 1964).

The study of the anaesthetic state can be performed at several levels. For example, we can study the site of action, or we can study the mechanisms by which anaesthetics produce their characteristic effect.

The theories of anaesthesia can be divided into two groups a) biochemical and b) physico-chemical. They must not be confused with correlations of physical properties of the anaesthetic agents with their clinical potency, or with the depression of some cellular function not necessarily related to anaesthesia.

a) Biochemical theories

Claude Bernard (1875) noted that frog muscles immersed in a solution containing chloroform became opaque,

an observation suggesting a reversible semicoagulation of nerve cell proteins. Later Moore and Roaf (1905) re-affirmed the belief of anaesthetic-protein interaction. However, Salkowski (1888) had demonstrated that chloroform at clinical concentrations did not cause precipitation of proteins, not even after an exposure lasting several months.

Asphyxia was considered to be the cause of anaesthesia by Verworn and co-workers (1912) against the obvious differences between these two phenomena reported earlier by Snow (1847) or the observations made by Claude Bernard (1875). Later it was demonstrated that pigeons - which as in all birds, have a high metabolic rate - could recover from various days of barbiturate anaesthesia (Ellis, 1923).

Depression of the metabolic rate of neuronal elements as explanatory for the anaesthetic state is a suggestive hypothesis (Quastel 1939). Nembutal and ether decrease O_2 consumption of brain slabs in vitro. This effect is reversible with the barbiturate but not with the latter. Moreover, the concentrations of anaesthetics required to depress oxygen consumption were greater than those needed in clinical anaesthesia, an observation confirmed in the sympathetic ganglion (Garfield, Alper, Gillis and Flacke, 1968). Furthermore, the use of anaesthetics

at concentrations associated with C.N.S. depression do not produce metabolic changes in vitro nor do they affect any enzymatic process.

In vitro, barbiturates suppress respiration of brain slices in the presence of glucose or pyruvate but not in the presence of succinate. This effect requires concentrations much higher than those used clinically to produce anaesthesia. However, when the tissue is stimulated or the study is made on mitochondrial respiration, the barbiturate levels for both effects are in close agreement (Brody and Bain, 1954; Quastel, 1965).

Ethanol and some other alcohols have no effect on mitochondrial respiration; they do not uncouple oxidative phosphorylation which is affected by anaesthetic concentrations of ether in mitochondria from rat brains (Quastel, 1965).

It must be realized that anaesthetics also affect cell metabolism: i.e. ATP formation, which among other things, suppresses the synthesis of acetylcholine. But, this is accepted as an effect and not a cause of anaesthesia (Paton and Speden, 1965). On the other hand narcotic concentrations of amytal have little effect on the ATP-ase suspected of being involved in the active transport of Na^+ and K^+ across membranes (Quastel, 1965).

b) Physico-chemical theories

A theory involving cell permeability was proposed by Dubois (1885) who suggested that anaesthesia was caused by cell dehydration, as observed with high concentrations of chloroform in some plants. However, experiments with dehydrated frog muscle demonstrated an increase in excitability rather than the expected depression (Langendorf, 1891). Winterstein (1926) on the other hand demonstrated that clinical concentrations of narcotics reduce the permeability of the membrane to water, while toxic anaesthetic concentrations increased this permeability. Later Höber (1907) suggested that the adsorption of anaesthetics to the cell membrane depressed its permeability. Lillie (1909), in one of the earliest observations on trans-membrane ionic movement, showed that Arenicola Larvae exposed to several anaesthetics could not adsorb or take-up NaCl. He also demonstrated that narcotics did not alter water permeability of Arbacia eggs except when fertilized (1918). Urethane was shown to decrease the rate of Cl^- loss for erythrocytes placed in isotonic solutions of sugar and sulphate (Siebeck, 1922). Davson (1940) showed that, in cat's erythrocytes, narcotics increased potassium while inhibiting sodium permeability.

The earlier theories of anaesthesia often described sites of action rather than mechanisms. Traube

(1904) and Winterstein (1926) considered that narcotics acted by adsorption to the surface of the cells, decreasing the permeability to water and salts owing to the close agreement between narcotic strength and surface activity present in these drugs. Mayer and Overton (1889, 1901) believed in the incorporation of the anaesthetic into the C.N.S. lipids. This idea was based on the correlation between anaesthetic potency and water to the olive oil partition coefficient. Their theory became widely accepted. Lillie (1923) considered that the two concepts, surface adsorption and lipid solubility, were compatible processes that could contribute to the total effect.

Some authors (Loewe, 1913) combined the lipid solubility with the adsorption idea, while others suggested a more radical action of anaesthetics in dissolving membrane lipids (Bibra and Harless, 1847). None of these theories explained how anaesthesia was produced although it was implicitly accepted that the excitability of neurones was depressed.

Höber (1945) suggested that polar groups confer to narcotic molecules surface activity that could inactivate enzymes. In other words, a physico-chemical interaction producing a metabolic effect.

Henderson (1930) tried to propose a mechanism of action when he wrote: "but before we accept this theory

(permeability) is it fair to ask what substances are prevented by the narcotic from entering the neurone, certainly not oxygen. Then is it ions. Is it not much simpler to think with Freundlich and Rona, for example, that the adsorbed narcotic decreases the surface charge available for the production of potential difference, that there is a stabilization as suggested by Lillie?"

The better understanding of the excitable process has permitted to visualize the mode of action of anaesthetics as an interference with the membrane ionic conductance. Still they may act on the surface of neurones or they may act by incorporation into their membrane. In this respect two modern theories have replaced the old ones: the molecular and the electrostatic theories.

The idea that anaesthetic agents may prevent the ionic movements involved in excitation has been proposed by several investigators. Pauling (1961, 1964) and Millar (1961) have given theoretical support to these hypotheses. "Brain waves" (consciousness) would be abolished by the presence of microcrystals (clathrates) which would interfere with the motion of ions or with some chemical reaction (neurotransmitter) involved in "electrical oscillations" (Pauling 1964). In other words obstructing the ionic process responsible for the generation and/or transmission of action potentials. Non-hydrogen bonding

anaesthetics (Xe, chloroform, halothane, N_2O) would act through a mechanism similar to that of stabilizer agents (Featherstone and Muehlbecher, 1963).

Catchpool (1966), one of the defendents of this theory, proposed that: "they (the crystals) would interfere with the movements of ions in the synapses and in neurones, in such a way as to increase the impedance of the reverberating electric oscillations, thus reducing the electrical activity to the point at which consciousness is lost. Hydrates may also inertly lodge in key enzymes sites on proteins, preventing reactant molecules from coming close enough to react with each other".

Expanding this theory, one could say that these crystals interfere with membrane ionic conductance including facilitation of the movement of cations. It could initially promote K efflux (hyperpolarization) and at higher concentrations Na influx (depolarization). A double effect proposed for various inhalation agents (Chalazonitis, 1967; Shapovalov, 1964) and observed on artificial membranes (Bangham, Standish and Miller, 1965). Whether clathrates are needed to alter the conductance of the membrane, or this effect can be obtained by the anaesthetic molecules alone, is not known. Some "inert" gases (Xe) are capable of binding to protein molecules (Schoenborn, 1968).

The electrostatic theory supported by McIlwain

(1962) Bangham, Standish and Miller (1965) and Blaustein and Goldman (1966b) is a modern version of the adsorption idea. Anaesthetics would alter the surface charge of the membrane and therefore its ionic permeability. Obviously, it is based on the idea that the membrane has a fixed positive charge (Teorell, 1956; Tasaki and Singer, 1966; Stein, 1967).

It is mainly due to Claude Bernard's original hypothesis, that most investigators approach the study of anaesthesia searching for a single mode of action. They expect that all anaesthetics, regardless of their physico-chemical properties, act on the central nervous system through an identical mechanism. But, as we will discuss later, different anaesthetics have different patterns of C.N.S. depression just as they have different physico-chemical properties.

C - NEUROPHYSIOLOGY AND ANAESTHESIA

Depression of the C.N.S. characterizes the anaesthetic state. Several sites and sequences of depression have been proposed; they can be reduced to a) hierarchic depression, b) depression of some centre of consciousness and c) depression of synaptic transmission. These effects can be followed by modifications of the EEG and evoked potentials. The sequential and functional changes are not exclusive of each other, rather they describe preferential sites and levels at which anaesthetics might act.

a) Hierarchic depression

Due to the great influence in the field of neurology of the concept of "levels of function" proposed by Hughlings-Jackson, anaesthetics were supposed to act by a "standard sequence of neuronal depression" (Harris, 1951). It is true that there is a pattern of functional depression of the C.N.S., but this observation cannot be interpreted as a functional dependence on precise anatomical localization. The idea of a departmental C.N.S. has been changed for that of an integrated C.N.S. (Sherrington, 1906).

The sequential hypothesis explained amnesia as a preferential depression of cells in the frontal associ-

ation area. Stupor would indicate suppression of the remaining areas of the cortex. Hyper-reflexia would indicate release of hypothalamic control from the cortex and loss of the heat-regulating mechanism would indicate complete hypothalamic depression.

We cannot completely discard the correlation between function and precise anatomical localization. However, a more reasonable approach to the mechanism of action of anaesthetics is to study the effect of these agents on basic neuronal functions such as synaptic transmission, excitation, inhibition or nerve transmission. Once this knowledge is acquired it will be possible to study more complex neuronal functions. Finally we might localize preferential sites of action, not necessarily defined by anatomical boundaries, but by the presence of a common neurotransmitter or a specific membrane characteristic.

b) Depression of the centre of consciousness

Present ideas on the neurophysiology of anaesthesia are based on the works of Moruzzi and Magoun (1949) on the reticular formation, Larrabee and Posternak (1952) on synaptic transmission in sympathetic ganglia and French and co-workers (1953a, b) on action potentials evoked in the thalamus and cortex.

Moruzzi and Magoun and French et al. (1953a) identified a "cephalically directed" brain stem system

formed by a series of reticular relays arriving at the cortex, at least in part, through the diffuse thalamic projecting system. High frequency stimulation of this system leads to desynchronization of the EEG (electrocortical arousal). Shortly after this discovery, French et al. (1953b) demonstrated that Nembutal and ether had a selective depressant action on responses being transmitted through this "central pathway" (ascending reticular system) and not on those transmitted through the classic medial lemniscal system. An observation later confirmed with cyclopropane (Davis, Collins, Randt and Dillon, 1957) and more recently suggested as caused by a preferential depression of the input carried by small fibres (Randt, Collins and Davis, 1958; Randt and Collins, 1959).

These pioneer observations were followed by an intense study on the pharmacology of the reticular formation. It was demonstrated that perfusion of this region with blood containing barbiturates suppressed the arousal response (Magni, Moruzzi, Rossi and Zanchetti, 1961). Further confirmation on the depressant effect of barbiturates upon R F. arousal were made on evoked responses (King, Naquet and Magoun, 1955; King, 1956) and on unit activity (Schlag, 1956; Tishehenko and Shapovalov, 1967). Responses of R.F. neurones could be evoked through varied types of sensory stimuli (Bell, Sierra, Buendia and Segundo, 1964).

However, electrical stimulation of the reticular formation could also induce inhibition of flexor motoneurones (Gernandt and Thulin, 1954; Llinas and Terzuolo, 1965). In addition to excitatory areas, the R.F. has been linked to the main sleep patterns (Candia, Rossi and Sekino, 1967; Jouvet, 1967). Depression of the reticular formation could also imply a state of disinhibition; a complicating effect for those who propose its non-specific depression as the cause of anaesthesia.

French et al. (1953b) considered that the multisynaptic nature of the reticular formation explained its vulnerability to anaesthetics; an hypothesis supported by the experiments of Wikler (1945) and Larrabee and Posternak (1952).

The belief that the anaesthetic state is the result of cortical de-afferentiation had been presented before by Bremer (1935) but was inconsistent with the presence of evoked potentials in the cortex under deep barbiturate anaesthesia (Forbes and Morrison, 1939; Forbes, Battista, Chatfield and Garcia, 1949). Sherrington (1906) and Bremer (1937) had also pointed out that multisynaptic networks appeared to be more susceptible to the effects of anaesthetics (they had no evidence to support their synaptic role). This effect was suspected but not convincingly presented by Heinbecker and Bartley (1940) who said:

"synapses of effector mechanism (bouton type?) such as those concerned in respiration are relatively unaffected by concentrations of anaesthetic agents which completely block synapses of afferent pathways". They suggested, from their observations on turtle hearts, that Nembutal and ether acted as "intrinsic inhibitory" agents. Furthermore they proposed the afferent terminals as the site of action of these two anaesthetics.

c) Depression of synaptic transmission

Larrabee and Posternak (1952) demonstrated the greater depressive effect of Nembutal and ether on the synaptic transmission of sympathetic ganglion as against their depression of nerve fibres (10:1 and 3:1, respectively). These authors pointed to the high synaptic selectivity of Nembutal when compared with ether. On the other hand, it cannot be compared with the highly specific effect of nicotine (1,000:1). Cocaine was equally effective in blocking axonal or synaptic transmission, while urethane had no effect on the latter, at anaesthetic concentrations; an observation recently made with halothane (Li, Gamble and Esten, 1968). Despite these observations it is common to refer to "all anaesthetics" as selective depressants of synaptic transmission.

The concluding remarks of the paper by Larrabee and Posternak are well worth quoting in full: "From this

discussion it becomes apparent that on of the tasks of future investigations is to determine whether anaesthetics act uniformly on all parts of a neurone, or whether certain regions associated with synapses, such as presynaptic endings or cell bodies or dendrites are more readily depressed. We have demonstrated that selective block of synaptic processes unquestionably occurs. The underlying mechanisms, however, remain obscure". After 16 years they still remain obscure. Recent attempts to localize the site of action of anaesthetics on the spinal cord monosynaptic reflex have been inconclusive. They seem to agree on three points:

(1) There is a depression of excitatory post-synaptic potentials (EPSPs) (Loyning, Oshima and Yokota, 1964; Somjen and Gill, 1963; Shapovalov, 1964).

(2) There is no significant change in the excitability of motoneurones to direct electrical excitation (Eccles, Løyning and Oshima, 1966 Somjen, 1967), and

(3) Presynaptic inhibition is potentiated by anaesthetics (Eccles, Schmidt and Willis, 1963; Schmidt, 1963; Miyahara, Esplin and Zablocka, 1966).

The excitability of the afferent terminals has been studied by indirect methods (Somjen, 1963) and by

Løyning et al. (1964). The former author found no significant changes with either ether or Nembutal. The latter considers that thiamylal anaesthesia is due to a failure in the presynaptic release of neurotransmitter.

Studies on the effect of anaesthetics on the post-synaptic membrane have not been done in a systematic form at those synapses in which the neurotransmitter is known. Procaine depresses the post-synaptic response to ACh in Renshaw cells (Curtis and Phillis, 1960) and at frog's myoneural junction (del Castillo & Katz, 1957) (for a presynaptic effect see Usubiaga and Standaert, 1968). Chloralose, which is considered as a blocker of ACh release by Mitchell (1963) but not by Phillis (1968), does not depress the response of Renshaw cells to iontophoretic ACh (Biscoe and Krnjević, 1963). Halothane and ether reduce the response to ACh in cat's myoneural junction (Karis, Gissen and Nastuk, 1967). Other studies by Krnjević and Phillis (1963), Salmoiraghi and Steiner (1963) and McCance and Phillis (1964) suggested that Nembutal and procaine produced a post-synaptic depression on cortical, caudate and cerebellar units respectively. Bloom, Costa and Salmoiraghi (1965) believe that Nembutal selectively depresses cholinergic excitatory receptors.

The depression of the spinal monosynaptic reflex by anaesthetics agrees with their induced muscular

paralysis (de Jong, Hershey and Wagman, 1967). However, during light anaesthesia there is an active contraction of the expiratory muscles (Freund, Roos and Dodd, 1964) that seems to be mediated by excitation of gamma motoneurones (Diete-Spift, Dodsworth and Pascoe, 1962).

Modifications of the EEG and evoked potentials

Changes in C.N.S. activity as dramatic as those induced by anaesthetic drugs, were expected to have a predictable effect on the EEG (Gibbs, Gibbs and Lennox, 1937). Indeed, such is the case and quite reasonably this has been one of the fields of study of electroencephalography (Brazier, 1954; Faulconer and Bickford, 1960). However, not all anaesthetics have a similar effect on the EEG nor is the loss of consciousness associated with a standard electrical pattern (Ether: Faulconer, 1952; Nitrous oxide: Faulconer, Pender and Bickford, 1948; Hydroxybutyrate: Metcalf, Robert and Stripe, 1966; Cyclopropane: Possati, Faulconer, Bickford and Hunter, 1953; Chloroform and halothane: Thomas, McKrell and Conner, 1961). Logically these results indicate some differences in the mechanism of action of the various anaesthetic agents. Furthermore, the EEG electrical arousal and its behavioural correlation are known to differ under various circumstances (Wikler, 1952).

In general, a progressive increase in concentration

of anaesthetic agents leads to an increase in the voltage and a decrease in the frequency of the EEG (synchronization) before final suppression of all electrical activity; CO_2 accelerates this sequence (Rubin and Freeman, 1940).

The beginning of anaesthesia is marked by a transient desynchronization of the alpha rhythm (Faulconer and Bickford, 1960). Hypoxia and hypercarbia have a similar effect (Preswick, Reivich and Hill, 1965). Unconsciousness, however, may be established with different EEG patterns (Creutzfeldt, Bark and Fromm, 1961). Under barbiturates the appearance of a high degree of synchronization, known as barbituric spindles, marks the onset of unconsciousness (Forbes, Merlis, Henriksen, Burleigh and Jiusto, 1956). Hypothermia has an effect similar to that of the inhalation agents (Pearcy and Virtue, 1960).

The effect of anaesthetics on evoked potentials in the somatosensory cortex was first described by Derbyshire, Rempel, Forbes and Lambert (1936), it reveals a preferential depression of the later components, or that part of the response which is believed to originate in the stimulation of non-specific afferent pathways (Domino, 1967). In contrast, the first components which represent activation of the specific afferent pathways are enhanced during the initial stages of anaesthesia (Abrahamian, Allison, Goff and Rosner, 1963). However, these observations

change according to the various anaesthetics (Hertz, Fraling, Niedner and Farber, 1967).

It may be concluded that the C.N.S. is more vulnerable than other systems to the effect of anaesthetics because of its functional dependence on synaptic transmission; a process depressed by concentrations of these drugs capable of producing clinical anaesthesia.

However, there is poor knowledge on the site of action, in the C.N.S. - pre- or post-synaptic -, where these drugs act, or the mechanism by which they may affect a given synaptic structure. Furthermore, it is not known if the selective depression of some neuronal circuits is needed in order to produce the anaesthetic state.

D - CUNEATE NUCLEUS

The experiments on synaptic transmission reported in this thesis were made on the cuneate nucleus of the cat. This nucleus represents the first synaptic relay of the main sensory pathway originating in the forelimb and terminating in the cerebral cortex. This pathway is also known as the dorsal column-medial lemniscal system. Most of the axons of cuneate relay cells form part of the medial lemniscus that ends in the VPL nucleus of the thalamus. Other axons and/or collaterals terminate in the cerebellum. This latter observation is of importance in the identification of relay cells since some of these units might not be evoked by antidromic stimulation of the medial lemniscus (Gordon and Seed, 1961).

The cuneate nucleus is located in the dorsum of the medulla, has a length of approximately 10 mm rostrocaudal and a thickness, or dorso-ventral diameter of 2 mm at its maximum. The terminal fibres of the dorsal columns cover the dorsal surface of the nucleus before they bend, at almost a right angle, to terminate in the nucleus itself. The two cuneates are separated from each other by the more medial gracilis nuclei.

Neurones in the cuneate may be divided in two functionally different groups (Andersen, Eccles, Schmidt

and Yokota, 1964): a) relay cells with projections to the thalamus and the cerebellum and b) interneurones. The data presented in this work are limited by this means as cuneo-thalamic relay cells; and to "interneurones" receiving synaptic excitation from the pyramidal tract (Towe and Jabbur, 1961; Kuypers and Tuerk, 1964). The internuncial units frequently receive collaterals from one or more peripheral nerves (Andersen et al., 1964).

The cuneate neurones can be divided into three groups: a) large marginal cells, b) large round cells organized in clusters or nests and c) small stellar-type cells found singly or in clusters. The axons of all three groups arise as a single unit from the soma, and after giving some small collaterals, penetrate into the white matter located at the base of the nucleus; the marginal cells send axons to the dorsal part (Ramon y Cajal, 1952).

The afferent terminals from the dorsal columns form thick arborizations of 1 μ final fibres, and terminal boutons of an average diamater of 1.8 μ - some can be as large as 8 μ (Walberg, 1966). The majority of these boutons make contact with similar synaptic structures located on the dendrites, or other axons; only 10% appear to synapse directly with the soma of cuneate cells. However, 80% of boutons are still present after section of the dorsal columns at C_3 which indicates the presence of other terminals

arriving from a) structures above C_3, b) pyramidal tract (smaller boutons) and c) internuncial units (Walberg, 1966).

Axo-axonal and axo-axo-dendritic synaptic junctions have been identified in this nucleus (Walberg, 1965). Furthermore the type of synaptic vesicles found in the axon-axon junction belong to the large elongated type described as inhibitory by Uchizono (1966) and Bodian (1966). According to Gordon and Paine (1960) and Gordon and Jukes (1964b) units in the rostral and caudal regions of the gracilis nucleus have larger receptive fields than those in the middle of the long axis; the latter have a greater inhibitory input. The data presented in this thesis were obtained almost exclusively from the middle-lateral part of the cuneate nucleus.

Schwartz (1965) investigated the resting activity and the morphological-functional correlation of cuneate units. She found that hair units were round and organized in nests in the superficial portions of the nucleus. Proprioceptive units were of the multipolar type found mostly single and in the deeper portions. The resting activity of hair and touch units consisted of doublets discharges at irregular intervals. Proprioceptive field neurones had a more regular discharge pattern with rare high frequency discharges. The mean resting discharge of cuneate cells was reduced 25 to 60% following de-afferentiation of the

nucleus. However, some of the hair units showed an increase as if an inhibitory input had been suppressed.

First order somatic afferent fibres are divided according to their diameter, myelin sheath and function in several groups (Ruch, Patton, Woodbury and Towe, 1965). Their skin receptors have been classified by Iggo (1962) in slowly and rapidly adapting units; both of them connected to myelinated fibres and mostly conducting in the A-delta speed range. The existence of specialized nociceptive receptors has been a controversial subject. Its existence has been denied (Sinclair, 1955; Weddell, 1955) or suspected (Von Frey, 1894; Bishop, 1946; Pearson, 1952). However, Burgess and Perl (1967) have demonstrated only recently the existence of afferent fibres responding specifically to noxious stimulation of the skin. These were small A-delta fibres with large receptor fields. These receptors, as well as their afferent fibres, are not affected by anaesthetics at clinical concentrations (de Jong and Nace, 1967).

The somato sensory cortex receives the peripheral afferents by way of two fast conducting systems: the spino-cervical pathway and the dorsal columns-medial lemniscal system. Some sensory modalities are represented on both pathways (Taub and Bishop, 1965), but several differences have been reported; fibres in the spino-cervical tract are

smaller while transmitting convergent input from hair and touch receptors (Perl, Whitlock and Gentry, 1962; Mendell 1966; Wall, 1967), in the dorsal column system this convergence has not been observed. Units responding to joint displacements are present only in the latter system (Wall, 1961; Bowsher and Albe-Fessard, 1967). Finally, only the spinocervical tract (and not the dorsal columns) carries input from nociceptors (Iggo, 1962; Burgess and Perl, 1967).

Primary afferent fibres on entering the dorsal horn of the spinal cord and the dorsal column nuclei have reciprocal presynaptic inhibitory effects (Andersen, Eccles, Schmidt and Yokota, 1964 Eccles, 1964; Wall, 1964). The inhibitory effect of large A fibres on small A and C fibres is supposed to control painful stimuli from entering (gate) the spinal cord to excite cells in the lamina IV (T cells) (Melzack and Wall, 1965). The spinocervical tract originates in these units (Rexed, 1964).

Mendell and Wall (1964) considered the inhibitory effect of large fibres to be mediated through depolarization of afferent terminals - closing the gate or presynaptic inhibition. Small C fibres would hyperpolarize these terminals - opening the gate. However, Zimmermann (1968) has recently denied the hyperpolarizing effect of the latter, instead they also depolarize the terminals.

The study of afferent input control in any of these two main pathways is of importance in the understanding of the role of anaesthetics on the depression of synaptic transmission in the central nervous system.

The dorsal column-nuclei (cuneate and gracilis) have been studied in some detail. The peculiar firing characteristics of cuneate neurones to afferent input (Amassian and de Vito, 1957; Galindo, Schwartz and Krnjević, 1968) is a sensitive parameter that can be used to detect the action of anaesthetic drugs (Galindo, 1967). Presynaptic inhibition represents a powerful control of synaptic transmission in these nuclei (Andersen et al., 1964), its pharmacology appears similar to that observed in the spinal cord (Boyd, Meritt and Gardner, 1966; Banna and Jabbur, 1968).

The possibility that glutamate, GABA and/or glycine are neurotransmitters in this nucleus (Galindo, Schwartz and Krnjević, 1967) offers an hypothesis, as well as a tool to study the effect of various compounds on the post-synaptic membrane. The histology and neuronal connections of the cuneate are well known. It receives descending input from the somato-sensory cortex (Kuypers and Tuerk, 1964; Walberg, 1965), mostly from the contralateral but also from the ipsilater hemisphere (Towe and Jabbur, 1961; Gordon and Jukes, 1964b). This input induces

pre- and post-synaptic inhibition upon cuneothalamic units (Andersen, Eccles, Oshima and Schmidt, 1964) through excitation of interneurones (Walberg, 1965). This effect is mainly localized at the base and middle third of the nucleus (Gordon and Paine, 1960). These nuclei have also a well mapped exteroceptive organization (Gordon and Jukes, 1964a).

It has been possible in this thesis to study the effects of three anaesthetics (procaine, Nembutal and halothane) on the afferent terminals of the dorsal column fibres, the post-synaptic membrane and trans-synaptic excitation of cuneate relay cells and interneurones, as well as synaptic inhibition in the cuneate nucleus of the cat. The results of this work may help to differentiate anaesthetic agents by their single or multiple site of action on the synaptic elements; it is also expected that this investigation will give an experimental background for a discussion on the possible mechanisms of anaesthesia.

METHOD

A GENERAL OUTLINE

B SURGICAL PREPARATION

C ELECTROPHYSIOLOGICAL METHODS

- a) Microiontophoresis
- b) Electro-osmosis
- c) Pressure

D DRUGS

E ELECTRONIC EQUIPMENT - ELECTRODES

- a) Recording
- b) Stimulation
- c) Preparation of the five-barrelled micro-pipette

F ANIMAL CARE - CONDUCTION OF EXPERIMENTS

G STUDIES ON SYNAPTIC TRANSMISSION

- a) Synaptic efficiency
- b) Post-synaptic effects
- c) Presynaptic afferent terminals
- d) Effect according to cell function
- e) Studies on synaptic inhibition

A - GENERAL OUTLINE

The work analyzed here is based on observations made in the pericruciate cortex and the cuneate nucleus of the cat. Synaptic transmission in the nucleus was studied using an experimental preparation developed as part of this research project. Three different anaesthetics: procaine, pentobarbitone and halothane, were administered locally and systemically to determine simultaneously their effects on afferent terminals and postsynaptic membrane. This experimental preparation represents the combination of several techniques previously developed, evaluated and accepted as neurophysiological tools.

Sherrington (1898) was the first to study the decerebrated state by removing the brain above the tentorium. The ischaemic decerebration was later developed by Pollock and Davis (1930). This is the first time that the combination of these two techniques has been used for the purposes described later.

The technique of microiontophoresis was initially applied by Nastuk (1953) and del Castillo and Katz (1957) at the myoneural junction. Later Curtis and Eccles (1958) developed the multibarrelled pipette for the simultaneous study of several drugs. The use of this pipette for

recording and stimulation of synaptic structures was first applied by Curtis and Ryall (1966) in the spinal cord. However, the identification of afferent terminals by stimulation of central structures using single electrodes is known as Wall's technique (1958), but actually it was first described by Edisen (1957) to study conduction velocity, refractoriness and sensitivity to hypoxia of the dorsal root intraspinal endings.

The use of a perfusion cup to circulate solution saturated at various concentrations of halothane, developed during these experiments, makes possible the administration of non ionizable inhalation anaesthetics to neurones located close to the surface of the C.N.S. without the artifacts introduced by the conventional methods of systemic administration.

TABLE 1

Total experiments	91	Cuneate	Pericruciate cortex
Decerebrated	59	59	--
Nembutal anaesthesia	22	17	16
Chloralose anaesthesia	10	8	6

Interspike interval distribution and number of spikes in a burst	47 cells - in 4 cats under chloralose Joint units 6 - Hair 26 - Touch 15 33 cells in 4 decerebrated cats Joint units 5 - Hair 18 - Touch 10

Studies on	Total	cuneate	cortex	Nembutal	procaine	halothane
Post-synaptic firing depression	41	20	21	31	38	25
Afferent terminals	42	42	--	33	12	18
Synaptic transmission	50	50	--	42	10	35
Synaptic inhibition	23	23		9	--	12

B - SURGICAL PREPARATION

A total of 91 successful experiments on cats are reported. Nembutal (sodium pentobarbitone) was used as the principal anaesthetic in 22 experiments. Chloralose in 10. In 59, ether was given at the beginning of the surgical preparation to be discontinued after decerebration. Of this last group no paralyzing agent was used in 12 cases, small amounts of succinylcholine were administred to 15, while initial doses of curare were given to 32 (Table 1)

The experiments were performed on adult cats of either sex and with an approximate weight of 2.5 Kg. Plastic catheters were placed in the femoral artery and femoral vein. The former was connected to a manometer. The latter was used for the administration of various drugs and solutions.

In the preliminary 15 experiments an ample left fronto-parietal craniotomy was performed with the head of the animal fixed in a bi-auricular holder. The dura and the arachnoid were removed from the pericruciate area for electrode penetration. A heating pad thermostatically regulated by a rectal probe was used to maintain a central temperature of $36^{o}C$ ($\pm 2^{o}$).

In 76 cats the muscles of the neck were separated. Part of the occipital bone and the posterior surface of

FIG. 1: Surgical exposure of the cuneate nucleus.

FIG. 2: Surgical exposure of the cuneate nucleus. Note the cup on the surface of the nucleus and the five-barrelled electrode ready for penetration.

the atlas were removed to expose the upper spinal cord and lower medulla (Fig. 1). The dura and arachnoid were removed, as in the cuneate, under a dissecting microscope. A small area of the pia over the left cuneate nucleus was removed for later penetration of micropipettes (Fig. 2). Decerebration was performed in 59 of these animals using the following technique developed during the course of these experiments: anaesthesia was induced with a mixture of ethyl chloride and then of ether given through a face mask. Once the animal became unresponsive to painful stimuli a metallic cannula was passed through a tracheostomy and the administration of ether continued with the aid of a special adapter (Fig. 3). Both common carotid arteries were tied, the superficial muscles, the esophagus and trachea were sectioned between ligatures. The rostral part of these tissues was pulled back; the structures ventral to the skull and the first vertebrae were exposed with the aid of a retractor.

The two muscles, longus capitis and rectus capitis anterior minor were separated from the surface of the occipital bone at the base of the skull. A five millimeter diameter hole was drilled between the two bullae tympani. The dura was removed and a metallic clip placed on the basilar artery* (Fig.3). The objective of this

* By sectioning the brain stem, above the pons, this technique can be utilized, leaving the brain in place, as a "cerveau isolé" preparation.

FIG. 3: Exposure of the basilar artery and special adaptor for ether administration.

FIG. 3: Exposure of the basilar artery and special adaptor for ether administration.

ligature was to minimize blood loss during subsequent surgical removal of the brain above the bony tentorium through an ample craniotomy. This minimal bleeding made possible to localize, by direct vision, the medial lemniscus and the pyramidal tract at the point of section. It also helped to maintain most of the experimental preparations in good condtion throughout the experiments (Fig. 4).

The anaesthesia was discontinued after decerebration. The left superficial radial and the median nerves were dissected and mounted on bipolar siiver wire electrodes specially adapted to a plastic cuff so as to make a "minipool" of mineral oil.

Artificial respiration, by means of a "Palmer" pump, was used whenever paralyzing agents were administered. Oxygen at various concentrations was given as required. A bilateral thoracotomy was performed to reduce respiratory movements thus facilitating single unit recording (Fig. 5).

Anaesthetics were administered using the following techniques: a) microiontophoresis, b) electroosmosis, c) pressure, d) local perfusion and e) systemic administration (intravenous; Nembutal and chloralose, inhalation: halothane).

FIG. 4: Site of decerebration. Section of the brain stem at the level of the mesencephalon. Green spots mark the medial lemniscus, pyramidal tract and the aqueduct.

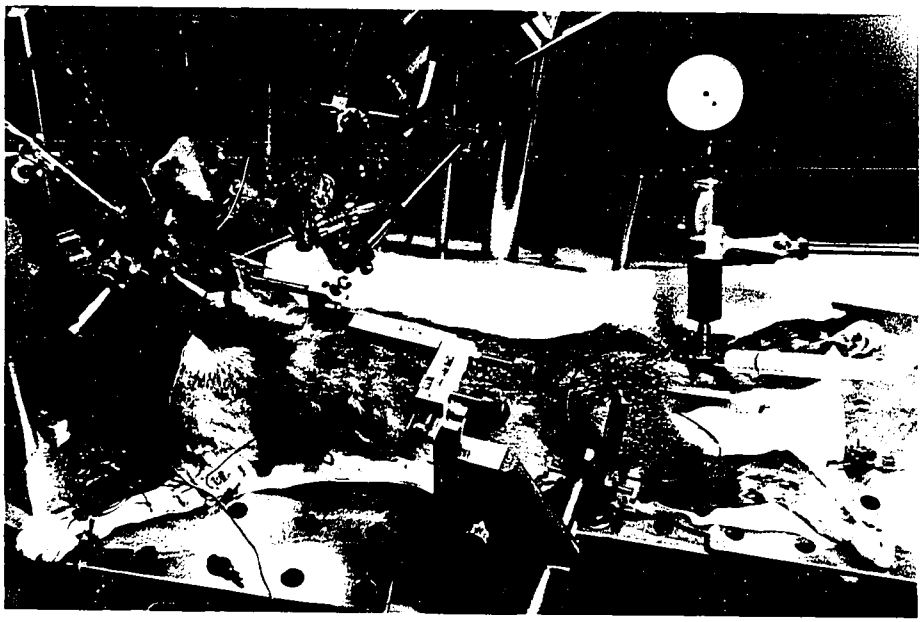

FIG. 5: Full view of the experimental preparation. Left forelimb electrodes on the superficial radial and median nerves. Upper electrodes, inside the cranial cavity, are located on the medial lemniscus and the pyramidal tract.

C - ELECTROPHYSIOLOGICAL METHODS

a) Microiontophoresis

The microiontophoretic technique of drug administration is based on the release of ionized chemical substances from fine glass pipettes by an electrical current. This movement can be controlled by the magnitude of the potential gradient applied across the conducting solution inside the pipette. If the solution is made positive relative to the tissue, cations will be carried out of the tip as a cationic current. If the tip is made relatively negative, anions are then carried out by anionic current. In order to prevent spontaneous release of active ionic compounds, a small current of opposite polarity to that of the active ion is permanently applied to the electrode. This current is known as the "backing current". Alternatively the micro-tap technique for controlling the release of drugs can be used (Comis, Evans and Whitfield, 1964).

The amount of a given ion released by iontophoresis can be calculated by the following equation:

$$M = \frac{n I}{Z F} \qquad (1)$$

where: M = moles/sec or ionic flux, n the transport number, I the current, F Faraday's constant and Z the valence.

Thus, the ionic flux is not directly known from the current flow since the exact value of n is not known. However, for a given pipette, the flux is usually proportional to the strength of the current (Krnjević, Mitchell and Szerb, 1963), consequently it is reasonable to study the relative activity of a given drug at different current strengths.

Except in the immediate vicinity of the tip (Curtis, 1964) once the ions are liberated, the potential gradient in the tissue is too insignificant to affect their distribution in the external environment. The concentration that can be obtained depends upon the rate of ejection and the characteristics of the extracellular (or intracellular) space. The concentration at a given distance is governed by diffusion laws. In an infinite homogenous medium, a steady efflux (M) from a point source should theoretically give the following distribution of concentration with time and with distance:

$$C = \frac{10^7 M}{4\pi x D} \operatorname{erfc} \frac{x.10^{-4}}{\sqrt{4Dt}} \qquad (2)$$

C is the concentration in moles/litre at a distance (x) from the tip at time t in seconds after the beginning of the ejection at a constant rate M moles/sec. D is the diffusion coefficient of the substance in the external medium. For long periods of time this equation can be

reduced to

$$C = \frac{10^7 M}{4\pi \times D} \qquad (3)$$

b) Electro-osmosis

The application of a potential gradient and the resultant current across a microelectrode not only releases ions by iontophoresis but induces an electrokinetic flow of solvent, which may also carry out inert or charged particles. This electro-osmotic ejection depends upon the concentration of the solution and its nature, as well as the type of glass used in the fabrication of the pipette.

$$V = \frac{r^2 \zeta e E}{4n\pi (300)^2} \qquad (4)$$

where V is the volume of liquid transported per sec in ml through a tube of radius r in cm. E is the applied potential gradient in volts and ζ is the zeta or electrokinetic potential (volts); e is the dielectric constant of the solvent and n is the coefficient of viscosity of the solution. This equation can be simplified to:

$$V = \pi r^2 u E \qquad (5)$$

where u is the electro-osmotic mobility in cm per volt per sec. For a micropipette, $V = \rho Iu$ - where I = current passed and ρ the specific resistivity of internal solution.

The contribution of electro-osmosis to the total amount of drug released when a potential gradient is applied

to a pipette is negligible in concentrated solutions as demonstrated by Krnjević et al. (1965). However, electro-osmosis can be used as the principal method of release when working with very low concentrations (NaCl $\leqslant$ 0.01 M) and non-ionizing drugs such as the inhalation anaesthetics. These solutions are positively charged with respect to Pyrex glass (Curtis, Phillis and Watkins, 1959), and they are released by cationic currents. However, it is difficult to determine the actual amount being released, a circumstance that prevented us from using this technique, except for a few experiments made to assess its possibilities and limitations.

c) Pressure

The use of pressure to eject drugs from micropipettes is a method that has been used by several investigators (Hodgkin and Keynes, 1956; Brooks, Curtis and Eccles, 1957; Price, Price and Morse, 1965). However, owing to the high resistance offered by electrodes of 1 or 2 μ diameter, mechanical displacements and possible damage to the cell by the volume of solvent being injected, this technique was used only in a few experiments that confirmed these theoretical disadvantages.

Iontophoresis, electro-osmosis and pressure were used in conjunction with the five-barrelled micropipette. The central barrel was filled with a 2.7 M solution of NaCl

for recording (or stimulation in some experiments). The other four barrels were filled with solutions of various compounds. In most pipettes one of these barrels had a 1 M solution of NaCl which was used to determine current artifacts. Halothane was injected by pressure from a saturated solution of 0.01 M NaCl placed in the central barrel. A lateral barrel was then used for recording.

D - DRUGS

Na L-glutamate, 2 M pH 6.5 (British Drug Houses), - current.

γ-aminobutyric acid (GABA), 1 M pH 2.4 and 5.5 (California Biochemicals), + current.

Glycine, 1 M pH 4.0 (Fisher), + current.

Gallamine Triethiodide, 0.5 M pH 6.3 (Poulenc), + current.

Strychnine Sulphate, 0.02 M pH 7.4 (British Drug Houses), + current.

Picrotoxin, 0.02 M pH 9.5 (British Drug Houses), - current.

Sodium pentobarbitone (Nembutal), 0.2 M pH 9.5 (Abbott), - current.

Procaine Hydrochloride, 1 M pH 5.4 (Abbott), + current.

Veratrine, 0.2 M pH 3.3 (British Drug Houses), - current.

Tøbocurarine chloride, 0.02 M pH 6.3 (Borroughs Wellcome), + current.

Succinylcholine chloride, 0.5 M pH 6.3 (Brickman and Company), + current.

Halothane B.P. (Hoechst).

The pH of these substances was adjusted with 0.1 M solutions of HCl or NaOH as required.

Halothane was vaporized by passing 4 L of oxygen per min through a calibrated Fluotec vaporizer (Cyprane Limited, England) and was delivered to the cat in a continuous flow. The same technique was used to equilibrate mammalian ringer

solutions at various concentrations. Once equilibration was achieved the solution was passed continuously through a plastic cup resting on the surface of the cuneate nucleus. The five-barrelled micropipette penetrated the tissue through the centre of this cup (Fig. 6).

Gases such as halothane are rapidly removed from the tissue by its blood supply, at a rate which can reasonably be assumed proportional to the tissue concentration (y). The basic diffusion equation is then

$$\frac{dy}{dt} = D \frac{d^2y}{dx^2} - Ky$$

where D is the appropriate diffusion coefficient, and K the coefficient of removal.

In the steady state, this equation becomes

$$D \frac{d^2y}{dx^2} - Ky = 0$$

For a semi-infinite solid, and a constant surface concentration (y_o), the solution is

$$y/y_o = \exp(-x \sqrt{\frac{K}{D}}) \quad \text{(Crank, 1956)}$$

If y_o is the concentration of gas in the superfusing saline, a correction is required for the saline-brain partition coefficient (α). Then,

$$y/y_o = \alpha . \exp(-x \sqrt{\frac{K}{D}})$$

Assuming complete equilibration between the blood and the tissue, we have

$$K = \beta \dot{V} \quad (\beta \approx 1/\alpha)$$

where β is the steady state partition coefficient between brain and blood, and $\dot{V}$ is the rate of blood flow (for the brain, the mean value of $\dot{V}$ is 50 ml/100g/min or 0.008 ml/g/sec (Cohen et al., 1964); for halothane, $\alpha = 2.6$ and $D = 10^{-5}$ cm/sec (Larson, 1963)). We can therefore easily estimate the likely concentration of halothane at various depths, as a percentage of the concentration in the superfusing fluid.

Values of y/y_o for different values of x are plotted in Fig. 6a. One can see that within the range of depth where most of the cuneate cells were recorded (shown by arrow), the halothane concentration was likely to be within 75-150% of the surface concentration.

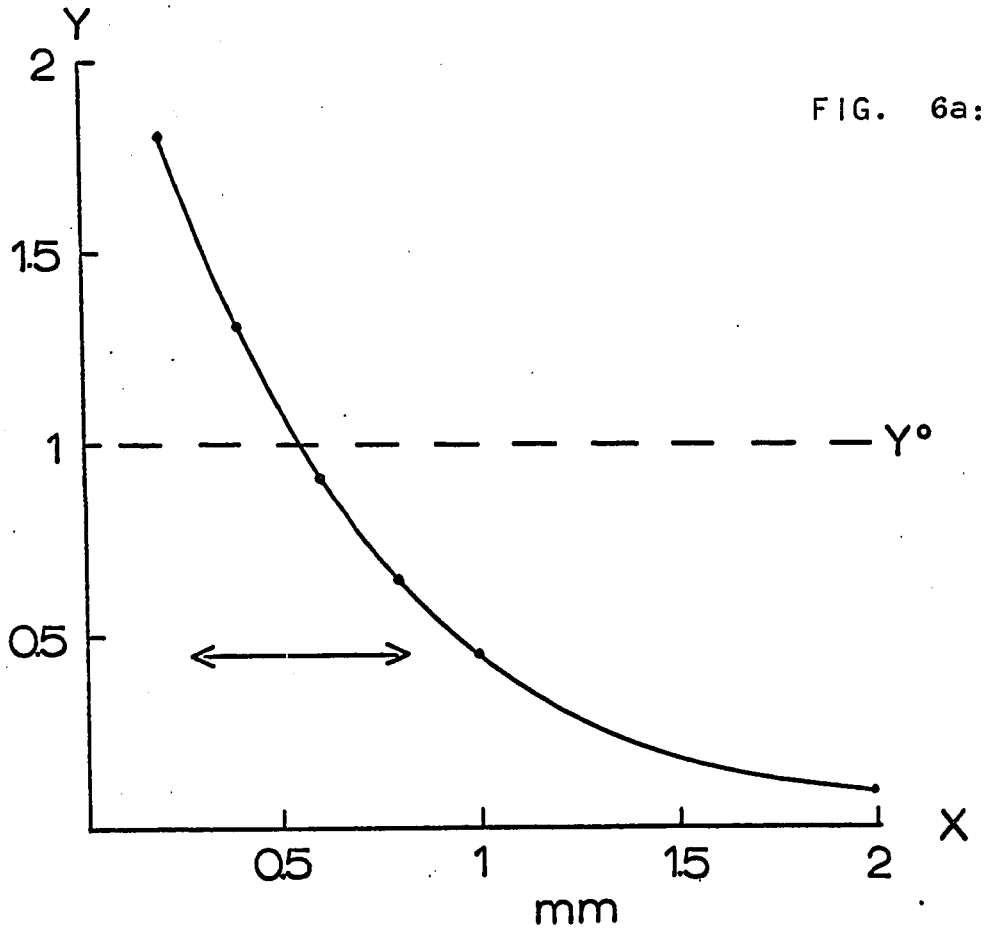

FIG. 6a:

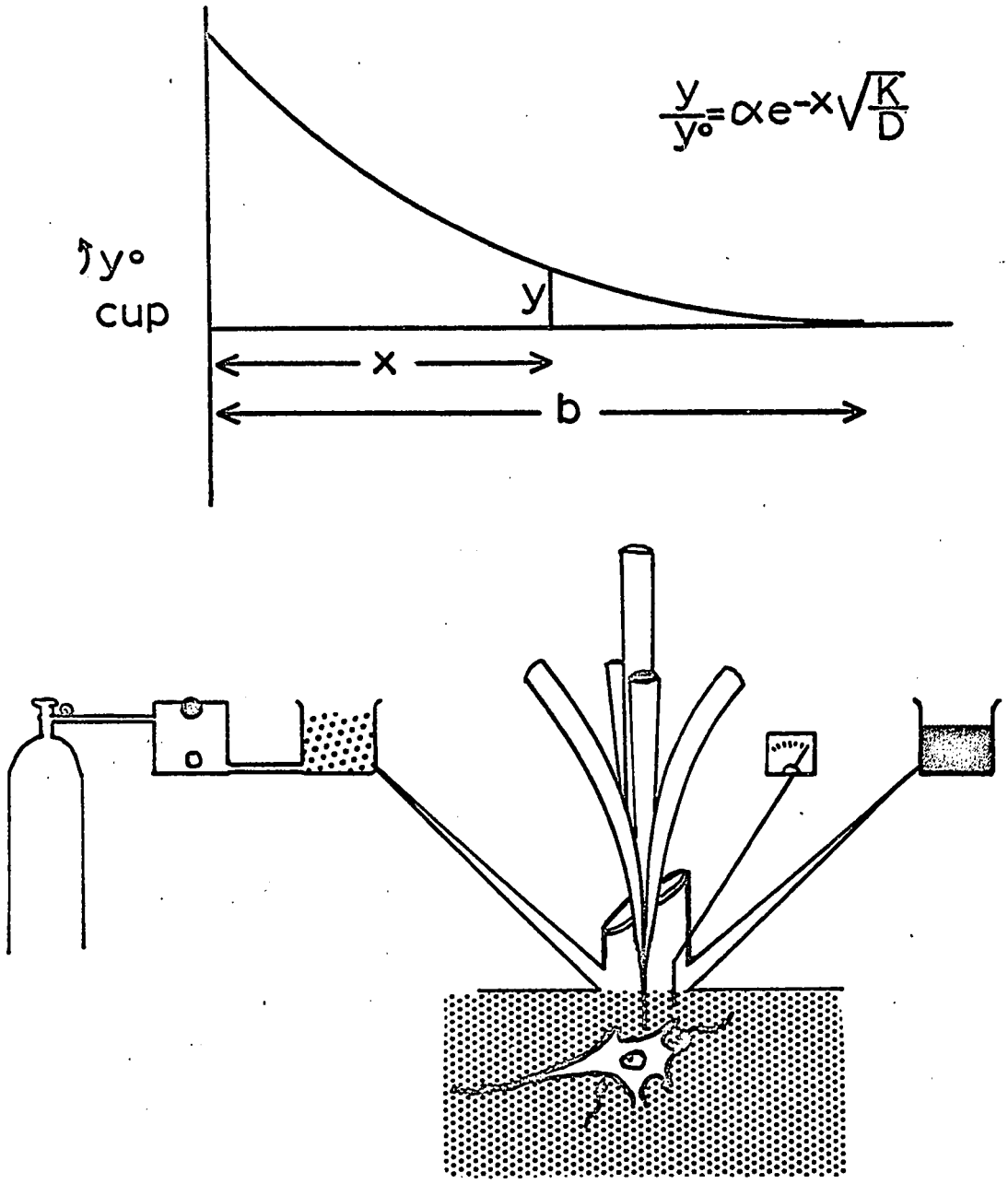

FIG. 6: Perfusion cup and equation to calculate tissue concentrations of halothane (y) at a distance (x) from the surface where $y = y_o$ at $x = 0$ At a distance b, $y = 0$.

E - ELECTRONIC EQUIPMENT - ELECTRODES

a) Recording

The recording was made in the cuneate nucleus, the medial lemniscus and the superificial radial nerve. The signals were fed into a cathode follower and then into a Tektronix 122 AC pre-amplifier with a time constant of 2 msec; final display was made on a 502 Tektronix cathode ray oscilloscope (Fig. 7).

The recordings from the medial lemniscus and the superficial radial nerve were made with bipolar silver electrodes (the portion of nerve between them was crushed for monophasic recording); the signals were led on the same electronic circuit except for the cathode follower omitted for these recordings.

The action potentials were displayed on the oscilloscope and photographed with a Grass Camera C4. For single unit analysis the rate of discharge was also integrated and recorded on paper by an oscillo/riter (Texas Inst. Inc.).

For the analysis of inter-spike intervals, burst discharges and post-stimulus histograms, the gated action potentials were fed into a 100 bin digital computer (Burns, Ferch and Mandl, 1965) (Ferch Electronics) and simultaneously displayed on a second oscilloscope; the histograms

were recorded on paper as described above.

Compound action potentials on single or superimposed sweeps were photographed. Their latency, duration and amplitude were measured.

b) Stimulation

Stimulating electrodes were placed on the superficial and median nerves, the medial lemniscus, the pyramidal tract (in a few experiments they were placed on the somatosensory cortex) and the cuneate nucleus.

Peripheral nerves and the medial lemniscus were stimulated with bipolar silver electrodes delivering electrical pulses of 0.1 and 0.02 msec duration respectively, at a variable voltage and frequency.

The pyramidal tract was stimulated with one per second bursts of 3 to 6 pulses (0.5 msec duration) at a frequency of 500 to 1000 per second, delivered through bipolar silver electrodes. The voltage was adjustable; single pulses of 1 to 2 msec duration were used in some experiments.

Cuneate Nucleus: a special switch was used for recording or stimulation from the cuneate nucleus through the central barrel of the micropipette. A 2 to 10 MΩ resistor in series with the electrode was used to reduce the effect of changes in electrode impedance (Fig.7).

The electrical stimulators used were the Grass

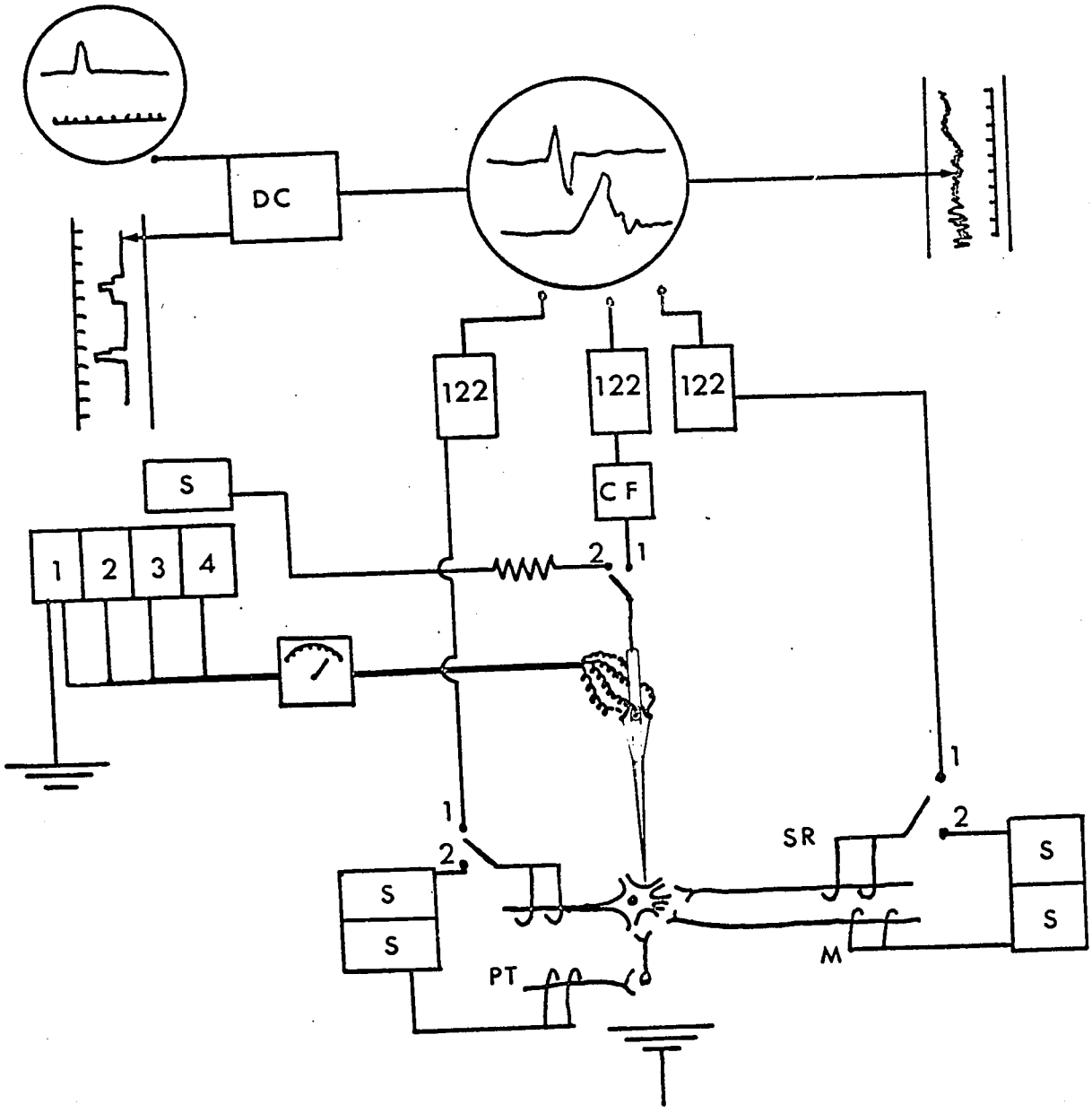

FIG. 7: Diagram of the stimulating, recording and amplifying arrangements used in the experiments on cuneate nucleus synaptic transmission. s = stimulator; C.F. = cathode follower; D.C. = Digital computer; 1,2,3,4. - iontophoretic unit.

SD 5, and Tektronix combination 162 and 161 wave form and pulse generators. In a few experiments a special arrangement was used to deliver triangular pulses of variable rate of rise (ramps), to study the rate of accomodation of afferent terminals.

A four-channel polarizing unit was used for the iontophoretic release of the various drugs. The iontophoretic currents generated in these circuits were measured with an electronic galvanometer (John Fluke Co.). The cat was grounded with a silver chloride electrode attached to the muscle of the neck.

c) Preparation of the five-barrelled micropipettes (Fig.8)

The electrodes were drawn out with a commercial puller (Narishige) and their tips broken to a 5 to 10 μ diameter, then immersed in distilled water and boiled until all air had been expelled from the various barrels. Once the electrode was at room temperature the water was sucked from each barrel with the aid of a small polyethylene tube. The central and one lateral barrel were filled with 2.7 M NaCl, the other three filled with various combinations of drugs according to the experiment. Color codes were used to identify these drugs. The electrodes were placed in a dark box which was kept at 4 to 10^{o}C. 24 to 48 hours to allow for the diffusion of the drug to the tip of the electrode.

These pipettes were used in no more than two different animals and stored for no more than one week. The resistance of each barrel was measured and recorded before and after its use.

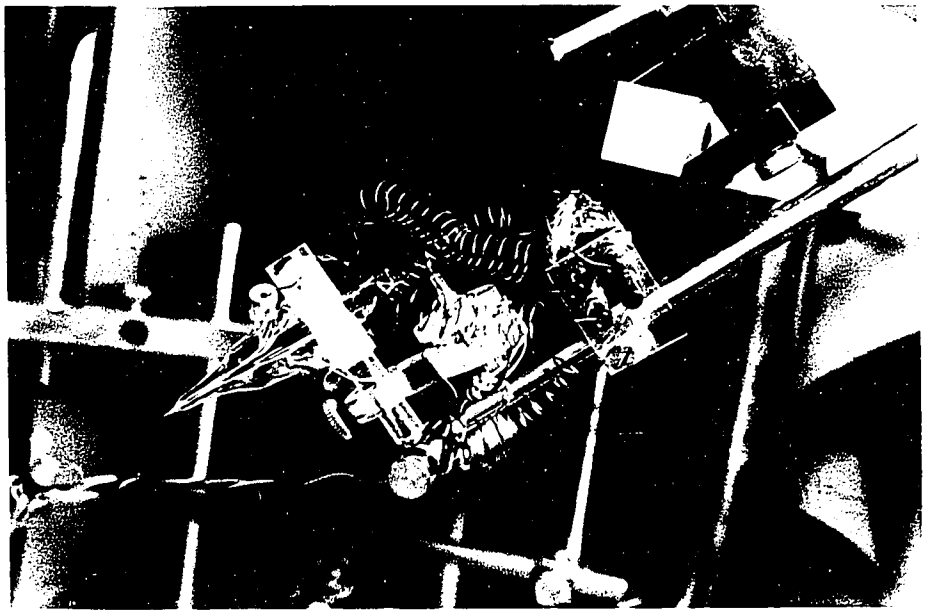

FIG. 8: View of the five-barrelled micropipette and part of the cathode follower.

F - ANIMAL CARE - CONDUCTION OF EXPERIMENTS

Special care was taken to maintain the cats in a good general condition during the experiments. Most animals had a mean blood pressure of 90 mm Hg. Hypotension was corrected when indicated by one or more of the following treatments: a) continuous intravenous administration of nor-adrenaline, b) correcting metabolic acidosis with sodium bicarbonate, c) oxygen-enriched respiratory input, d) infusion of plasma expanders (Subtosan, Poulenc) and as fast and careful surgical preparation as was possible. Whenever the arterial blood pressure could not be maintained over 60 mm Hg the experiments was discontinued.

Two paralysing agents were used, tubocurarine and succinylcholine. Tubocurarine in single doses of 0.5 to 1 mg/Kg every 30 to 60 minutes during the first six hours. After this time only negligible further doses were required. Unfortunately, it lowered the blood pressure below 90 mm Hg in most animals thereby increasing the need for vasopressors. Furthermore tubocurarine depressed the afferent terminals when given systemically or iontophoretically. Its depressant effect on the efferent terminals has been reported (Standaert, 1963).

Succinylcholine was given by continuous intra-

venous injections of a solution of 1 mg/ml, at a rate of 0.2 to 0.5 ml per minute. It offered some advantages over tubocurarine. The blood pressure was well maintained, while the experimental results showed no central effects. This paralyzing agent has been shown to depress motor nerve terminals (Standaert and Adams, 1965) while it is suspected of depolarizing afferent terminals in the spinal cord through activation of muscle spindles (Cook, Neilson and Brookhart, 1965). The observations reported in this thesis were made on the terminals of a pure sensory (skin) nerve - the superficial radial.

G - STUDIES ON SYNAPTIC TRANSMISSION

a) Synaptic efficiency

This efficiency was determined by the following criteria: a) changes in the main response of cuneate cells to peripheral stimulations (touch, pressure, joint movements, hair displacement), b) changes in the medial lemniscal action potential evoked by cuneate stimulation, c) changes in the action potential of cuneate cells during electrical stimulation of peripheral nerves (superficial radial or the median nerves) and/or medial lemniscus (Fig. 9).

b) Post-synaptic effects

The effect of anaesthetics and other substances on the post-synaptic membrane were assessed by the changes induced in the response of the cells to iontophoretic ACh and glutamate. These observations were made in both cortical and cuneate neurones.

c) Presynaptic afferent terminals

These studies were made in the cuneate nucleus (Fig. 9); a five-barrelled micropipette was introduced to a depth between 300 μ to 1.500 μ so as to locate the afferent terminals of the superficial radial nerve. These structures were differentiated from cell units by the positive response to high frequency stimulation , and from dorsal column

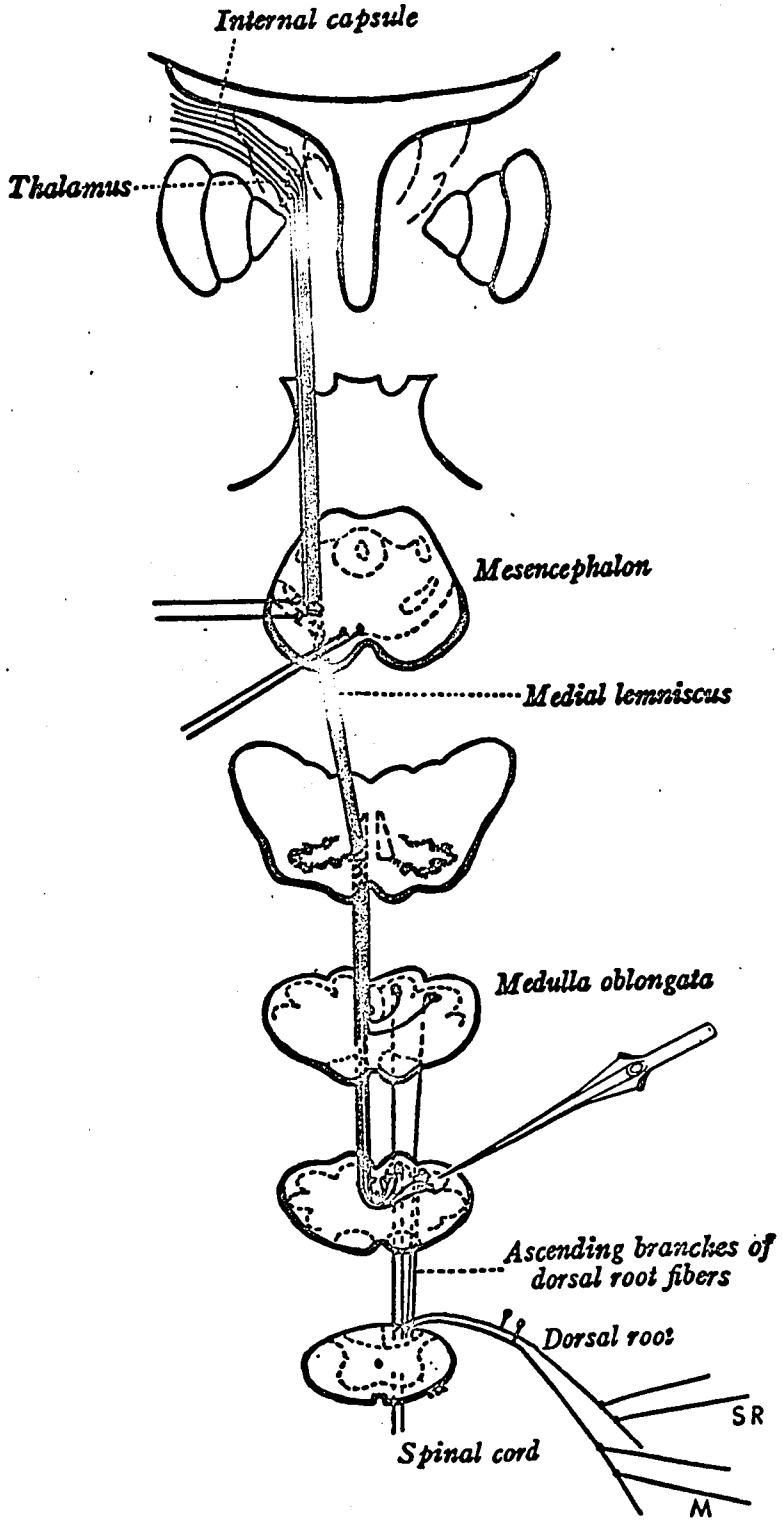

FIG. 9: Diagram of the experimental preparation. Electrodes on the mesencephalon represent the site of stimulation of the medial lemniscus and the pyramidal tract. (Fig. 260 from Ranson, S.W. and Clark, S.L. The Anatomy of The Nervous System, 9th edn, p.353. Philadelphia: Saunders.)

fibres by the lesser resistance to hypoxia as shown in Figure 30. The central barrel was used for stimulation. The other four barrels were used for the microiontophoretic release of several substances. Also studied were the effects produced by the systemic administration of Nembutal, halothane, curare, succinylcholine, strychnine and picrotoxin.

d) Effect according to cell function

Two populations of cells were studied: cuneothalamic relay cells and interneurones. The former were identified by their antidromic response to medial lemniscal stimulation. The latter by their trans-synaptic response to pyramidal tract stimulation and unresponsiveness to medial lemniscal stimulation (Andersen, Eccles, Schmidt and Yokota, 1964).

e) Studies on synaptic inhibition

The effects of the systemic administration of Nembutal, halothane, strychnine and picrotoxin were observed. Possible pre- and post-synaptic inhibitions were studied using the following methods: a) Depression of the trans-synaptic response of one nerve, the superficial radial, while stimulating a second one, the median. Trans-synaptic action potentials were recorded from the cuneate nucleus and/or the medial lemniscus. b) Cuneate stimulation: median nerve conditioning. Inhibition was determined

by changes in the medial lemniscal action potential. The changes observed with either method were plotted as percentage of control at various intervals after a conditioning pulse. c) Post-synaptic inhibition curves determined by the inhibitory effect of peripheral pulses on cuneate cells being stimulated by microiontophoretic administration of glutamate. Post-stimulus histograms as well as photographic records were used for this determination. The effects of picrotoxin and strychnine on synaptic inhibition were observed following their systemic and/or iontophoretic administration.

RESULTS

A EFFECTS ON SYNAPTIC TRANSMISSION THROUGH CUNEATE NUCLEUS

a) Excitation of cuneate cells by stimulation of peripheral receptors

Procaine

Ca^{++}

Sodium Pentobarbitone (Nembutal)

Halothane

b) Indirect electrical stimulation of cuneate cells (orthodromic and antidromic)

Procaine

Sodium Pentobarbitone (Nembutal)

Halothane

DISCUSSION

c) Direct electrical stimulation of the cuneate nucleus

Procaine

Nembutal

Halothane

DISCUSSION

B EFFECTS ON THE POST-SYNAPTIC MEMBRANE OF CUNEATE CELLS

a) Microiontophoretic, electro-osmotic and

A - EFFECTS ON SYNAPTIC TRANSMISSION THROUGH CUNEATE NUCLEUS

a) Excitation of cuneate cells by stimulation of peripheral receptors

These receptors were classified as: hair, touch and proprioceptive, according to the response of cuneate neurones to the various modalities of stimulation; a) a jet of air, b) a weight applied upon the receptive area, or, c) the displacement of a joint (Amassian and de Vito, 1957; Schwartz, 1965; Galindo, Krnjević and Schwartz, 1967).

The activity of these cells was analysed as follows: 1) by their firing rate, 2) by the number of spikes forming a continuous discharge or burst and 3) by the distribution of interspike intervals (IID).

Procaine: This local anaesthetic was administered iontophoretically in 8 experiments, four of them under chloralose anaesthesia (80 mg/Kg I.V.) and the others on decerebrated cats. There were no significant differences between these two groups. A total of 11 joint units, 25 touch units and 44 hair cells were analyzed. Procaine was released for a period of 10 to 30 seconds at strengths between 10 and 100 nA. The initial effect of this anaesthetic was to reduce the number of spikes in a given burst

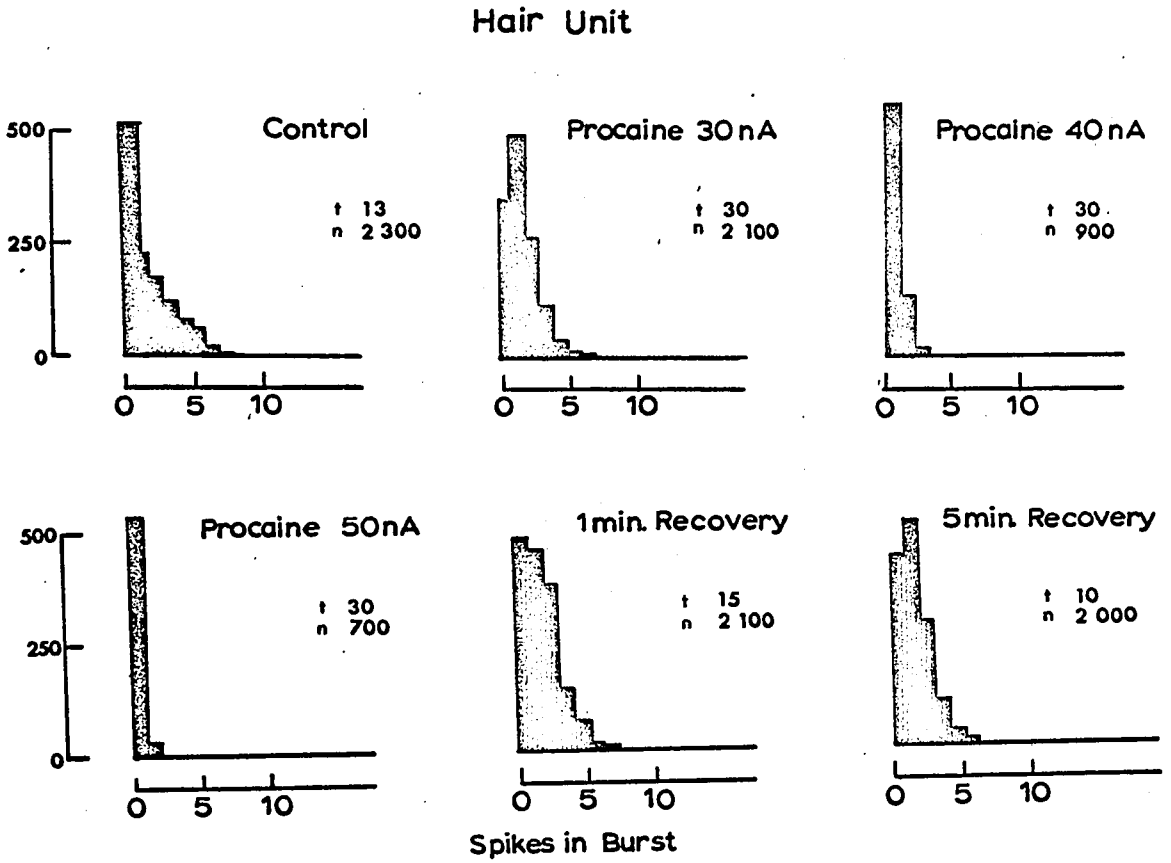

FIG. 10: Effect of procaine on the number of spikes forming a burst. Ordinate: number of ~~spikes~~ bursts; abscissa: number of spikes in a single burst. n = total number of spikes during t - time in seconds. Procaine reduces the number of spikes in a given burst. Observations made in the cuneate nucleus of a cat under chloralose anaesthesia (80 mg/Kg).

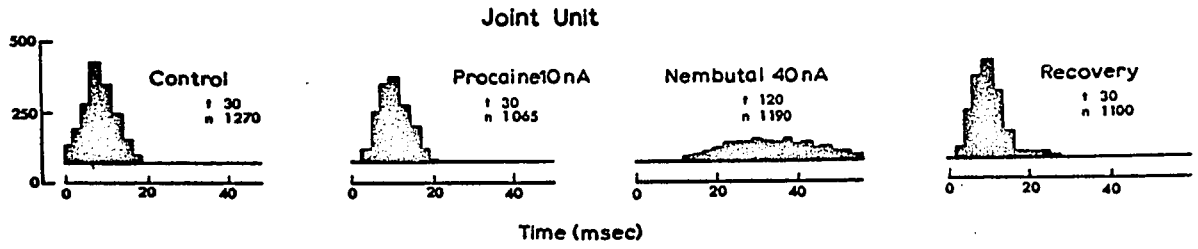

FIG. 11: Interspike interval distribution and the effect of procaine and Nembutal. Ordinate: number of spikes; abscissa: time in msec between two spikes. n = total number of spikes during time t in seconds. Unit activity was evoked by flexion of the wrist. Decerebrated cat.

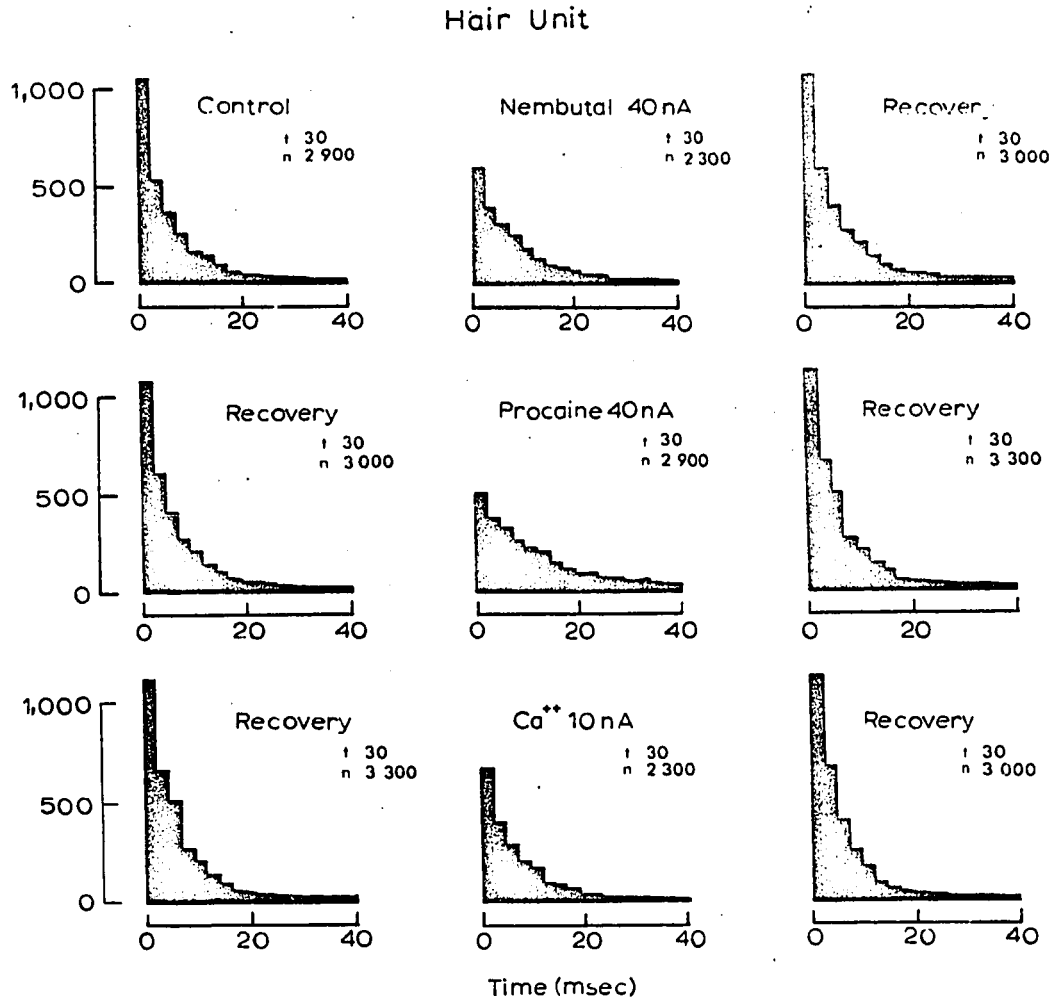

FIG. 12: Interspike interval distribution histogram of a hair unit peripherally evoked by a continuous jet of air. Note the difference in interspike interval distribution with the proprioceptive unit (Fig. 11). (See Galindo, Krnjević and Schwartz, 1968).
Nembutal, procaine and Ca^{++} displace the histogram to the right or in favour of longer interspike intervals. This parameter is the first to be affected by anaesthetics.
t = time. n = number of spikes. Observations made in the cuneate nucleus of a decerebrated cat.

TABLE 2

A

# of cells	Place	Glut nA	S.E.	Procaine nA	S.E.	# of cells	Glut nA	S.E.	Nembutal nA	S.E.
11	c. cortex	79.0	8.8	36.8	10.4	25	76.4	5.7	69.2	6.6
12	cuneate-hair	123.2	35.7	28.1	5.4	10	131.0	25.5	55.0	12.2
8	cuneate-others	151.2	33.9	38.7	7.3	25	57.2	7.6	45.2	6.4

B.

# of cells	Nembutal nA	S.E.	Procaine nA	S.E.	P<
13	113.07	19.9	33.4	4.9	.01

C.

# of cells	GABA nA	S.E.	Glycine nA	S.E.	P<	ANAESTHESIA
10	60.0	11.9	18.8	4.7	.01	Decerebrated
10	19.0	3.7	7.0	2.1	-	Nembutal 30 mg/Kg
17	25.2	7.2	23.3	7.9	-	chloralose 80 mg/Kg

The information in this table is derived from a maximum of two cells per any given experiment. Different electrode for each unit analyzed. In A are compared the ratio of procaine to glutamate and Nembutal to glutamate. That is the current strength of the two anaesthetics needed to suppress the post-synaptic excitation induced by the amino acid. In B are compared the effectiveness of Nembutal and procaine on the same cuneate neurones. These are equipotent doses needed to suppress a similar level of glutamate excitation. In C are compared the effectiveness of GABA and glycine according to the type of anaesthesia.

(Fig. 10) and to change the shape of the IID (inter-spike interval distribution) (Fig. 11). The reduction in mean firing rate was less apparent; but higher current strengths had a marked effect on this latter parameter.

Ca^{++}: This ion had an effect similar to that of procaine (Fig. 12); it was tested in six experiments for a total of 15 cells; four under chloralose anaesthesia and two decerebrated preparations. The main difference with procaine was the presence of larger spikes under Ca^{++} administration just before their disappearance.

Sodium Pentobarbitone (Nembutal): Its systemic administration (10-20 mg/Kg) facilitated the action of its iontophoretic release. This anaesthetic reduced the firing rate, the number of spikes in a burst and lengthened the inter-spike intervals (Figs. 11-12). These effects were similar to those of procaine, but a larger dose of Nembutal was required to induce the same depression (Table 2). The results with Nembutal were difficult to reproduce in all animals. This lack of consistency could be explained by a combination of factors: a) the pH of the pentobarbitone solution had to be adjusted between 9-10: if higher, (>11) the tip of the electrode disintegrated too rapidly for use; if much lower (<9) the anaesthetic tended to precipitate. b) The use of current higher than 100 nA (-). The

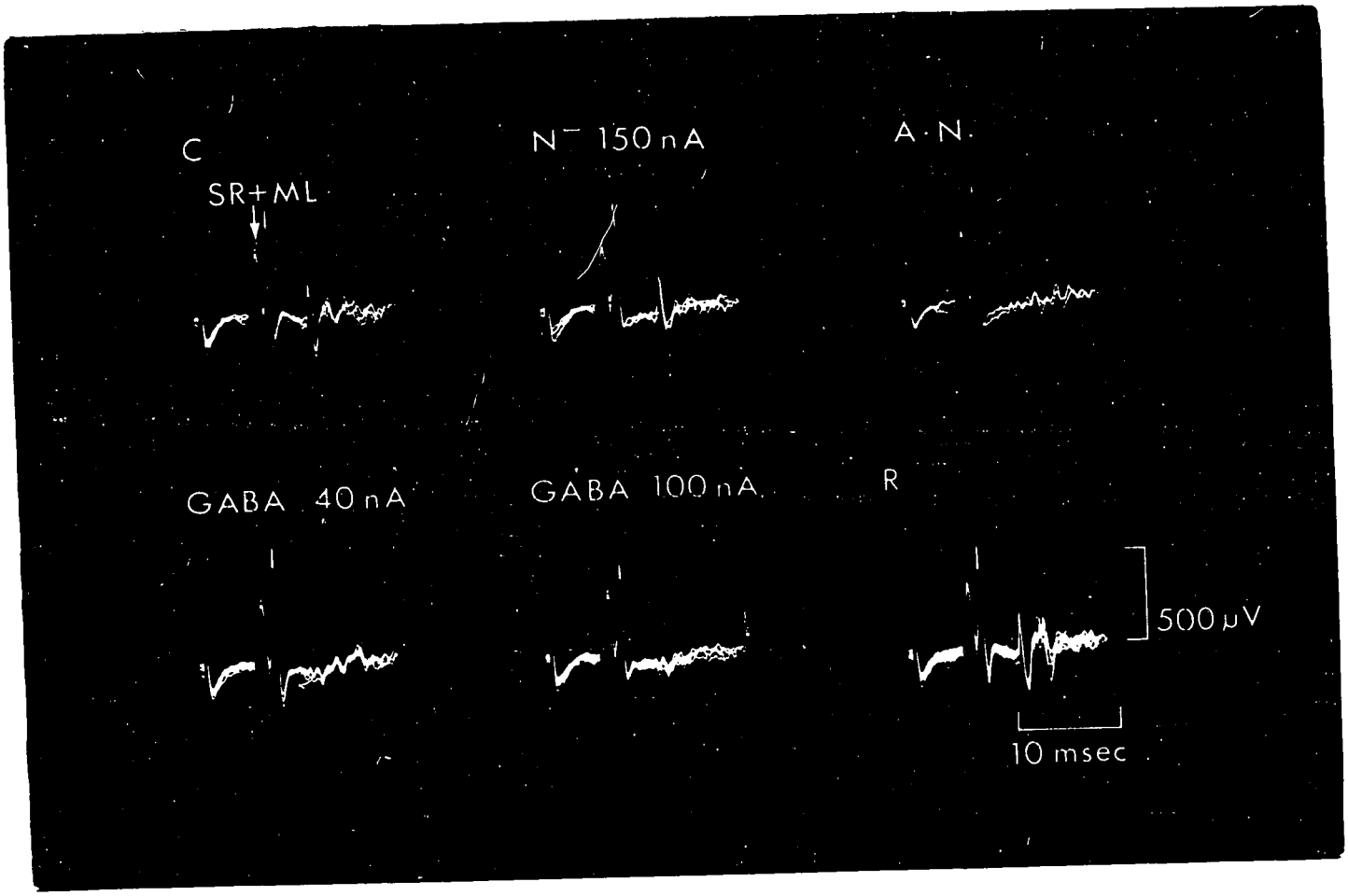

FIG. 13: Effect of Nembutal and GABA on a cuneate relay cell being evoked simultaneously from the superficial radial nerve (S.R.) and antidromically from the medial lemniscus (M.L.).
The effect of Nembutal (N - 150 nA) is seen on the orthodromic action potential only immediately after the releasing current is turned off (A.N.).
GABA is more effective on the orthodromic spike.
The antidromic spike increases in size initially (40 nA) suggesting hyperpolarizing effect.
Several traces are superimposed in each record.
Records from the cuneate nucleus of a decerebrated cat.

negative polarity tends to offset the effect of Nembutal owing to its depolarizing effect. The effectiveness of the anaesthetic could be demonstrated only immediately after cessation of the releasing current (Fig. 13).

c) Impurities and dirt from water used to prepare solutions. This was partly controlled by filtering the distilled water with a 0.2 μ millipore tissue.

The depression of synaptic transmission induced by Nembutal took 20 to 40 sec to reach its maximum and a similar time to disappear. In a few (6) experiments thiopental (Pentothal) was administered iontophoretically (50-150 nA) with similar results.

Halothane: This agent was studied in 25 experiments. Its effect appeared after more than 7 min of its administration by inhalation and persisted for more than 20 minutes after its cessation. When the anaesthetic was given through the cup both times were shorter. The results were inconsistent when it was administered randomly to various types of cuneate neurones. The rate of firing decreased with some cells or rose with others. This action was not related to the concentration (when given by inhalation or through the cup at 1-2% v/v). The same unpredictable results were observed on the inter-spike interval analysis and in the shape of the burst. It was later realized that these confusing effects could be explained by functional differences

in the population of cuneate neurones. Identification of units was then made to test this idea, the results are presented on page 77.

b) Indirect electrical stimulation of cuneate cells (Orthodromic and antidromic)

This part refers to the effects of the anaesthetics on cuneothalamic relay cells. Two stimuli were applied simultaneously to activate the same unit, one to a peripheral nerve for trans-synaptic excitation and the other to the medial lemniscus for antidromic invasion of relay units. The strength of stimulation was adjusted just above threshold. Collision of the two action potentials was used to ascertain the single origin of the spikes.

The antidromic action potential had a delay of less than 2 msec while that of the orthodromic was between 4 and 6 msec. There were no significant changes in conduction velocity after the intravenous injection of Nembutal (30 mg/Kg) or the inhalation of halothane (2%).

Procaine: The iontophoretic release of this anaesthetic (10 to 100 nA), which was studied in 15 experiments for a total of 57 cells, depressed both the antidromic and the orthodromic action potentials at equal releasing currents (84.5-85.2 nA, respectively, $P < 0.1$, Fig. 14). On several occasions the antidromic spike was depressed

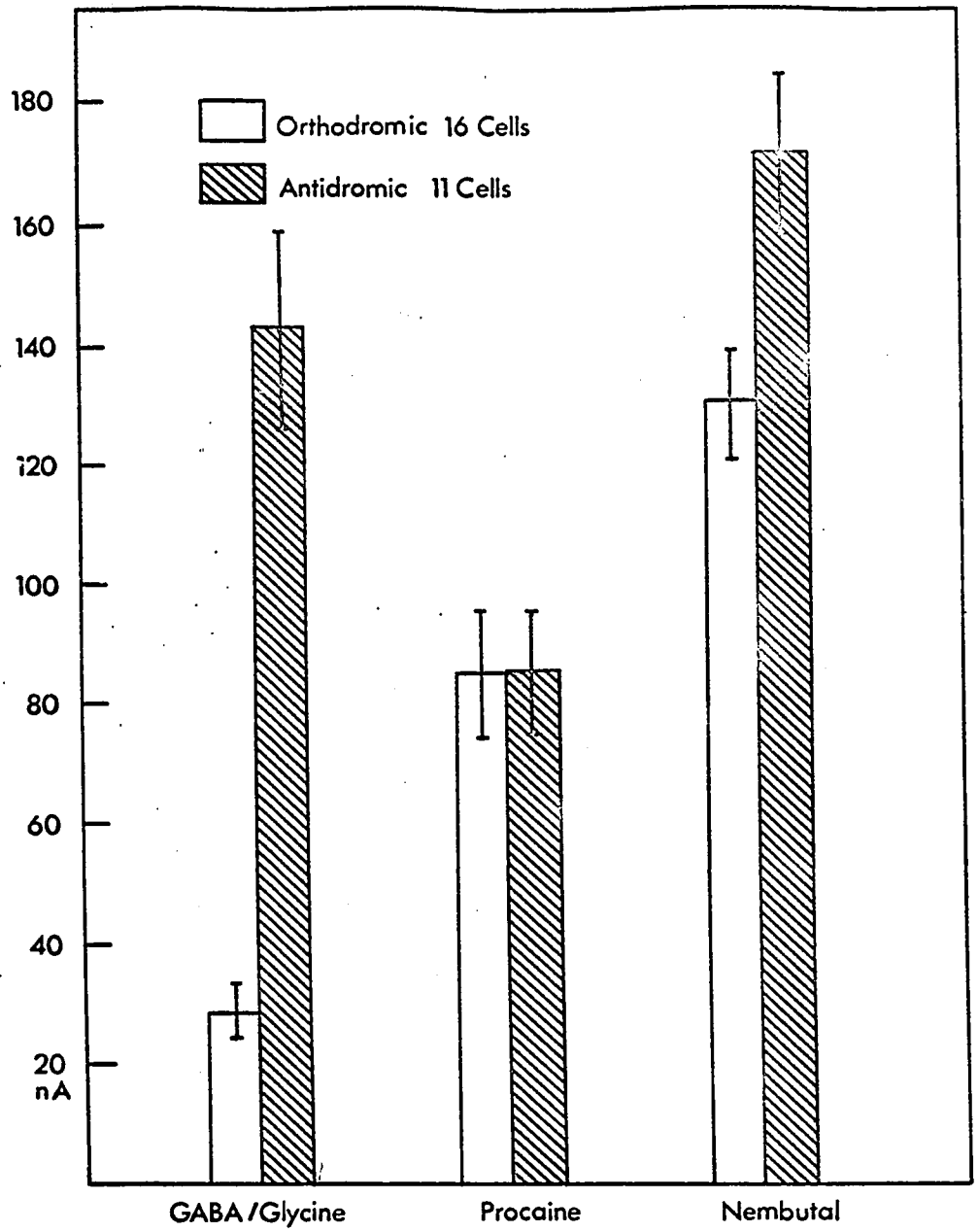

FIG. 14: Effects of GABA or glycine, procaine and Nembutal on both orthodromic and antidromic action potentials of cuneate relay cells. GABA or glycine are five times more effective in depressing orthodromically evoked action potentials than in depressing the antidromic invasion of the same units ($P < 0.01$). Nembutal is also more effective in suppressing trans-synaptic invasion of relay units ($P < 0.05$). However, the difference in dose needed to suppress this latter response on the antidromic invasion is smaller (25%). Procaine is equally effective on both of them. Observations made on decerebrated cats.

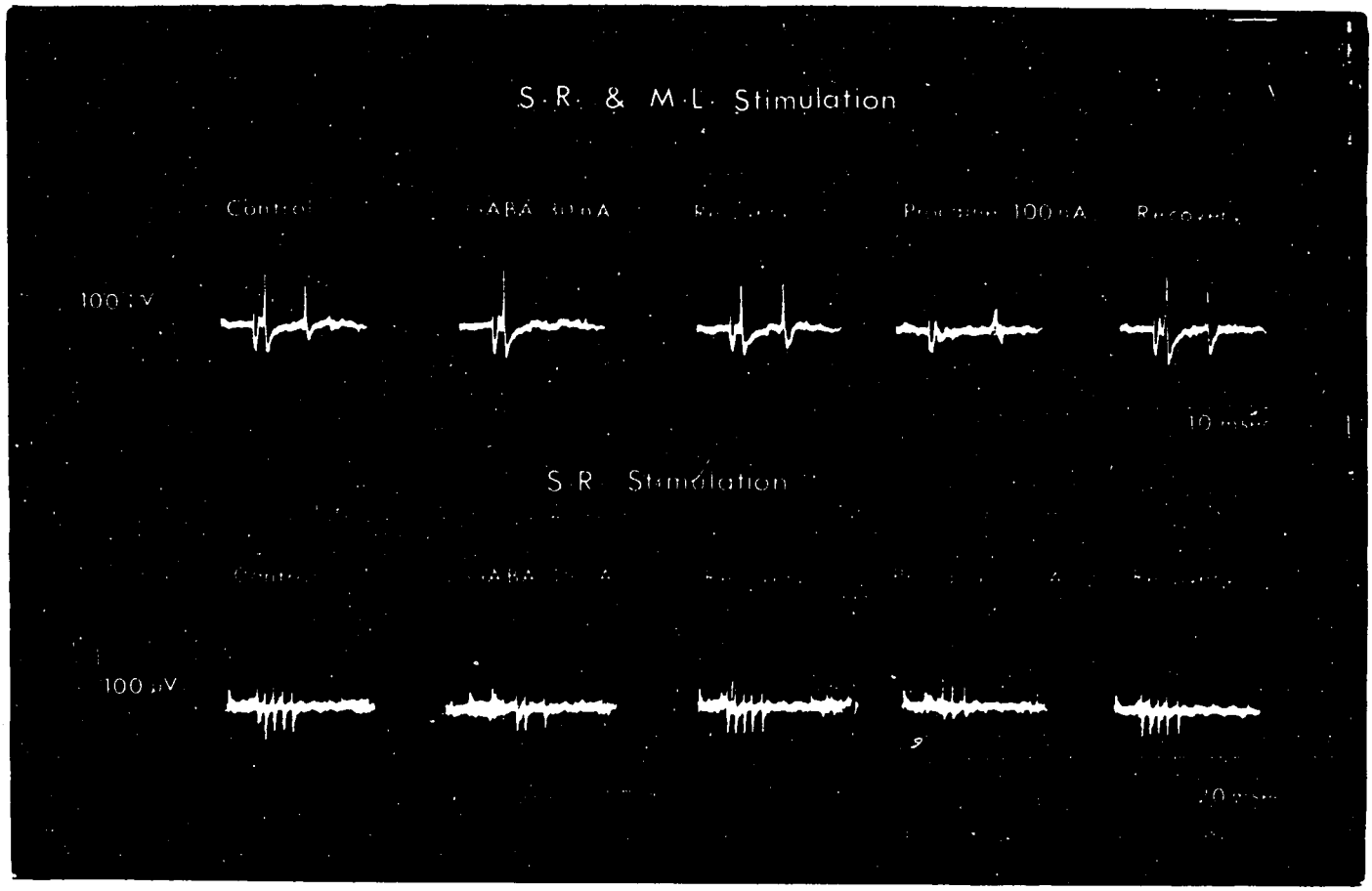

FIG. 15: Upper line, simultaneous ortho- and antidromic stimulation of a cuneothalamic unit. GABA is more effective in blocking the former while procaine is equally effective on both. Lower row of photographs shows the effect of these two drugs on burst activity evoked from the periphery. S.R. = superficial radial nerve. M.L. = medial lemniscus. Recording from the cuneate nucleus through the central barrel of a five-barrelled micropipette. Microiontophoretic release of GABA and procaine. Decerebrated cat.

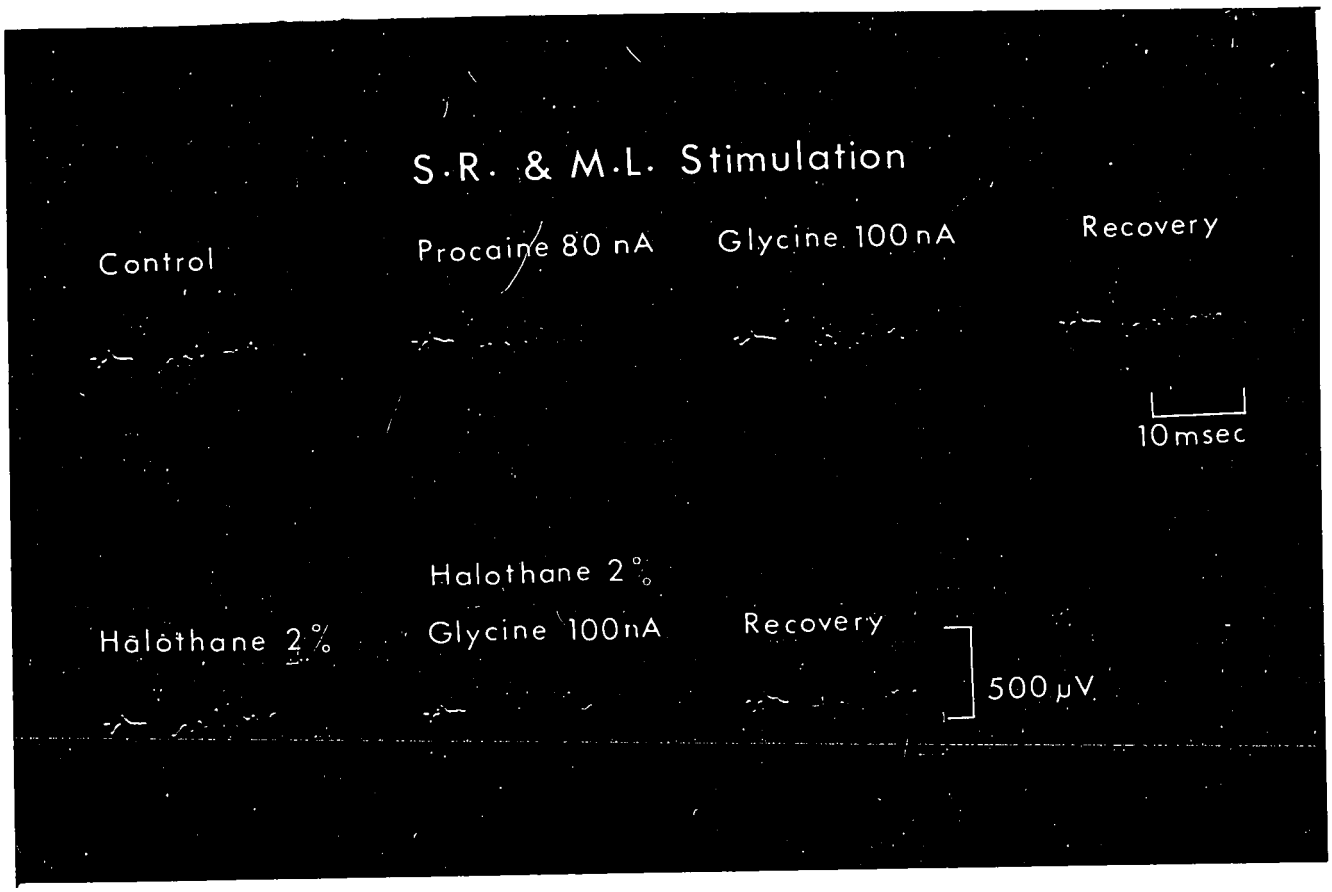

FIG. 16: Simultaneous stimulation of a cuneothalamic unit from the superficial radial nerve (S.R.) and the medial lemniscus (M.L.). Halothane abolishes the orthodromic response of relay cells when given at concentrations of 2% or more. The effect of glycine is easily observed during halothane administration. Recording from the cuneate nucleus in a decerebrated cat.

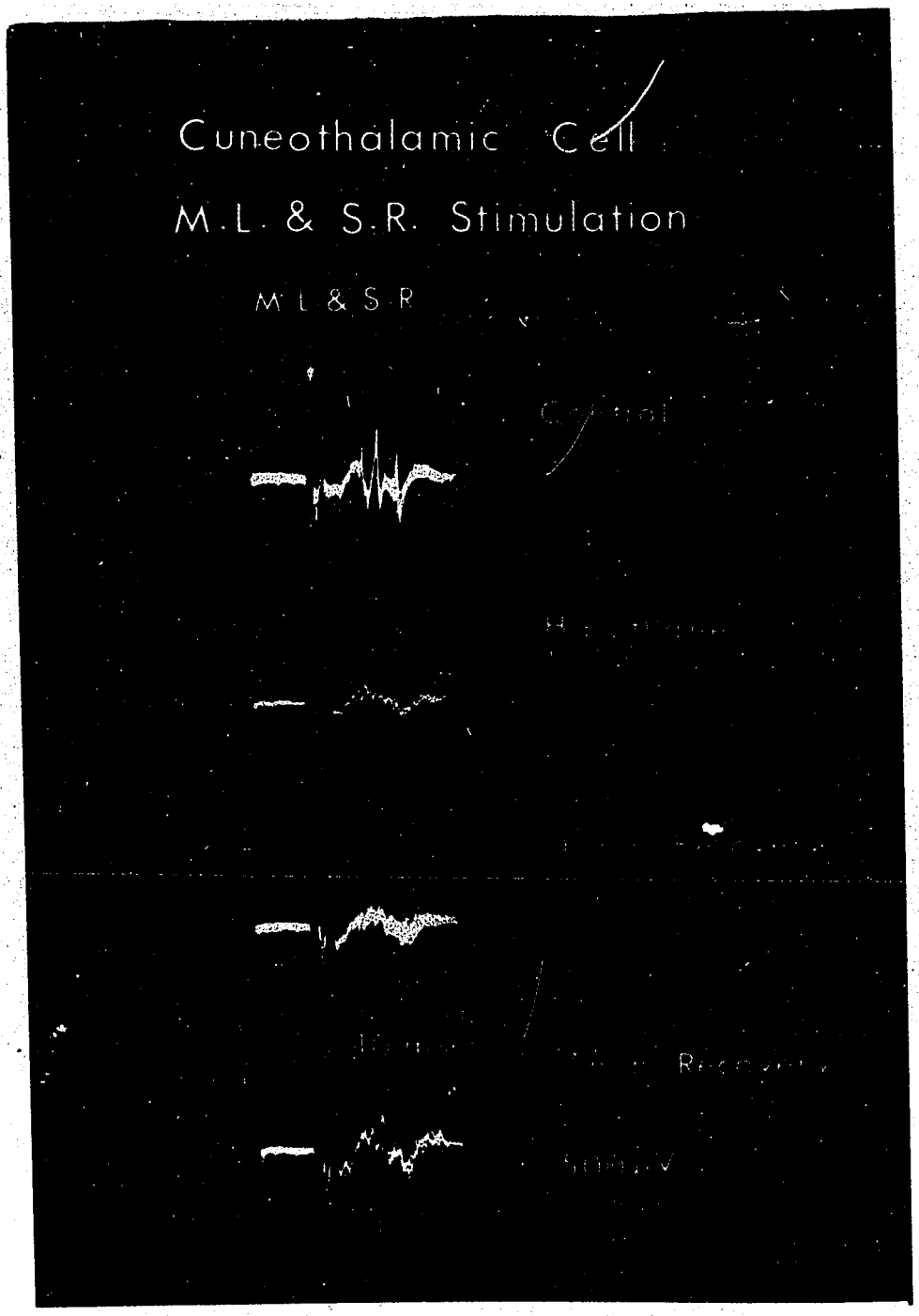

FIG. 17: Effect of 3% halothane - given through the cup - on a cuneothalamic relay cell being evoked simultaneously from the medial lemniscus (M.L.) and the superficial radial nerve (S.R.). The antidromic action potential is first increased in amplitude (not shown) and subsequently reduced, its duration becomes longer. The reverse sequence is observed during the recovery period (shown at 4 min). This effect suggests a hyperpolarizing mechanism. Orthodromic action potentials are easily depressed by halothane. Recording from the cuneate nucleus, decerebrated cat, suprathreshold stimuli. Several traces are superimposed.

first (Fig. 15).

Nembutal (Sodium pentobarbitone): This barbiturate was studied in 27 experiments for a total of 63 units. It consistently depressed the orthodromic spike first and only by increasing the strength of the current by more than 25% ($P < 0.05$) was it possible to suppress the antidromic spike (Figs. 13, 14). Its systemic administration (20 mg/Kg) was effective in depressing orthodromic action potentials but had no effect on the antidromic spikes.

Halothane: This agent was studied in 20 experiments for a total of 56 units. The results were similar regardless of which of two methods was used: inhalation or local perfusion. Halothane up to 2% (v/v) for more than 4 min of administration depressed synaptic transmission (cuneothalamic cells) but had no effect on the antidromic invasion (Figs. 16, 17). This effect was reversible after short periods (10-20 min) of administration. In most experiments halothane was given through the perfusion cup to avoid systemic changes in blood pressure.

DISCUSSION

These results indicate that the mode of action of procaine, Nembutal and halothane differ from each other. Procaine is equally effective in blocking the antidromic or the trans-synaptic stimulation of cuneothalamic cells, an effect consistent with its non-specific action as a

blocker of Na conductance (Shanes, 1958; Taylor, 1959).

Nembutal is more effective in blocking the trans-synaptic excitation of cuneate neurones; at higher doses it blocks their response to antidromic stimulation. Halothane, at anaesthetic concentrations, depresses only the orthodromic invasion of cuneothalamic units.

Similar differences have been reported before. Lorente de Nó (1947) described the effect of cocaine and ether on frog's nerve excitability. The blockade of nerve conduction produced by cocaine did not affect the resting membrane potential while ether depolarized the nerve. Furthermore, the depolarization caused by ether was different from that produced by anoxia, a difference previously suspected by Biederman (1895).

Larrabee and Posternak (1952) and Larrabee and Holaday (1952) clearly demonstrated the existence of various mechanisms responsible for the blockade of synaptic transmission in sympathetic ganglia. Nembutal preferentially depressed synaptic transmission. Cocaine was equally effective in blocking synaptic transmission and nerve conduction. Urethane, on the other hand, appeared more effective in blocking the latter.

More recently Herz, Fralig, Niedner and Farber (1967) demonstrated differences in the effect of various anaesthetics on auditory evoked potentials recorded from

the sensory cortex of cats.

Despite this experimental evidence there is a tendency to consider the depression of synaptic transmission produced by anaesthetics as a phenomenon having only one site and one mechanism of action (Loyning et al., 1964; Somjen et al., 1963; 1967). This tendency is supported by the depression of excitability in motoneurones observed with ether and Nembutal (Somjen and Gill, 1963; Shapovalov, 1964). This depression has been considered to have a similar mechanism to that of the blockade of nerve transmission induced by procaine, an agent that characterizes anaesthetics for a large group of investigatores (Inoue and Frank, 1962, 1965; Feinstein, 1964; Blaustein and Goldman, 1966; Frank, 1968).

Shanes (1958) called procaine a "stabilizer" because it prevents the increase of Na conductance. This effect was demonstrated by Taylor (1959) on the squid axon, but as he recognized, more experimental work is needed before one could understand the processes controlling the Na and K membrane conductances; even depression of membrane excitability may still be produced by several mechanisms. Tetrodotoxin is a specific blocker of Na conductance but hardly qualifies as a safe anaesthetic agent (Moore and Narahashi, 1967 and Blankeship,1968). Moreover, the excitability of neurones can be depressed also

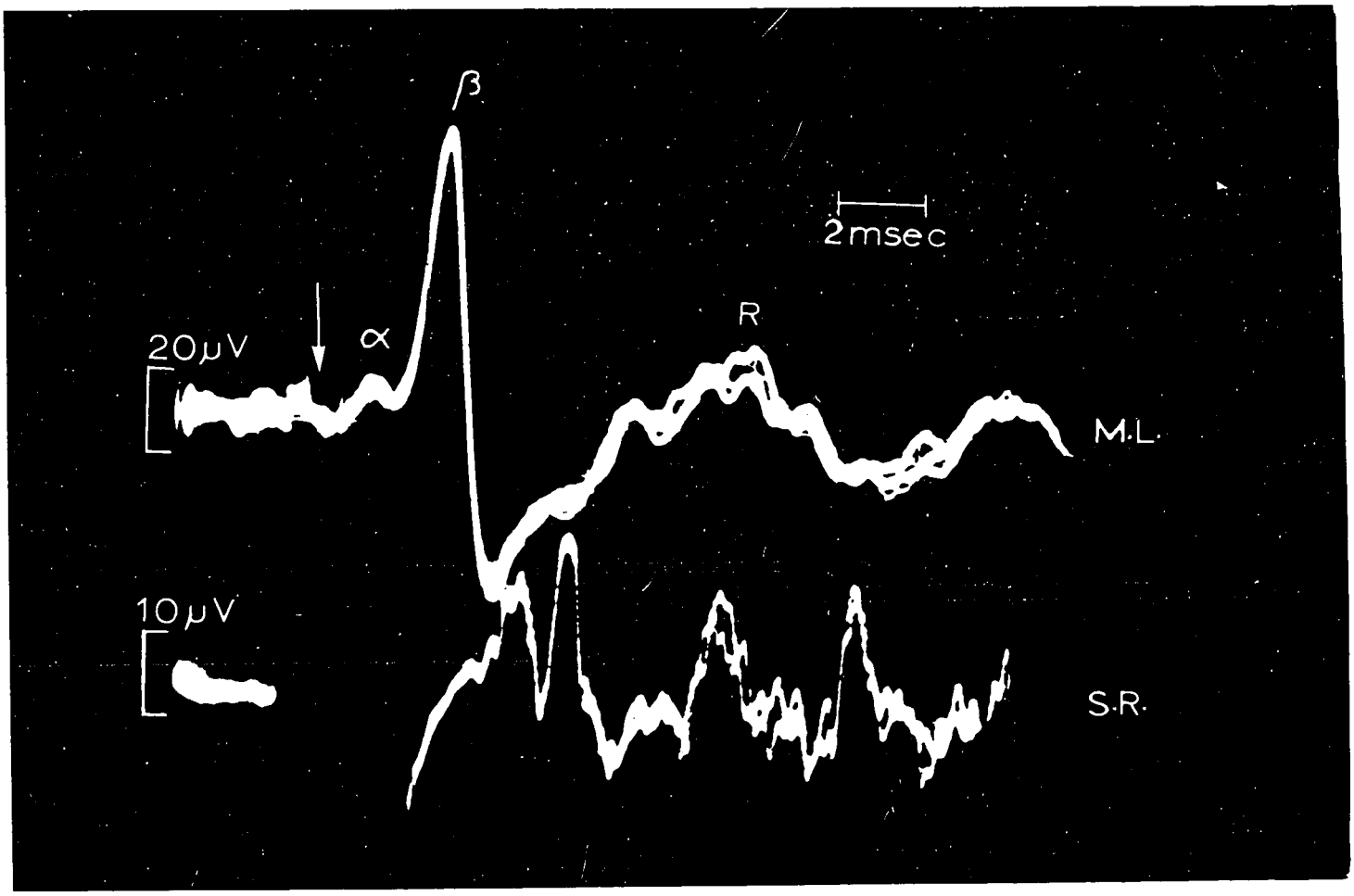

FIG. 18: Upper trace - orthodromic action potential recorded in the medial lemniscus. Lower trace - antidromic action potential recorded in the superficial radial nerve. Cuneate nucleus stimulation. ∝, β and R components as described in text. Several traces superimposed. Decerebrated cat at 34°C.

by increased inhibitory input acting pre- or post-synaptically upon them. (Granit, Kellerth and Williams, 1964).

c) Direct electrical stimulation of the cuneate nucleus

The cuneate nucleus was stimulated through the central electrode of the 5-barrelled micropipette, thus activating the terminals and the dendrites of dorsal columns fibres and cuneate neurones, respectively. This method made it possible to study simultaneously synaptic transmission and the excitability of presynaptic terminals. The results of the latter are discussed elsewhere (page 73). In this part are presented the effects of anaesthetics on the action potential recorded orthodromically in the medial lemniscus.

This action potential (Fig. 18) can be divided into three components - α, β and R (Andersen, Eccles, Oshima and Schmidt, 1964). The α component has a latency of less than 2 msec and appears only when the stimulating electrode is located in the upper 1/3 of the cuneate nucleus and deeper than 1.5 mm. It is considered to be generated by direct stimulation of relay cells. The β component is believed to correspond to the trans-synaptic stimulation of cuneate neurones. The R component is formed by a series of 2, 3 or more small waves that appear after the β spike. These waves correspond to the dorsal column reflex (Fig.18).

Procaine: This anaesthetic was studied in 10 experiments. It was a potent depressant of synaptic transmission when given iontophoretically at strengths between 50 and 200 nA (∝, β and R). Its effect lasted for a period of 2 minutes following the end of its administration.

Nembutal: The systemic administration of Nembutal (2-30 mg/Kg), which was studied in 22 experiments, reduced the size of the medial lemniscus compound action potentials (10 to 50% respectively) (β and R). Its effect was manifested with doses as low as 2 mg/Kg (Figs. 19, 20). The usual anaesthetic dose is 30-40 mg/Kg.

Halothane: Inhalation of 1-2% (v/v) halothane - studied in 45 experiments - reduced the trans-synaptic action potential (β and R). This effect was manifested 7 min from the beginning of its administration, it was reversible after short periods of administration (10-20 min); complete recovery took 30 min. The effect was small but consistent in all experiments (Figs. 21, 22).

DISCUSSION

It is clear from these experiments that the three anaesthetics investigated depressed synaptic transmission in the dorsal columns medial lemniscus pathway. However, these results do not clarify their site of action, nor do they suggest the possible mechanisms.

Subsequent experiments were planned to study

their effect on a) the post-synaptic membrane, b) the afferent terminals, c) on cuneate neurones according to their function, and finally, d) on the inhibitory process.

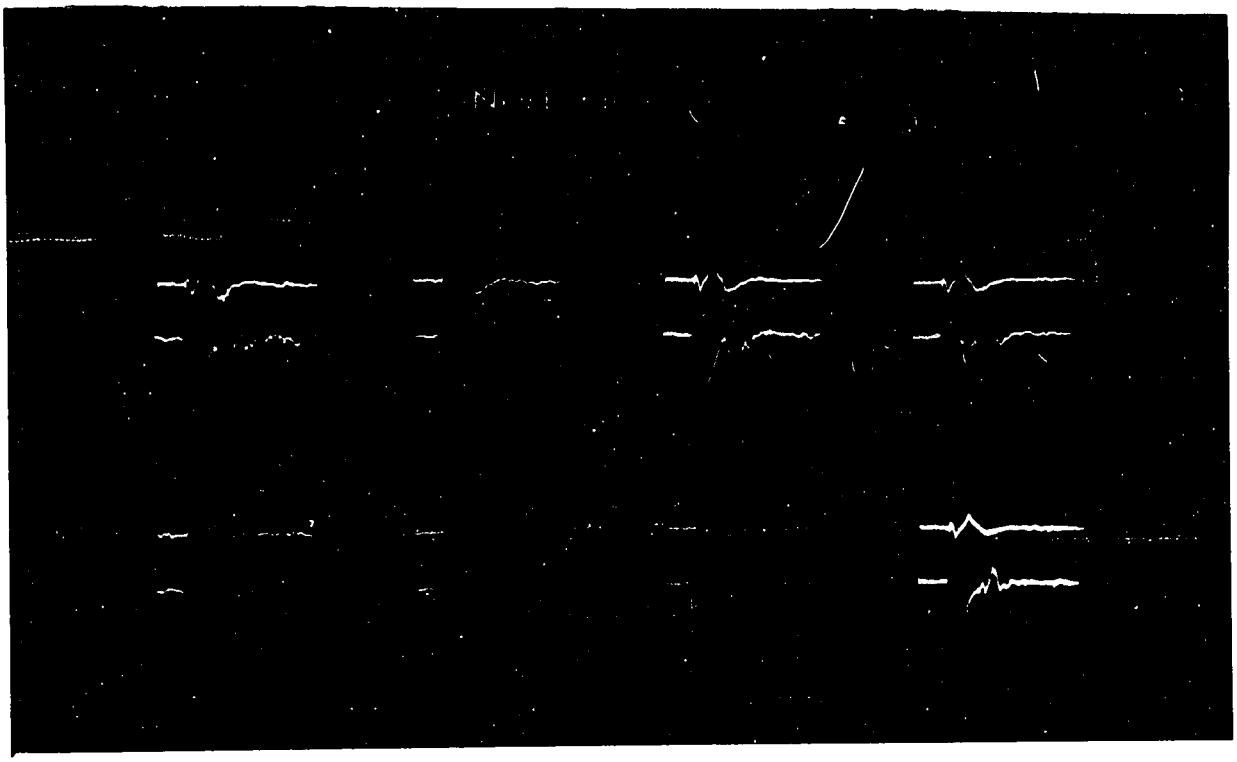

FIG. 19: Effect of intravenous Nembutal on the trans-synaptic action potential recorded from the medial lemniscus (upper traces) and the anti-dromic action potential recorded from the superficial radial nerve (lower traces). Cuneate nucleus stimulation - 1/sec, 0.02 msec duration and at 80 and 60 volts strength. One ot several traces superimposed in the various photographs. The depression produced by Nembutal is present following a small intra-venous dose (2 mg/Kg). Decerebrated cat.

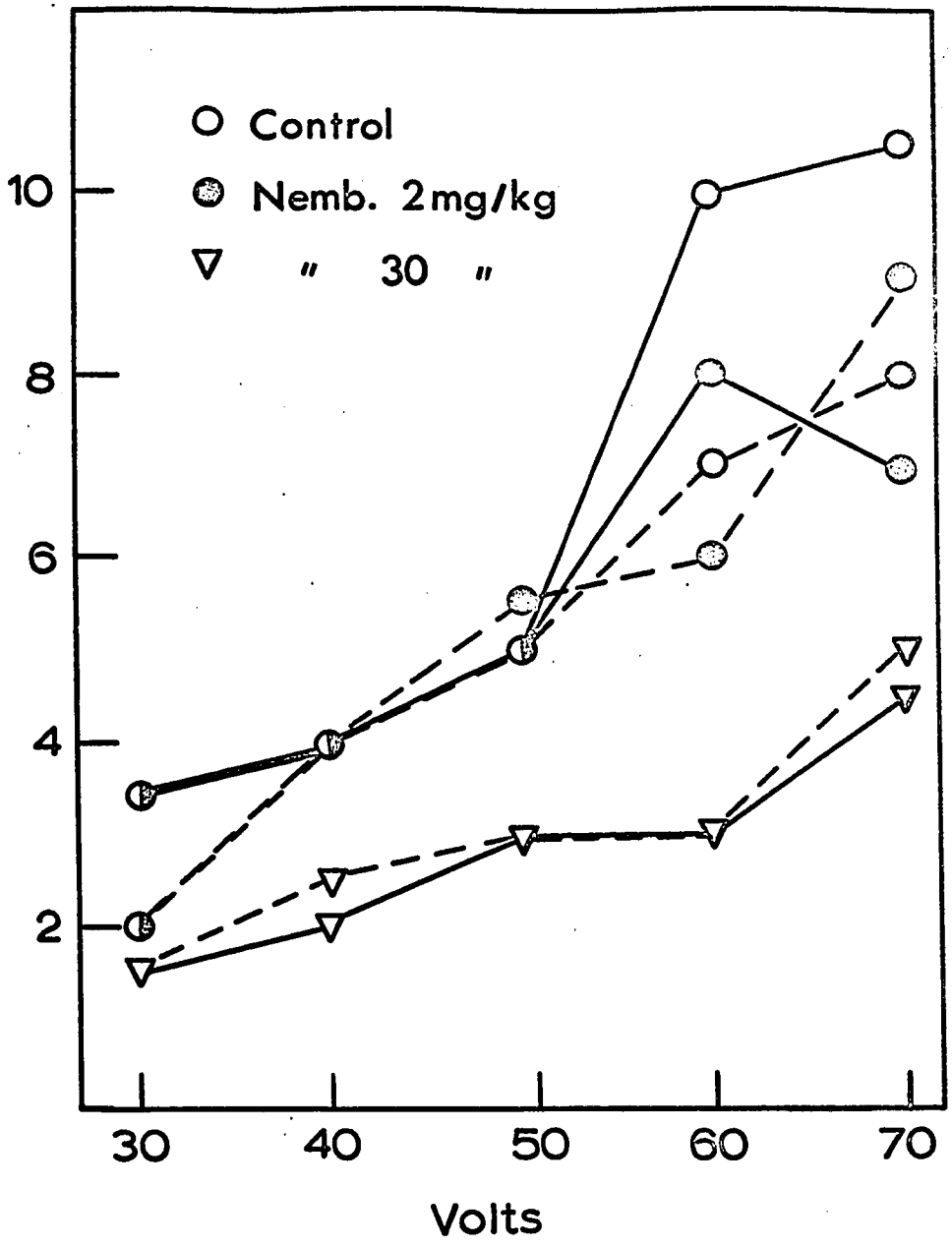

FIG. 20: Effect of Nembutal on afferent terminal (broken line) and trans-synaptic action potential (continuous line). In the ordinate: the size of the spikes in arbitrary units. In the abscissa: the strength of the stimulus applied to the cuneate nucleus (duration 0.02 msec).

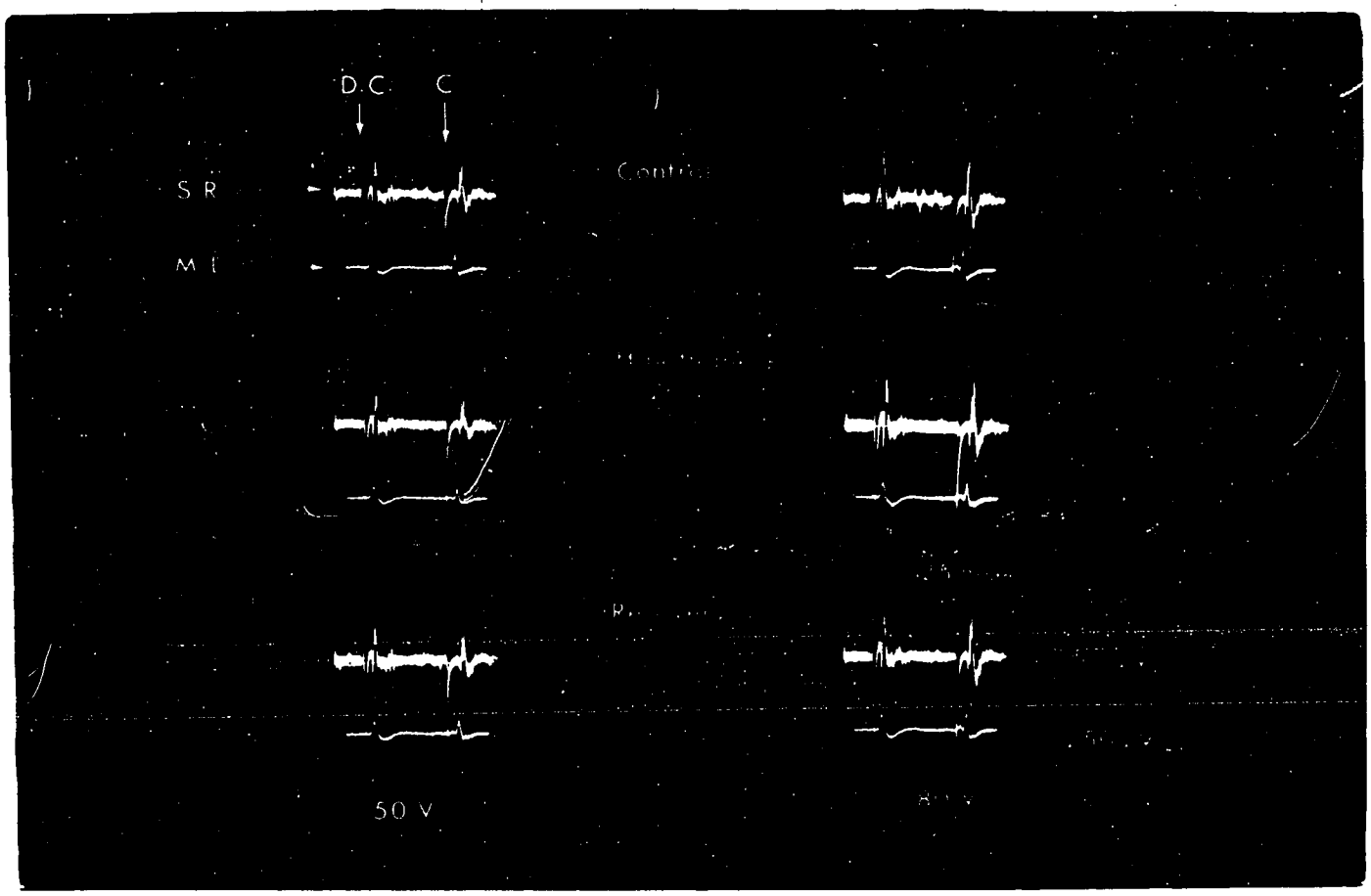

FIG. 21: Effect of halothane on synaptic transmission (M.L. = medial lemniscus), afferent terminals (C) and dorsal columns fibres (D.C.). Several traces superimposed. Responses evoked from C. and D.C. are recorded antidromically in the superficial radial nerve (S.R.) and orthodromically (in M.L.). Halothane has no significant effect on the dorsal columns fibres, it depresses the terminals at lower stimulating voltages (50 V) while it has no effect or increases their excitability at higher voltages (80 V). Duration of stimuli is 0.02 msec.

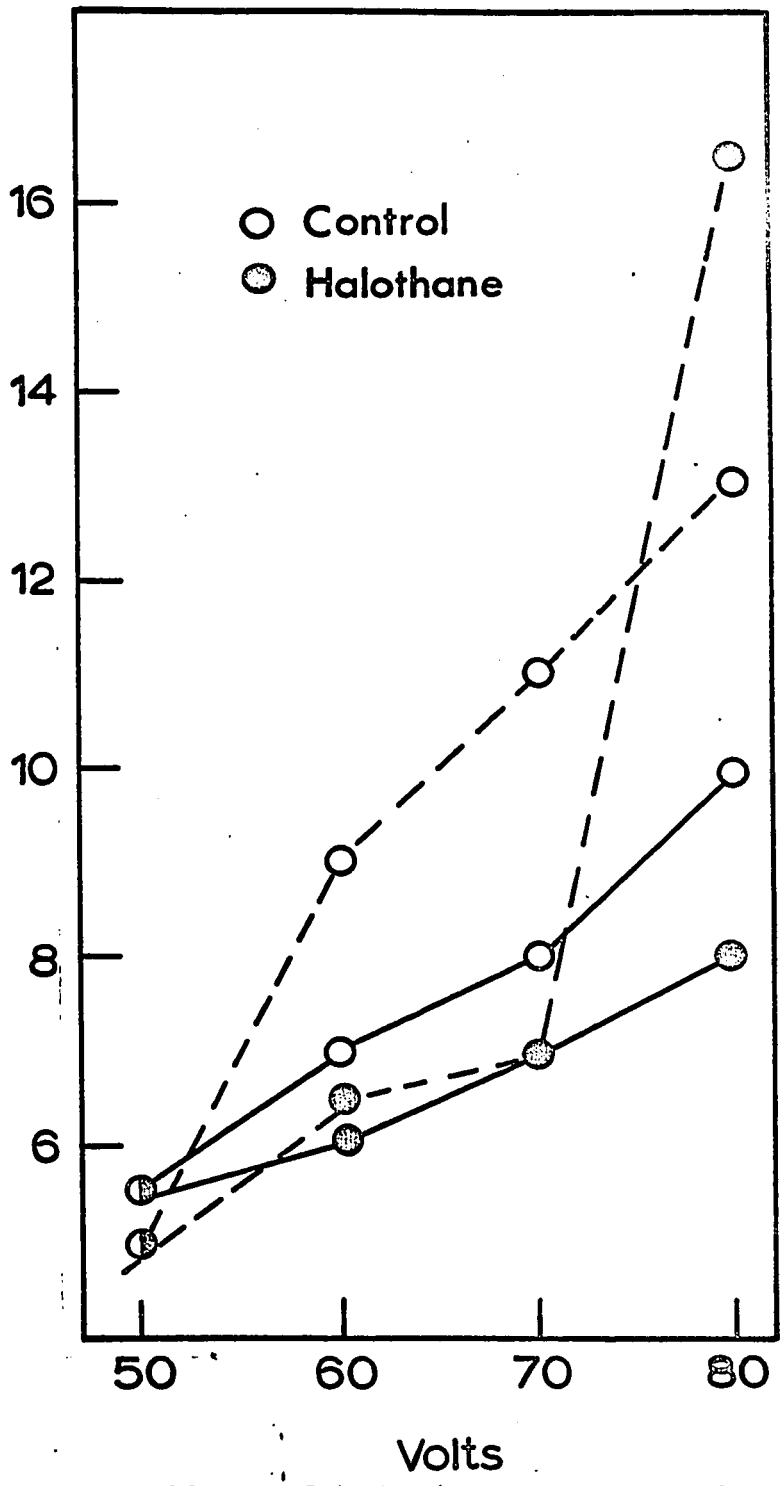

FIG. 22: Effect of halothane on synaptic transmission (continuous line) and afferent terminals (broken lines). Ordinate: spike size. Abscissa: strength of cuneate nucleus stimulation (0.02 msec pulse duration). Halothane depresses synaptic transmission along the dorsal columns - medial lemniscus pathway. Its effect on the terminals depends on the strength of stimulation.

B - EFFECTS ON THE POST-SYNAPTIC MEMBRANE OF CUNEATE CELLS

This effect was determined by observing the action of the various anaesthetics on the glutamate-induced firing of cuneate neurones and the ACh-induced activity of cortical units. The use of glutamate as an agent to study the effect of anaesthetics on the post-synaptic membrane is justified even if its function as a neurotransmitter has not been established. The available information derived from the myoneural junction in crustacea (Takeuchi and Takeuchi, 1964) and the giant Mauthner cells of gold-fish (Diamond, 1968) supports this assumption. The action of glutamate in the cuneate appears to be restricted to specialized sites on the post-synaptic membrane (Galindo, Krnjević and Schwartz, 1968). The depression of its excitatory action may be considered a post-synaptic effect.

These studies were made as follows: the unit was excited first by the minimum amount of glutamate that could be accurately reproduced. Various manners of testing were used; the one adopted during these experiments was that of short glutamate applications - 5 to 20 seconds - repeated at fixed intervals - 20 to 60 seconds. "Control" was defined as the response of a given unit to the lowest glutamate current applied for two or more of these periods.

This reproduceability was possible only in few cells in any given experiment (approximately 20% of the units). Anaesthetics, GABA or glycine were then given until complete suppression of the glutamate (or ACh) response. This was considered as the first "point" of the "effectiveness" of the former compounds. A second point was determined by doubling the glutamate current a third and subsequent points were determined by doubling again the current of glutamate. The plotting of these points gave an initial ratio, of variable slope, followed by a "plateau" suggestive of "saturation of receptor sites" (Figs. 24, 26, 27).

a) Microiontophoretic, electro-osmotic and pressure release

Procaine: This anaesthetic depressed the post-synaptic membrane of cortical as well as cuneate neurones when studied in 21 experiments. Its effect started during the first five seconds of its administration, it disappeared in the same period of time after its cessation. Often, there was a period of approximately 20 seconds of hyper-excitability immediately after the end of its administration (Figs. 23, 24, Table 2).

Nembutal: This barbiturate was studied in 26 experiments. Its mode of action was similar to that of procaine; however, its maximum effect took 20 to 40 seconds to appear with a similar period to wear off. There was no post-application

rebound of excitation (Fig. 24).

Both procaine and Nembutal were applied for a few seconds (10-30) preceding a given dose (current strength) of glutamate that was administered for fixed periods of time (5-20 seconds) as explained above.

Halothane: The effects of this gas were not consistent. Illustrated in Fig. 25 is one of the few examples in which halothane depressed the post-synaptic membrane of cuneate neurones. In some cells halothane had no effect, or there was an irreversible depression, or the units accelerated their firing rate. These methods of halothane administration were abandoned owing to the impossibility of dose control.

Cholinoceptive units: A total of 12 cortical units were excited by both ACh and glutamate. Procaine (40-100 nA) and Nembutal (80-200 nA) were equally effective in depressing their induced activity.

b) Systemic administration

Nembutal: Intravenous Nembutal (10 mg/Kg), studied in 5 experiments, depressed post-synaptic excitability. This effect was manifested during the first minute of its administration, it was not related to blood pressure changes since the pressure was artificially controlled by infusion of nor-adrenaline.

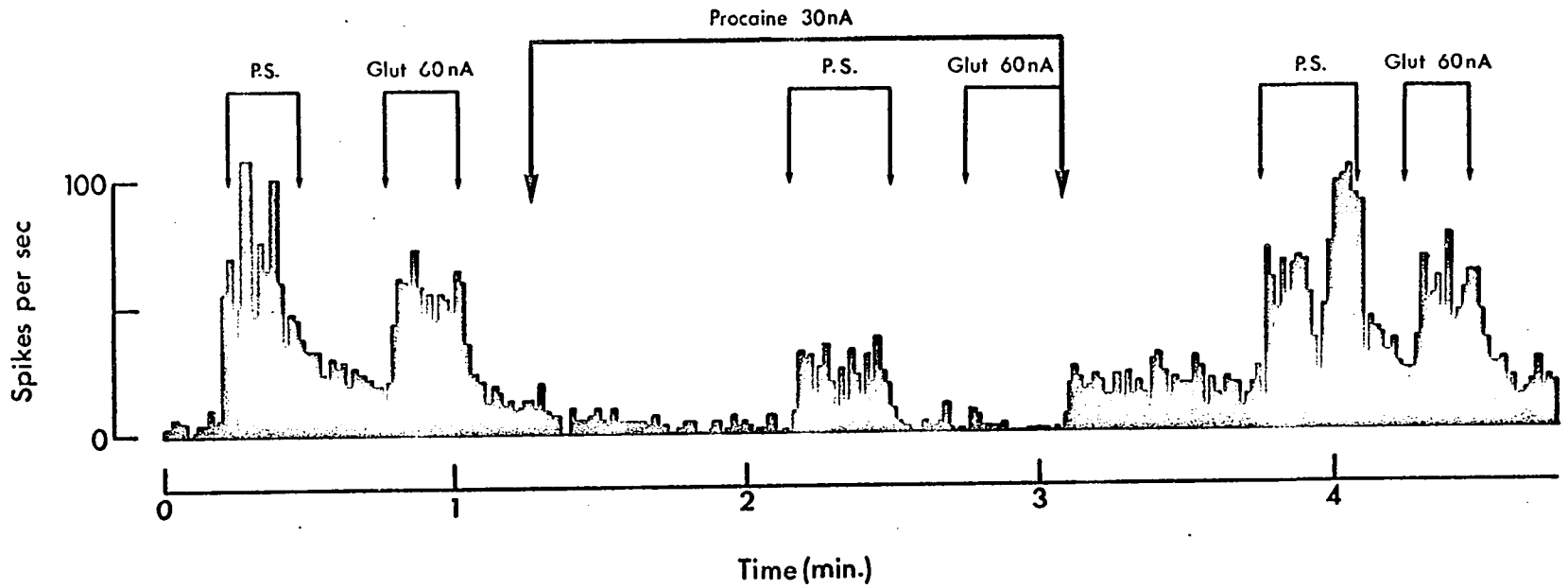

FIG. 23: The iontophoretic release of procaine depresses both the post-synaptic response to glutamate (Glut) and the trans-synaptic response to a jet of air applied at the periphery (P.S.). There is a transient period of hyperexcitability following the cessation of procaine administration. Records taken from the cuneate nucleus of a decerebrated cat using the central barrel of a five-barrelled micropipette. Glutamate and procaine were released iontophoretically through two other barrels.

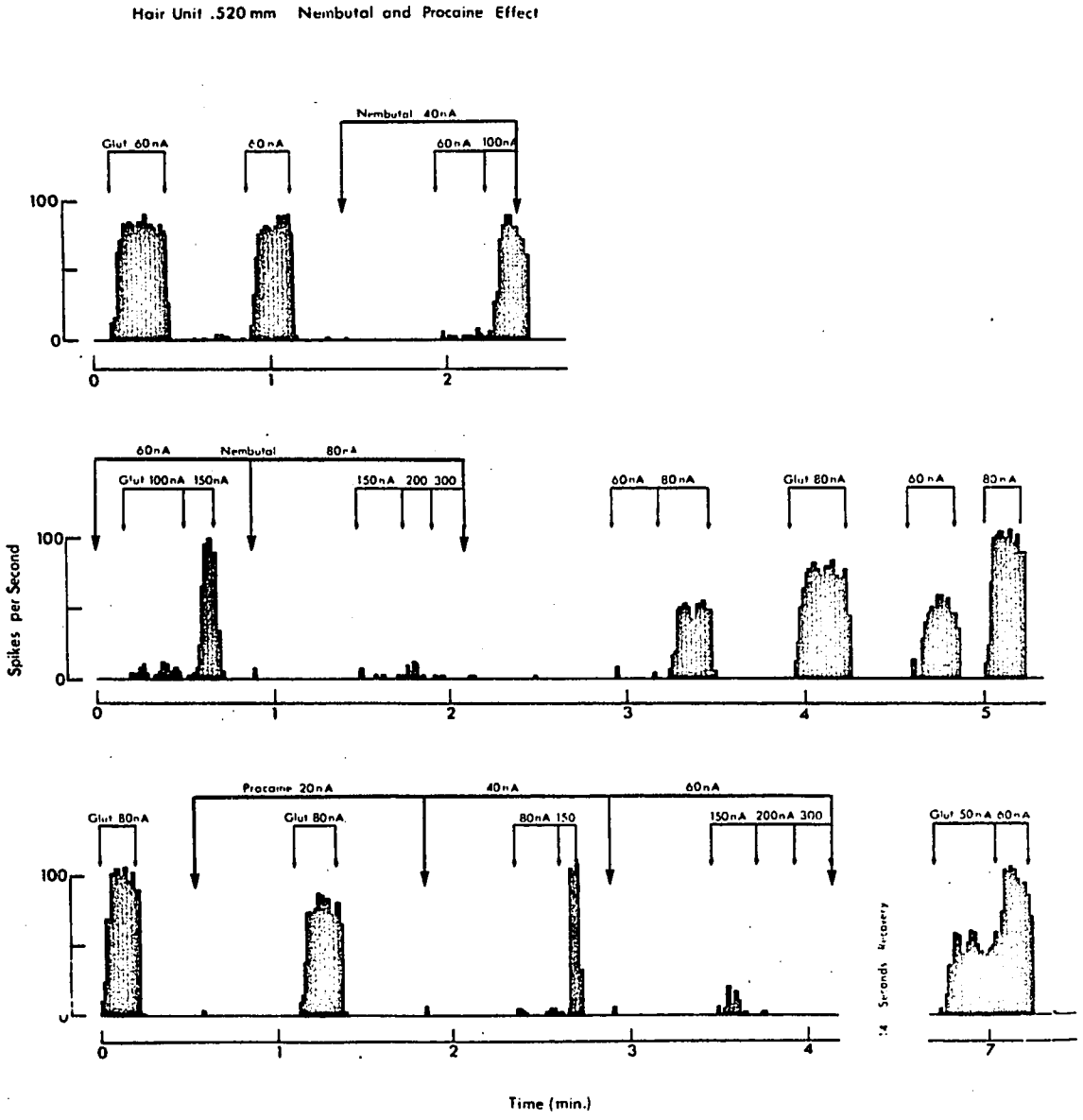

FIG. 24: The post-synaptic response to glutamate is blocked by both procaine and Nembutal. The type of interaction between these two anaesthetics and glutamate suggests a saturation of receptor sites. Complete saturation with Nembutal is at 80 nA and with procaine at 60 nA. Note the slower recovery after Nembutal than after procaine. The figure should be read as a continuous record of 16 minutes duration. Five barrelled micropipette was used for recording and drug administration in the cuneate nucleus. Decerebrated cat.

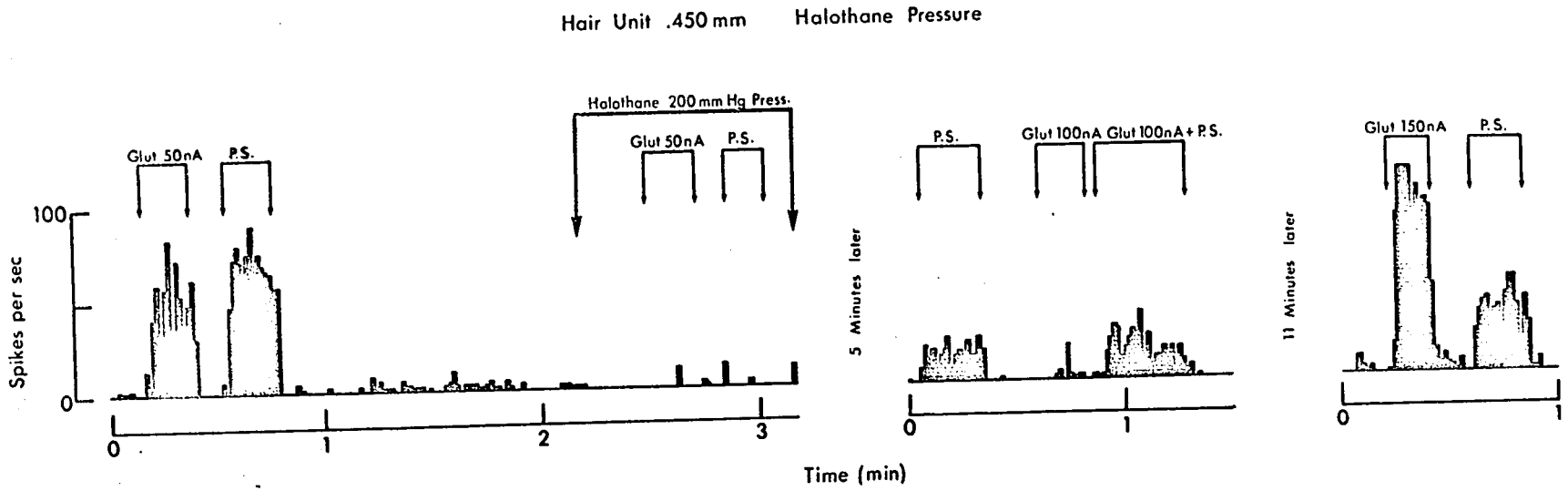

FIG. 25: Example of depression of the post-synaptic membrane by halothane. Recording through one lateral barrel of a five-barrelled micropipette located in the cuneate nucleus. Halothane was released by applying pressure to the central barrel of this pipette filled with the undiluted anaesthetic. A jet of air was used as peripheral stimulation (P.S.). Glutamate (Glut) was released iontophoretically. Decerebrated cat.

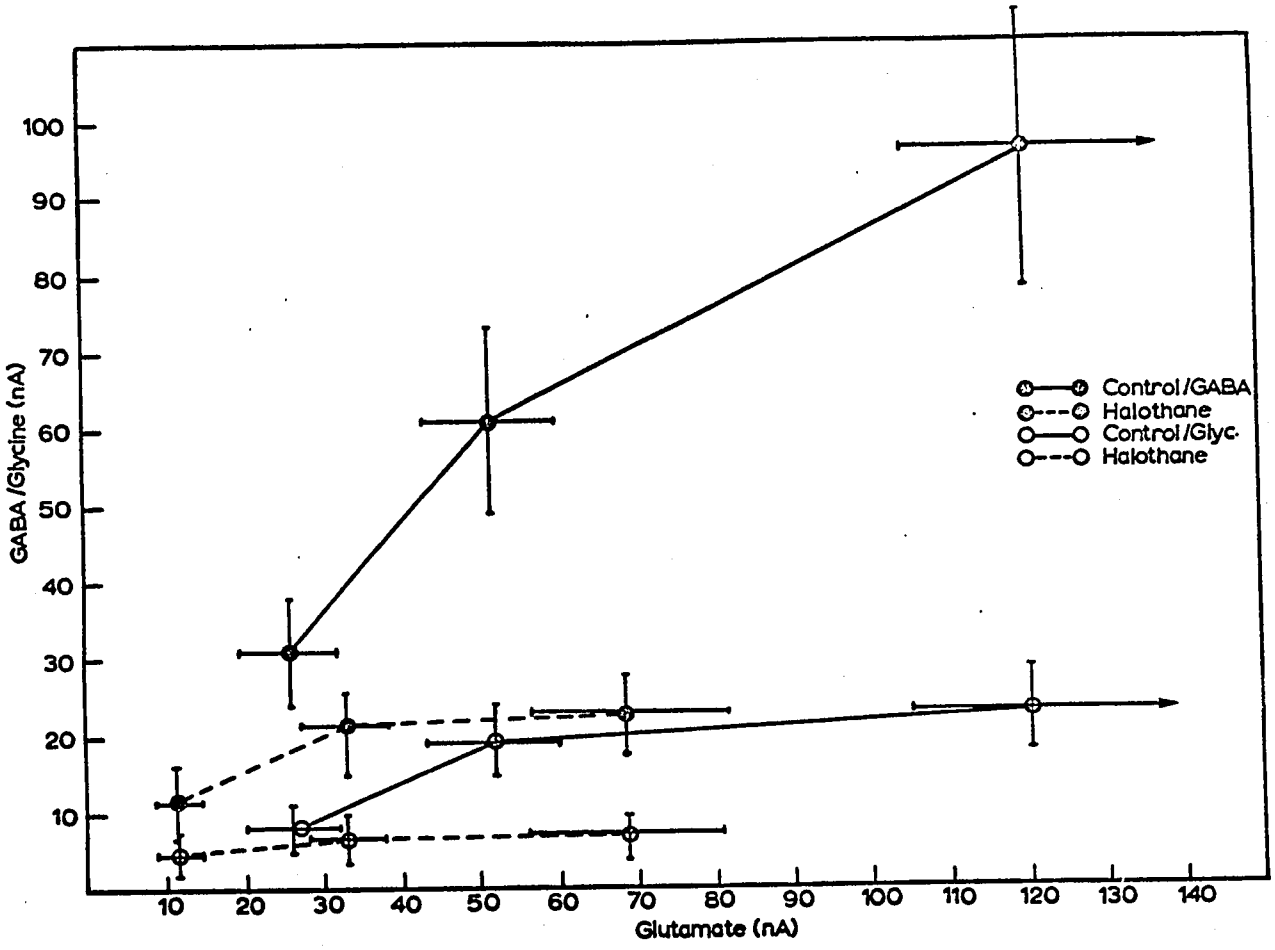

FIG. 26: Effect of halothane on the post-synaptic response to glutamate and its depression by GABA and glycine ± 1 S.E. Ten neurones were studied before and ten different units after 2% halothane (inhalation). Systemic blood pressure maintained at 90 mm Hg. (Noradrenaline). Halothane reduced the amount of GABA and glycine needed to abolish a given strength of glutamate excitation. The threshold for this latter amino acid was also reduced. These results suggest that, at this anaesthetic concentration, halothane lacks post-synaptic depressant properties. Observations made in the cuneate nucleus of a decrebrated cat.

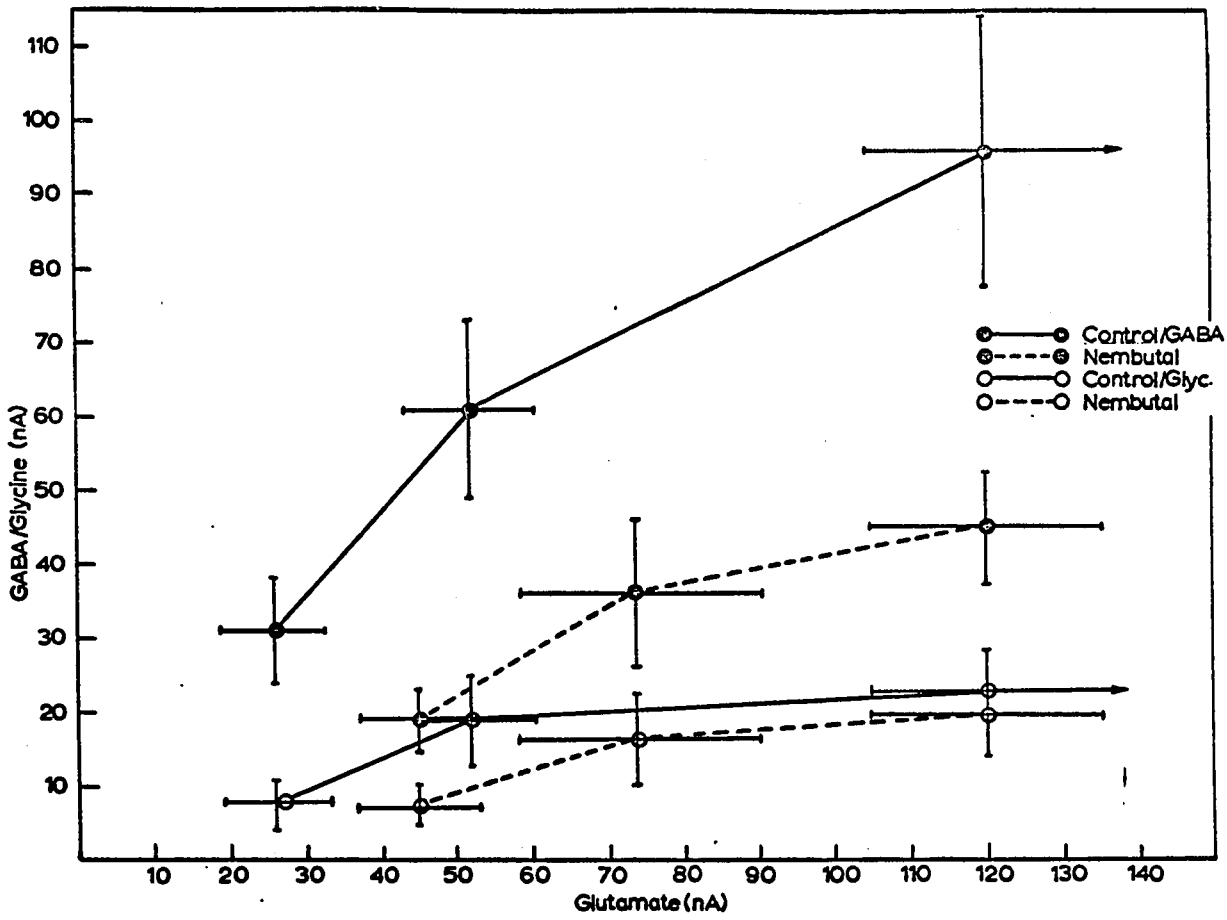

FIG. 27: Effect of nembutal on the post-synaptic response to glutamate and its depression by GABA and glycine ± 1 S.E. Ten neurones were studied before and ten different units after 20 mg/Kg of intravenous Nembutal. Blood pressure maintained at 90 mg/Kg (nor-adrenaline). Nembutal reduced the effective amount of GABA needed to abolish a given strength of glutamate excitation. It had no effect on glycine effectiveness. The threshold for glutamate was raised by the anaesthetic. These results are consistent with a post-synaptic depression induced by the anaesthetic. Records taken in the cuneate nucleus of a decerebrated cat.

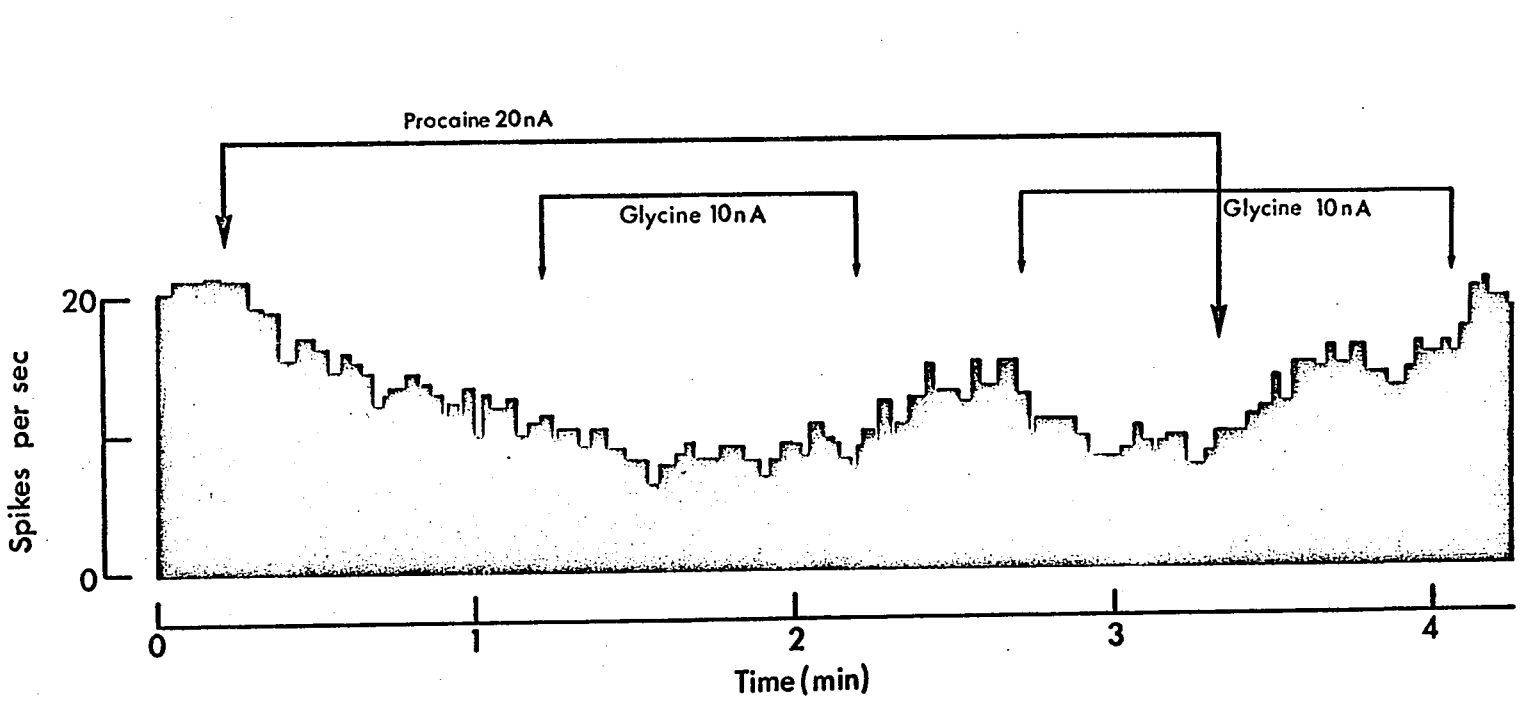

FIG. 28: The effect of the combination of procaine and glycine is additive. Observations made in the cuneate nucleus of a decerebrated cat. Both drugs were released iontophoretically. Recording through the central barrel of a five-barrelled micropipette.

Halothane: The inhalation of halothane (1-2% v/v), for more than seven minutes, had no effect or increased the responses of cuneate and cortical neurones to the administration of glutamate. These observations were made in 15 experiments.

The effects of Nembutal and halothane, on the post-synaptic membrane, were studied in two ways; a) the post-synaptic response to glutamate was tested before and after the systemic administration of these two anaesthetics, and b) in other experiments the glutamate threshold was determined in several cells before, and in several different cells after their systemic administration; the results were compared statistically (Fig. 26, 27). They were consistent with the initial observations, Nembutal depressed while halothane had no effect on the post-synaptic membrane.

c) Interaction with inhibitory transmitters

It was assumed that GABA and glycine represented the most likely inhibitory transmitter in the cuneate nucleus (Galindo, Krnjević and Schwartz, 1967). Procaine and Nembutal when combined with them, had simply additive effect (Fig. 28). However, in a few instances Nembutal increased the effectiveness of GABA. In three different experiments the same cuneate cell was depressed with both amino acids before and after the administration of 20 mg/Kg of Nembutal. Glycine was more effective than GABA before

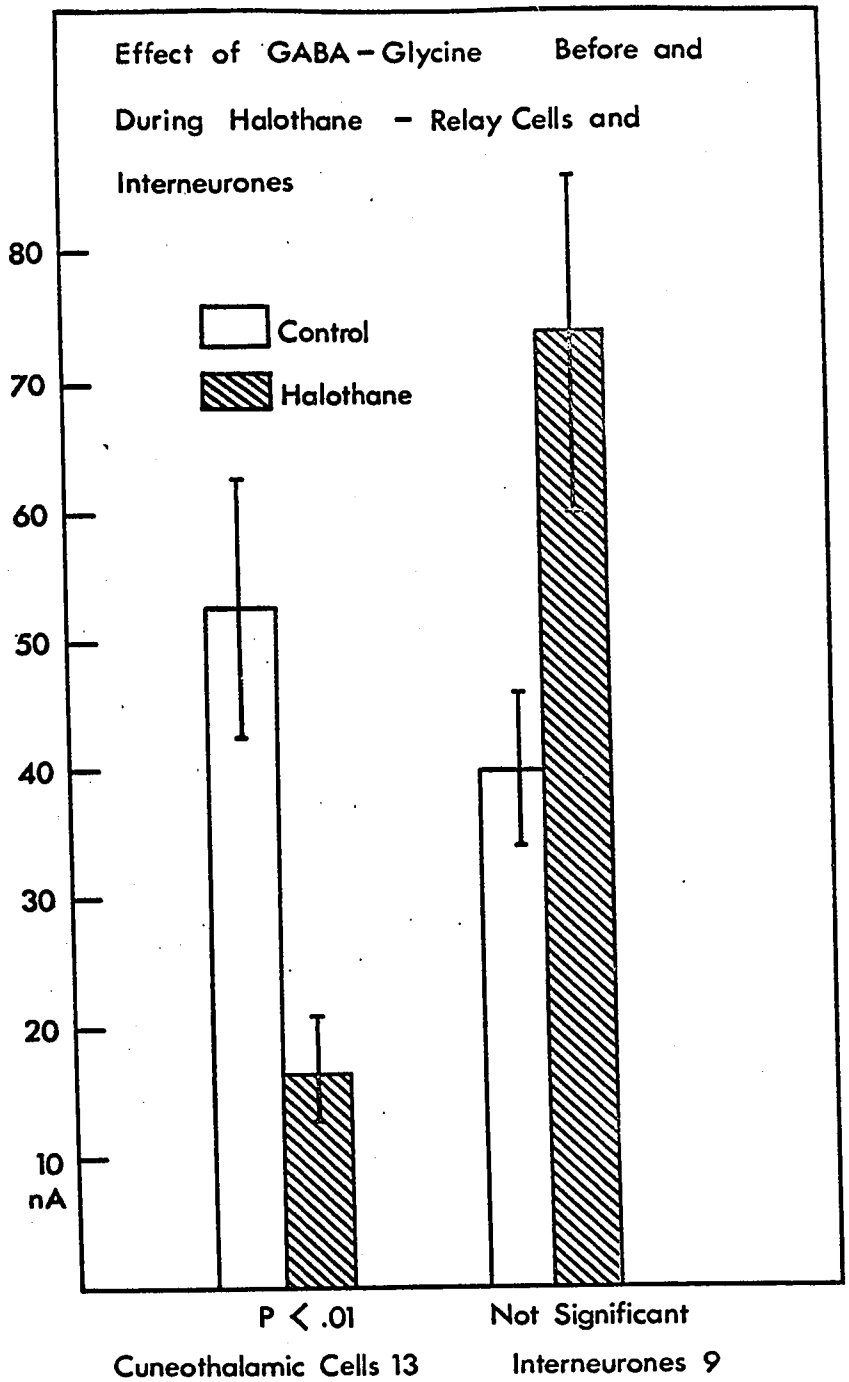

FIG. 29: Effect of halothane on the effectiveness of GABA or glycine. Evoked responses of relay cells and interneurones (identification and electrical stimulation as discussed in the text). 2% halothane (cup) reduced the need for the amino acids ($P<0.01$) on the cuneothalamic units while it tended to increase it on the interneurones (not significant) $\pm$ 1 S.E. Numbers on the left indicate the strength, in nA, of the GABA or glycine releasing current needed to depress trans-synaptic unit stimulation. Observations made on decerebrated cats.

Nembutal (20 vs 25 nA). After its administration GABA became relatively more effective (18 vs 15 nA). In an other experiment a group of 10 cells were observed before and after Nembutal. GABA became more effective after the administration of the anaesthetic. The action of glycine was unchanged (Fig. 27).

Halothane: In 23 experiments, halothane (2% for a minimum of seven minutes) potentiated the effect of GABA and glycine both on cortical units and in the cuneate nucleus. In this latter place there was a potentiation with cuneothalamic cells but not with interneurones, which showed no change or a decrease in effectiveness (Fig. 29).

DISCUSSION

These results offer a further confirmation of the differences among the three anaesthetics being studied. The depression produced by procaine and Nembutal contrasted with the lack of such action during halothane administration. On the other hand, this latter anaesthetic potentiated the effects of GABA and glycine, while the former two had only additive effects with these amino acids.

Nembutal preferentially depresses sites accessible to excitatory transmitters, as suggested by its greater effectivness in blocking the orthodromic action potentials. Higher doses of Nembutal may affect the excitable process in a manner similar to that of procaine.

The blockade of the antidromic spike supports this idea; but the depression of sympathetic nerves is a more conclusive proof of its local anaesthetic properties (Larrabee and Posternak, 1952). Thiopental at high concentrations acts also as a local anaesthetic (Schoepfle, 1957).

Procaine depresses the excitability of the post-synaptic membrane both non-specifically and by interacting with membrane receptors. It has been demonstrated that procaine and atropine block the generation of the action potential owing to a direct effect upon the spike generating mechanism but not on the post-synaptic potentials, both EPSP and IPSPs (Curtis and Phillis, 1960). These observations indicate "that the manner in which sodium ion passes through the membrane during the EPSP is different from that in which it does during the spike". However, in Renshaw cells, procaine acts preferentially on the cholinergic receptors, an action in line with the findings of del Castillo and Katz (1957) at the myoneural junction. It is interesting to note that procaine and ACh seem to compete for the same membrane sites. This suggestion is based on their antagonistic effect on nerve excitability (Bloom and Schoepfle, 1963).

Halothane does not depress the post-synaptic membrane of cuneate neurones at anaesthetic concentrations. However, experiments by Karis and co-workers (1967) with

ether and halothane, on the frog sartorius muscle sciatic nerve preparation in vitro, indicate that these agents depress the response of the post-synaptic membrane to the iontophoretic release of acetylcholine. There is no change in the resting membrane potential nor in the threshold for direct stimulation of the muscle. Apparently these results are at variance with our own. However, the transmitters involved are different. Their observations were made on frog's myoneural junction at room temperature, ours in cats at 36°C. Moreover, the anaesthetic concentrations used by them were higher than those needed to depress synaptic transmission elesewhere in the C.N.S. Our experiments indicate that high concentrations of halothane, as when released under pressure or electro-osmosis, might also depress the post-synaptic membrane.

A depression of excitatory post-synaptic potential (EPSP) could be explained by either a curare-like effect in which the anaesthetic would occupy the receptor site, or by changes which alter the ionic conductance of the membrane. The latter effect can be mediated by direct membrane interaction or by increased inhibitory input. Further experiments with halothane supported its role as a potentiator of inhibition.

The depression of sympathetic ganglionic transmission by cyclopropane, ether and halothane (Biscoe and

and Millar, 1966a) in the presence of increased pre- and post-ganglionic activity (Millar and Biscoe, 1966b) appears paradoxical, especially in the presence of a reduced autonomic reflex activity observed by the same investigators (Biscoe and Millar, 1964, 1966b). These observations suggest the involvement of effector sites as well as transmitter release. An experimental technique that can differentiate between the various neuronal constituents is needed to clarify the above observations. However, more recently Li et al. (1968) have presented evidence against the depression of synaptic transmission caused by halothane in the sympathetic ganglion. This observation is consistent with a lack of inhibitory input converging into this synapse.

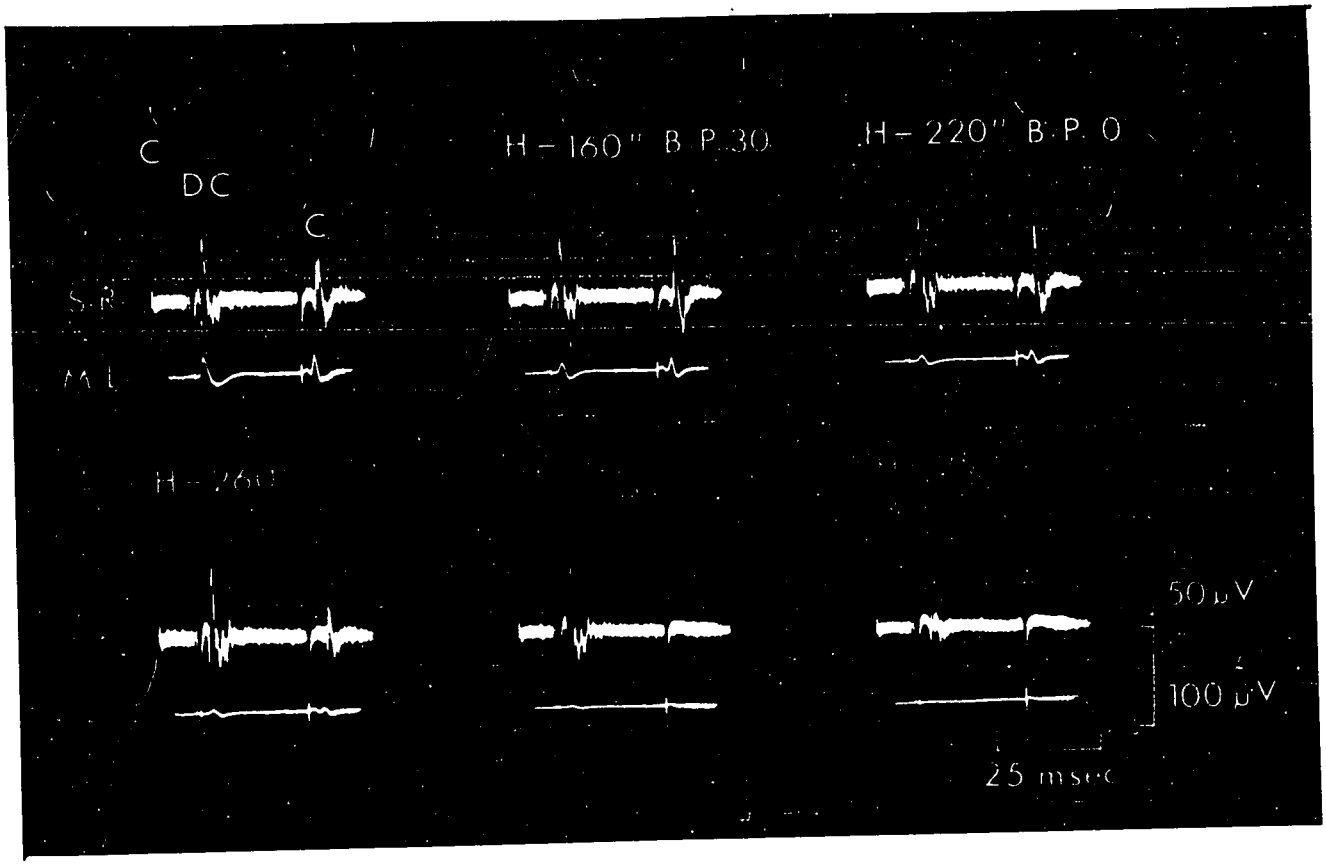

FIG. 30: Effect of hypoxia (H) on the cuneate nucleus afferent terminals (C) and dorsal columns fibres (D.C.). Cuneate nucleus (0.63 mm deep) and dorsal columns stimulation with 2 separate microelectrodes (1/sec - 50 V - 0.02 msec). Recording from the superficial radial nerve (S.R.) antidromic, and orthodromically from the medial lemniscus. Several traces superimposed. Note the reduction in synaptic transmission (M.L.) and increase in excitability of the terminals indicative of a reduced transmitter output (H - 160"). The terminals are completely depressed at 280 seconds while the dorsal columns are still conducting at 25 minutes. This criterion was used to differentiate the terminals from nerve fibres.
B.P. = blood pressure.

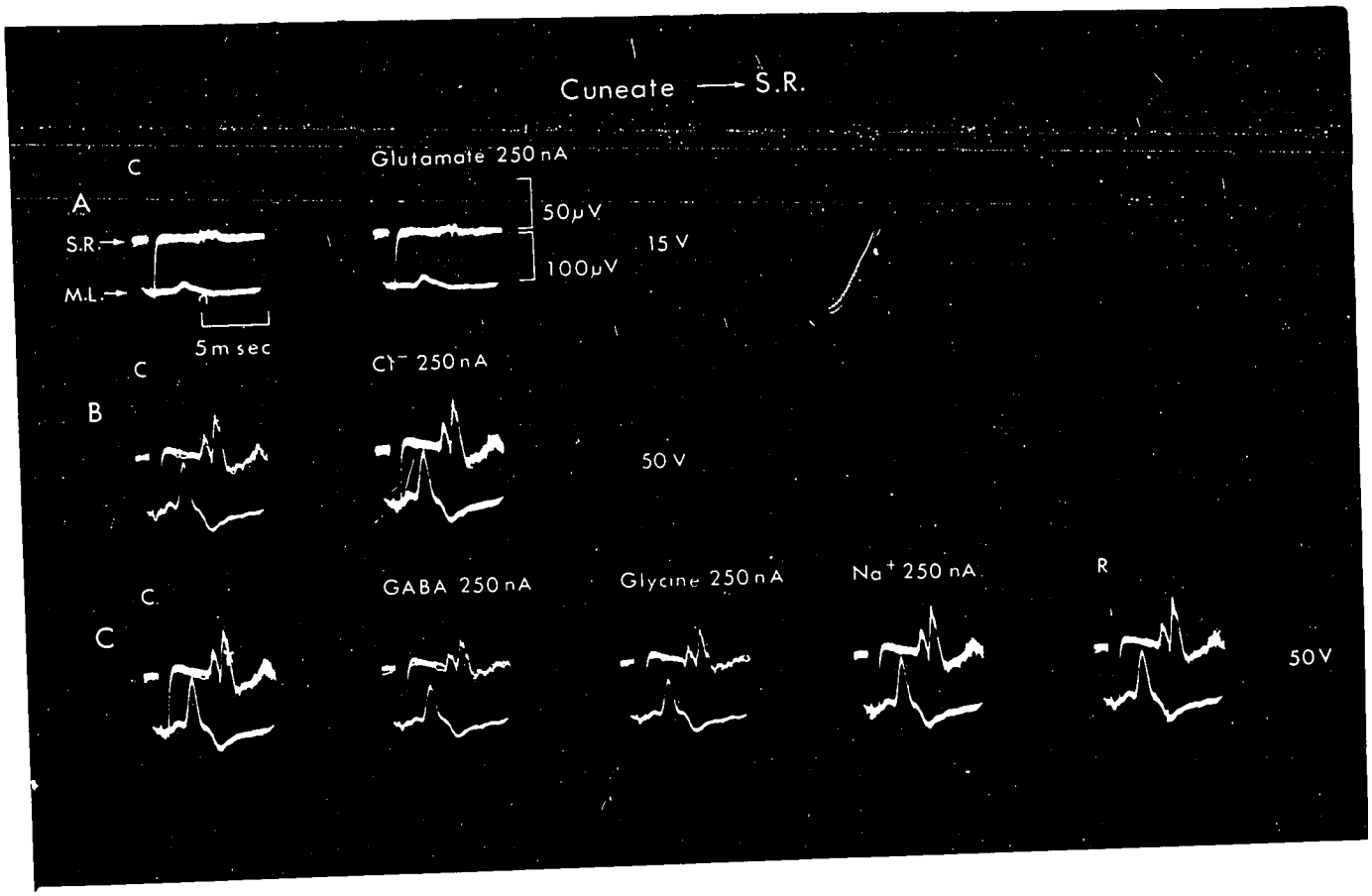

FIG. 31: Effect of GABA, glycine, glutamate, positive and negative currents on the terminals (S.R.) and synaptic transmission (M.L.). Cuneate stimulation 1/sec, 0.01 msec antidromic (S.R.) and orthodromic recording (M.L.). Glutamate had no effect on the terminals or the trans-synaptic spike. R: recovery. Current artifacts, electrode coupling, and changing electrode impedance are frequently observed. Several traces superimposed.

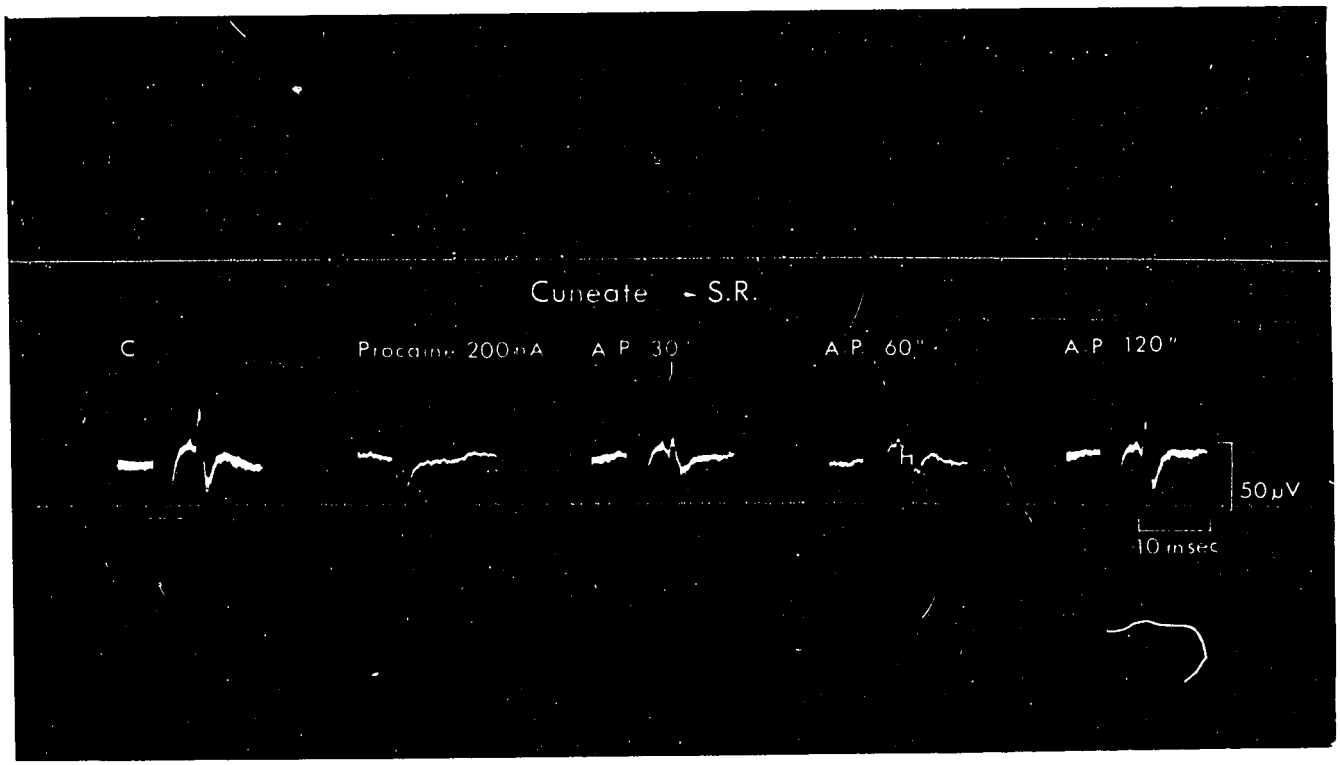

FIG. 32: Procaine suppresses the antidromic action potential indicating a strong effect on the afferent terinals of the superficial radial nerve in the cuneate. Recovery after procaine (A.P.) takes between 1 and 2 minutes. Several traces superimposed.

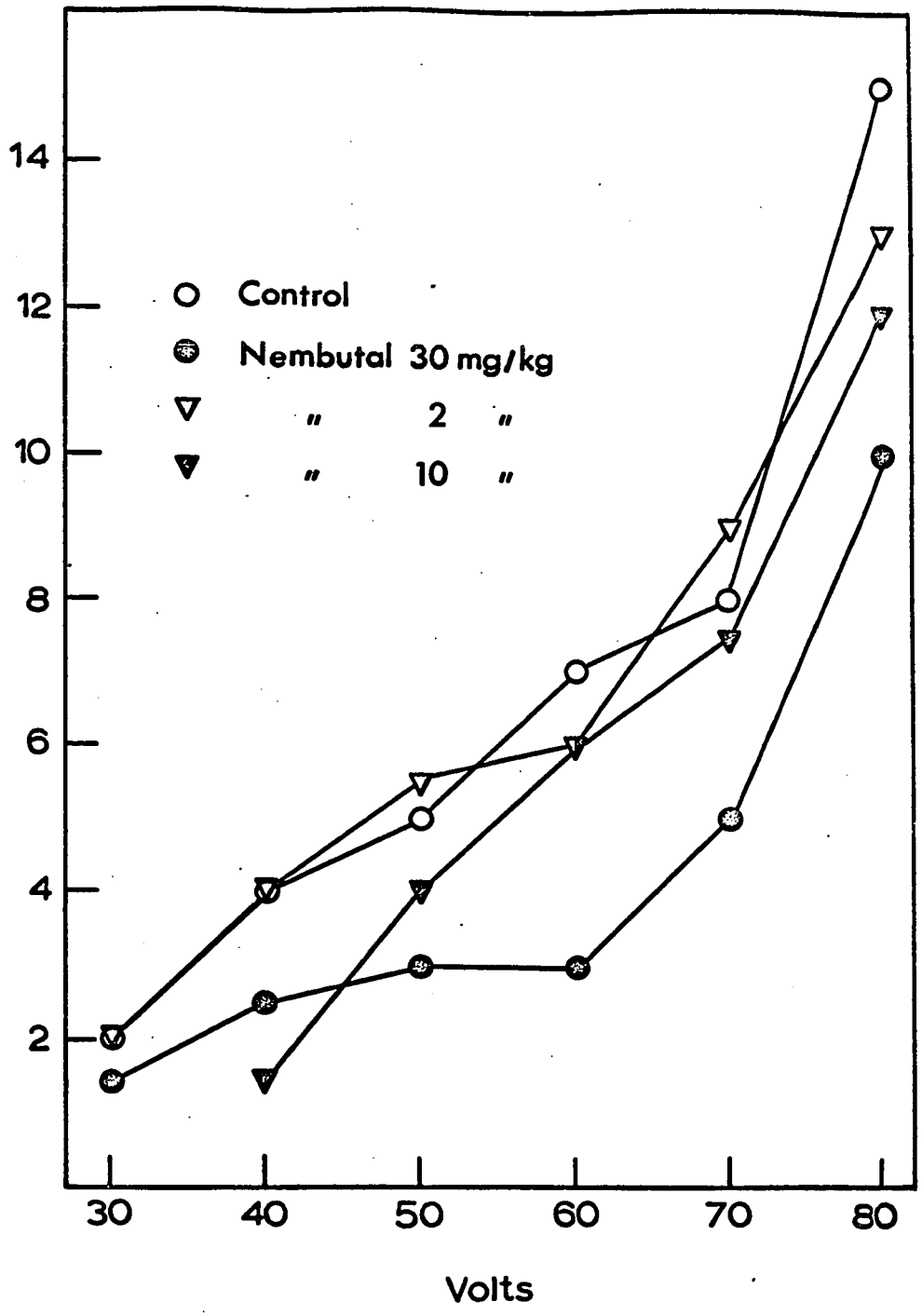

FIG. 33: Effect of Nembutal on the afferent terminals. Ordinate: spike size in arbitrary units. Abscissa: strength in volts of cuneate nucleus stimulation - 1/sec and 0.02 msec pulses. Nembutal depresses the size of the antidromic spike recorded from the superficial radial nerve at doses as low as 2 mg/Kg.

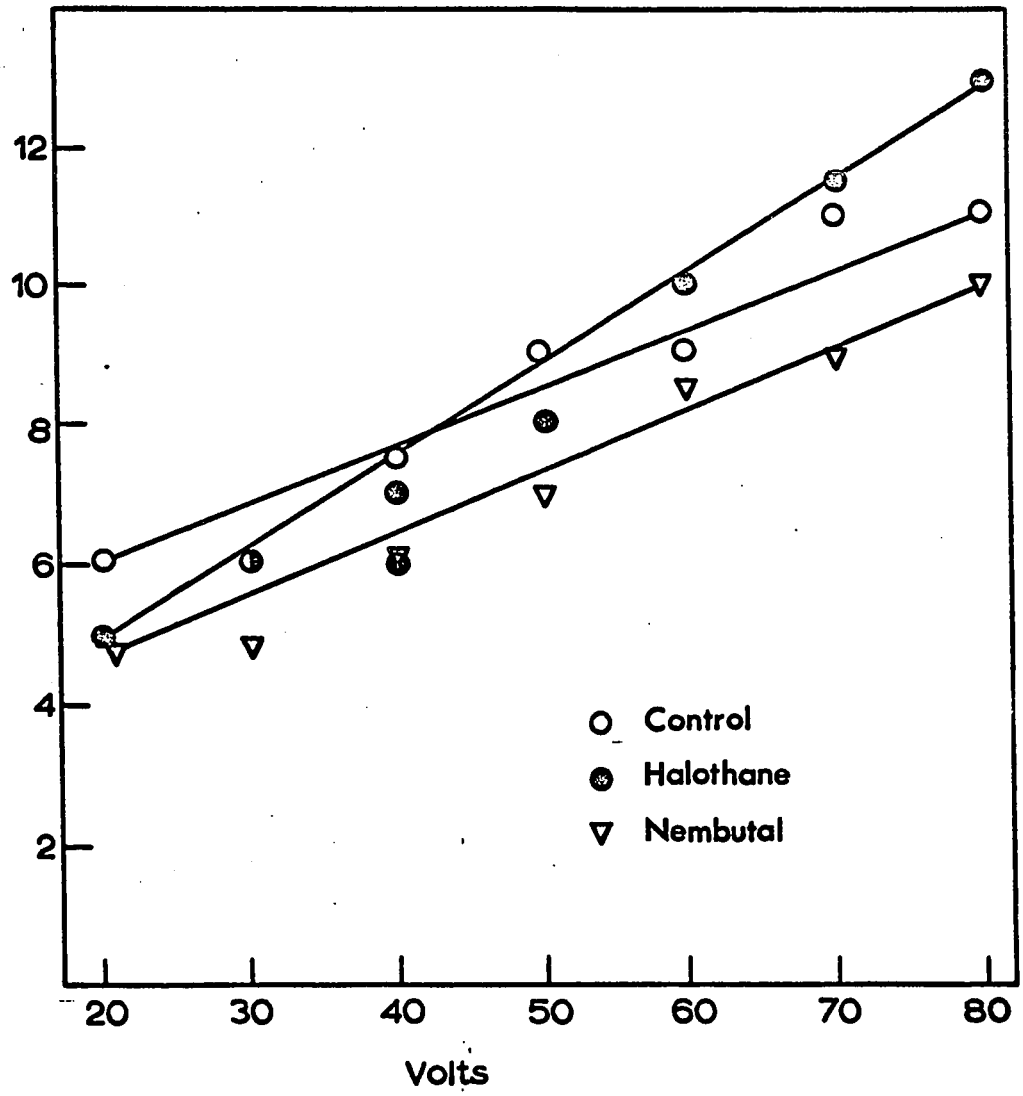

FIG. 34: Comparison between the effects of halothane and Nembutal on the afferent terminals. Cuneate nucleus stimulation 1/sec - 0.02 msec pulses. Antidromic recording from the superficial radial nerve. Ordinate: spike size in arbitrary units. Abscissa: strength of stimulation. Halothane increases the excitability of these endings at high voltages of stimulation, but it depresses them at low voltages. Nembutal has only a depressant effect.

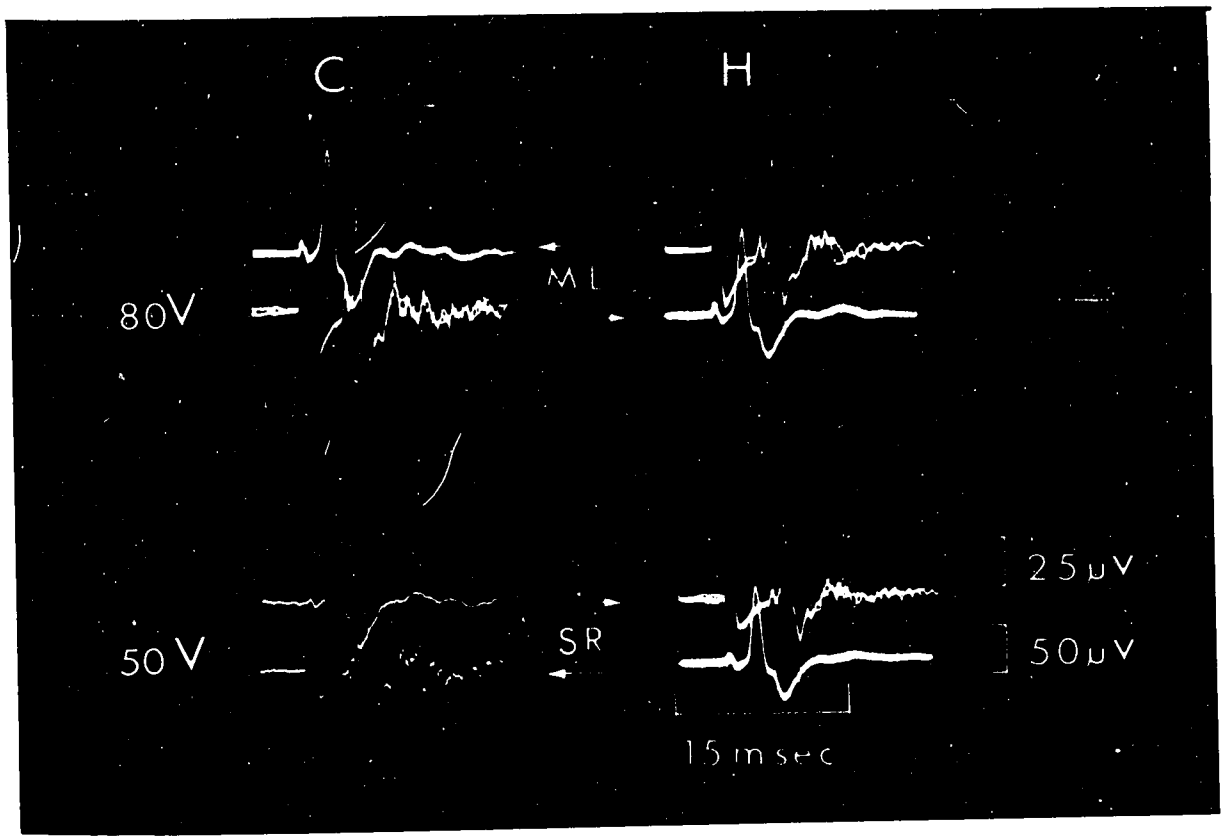

FIG. 35: Effect of 2% halothane (H) inhalation on the cuneate nucleus synaptic transmission (ML) and superficial radial nerve afferent terminals (SR) at two different voltages of cuneate nucleus stimulation (1/sec - 0.01 msec). Several traces superimposed. Note the changes in the position of the two traces. Halothane tends to increase the excitability of the afferent terminals while it depresses synaptic transmission as well as the dorsal column reflex. Control: C.
Results from other experiments indicate that this anaesthetic has no depressant effect on the postsynaptic membrane at anaesthetic concentrations (2% v/v). On the other hand, halothane potentiates the depression produced by GABA and glycine as well as increases the intensity and duration of synaptic inhibition. All these results suggest that this agent facilitates the inhibitory process in the cuneate nucleus.

C - EFFECT ON THE AFFERENT TERMINALS

The axons of the dorsal root ganglion cells terminate in the cuneate nucleus in small 1 μ endings (Walberg, 1966). The "bouton terminal" is enlarged and deprived of the myelin sheath (Walberg, 1965). Similar endings in the spinal cord have a longer refractory period and their vulnerability to hypoxia is several times greater than the rest of the nerve fibre (Edisen, 1957). This latter property is useful for its identification as seen in Figure 30 where the antidromic action potentials were evoked by simultaneously stimulating the cuneate nucleus and the dorsal columns using two different electrodes. The response from the terminals disappeared after 4 min of hypoxia while a response could be elicited from the dorsal columns 35 min later. This is a susceptibility proper of the terminals and not of non-myelinated fibres (Ruch et al.,1965).

Most endings of the superficial radial nerve (the only terminals studied in this work) are located from 200 μ to 700 μ from the surface in the middle lateral half of the cuneate. Changes in the size of the antidromic action potential indicate their state of excitability (Wall, 1958).

Procaine: The administration of iontophoretic procaine

was studied in 12 experiments. As expected this local anaesthetic (150-200 nA) suppressed the antidromic spike evoked at 2 times threshold strength (1/sec - 0.02 msec); the recovery took one to two minutes (Fig. 32).

Nembutal: This barbiturate was studied in 23 experiments. The results with Nembutal were different depending on whether it was released iontophoretically or given intravenously. There was no effect or a slight increase in the size of the antidromic action potential when the anaesthetic was released locally (100-200 nA). On the contrary, there was a consistent depression following its intravenous administration (2-30 mg/Kg) (Figs. 19, 20, 33, 34). This depression was related to the dose administered. The blood pressure was maintained at control levels by the intravenous administration of nor-adrenaline.

Halothane: The administration of halothane was studied in 18 experiments. Its inhalation (1-2% v/v for more than 7 min) increased the excitability of the terminals, however, they were depressed when the strength of stimulation was just above threshold (Figs, 22, 34, 35). The excitability returned to control values after short periods of administration (10-20 min). The blood pressure was maintained at control levels throughout the experiments by the use of intravenous nor-adrenaline.

Other substances: Veratrine (6 experiments) was released

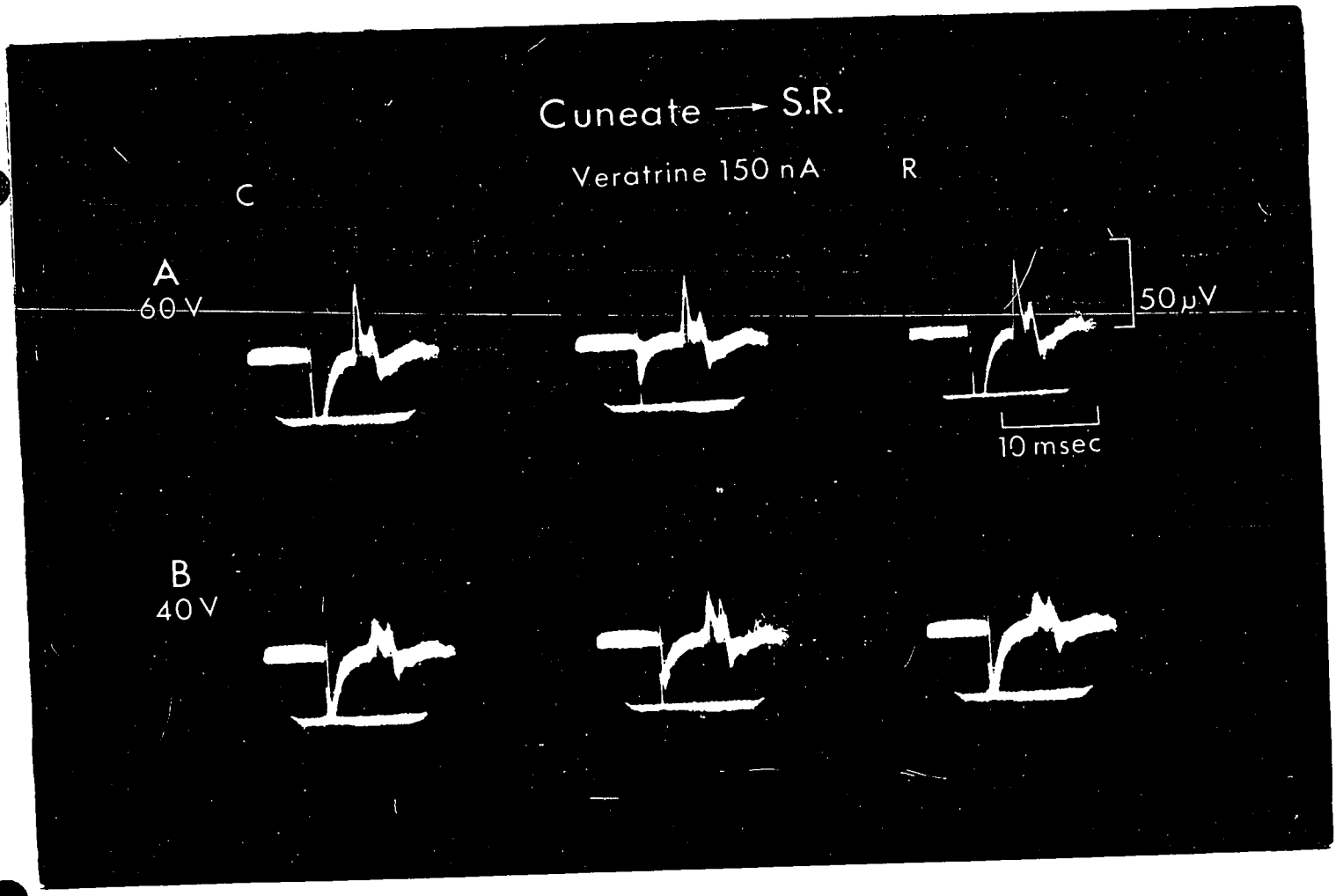

FIG. 36: Effect of veratrine on the terminals. This effect is easier to detect at lower stimulating strengths (40 V). C = control. R = recovery. Cuneate stimulation 1/sec - 0.02 msec. Several traces were superimposed. Decerebrated cat.

iontophoretically (100-200 nA) and used as a reference for depolarizing changes. There was an increase in the size of the antidromic spike indicating a similar change in the excitability of the afferent terminals. This effect could be seen at near threshold voltages of stimulation, it disappeared at high voltages (Fig. 36). Increases in excitability must be observed at stimulus strength close to threshold; on the other hand, decreases in excitability can be observed in a wider range.

GABA and glycine: These amino acids were tested on 23 experiments. GABA consistently depressed the terminals (Fig. 31). Glycine was less effective.

Glutamate: This amino acid was studied by iontophoretic release in 25 experiments. It had no significant effect on the excitability of the terminals at doses up to 300 nA (Fig. 31).

Curare and Flaxedil: Both of these agents slightly depressed the excitability of the endings (200 nA) (Fig. 37). Curare was also depressant when given intravenously (0.5 to 1.0 mg/Kg).

Succinylcholine: This paralitic agent had no effect on the size of the antidromic spike when given intravenously (1 to 5 mg/Kg).

Picrotoxin: This drug was studied in 21 experiments. It increased the excitability of the terminals to direct electrical stimulation when given intravenously in doses of 1-2 mg/Kg (Fig. 38). The iontophoretic release had no significant effect. It blocked the depression produced

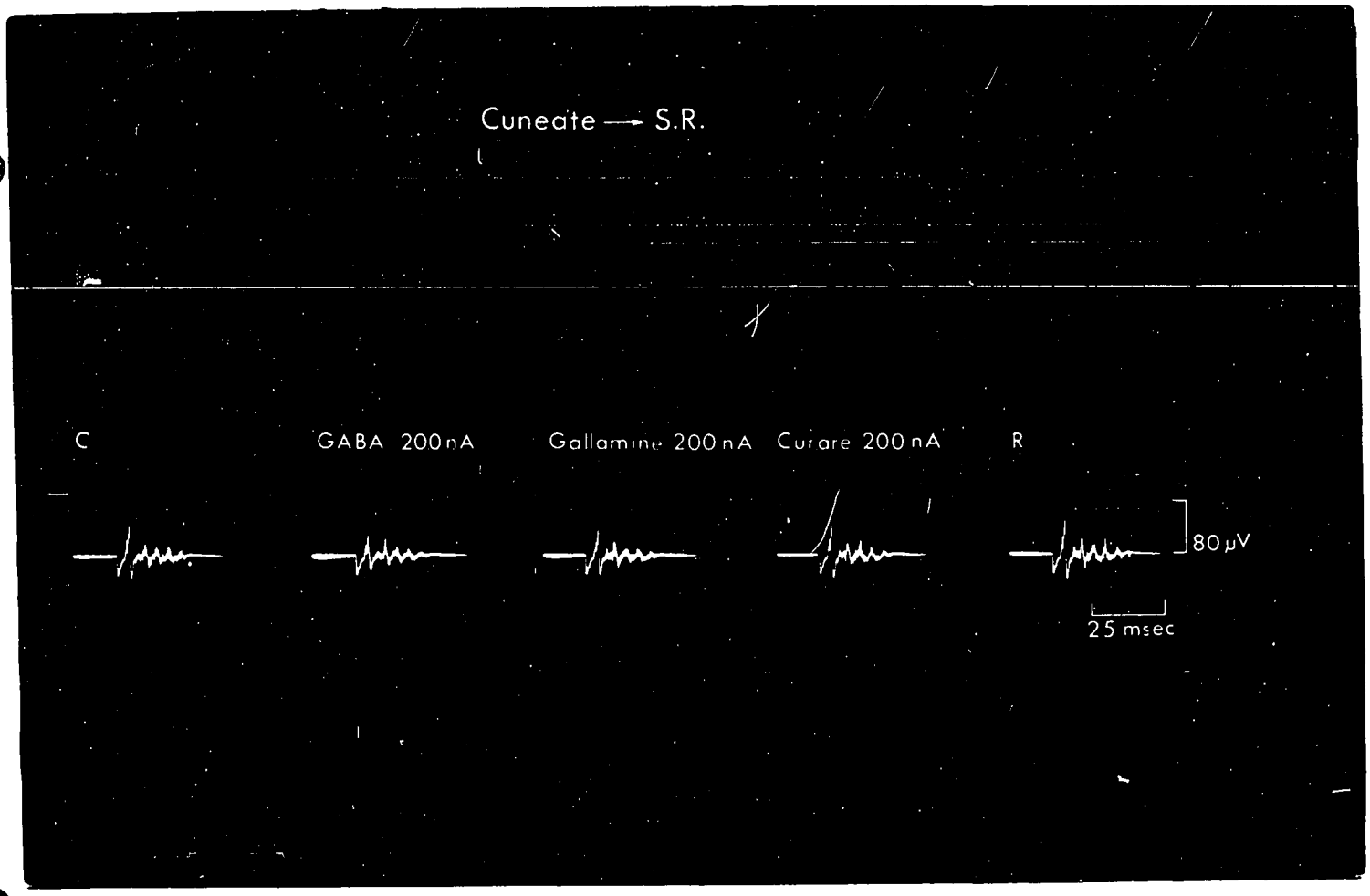

FIG. 37: Effect of GABA, gallamine and tubocurare on the afferent terminals of superficial radial nerve in the cuneate. Note the slight depression produced by gallamine and curare on the antidromic spike and the dorsal column reflex. Cuneate nucleus stimulation - 1/sec - 0.02 msec. Antidromic recording from the superficial radial nerve. Several traces superimposed. C = control. R = recovery. Decerebrated cat.

by GABA (Fig. 39) but not that of glycine.

Strychnine: This convulsant was studied in 19 experiments. It had no direct effect on the direct stimulation of the terminals regardless of the method of administration: iontophoretic (100-250 nA) or intravenous (0.1 to 0.2 mg/Kg).

Hypoxia: The effects of hypoxia were studied in 11 experiments. Administration of 5% 0_2 in 95% N_2 or N_2O initially increased the excitability; further severe hypoxia reduced the size of the antidromic action potential (Figs. 40, 42). This was used as a test to confirm the localization of the stimulating electrode as discussed above, Hypoxia must depress the release of transmitter at a time when the trans-synaptic spike was greatly reduced (Fig. 30). (It is known that the electrical excitability of the post-synaptic membrane is resistant to hypoxia (Eccles, Løyning and Oshima, 1966).

DISCUSSION

The afferent terminals have an important role in the synaptic process, reduction in their effectiveness is manifested by depression of synaptic transmission.

Previous studies on the effect of anaesthetics on these structures have not been made in a systematic way using appropriate techniques. Loyning, Oshima and Yokota

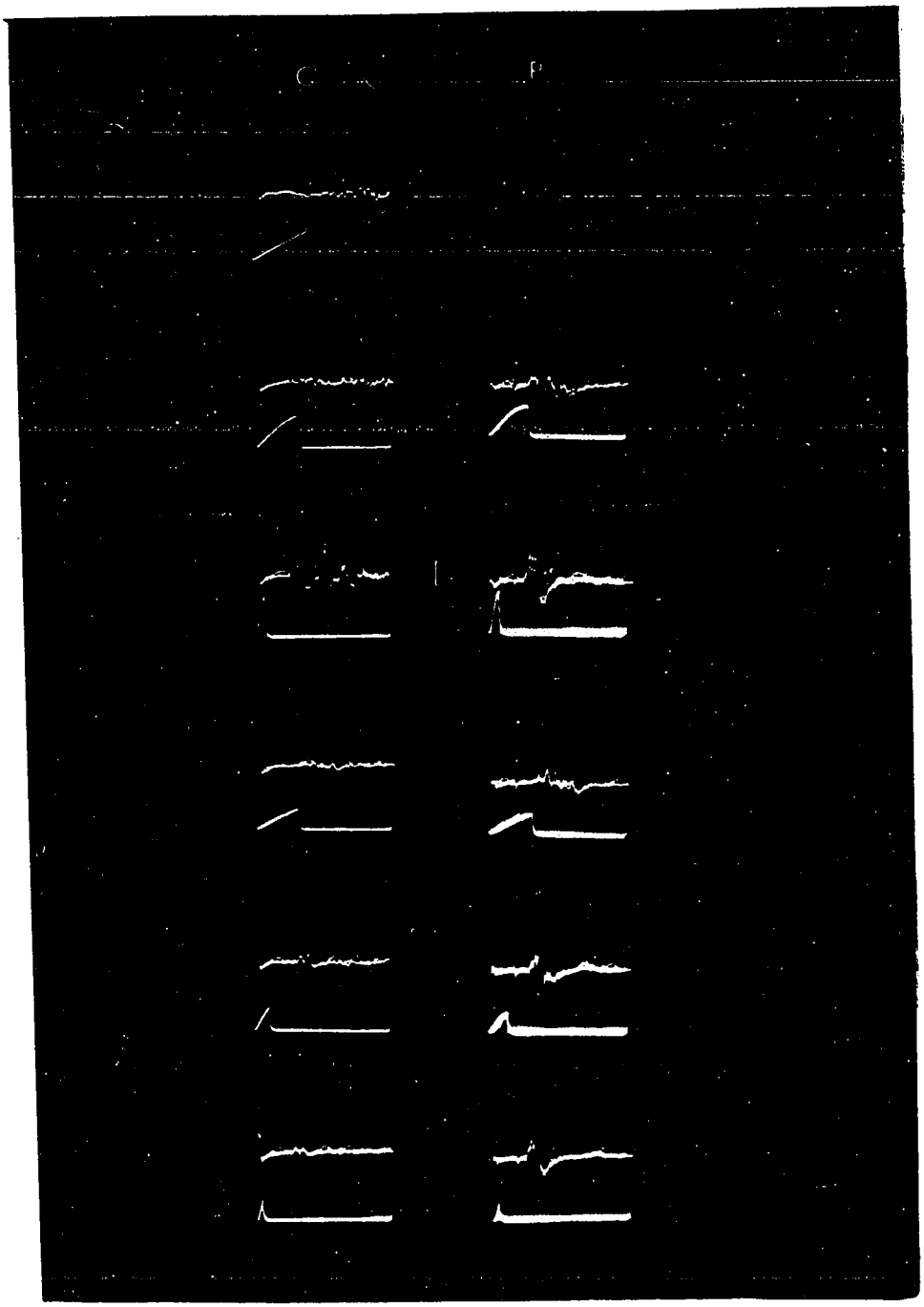

FIG. 38: Picrotoxin increases the excitability of the terminals and depresses the dorsal column reflex. Effect of various rates of rise on the cuneate nucleus stimulating pulse (1/sec). Left column: control. Right column: after 2 mg/Kg of picrotoxin. Antidromic recording from the superficial radial nerve. Several traces superimposed. Decerebrated cat.

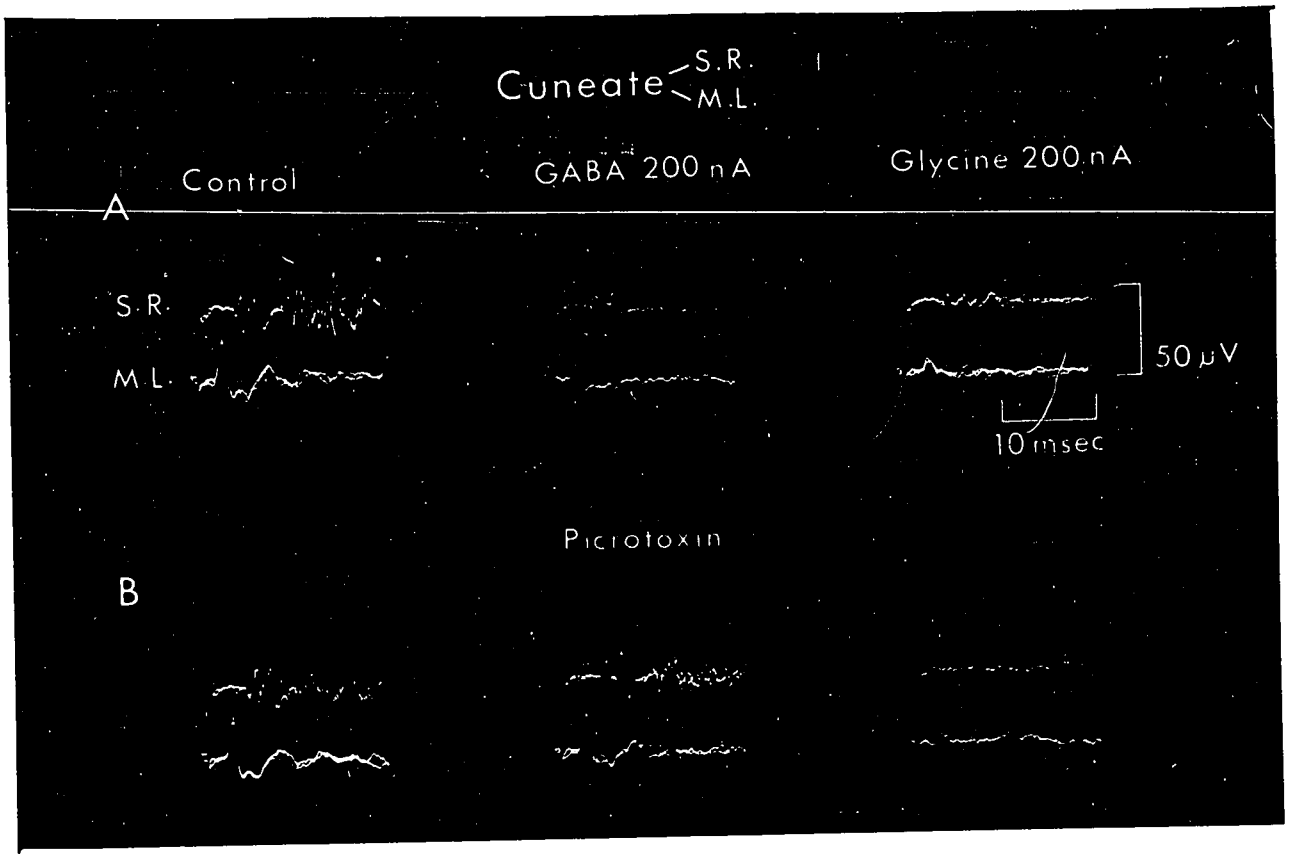

FIG. 39: Specific blockade of GABA-depression by iontophoretic picrotoxin (- 60 nA). Cuneate nucleus stimulation - 1/sec, 50 V, 0.02 msec. Several traces superimposed. Antidromic recording from the superficial radial nerve (S.R.) and orthodromic recording from the medial lemniscus. Decerebrated cat.

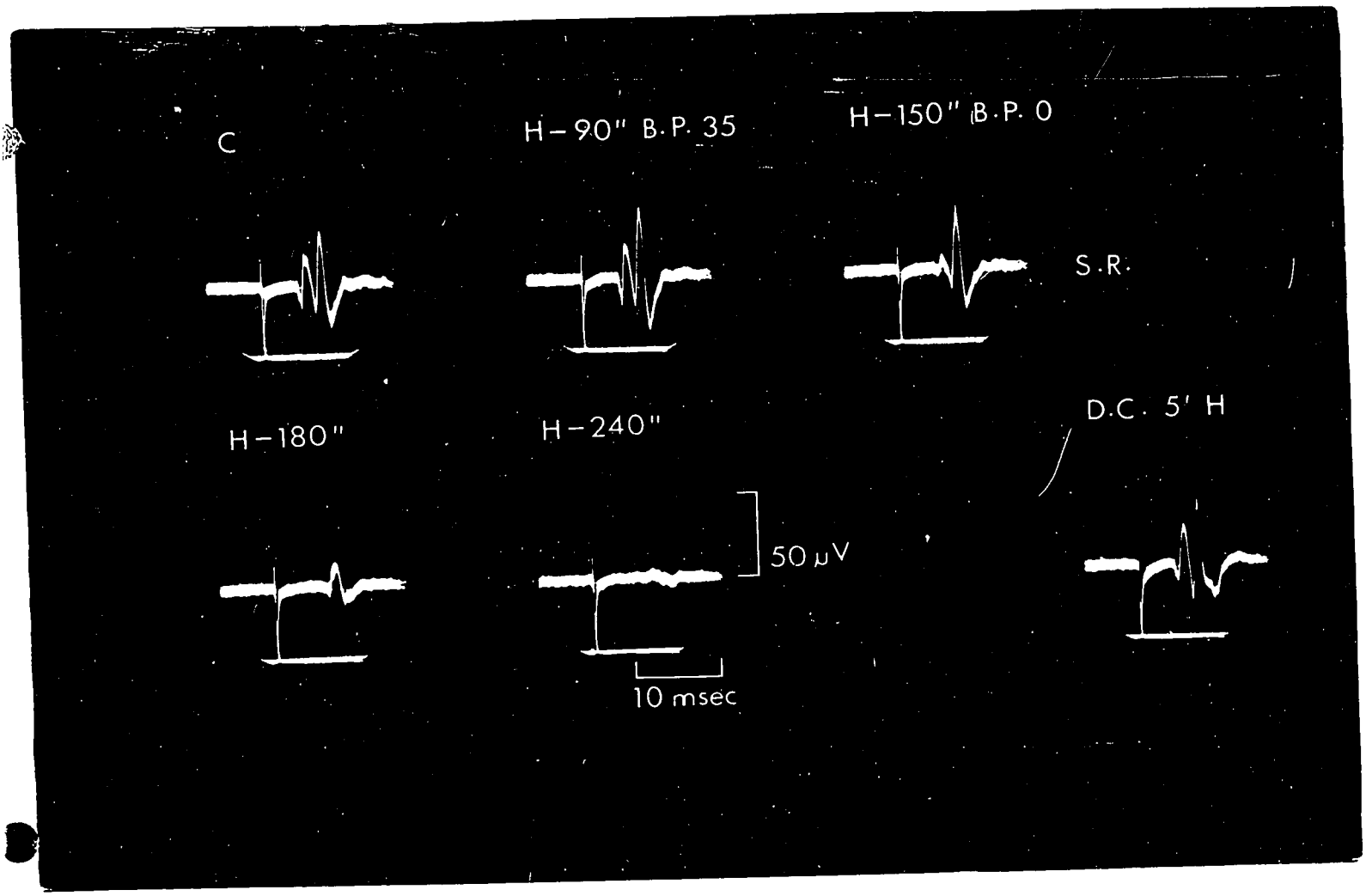

FIG. 40: Effect of hypoxia on the afferent terminals of the superficial radial nerve (C to H - 240") and the dorsal column fibres (D.C. 5' H). Cuneate nucleus stimulation - 1/sec, 0.02 msec - 60 V. Several traces superimposed.
The excitability of the terminals increases during the first 90 sec of hypoxia - blood pressure 35 mm Hg (H - 90" B.P. 35). The fast conducting fibres (first spike) are the first to be depressed (H - 150" B.P. 0). Total suppression of the action potentials originating at the terminals occurs 240 sec after the beginning of hypoxia (H - 240"). The same electrode was then moved to the dorsal columns where the stimulus was maintained unchanged. The fibres could be excited 5 min after the beginning of hypoxia (D.C. 5') or 1 min after the terminals were unexcitable. This response continued for more than 20 min. Observations made in a decerebrated cat. Hypoxia induced by inhalation of 100% Nitrogen.

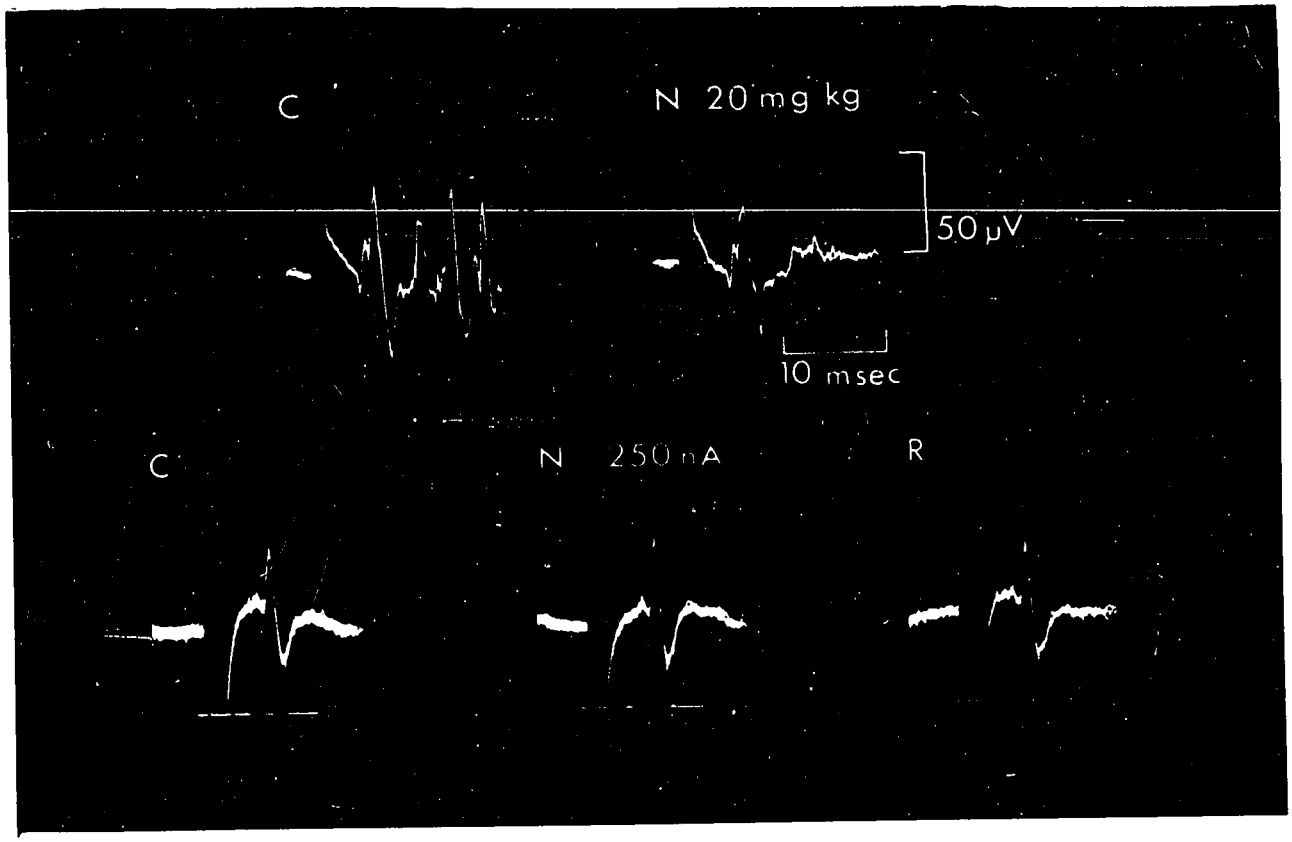

FIG. 41: Effect of Nembutal on the afferent terminals of the superficial radial nerve in the cuneate nucleus. Upper trace recorded in a cat at 34°C shows the submaximal antidromic action potential (first spike) and the dorsal column reflex (3 last spikes). Several traces superimposed. Intravenous Nembutal suppresses the latter and depresses the former - lower trace recorded in a different cat at 36°C. Microiontophoretic Nembutal increases the size of the antidromic spike. Stimulation of cuneate nucleus - 60 V, 0.02 msec at 1/sec. Recording from the superficial radial nerve. C = control. N = Nembutal. R = recovery.

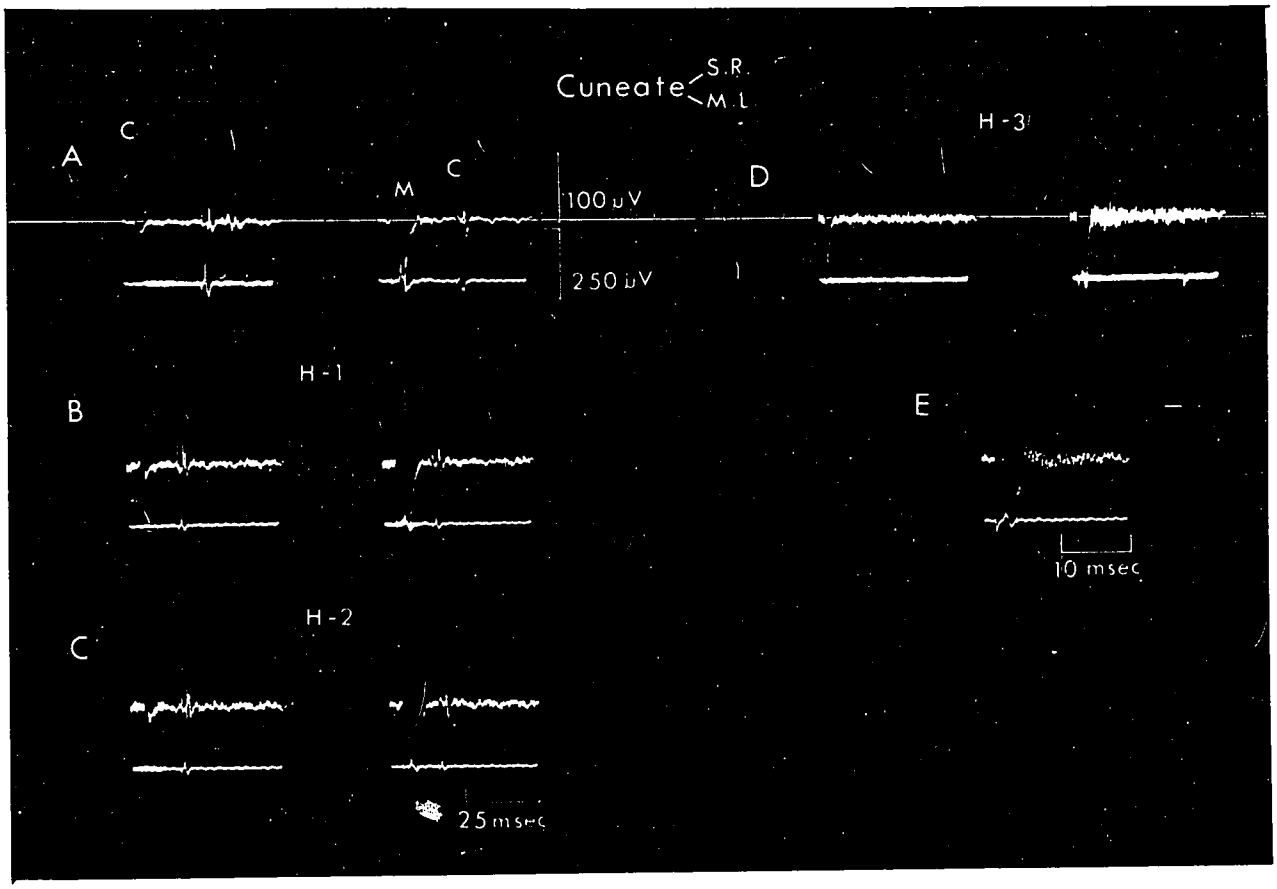

FIG. 42: Effect of progressive hypoxia on the interaction between terminals. Stimulation in the cuneate nucleus 50 V, 0.02 msec pulses at 1/sec. Antidromic spike record from the superficial radial nerve (upper trace) and orthodromic trans-synaptic from the medial lemniscus (lower trace). Several traces superimposed. Conditioning pulse to the median nerve. A - control before the beginning of hypoxia. B - severe hypoxia causing depression of the medial lemniscal spike. Note D.C. reflex still present. C - after further hypoxia, the interaction of the terminals decreases the size of the antidromic spikes, as expected from partially depolarized terminals. D - trans-synaptic action potential is barely present but there is a greatly increased dorsal column reflex. Latencies get shorter. E - same as D but with faster sweep. The first electrical artifact on the left column was produced by sub-threshold stimulation of the median nerve.

(1964) considered them as the site of action of thiamylal, but Somjen (1963) believed that they are not affected by these drugs. Both groups of investigators based their affirmation on indirect observations (field potentials). I have used a more direct experimental method to study the excitability of these endings (Edisen, 1957; Wall, 1958).

It must be pointed out that the technique of stimulation through the central barrel of a five-barrelled micropipette, while releasing drugs and other compounds through the other four barrels, may be affected by three possible major problems: a) changes in electrode resistance, b) current artifacts and c) electrode coupling.

The first and last of them were minimized by placing a high resistance in series with the stimulating electrode. The resistance of the former (10 MΩ - ~~05~~ 50 MΩ) being 10 times as large as the initial resistance of the electrode in vitro. This gives almost constant current stimuli. Current artifacts were discarded by using control currents through a barrel filled with sodium chloride whenever an effect was observed with a testing substance. However, owing to the technical difficulties in recording the current strength of the short pulses (0.02 msec) used, these results have been presented only in a qualitative form and should be accepted with reserve.

It is unlikely that the results observed with the iontophoretic release of GABA, glycine, picrotoxin, strychnine, and other substances on the antidromic spike were entirely the result of the above pitfalls since: a) the effect of these substances were consistent and reproducible under various conditions in a large number of experiments, b) there were differences between the effects on the dorsal column fibres and the terminals, c) a minimum of two electrodes were used per experiment and d) the various solutions of the compounds being tested were made to be used in no more than five experiments.

Intravenous Nembutal depressed terminal axons (Fig. 41) while halothane had no effect or slightly increased their excitability. None of these agents affected the antidromic action potential generated in the dorsal columns. Procaine, as expected, was equally effective in suppressing the action potential originating at the terminals and/or the dorsal columns.

The lack of a consistent response or even weak excitation observed with iontophoretic Nembutal is difficult to understand. It could be due to an electrical artifact; the iontophoretic release of negatively charged depressant substances tends to mask their true effect as observed with the same anaesthetic on the post-synaptic membrane (Fig. 13). A possible explanation could be found

in the work of Sato and co-workers (1967). For these authors Nembutal in high concentration would increase the permeability of the membrane to K. Consequently, it might reduce the excitability of neuronal elements while increasing their susceptibility to changes in extracellular K, known to increase with the arrival of nerve impulses (Orkand, Nicholls and Kuffler, 1966).

Thus, this anaesthetic depresses the terminals as well as their liberation of potassium. However, since this ion becomes more effective on these structures, their increased interaction is manifested by a dorsal root potential (DRP) of greater duration and amplitude, an effect observed by Eccles et al. (1963) and Schmidt (1963).

D - CUNEATE NEURONES

Two types of functionally different cells were identified in the cuneate nucleus. Cuneothalamic relay cells (R) and interneurones (I). Their possible function and how they react to pharmacological agents is the subject of this part of the research project.

Identification

Not all neurones can be identified beyond reasonable doubt but we can positively identify some of the cuneothalamic relay cells and some of the interneurones.

Units responding to medial lemniscal electrical pulses with a fixed latency shorter than 2 msec and able to follow high frequencies of stimulation were considered as antidromically activated relay cells (cf. Gordon and Seed, 1961). On the other hand units activated from the pyramidal tract (and not from the medial lemniscus) with a latency longer than 2 msec, were classified as interneurones. These criteria have been applied by Andersen et al. (1964) and Gordon and Jukes (1964b). However, interneurones that did not respond to pyramidal tract stimulation but were excited from one or several peripheral nerves and not from the medial lemniscus were left as unidentified units owing to the possibility of being relay cells not projecting to the medial lemniscus or alternatively

projecting to this tract but no being excited for technical reasons (Gordon and Seed, 1961).

Trans-synaptic stimulation of interneurones was easily depressed by iontophoretic procaine (10-20 nA) or Nembutal (50-150 nA). The systemic administration of the latter (20 mg/Kg) increased their threshold by more than 50%. The inhalation of halothane (2%) had no depressant effect on interneurones, if anything their spontaneous activity increased during its inhalation (Fig. 43). The depression produced by iontophoretic GABA and glycine on internuncial cells was not significantly affected by halothane (Fig. 29).

Post-synaptic inhibition was studied in cells excited by iontophoretic glutamate (10 nA) while a peripheral nerve was being stimulated. The effect of this stimulus on the glutamate response was plotted as a post-stimulus histogram. In relay cells (25 units in four experiments) this pulse produced an inhibition with a duration anywhere between 20 and 200 msec (Fig. 44). It could be reduced to 10-20 msec by iontophoretic picrotoxin (50-100 nA given for 3 min prior to the test). No such pause was observed in the firing of interneurones (more than 20 units in four experiments). On the contrary their firing rate was accelerated by the stimulus (Fig. 45). It is concluded (cf. Andersen and co-workers, 1964), that these cells

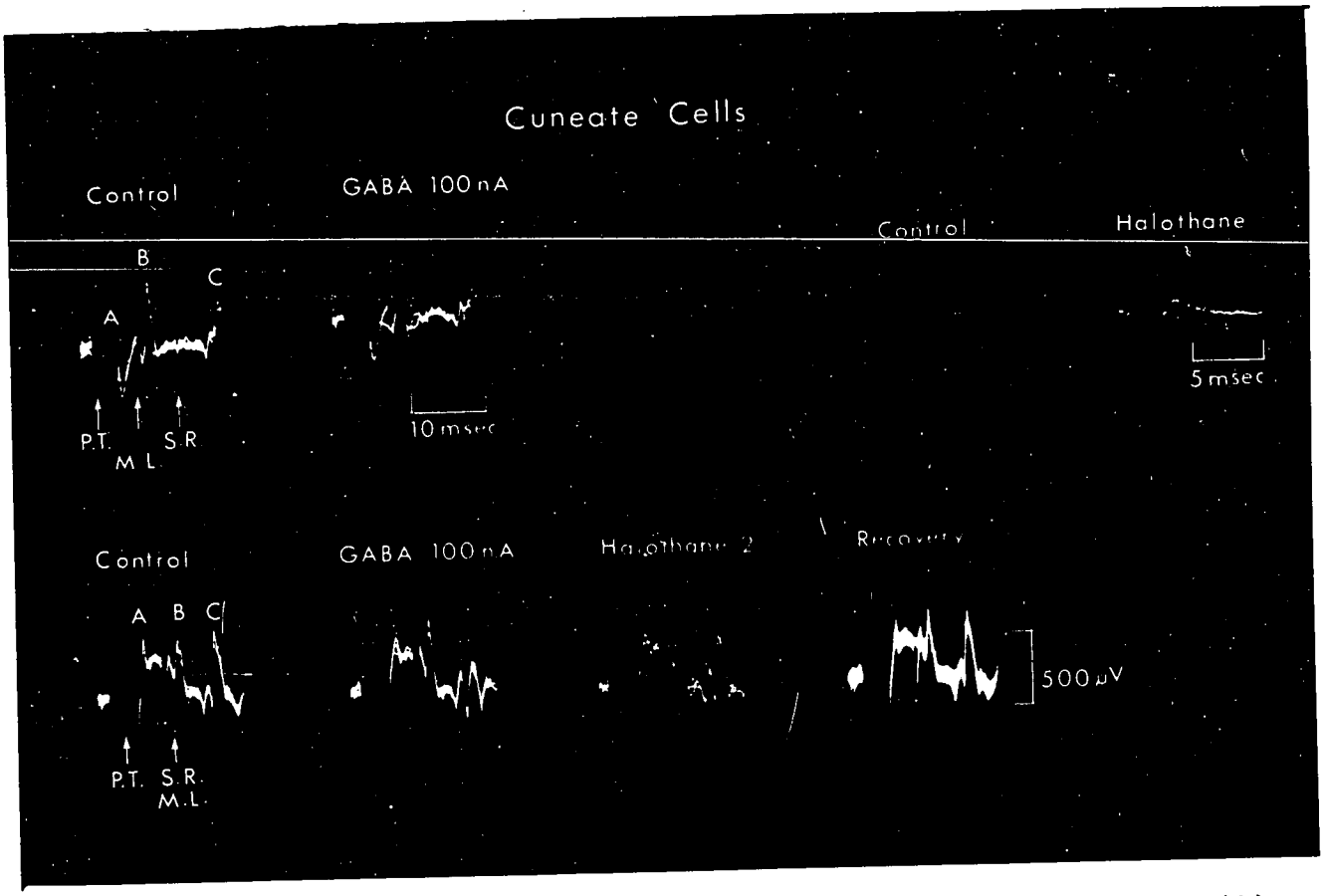

FIG. 43: Effect of halothane and GABA on an interneurone (A) being excited from the pyramidal tract and a cuneothalamic cell. The latter was excited antidromically from medial lemniscus (B) and orthodromically from the superficial radial nerve (C). Upper right corner same interneurone as in lower traces. Upper left hand traces are from different units. Several traces superimposed.
GABA blocks more effectively the orthodromic responses of relay cell than the evoked activity of the interneurone (orthodromic). Halothane (2%) depresses the trans-synaptic activity of the relay units but not that of interneurones. Both GABA and halothane have less effect on the antidromic spike. Note the increased amplitude of this action potential during the administration of these agents (GABA released iontophoretically. Halothane applied on the surface of the cuneate by cup). This effect suggests a common mechanism of action, possibly by hyperpolarization. P.T. = pyramidal tract stimulation by burst of 3-6 stimuli (3 V, 0.1 msec at 500/sec). M.L. = medial lemniscal stimulation (20 V, 0.05 msec, frequency of stimulation 1/sec). S.R. = superficial radial nerve at twice threshold.

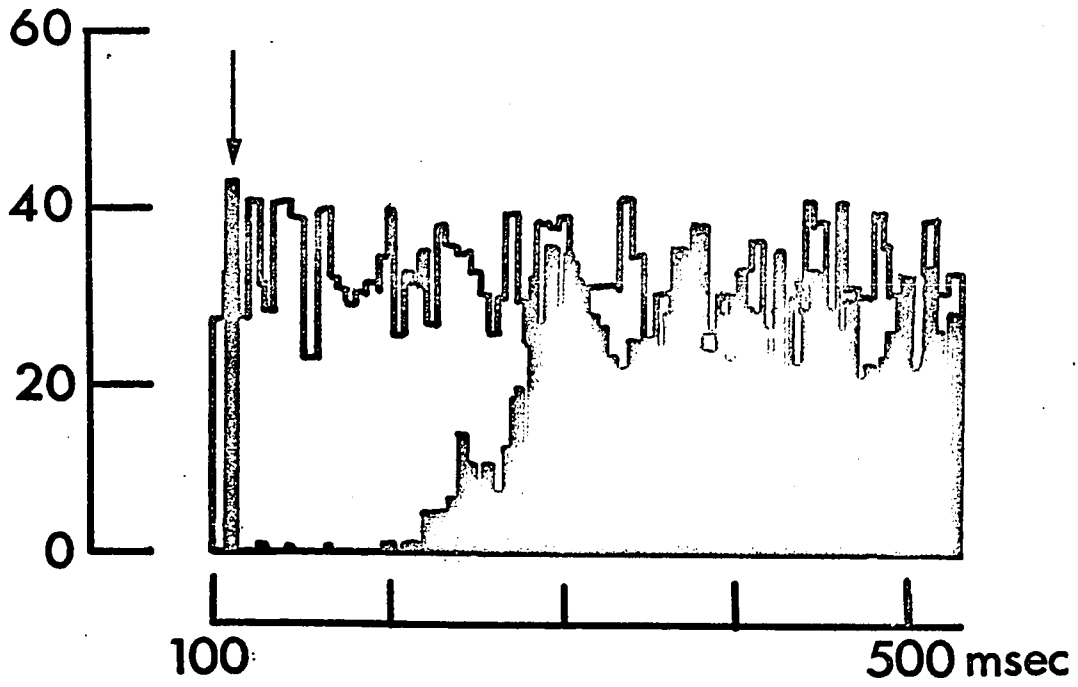

FIG. 44: Shaded area is post-stimulus histogram of a cuneothalamic neurone being stimulated by glutamate (10 nA). The depression observed after the stimulus to the median nerve can be considered as post-synaptic inhibition; it is increased by 2% halothane and reduced by 2 mg/Kg picrotoxin. Ordinate: number of spikes. Stimulus to the median nerve - 1/sec, 0.2 msec, 6 V. This type of inhibition is not observed on interneurones. White area represents the control firing of this unit. A five-barrelled micropipette was used for recording and iontophoretic glutamate release.

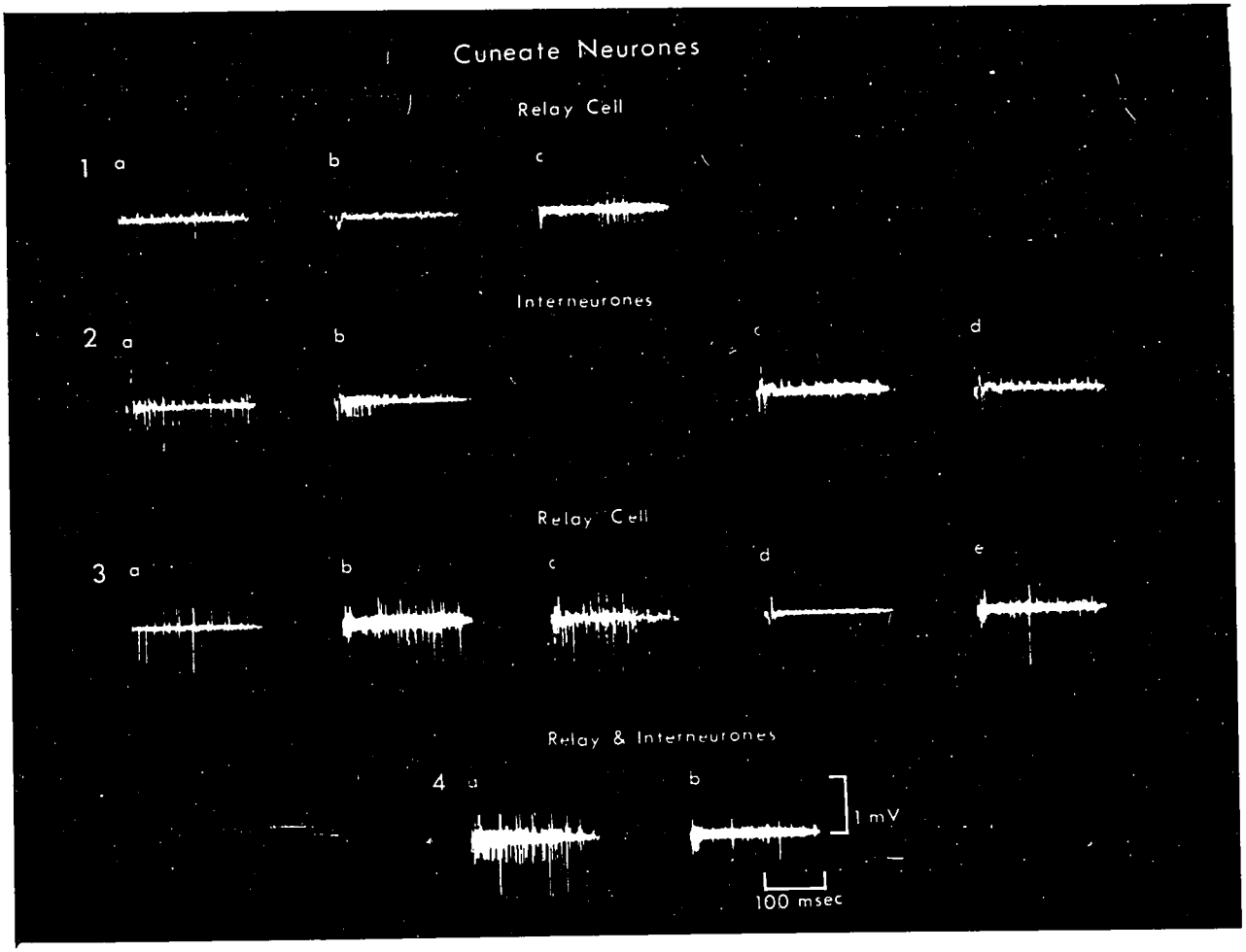

FIG. 45: Lack of post-synaptic inhibition on interneurones. Cells excited with glutamate (10 nA). 1a - control relay cell. 1b - post-stimulus depression by an electrical pulse on the superficial radial nerve. 1c - reduction of this inhibition by iontophoretic picrotoxin (50 nA). 2a - lack of post-stimulus depression (inhibition) in an interneurone. 2b - picrotoxin effect. 2c and d - two different interneurones without post-stimulus depression. 3a - relay cell being stimulated with glutamate (10 nA). 3b - post-stimulus inhibition. 3c - picrotoxin (40 nA) suppresses this inhibition. 3d - strychnine (90 nA) depresses cell firing. 3e - recovery - glutamate evoked activity. 4a and b - two pairs of interneurones and relay cell being stimulated simultaneously. Absence of post-stimulus inhibition identifies the interneurones. Several traces superimposed.

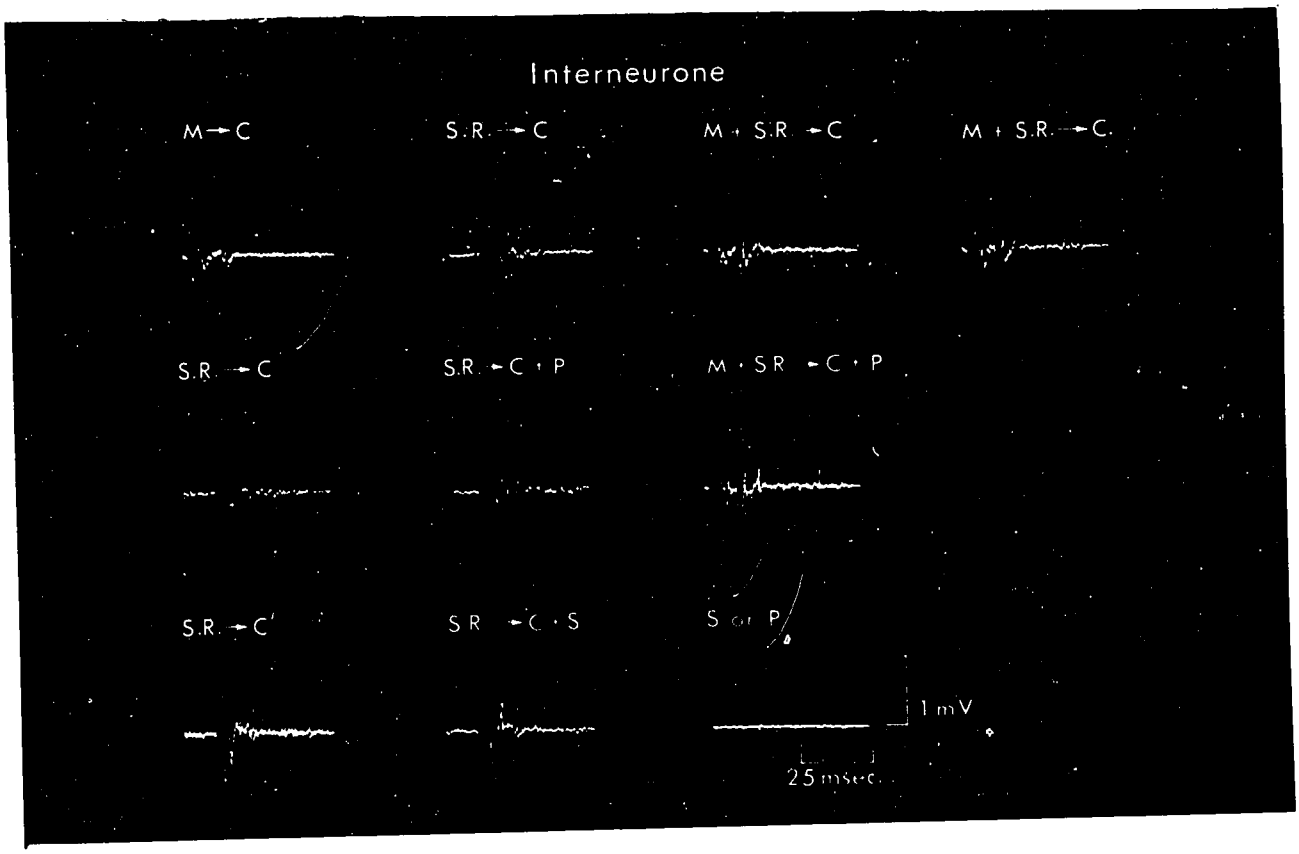

FIG. 46: Absence of pre- or post-synaptic inhibition in an interneurone. Cell evoked from median (M) and superficial radial (S.R.) nerves stimulation. The inconsistent depression observed by combined nerve stimulation is not affected by picrotoxin (P) ~~suggesting the lack of presynaptic inhibition~~. This depression is likely due to post-activation depression. Strychnine (S) depresses the glutamate evoked activity. Strychnine or picrotoxin have no effect by themselves as seen in the last lower trace (S or P).

receive little if any post-synaptic inhibitory input from the periphery.

When two pulses, one to the median and the other to the superficial radial nerve, were delivered 10 to 40 msec apart, inhibition appeared following the second stimulus. This inhibition was not affected by picrotoxin as occurred with the relay cells (Fig. 46). Both stimuli excited the interneurone when applied alone, thus this depression was either a) presynaptic inhibition mediated by depolarization of the terminals or b) post-activation depression. The fact that picrotoxin did not suppress it is in favour of a post-activation depression.

It is suggested that the lack of post-synaptic inhibition could serve as a simpler method to identify interneurones if proven to be true in a greater population of internuncial elements.

DISCUSSION

Function of cuneate neurones

On the bottom of Figure 45 are illustrated two units recorded simultaneously - the one is a relay cell and shows post-synaptic inhibition from the periphery; the other is an interneurone and shows no such inhibition. The firing of this latter coincides with the post-stimulus pause suggesting a relation to this phenomenon. A similar observation on post-stimulus pattern of discharge has

been made recently in the thalamus (Marco, Brown and Rouse, 1967). Interneurones are considered to mediate inhibition in the spinal cord (Wall, 1967; Eccles, 1964). Their selective destruction, after 40-50 minutes of spinal asphyxia, produces a state of rigidity (Gelfan and Tarlov, 1956) considered to be mediated by the uninhibited activity of motoneurones. Interneurones are suspected of mediating inhibition in the cuneate nucleus (Andersen, Eccles and Schmidt, 1962) an idea supported by the histological work of Walberg (1965) (Fig. 47).

The present study clearly indicates that these two types of units are functionally and pharmacologically different. Procaine and Nembutal depress both relay cells and interneurones, but halothane appears to mediate its depressant action on the former by facilitating the inhibitory effect of interneurones. Previous attempts to differentiate the susceptibility of neurones to anaesthetics were supposedly based on size. Somjen, Carpenter and Henneman (1965) classified motoneurones by their spike heights and suggested that small units were easier to depress. However, the pharmacology of neurones, based on size, has less significance than when it is based on function.

E - EFFECTS ON SYNAPTIC INHIBITION

Post-synaptic inhibition has already been discussed (page 78).

Synaptic inhibition in general (comprising pre- and post-synaptic inhibition) was studied as follows:

a) Inhibitory effect of median nerve conditioning pulses on the superficial radial nerve trans-synaptic action potentials recorded in the cuneate nucleus or the medial lemniscus.

b) Inhibitory effect of median nerve conditioning pulses on the trans-synaptic action potential originated by direct stimulation of the cuneate nucleus.

Changes in the size of the β component defined previously, page 59) of the medial lemniscus action potential were compared with control observations. The results were plotted as percentage changes at different time intervals (in milliseconds) after the conditioning pulse.

The size of the trans-synaptic action potential recorded in the medial lemniscus, during cuneate stimulation, could be reduced (inhibition) by a conditioning pulse to one peripheral nerve (median). The length of this inhibition lasted between 60 and 300 msec - tests made at a frequency of more than 2/sec were misleading as the inhibition became shorter at higher frequencies.

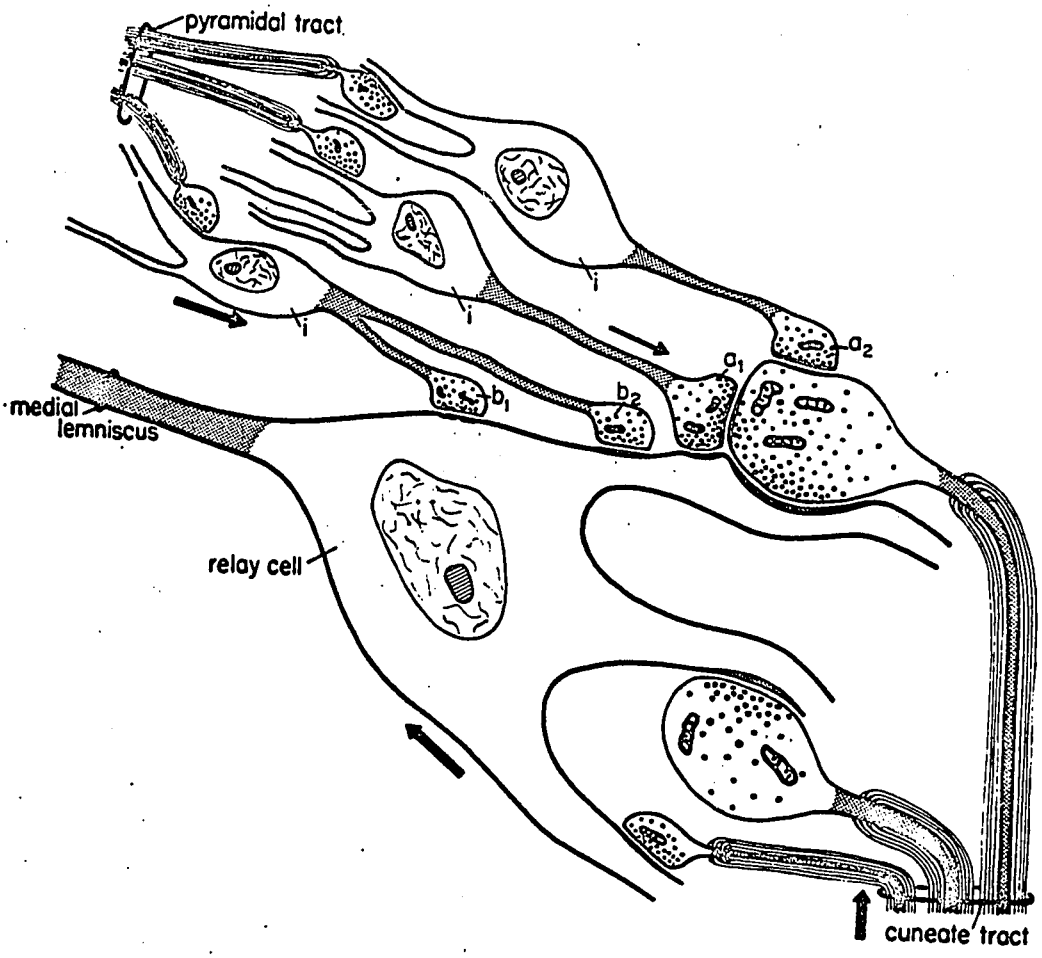

FIG. 47: Diagram showing the supposed pattern of synaptic contacts in the cuneate nucleus. The large and probably also some small axon terminals of the fibers of the cuneate fascicle make contact with dendrites of relay cells. The small terminals of the pyramidal tract fibers have synapses with dendrites of interneurons (i). The axon terminals of the interneurons synapse with soma and dendrites of relay cells (b_1 and b_2). Some of these terminals may also be derived from cells of the adjacent reticular formation. Some of the axon terminals of interneurons which make axodendritic synapses are in addition in contact with large axon terminals of fibers of the cuneate fascicle. This is an axoaxonic synapse (a_1). Other terminals of the fibers of the interneurons are in contact only with terminals of the fibers of the cuneate fascicle, this axoaxonic synapse is shown at a_2 (Walberg, 1965).

The strength of the conditioning pulse was an important parameter (Fig. 48) when the test stimulus originated in the cuneate nucleus but not when it originated in the superficial radial nerve.

Nembutal: (6 experiments) Intravenous Nembutal (2-30 mg/Kg) shortened the duration and decreased the intensity of synaptic inhibition. The minimum effect was observed when 2 mg/Kg were given and the maximum following the administration of 10 mg/Kg (Figs. 49, 50).

Halothane: (7 experiments) The inhalation of this anaesthetic (1-2%, artificially maintained blood pressure) increased the duration and the intensity of synaptic inhibition by more than 20% (Figs. 50, 51). The effect of halothane was maximal 5 to 10 min after the beginning of its administration. Recovery from this effect was observed at more than 30 min of its cessation. The anaesthetic was given for periods of 20 to 30 min.

Picrotoxin: (5 experiments) 2 mg/Kg of this drug greatly reduced both duration and intensity of synaptic inhibition. Given iontophoretically it blocked the effects of GABA (but not of glycine) on terminals and post-synaptic membranes (Figs. 52, 53, 54, 55).

Strychnine: (6 experiments) Intravenous administration of 0.1 to 0.2 mg/Kg had no effect or increased both duration and intensity of inhibition in three experiments in which

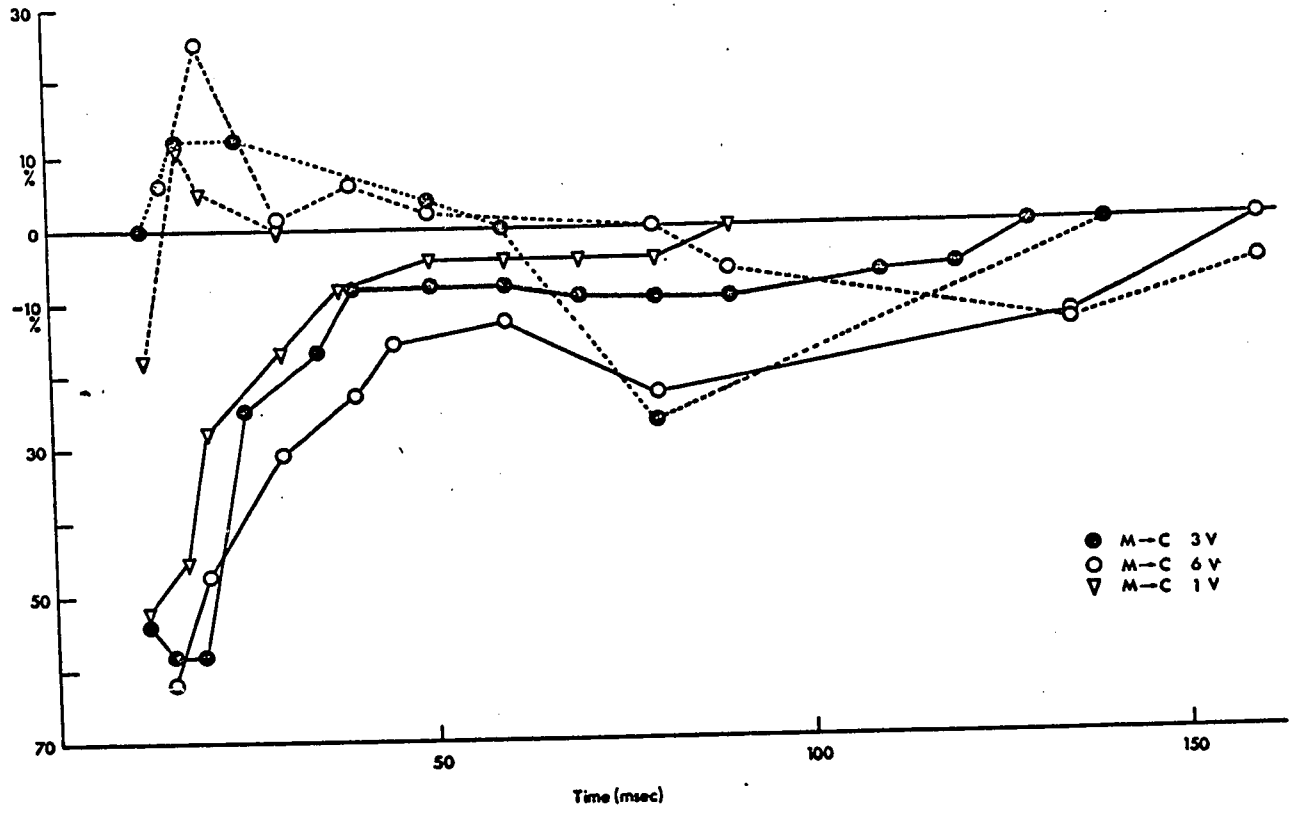

FIG. 48: Excitability changes of the superficial radial afferent terminals (broken lines) and synaptic inhibition of β spike (solid lines) at various voltages of median nerve conditioning pulse. Test pulses - 1/sec, 0.02 msec, 50 V - to the cuneate nucleus of a decerebrated cat. Both changes are dependent on the strength of the conditioning pulse. Ordinate: percentage change (±) from control of the action potential size. Abscissa: time in msec between the conditioning and the test pulses.

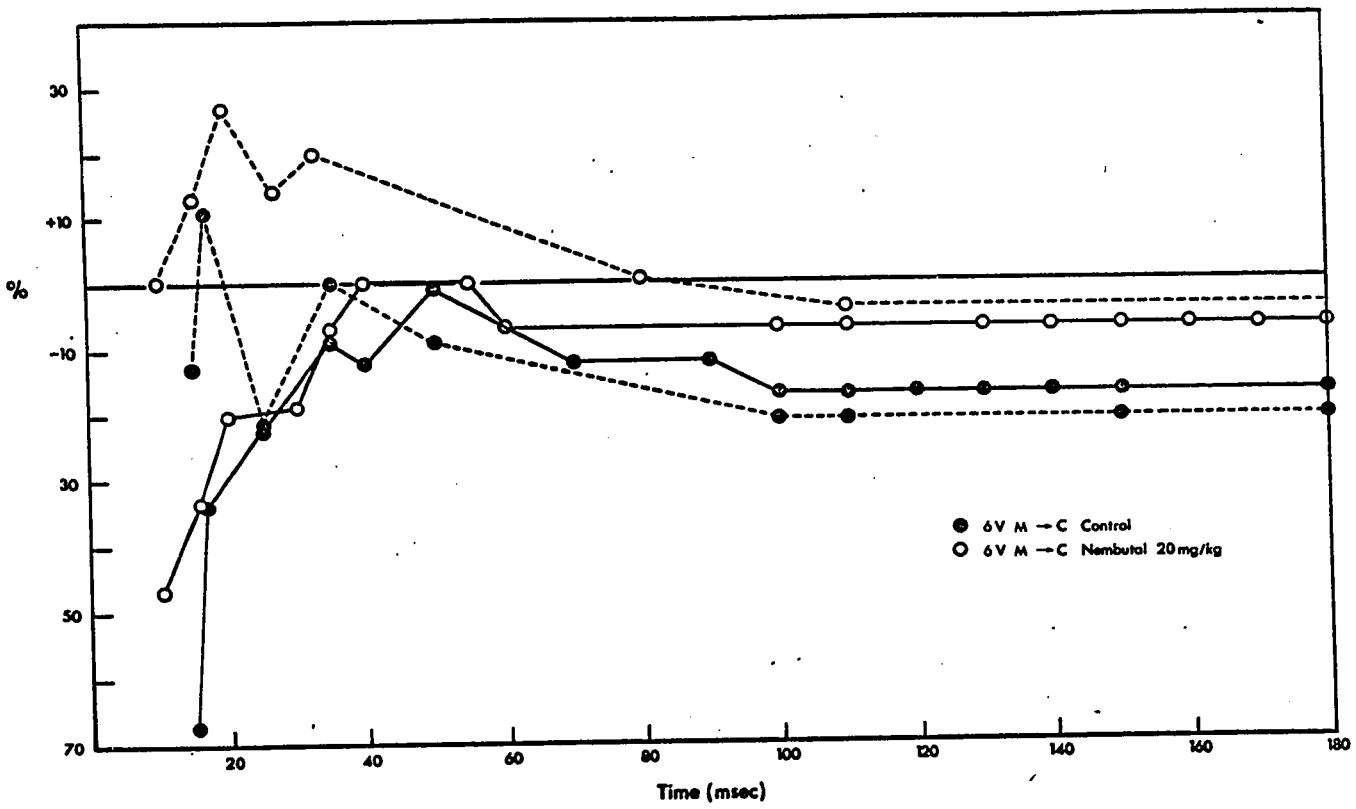

FIG. 49: Effect of intravenous Nembutal (20 mg/Kg) on the excitability of the superficial radial terminals (broken lines) and synaptic inhibition (β spike in the medial lemniscus). The anaesthetic reduces the intensity and duration of synaptic inhibition while increasing the interaction between terminals. This latter observation is consistent with the prolongation of the dorsal root potential (DRP) (Schmidt, 1963). Cuneate nucleus in a decerebrated cat.

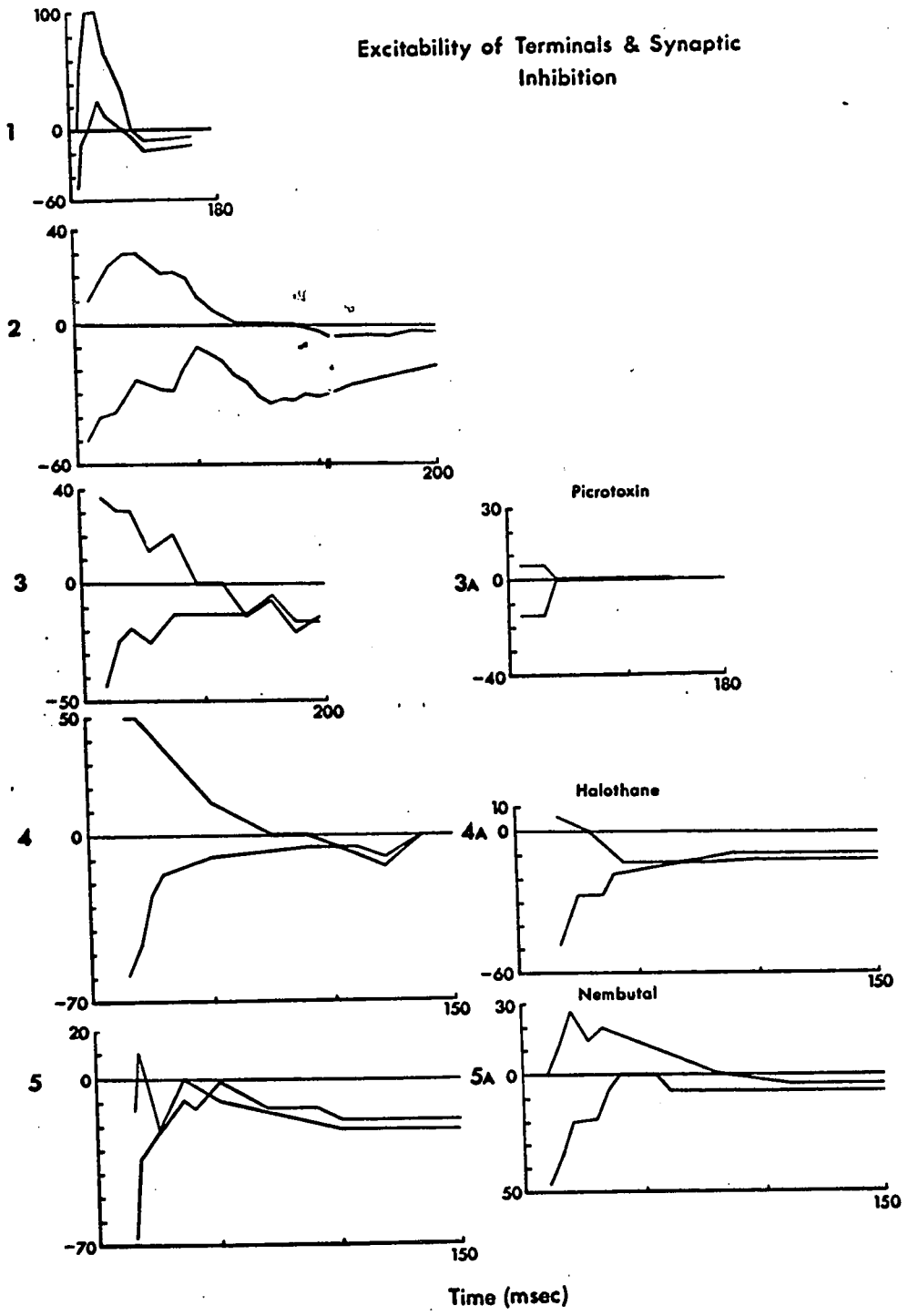

FIG. 50: Differences in the time course between the excitability changes of the superficial radial nerve terminals (upper traces) and cuneate nucleus synaptic inhibition of the β spike (lower traces). Controls in left hand column. Note the changes in the time courses following the administration of intravenous picrotoxin (2 mg/Kg), Nembutal (20 mg/Kg) and the inhalation of 2% halothane. Records from five different decerebrated cats.

This figure includes graphs also shown in greater detail in figures 48, 49, 51.

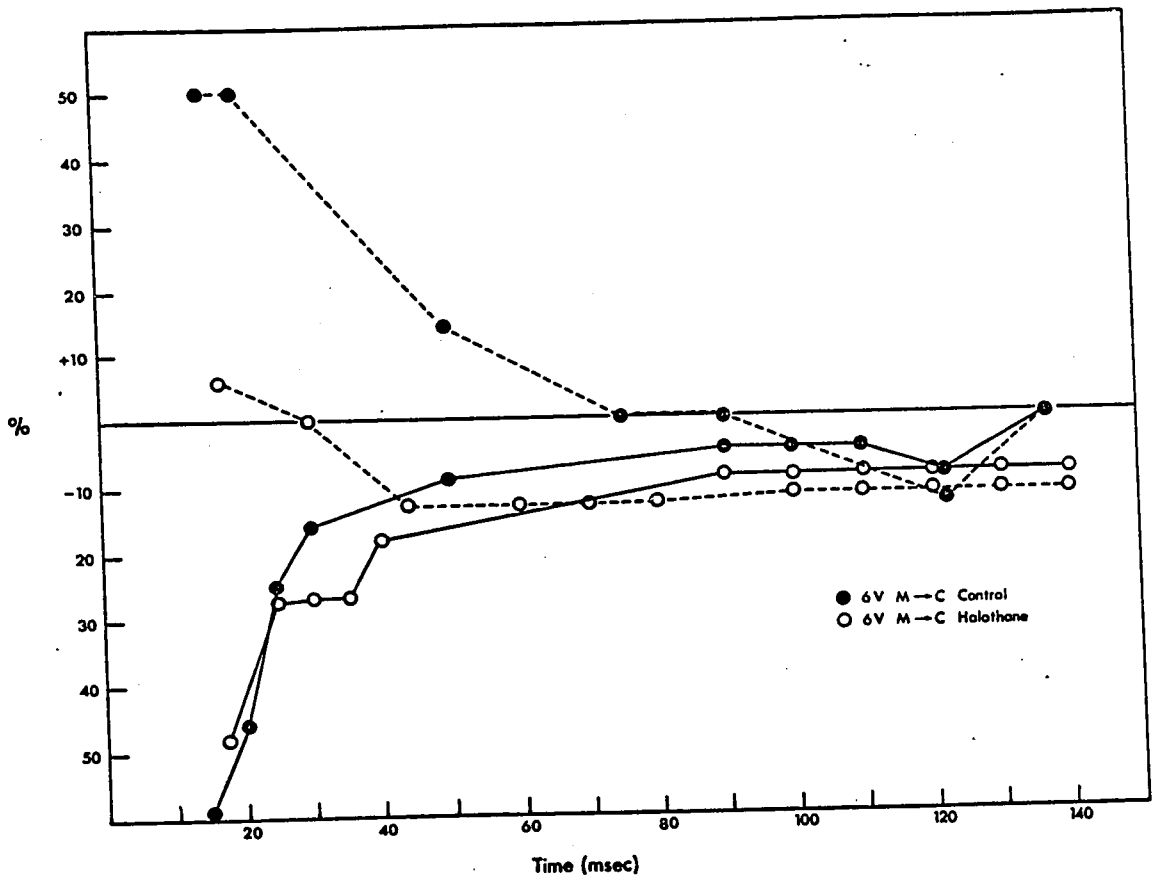

FIG. 51: Effect of the inhalation of 2% halothane on the excitability changes of the superficial radial nerve terminals (broken lines) and cuneate nucleus synaptic inhibition (β spike) induced by a conditioning pulse to the median nerve (M) - 0.2 msec, 6 V - at various preceding intervals (in msec) to a testing pulse to the cuneate nucleus (C) 1/sec, 0.02 msec, 40 V. Halothane reduces the interaction between terminals while increasing synaptic inhibition. Decerebrated cat.

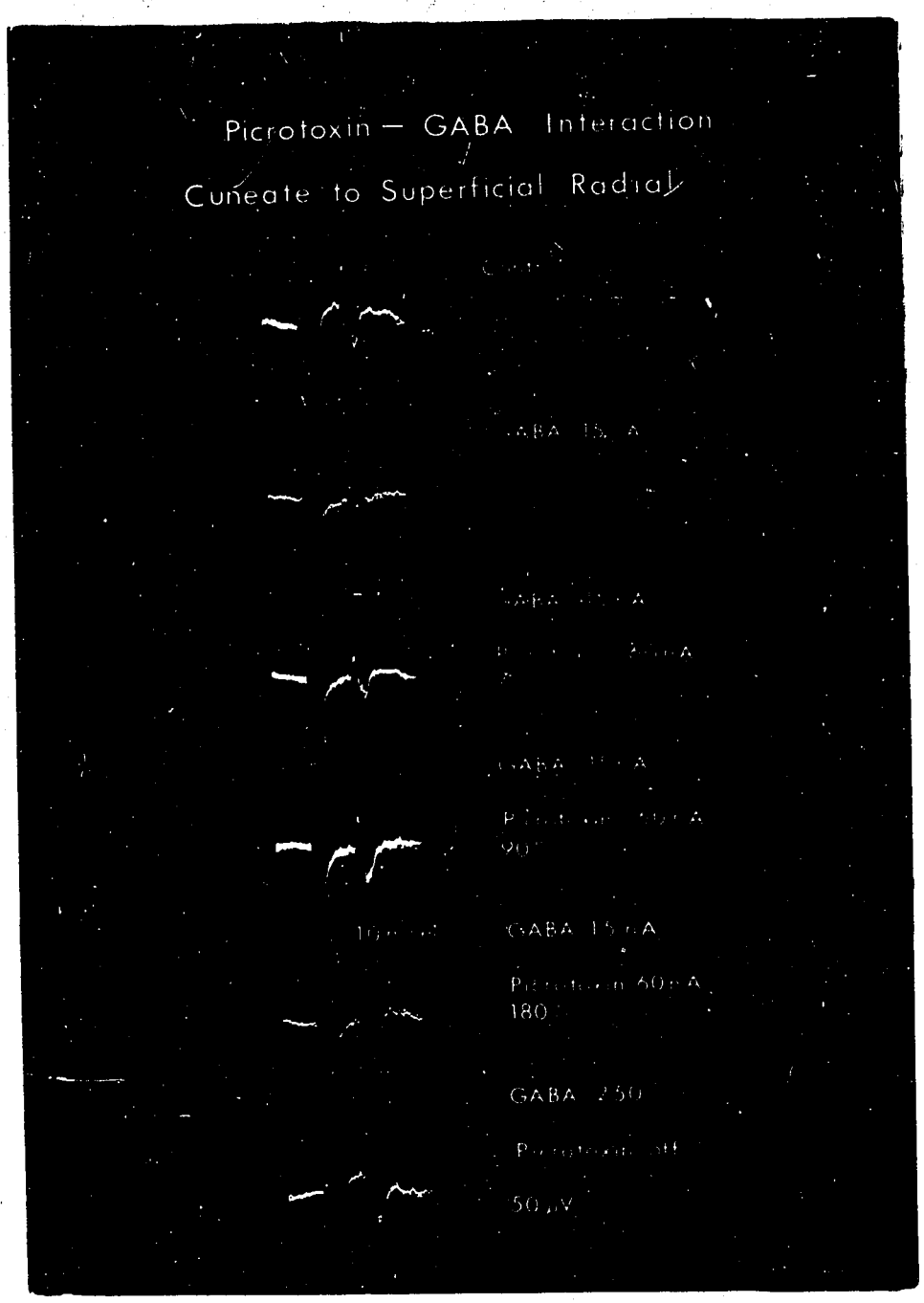

FIG. 52: The complete blockade of GABA-depression on the afferent terminals of the cuneate nucleus, by picrotoxin takes up to 3 minutes. It is present after its cessation for 5 to 10 more minutes. The lowermost trace shows the magnitude of picrotoxin effect immediately after its application, the antidromic spike is now resistant to a GABA dose 16 times the one used before the administration of picrotoxin (15 nA). Cuneate stimulation - 1/sec - 0.02 msec - 50 V. Several traces superimposed. Recording from the superficial radial nerve. Decerebrated cat.

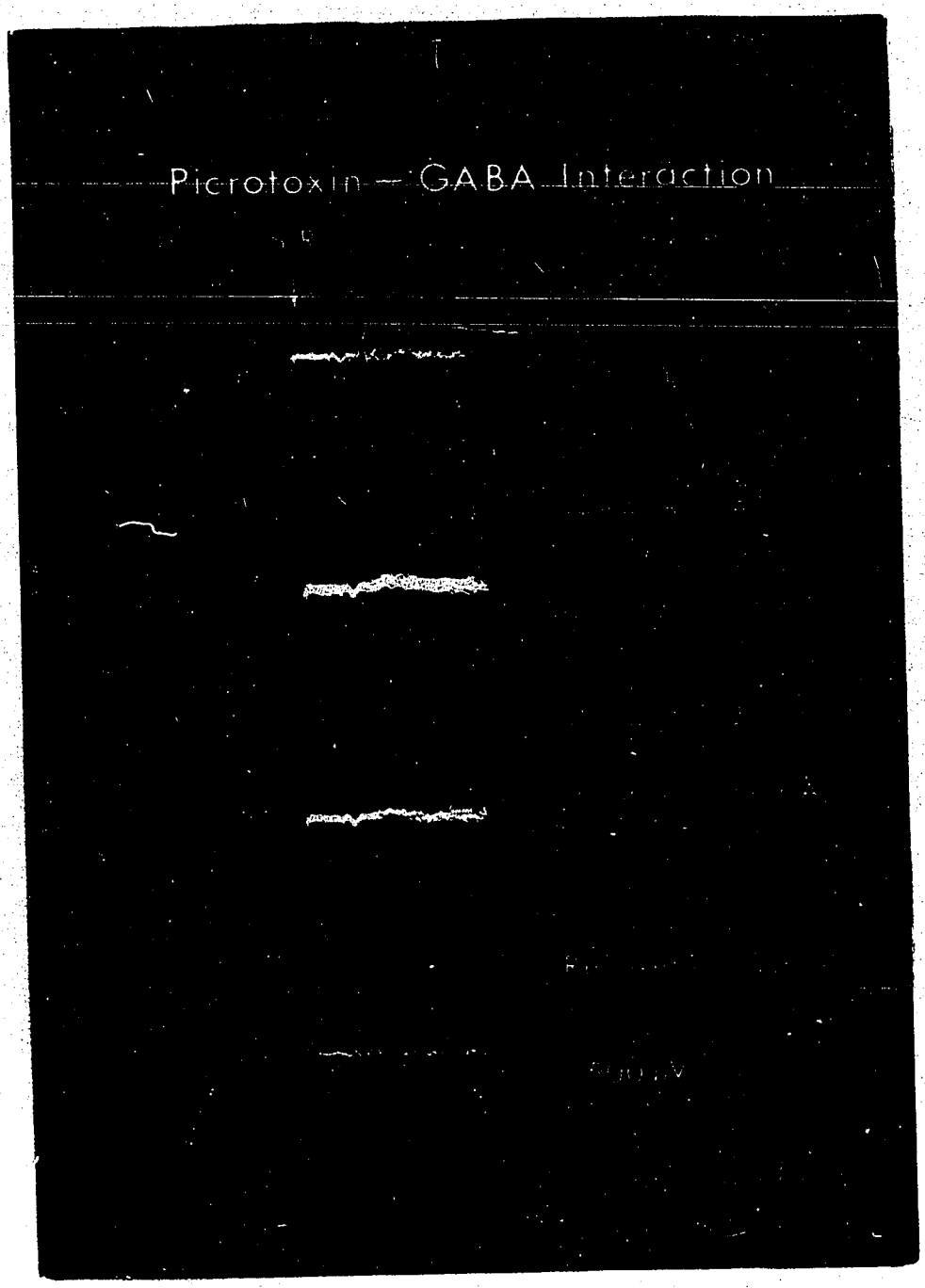

FIG. 53: Blockade of the depression produced by GABA during the release of picrotoxin. The arrow indicates the peripheral stimulation of the superficial radial nerve - 4/sec - 0.2 msec - 6 V - recording and microiontophoretic release of GABA and picrotoxin through a five-barrelled pipette placed in the cuneate nucleus. Decerebrated cat. Several traces superimposed.

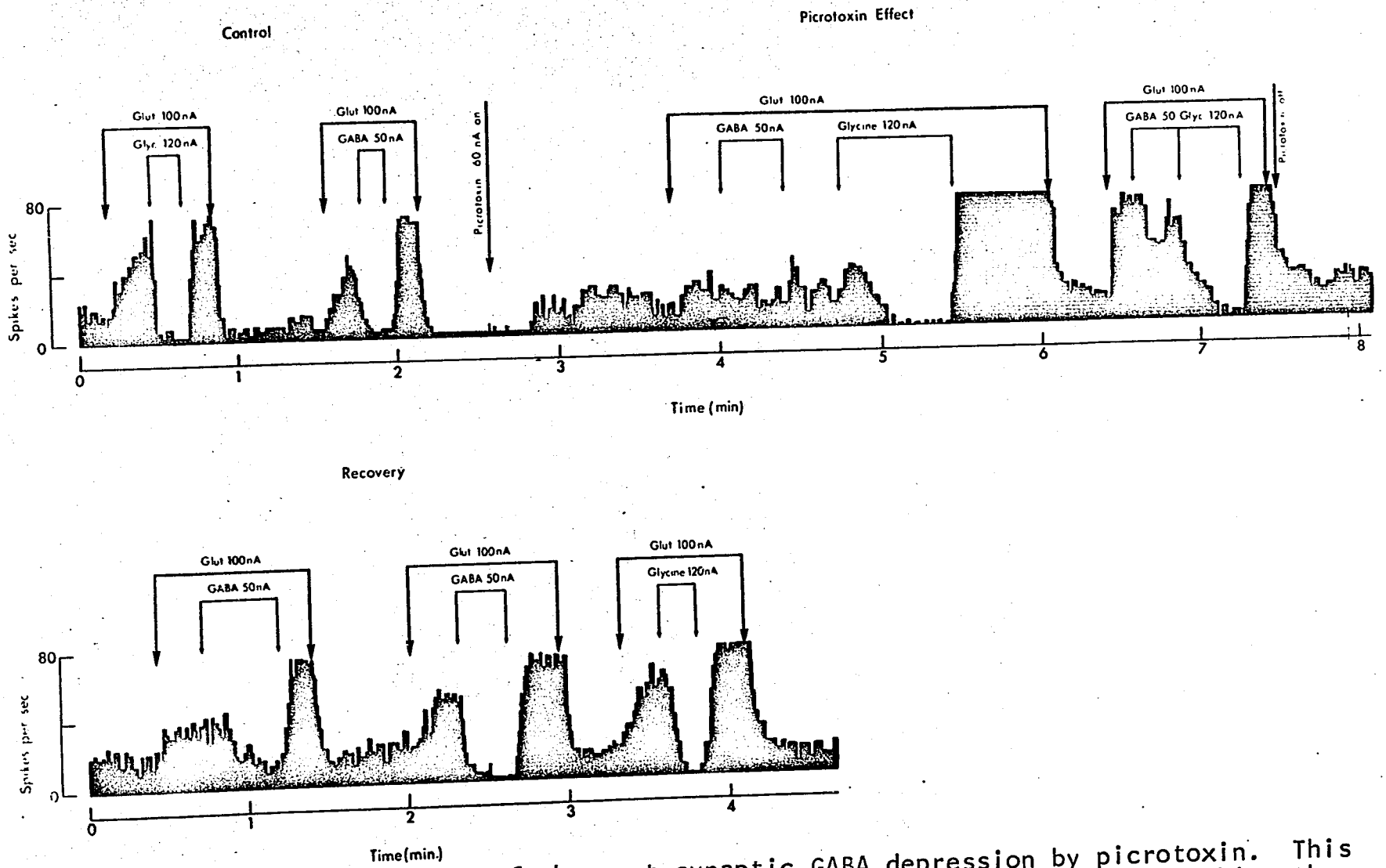

FIG. 54: Specific blockade of the post-synaptic GABA depression by picrotoxin. This latter substance interferes with the cell response to glutamate making the interpretation of these results difficult. However, some observations are consistent with the GABA-picrotoxin interaction, as demonstrated by other methods. Recording from the central barrel of a five-barrelled micropipette placed on the cuneate nucleus of a decerebrated cat. Iontophoretic release of GABA, glycine, glutamate and picrotoxin made through the lateral barrels.

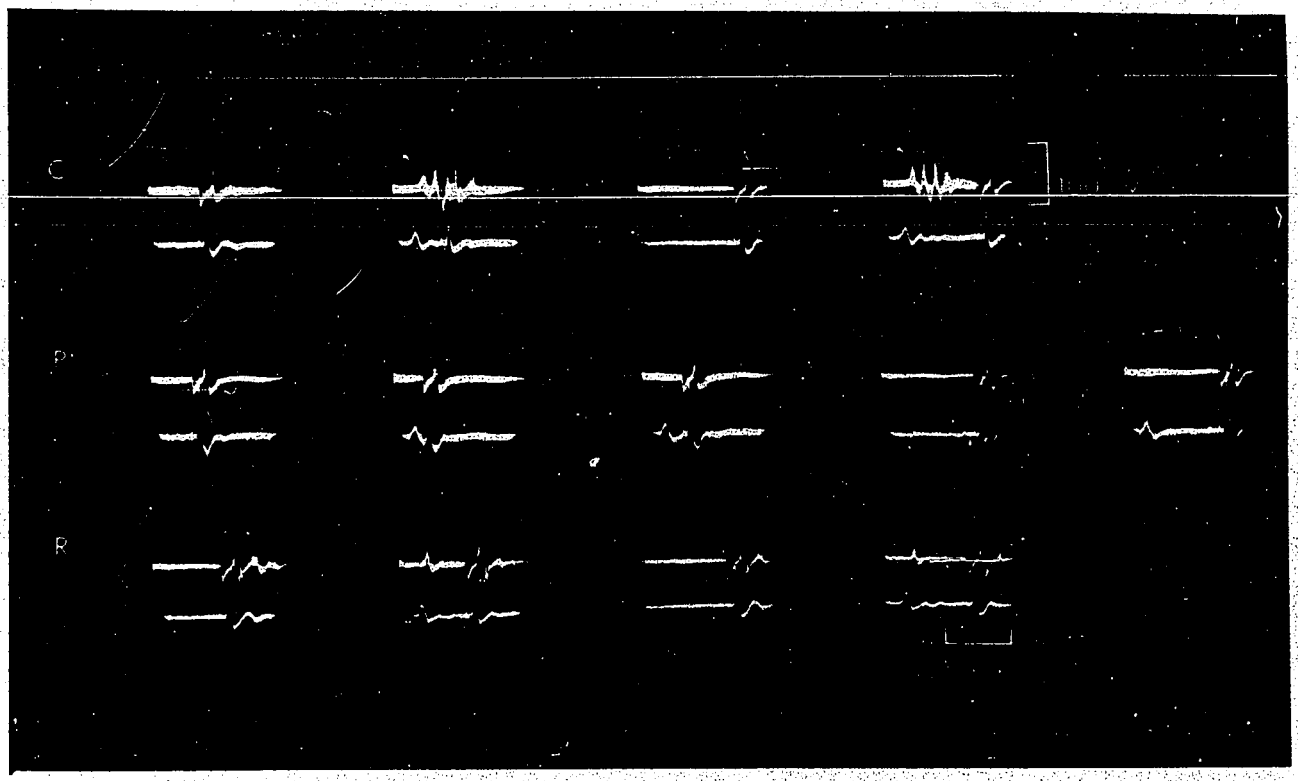

FIG. 55: Effect of intravenous picrotoxin (2 mg/Kg) (P) on the excitability of the afferent terminals, the dorsal column reflex and cuneate nucleus synaptic inhibition. Cuneate nucleus stimulation - 1/sec - 40 V - 0.02 msec. Several traces superimposed. Recording antidromically from the superficial radial nerve (upper traces) and orthodromically from the medial lemniscus (lower traces). Arrows on b and d indicate the dorsal column reflex evoked by the conditioning pulse on the median nerve - 0.2 msec, 6 V - Picrotoxin (P) increases the excitability of the terminals (P row), abolishes the dorsal column reflex and depresses the synaptic inhibition in the nucleus. Lower row, recovery after 3 hours. Observations made ina decerebrated cat.

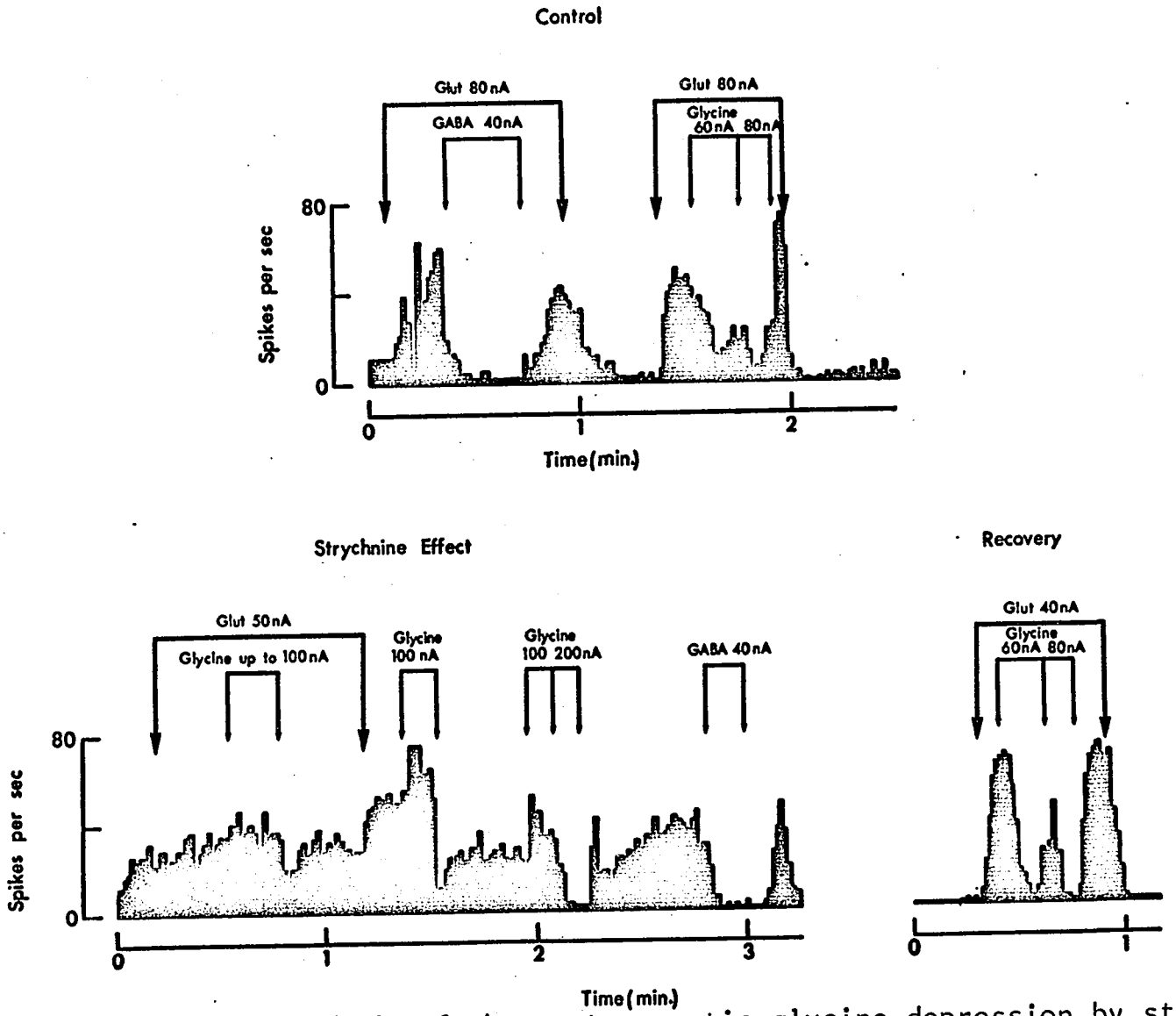

FIG. 56: Specific blockade of the post-synaptic glycine depression by strychnine. Strychnine depresses the effect of glutamate (as observed in the cerebral cortex by Phillis and York (1967)). GABA is equally effective before and during strychnine administration - it was released iontophoretically (90 nA) during the period marked as "strychnine effect". Note the excitatory effect of glycine during administration of strychnine. Five-barrelled micropipette inserted in the cuneate nucleus of a decerebrated cat.

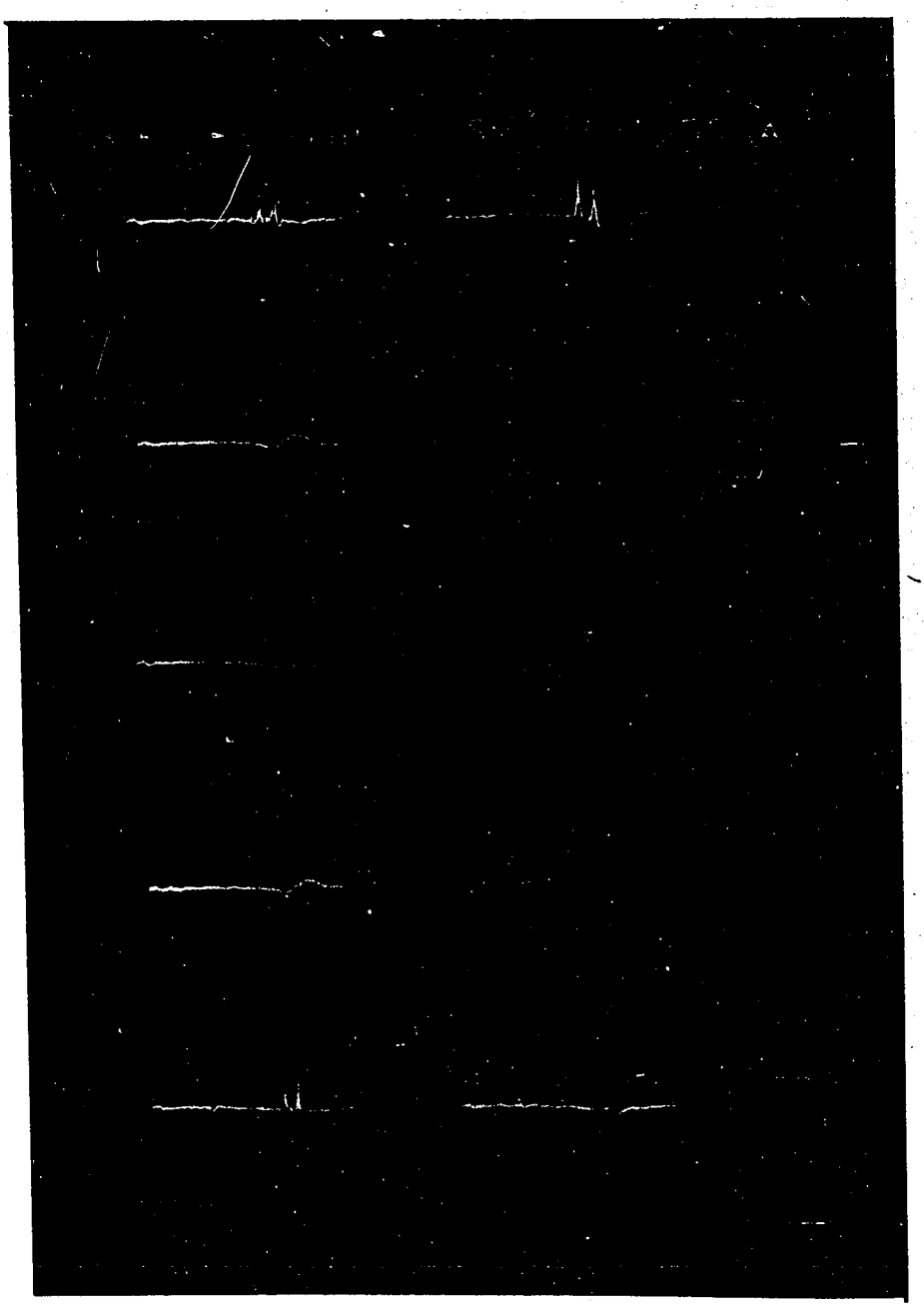

FIG. 57: Specific blockade of glycine depression by strychnine. The depression produced by GABA is not affected. The arrow indicates the stimulation on the superficial radial nerve (S.R.) - 4/sec - 0.2 msec - 3V - recording through the central barrel of a five barrelled micropipette placed in the cuneate nucleus. Strychnine, glycine and GABA were released iontophoretically from other barrels.

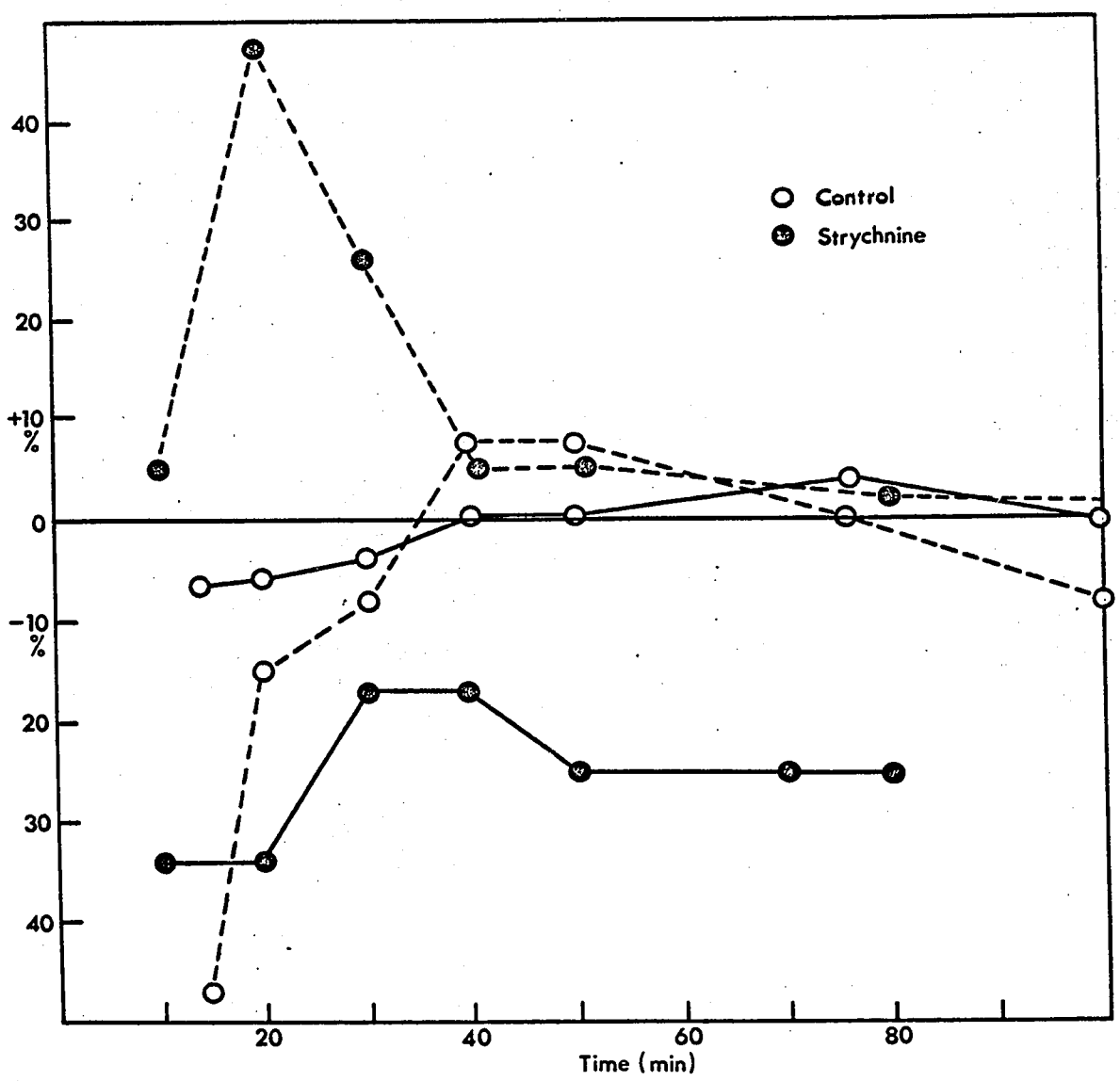

FIG. 58: Effect of intravenous strychnine (0.2 mg/Kg) on the intensity of synaptic inhibition (β spike - continuous line) and the interaction of the afferent terminals (broken lines). Test stimulus applied into the cuneate nucleus 1/sec, 0.02 msec, 50 V - recording from the superficial radial nerve and the medial lemniscus. Ordinate: percentage changes from control induced by a conditioning pulse to the median nerve - 0.2 msec, 6 V - at various preceding intervals in msec (abscissa). Strychnine increases both synaptic inhibition and the interaction of the terminals.

this study was made (Fig. 58). When released by iontophoresis this drug blocked the effect of glycine, not the effect of GABA, on the post-synaptic membrane (Figs. 56, 57).

The post-synaptic inhibition observed in cuneothalamic cells was reduced by the iontophoretic release of picrotoxin, as discussed before, but it was not affected by iontophoretic strychnine (50-150 nA for up to 5 min of administration) (Fig. 45).

In only one experiment the interaction between picrotoxin and halothane was studied. The anaesthetic (2%) reduced the blockade of inhibition induced by intravenous picrotoxin (1 mg/Kg).

It must be stressed that the results on the post-synaptic interactions between GABA and glycine on the one hand and picrotoxin and strychnine on the other, using the glutamate depression criteria, presents problems of interpretation. Strychnine depresses the effect of glutamate, an observation also made by Phillis and York in the cortex (1967) (Fig. 56). Picrotoxin makes some units less and others more excitable to this amino acid (Fig. 54). Finally, the cells response to continuous glutamate administration is seldom steady and reproducible. The tests with this amino acid should be made for short and equally spaced periods of time as seen in Fig. 24.

DISCUSSION

The role of inhibition on the anaesthetic state was suggested by Dworkin, Raginsky and Bourne (1937) and by Heinbecker and Bartley (1940). However, it was not clear what they mean by "inhibition"; certainly it was not similar to modern concepts.

Eccles *et al.* (1963) considered that small doses of Nembutal potentiate presynaptic inhibition while larger doses have a depressant effect on this function. Their data are contradictory and tend to support the latter concept. Schmidt (1963), working with frog's spinal cord *in vitro*, correlated amplitude and duration of the dorsal root potential with the intensity of presynaptic inhibition. His results indicate that light barbiturate, ether, urethane and chloral-hydrate anaesthesia, potentiate inhibition while deep anaesthesia depresses this process. These results have been partially challenged by Miyahara, Esplin and Zablocka (1966) who found only potentiation with ether as well as nitrous oxide, chloroform, chloralose, Nembutal and small doses of procaine.

Presynaptic inhibition has been linked to the dorsal root potential (Eccles et al., 1963). Nembutal prolongs the duration of the latter (Schmidt, 1963; Eccles et al., 1963); whether it potentiates or depresses presynaptic inhibition remains to be clarified. Several authors

have presented indirect evidence of a reduced inhibition acting on unit activity during its administration (Valdman, 1967; Tishehenko and Shapovalov, 1967). Similar observations have been made in the visual cortex (Brazier, 1958; Kimura, 1962; Perlman, 1963; Kondrat'eva, 1967). The results presented in this thesis support these observations. However, they do not support the causal relationship between the DRP and presynaptic inhibition. Moreover in some of the studies on presynaptic inhibition one could criticize this relation, as well as the definition and nature of presynaptic inhibition itself.

One of two alternatives exist: a) presynaptic inhibition and the dorsal root potential (DRP) are caused by the same phenomenon - depolarization of afferent fibres - or b) these are two coincident phenomena not directly related.

a) If it is accepted that the same effect producing DRP causes presynaptic inhibition it is likely that presynaptic inhibition is mediated by depolarization of the afferent terminals as originally proposed by Renshaw (1946). In that case:

1) It is the result of direct interaction of afferent fibres as proposed by Barron and Matthews (1938), Lloyd and McIntyre (1948), Renshaw (1946) and supported by the observations of Marazzi and Lorente de Nó (1944)

and Katz and Schmitt (1940) or

2) It is produced by the discharge of interneurones as suggested by Eccles, Schmidt and Willis (1963) and Wall (1964).

If it is mediated by a direct interaction of terminals mephenesin should be ineffective on them. But it is known that this drug selectively depresses interneurones as well as presynaptic inhibition (Llinas and Terzuolo, 1965). If it is mediated by interneurones, Nembutal and mephenesin should depress both the DRP and presynaptic inhibition. But, as discussed above, the DRP is increased by Nembutal, suggesting that afferent fibre depolarization does not depend on interneurones. The same authors defending the role of interneurones on presynaptic inhibition consider that this barbiturate potentiates this type of inhibition - an effect inconsistent with their hypothesis. They explain this paradoxical result by postulating the depression, by the barbiturate, of the enzyme in charge of transmitter removal; an unlikely hypothesis in the presence of an undepressed parent cell (Eccles et al., 1963). Alternatively, Wall (1964, 1967) makes a special and equally unlikely concession by which some small inhibitory interneurones are not affected by anaesthesia.

b) In view of the experimental results discussed above

and what is known on the pharmacology of the DRP and presynaptic inhibition it is more reasonable to propose that these are two coincidental phenomena not directly related. It can be suggested that: 1) the DRP is caused by a direct interaction of afferent terminals and 2) synaptic inhibition is mediated by stimulation of interneurones. To accept the latter it must be postulated that their transmitter acts by depressing rather than exciting these terminals, as suggested by these experiments. Under this hypothesis the DRP and synaptic inhibition could change in opposite directions, as seen previously, without calling for esoteric explanations.

The possibility of a difference between the pharmacological properties of the spinal neurones and those of the dorsal column nuclei cannot be discarded. Inhibitory spinal interneurones are known to receive both inhibitory and excitatory stimuli (Hongo, Jankowska and Lundberg, 1960; Preston and Whitlock, 1960) an effect not yet seen in the cuneate (see page 78). However, there is indirect evidence of a similar pharmacology in both structures (Boyd, Meritt and Gardner, 1966; Banna and Jabbur, 1968).

F - DORSAL COLUMN REFLEX AND INTERACTION OF AFFERENT TERMINALS

The dorsal column reflex corresponds to what is known as the dorsal root reflex in the spinal cord. The arrival of a nervous impulse through a given fibre induces a change in the adjacent fibres. This change is manifested by a depolarizing potential detected in the same or neighbouring structures. Its propagation is electrotonic, having a sharp decay from the site of origin. This phenomenon was discussed in the preceding section, it is known as the dorsal root potential (Barron and Matthews, 1938; Lloyd and McIntyre, 1948).

A recording electrode located on a peripheral nerve detects antidromic action potentials induced by the stimulation of an adjacent nerve or the same nerve (Toennies, 1938). These spikes constitute the dorsal root or dorsal column reflex, they are part of the dorsal root potential (Tregear, 1958).

Interaction between afferent fibres can be detected by changes in excitability of the terminals of a given nerve as affected by a conditioning pulse to a second nerve. In the present study, the cuneate nucleus was stimulated directly to detect changes in excitability of the terminals of the superficial radial nerve (sensory), produced

by a conditioning pulse in the median nerve (mixed nerve), applied at various preceding intervals. The results are plotted as percentage changes from control, as a function of time, in msecs after a median nerve conditioning pulse.

The systemic administration of Nembutal (10-30 mg/Kg) or halothane (2% for more than 7 min) reduced the dorsal column reflex (Fig. 41); however, the terminals became less excitable with Nembutal and more excitable with halothane. Intravenous picrotoxin (1-2 mg/Kg) decreased the dorsal column reflex but increased the excitability of the endings (Fig. 55). On the other hand, strychnine (0.1-0.2 mg/Kg) had no effect on the excitability of the terminals but greatly increased the dorsal column reflex. A conditioning pulse to the median nerve induced a reflex response in the superficial radial nerve, it increased the excitability of the terminals on the latter nerve while suppressing its reflex response.

Hypoxia increased the dorsal column reflex; the interaction of the terminals was manifested by either an increase or a decrease in the size of the antidromic spike (Fig. 42). The D.C.R. was maximal when severe hypoxia had interrupted synaptic transmission. Further hypoxia abolished all activity originating at the terminals (Fig. 42).

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DISCUSSION

The dorsal root (or column) reflex originates in or near the afferent terminals (Toennies, 1938). Its disappearance coincides with that of the antidromic action potential initiated at these structures. It is attributed either to direct fibre interaction (Barron and Matthews, 1938) or to the activity of internuncial neurones (Toennies, 1938; Eccles et al., 1964). The latter hypothesis is unlikely according to the preceding discussion (page 86).

It can be postulated that changes in the reflex excitability of afferent fibres depend upon a) excitability of the afferent fibres themselves, b) the amount of potassium released per nerve impulse (Orkand, Nicholls and Kuffler, 1966; Rang and Ritchie, 1968), c) the permeability of the terminals to this ion and d) the effect of inhibitory input acting through axo-axonic synapses (Walberg, 1965).

A conditioning pulse to one peripheral nerve (the median) produces an increase in the excitability of the afferent terminals of another closely related nerve (the superficial radial). This effect is measured by the increase in the size of the submaximal action potential induced antidromically in the peripheral nerve by direct electrical stimulation of its intraspinal terminal branches. On the other hand, this conditioning pulse depresses or

abolishes the reflex discharge appearing after a preceding direct stimulus.

Apparently, the increase in excitability to direct stimulation conflicts with the reflex depression. However, it can be argued that the former effect reflects the increase in extracellular potassium induced by the conditioning pulse, while the depression of the dorsal root reflex is produced by an increased inhibitory input known to occur following the conditioning stimulus (see page 81).

The pharmacology of the dorsal root (or column reflex) supports these assumptions. Halothane tends to increase the excitability of the terminals while suppressing the reflex. The effect on the terminals is in line with its lack of post-synaptic depression; the reflex depression could be explained by an increased inhibitory input.

Nembutal depresses in the same proportion both the direct excitability of the terminals and the dorsal root reflex. This effect is consistent with a non-specific depression of excitation attributed to this anaesthetic (Krnjević and Phillis, 1963; McCance and Phillis, 1964; Bloom, Costa and Salmoiraghi, 1965) and supported by these experiments. There is less release of potassium by the terminals, as well as less reflex response. However, the effect of this ion is prolonged as confirmed by the

longer duration of both the dorsal root potential (Schmidt, 1963) and the interaction of the terminals.

Hypoxia increases the excitability of the terminals to direct stimulation; it also increases their reflex discharge. These two effects are easily explained by the depolarizing action and the synaptic depression (of inhibitory input) observed in these experiments.

The effect of picrotoxin suggests that this compound acts directly on these endings increasing their excitability to direct stimulation. On the other hand, it may reduce either their permeability to potassium, or the amount of this ion released per nerve impulse, thus explaining the depression of the reflex (Fig. 55). Further support for these latter effects is derived from the reduced interaction of afferent terminals observed following its intravenous administration. An increased inhibitory input, suppressing the dorsal root reflex, is ruled out owing to the pharmacology of this drug.

Strychnine does not affect the excitability of the terminals to direct stimulation but increases their reflex discharge as well as their interaction. These results may be explained by a) direct effect on the terminals, increasing their permeability to potassium, b) depression of inhibition, or c) greater release of potassium per nerve impulse. More experiments are needed to

test these hypotheses.

Finally, the intravenous administration of a local anaesthetic agent (lidocaine (xylocaine) 5 mg/Kg) selectively depressed the reflex discharge with little effect to the direct stimulation of the terminals (Galindo, unpublished observations). This action suggests a greater sensitivity of the reflex discharge to changes in excitability since it is reasonable to expect from this anaesthetic a non-specific depression of these structures to both direct and reflex stimulation.

Further discussion on the physiology and pharmacology of the dorsal root reflex must take into consideration the various factors that may affect the excitable process at these fine non-myelinated endings, and the excitability of the synaptic boutons. The suggestions presented above, with respect to potassium permeability changes and direct interaction of these structures, is a simplification of a complex process.

GENERAL DISCUSSION

STABILIZATION

SPECIFIC INTERACTION

INHIBITION

DEPOLARIZATION

The development of the experimental preparation used in this research work permitted the study of pre- and post-synaptic elements in the first relay nucleus of an afferent pathway. This technique made also possible the identification of two functionally different cuneate neurones.

Although the cuneate nucleus synapse is not in a pathway carrying painful stimuli, it is reasonable to postulate that the effects observed in these experiments apply also to other synapses in the central nervous system involved in pain perception and where the various elements, terminals, interneurones and post-synaptic structures are likely to have similar membrane characterisitics to those of cuneate elements.

The concept of facilitation of inhibition, as a possible mechanism of anaesthesia was tested by observing the physiology and pharmacology of this phenomenon. Synaptic transmission in the cuneate appears to have pre- and post-synaptic inhibitory inputs similar to those observed in the spinal cord monosynaptic reflex (Andersen, Eccles and Schmidt, 1962; Andersen, Eccles, Oshima and Schmidt, 1964).

Post-synaptic inhibition is characterized by the presence of inhibitory post-synaptic potentials (Eccles, 1964). Presynaptic inhibition is more difficult to assess.

Frank (1959) postulated the existence of an inhibition acting at a distance from an intracellular electrode and probably located on remote dendrites. The work of Granit, Kellerth and co-workers (Granit, Kellerth and Williams, 1964; Kellerth and Szomiski, 1966) suggested the possibility of a post-synaptic inhibition occurring at the dendrites but too remote to be manifested by hyperpolarizing post-synaptic potentials. Rall (1967) (Rall, Burke, Smith, Nelson and Frank, 1967) has presented a mathematical model that explains why this type of inhibition might not be observed by intracellular electrodes. More recently, the studies of Diamond (1968) on Mauthner neurones, have demonstrated the existence of GABA sensitive dendritic sites capable of producing an inhibition, remote enough to be confused with a pre-synaptic phenomenon.

For Granit and co-workers (1964) it is difficult to identify post-synaptic inhibition based on conductance changes and inhibitory post-synaptic potentials, in neurones under the influence of both inhibitory and excitatory inputs. They studied, in addition, the reductions in the firing rate of a motoneurone stimulated by trans-membrane currents, the presence of synaptic noise and the hyperpolarization of membrane potential.

Of these criteria the reduction in firing rate was by itself the most reliable. An inhibition based only

on this parameter was considered as "remote". Evaluation of inhibition by the size of the EPSP in combination with shifts in the membrane potential could lead to false conclusions.

Eccles and Krnjević (1959) recording intracellularly from afferent nerve fibres confirmed previous observations (Barron and Matthews, 1938) of primary afferent fibre depolarization, which were later related to presynaptic inhibition (Eccles, Kostyuk andSchmidt, 1962; Eccles, Eccles and Magni, 1961) as originally suggested by Renshaw (1946) - this correlation is based on the reduction of transmitter released from partially depolarized terminals (Hubbard and Willis, 1962). - The original experiments were made in the spinal cord; recently they have been confirmed in the cuneate nucleus and in other afferent relay nuclei (Stewart, Scibetta and King, 1967; Rudomin, 1967; Shende and King, 1967; Cesa-Bianchi, Maneia and Sotgiu, 1968). Wall (1964; 1967) explains this depolarization through reverberating circuits of interneurones. Eccles believes that these cells liberate a depolarizing neuro-transmitter that stays at the terminals for long periods of time; GABA has been suggested as this transmitter (Eccles, Schmidt and Willis, 1963).

The mechanisms of inhibition have been investigated in the cuneate nucleus, where GABA and glycine could

be inhibitory transmitters (Galindo, Krnjević and Schwartz, 1967) the interaction getween GABA, glycine picrotoxin and strychnine was studied; these last two substances have been considered specific antogonists of pre- and post-synaptic inhibition respectively (Eccles, et al., 1963).

The results from these experiments indicate that:

1) Both GABA and glycine strongly depress a) the excitability of afferent terminals, b) synaptic transmission and c) post-synaptic responses of cuneate cells to the iontophoretic release of glutamate. (Glutamate has no detectable effect on the terminals). The actions of GABA and glycine are reduced or completely blocked by picrotoxin and strychnine, respectively. This latter interaction (strychnine-glycine) has also been observed in the spinal cord (Curtis, Hösli, Johnston and Johnston, 1967, 1968; Werman, Davidoff and Aprison, 1968). While GABA-picrotoxin interaction has been demonstrated in the crayfish neuromuscular junction (Robbins and Van der Kloot, 1958). These effects appear to be specific since picrotoxin does not suppress glycine-induced inhibition nor does strychnine modify the action of GABA.

2) Synaptic inhibition and the depolarization of afferent terminals (as shown by an increased excitability) have a

similar time course under barbiturate anaesthesia, or following the administration of picrotoxin. However, the time courses are different in the decerebrated, unanaesthetized animal. Nembutal and picrotoxin depress synaptic inhibition. Nembutal reduces the excitability of afferent terminals but prolongs their interaction. Picrotoxin, on the other hand, makes the endings more excitable while diminishing their interaction. This action is identical to that of halothane, but unlike picrotoxin, this anaesthetitic increases synaptic inhibition.

3) Strychnine does not depress synaptic inhibition; on the contrary, it tends to increase its amplitude and duration.

4) Dorsal column reflex discharges and excitatory interaction between afferent terminals can be seen during severe hypoxia when synaptic transmission is no longer observed or is greatly reduced.

The hypothesis that afferent fibre depolarization can explain presynaptic inhibition is based on the similar time course of both events and on the pharmacological blockade of presynaptic inhibition by picrotoxin (Eccles et al., 1963). But, the time courses do not follow the expected changes after Nembutal, halothane or picrotoxin administration.

On the other hand the specific picrotoxin-GABA

interaction is consistent with the existence of an inhibitory transmitter similar to GABA. This amino acid appears to mediate pre- and post-synaptic inhibition in crustacea (Kuffler and Edwards, 1958; Dudel and Kuffler, 1961; Takeuchi and Takeuchi, 1966a, b), and post-synaptic inhibition in the mammalian cerebral cortex (Krnjević and Schwartz, 1967) and the medulla oblongata (Obata, Ito, Ochi and Sato, 1967). GABA has been suggested as the transmitter mediating presynaptic inhibition through a depolarizing effect (Schmidt, 1963). However, Curtis and Ryall (1966) demonstrated that GABA depresses the excitability of the afferent terminals in the spinal cord just as it does in the cuneate.

The present experiments demonstrate that presynaptic inhibition is possibly mediated by a transmitter that, like GABA, blocks the invasion of terminals by afferent impulses (Dudel and Kuffler, 1961; Takeuchi and Takeuchi, 1966a, b). Fig. 59 illustrates a late depression in the size of the antidromic spike induced by a conditioning pulse, a similar depression is obtained by the iontophoretic release of GABA. The increase in excitability of the terminals can be explained by a direct interaction of afferent fibres (Barron and Matthews, 1938; Renshaw, 1946; Lloyd and McIntyre, 1948) produced by an accumulation of extracellular K released by nerve impulses (Orkand,

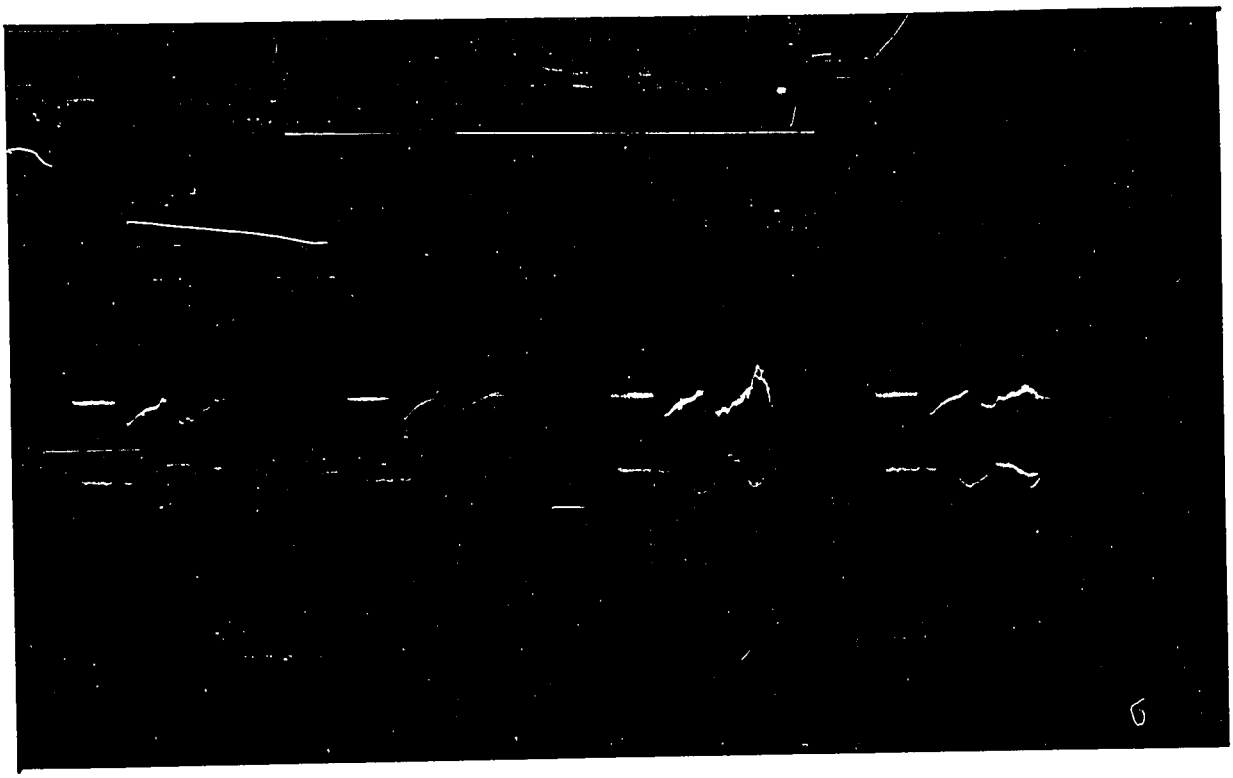

FIG. 59: Effect of a conditioning stimulus (S) and iontophoretic GABA (G) (200 nA) on the antidromic action potential (upper traces) recorded from the superficial radial nerve, and the orthodromic action potential (lower traces) recorded from the medial lemniscus. Several traces superimposed. Cuneate nucleus stimulation 1/sec - 50 V - 0.02 msec. Median nerve conditioning stimulus 135 msec delay, 6 V, 0.1 msec. Note the effect of both in abolishing the dorsal column reflex - second spike. Controls records (C). At this conditioning interval the afferent terminals are little affected by the conditioning pulse, in most unanaesthetized animals they are depreseed. Synaptic transmission is consistently reduced. GABA can mimic this effect.

Nicholls and Kuffler, 1966; Rang and Ritchie, 1968). The activity of interneurones may not be needed to explain the dorsal column reflex nor afferent fibres interaction (Carlson, 1964), moreover, progressive depression of synaptic transmission by hypoxia potentiates these two phenomena; this observation is hardly consistent with an interneurone-mediated depolarization.

The inhibitory effect of a conditioning peripheral stimulus on the lemniscal or cuneate response to stimulation of a second nerve, could be mediated by two types of transmitters: one like glycine and readily blocked by strychnine (Curtis, Hösli, Johnston and Johnston, 1967, 1968) and the other similar to GABA and readily blocked by picrotoxin. The absence of any clear action of strychnine suggests only a minor role for the glycine-type substances; on the other hand, the marked effect of picrotoxin indicates that the GABA-like compounds are more likely to be concerned in this process.

GABA could act pre- and post-synaptically. Post-synaptically on the cell soma, or on the dendrites producing a "remote" inhibition. Presynaptically, as suggested by Walberg's electromicrographs (1965) GABA could block afferent impulses at the terminals through a mechanism analogous to presynaptic inhibition at the myoneural junction of the crayfish (Dudel and Kuffler, 1961, Takeuchi and

Takeuchi, 1966b).

The observations of Walberg (1965), with the electron microscope, according to which synaptic boutons of interneurones axons make simultaneous synapses with an afferent terminal and a dendrite from a cuneothalamic cell (Fig. 47) does not support the hypothesis of presynaptic depolarization mediated by interneurones and responsible for inhibition. A similar observation has been made in the spinal cord (Ralston, 1968). However, it can be postulated that either the same transmitter has a different effect at both places or two different transmitters are released simultaneously. Both alternative are remotely possible; no example of such behaviour has been described in the C.N.S. of mammals.* Moreover, electron microscopic studies indicate the presence of only one type of synaptic vesicles on these synapses and they happen to be the ones postulated to be inhibitory (Bodian, 1966; Uchizono, 1966; Ralston, 1968).

The depression of interneurones and synaptic inhibition by Nembutal, is a consistent finding that gives support to the hypothesis that presynaptic inhibition

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*However, a double effect by the same transmitter was recently described in Aplysia (Wachtel and Kandel, 1967), the difference appeared to be related to the frequency of stimulation.

is mediated by interneurones. Further support for this hypothesis is derived from the facilitation of inhibition, and the excitation of interneurones, observed during the administration of halothane.

The results of this work demonstrate that although synaptic transmission (along the dorsal columns-medial lemnicus pathway) is depressed by the three anaesthetics investigated, their site of action and their possible mechanisms are different. Procaine appears as a non-specific depressant of pre- and post-synaptic elements. Halothane on the other hand facilitates synaptic inhibition having no depressant effect upon pre- or post-synaptic structures. Nembutal preferentially depresses the post-synaptic membrane. These sites of action are likely to be related to differences in effect of these agents upon excitable membranes and possibly to their known clinical manifestations.

A reasonable approach to the understanding of the mechanism of anaesthesia is then to study the excitable process and the various manners in which it can be affected.

The mechanism that generates the action potential momentarily increases sodium conductance; this process is different from the one that generates the excitatory post-synaptic potentials (EPSP), although Na permeability changes are involved in both. Excitatory post-synaptic potentials are chemically mediated; this implies the

existence of specific receptor sites (Cavallito, 1967) in the post-synaptic membrane that can be activated by compounds having a particular molecular structure (transmitters), similarly they can be inactivated or "occupied" by compounds sharing a given configuration. This type of blockade is known as a "specific interaction"

It is likely that anaesthetics have a preferential effect on excitable membrane possibly mediated by a physicochemical interaction that modifies its ionic permeability.

STABILIZATION: The concept of stabilization refers to the action of those drugs and special circumstances which decrease membrane permeability to Na and K ions, with no significant changes in resting membrane potential.

Blaustein and Goldman (1966b) visualize the mechanism of action of procaine as an ionic interaction on the surface of the membrane by which the anaesthetic displaces Ca^{++} (see Koketsu and Nishi, 1968). This mode of action satisfies the competitive relationship between the ion and the drug while both of them stabilize the membrane. Recent observations on mitochondrial membranes support this idea (Chance, Mela and Harris, 1968).

Stabilization is a non-specific phenomenon which affects all excitable membrane and synaptic sites (both excitatory and inhibitory). An anaesthetic operating

through this mechanism when given systemically depresses excitation, the excitation that initiates inhibition and inhibition per se. The result of such an action in a given neurological function could indicate its balance between excitation and inhibition.

SPECIFIC INTERACTION: It refers to those compounds that block the generation of post-synaptic potentials but which do not interfere with the generation of the action potential. Curare, atropine and nicotine are examples of this kind of interaction. They block the effect of ACh at various functionally different receptor sites. Nembutal can be considered as having some specific interaction (depression) with cholinergic (Krnjević, 1967) and amino acidic (glutamate) receptors mediating post-synaptic excitation, while having no effect on inhibitory receptor sites. (It may facilitate the effect of GABA). This agent has also stabilizing properties. For Blaustein and Goldman (1966b) Nembutal interacts with membrane Ca^{++} stabilizing the membrane; for Sato and co-workers (1967) it will increase K conductance. These two hypotheses are not exclusive of each other.

INHIBITION: This concept refers to those compounds that selectively increase membrane permeability (conductance) to K or Cl, driving the membrane potential to a new equilibrium above or below the resting level

(Eccles, 1964). Changes in membrane charge (ζ potential) produced by some physico-chemical interaction of the anaesthetic molecules with the membrane could facilitate this particular ionic movement (Bangham, Standish and Miller, 1965). Halothane anaesthesia could operate through this mechanism.

DEPOLARIZATION: It is the opposite of stabilization, drugs producing this effect are also called labilizers (Shanes, 1958). They act by increasing the conductance to all ions. Veratrine alkaloids are representative of this group. Its effect is blocked by stabilizers but not by inhibitory substances.

Lorente de Nó (1947) demonstrated that ether can block nerve transmission through depolarization; an observation that led some investigators to consider depolarization as the mechanism responsible for anaesthesia with a large group of inhalation agents (Van Harreveld, 1947; Carpenter, 1954; Wyke, 1960; Bennet and Hayward, 1967).

Anaesthesia can be produced by one or a combination of the above mechanisms. Drugs that produce unconsciousness and analgesia can be classified by their dominant mode of action. Whether they have clinical applications or not, depends on other factors not considered in this work.

"The lack of structural specificity in anaesthetics

the reversible nature of the anaesthetic process, and such correlations as there are with physical properties have been taken as indications that anaesthesia is produced by a "physical" rather than a chemical mechanism. All evidence would indicate that anaesthesia does not depend upon participation of the drug itself in chemical reaction at least in the generally accepted sense of the word" - (Butler, 1950).

Moreover, we must be careful in not drawing conclusions from the correlation of some physico-chemical properties of anaesthetics and their biological effect (such as their teratogenic action (Smith, Gaub and Moya, 1965; Andersen, 1968) since they may not be involved in its mechanism of action; not in concluding that the effects of a given anaesthetic on the various systems are due to the same physico-chemical properties.

Finally, since anaesthetics are capable of interfering with ionic movement at the membrane level, a basic function of membranes in general, it is not surprising that they can alter a variety of cell functions in other systems, functions not directly related to the anaesthetic state, but dependent on trans-membrane ionic exchange such as the effects on Na movements across frog's skin (Gottlieb and Savran, 1967) or the interference with glucose transport in red cells (Greene and Cervenzio, 1967).

Anaesthetics, then, depress synaptic transmission by a physico-chemical phenomenon that interacts with excitable membranes altering their functional ionic permeability. This effect can be also obtained with a variety of compounds lacking any clinical application.

SUMMARY

The work analysed in this thesis was designed to study the effect of three different anaesthetic agents on synaptic transmission through an afferent relay station - the cuneate nucleus.

The experiments were performed on 91 cats; in 59 cases in the absence of local or general anaesthetics, decerebrated by the removal of the supratentorial portion of the brain.

Five-barrelled micropipettes were inserted into the cuneate nucleus. The central barrel, filled with 2.7 M sodium chloride was used for recording or stimulation of the various synaptic elements. The other four barrels were used for the administration of various substances, including anaesthetic agents.

The afferent terminals of the dorsal columns fibres were excited by a microelectrode inserted into the cuneate nucleus, the antidromic nerve spike being recorded at the periphery from the superficial radial nerve.

Cuneate neurones were excited directly by the iontophoretic release of an excitatory amino acid, glutamate; their discharge was recorded through the central barrel of the multibarrelled micropipette.

Several tests were used to study synaptic transmission through the nucleus such as the response of cuneate

neurones and the medial lemniscus compound action potential to electrical stimulation of a) peripheral nerves, b) dorsal columns, or c) the cuneate nucleus.

Synaptic inhibition in the nucleus was studied by observing a) changes in the post-stimulus time histogram of cuneate neurones being stimulated directly by glutamate, b) the inhibitory effect of a conditioning pulse on the amplitudes of the medial lemniscal action potential, evoked by direct electrical stimulation of the cuneate nucleus.

The effects of GABA and glycine on the post-synaptic membrane and on the afferent terminals were studied. The interaction between these amino acids, and picrotoxin or strychnine was also studied.

The time course of changes in the excitability of afferent terminals was determined simultaneously with that of synaptic inhibition. The dorsal column reflex changes were correlated with the excitability of the terminals.

Finally, a comparison was made between the pharmacological properties of two functionally distinct groups of cuneate neurones; the cuneothalamic relay cells and other cells ("interneurones").

The results of these experiments indicate that:

1. Procaine depresses afferent terminals, the antidromic invasion of relay cells, and the post-synaptic

membrane of all cells.

2. Nembutal preferentially depresses the post-synaptic membrane of all cells; it depresses the duration and intensity of synaptic inhibition. It also reduces the excitability of the terminals and the dorsal root reflex.

3. Halothane depresses synaptic transmission in the dorsal column-medial lemniscal tract. It does not depress the trans-synaptic excitation of "interneurones". Halothane has little effect on the excitability of the afferent terminals and potentiates synaptic inhibition as well as the actions of GABA and glycine. It has no effect on the post-synaptic membrane.

These results are consistent with the idea of multiple mechanisms of action responsible for the synaptic disruption observed during the administration of anaesthetics. These mechanisms could be in turn explained through functional changes in membrane conductance to various ions.

In brief, procaine acts as a non-specific "stabilizer" of all excitable membrane, Nembutal as a depressor of post-synaptic excitation, and halothane as a potentiator of the inhibitory process.

Further experiments on synaptic inhibition and the function of interneurones support these conclusions.

They also suggest the possibility that GABA may act as neurotransmitter in the cuneate nucleus.

Present ideas on presynaptic inhibition were discussed according to the findings of this work. These observations do not agree with the widely accepted hypothesis that afferent terminal depolarization can explain this phenomenon; rather they suggest a mechanism of presynaptic inhibition similar to that described in the crayfish neuromuscular junction. This assumption is supported by the similarity of action betwen GABA and the hypothetic inhibitory transmitter of the cuneate nucleus. It is further suggested by the specific antagonism between the action of picrotoxin and GABA on the various structures of the nucleus as well as the depression of synaptic inhibition induced by picrotoxin.

CLAIMS TO ORIGINALITY

The work presented in this thesis is original in its totality; it represents the first comprehensive study on the effect of anaesthetics on synaptic transmission in the C.N.S.

The following points summarize my claims to originality:

1. The development of a surgical preparation which combines two techniques of decerebration. This new preparation, because of minimal blood loss, made possible to maintain the cats in good systemic conditions throughout the experiments, it also facilitated the reliable identification of the medial lemniscus and the pyramidal tract.

2. The application of the surface "perfusion" for the topical administration of inhalation volatile anaesthetics to neurones located in the cuneate nucleus.

3. The suggestion that a mechanism of anaesthesia may be through the potentiation of inhibition.

4. The first report on the direct effect of anaesthetics on the afferent terminals of the dorsal column fibres.

5. The simultaneous observation of the effects of Nembutal or halothane on the excitability of afferent

terminals and on synaptic inhibition.

6. A systematic study of the pharmacological and physiological properties of functionally different types of cuneate neurones.

7. A description of the effects of picrotoxin, strychnine, GABA, glycine and hypoxia on the excitability of the dorsal column afferent terminals and on the antidromic dorsal column reflex discharge.

8. The use of hypoxia as a tool in the study of presynaptic inhibition.

APPENDIX

After the completion of this thesis, the difficulties in interpreting the results observed during stimulation of afferent terminals (Wall, 1958) became apparent. With this technique one could stimulate both the non-myelinated portion of these endings, not functionally involved in synaptic transmission, and the terminal boutons, directly involved in this process. It is reasonable to suspect that neurotransmitters may not affect the non-myelinated fibre, thus having "no effect" on "the terminals", if the stimulating electrode is not accurately located. Only intracellular recordings, from the boutons, would selectively detect actions upon these endings.

Furthermore, the iontophoretic release of various substances using Curtis and Ryall's (1966) technique introduces electrical artifacts. Measurements of the stimulating current - delivered through the central barrel of the five-barrelled micropipette - showed a reduction in the strength of the latest part of the pulse when $GABA^+$ or $glycine^+$ were simultaneously released by iontophoresis. This reduction was not observed during the release of sodium ions, normally used as a "control". These artifacts were seen with pulses of more than 0.1 msec. They could not be demonstrated with stimulating pulses of 0.02 msec.

The capacitive artifact made impossible measurements of current in the short pulses, used throughout this thesis for investigations on the terminals.

Nine more experiments were performed in order to study further the effects of GABA, glycine, glutamate and K^+ (KCl) on the afferent terminals. In four of them GABA was administered iontophoretically, as before, but careful controls were made of the stimulating current in long pulses (0.1 msec). In the other experiments the cuneate nucleus, on the one hand, and the dorsal columns, on the other hand, were stimulated using two glass micropipettes filled with 2.7 M NaCl. Antidromic recording was made from the superficial radial nerve. Orthodromic recording was made from the medial lemniscus. GABA, glycine, glutamate and KCl (0.1 M) were given by continuous superfusion of the dorsal surface of the medulla (including the region of the spinal cord and the dorsal columns).

The latest part of the stimulating pulse was reduced in amplitude by the release of GABA through one of the lateral barrels of the five-barrelled pipette. However, the reduction in the amplitude of the action potentials appeared greater than could be accounted for by the change in the stimulating pulse.

GABA when given by continuous superfusion of the cuneate nucleus (0.1 M in Ringer locke), depressed both

synaptic transmission and the excitability of the terminals; it increased the dorsal column reflex. This amino acid had no significant effect on the excitability of the dorsal column fibres. Intravenous picrotoxin (2 mg/Kg) blocked the effects of GABA. Potassium chloride depolarized both the afferent terminals and the dorsal column fibres. The observations with glycine and glutamate are not conclusive enough, further experiments are required with these amino acids.

These results give further support to previous discussions; they indicate the need for caution in the interpretation of changes in excitability of "the terminals", as determined by the electrical stimulation of the cuneate nucleus. The greater dorsal column reflex, observed during the superfusion with GABA, could be explained by increased permeability to potassium at the terminals.

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