THE CLINICAL NEUROPHYSIOLOGY OF ORGANOPHOSPHATE POISONING

by

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Preface

The studies reported in this thesis arose out of specific research requirements of the Defence Medical Services. There has been increasing interest in providing protection to persons exposed to the risk of organophosphate toxicity by pretreatment with the carbamate pyridostigmine. Although this drug has been in clinical use for many years as a treatment for myasthenia gravis its actions in low doseage on normal subjects were relatively unknown. The drug has also been used in clinical anaesthesia to reverse the actions of non - depolarizing relaxants. The possible effects of its action in this mode if given before the relaxant were equally obscure. Experiments are therefore reported which investigate some of the neurophysiological actions in man of the organophosphate sarin and their modification by pyridostigmine. In addition the consequences of pyridostigmine pretreatment for muscle relaxation in anaesthesia are examined. Because the topic is by nature multidisciplinary, I have tried to review developments in anticholinesterase chemistry together with some of the theory and monitoring of neuromuscular transmission. In addition, the technique of single fibre electromyography, which may be unfamiliar, is described in some detail.

I would like to acknowledge the many people who have provided help and advice for this project. In particular I thank Dr. E.M. Sedgwick for introducing an anaesthetist to clinical neurophysiology and for giving more help and encouragement than could reasonably be expected of a clinical supervisor over three years. I would also like to acknowledge the help provided by many members of the staff of the Chemical Defence Establishment at Porton Down. Among these Mr. R. White and Dr. R.I. Gleadle of the Clinical Studies Unit deserve special mention for organizing volunteers and analytical facilities.

The statistical analyses were performed by Mr. N. Cross who gave helpful advice about experimental design. All the conventional and single fibre electromyography recordings reported in this study were made by the author. I am grateful to my anaesthetic colleague Dr. G. Turner for invaluable assistance during the isolated forearm experiments. Finally, I would like to thank the Medical Director General, Royal Navy for allowing me to pursue the studies and the Institute of Naval Medicine for supplying much of the specialized equipment used. The varied scientific and clinical backgrounds of those acknowledged indicates the scope of the studies reported which do not fit neatly into any one particular discipline. I hope the work will prove of interest to an equally wide range of readers and promote further co - operation between those involved in the basic and clinical sciences.

To my wife and colleague
Dr. Marian Barry
in appreciation of her patience and encouragement

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UNIVERSITY OF SOUTHAMPTON

ABSTRACT

FACULTY OF MEDICINE

MEDICINE 1

Doctor of Medicine

THE CLINICAL NEUROPHYSIOLOGY OF ORGANOPHOSPHATE POISONING

by

David James Baker

Clinical neurophysiological studies are presented of the effects of oral pyridostigmine bromide on normal neuromuscular transmission. This drug has recently been identified as providing an effective prophylaxis against accidental exposure to organophosphate anticholinesterases. In addition, it has also found use in clinical anaesthesia to reverse the effects of non - depolarizing muscle relaxants.

Experiments are reported which investigate the action of pyridostigmine and low doses of the organophosphate sarin on single fibre electromyography (SFEMG) in man. Further studies examine the actions of pretreatment with pyridostigmine on muscle relaxation produced by the non - depolarizing relaxant alcuronium in the isolated human forearm.

Pyridostigmine produces little change in SFEMG but jitter increases were detected after sarin exposure. In the isolated forearm pyridostigmine pretreatment did not affect degree of relaxation produced by alcuronium. The degree of fade produced on repetitive stimulation differed between onset and recovery of relaxation. This relationship may be affected by pyridostigmine. The results are discussed in relation to recent knowledge of the electrophysiological and structural actions of anticholinesterases at the skeletal neuromuscular junction and to hypotheses of the mechanism of neuromuscular fade.

CHAPTER 1: Organophosphate Compounds; Pharmacology and Clinical Effects

1.1 Discovery and development

Few classes of organic compound have received such extensive study as the organophosphates. The systematic study was begun by Schrader in 1935 and in subsequent years over 60,000 organophosphates have been synthesized and investigated (Holmstedt, 1963). The first recorded synthesis of an organophosphate was by de Clermont in 1861. The compound was tetra ethyl pyrophosphate (TEPP), and its discoverer was fortunate to survive to the age of ninety, since one test in his investigations was to taste the substance. TEPP, like all organophosphates was later shown to be extremely toxic. The interest shown in organophosphates eighty years later was initially because of the realisation of possibility of insecticidal properties, and subsequently because of their development as chemical weapons. Tabun (GA) and sarin (GB) were synthesized by Schrader at IG Farben in Germany shortly before the Second World War, and were then subjected to such a secret development and production programme that the Allies had no clue of the existence of the so - called nerve gases even at the end of the war. A factory in Upper Silesia which had produced several thousand tons of GA was captured intact by the Red Army and transported back to the USSR. At the same time both the United States and Great Britain commenced intensive research programmes in organophosphates. The escalation in development of nerve gases and

research into protection against their effects has continued to the present day (Harris and Paxman, 1984).

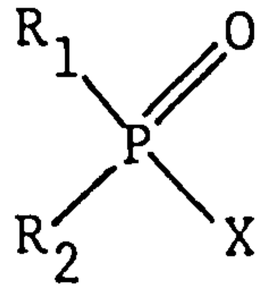
Apart from the interest in organophosphates as military weapons, the past thirty years have seen an unprecedented growth in their use as insecticides, stimulating the production of certain compounds such as malathion which are degraded by higher organisms while remaining toxic to arthropods. Currently many hundreds of thousands of tons of organophosphates are used in this way throughout the world.

The chemistry and pharmacology of the organophosphate compounds has been reviewed extensively, notably by Holmstedt (1959) who has produced a systematic classification (1963) and by Koelle (1963).

In the remainder of this chapter, an outline is given of the chemistry and pharmacology of the organophosphates, with particular emphasis on GB, the agent used in the studies which are reported in this thesis. The clinical signs and symptoms of organophosphate poisoning are then considered, together with the pathophysiology and current treatment practice.

1.2 The structure and chemistry of the organophosphates

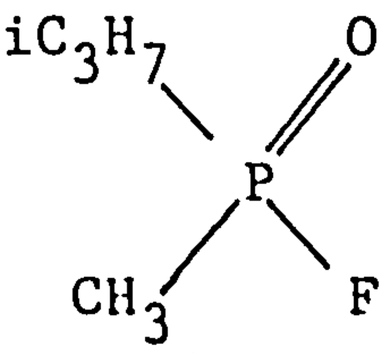
The general formula of the organophosphates is:



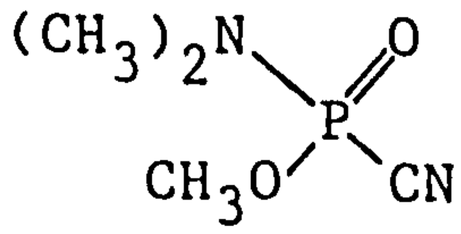
R1 and R2 are organic groups which are capable of almost infinite variation. They may for example be alkyl or aryl groups, alcohols, phenols, mercaptans or amides. The enormous number of each of these

individual subgroups which exist gives an idea of the scope for producing many thousands of organophosphates. X may be fluorine, as in the case of GB, paranitrophenol, phosphates, cyanide or isocyanate, enol, carboxyate, or any phenoxy or thiophenoxy group.
The formula of GB (Isopropyl methyl phosphonofluoridate) which was used in experiments reported later in this study is:

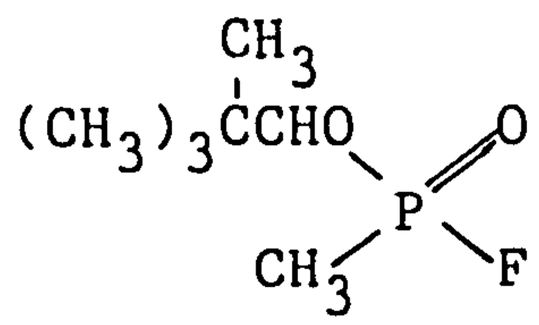
Sarin (GB)



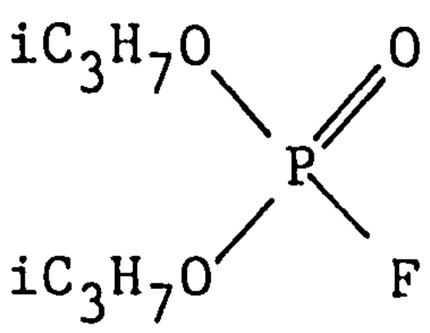
The other widely studied nerve agents are tabun (GA) and soman (GD). Di - isopropyl fluorophosphate (DFP) and paraoxon are organophosphates which have been used extensively in laboratory studies. The formulae of these compounds are as follows:



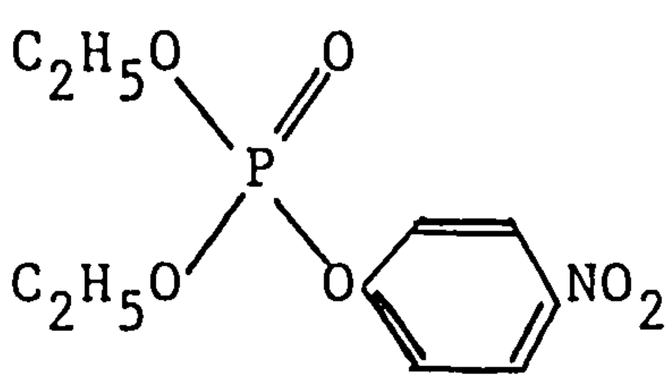
GA



GD



DFP



Paraoxon

All these compounds are not in fact gases as their popular name suggests, but liquids with high vapour pressure. They vary in volatility over a range similar to that between petrol and heavy lubricating oil as the size of the R1 and R2 groups increases. None freeze until -40 deg C. They are all pale yellow

or colourless in appearance and are essentially odourless. All the nerve agents are soluble in water, being broken down slowly by hydrolysis. In the body the hydrolysis is rapid and is catalysed by the phosphorylphosphatase group of enzymes.
Organophosphates are rapidly broken down by strong alkalis and by oxidation. Their destruction by bleach (sodium hypochlorite) is the basis of military decontamination procedures.

1.3 The anticholinesterase effects of organophosphates

Organophosphates bind strongly and largely irreversibly to both acetyl cholinesterase (AChE) in cholinergic synapses and to butaryl cholinesterase (BuChE) which is found in plasma and in the central nervous system. AChE, which is considered further in section 3.4 is essential to the function of all cholinergic synapses whether skeletal or autonomic. The reaction with the enzyme is the key to the considerable number of pharmacological actions possessed by organophosphates, although other biochemical properties of these compounds have recently been recognised as important (Marquis, 1985). The anticholinesterase pharmacology of organophosphates has been reviewed by Hobbiger (1975). The clinical expressions of this widespread modification of cholinergic function are discussed in section 1.5.

1.3.1 The combination of organophosphates with AChE

The interaction between acetyl choline (ACh) the normal substrate of AChE, and the enzyme was investigated thirty years ago. The reaction mechanism shown in figure 1.1 has been proposed (Goodman

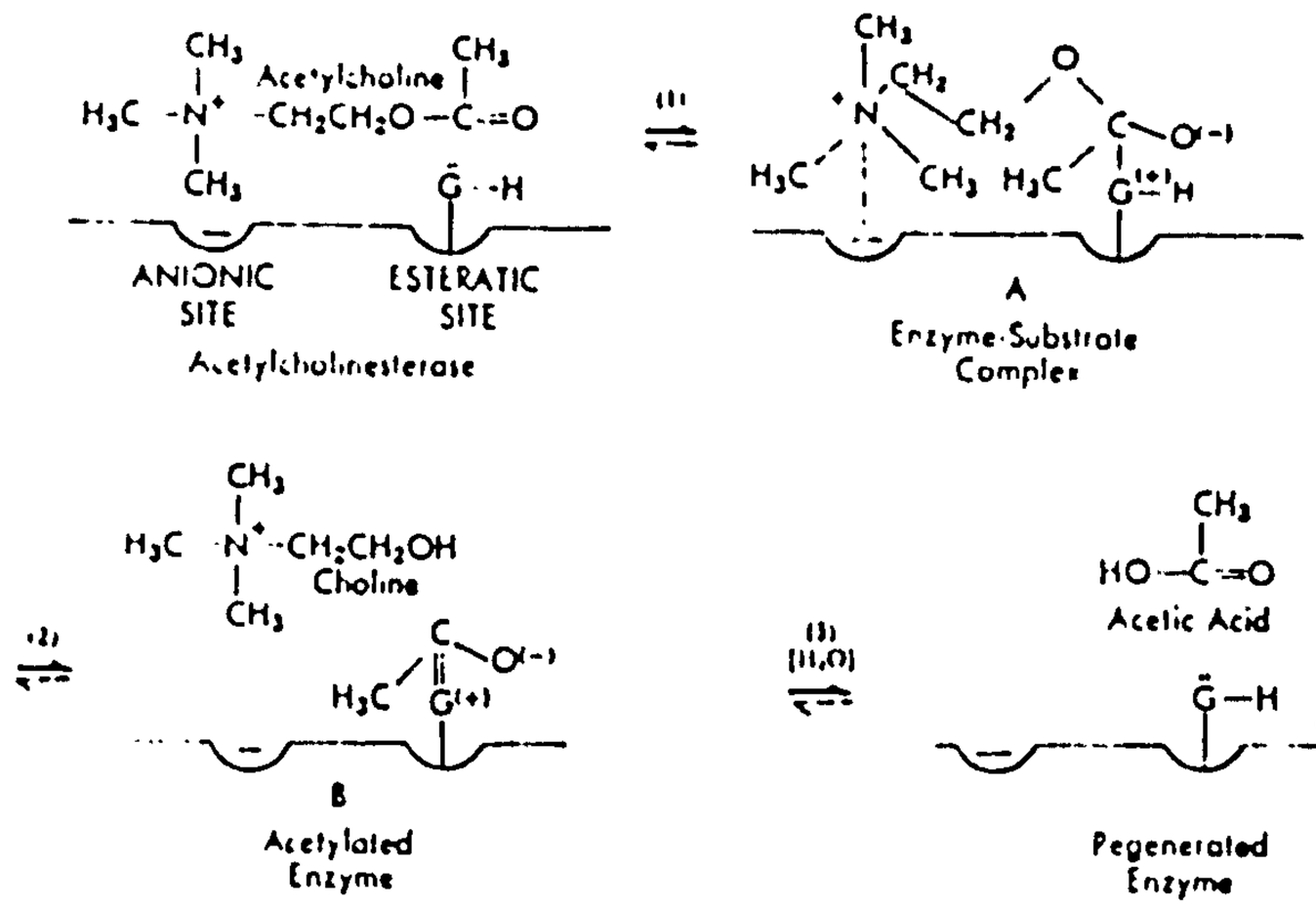


Fig. 1.1 Stages in the hydrolysis of acetyl choline by acetylcholinesterase (from Goodman and Gillman, 1980)

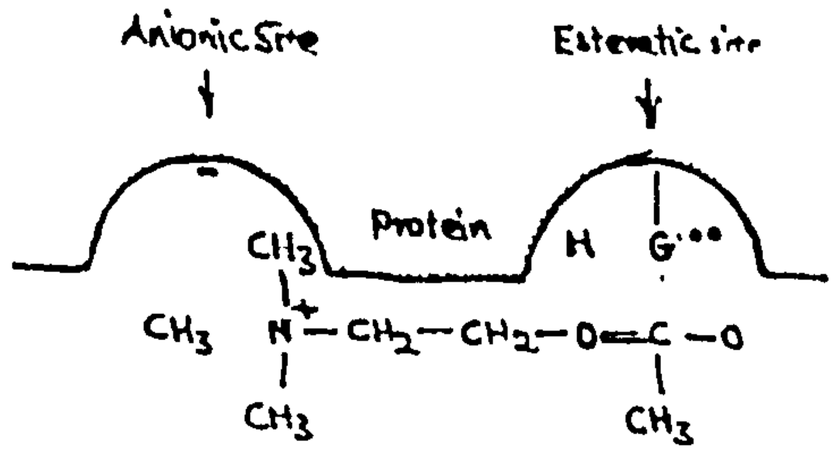


Fig. 1.2 Binding of ACh to AChE. The covalent binding at the esteratic site is between carboxyl carbon and the basic group on the enzyme (G). Binding at the anionic site takes place by electrostatic forces.
(from Goodman and Gillman, 1980)

and Gillman, 1980). The ACh molecule is thought to be bound to the enzyme at two sites shown in figure 1.2. These are (1) an anionic site, which is probably a dissociated carboxyl group where electrostatic binding occurs with the cationic N+ atom of the choline moiety and (2) an esteratic site consisting of a basic group and a protonated acidic group which forms a covalent linkage with the electrophilic group of the ester. Further strengthening of the link between ACh and the enzyme is achieved by Van der Waals forces. The next stage in the reaction splits off the alcoholic portion, choline, leaving an acetylated esteratic site. This reacts very rapidly with water, producing acetic acid and regenerating the original enzyme. The whole reaction sequence, although theoretically reversible, is driven by its dissociation constants to the right. Thus the action of ACh at the postjunctional receptor sites of the neuromuscular junction is terminated within a few hundred usec. In addition to combining with its normal substate AChE reacts with anticholinesterase compounds, of which organophosphates are one class. The carbamate anticholinesterases which form a reversible link with AChE are discussed in chapter 2. They differ essentially in their combination with AChE in that the carbamylated enzyme (fig 1.3) reacts with water at less than a millionth of the rate of the enzyme - ACh complex.

The reaction of organophosphate compounds with AChE takes place at the esteratic site. Figure 1.4 shows DFP undergoing such a reaction. The phosphorylated enzyme so formed is extremely stable. Any subsequent hydrolysis that does occur depends on whether the attached alkyl groups are methyl or ethyl. Following combination with GB, significant regeneration of the enzyme will take place over several hours. If the attached R groups are bigger, as in the case of GD, no significant spontaneous hydrolysis will occur. The degree of hydrolysis depends, for all

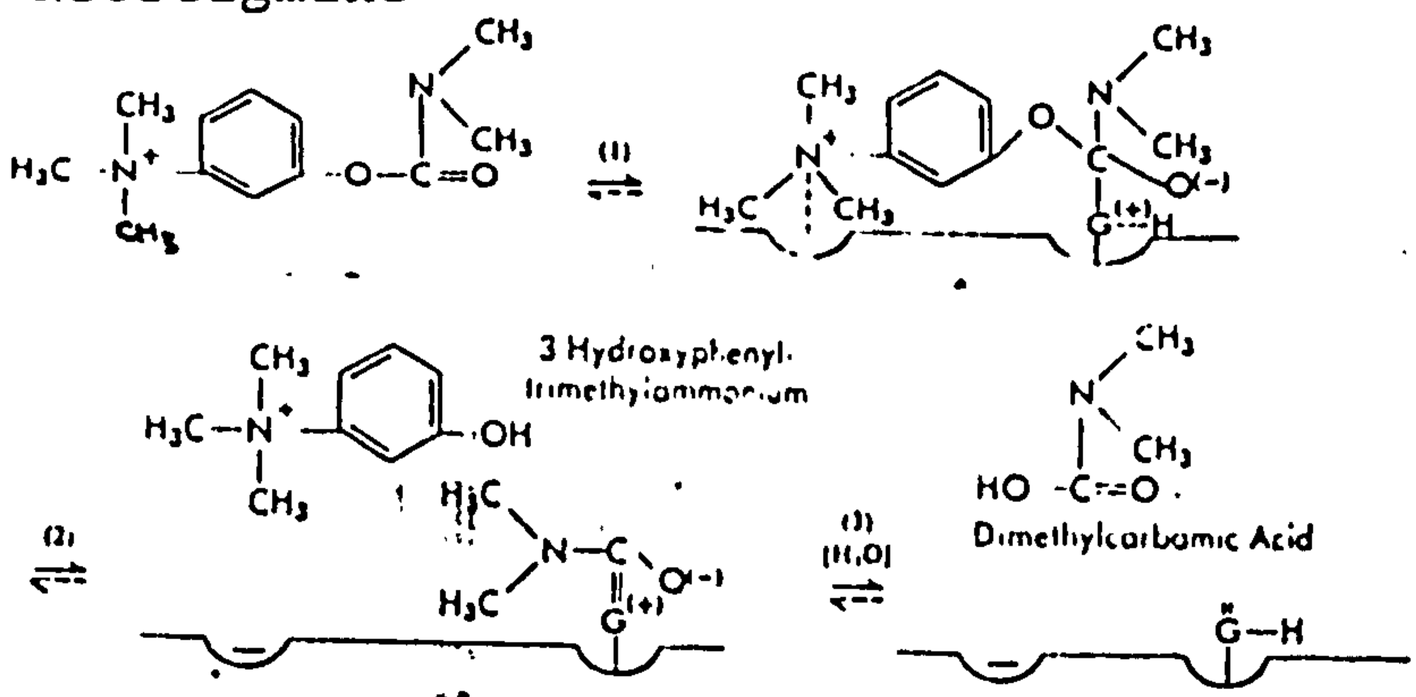


Fig. 1.3 Reaction of neostigmine with AChE. The first two steps are similar to the binding of ACh but step 3 takes place at less than a millionth of the rate of the hydrolysis of ACh (from Goodman and Gillman, 1980)

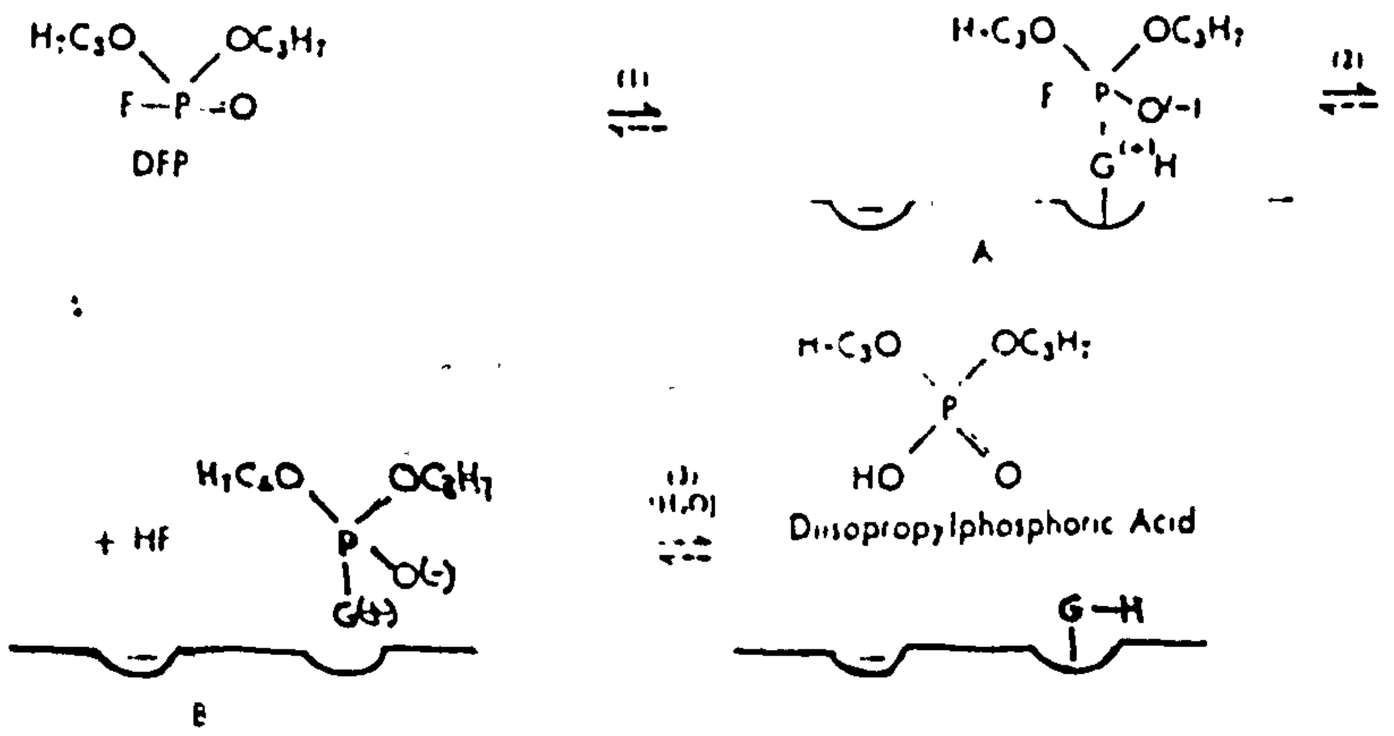


Fig. 1.4 Reaction of the organophosphate DFP with AChE. Binding takes place at the esteratic site. Little spontaneous regeneration of diisopropylphosphoryl AChE takes place (3). With OP compounds having bigger R groups regeneration is absent.
(from Goodman and Gillman, 1980)

organophosphates, on the nature of R1 and R2. Thus two compounds with different X but the same R1 and R2 groups in their structures will regenerate enzyme at much the same rate (Aldridge et al, 1953). It has been shown that the rate of alkaline hydrolysis of organophosphate compounds, mentioned above is related to their effectiveness as inhibitors (Andrews et al, 1952).

1.3.2 Reactivation of the phosphorylated enzyme.

The degree of hydrolysis of the phosphorylated AChE was stated above to be negligible when the R1 and R2 groups were bigger than ethyl. However, Wilson (1951) noted that hydroxylamine (NH2OH) reactivated organophosphoryl AChE more rapidly than water. This observation was extended by several workers to discover that substituted hydroxamic acids (RCONHOH) were more effective at reactivation. Later, the synthesis of the oximes, of which pralidoxime (pyridine - 2 aldoxime methiodide, P2S) is the major example, provided an effective means of reversing the OP - AChE complex. P2S became a major drug used in the treatment of nerve agent poisoning. It is only effective if used a short time after exposure to an organophosphate. This is because the phosphorylated enzyme complex undergoes an 'ageing' process which is probably due to the splitting off of one alkyl or alkoxy group, leaving a more stable monoalkyl or monoalkoxy - phosphoryl AChE (Berends et al, 1959). The regeneration process of the complexed AChE by oximes proceeds according to the reaction scheme shown in figure 1.5. It may be seen that reaction takes place directly with the alkylphosphorylated enzyme to free the active unit. In the case of quaternary ammonium reactivators such as pralidoxime, the rate of combination is greatly enhanced by electrostatic forces between the quaternary nitrogen atom and the

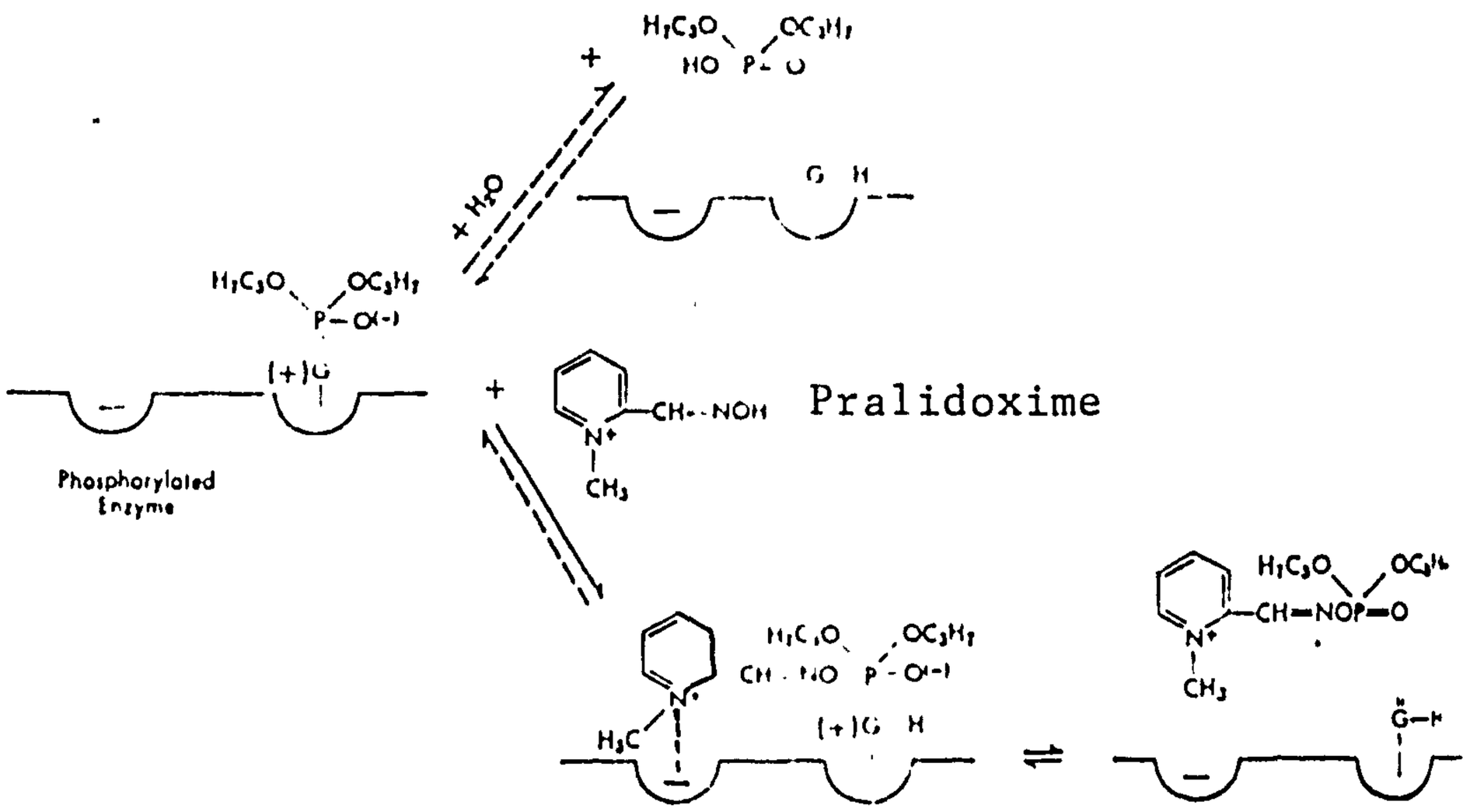


Fig. 1.5 Reactivation of phosphorylated AChE by pralidoxime. The reaction only takes place if the phsophorylation is recent (see text) (from Goodman and Gillman, 1980)

anionic site. The velocity of reactivation of phosphorylated AChE by a given oxime or hydroxamic acid follows the same sequence as the order for spontaneous hydrolytic reactivation. Thus the reactivation of dimethylphosphoryl AChE > diethylphosphoryl AChE > diisopropyl AChE.

1.4 The applied pharmacology of organophosphates

1.4.1 Muscarinic and nicotinic effects

The classical action of organophosphates, in common with other anticholinesterases, is to inhibit the breakdown of acetyl choline (ACh) at cholinergic synapses. These are distributed both in the peripheral and central nervous systems and are grouped into muscarinic and nicotinic sites. The central nervous system contains a further subgrouping of M2 muscarinic sites. This fundamental view of cholinergic transmission, proposed by Langley (1905) is reviewed by Goodman and Gillman (1980). The effect of build up of ACh at the muscarinic sites centrally is to cause activation of the many cholinergic pathways leading to convulsions and the depression of the respiratory centres in the medulla and midbrain. Peripherally, the excited muscarinic synapses cause the release of salivary secretions, bronchoconstriction, contraction of smooth muscle in the bladder and bowel, and bradycardia. In addition there is loss of accomodation and miosis due to effects on muscarinic receptors in the ciliary muscle and sphincter pupillae. Muscarinic actions of ACh are seen only in the parasympathetic nervous system and are characteristically antagonised by atropine. At nicotinic sites there is activation of presympathetic ganglia leading to increased vascular tone and peripheral weakness from the action at the

skeletal neuromuscular junction (SKNMJ).
The clinical consequences of the muscarinic and nicotinic pharmacological action of anticholinesterases are considered in a later section.

1.4.2 Actions of organophosphates at the neuromuscular junction

Since the early observation (Lovatt - Evans, 1951) that organophosphates caused potentiation of the single stimulated twitch in a nerve - muscle preparation, the effects of organophosphates at the neuromuscular junction have been studied in considerable detail (for reviews see Karczmar, 1967 and Hobbiger, 1976). The effects at this site were recognised to be a nicotinic cholinergic effect. In common with other anticholinesterases, organophosphates were shown to be causing a prolongation of both the miniature end plate potential (MEPP) and the end plate potential (EPP) at the post junctional membrane (Eccles et al, 1942).
The investigations into the actions of organophosphates at neuromuscular junction have attempted to explain the following phenomena

(1) potentiation of the single twitch
(2) faciculations
(3) fade of the muscle response to tetanic stimuli, leading to total block

1.4.2.1 Twitch potentiation and fasciculation

Potentiation of the single twitch response and fasciculation have received careful study and the present position of understanding has been summarized by Hobbiger (1976). Twitch potentiation and

fasciculation both appear to be the consequence of changes occuring in the nerve terminal and the first node of Ranvier. Following the arrival of an orthodromic impulse these sites initiate repetitive EPP in individual motor units by an axon reflex. This can only be the consequence of a presynaptic action of ACh (as a result of AChE inhibition) of organophosphate compounds and Hobbiger considers that all the experimental evidence is consistent with the same explanation for carbamates. However, both these anticholinesterases are known to have marked electrophysiological efects which may be important at frequencies of stimulation other than those used for single twitch studies. The repetitive firing phenomenon is one example of an organophosphate action which may not be related to its ability to inhibit AChE.

1.4.2.2 Neuromuscular block

The failure of neuromuscular transmission associated with toxic doses of organophosphate may be compared with the neuromuscular block produced by depolarizing muscle relaxants such as succinyl choline (Zaimis, 1975). At the post junctional site, the accumulation of ACh was originally thought to cause a persistent depolarization allowing no further generation of a muscle action potential. However, Thesleff (1959) showed that this was not the case. The post junctional membrane depolarization returns to normal very quickly, but the block persists in the surrounding muscle membrane. This means that the local potential change produced by a large dose of ACh gradually declines, even though the ACh concentration in the vicinity of the end plate is high. In other words, the receptors appear to become refractory to the actions of ACh (Zaimis, 1975).
In reviewing the known actions of organophosphates in

1959, Holmstedt (Holmstedt, 1959) questioned whether the agents complied exactly with the above sequence in causing neuromuscular block, or whether they might have effects other than those related to inhibition of AChE (section 1.3.3). The whole question of whether organophosphates cause neuromuscular block entirely by AChE inhibition has again been challenged recently by Albuquerque (1983) who questioned whether there is enough releasable ACh in the nerve terminal to cause a sustained depolarization block.

1.4.2.3 Neuromuscular effects of organophosphates in relation to degree of AChE inhibition

Experiments conducted by Berry and Lovatt Evans (1951) and Burgen and Hobbiger (1951) studied the nature of neuromuscular block produced by several anticholinesterase compounds in the isolated rat hemidiaphragm preparation.

It was clear that the block was reversible with carbamates and less so with certain organophosphates (e.g. TEPP). With other agents, such as the the organophosphate DFP, no washing - related recovery was possible if exposure to the agent was prolonged. A puzzling feature of this work was the fact that following carbamate exposure and washing there was recovery of the AChE level to correspond to the recovery of function, whereas in some cases with DFP recovery was sufficient to pass an 80 stimulus tetanus with no apparent recovery of the enzyme. It should be stated that the methods for determining the tissue levels of AChE were less sophisticated at that time than at present. Thus difficulties in analysis of enzyme levels may provide answers to apparently anomalous results. Inaccuracy in enzyme determination may also provide some explanation for the findings of Barnes and Duff (1953) who examined twitch changes in the isolated rat hemidiaphragm at different levels of

AChE. Using TEPP, DFP and paraoxon these authors showed that the response to indirect stimulation went through a whole series of changes as the AChE was progressively inhibited. It was found impossible to block the response to single stimuli even at high degrees of inhibition but that response to a tetanic stimulus failed. Calculations showed that fasciculation and enhanced response to single stimuli took place while the AChE level was reduced from 50% to 10% of normal. At 10% of normal these responses disappeared, together with the power to sustain a tetanic stimulation of 50Hz for 5 seconds. However, during recovery of the muscle preparation, after removal of the inhibitor, calculations suggested that muscle power returned when only 5% of the BuChE and no AChE was present. It should be emphasized that predicted levels of enzyme were used based on AChE kinetic data from homogenised preparations.

Van der Meer and Meter (1958) studied the effects of DFP on the rat phrenic nerve - diaphragm preparation. At 0.5 Hz contractions were enhanced by the addition of DFP. This occurred at muscle AChE levels which were 20% of normal. When contraction had returned to normal practically complete inhibition of the AChE was noted. In unstimulated preparations, spontaneous contractions were seen during the periods in which otherwise the enhanced contractions would have occured. Figure 1.7 summarizes the knowledge of actions of organophosphates at various degrees of AChE inhibition.

The studies quoted indicate that the neuromuscular effects of organophosphates occur broadly over a range of high degrees of inhibition of AChE but that the actual responses at set enzyme levels are difficult to specify because of the difficulty of enzyme assay.

1.4.3 The effects of organophosphates on the respiratory centre

Before reviewing the clinical signs and symptoms of organophosphate poisoning the effects of the agents on the respiratory centre must be considered. These are only part of the previously mentioned widespread activity of organophosphates in the cholinergic central nervous system which form a large topic outside the scope of this thesis (for a review see Karczmar, 1984). GB has a marked effect on the respiratory centre of several species which precedes the development of peripheral neuromuscular failure. However, it does not share this distribution of effect with all organophosphates. GA, paraoxon and TEPP for example cause rapid development of neuromuscular block before the establishment of central respiratory failure and are associated with an initial phrenic nerve discharge from central stimulation by asphyxia and vagal or other afferent nerve discharge (de Candole et al, 1953). This stimulation however is usually insufficient to overcome the gradual depression of phrenic nerve discharges which results from the direct effect of the agents on the respiratory centre.

Almost all the studies of organophosphates on the respiratory centre have been performed using animal models in which there is wide species difference. Also, animal studies of the contribution of central depression to overall respiratory failure have been complicated by the possibility that recorded central changes may be secondary to peripheral ones. In human studies using GB (chapter 7), the exposure doses are so small that central respiratory depression can effectively be ignored. The effects of organophosphates on the respiratory centre are mediated through muscarinic receptors. Blockade of these sites by atropine is limited by the ability of

AChE (%normal)*	Peripheral Effects		Central Effects#
	Nicotinic	Muscarinic	
85 - 50		miosis loss of accomodation rhinorrhoea lachrymation	giddiness and restlessness emotional lability headache nightmares(with chronic toxicity)
	slight rise in BP	dyspnoea(subjective tightning of chest)	Difficulty in concentration
		progressive bronchospasm	
40 - 20		nausea vomiting abdominal cramps	drowsiness slurred speach
10	fasciculation and progressive weakness	involuntary defecation and micturition	bursts of slow wave activity on EEG
			progressive failure of medullary respiratory centres
0	paralysis of respiratory muscles		coma

Fig. 1.6 Pharmacological and clinical effects with degree of AChE inhibition by organophosphates.
Note: * AChE levels are only approximate due to changes in estimation techniques over forty years of laboratory and clinical investigation
central effects vary with organophosphate and species; terminal respiratory failure may thus be central, peripheral or both

that compound to cross the blood brain barrier. To be effective centrally atropine must be given in a higher dose than that required for blockade of peripheral muscarinic sites (Wadia et al, 1974).

1.5 Clinical signs and symptoms of organophosphate poisoning

The signs and symptoms of nerve agent poisoning were investigated in volunteers during the late forties and early fifties by Grob and Harvey (1953). The same authors have also investigated specifically the human effects of sarin (Grob and Harvey, 1958). With the advent of stricter controls on volunteer experiments, few further studies have been carried out since then, although clinical evidence has been gathered retrospectively from cases of accidental and self - poisoning with organophosphate insecticides (Wadia et al, 1974). The respiratory actions of organophosphate toxicity were the subject of a recent symposium organised by the United States Army (Newball (ed.), 1983).

1.5.1 Absorption

Organophosphates may be absorbed in many ways. Inhalation and absorption of the vapour through mucous membranes are obvious routes, but the penetration through the dermis is considerable and is important. Absorption may produce local effects on the tissues through which it passes and these are most noticeable in the respiratory tract and eyes. The respiratory tract is known to be the most rapid and complete of all possible routes and it has been observed that an equal amount of compound may be more toxic in the vapour than the gaseous phase (Punte et al, 1958).

1.5.2 Local effects of vapour

The earliest effect of local absorption of vapour which follows minimal sympomatic exposure is miosis. This is felt subjectively as a sensation of

pressure and heaviness in and behind the eyes. The aching is attributed to ciliary spasm which may occasionally be accompanied by nausea and vomiting. Exposed subjects will often report photophobia and dark sensitivity, even after exposure to small concentrations of agent, when the symptoms will take two or three days to subside. The effects of local absorption of the respiratory tract are to produce a watery nasal discharge, nasal hyperaemia, a sensation of tightness in the chest and occasionally expiratory wheezing indicating bronchoconstriction and increased bronchial secretion (Grob, 1956)

1.5.3 Systemic effects

At about six times the absorption of a dose of organophosphate sufficient to cause minimal symptomatic effects systemic effects will become apparent. At 15 to 20 times the minimal symptomatic dose serious incapacitation occurs. The period between the exposure and the onset of symptoms depends on the degree of exposure and intervals of between a few minutes and up to half an hour have been noticed. The duration of symptoms depends again on the dose and may be between several hours and several days for modest doses of agent. However, during recovery symptoms may recur intermittently, especially following exertion.

1.5.4 Clinical consequences of ACh increase at muscarinic sites

The local effects of bronchoconstriction, hypersecretion and miosis are muscarinic effects. After substantial exposure the bronchoconstriction and secretion may become considerably worse, leading to further decreased lung compliance and airway blocking.

Laryngeal spasm may become serious and cyanosis and hypoxia may become severe. At this stage, which occurs at 30 to 50 times the minimal symptomatic exposure, gastrointestinal symptoms of increased peristalsis, abdominal cramps, vomiting, diarrhoea, tenesmus and involuntary defecation occur. In addition, the casualty may be noticed to be bathed in sweat, have increased lacrimation, urinary frequency and occasional slight bradycardia, although this is not always as severe as might be expected.

1.5.5 Clinical manifestations of ACh increase at nicotinic sites

The earliest detectable effects of organophosphates on muscle are increased fatigability and mild generalised weakness which is increased by exertion. It is important at this stage that all excess physical activity is avoided since it may considerably increase the toxicity of the agents (Punte et al, 1953).

At more severe levels of poisoning, involuntary muscle twitching, fasciculation and cramps occur. The fasciculation usually occurs first in the facial muscles and may become generalised. At the final stage of poisoning, severe weakness occurs in all muscles including the respiratory muscles, which finally fail completely, leading to hypoxia and death unless intermittent positive pressure ventilation is started.

1.5.6 Clinical effects on the central nervous system

All organophosphates cross the blood - brain barrier to a significant extent. Because all of the many cholinergic pathways in the brain are affected by organophosphate intoxication the symptoms and signs

are numerous (Grob, 1956; Grob et al, 1947). Sleep disturbances with excessive dreaming and nightmares may occur and have been detected in workers involved in sarin production suffering from accidental chronic exposure to the agent (Duffy and Burchfiel, 1979). In this study EEG abnormalities were detected. Other psychological symptoms include inability to concentrate, impairment of memory and slowing of reactions. The earliest symptoms include tension, anxiety, restlessness and giddiness. Impotence may also be reported in chronic low dose exposure. At severe levels of intoxication there may be marked speech impairment, with slurring of words and multiple repetition of the last syllable. Convulsions and coma may ensue prior to death.

1.5.7 Myopathic effects of chronic low level exposure to organophosphates.

The low level exposures discussed in the previous section provided evidence that organophosphates may cause clinical damage in doses which hitherto had not been recognised as being toxic. Neuromuscular effects of low doses of organophosphates in animals are considered in chapter 4. However one epidemiological and experimental study of the development of unexplained myopia in Japanese children exposed to such doses has indicated that structural changes in the highly cholinergic ciliary muscle were responsible. The condition, known as Saku disease, was produced in beagles where electronmicroscopic studies of the ciliary muscle showed almost amorphous changes in some muscle fibres after two years exposure to organophosphates sufficient to reduce AChE activity to 40% of normal (Ishikawa and Miyata, 1980). As will be seen later in this study, low dose organophosphate exposure may prove to be of more clinical importance than was previously realised.

1.5.8 Causes of death in organophosphate poisoning

The cause of death in severe organophosphate intoxication is a combination of several factors which are:

1. CNS: (a) failure of the respiratory centre in the medulla oblongata (b) generalised cortical activity causing convulsions which aggrevate the hypoxia;

2. Respiratory System: (a) neuromuscular block with early involvement of the respiratory muscles (b) increased bronchospasm and secretion;

3. Circulatory Effects: bradycardia with decreased cardiac output and peripheral vascular effects.

1.5.9 The development of respiratory failure after organophosphate poisoning

Many of the pharmacological events mentioned in the previous sections combine to produce an overall picture of respiratory failure. The action of organophosphate on the respiratory centre leads to failure of the centrally generated nerve impulses to reach the peripheral muscles of respiration. This action was always thought to be more important than the peripheral neuromuscular blockade in bringing about failure of respiration. However, the relative effects are highly dependent on the agent used and the species which is being studied (Albuquerque, 1983). Blockage of the upper respiratory tract is a combination of the increase in secretions from the salivary glands and the flaccid paralysis of the pharyngeal musculature. Laryngospasm is a possible serious complication during the early stages of acute

poisoning. In addition, there is a hazard of the airway becoming filled with vomitus from the actions of the agent on the gastrointestinal tract. Below the larynx, the trachea fills with secretions and there is severe bronchospasm leading to a marked decrease in pulmonary compliance (the degree of the distension of the lungs for each unit of ventilating pressure applied). At the level of the peripheral respiratory musculature there is progressive weakness from the depolarization block leading to the cessation of sustained respiratory activity. The processes mentioned all combine to give ventilatory failure with hypoxaemia and acidosis. In combination with the effects on the heart causing severe bradycardia the usual outcome of severe organophosphate poisoning is asystolic cardiac arrest.

1.6 The clinical management of organophosphate poisoning

The foundation of the clinical management of organophosphate poisoning is aggresive first aid treatment, using repeated doses of atropine to counter the muscarinic effects and oximes to regenerate the bound AChE. In addition airway management with endotracheal intubation, suction and intermittent positive pressure ventilation is usually necessary. The longer term management of organophosphate intoxication is an intensive care unit problem, which may involve several weeks of ventilation with associated intravenous fluid therapy, renal and inotropic support. Huge doses of atropine may be required to counter the muscarinic effects, particularly on the vagally mediated bradycardia. The prognosis is usually poor (Wadia et al, 1974). The prognosis can theoretically be improved considerably by pretreatment of subjects who may be exposed to OP with pyridostigmine. This causes

formation of a reversible carbamate - AChE complex with the enzyme which is not subject to organophosphate attack. Thus even after exposure to OP concentrations sufficient to bind AChE irreversibly, a sequestered reserve of AChE is available through the reversible breakdown of the pyridostigmine complex over the subsequent 48 - 72 hours. However, during this period intensive life support measures will be required until the normal processes of neuromuscular transmission have been restored. The use of pyridostigmine in this way and some of the screening studies for its administration to fit subjects are considered later in the next chapter.

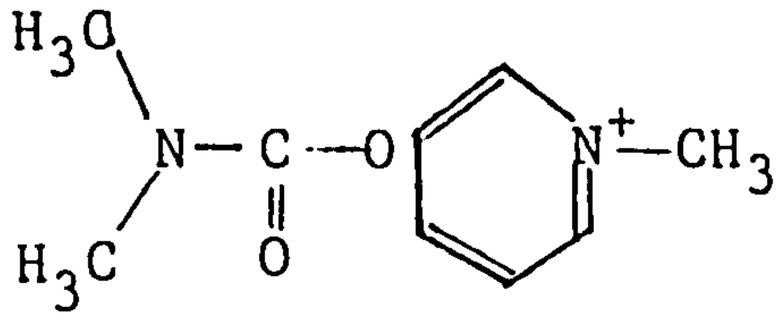
CHAPTER 2: The Pharmacology of Pyridostigmine

2.1 Introduction

Chapter 1 described the chemistry and pharmacology of the organophosphates, one of the most important classes of anticholinesterase compounds. Equally important, both as pesticides and in medicine are the carbamate anticholinesterases. This chapter reviews the essential pharmacology and uses of the carbamate pyridostigmine which belongs to this group.

2.2 Chemistry

The formula of pyridostigmine is:



Structurally the compound is the dimethylcarbamate ester of 3 - hydroxy - 1 - methylpyridinium bromide which was first synthesized by the Hoffman La Roche laboratories in Basle in 1945. It is the pyridine analogue of an earlier synthetic anticholinesterase neostigmine which has the following structure:

Both these compounds, together with physostigmine which occurs naturally in the Calabar bean, are classed as reversible anticholinesterases because of the nature of the complex they form with AChE. From the formulae it is seen that pyridostigmine and neostigmine both contain quaternary nitrogen atoms which restricts their movement across membranes and is thought to account for their binding to the enzyme.

2.3 Anticholinesterase action of pyridostigmine

In section 1.3.1 the interaction of the organophosphates at the anionic and esteratic sites of AChE was considered. Figure 1.3 indicated how carbamates like pyridostigmine and neostigmine react with the enzyme at the same site.

The reversibility of the process, known as carbamolyation lies in the final stage. Although the carbamolylated enzyme produced by neostigmine and related ammonium or amino - carbamyl esters, including pyridostigmine, does react with water to give the regenerated enzyme, it does so at a rate less than one millionth of that of the acetylated complex formed by ACh. Nevertheless, the reaction takes place within a biologically useful timescale and is the basis of the use of carbamates in protection against nerve agents (section 2.4.3). In the case of the organophosphate anticholinesterases, the complex formed with the enzyme is in most cases hydrolysed to a negligible extent and may be regarded as being irreversible. The actions of pyridostigmine described here take place at the post junctional membrane of the neuromuscular junction and represent the classical

view of the effects of the compound. The actions of AChE at this site are considered further in section 3.4. However, the long term action of pyridostigmine produces changes at both the pre and post junctional sites. These are the subject of the review in section 4.3.

2.4 Uses of pyridostigmine

2.4.1 The treatment of myasthenia gravis

For over 30 years pyridostigmine has been used in clinical practice as an orally active anticholinesterase to treat the symptoms of myasthenia gravis (Goodman and Gillman, 1980). In this condition, there is an immunologically determined loss of ACh receptor sites at the post junctional membrane (section 12.1.1). ACh released normally is therefore less efficient at generating an end plate potential since fewer ion channels are available at any point in time for depolarization of the post junctional membrane. This situation leads to failure of generation of a muscle action potential in some muscle fibres which is detected clinically as progressive weakness. By occupying a proportion of the active sites on the AChE, pyridostigmine and the other carbamate anticholinesterases can improve transmission by effectively extending the lifespan of any ACh molecule before hydrolysis. Since attachment to the receptor is a competitive process, which is therefore concentration dependent, any increase of the ACh concentration at the post junctional site makes occupation of the existing receptors more likely and so improves the chances of generating a useful end plate potential.

The average daily oral dose of pyridostigmine used to treat myasthenia gravis is about 600 mg (section

2.4.1.2), which can be given in intravenous or intramuscular doses of 1/30 of the oral dose. At this level the side effects of the drug are usually minimal.

2.4.1.1 Overdoseage of pyridostigmine during treatment of myasthenia gravis

The effects of overdoseage by pyridostigmine can be classified in terms of actions at the classical muscarinic and nicotinic cholinergic synapses. Muscarinic side effects include nausea, vomiting, diarrhoea, abdominal cramps, increased peristalsis, increased salivation, increased bronchial secretions, miosis and heavy perspiration. The nicotinic effects are manifest as muscle cramps, fasciculations and weakness and give rise to the 'cholinergic crisis' sometimes seen in overtreated myasthenics. In addition to these effects which can be ascribed to build up of ACh, pyridostigmine has been shown to cause changes in the morphology and function of the neuromuscular endplate which are discussed in section 4.3. Clinically, in a patient with myasthenia gravis the differentiation of muscle weakness due to the disease process from that due to overtreatment with pyridostigmine can often be difficult.

2.4.1.2 Clinical experience with pyridostigmine

Neostigmine was originally used to treat myasthenia gravis but over the past 30 years considerable experience has been gained clinically in the use of pyridostigmine and several authors have commented on efectiveness and side effects of the drug. Siebert(1953) stated that in a therapeutic trial of the drug at doses ranging between 240 - 730

mg /day in 23 myasthenic patients undesirable side effects were mild and rare. Schwab and Timberlake (1954) replaced neostigmine with pyridostigmine in 50 myasthenic patients under treatment. They found that even with doses as high as 1620 mg/day disagreable intestinal side effects were absent, but that in some cases the drug was not so effective as neostigmine. Tether (1956) reported on a study of 165 myasthenic patients who were treated with pyridostigmine at daily doses of 60 - 6000 mg/day. He noted that there were no distressing side effects even in patients who had used the drug for as long as 17 months. However, the side effects listed above were noted, and mild vertiginous visual disturbance and speech impairment were reported. Further studies on series of myasthenic patients reported by Schwab et al (1957) and Osserman et al (1958) confirmed the clinical opinion that pyridostigmine was an effective drug for use in myasthenia gravis and relatively free from side effects, except in the case of frank overdoseage. In this situation the muscarinic side effects were apparent before the nicotinic.

These clinical studies have provided a large amount of information about the effects of pyridostigmine given over a long period. A further body of information about the effects of pyridostigmine is avaiable from studies of its use in clinical anaesthesia.

2.4.2 The use of pyridostigmine in clinical anaesthesia

Modern balanced anaesthesia requires controlled muscle relaxation for intermittent positive pressure ventilation and facilitation of surgery. These conditions are usually provided by the use of non - depolarizing relaxants such as curare, pancuronium or more recently, vecuronium. At the end of operation residual paralysis must be reversed,

prior to re-establishing spontaneous ventilation. The drugs mentioned act by combination with the acetyl choline receptor (AChR) making it unavailable for the conformational change produced by ACh which opens the ion channel (section 3.3). Since this process is concentration - dependent the use of an anticholinesterase may be expected to build up the level of ACh at the post junctional membrane and overcome the neuromuscular block. The drug most often used to achieve this is neostigmine, administered intravenously in doses between 2.5 and 5mg, after atropine which is given to block the considerable muscarinic effect of bradycardia.

Pyridostigmine, given intravenously in doses of 10 - 20 mg, has been investigated by several workers as a possible alternative to the use of neostigmine. It was hoped that the drug might have less action on the heart. Katz (1967) found pyridostigmine to be an effective anatagonist of curare in 104 patients although atropine was still necessary to prevent excessive salivation and bradycardia. Fogdall and Miller (1973), Ravin (1975) and Gyermek (1977) confirmed the efficacy of pyridostigmine in reversing neuromuscular block produced by curare and pancuronium. The last study contained the interesting observation that the onset of action of pyridostigmine, usually slower than neostigmine (Miller et al, 1974) was accelerated by the prior use of edrophonium, a carbamate which only combines at the anionic site of the AChE. None of these studies and others, which have been concerned with the effectiveness of pyridostigmine and its capacity to produce muscarinic effects such as cardiac dysrhthmias, have reported any neuromuscular dysfunction as a result of nicotinic effects of overdose. In theory however, as in the case of organophosphate poisoning, overdose of pyridostigmine could lead to such an excess of ACh that depolarization could occur, leading itself to muscle

weakness. Such a finding has been reported by Payne et al (1980) for neostigmine.

2.4.3 The use of pyridostigmine in protection against the toxic effects of organophosphates

The reaction of anticholinesterases with AChE was considered in section 1.3.1. In the case of organophosphate anticholinesterases, the complex formed is irreversible due to the negligible degree of hydrolysis occurring at the final stage. Conventionally, the production of this stage is retarded by the use of oximes (section 1.3.2). The combination of atropine and the oxime P2S have for many years been the mainstay of treatment of organophosphate poisoning (Anon., 1972). The nerve agent GD (section 1.2) was found however not to respond to conventional oxime/atropine treatment (Loomis and Salafsky, 1963; Heilbron and Tolagen, 1965) and consideration was given to the problem of preventing and treating poisoning with this and other similar agents. The observation by Koster (1946) that whole body protection against the organophosphate DFP was given by physostigmine prompted further studies of the possible protective effects of carbamates. Berry and Davies (1970) reported that pretreatment of guinea pigs by physostigmine improved their survival rate when exposed to GD. It was suggested by these authors that the protective action of the carbamate might be explained according to the scheme shown in figure 2.1. When the animals are treated with physostigmine, a certain proportion of the AChE is complexed reversibly by the carbamate. If the animal is then exposed to nerve agent the free enzyme is attacked in the usual way producing an irreversible complex. However, the proportion of the AChE which is bound to the carbamate is not attacked by the organophosphate. After the poisoning episode, any free organophosphate is rapidly

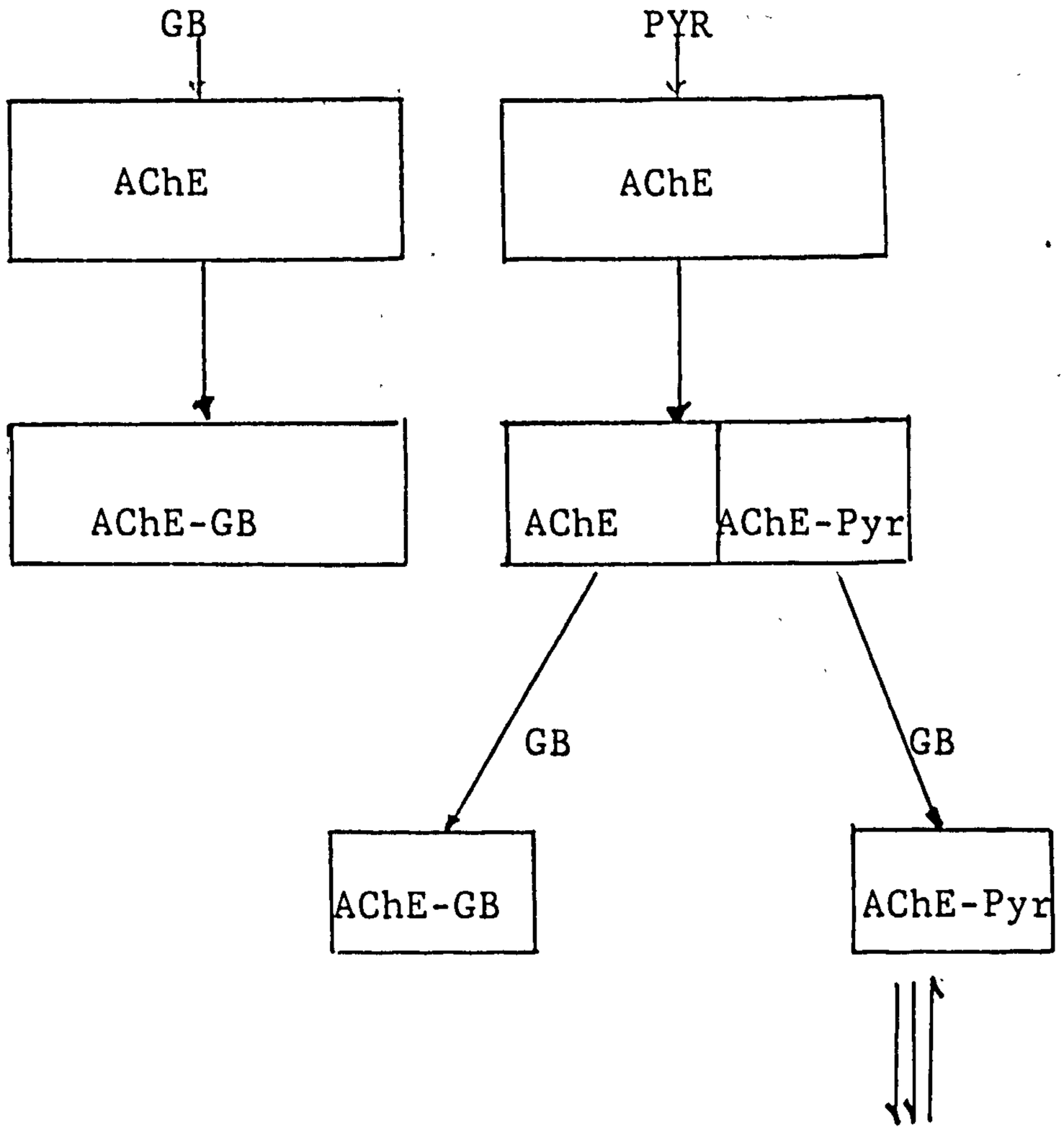


Fig.2-1 Hypothesis for the protective action of pyridostigmine on AChE against GB. Exposure of the enzyme to organophosphate produces an irreversible phosphorylation. Pretreatment by pyridostigmine produces 30 - 40% carbamylation of the enzyme which is resistant to subsequent exposure to GB. Regeneration of AChE takes place over 48 hours. The quantities of AChE regenerated are adequate to maintain normal nicotinic and muscarinic synaptic transmission.

hydrolysed in the body to inactive products. Meanwhile, the reversible carbamate - AChE complex breaks up over a short time span releasing free enzyme which is then available for its normal function. The hypothesis depends on the spontaneous regeneration occuring at a slower rate than the hydrolysis of the organophosphate in plasma. This reaction is normally very fast (section 1.2). The protective action of physostigmine was found to be the property of other carbamates including pyridostigmine (Gordon and Leadbeater 1977). In a futher study Gordon et al (1978) found a wide species difference in the degree of protection afforded by carbamates against organophosphates. Guinea pigs were found to be better protected than rabbits. Both these animals were protected to a much greater extent than rats. The protective action of carbamates was found to extend for 3 - 4 hours after intramuscular injection and pyridostigmine was found to have the longest action. It was also found that the dose of prophylactic carbamate was not critical, significant protection being afforded to the animal by about a quarter of the maximum dose that could be given with signs of cholinergic crisis still absent.

Because of the considerable amount of clinical pharmacological information known about pyridostigmine and because, being a quaternary drug it was unlikely to cross the blood - brain barrier to any extent, the Chemical Defence Establishment at Porton Down decided to study the protective actions of pyridostigmine extensively in primates and man. Pyridostigmine also possessed the considerable advantage of having had a product licence for several years (Mestinon, Roche). Studies on the protective action of pyridostigmine in primates have been reported by Dirnhuber et al (1979). This work indicated that rhesus monkeys and marmosets could be protected against fifteen times the subcutaneous LD50 dose of GD by pretreatment with pyridostigmine and atropine. The same studies showed

that pyridostigmine was equally active when given by the oral route. The duration of the protective effect following a single intragastric dose was found to be at least 16 hours in GD poisoned monkeys. This protection was achieved with a peak AChE inhibition of 40% at 3 hours after dosing falling to 10% at 16 hours.

The studies of the actions of pyridostigmine on 100 fit volunteers have been summarised by Gall (1981). The dose of drug tested was 30 mg 8 hourly. After two days on this regime peak blood levels of 35 ug/ml pyridostigmine were measured at 2.5 hours after dosing, and the inhibition of AChE was 42%. In this study the drug was administered for as long as four weeks. Side effects noted were slight increase in flatus, occasional looseness of the bowels and a slowing of the pulse by five beats/min both at rest and at exercise. No neuromuscular neurological changes could be detected and no effects were found on visual parameters including pupil size. There were no changes in physiological or blood parameters and no detectable changes in psychological or cognitive functions.

2.5 Conclusions

The preceeding sections have shown that pyridostigmine has been a useful drug in many clinical areas over a long period since it was first synthesized. It has a wide therapeutic dose range and large doses have been given to patients with myasthenia gravis for long periods without apparent ill effect. The drug has received attention as a means of reversing muscle relaxation in general anaesthesia and as a prophylaxis against nerve agent poisoning. Studies of the latter use are reported in chapter 8.

CHAPTER 3: Recent Developments in the Theory of Neuromuscular Transmission

3.1 Introduction

The transmission of nerve impulses to muscle fibres has been one of the most studied phenomena in pharmacology (for an extensive review see Zaimis (ed), 1975). Nevertheless, important new work is still in progress in identifying the structure of the ACh receptor sites, the manner of release of ACh from the nerve terminal and the explanation of fade in the partially curarized muscle. Some of the recent developments in this field are reviewed in this chapter.

The transmission of controlling impulses from a terminal motor nerve to its muscle fibre takes place through a specialized region of the sarcolemma called the skeletal neuromuscular junction (SKNMJ) or motor end plate. Each SKNMJ is found at approximately the mid - point of a muscle fibre (the motor point). The structure of the end plate has recently been reviewed by Duchen and Gale (1985) and is shown diagrammatically in figure 3.1. The non - myelinated terminal nerve fibre resolves into the nerve terminal which is supported by a Schwann cell. The terminal is separated by about 60 nm from the postsynaptic sarcolemma, or post junctional membrane. This is organised into invaginations called junctional folds. The depth, density and degree of branching of these folds depends on the type of muscle fibre controlled. In mammalian muscle, junctional folds are more

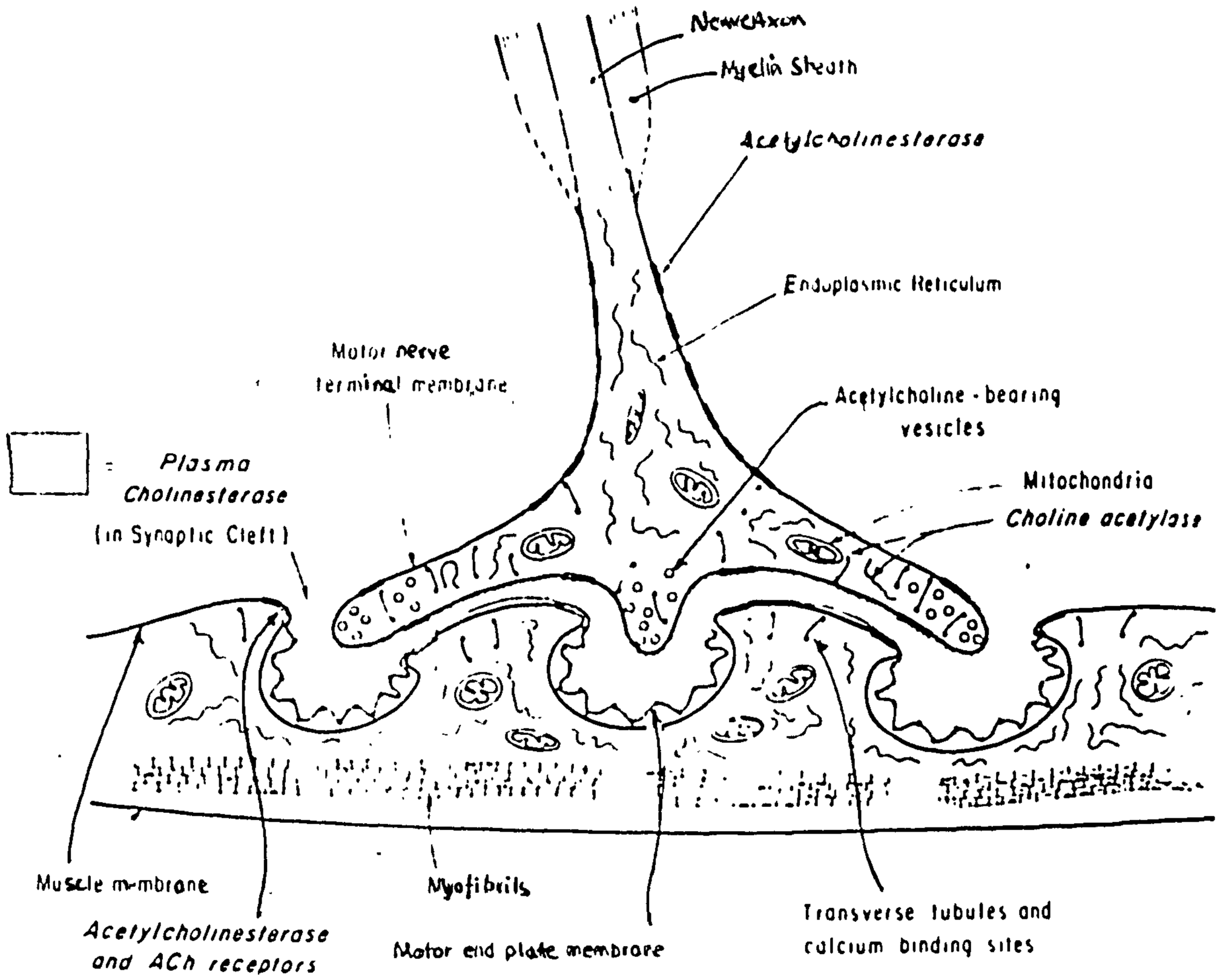


Fig. 3.1 Structure of the neuromuscular end plate (from Ali and Savarese, 1976)

numerous, deeper and more branched in the type 2 (fast) muscle fibres than in the type 1 (slow) fibres. Bowden and Duchen (1976) have reviewed the difference between these two types (section 6.9.2). The whole end plate structure is circular in shape and is sealed at the edge by basal lamina of the Schwann cell fused with that of the sarcolemma. The basal lamina extends into the synaptic cleft between the nerve terminal and the postjunctional folds.

The receptor sites for acetyl choline, released from the nerve terminal are located on the shoulders of the post junctional folds. These are considered further in section 3.3. Transmission of an impulse at the neuromuscular junction involves the following stages:

(1) arrival of the impulse at the nerve terminal

(2) release of ACh from the nerve terminal into the synaptic cleft

(3) alteration of membrane permeability at the postsynaptic membrane following occupation of special receptor sites (cholinoceptors) by ACh leading to the generation of an end plate potential (EPP)

(4) initiation by the EPP, after reaching a critical amplitude of 10 to 20 mV of a propagated muscle action potential which triggers the contraction process in the muscle fibre

(5) rapid termination of the action of ACh at the post junctional membrane by AChE.

These stages are those of the classical theory of cholinergic neuromuscular transmission. The essential concept of the EPP being generated by the passage of ions through the ion channels created by opened receptor sites remains unaltered in present theory. However, it has now been recognised that the modification of the ion flow at the post junctional receptor is possible by factors unconnected with the attachment of ACh to the receptor site. The phenomenon of ion channel blockade is considered in section 3.5.6.

3.2 Release of transmitter at the neuromuscular junction

3.2.1 Introduction

The central concept of neuromuscular transmission, stated in section 3.1, is that acetyl choline is stored, mobilized and released from the nerve terminal to pass across the synaptic gap to the post junctional membrane. Over the past fifteen years, the processes controlling the release of ACh have attracted increasing attention. The relationship between the descending nerve impulse and the actual release of ACh from the nerve terminal is still unclear. The following sections summarize the present state of the morphological and functional knowledge relating to ACh release and indicate the way in which drugs may interfere with the process.

3.2.2 Nerve terminal morphology and the quantum hypothesis

The observations by Fatt and Katz (1951) of small depolarization potentials at the neuromuscular junction were the foundation of the quantum hypothesis of ACh release. These miniature end plate potentials (MEPP) were about 1/100 of the size of the EPP which depolarizes the end plate and generates the muscle action potential. MEPP were found to be similar to EPP in their time characteristics and in the way they were affected by drugs. From statistical analyses (Martin 1966) it was concluded that the MEPP was the result of a basic unit of ACh release in neuromuscular transmission, and that the amplitude of EPP varied in units which are multiples of the MEPP. MEPP are too

large to be produced by one molecule of ACh and it was therefore thought that they were produced by the release of a discreet release of a packet or quantum of ACh (Boyd and Martin, 1956). At the same time, early electron microscopic studies (Palade, 1954; Palay, 1954; Robertson, 1956) showed the presence in the nerve terminal of small round structures which were termed vesicles. The vesicles were all accumulated near the synaptic surface of the nerve ending or terminal, and del Castillo and Katz (1954) proposed that the vesicle was the morphological correlate of the ACh quantum. Thus, release of several hundred quanta was thought to produce an EPP (Martin, 1966). The discovery of vesicles influenced hypotheses of ACh release and there have been many subsequent attempts to correlate the known physiological events at the end plate with morphological changes during the release of ACh. Heuser and Krause (1973) showed that the vesicles were arranged in triangular aggregates with the apex opposite a convolution in the post junctional membrane (see fig 3.2). Overall, the vesicles were seen to be congregated in the portion of the nerve terminal nearest the junctional surface with the microtubules mitochondria and support structures aligned towards the other side. The apex of the triangle was aligned towards the postjunctional cleft which had been shown to be rich in ACh receptors (AChR) (Land, Salpeter and Salpeter, 1980). The prejunctional accumulations of vesicles were called 'active zones.' These zones were about 50 nm wide. Each nerve ending was found to contain about 500 to 1000 active zones (Peper et al, 1974; Cecarelli and Hurlbut, 1980). By means of the scanning electron microscope and freeze fracture technique attempts were made to identify vesicles in the act of discharging ACh into the synaptic cleft. Heuser et al (1979) noted pits alongside the active zone following stimulation of the nerve terminal which were thought to be the remains of discharged vesicles.

Fig. 3.2 Longitudional section of the frog neuromuscular junction. The nerve ending is in the centre, between the Schwann cell sheath (top) and the postjunctional membrane (botoom). Vesicles are concentrated in active zones (large arrow) opposite postjunctional clefts (from Heuser and Reese, 1973)

These authors noted that the number of pits always increased with time after stimulation and that they were almost always in rows. Heuser and Reece (1981) showed an increase in the number of pits in an active zone with time after the stimulus arrived the nerve terminal.

3.2.3 Re-use and synthesis of vesicles.

After the release of ACh, which is considered in detail below, the fate of the vesicle is of some interest. Most workers feel that a system for recycling the membrane must exist in the nerve terminal. Since the inner surface of the vesicles is greater than the area of the terminal itself, it is thought that the vesicles must flatten after discharge and disappear within the cytoplasm, rather than becoming continuous with the nerve terminal surface. Thus there must be some form of recycling of membrane material, since new vesicular membrane cannot be synthesized within the terminal. Instead, synthesis must take place within the cell body and the products then transported to the nerve terminal along the axoplasm. Photographic evidence of membrane recycling has been provided by several investigators. Heuser and Reece (1981) published a carefully timed sequence of vesicular release which seems to show vesicles in the act of fusing and flattening.

3.2.4 The mechanism of ACh release.

So far we have considered morphological evidence of ACh release. What is the mechanism linking the arrival of a nerve action potential at the terminal with the release of ACh - containing vesicles? This subject has been reviewed by Standaert (1982). The key ion involved in this process has been

shown to be calcium (Katz and Miledi, 1965, 1967, 1969 a and b). The number of quanta released by a stimulated nerve terminal is greatly influenced by the concentration of ionised calcium in the extracellular fluid. Dodge and Rahamimoff (1967) showed that change in the quantal content (the number of constituent quanta) in the EPP is proportional to the fourth power of the change in the Ca2+ concentration. If Ca2+ is absent from the extracellular fluid, no ACh release can take place even if depolarization is present at the nerve terminal. Normally, the calcium concentration within a nerve is very low since there are efficient mechanisms for scavenging any ions which may enter. However, quantal release of ACh is dependent not only on the outside concentration of Ca2+ but also the length of time that elapses during which Ca2+ flows in. In other words, the quantal content of the EPP is a function of the total number of Ca2+ ions present in the terminal after a nerve is stimulated. Calcium influx is usually balanced by K+ efflux. The calcium current starts at the time when the action potential approaches its maximum depolarization.

3.2.5 Calcium channels into the nerve terminal

Ca2+ is presumed to enter the nerve via special proteins that form channels in the nerve membrane. The key channels related to ACh release may be located in the active zones (Cecarelli et al, 1979). The mechanism of opening this channel, may be direct (Katz and Miledi, 1964) or via cyclic AMP which is formed following the arrival of the nerve action potential at the nerve terminal.

3.2.6 Cyclic AMP and ACh release

There are several steps in the process between the arrival of the nerve action potential and the release of the vesicles. Not all the stages of this excitation - secretion process are known but recent research has shown that cyclic AMP may have an important role. Experiments have been conducted using the lipid soluble analogue of cyclic AMP, 2,3 di-butyl AMP (a more stable experimental substrate) which indicate that cyclic AMP promotes the entry of ions into the nerve terminal giving a stimulus bound response. The passage of calcium ions inwards as a result of cAMP action has been confirmed by several investigators (For review of this subject see Standaert and Dretchen, 1979 and 1981). Modification of the calcium ion concentration has lent support to the hypothesis. Thus Standaert and Dretchen have confirmed that the neural effects of cAMP are not changed by tetrodotoxin which acts upon sodium channels but are antagonised by several Ca2+ ion flow antagonists including verapamil. They are also modified when the external Ca2+ concentration is altered using chelating agents.

Further evidence concerning the importance of cAMP in ACh release comes from a study of the metabolism of the molecule. cAMP is metabolized by phosphodiesterase (see fig 3.3). Drugs which can inhibit this enzyme have a considerable effect on the action of cAMP whose concentration increases, and on the subsequent release of ACh. This point is discussed further in section 3.2.7.

Considering the formation of cAMP Standaert and Dretchen postulate that the adenylate cyclase in the nerve membrane is activated when an action potential reaches the nerve terminal. This enzyme is directly responsible for the production of cAMP. The activation may be direct, as a result of the action of the electric field. An alternative explanation is

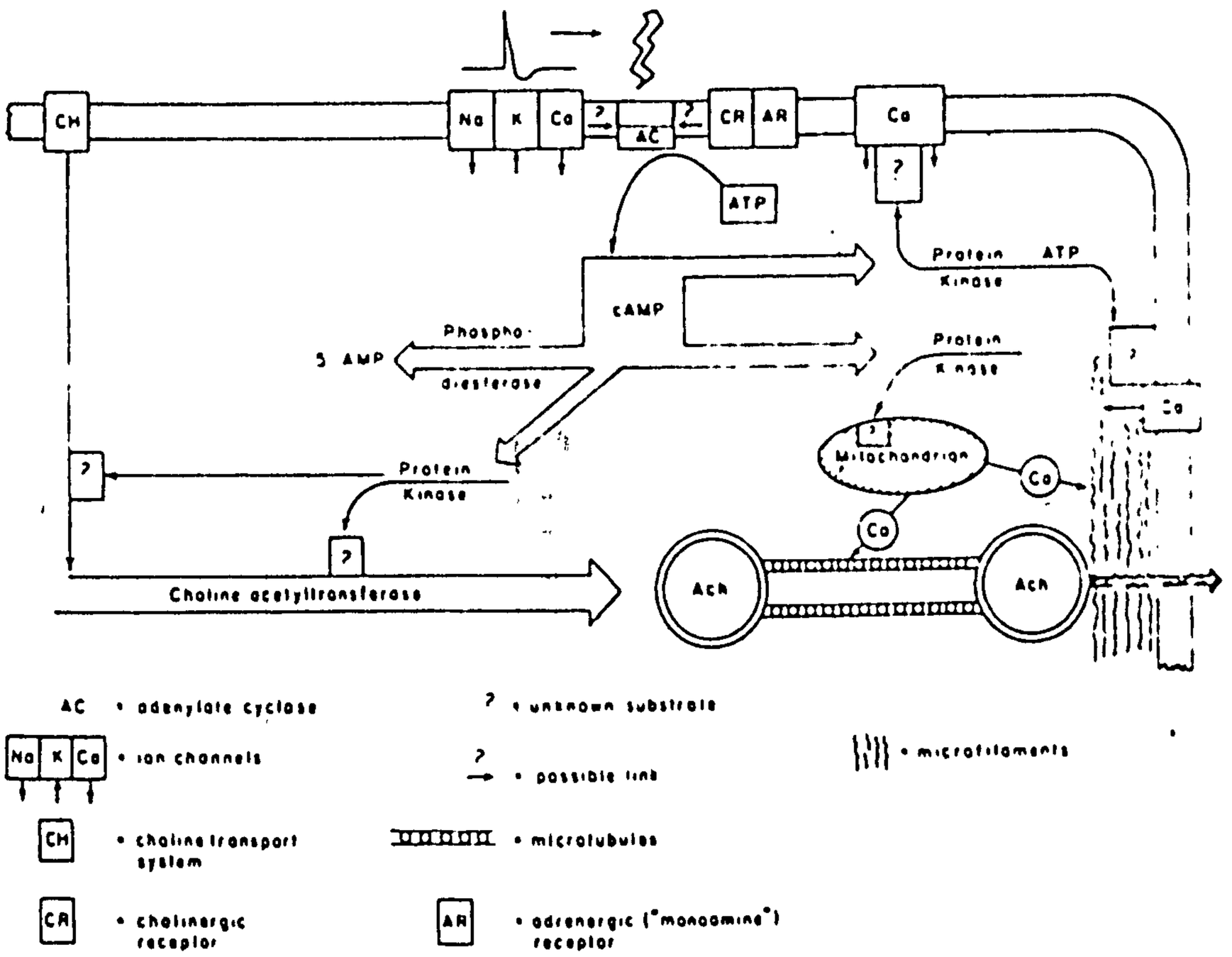


Fig. 3.3 Role of cAMP in ACh release (after Standaert and Dretchen, 1979)

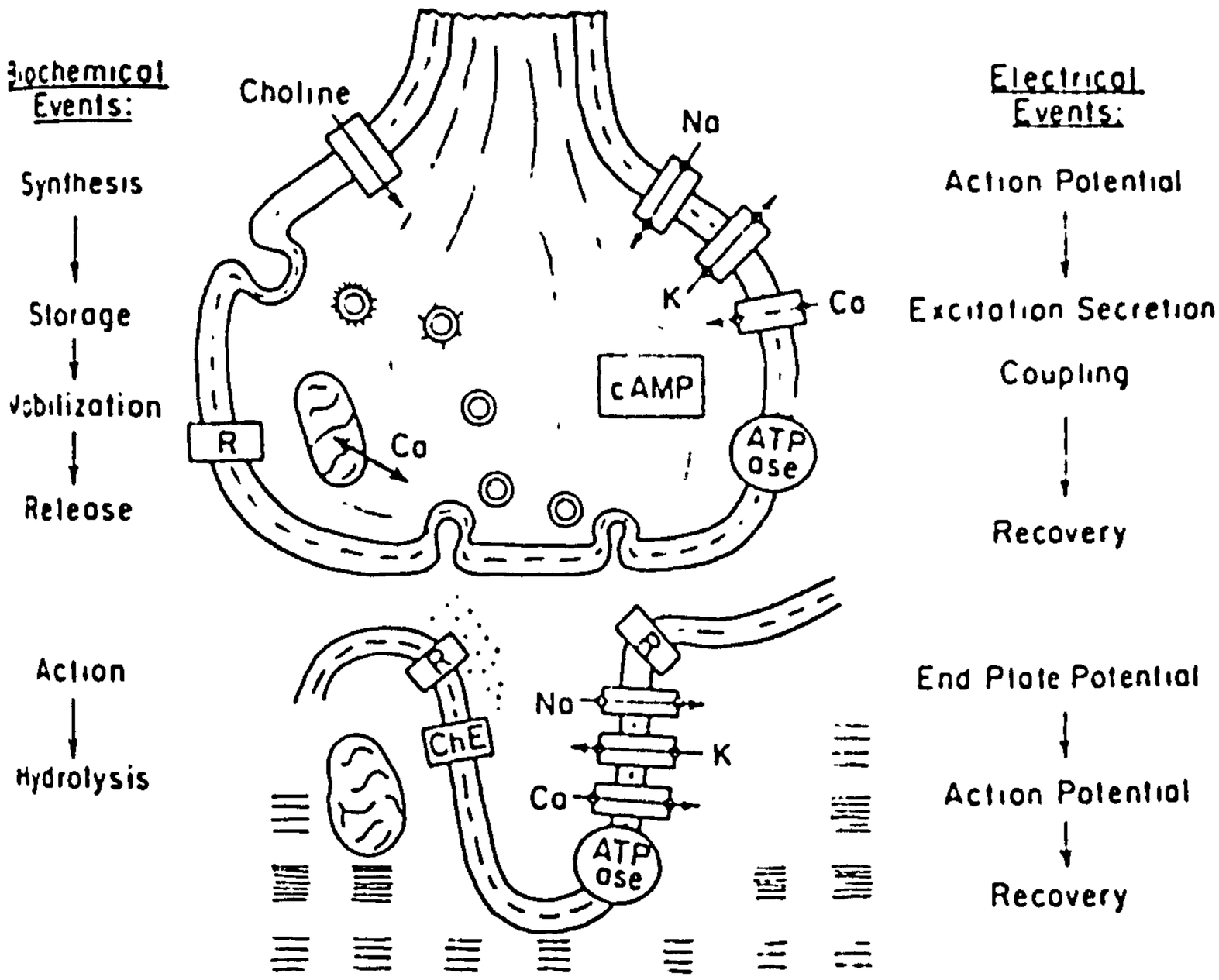


Fig. 3.4 Schematic representation of the motor nerve terminal and end plate depicting the major chemical and electrical events in neuromuscular transmission
(after Standaert and Dretchen, 1979)

that a small amount of Ca2+ enters with an action potential - related surge of Na+ into the axon, reacting with the protein calmodulin to activate the cyclase. Activated adenylate cyclase in turn forms cAMP which, through protein kinases causes phosphorylation of other proteins. This causes an opening of ion channels through which the Ca2+ can then pass. Jones (1985) has reviewed the evidence pointing towards the important action of calmodulin as a modulating substance to the action of calcium. This protein is a single chain molecule with a molecular weight of about 16,500. There are four Ca2+ binding sites which cause a change in the shape of the protein and activate it. The activated form modulates several calcium - dependent processes including vesicle aggregation.

Through the cAMP mechanism therefore, the arrival of an action potential at the nerve ending depolarizes it and allows Ca2+ to enter. The calcium causes ACh containing vesicles to move up to the terminal membrane, fusing with it to release their contents into the synaptic cleft. The details of the mechanism of this stage of the release process are unknown. After releasing the ACh the vesicles flatten and fuse with the membrane surface. The Ca2+ which has entered the nerve terminal is captured by proteins in the endoplasmic reticulum and sequestered there or in the mitochondria until it is moved out of the terminal by the Na+/Ca2+ antiporter system (a scavenging ionic pump) or by ATPase. The sodium flux which was associated with the original action potential may diffuse into the cytoplasm to influence intracellular events but eventually it is removed by ATPase. These processes are summarized in figure 3.4.

This description summarizes the current thinking about ACh release but there several unanswered problems. The released vesicles for example, are usually those which have most recently been synthesised. Not all the ACh in the nerve terminal is contained in

vesicles. This has been established by several workers. Mitchell and Silver (1963) concluded that no more than 2% of the ACh released from unstimulated preparations could correspond to the genesis of MEPP. Fletcher and Forrester (1975) found that at least 98% of the ACh released from a resting nerve ending came from non - vesicular origins. Vizi and Vyscocil (1979) reported that non - vesicular release continues during stimulation but Katz and Miledi (1981) were not able to detect a significant contribution of non - vesicular ACh to stimulus - evoked transmission. The role of ACh leakage therefore remains unclear. Recently Dunant (1985) has reviewed the whole question of ACh release and modified the quantal hypothesis to take account of such 'abnormal' modes of transmitter release.

3.2.7 Drugs and ACh release at the nerve terminal

The preceeding description of events accompanying the release of ACh at the nerve terminal indicated several points at which drugs affecting the process could act. Reduction of external Ca2+ concentration is well known in causing neuromuscular block. That calcium concentration within the nerve terminal may play a critical role was indicated by the studies of Vizi et al (1977) of the actions of 2 and 4 amino pyridine (2AP, 4AP) on ACh release. Both drugs were found to increase the rate of transmitter release at rest in the Auerbach plexus of the guinea pig ileum at both high (10Hz) and low (0.5Hz) frequency stimulation. At the lower frequency of stimulation, the potentiation of ACh release was much greater than at the higher rate. These workers showed that 4-AP was able to increase ACh release even in the absence of detectable Ca2+ outside the nerve. However, the presence of a chelating agent which would be expected to remove all traces of Ca2+ supresses ACh release

altogether. It appears that 4-AP lowers the demand of the nerve terminal for Ca2+ required in the excitation - secretion process. However, the chelating agent experiment showed that a small concentration of Ca2+ outside the nerve is presumed essential for the action of 4-AP. 4-AP itself has been shown to increase quantal content of the EPP.

The drug modification of the cAMP link in the release process was mentioned in the previous section. Thus drugs can affect both the synthesis and metabolism of cAMP. In the synthesis adenylate cyclase plays an important role. This enzyme can be induced by sodium fluoride and antagonised by alloxan.

Phosphodiesterase is the enzyme responsible for the breakdown of cAMP therefore metabolism of cAMP can be modified by any drug which can attack phosphodiesterase. This explains the action of several quite different separate drugs such as azothiaprine and theophylline in affecting neuromuscular transmission.

The inhibition of cAMP phosphodiesterase by physostigmine and theophylline has been studied by Standaert and Dretchen (1981). Both these drugs are known to produce fasciculations, potentiate the twitch response, antagonise dTC block and potentiate the block produced by suxamethonium. Physostigmine is a known carbamate AChE inhibitor and the original explanation for these findings was to relate them to the cholinergic mechanism. However, theophylline has no action on this system and so the authors tested the hypothesis that the two drugs might be acting through phosphodiesterase inhibition. It was found that both compounds inhibited phosphodiesterase in a similar way. Other AChE inhibitors, including the other carbamates do not share this action. It is not yet known how many of the actions of physostigmine can be attributed to this hitherto unrealised action on the cAMP system. The action of carbamates in modifying ACh release is considered in section 4.3.3. A summary

of drug - related modification of ACh release is shown in figure 3.5.

3.3 The Acetyl Choline Receptor

The previous section discussed the release of the ACh transmitter from the nerve terminal. Next, the interaction of ACh with the receptor sites will be considered in the light of recent evidence.

The concept that drugs acting on the neuromuscular junction do so at specific receptor sites has remained central to the theory of neuromuscular transmission since it was first proposed by Langley in 1906 (section 1.4.6). The advent of techniques such as interference optical and electron microscopy have clarified the nature of the special receptor sites and their functional role. From the initial idea of one type of receptor being responsible for events at the junction, the concept has now been developed to encompass the possibility of the existence of other AChR, sited prejunctionally.

The detailed study of the distribution and structure of the AChR has been carried out in the identical receptor found in the electric organ of certain fish of the Torpedo family (for a review of the AChR see Dreyer, 1982). The receptors are seen under electron microscopy as discrete particles, about 10 nm in diameter, and having a density of 7,500 /um2 on the shoulders of the junctional folds. This position places the receptors exactly opposite the ACh release sites in the nerve terminal. Studies using the snake venom alpha bungarotoxin, which binds irreversibly to AChR, have shown that between 2 and 4 binding sites for ACh are available at each receptor.

Ultrastructural studies of AChR have shown an integral membrane protein structure extending about 1.5 nm into the intracellular and 5nm into the extracellular space

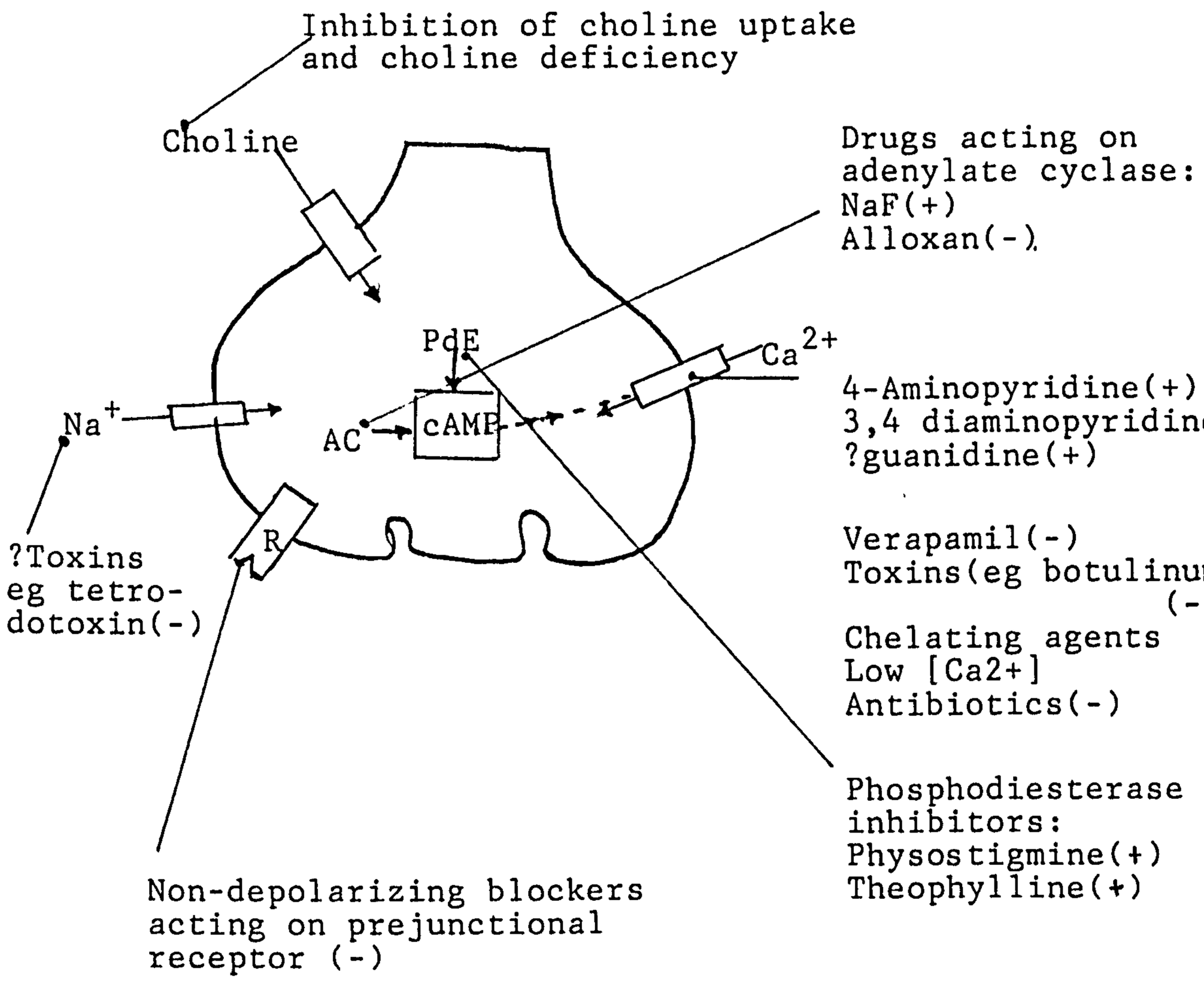


Fig. 3.5 Summary of the main processes in ACh release and drugs which affect them. The final action on release of transmitter is indicated by the sign in brackets.

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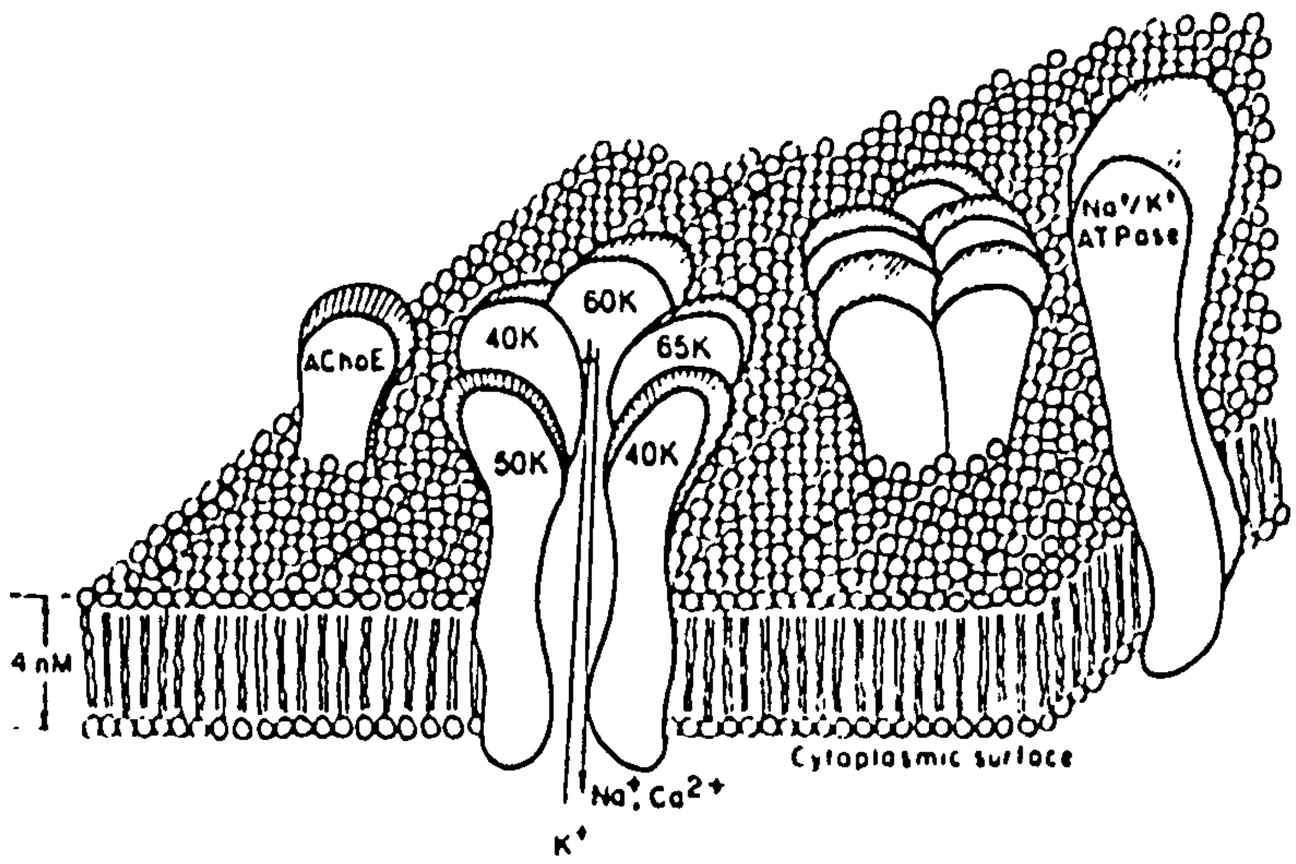
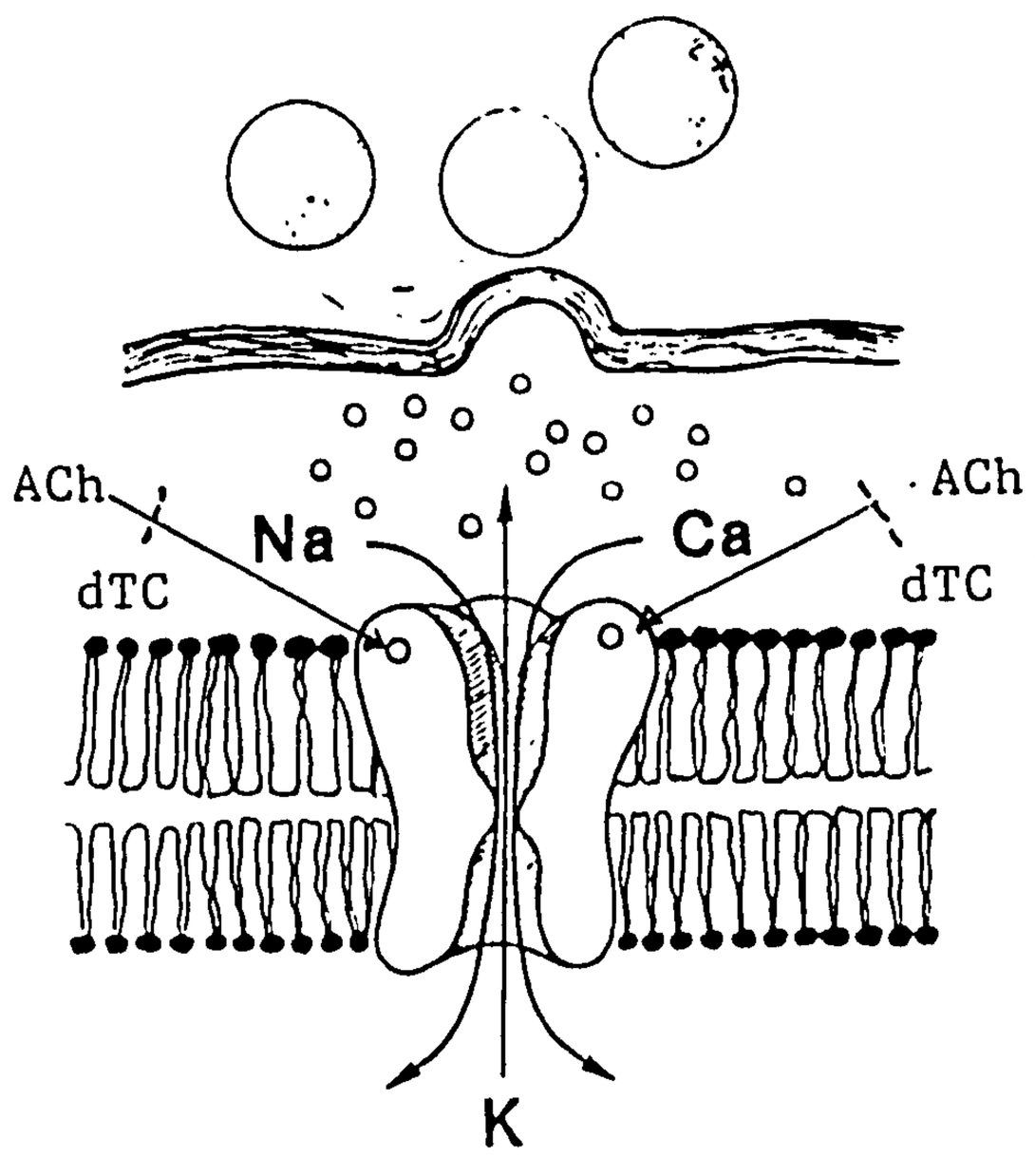
(see figure 3.6). The total molecular weight is about 250,000 Daltons. The receptor has been shown to comprise five glycoprotein units, two having a molecular weight of 40,000 (alpha) and the remainder of 50,000, 60,000 and 65,000 Daltons (beta, gamma and delta). This gives the protein structure a funnel shape with the wider end facing the nerve terminal. ACh attaches to the alpha units and causes a conformational change which opens the funnel to allow the passage of sodium and potassium. This passage through the receptor is known as the ion channel and to open it both the alpha units must attach to ACh molecules simultaneously. If one alpha site attaches competitively to a drug such as curare the channel cannot open and the site is effectively blocked. This process is shown diagrammaticaly in figure 3.7. In summary, the action of ACh at the postjunctional site is brought about by a brief increase in its concentration which is sufficient to open about 2000 ion channels within 300 usec. This process is terminated by the action of acetylcholinesterase (AChE) which is sited on the post junctional folds and hydrolyses the ACh. The modification of this process by AChE and drugs will now be considered.



Sketch of receptor/ionophores and some proteins in postjunctional membrane. The centre figure labels the five subunits of the receptor protein and is cut away to show its ion channel; another unlabelled, receptor is to the right. Acetylcholinesterase (ACho E) and sodium potassium ATPase are also sketched.

Fig. 3.6

Ultrastructure of the ACh receptor (from Standaert, 1983)



Acetylcholine, as released from a vesicle in the motor nerve ending at the top, activates ion conductance through a postjunctional nicotinic receptor. Open circles represent acetylcholine.

Fig. 3.7 Normal ion flow after the attachment of ACh to the alpha units of the AChR. Block of one of these sites by a blocking drug such as curare causes closure of the channel (from Standaert, 1983)

3.4 Acetylcholinesterase and the neuromuscular junction

3.4.1 The location of AChE

Given that the transmission rate across the SKNMJ is between 5 and 50 Hz it is clear that removal of ACh from the receptor sites must be swift and efficient. This is achieved by the enzyme acetyl cholinesterase (EC.3.1.1.7, AChE) which is found on the post junctional membrane. There are at least three forms of this enzyme characterised by the sedimentation numbers 4S, 10S and 16S (Hall, 1973). The enzyme facilitates hydrolysis of the ACh to give choline and acetic acid in concentrations which are inert with respect to neuromuscular transmission (see figure 3.8). The localization and actions of AChE have been studied extensively. Detailed histochemical studies (for a review see Koelle et al, 1968) have shown that in the SKNMJ the enzyme is located on the shoulders of the secondary synaptic folds. It is thus conveniently placed for catalysing the hydrolysis of ACh at the receptor sites.

AChE is also found in red blood cells where its concentration may conveniently be measured (Ellman et al, 1960). Following irreversible inhibition of the enzyme by organophosphate (section 1.3) red cell AChE levels may take up to three months to return to normal. The turnover in the SKNMJ in such a situation is unknown.

The details of the action of AChE on ACh hydrolysis have been reviewed by Hobbiger (1976). There are at least 10 AChE sites and 10 AChR available for each molecule of ACh released by a single nerve impulse. Hobbiger points out that with these ratios, even if ACh only reached 10% of the available receptor sites and its concentration were so high as to inhibit its

own hydrolysis to an extent, AChE should still be able to lower the concentration of ACh markedly within a millisecond provided AChR and AChE are evenly distributed and the rates of hydrolysis of ACh determined *in vitro* still apply. Furthermore, even if 90% of the AChE sites are blocked there should still be enough uninhibited enzyme available to prevent the accumulation of ACh released at random or by low rates of nerve stimulation. The physiological consequence of reduction in the rate of hydrolysis of ACh is a modification in the shape of the EPP.

3.4.2 Modification of EPP characteristics by anticholinesterases

3.4.2.1 Study of EPP

The inhibition of AChE prolongs the post synaptic action of ACh causing a change in the shape of the EPP. The study of the way the EPP controls a muscle fibre is not easy (for reviews see Katz, 1966; Bowman, 1980). One problem is that the EPP can only be recorded if the muscle action potential triggered by it is eliminated. This can by achieved in a variety of ways such as cutting the fibre on either side of the end plate or blocking a proportion of the postjunctional receptor sites with dTC so that the muscle action potential is not generated (Glavinovic, 1979). However, observations of the effects of anticholinesterases on the shape of the EPP in the presence of dTC may not be the same as those that would be recorded in its absence. Another method of studying the EPP characteristics is to use a pair of nerve stimuli so timed that the second falls within the period of refractivity of the muscle fibre so that the second induced EPP can be studies without muscle fibre contraction interference (Eccles et al, 1941).

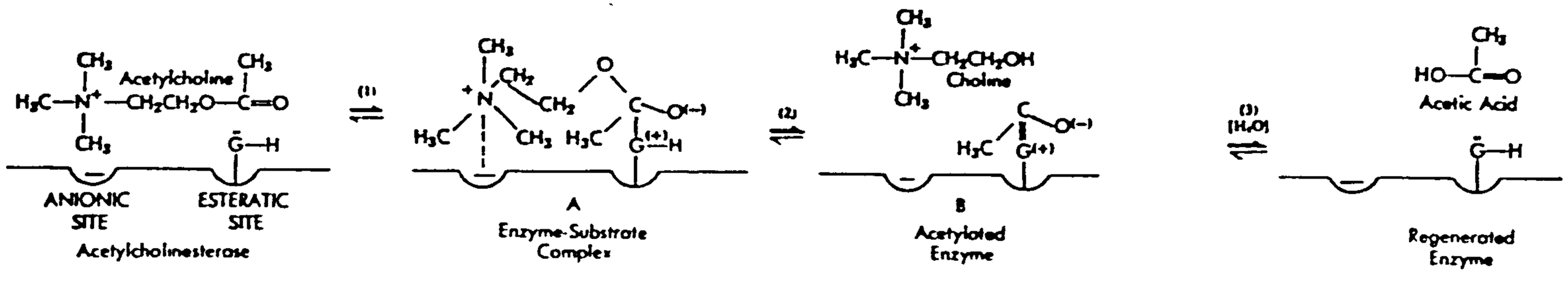
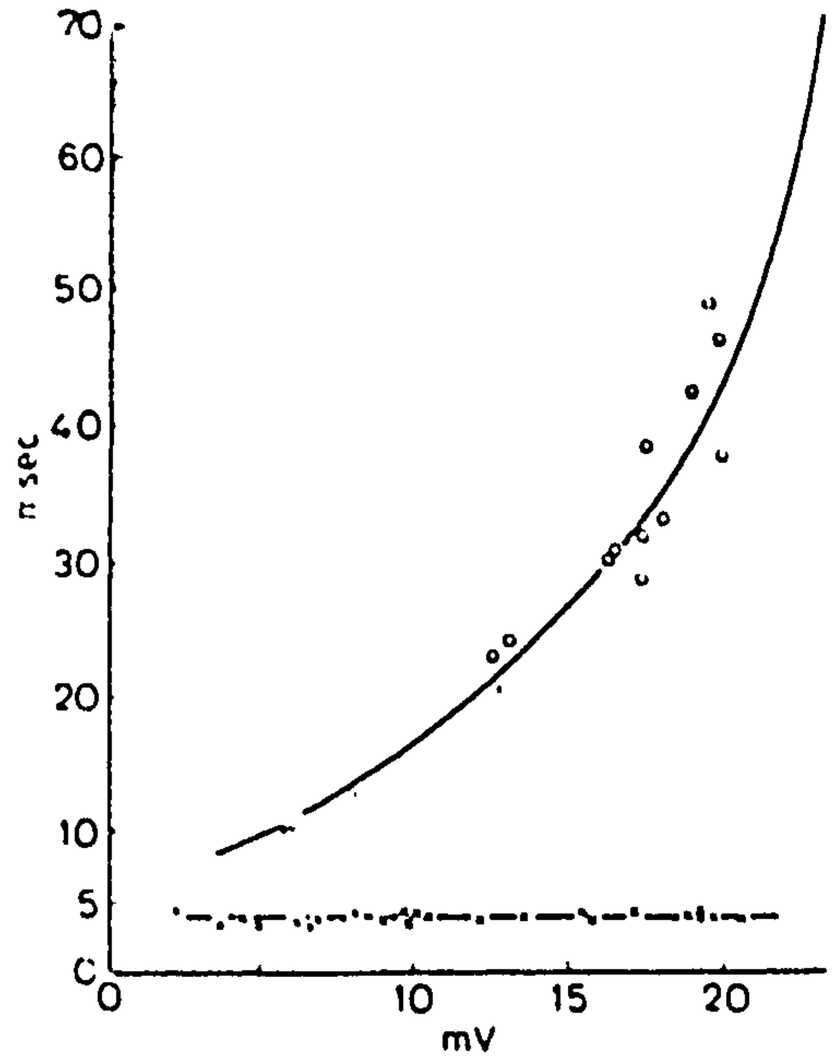


Fig. 3.8 The hydrolysis of acetyl choline by the enzyme acetylcholinesterase
The substrate ACh combines with an active unit of the enzyme to form a complex (A) by electrostatic attraction between the N^+ atom of the choline moiety and the anionic site of the enzyme, and the electrophilic C atom of the carboxyl group and a protonated acidic group of the esteratic site. Choline is then split off (step 2) leaving the acetylated enzyme (B) which reacts rapidly with water (step 3) to produce acetic acid and the regenerated active enzyme
(from Goodman and Gillman, 1980)

3.4.2.2 EPP duration and anticholinesterases

In their early studies Eccles et al (1942) noted that the EPP was always prolonged in the presence of physostigmine. This observation was confirmed later using intracellular electrodes to study neostigmine by Kuba and Tomita (1971). In their studies the muscle action potential was eliminated by either cutting the muscle fibre as mentioned above, or using tetrodotoxin. This toxin eliminates muscle contraction by blocking the propagation of the muscle fibre action potential. In a tetrodotoxin - treated rat diaphragm preparation these authors were able to demonstrate that neostigmine markedly increased the amplitude and duration of EPP to 20 mV or more (see figure 3.9). The rate of rise of the EPP was not presented in this study.

Increase in the duration of the EPP in the presence of an anticholinesterase was also demonstrated by Barstad and Lilleheil (1968). In their experiments, neostigmine, physostigmine and DFP increased both the amplitude and duration of EPP in the isolated rat diaphragm following the application of a single stimulus to the phrenic nerve. These effects were fully reversed by obidoxime which reactivated the di - isopropyl phosphoryl - AChE formed by the organophosphate. Further evidence that anticholinesterases prolong EPP duration was presented by Blaber (1972) using edrophonium. Here, the effect demonstrated was concentration - dependent. In a concentration of 0.1 uMole/l the drug increased the amplitude of the EPP without affecting the time course. With concentrations of 1 uMole/l and higher the amplitude was increased and the rise time and half decay time were prolonged. This effect on the rise time is suprising and is not what might be predicted from a purely post junctional action of the



Effect of neostigmine (3 μmol l.⁻¹ ≈ 1 μg ml) on the amplitude (in mV) and half-decay time (in msec) of e.p.p.s triggered by application of graded pulses to the nerve terminal in the rat isolated nerve-diaphragm preparation bathed in oxygenated Krebs solution containing 1 μg tetrodotoxin ml⁻¹. Crosses represent results in the absence of neostigmine and open circles results obtained in its presence

Fig. 3.9 Action of neostigmine on the amplitude and duration of the EPP (from Kuba and Tomita, 1971)

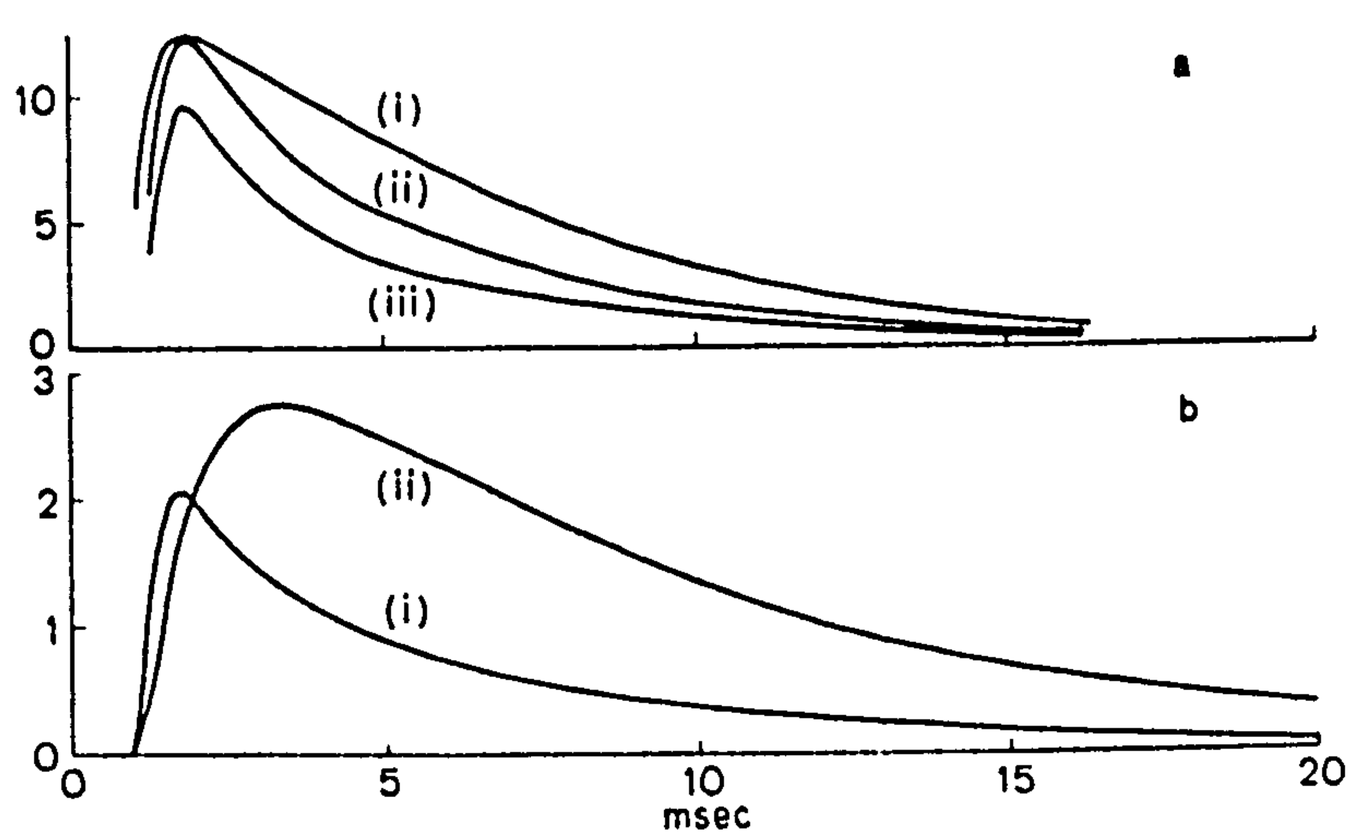


Fig. 21 a and b. Effect of tubocurarine and physostigmine on the e.p.p. of the soleus muscle in the cat. (a) Two successive e.p.p.s are evoked, the first and second being separated by 1.6-msec. i: normal muscle; ii and iii: after successive doses of tubocurarine. (b) e.p.p. produced by single nerve volley in same experiment. i: after a dose of tubocurarine. ii: after doses of tubocurarine and physostigmine; the rate of rise is slower than in (i) because of deeper curarisation. Ordinate: percentage of maximum spike potential. Abscissa: time from nerve stimulus.

Fig. 3.10 Physostigmine action raising the amplitude of the EPP and prolonging the rise and decay time (from Eccles et al, 1942)

anticholinesterase in prolonging ACh life.
Further information about the rate of rise and duration of the EPP comes from a number of studies whose primary purpose was to demonstrate the anticurare action of anticholinesterases. The classical theory of the action of curare centered around its action as a competitive antagonist for post synaptic cholinoceptors, thus reducing the end plate potential. The work of Eccles et al (1941, 1942) showed that when the concentration of dTC at the end plate was such that the EPP no longer reached the critical amplitude (10 - 20 mV) required to trigger a propagated muscle action potential, a nerve impulse arriving at the nerve terminal failed to cause the muscle fibre to contract. The extent of the neuromuscular block was found to be proportional to the number of motor end plates which had ceased to transmit impulses. Reversal of this blockade was recognised to be the characteristic of a large number of anticholinesterases. Explanation of this finding in terms of increase in concentration lead to more studies which gave information about the EPP shape. Eccles observed that in cat soleus physostigmine given after dTC raised the amplitude of the EPP and prolonged its rise and decay time (see figure 3.10). Boyd and Martin (1956) and Blaber and Christ(1967) found similar results with 0.3 uMole/l and 0.1 uMole/l neostigmine respectively in the cat tenuissimus muscle.
Anticholinesterases are thus seen to have a marked effect on the amplitude and shape of the EPP. In biochemical terms this reflects conformational change in the AChR. The effect is normally short term and the SKNMJ regains its former morphology. There is however a possibility of long term effects on the AChR induced by prolonged depolarization. This is discussed further in chapter 4.

3.4.2.3 Measurements of EPP in man

Direct measurements of the end plate potential in humans have been published, but the work of Elmqvist and his colleagues (1964) provided EPP data from and isolated human intercostal nerve - muscle preparation. In their studies, muscle taken surgically from controls and subjects with myasthenia gravis was set up in a perfusion bath and stimulated at frequencies of 2 Hz or more. It should be noted that the muscle taken from the myasthenic patients in this study had been treated with anticholinesterases for long periods. Only the first few stimuli elicited contractions in the majority of patients. EPP could be recorded at end plates after muscle contractions had ceased and were found to be of normal time course. No attempt was made in this study to block the muscle fibre contraction by cutting or using dTC. When EPP were recorded in the myasthenic muscle, (see fig 3.11) the amplitude of the biggest EPP which were subthreshold varied between different junctions but was in the range 7 - 20 mV which is normal for mammalian muscle. In other fibres even the first nerve stimulus did not result in a suprathreshold EPP. Examination of the MEPP in this preparation showed that spontaneously released MEPP in myasthenic muscle were of a smaller size than normal and had a mean amplitude of 0.2 mV, one fifth of normal. Using the method of del Castillo and Katz (1954) it was found that the quantal components of EPP in this study corresponded closely to the MEPP amplitude. Calculations of the quantum content of EPP at various frequencies showed these to be similar to normal junctions. As the authors were unable to demonstrate any change in postsynaptic sensitivity to topically applied carbachol and decamethonium, which both mimic the action of ACh they concluded that there may be a reduced amount of ACh in the quanta released from the nerve terminals.

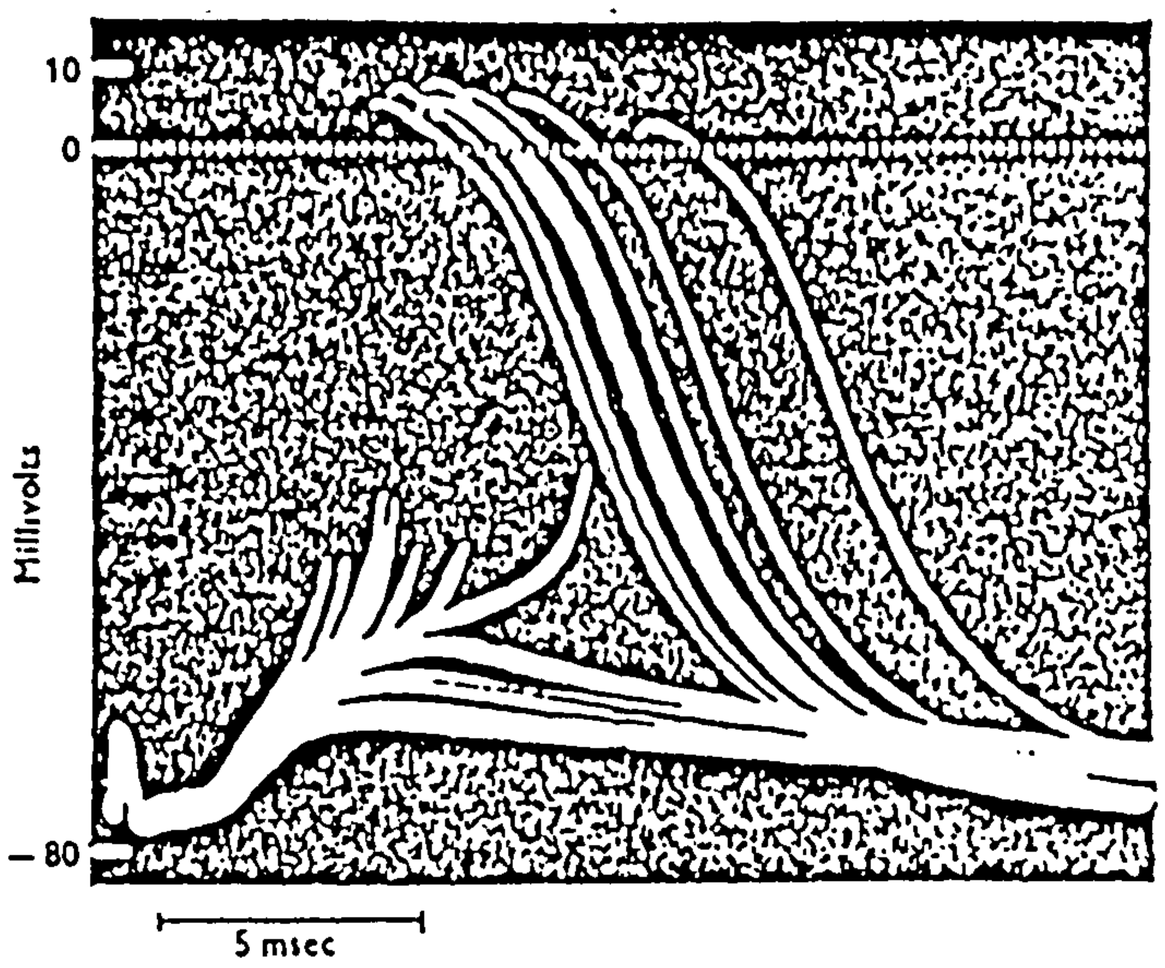


Fig. 3.11 EPP and action potentials recorded intracellularly near the end plate of a myasthenic muscle fibre (from Elmqvist et al, 1964)

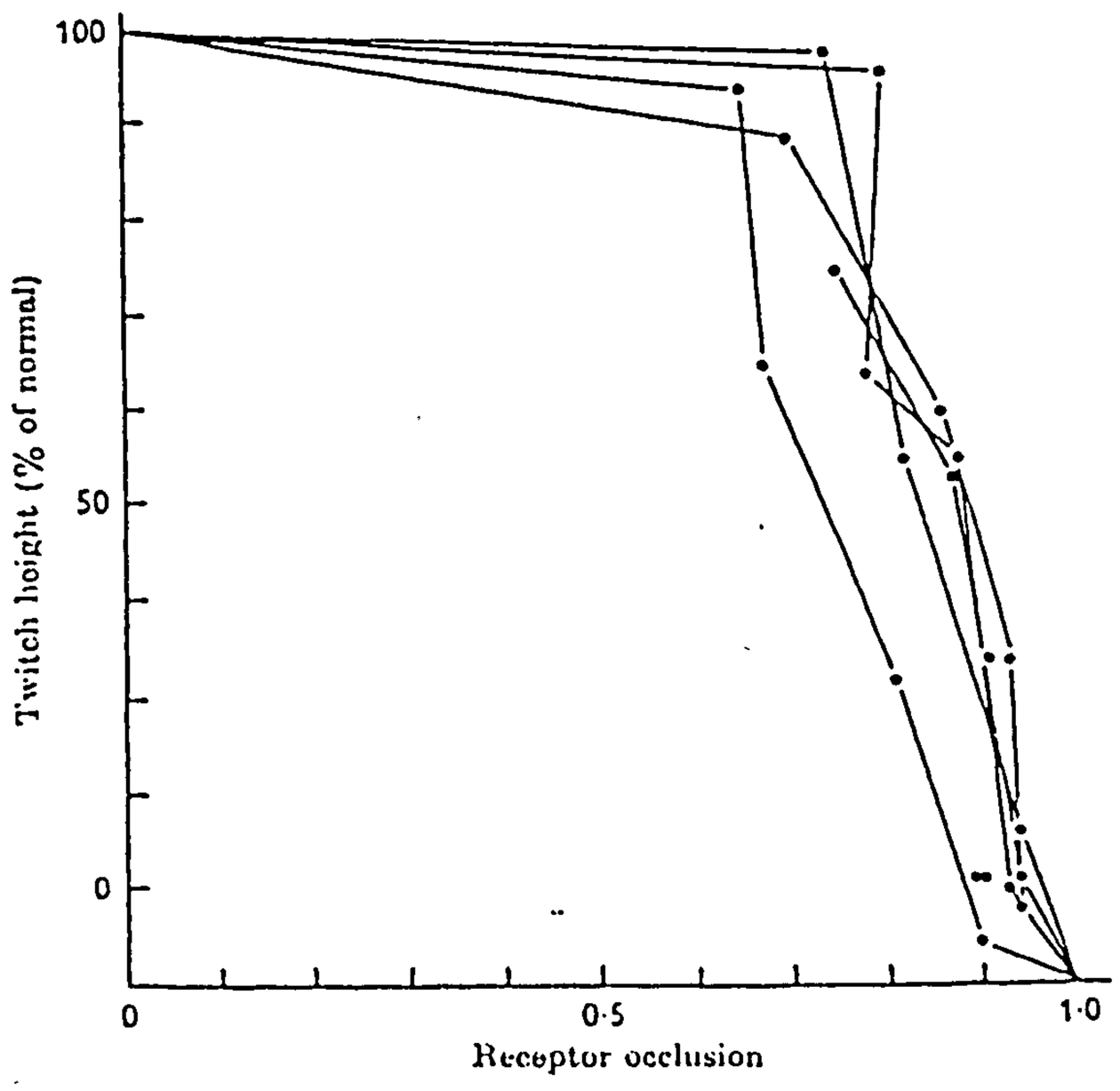


Fig. 3.12 Safety margin at the neuromuscular end plate. Relationship between twitch height and degree of receptor occlusion by a non-depolarizing blocking agent. The sharp downturn in the curve takes place once the safety margin for the fibres has been reached. Once some degree of block is obtained little increase in occupancy is needed to reach deep block. The technique used was the 'dose ratio' method (the ratio by which the dose of an agonist must be increased in the presence of an antagonist to match the original unantagonized response.
(from Paton and Waud, 1967)

3.5 New ideas concerning neuromuscular fade

3.5.1 Introduction

The phenomenon of a diminution of muscle response to repeated stimuli after administration of non - depolarizing relaxant drugs has been extensively studied (for reviews of the clinical aspects of this subject see Smith, 1975; Viby Moegensen, 1982 and Jones, 1985). The fade so produced is essentially a phenomenon occuring in the neuromuscular junction and is distinct from the diminished contraction produced by exhaustion of a muscle fibre after repeated stimulation in absence of a blocking drug. Explanations of neuromuscular fade were, for many years tied to the strict classical interpretation of the concept of chemically mediated neuromuscular transmission (section 3.1). Acetyl choline released from the nerve terminal was held to react only at the post junctional receptor site. Mobilization and release of the transmitter was thought to be a largely passive process. Over the past fifteen years it has become evident that this explanation of neuromuscular events, whilst being essentially correct, requires modification.

The original view concerning ACh release was that the output of transmitter reduced during high frequency stimulation of the nerve terminal. The reason that no apparent failure of neuromuscular transmission occurred was thought to be due to the considerable excess of receptor sites over the minimum required for transmission of the EPP (the 'safety margin,' Paton and Waud 1967, figure 3.12). The explanation of the action of non - depolarizing drugs such as curare was that, by blocking off the excess receptors providing the safety margin, they unmask the waning concentration of ACh in the postjunctional region by

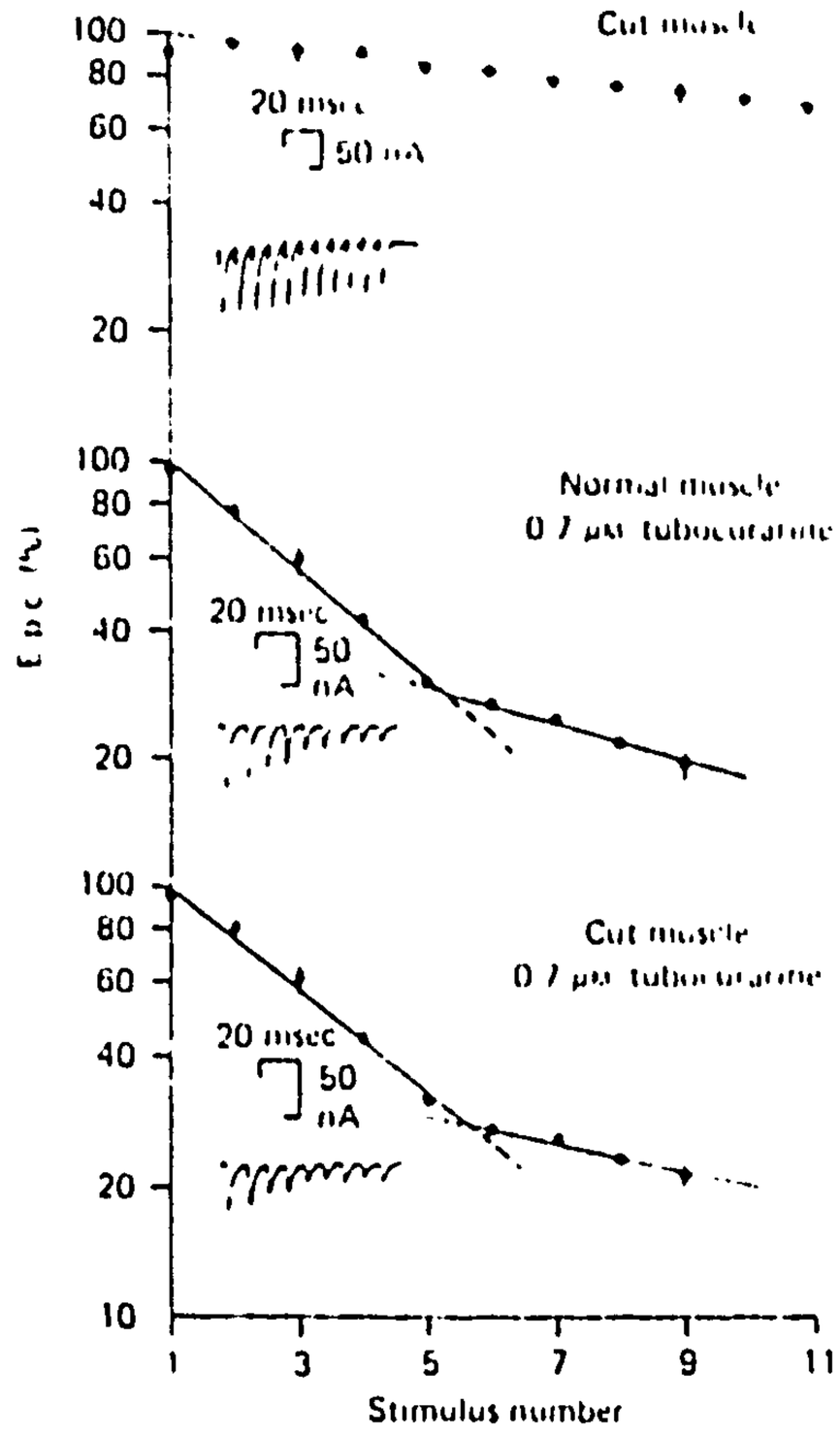
revealing muscle fibres that fail to fire. In clinical terms this is expressed by a diminishing response to repeated stimuli or fade.
Some of the recent modifications to the passive theory of neuromuscular fade are now considered.

3.5.2 The prejunctional effects of curare at the neuromuscular junction

Section 3.2 discussed findings from the technique of intracellular microelectrode recording of EPP. One of the first indications that non - depolarizing muscle relaxants might have actions at sites other than the postjunctional membrane came from the study of run down of EPP or endplate current (EPC) following repeated stimuli. Intracellular electrode study of the neuromuscular junction indicated the possibility that dTC might itself be causing the rundown of ACh release apart from its actions at the postjunctional membrane.
Section 3.4.2 pointed out that to record EPP at the neuromuscular junction measures must be taken to dissociate it from the muscle action potential it generates. This can be done by cutting the muscle fibres on either side of the junction, stretching them over an inflated baloon or treating them with a mixture of glycerol and dantrolene. The experiments of Glavinovic (1979) recorded EPC in voltage - clamped rat diaphragm fibres. In the voltage clamp technique, the normally variable EPP is fixed by application of an external potential and ionic flow through the channels of the ACh receptors is detected by current measurements. At a stimulation frequency of 150Hz there was relatively little diminution of EPC recorded from end plates not treated with dTC. However if dTC was used there was seen to be considerable run down in EPC even if a lower stimulation frequency of 100 Hz

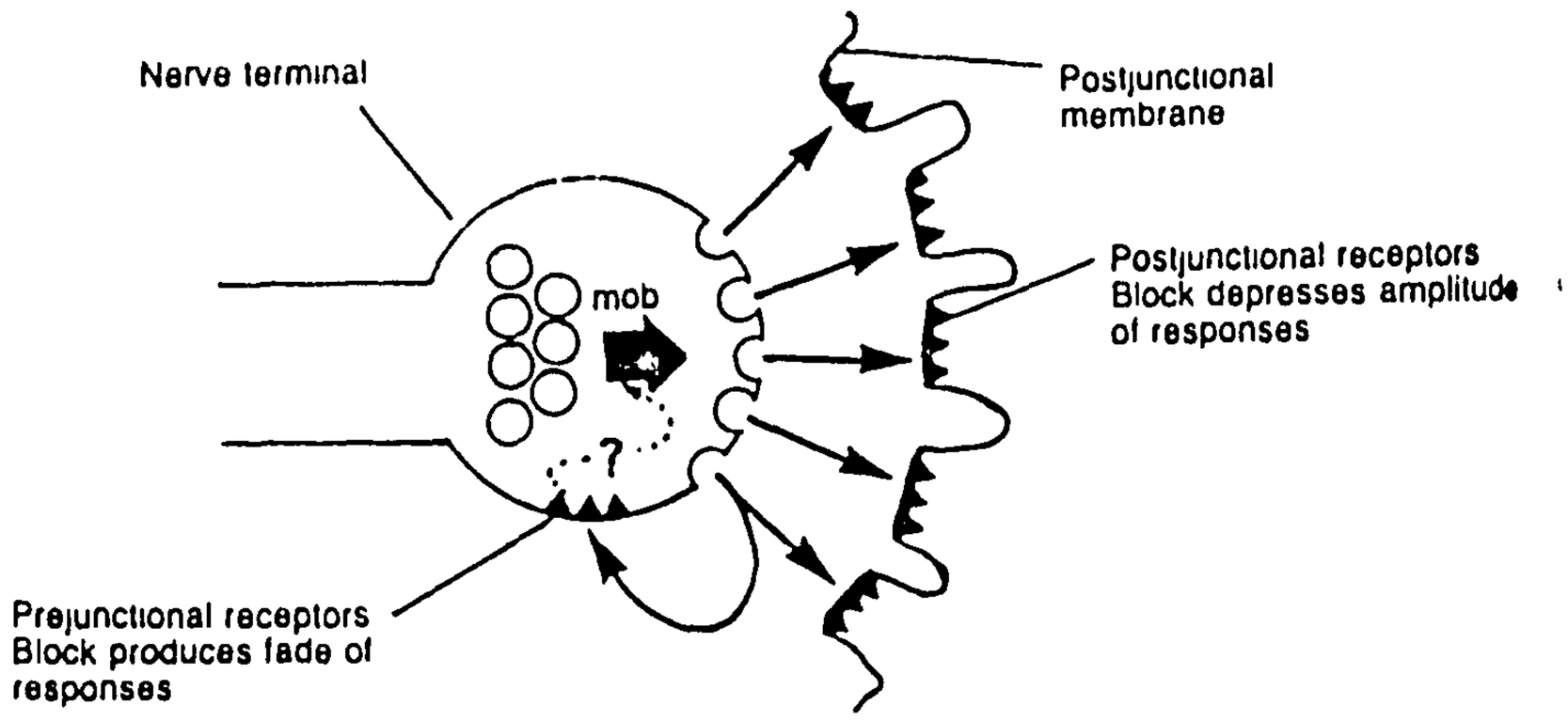
was used. These experiments indicated that dTC itself might be causing the run down in EPC (see fig 3.13). There has been some disagreement in the literature about the direct effect dTC has on measured ACh release. Blaber (1970), using decamethomium as an analogue of ACh, which is an unstable and difficult substance to handle experimentally, studied the parameters of the end plate potential in the cat tenuissimus muscle. The fibres were cut on either side of the end plate to prevent contraction and generation of muscle action potentials. Blaber found that decamethonium caused significant increase in the quantal content of the first EPP measured by intracellular electrode recording. This increase was caused by an increase in mobilization rate which in turn caused an increase in the readily available ACh store. DTC in low concentration was found to antagonise this effect. Other findings of this study were that Ca2+ reduced quantal content of the first EPP by 50% by reducing fractional release and that Na+ reduced ACh mobilization rate at the nerve terminal which in turn reduced the available store. However in the latter case fractional release was increased so that there was no significant change in the quantal content of the first EPP. From these observations Blaber concluded that there was a cholinoceptive receptor on the unmyelinated portion of the nerve terminal which probably controlled the influx of Na+ ions (see figure 3.14).

Other workers however (Chang et al, 1967; Krnjevic and Mitchell, 1961; and Straughan, 1960) have failed to detect diminution in the release of ACh by dTC. Bowman (1980) gives three reasons why this may have been the case: (1) the bioassay methods used may not have been sensitive enough to detect a small fall of in released transmitter; (2) collection of the transmitter was invariably carried out in the presence of an anticholinesterase drug to improve the sensitivity of the experiments; the possibility exists



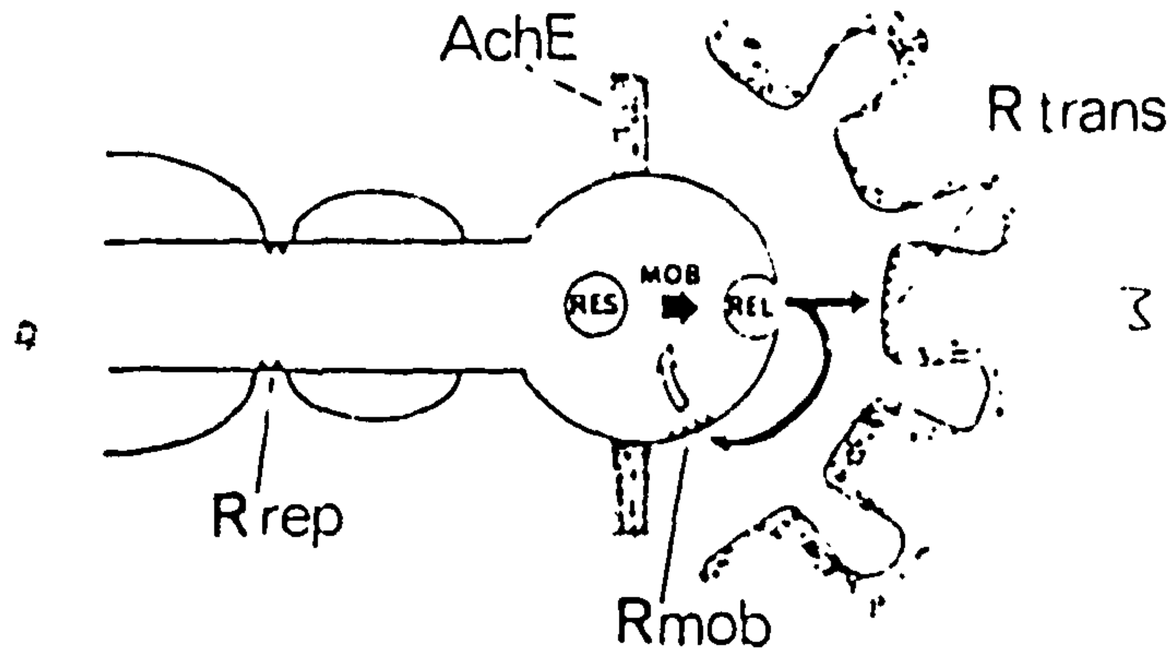
End-plate currents, generated by short tetanic train, plotted against stimulus number. The data represent the average of ten such tetanic trains. The trains were separated by at least 90 sec. The bath was held at room temperature (23° C). The traces of three such tetanic trains in the different experimental situations are also illustrated. Above: short tetanic stimulation (11 pulses at 150 Hz) of a cut muscle in a solution without blocking agents. The line represents the best fit from the 2nd to 11th responses, assuming an exponential decay. The responses are normalized to an extrapolated maximum at zero time. The standard errors (±) of the 3rd and 9th pulse are indicated. The second response is larger than the first, indicating early transient facilitation. Middle and below: short tetanic stimulation (9 pulses at 100 Hz) of a normal and a cut muscle respectively, both in 0·7 μM-D-tubocurarine. Note two phases of decay of e.p.c.s; the first line represents the best fit for the 1st to 5th responses and the second for the 6th to 9th, both assuming exponential decay.

Fig. 3.13 Curare causing a rundown in end plate current (from Glavinovic, 1979)



Diagrammatic representation of the proposed actions of released acetylcholine on prejunctional and postjunctional nicotinic cholinoceptors. Mobilization should be taken to include all those processes that serve to place acetylcholine in a readily releasable situation between nerve impulses, and not merely the movement of vesicles towards the terminal membrane, as suggested by the diagram. The '?' near the prejunctional receptors is intended to indicate that any second messenger involved is unknown. It should be noted that the prejunctional receptors are not those that mediate antidromic repetitive firing in motor nerves under some circumstances

Fig. 3.14 The prejunctional cholinergic feedback receptor



Suggested prejunctional feedback role of transmitter acetylcholine (AchE) in maintaining transmitter output during high frequency stimulation. The diagram represents a nerve ending and the postjunctional membrane of the motor end plate. Transmission is mediated by acetylcholine stimulating the postjunctional receptors (R trans). The released acetylcholine also stimulates prejunctional receptors (R mob), which facilitate mobilization (MOB) of vesicles from the reserve (RES) to the readily releasable (REL) store, so that output of acetylcholine can keep up with the demands of high frequency stimulation. Block of the prejunctional receptors involved (R mob) will cause tetanic fade. All the acetylcholine antagonists block postjunctional receptors (R trans) but their relative affinities for the prejunctional receptors (R mob), and therefore their abilities to produce tetanic fade, differ. alpha-Bungarotoxin has little affinity for R mob, whereas hexamethonium has relatively more affinity for R mob than for R trans. Tubocurarine and pancuronium fall between these extremes. A second population of prejunctional receptors (R rep) located at the first node of Ranvier are represented. These mediate depolarization of the axonal membrane, which may give rise to repetitive firing of the nerve fibers. One of the functions of junctional acetylcholinesterase may be to protect the axons from stimulation, through these receptors, by transmitter acetylcholine.

Fig. 3.15 Postulated positive ACh feedback mechanism (after Bowman, 1979)

that the anticholinesterase itself may modify the ACh release characteristics; (3) some of the ACh released from the nerve terminal is non - quantal (section 3.2.6). It is presumed that the non - quantal release comes from sites other than vesicles and it may not be concerned with transmission. Therefore even a pronounced fall off in the release of the fraction responsible for transmission may be undetectable by assay of the total release.

3.5.3 Fade and single response: animal and clinical studies

Further evidence for a prejunctional effect of non - depolarizing relaxants came from the studies of Bowman and Webb (1976) who investigated the effects of different relaxant drugs on fade and reduction of the single stimulus muscle response. Hexamethonium, dTC and pancuronium were all tested in a cat soleus preparation. All three drugs were found to produce tetanic fade but the degree of twitch depression that accompanied the fade was different in each case. Hexamethonium produced complete tetanic fade to zero with very little depression of the peak tetanic depression. Pancuronium on the other hand produced tetanic fade only in doses that also caused pronounced twitch and peak tetanic tension depression. Curare was found to lie midway between these two extremes. Other studies on fade and muscle single twitch response (Williams et al, 1980) during induction of clinical anaesthesia have confirmed the findings of Bowman and Webb. Williams et al studied onset of paralysis using the train of four technique (section 5.3.1) in twenty five patients undergoing general anaesthesia with relaxation produced by several relaxant drugs. The results indicated that, during induction of neuromuscular block the relationship

between amplitude of the recorded action potential and fade was not constant. When the amplitude of the first potential was reduced to 50% the mean neuromuscular decrement increased in the order pancuronium < dTC < fazadinium < gallamine. In other words, dTC and gallamine were found to have a more pronounced effect on fade than pancuronium and alcuronium which preferentially reduced the level of the first response.

Given that the reduction of single twitch tension or first response of the train of four by dTC and similar drugs can readily be explained by the classical theory of receptor occupancy at the post junctional membrane, the unexpected effects of different non - depolarizing blockers on fade requires an examination of other sites of action of this class of drug. Bowman (1980) has examined two possibilities (1) that the drugs act on the nerve endings to impair the release of ACh and (2) that high frequency stimulation causes the drug to exert a hitherto unexpected postjunctional action that becomes more pronounced as the stimulation increases.

3.5.4 The postulated prejunctional receptor

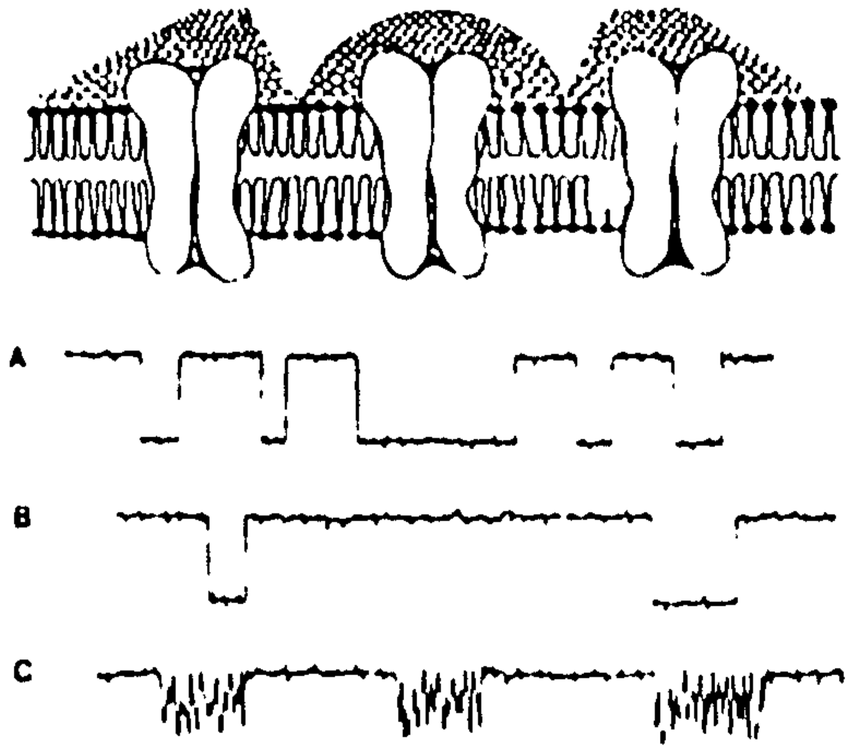
Figure 3.15 shows a mechanism, postulated by Bowman, for a prejunctional feedback mechanism for ACh to maintain transmitter output during high frequency stimulation. Rtrans represents the postjunctional receptors. When these are occupied by ACh, depolarization occurs and transmission takes place to the muscle fibre. On the nerve terminal are situated further receptors, Rmob. These are also thought to be stimulated by released ACh as part of a positive feedback mechanism. The Rmob receptors facilitate mobilization of ACh containing vesicles from the reserve (RES) store to the ready releasable (REL) store so that output of ACh can keep up with the demands of high frequency stimulation. Block of the

Rmob receptors by non - depolarizing relaxants such as dTC will give rise to fade on repeated stimulation. A further receptor Rrep is shown at the first node of Ranvier. This site is included in the expanded receptor theory to explain the repetitive firing that has been demonstrated to occur when anticholinesterases are applied to the nerve terminal (section 1.4.2.1).

3.5.5 Ion channel blocking and fade

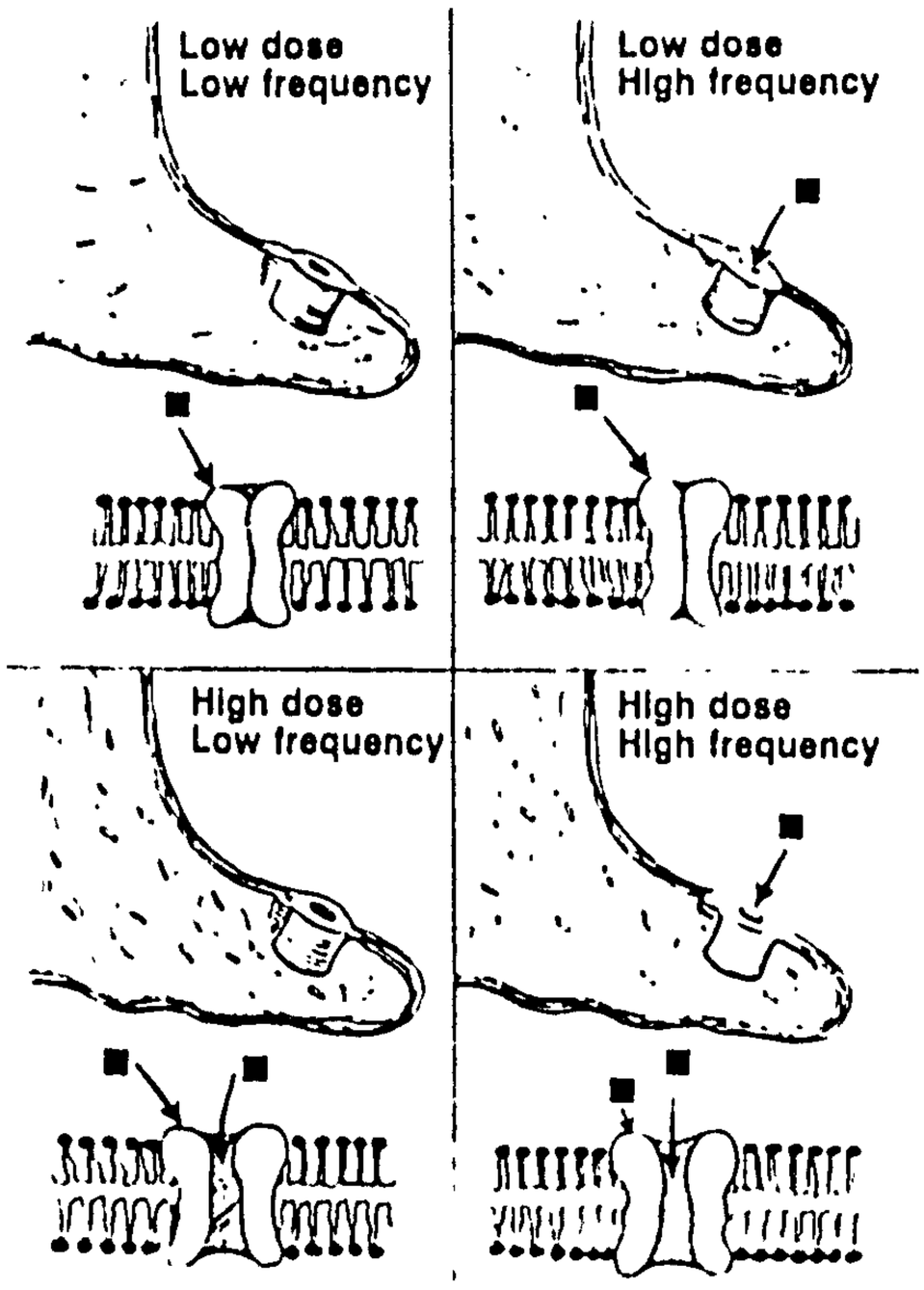
The main alternative to the prejunctional receptor theory of fade is found in the concept known ion channel blocking. The existence of this mode of interfering with ion flow through the post junctional was indicated by electrophysiological analysis using the patch clamp technique (Neher and Sakmann, 1975). In this method, a very fine pipette is used whose tip is just big enough to cover the area of one receptor site. The potential across the channel is held steady at 90 mV by an applied potential. ACh and other relaxant drugs can be applied to the receptor through the pipette so the site becomes an experimental model that can be controlled and recorded. If ACh is applied through the pipette, the channel opens. Normally this would give rise to a depolarization, but because the transmembrane potential is held, or clamped, the response is measured as a current flow as the ions move through the channel. Thus the action of ACh gives rise to a pattern shown in figure 3.16 where the passage of current is indicated.
In the presence of a nondepolarizing drug such as curare, the openings of the channel are seen to be less frequent because of the competitive nature of the drug against ACh in reaching the alpha glycoprotein sites. At high concentrations of curare however the current flow through an open channel is seen to be fluctuating, indicating that the ion channel is being

randomly blocked. Other drugs such as local anaesthetics, have been noted to have this property of generating noise in the current passage of an open ion channel. The explanation is thought to be that when the ion channel is in the open state, certain molecules in the vicinity, including those which may ordinarily react with the receptor site, can enter the channel and interrupt the ion flow by simple plugging and obstruction. Since the blocking molecule can leave as easily as it can enter, the nature of this channel block is transitory. The funnel shape of the channel is thought to explain why ion channel blockers do not pass all the way through the passage whereas smaller ions such as sodium and potassium can.
Considered over the whole endplate, the ion channel blocking produces a reduction of endplate current and ultimately a failure of transmission.
It should be noted from the preceeding discussion that ion channel blockade can only occur when the channel is in the open state. The potential ion channel blocking capability of any molecule depends on its structure and stearic hindrance. In the case of the muscle relaxants different drugs have different capabilities for combining with the AChR or as channel blockers. For example, gallamine is about equally active in both situations whereas pancuronium acts preferentially on the alpha receptor sites, presumably because of its bulky steroid structure (Standaert, 1982). Curare tends to act at the receptors in low doseage but as a channel blocker at high doses.
Because the action of channel blockers is restricted to open passages the process is said to be use - dependent. Hence considerations of the frequency of ACh release at the nerve terminal have bearing in discussion of the relevance of ion channel blocking at the neuromuscular junction.
Dreyer (1982) has summarised three important points about the phenomemon of ion channel blockade;
(1) the effect is intensified by all factors which



Patch-clamp technique with micropipette isolating a single acetylcholine receptor to record current flow (top). Traces in the lower part of the figure represent patch-clamp recordings of conductance through a single receptor in the presence of an agonist (A), tubocurarine and agonist (B), and a quaternary local anesthetic, QX 222, and agonist (C)

Fig. 3.16 Patch clamp technique and ion channel blockade (from Standaert, 1983)



Sites of action of tubocurarine as a function of dose and stimulation frequency (■) = tubocurarine molecule

Fig. 3.17 Relative importance of antagonist and ion channel blockade with dose of non - depolarizing blocker and frequency of stimulation (from Standaert, 1983)

can open more channels such as depolarising relaxants, AChE inhibitors and high frequency stimulation;

(2) normally, ion channel blockade produces only a relatively small effect on the peak amplitude of end plate responses; ordinary neuromuscular transmission with its high safety factor is unaffected unless this factor is reduced; in this situation, the small contribution of channel block may be sufficient to reduce the end plate response below the threshold for generating a muscle potential;

(3) drug - induced channel block cannot be antagonised by AChE inhibitors.

3.5.6 The importance of channel blockade at the postjunctional receptor

With the possibility of two separate drug actions by non - depolarizing relaxants at post-junctional receptor sites the question arises which might be expected to be more important, and under what conditions Standaert (1983) has summarized the theoretical situation for curare in terms of dose and frequency as shown in figure 3.17. At low doses, equivalent to those which produce minimal blockade of transmission, and at low frequencies, equivalent to one shock every few seconds, dTC would be expected to act predominantly at the receptor site to compete with ACh. At higher frequencies but still with low dose of curare, the relaxant affects the nerve terminal causing reduced ACh release. At higher concentrations of curare significant ion channel blocking can occur with low frequencies of stimulation, contributing an element of blockade which is not reversible by anticholinesterases. At both high dose and high frequency of stimulation, the situation is compounded by prejunctional effects.

3.5.7 Conclusions

The two major modifications of the classical theory of neuromuscular transmission, prejunctional receptors and ion channel blocking have been discussed. The case for prejunctional receptors has been argued most strongly by Bowman who criticises the ion channel blocking theory on the following grounds; (1) since, at steady state block the peak tension remains the same and is greater than the degree of fade at the end of the preceeding tetanus, the channel blocking antagonist apparently escapes temporarily from the ion channel during the brief interval between successive tetani; (2) neostigmine abolishes fade, although it might be expected that the increased channel opening by excess ACh would lead to a greater degree of channel blocking. Standaert's position is that ion channel blocking may operate in conjunction with conventional receptor occupancy of pre and post junctional receptors by acetyl choline. The key to the relative contributions appears to be the rate of stimulation.

It is clear from the information presented in this chapter that clinical monitoring of neuromuscular fade clinically will depend on many pharmacological and physiological factors. These have a major bearing on the monitoring of neuromuscular transmission following pyridostigmine pretreatment (section 2.4.3) which is considered later.

The mechanisms presented to explain fade may also have an effect on the rise time of the EPP itself. This point is considered further in chapter 10.

Chapter 4: Long Term Structural and Electrophysiological Effects of Anticholinesterases at the Skeletal Neuromuscular Junction

4.1 Long term changes produced by organophosphates

4.1.1 Introduction

Section 1.3.1 introduced the organophosphate compounds as largely irreversible inhibitors of AChE. In common with the reversible carbamate anticholinesterases such as neostigmine, pyridostigmine and physostigmine they reduce the activity of AChE at the post junctional membrane, giving rise to increased concentrations of acetyl choline. In addition to this action however there is evidence of other junctional changes induced by anticholinesterases.

The observation by Ariens et al (1969) that intravenous infusion of paraoxon (section 1.2) caused necrosis in the striated muscle of the albino rat prompted an extensive series of studies by Dettbarn and his colleagues (for a review see Wecker, Laskowski and Dettbarn, 1978) of the actions of paraoxon and other anticholinesterases on the degree of inhibition of AChE and the associated effects on the nerve terminal and postjunctional sites.

4.1.2 Structural and ultrastructural effects of organophosphates at the neuromuscular junction

4.1.2.1 Myopathic changes

Paraoxon (diethyl-p-nitrophenyl phosphate) which is an active metabolite of many organophosphate insecticides, has been used in most of the studies of induced myopathy considered here. Fenichel et al (1972) gave doses of 0.25 and 0.75 mg/Kg to rats intraperitoneally (i.p.) for up to 12 days. They showed that a histological picture of myopathy was produced in the quadriceps, soleus and gastrocnemius muscles. The earliest changes were non - specific and consisted of degeneration in the central zone surrounded by normal myofilaments, the so - called 'target fibre.' These changes are also seen in neuropathy, familial periodic paralysis, polymyositis and experimental tenotomy. They are distinct from the changes of denervation atrophy which causes a reduction in the number of myofilaments near the sarcolemma (McComas, 1975). The larger dose of paraoxon used by Fenichel et al produced a progressive myopathy up to a ceiling effect on the ninth day after injection. The early central zone changes progressed to a total loss of myofilaments in the centre of the fibre. The filaments adjacent to the central area were severely disorganised and the Z band fragmented. It was notable in this study that overall cytoarchitectural abnormalities were least severe beneath the sarcolemma. It will be shown later that this finding was not found to hold for the area directly beneath the end plate region. Apart from the central damage, the sarcoplasmic reticulum was mildly dilated but the mitochondria, basement membrane and fat droplets were normal. The focal changes

progressed to a generalised breakdown of fibre architecture characterised by a loss of staining quality and finally phagocytosis. Progressive spread with increasing severity was confirmed by longitudinal sections. The number of lesions was scored quantitatively per thousand fibres and was shown to be dose related. Two factors were found to modify this pattern: (1) denervation, which substituted a denervation atrophy for the myopathic changes and (2) hemicholinium, which reduces prejunctional ACh synthesis (McIntosh, 1956) and attenuates the severity of the myopathic lesions.

4.1.2.2 Changes in the end plate region

Laskowski et al (1973) considered the ultrastructural effects of paraoxon on rat diaphragm in more detail. These authors showed that this muscle, removed from rats who were sacrificed after 2 - 5 days of i.p. injections of paraoxon 0.2 mg /Kg (producing an AChE level 35% of normal) exhibited marked changes in ultrastructure of the end plates. The indication was that these were the primary sites for paraoxon in producing myopathy. After two days of paraoxon treatment some nerve terminals contained many coated vesicles and swollen mitochondria. The subsynaptic folds usually contained cleft vesicles of varying sizes. It was apparent that the muscle beneath the subsynaptic folds had lost its architectural organisation whereas the region of muscle away from the end plate was relatively unaffected. In this study, the changes noted could not be related to specific fibre type owing to poor differential staining.

In a further study (Laskowski et al, 1977) structural changes at the end plate were examined after repeated paraoxon injections. Fibres were studied using light and electron microscopy, in animals who had received

0.24 mg/Kg. paraoxon. This experiment showed that myopathic changes had already begun 30 minutes after the first injection. The initial myopathic changes were noted beneath the end plate, but after 6 hours, when the increased acetylcholine release had declined towards control levels, the pathological changes had progressed from the end plate to a small extent. Both electron and light microscopic changes were present at this stage and were seen to progress over the next 72 hours but at a much slower rate. The structural abnormalities confirmed were a disordered cytoarchitecture beneath the end plate, composed of dilated mitochondria and an expanded sarcoplasmic reticulum. The nerve terminals were noted to contain accumulations of coated vesicles and the widened subsynaptic folds contained membrane bound vesicles. Soleus was less severely affected than diaphragm, where the subsynaptic disorganisation continued to expand up to 72 hours after paraoxon. This study indicated that a critical degree (75%) of AChE inhibition was necessary to produce the myopathic changes and that the prejunctional increased transmitter release was associated with the development of the myopathy. Further, it was demonstrated that the oxime 2 PAM (pyridone-2-aldoxime methiodide) restored AChE activity, eliminated antidromic firing (section 1.4.2) and excess transmitter release, and attenuated the myopathy.
In their study published in 1978, Wecker, Kiauta and Dettbarn examined the relationship of AChE inhibition and the development of myopathy for paraoxon, neostigmine and pyridostigmine, both separately and in combination with respect to degree and duration of the inhibition. The time scale of the reversal of the myopathic effects by the oxime 2 PAM was also studied. The results again showed that paraoxon 0.23 mg/Kg, a dose sufficient to cause muscle fasciculations for two hours, produced a grouped muscle necrosis evident 24 hours after a single injection. 2 PAM was given

between 10 and 120 minutes after the organophosphate injection and during this time period variably reactivated phosphorylated AChE in both junctional and non - junctional regions of the rat diaphragm. The time interval between the injections of paraoxon and 2 PAM was found to be positively correlated to the severity of the myopathy. The myopathy was dose dependent and an 85% inhibition of AChE during the first hour after injection was found to be asociated with maximal fibre breakdown.

4.1.2.3 Relationship of structural changes to fibre type and action

Subsequent work (Wecker and Dettbarn, 1976) indicated that the changes might be fibre-type specific. Diaphragm, soleus and gastrocnemius muscles, which are all very distinct in their predominant fibre type (section 6.9.2), speed of contraction and inherent metabolism, were studied. Myopathic changes were shown to be more severe in diaphragm with its predominance of slow contracting (type 1, section 6.7.1) fibres. Soleus, which has a majority of intermediate fibres and gastrocnemius, which contains mainly fast fibres were less severely affected. The authors speculated that the changes might be related to the rate of neural activation of the muscle. Over a 24 hour period, diaphragm receives a larger number of impulses to its slow, repeatedly active fibres than gastrocnemius which is active only intermittently. In a further experiment in rats given paraoxon, immobilization of the limb muscle on one side was shown to produce protection against the subsequent development of the myopathy compared with a freely active contralateral limb.
A summary of myopathic changes after organophosphate exposure is given in table 4.1.a

4.1.3 Electrophysiological changes produced by organophosphates at the neuromuscular junction

4.1.3.1 Changes in MEPP and EPP activity

Together with the structural studies reviewed above, Laskowski and Dettbarn (1975) have examined the effects of paraoxon on the electrophysiology of the muscle end plate. In studies of rats given a single dose of 0.5 mg paraoxon i.p., the animals were sacrificed at periods after injection ranging from 30 minutes to 24 hours. Some had the diaphragms removed and analysed for AChE activity. From others, the diaphragms were removed and set up in vitro as a perfused preparation with the phrenic nerve attached. MEPP analysis was done using conventional microelectrode techniques (section 3.2). In addition, EPP were analysed using the standard variance technique (Martin 1966). It was found that following a single dose of paraoxon the MEPP frequency rose by a factor of 38 from 2.88 $\pm$ 0.23/sec to 109 $\pm$ 13.1/ sec. This increase was found to return to control levels six hours after the organophosphate injection. By contrast, the quantum content (the number of discrete ACh packages release from the nerve terminal) of the EPP was reduced from 137.8 $\pm$ 14 to 92.9 $\pm$ 8.0 and the quantum size (the amount of ACh in each package) doubled. Accompanying the increase in quantum size, the amplitude of the first EPP nearly doubled from 1.56 $\pm$ 0.13 to 2.49 $\pm$ 0.19 mV. The cholinesterase activity in diaphragms removed 30 minutes after giving paraoxon was 30% of normal. When the phosphorylated enzyme was reactivated with 2 PAM, the raised MEPP frequency was reduced significantly and antidromic activity was abolished. This action of

the oxime in reversing the effects of paraoxon was taken as evidence that paraoxon was acting through its cholinergic rather that a direct action. The fact that 2 PAM did not reduce the raised MEPP frequency completely to normal was attributed to its quaternary structure. The oxime would therefore be expected to reactivate only surface ChE, whereas the highly lipid soluble paraoxon inhibits both surface and axonal ChE and could therefore lead to a build up of ACh in the nerve terminal which would be unaffected by oxime.

4.1.3.2 Comparison of acute and chronic effects

In a further study (Carlson and Dettbarn 1983) the acute and chronic effects of paraoxon on spontaneous transmitter release were compared. Paraoxon (0.23 mg/Kg) was injected into rats i.p. daily for 14 days. MEPP frequency was determined from the isolated phrenic nerve - diaphragm preparation as outlined above, on the 3rd, 8th and 14th day of paraoxon injections and compared with the MEPP frequency measured 1.5 hours after the first injection. The single injection of paraoxon was again shown to elevate the frequency of MEPP to three times the control value in 34% of the fibres studied. The elevated frequencies were observed in areas of extensive muscle twitching and were shown to be associated with a high incidence of 'giant' MEPP (an MEPP having an amplitude more than two times that of a standard MEPP, Dunant, 1985).

Chronic exposure to paraoxon first reduced the overall MEPP frequency to below control level with many fibres showing no spontaneous activity after three daily injections. The authors were uncertain whether the latter observation was due to alteration in postsynaptic sensitivity (Chang et al, 1973) or to nerve terminal destruction, since very low MEPP frequencies were observed in the remaining active

fibres. During more prolonged paraoxon exposure, the MEPP frequency returned to control level and a greater percentage of the fibres studied exhibited spontaneous activity. The MEPP amplitude however remained depressed and only a few fibres showed increased MEPP frequency.

The implication of these observations is that there is a difference between the recovery of pre and post synaptic lesions caused by organophosphate exposure, an observation noted by Tiedt (1978) for neostigmine. The MEPP frequency elevating effect of single dose paraoxon was only noted again one week after stopping daily injections.

4.1.3.4 Mechanisms of electrophysiological changes produced by organophosphates

The apparent dual effect of paraoxon on the MEPP frequency after chronic administration lead to speculation about the action of the organophosphate at the nerve terminal site. A possible direct depolarizing effect of the drug on the terminal was discounted after examination of the potassium release curves for control, acute or chronically exposed preparations which were at variance with any observed depolarization which could be produced by ACh, paraoxon or excess potassium. The failure of increasing the potassium concentration to produce a rise in the MEPP frequency suggests that paraoxon increases the ratio of spontaneous quantal release by a mechanism which is independent of any depolarizing action of paraoxon. The association of paraoxon treated fibres with high frequency MEPP and with an increased incidence of giant MEPP suggested that the action of paraoxon on uniquantal release is connected in some way with presynaptic factors which give rise to giant potentials. Heuser (1974) has suggested that giant MEPP may be due to coalesced vesicles. These

waveforms are known to be produced by neurotoxins and 4 aminopyridine, presumably by increasing the duration of the calcium influx during the presynaptic action potential.

Further studies to investigate the mechanism of damage were made by Dettbarn (Dettbarn 1984) using soman (GD), an irreversible organophosphate (see section 1.2) and phospholine, an organophosphate which can exist in a tertiary and quaternary state. The tertiary state allows better membrane penetration than quaternary. Both these agents produced a progressive dose related necrosis in the fibres of rat skeletal muscle. The severity of the myopathy depended on a critical decrease in activity and duration of ACh inhibition. In this work, the inhibition of AChE was examined in some detail for extensor digitorum longus (EDL) and soleus. Soman was shown to have a greater effect upon the AChE of soleus than that of EDL. The number of muscle fibres affected was greatest after exposure to this agent, followed by tertiary phospholine, paraoxon and quaterary phospholine. Adult rat skeletal muscle has been shown to contain three major forms of AChE 4S, 10S and 16S (section 3.4.1). Non - endplate regions contain the 4S and 10S forms whereas the 16S form is found mainly in the end plate zone. In the soleus muscle quaternary phospholine was found to cause a significantly greater inhibition of the 16S, 10S and 4S forms than in the EDL. This allowed the conclusion that in soleus all three major molecular forms are more externally located than in EDL. Also, reduction of the 16S form to less than 50% may be critical enough to induce the mechanisms that lead to necrosis, since in soleus further reduction of the 16S activity by the tertiary phospholine did not cause an increase in the number of necrotic fibres. Repair of these necrotic fibres was noted within one week. In this study the presynaptic effects of the organophosphates were studied further by the use of atropine and gentamycin. These drugs

were studied in concentrations that did not block neuromuscular transmission and were found to attenuate the necrotic action of organophosphate by interaction with the presynaptic Ca 2+ uptake mechanism.
A summary of the electrophysiological changes produced by organophosphates is given in table 4.2a.

4.2 Carbamates and the neuromuscular junction: Morphological and neurophysiological effects

The anticholinesterase effects of carbamates were discussed in relation to pyridostigmine, in section 2.3. In parallel with the studies of the actions of organophosphates at the neuromuscular junction reviewed in the previous sections, several papers have been published which examine the ultrastructural and electrophysiological modifications caused by carbamate anticholinesterases.

4.2.1 Ultrastructural alterations caused by carbamates at the neuromuscular junction

4.2.1.1 Pre and post junctional changes

The observation (section 6.12.1.1) that patients with myasthenia gravis have a simplification of the post junctional regions and a decrease in the mean nerve terminal area prompted Engel et al (1973) to question whether these changes might be a result of long term treatment with carbamates rather than a pathological manifestation of the disease itself. In their experiments both red and white fibres of gastrocnemius, soleus and diaphragm were studied in rats who received neostigmine 0.05 mg/Kg subcutaneously (s.c.) initially, increasing to 0.4 mg/Kg over the next eight days and then continued at that level. The choice of these three muscles was made in view of the different proportions of muscle fibre type (section 6.9.2). The findings indicated that even after 140 days of treatment some end plates showed no abnormality. In others however the following structural changes were present after only 42 days:

(1) post synaptic vesicles were abundant in both red and white end plates; (2) there was degeneration of

post synaptic fibres in both soleus and diaphragm but not in gastrocnemius, indicating that red end plates are preferentially affected; (3) the post synaptic folds became atrophic and residues collected within widened synaptic clefts; (4) in the nerve terminal the mean mitochondrial area was increased and the presynaptic membrane facing the primary synaptic cleft was partially and sometimes completely covered by a Schwann cell process; (5) the mean synaptic vesicle count, the mean nerve terminal area and the mean postsynaptic area associated with a nerve terminal were not affected by neostigmine in either gastrocnemius or soleus end plates. These last findings are in contrast to the picture usually presented by myasthenia gravis (McComas, 1977). The study concluded that long term treatment with carbamates could lead to eventual side effects in neuromuscular transmission and possible refractoriness in the treatment of the disease. The study did not measure the degree of inhibition of AChE by neostigmine.

4.2.1.2 Effects of carbamate exposure on the number of AChR

Chang et al (1973) studied the influence of chronic neostigmine treatment on the number of AChR in rat diaphragm end plates using the technique of alpha bungarotoxin binding. This compound, extracted from the venom of the Taiwan banded krait, binds irreversibly with ACh receptors. Rats were given 0.1 mg neostigmine s.c. twice daily for seven days. After this time the number of receptors per end plate was reduced from 2.11 $\pm$ 0.12 X 10exp7 in controls to 1.22 $\pm$ 0.21 X 10exp7 in treated animals. The total content of ACh in the hemidiaphragm was found to be unchanged. This experiment is of particular importance, but unfortunately has not so far been repeated by any

groups studying organophosphate exposure in the same context.

Ward, Forbes and Johns (1975) found that in rats receiving 0.1 mg neostigmine/ day for 7, 30 and 100 days there was simplification of postsynaptic membranes with reduced post junctional folding and widened synaptic clefts. Moreover, animals treated for 100 days showed occasional multiple junctional regions. Not all structural effects demonstrated have been long term. Hudson et al (1978) showed that neostigmine causes morphological alterations within half an hour of injection increasing in severity with increasing treatment. A decreased synaptic vesicle density was noted within the motor terminal.

4.2.1.3 Myopathic changes

Myopathic changes similar to those noted by Dettbarn and his co - workers have also been noted for carbamates by Kawabuchi et al (1976). They found that ultrastructural observations in the rat soleus muscle, two hours after a single injection of neostigmine showed dramatic localised lesions in the areas of muscle fibres adjacent to the motor end plates. In the areas just below the end plate myofibrils were stretched and severely disorganised with Z - like dense material being increased in the sarcomeres. In the sarcoplasm many dense bodies, vacuoles and swollen mitochondria were also observed. Studies on rats repeatedly treated with neostigmine showed that the sarcoplasmic changes such as vacuoles, dense bodies and swollen mitochondria disappeared from the first week onwards, whereas focal lesions showing increased density of Z like material in the disorganised myofibrils could still be recognised up to the eighth week. At the end plate itself, the width of the primary synaptic cleft was found to range between 500 and 900 Angstroms. Secondary synaptic clefts were

also found to be widened. The authors remarked on the similarity of these carbamate - induced ultrastructural changes with those induced by DFP or paraoxon.
A summary of the structural effects induced by carbamates is shown in table 4.1b.

4.2.2 Electrophysiological actions of carbamates

4.2.2.1 Effects on MEPP and EPP

Table 4.2b summarizes the results of several studies on the effects of carbamates on miniature MEPP frequency and height together with quantal content and EPP size. Although dose magnitude and time schedules varied, all studies where MEPP amplitude was measured show that this parameter is reduced by carbamates. MEPP amplitude reflects the number of receptors at the post junctional site which are available for interaction with ACh. We have seen in the previous section that Chang et al (1973) showed directly that the number of ACh receptors was reduced by carbamates. In addition, these authors showed by organ bath analysis that following neostigmine 0.1 mg b.d. for 7 days ACh release was reduced prejunctionally. MEPP frequency and quantal content studies, reflecting information about ACh release from intracellular electrode measurements, are divided about the effects of carbamates. Roberts and Thesleff (1969) were unable to detect any overall change in MEPP frequency, although the EPP quantal content and the number of quanta available for ready release were reduced. In another study in rats given neostigmine, Engel et al (1973) found both MEPP frequency and quantal content to be unaffected but MEPP amplitude was reduced. Ward et al (1975) used challenge tests to estimate the prejunctional effect of neostigmine.

Normally guanidine, 2,4 di-nitrophenol and increasing K+ concentration increase the MEPP frequency. Neostigmine was found to inhibit these tests reversibly. Gillies and Allan (1977) studied both neostigmine and pyridostigmine and found that the rate of release of ACh quanta at high stimulation rates and the frequency of MEPP was reduced by both drugs. At the lower stimulation rate of 1/sec the number of quanta released was not altered by pyridostigmine but was reduced to 52% by neostigmime. This study showed that pyridostigmine reduced the MEPP amplitude to 54% of control, indicating its post junctional activity. Tiedt et al (1978) showed reduction in MEPP height and frequency, and reduced quantal content and EPP size in rats given neostigmine 0.1 mg b.d. for three days. The presynaptic effects (decreased MEPP frequency and decreased EPP quantal content) appeared maximal afer three days of neostigmine treatment but were almost completely recovered by 22 to 25 days of continuous treatment. MEPP amplitude however remained depressed after up to 106 days of treatment along with the severe morphological alterations of the postsynaptic membrane, including degeneration of the junctional fold crests and the appearance of vesicular debris in the widened synaptic clefts referred to previously (Hudson et al 1978).

4.2.2.2 Effects of carbamates on ion channel current

Using the technique of measuring the end plate current with a voltage patch clamp over an ACh receptor and the associated ion channel Albuquerque and his associates (Albuquerque et al, 1984) have extended the present knowledge of the electrophysiological actions of carbamates at the neuromuscular junction. In experiments carried out in both frog and rat muscle using pyridostigmine and

physostigmine these authors have shown both drugs to have effects at the post junctional site unrelated to their anticholinesterase activity. Physostigmine was noted to decrease the end plate current decay time constant suggesting that this drug possesses ion channel blocking properties with the channel in the open state (section 5.4). The experiments also suggested that pyridostigmine reacts with the ACh receptor as a weak agonist capable of inducing desensitisation alone and in conjunction with ACh.

4.3 The AChE inhibition link between organophosphate and carbamate anticholinesterases

The previous sections have shown that both organophosphates and carbamates are capable of inducing both ultrastructural and electrophysiological changes at the neuromuscular junction. Relationship of their actions to the degree of AChE inhibition is difficult in many cases because of the problem in estimating AChE activity in a reversible complex such as that formed by the carbamates. Thus Wecker, Kiauta and Dettbarn (1978) did not attempt estimates of AChE activity in their comparative study of physostigmine, neostigmine and paraoxon. It was found that administration of physostigmine and neostigmine in concentrations which caused 15 to 20 minutes of fasciculations caused little or no muscle necrosis. These findings were similar to the fasciculations caused by 0.11 mg/Kg of paraoxon which reduces AChE activity to 20% of normal. When given repeatedly (two to four administrations of between 0.1 and 0.3 mg/Kg) within a three hour period to prolong both the ChE inhibition and the visible nerve and muscle hyperactivity, physostigmine and neostigmine were found to induce a muscle necrosis qualitatively similar to paraoxon 0.23 mg/Kg.

The prophylactic effect of carbamates on the damage caused at the end plate by organophosphates is discussed elsewhere (section 2.4.3). The work of Wecker, Kiauta and Dettbarn (1978) also showed that prophylactic administration of the reversible inhibitors in doses which produce little or no neuromuscular hyperactivity is effective against induction of fibre necrosis by paraoxon. This may indicate an alternative explanation for the protective action other than reversible complexing of ACh with the carbamate.

4.4 Conclusions

The studies reviewed in this chapter have shown that both carbamate and organophosphate anticholinesterases have structural and electrophysiological effects at the neuromuscular junction. These effects are in addition to their normal action against AChE. It should be noted however that the experiments described have been conducted in rat and amphibian muscle and the relevance to human muscle is, at best, speculative. Both types of anticholinesterase affect nerve terminal and post junctional sites, leading to modified ACh release and fewer postjunctional receptors with which ACh can react. The interrelationship of pre and post junctional effects of the two anticholinesterases may be complicated and cause significant effects on EPP rise time and the subsequent generation of MAP. This possibility is considered further in chapter 10.

Authors	Study	Findings
ORGANOPHOSPHATES		
Ariens (1969)	iv paraoxon	Necrosis in striated muscle
Fenichel et al (1972)	ip paraoxon, 12 days	Quadriceps, gastrocnemius and soleus showed central zone degeneration; dose related progressive myopathy modified by hemicholinium
Laskowski et al (1973)	ip paraoxon 2 - 5 days; 0.2 mg / Kg (AChE 35%)	Nerve terminal changes after 2 days; loss of muscle architecture below subsynaptic folds
Wecker and Dettbarn(1976)	ip paraoxon	Myopathic lesions related to fibre type in severity; diaphr->soleus>gastrocnemius
Laskowski et al (1977)	paraoxon 0.24mg/Kg	Myopathic lesions evident at 30' after injection; initial changes below end plates but spreading at 6 hours; nerve terminal changes seen; critical AChE inhibition 75%
Wecker, Kiauta and Dettbarn (1978)	single injection of paraoxon 0.23 mg/Kg	Necrosis at 24 hours; reversible by 2 PAM
CARBAMATES		
Engel et al (1973)	neostigmine 0.4 mg/Kg, 8 days	Some muscle fibres of gastocnemius, soleus and diaphragm showed no change at 140 days; others showed post - synaptic fibre degeneration red > white
Chang et al (1973)	neostigmine, 0.1 mg/Kg sc for 7 days	Number of ACh receptors / end plate halved after 7 days
Ward, Forbes and Johns (1975)	neostigmine, 0.1 mg/Kg for 7, 30 and 100 days	Simplification of post - junctional membranes with reduced folding and widened synaptic clefts
Hudson et al (1978)	neostigmine 0.1 mg/Kg	Progressive post junctional changes; decreased synaptic vesicle density at the nerve terminal
Kawabuchi et al (1976)	long term neostigmine	Myopathic changes 2 hours after injection still visible after 8 weeks with repeated doses; widened primary cleft and similarity of changes to organophosphate myopathy

Table 4.1. Structural changes in rat neuromuscular end plate after treatment with organophosphate and carbamate anticholinesterases

Study	Drug	MEPP freq	MEPP amp	Quantal content	Quantal size	EPP size	Notes
CARBAMATES							
Roberts and Thesleff (1969)	Neostigmine 0.1 mg bd 5 - 7 days	→	↓	↓			No of quanta available for release reduced
Engel et al (1973)	Neostigmine 50 - 400 µg/Kg bd	→	↓	→			ACh release reduced
Ward et al (1975)	Neostigmine 0.1 mg/Kg	→	↓				Absence of |MEPP freq to challenge tests; MEPP shape unchanged
Gillies and Allen (1976)	Pyridostigmine 3.2 mg/day	↓	↓	→ *			*but reduced at high rate of stimulation Data here are at 1 Hz
	Neostigmine 0.8 mg/Kg/day	↓	↓	↓			
Tiedt et al (1978)	Neostigmine 0.1 mg bd	↓	↓	↓		↓	Electrophysiology normal after 25 days but structural alterations persisted to 100 days (see table 4.1
Duncan and Publicover (1979)	Neostigmine Edrophonium Physostigmine	↓					Frog study
ORGANOPHOSPHATES							
Laskowski and Dettbarn (1979)	Paraoxon	↑↑		↓	↑	↑	AChE activity 30% normal; MEPP freq normal 6 hr after exposure; MEPP change reversed by oxime
Carlsson and Dettbarn (1983)	Paraoxon 14 days 0.23 mg/Kg	↑ then ↓	↑ (giant MEPP)				Difference between acute and chronic effect

Table 4.2 Electrophysiological changes produced by organophosphates and carbamates: all studies are in rat except where indicated

CHAPTER 5: The Clinical Measurement of Muscle Activity

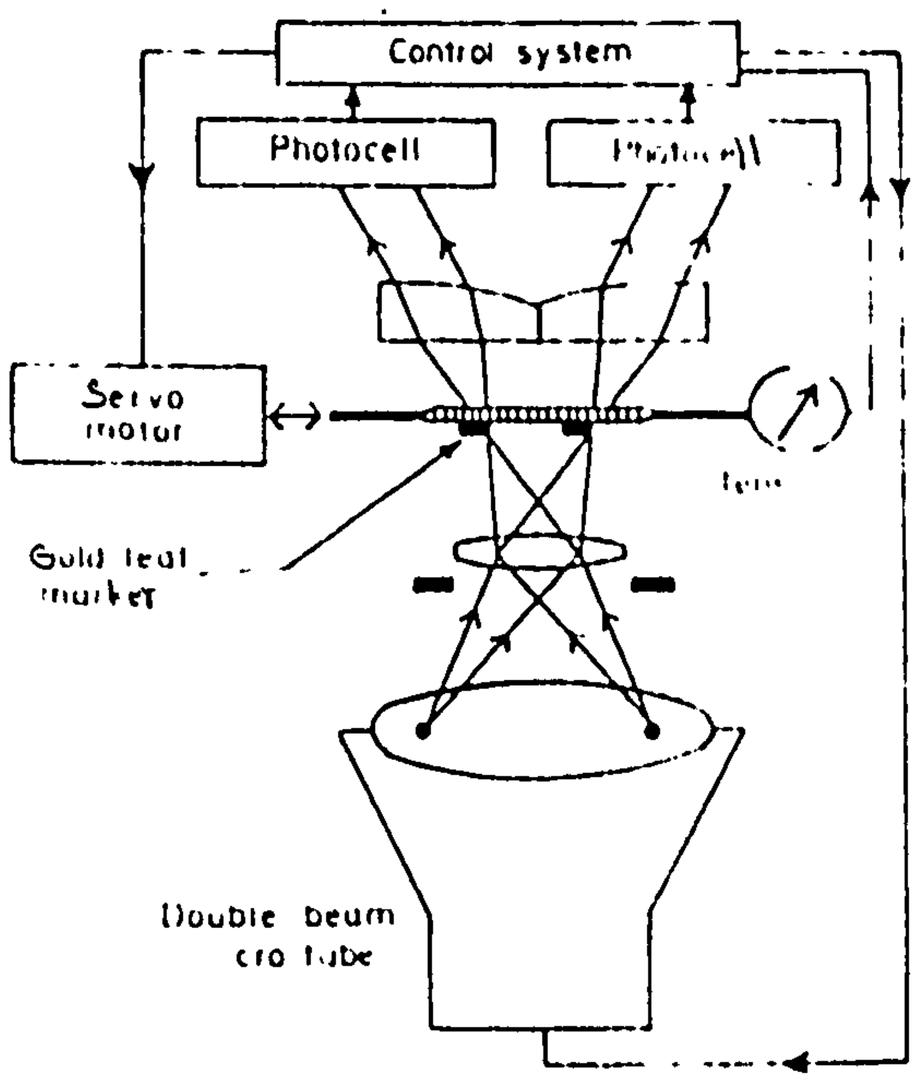
5.1 Introduction

The controlled measurement of muscular activity is important clinically in conditions where contraction may be modified by disease or drugs. In clinical neurophysiology decrement testing to repeated stimuli is standard practice in assessing conditions of muscle weakness. The growth in the use of muscle relaxants in anaesthetic practice has been accompanied by interest in measuring their action. Early assessments that were essentially non - invasive, such as measurement of grip strength, head raising, eye opening and tongue protrusion have been correlated with, and in some cases replaced by more accurate methods of recording muscle function. The field has been extensively reviewed (Ali and Savarese, 1976, 1983; Smith, 1976; Viby - Mogensen, 1982; Hughes, 1984; Jones, 1985). In addition to measuring the force produced by a stimulated muscle its activity can be followed by study of the resultant action potential (the electromyogram or EMG) from the individual potentials of the component fibres. This approach has also been applied in the field of anaesthesia.

5.2 Problems in measuring muscle activity: isometric and isotonic contraction

5.2.1 Experimental recording of muscle action

Measurement of the mechanical action of a muscle depends on whether the muscle is fixed or able to contract during the period of stimulation. This topic has been reviewed by McComas (1977). When a muscle is fixed and the tension developed is recorded without fibre shortening the contraction is said to be isometric. Most actions performed by muscles are under conditions where shortening of the fibres is possible. Such a contraction is called isotonic. During isotonic contraction muscles are able to shorten and lift the load applied to the tendon. Under these conditions external work is performed. Most experimental muscle recording however is under isometric conditions. Since the muscle is prevented from shortening it develops tension at the points of attachment of the origin and tendon. The biochemical mechanism of contraction, by the formation of bridges between the actin and myosin protein components of the muscle fibre, remains the same irrespective of the conditions of contraction. The degree of contraction produced in a single fibre depends on the amount of overlap of the actin filaments over the the central myosin rod. This is shown diagrammatically in figure 5.1. As the fibre is gradually stretched, the force becomes maximal and then gradually diminishes. This relationship was established by Gordon, Huxley and Julian (1966) using single muscle fibres marked with gold leaf. With the aid of a servo mechanism the contractile force developed could be measured with a strain gauge.



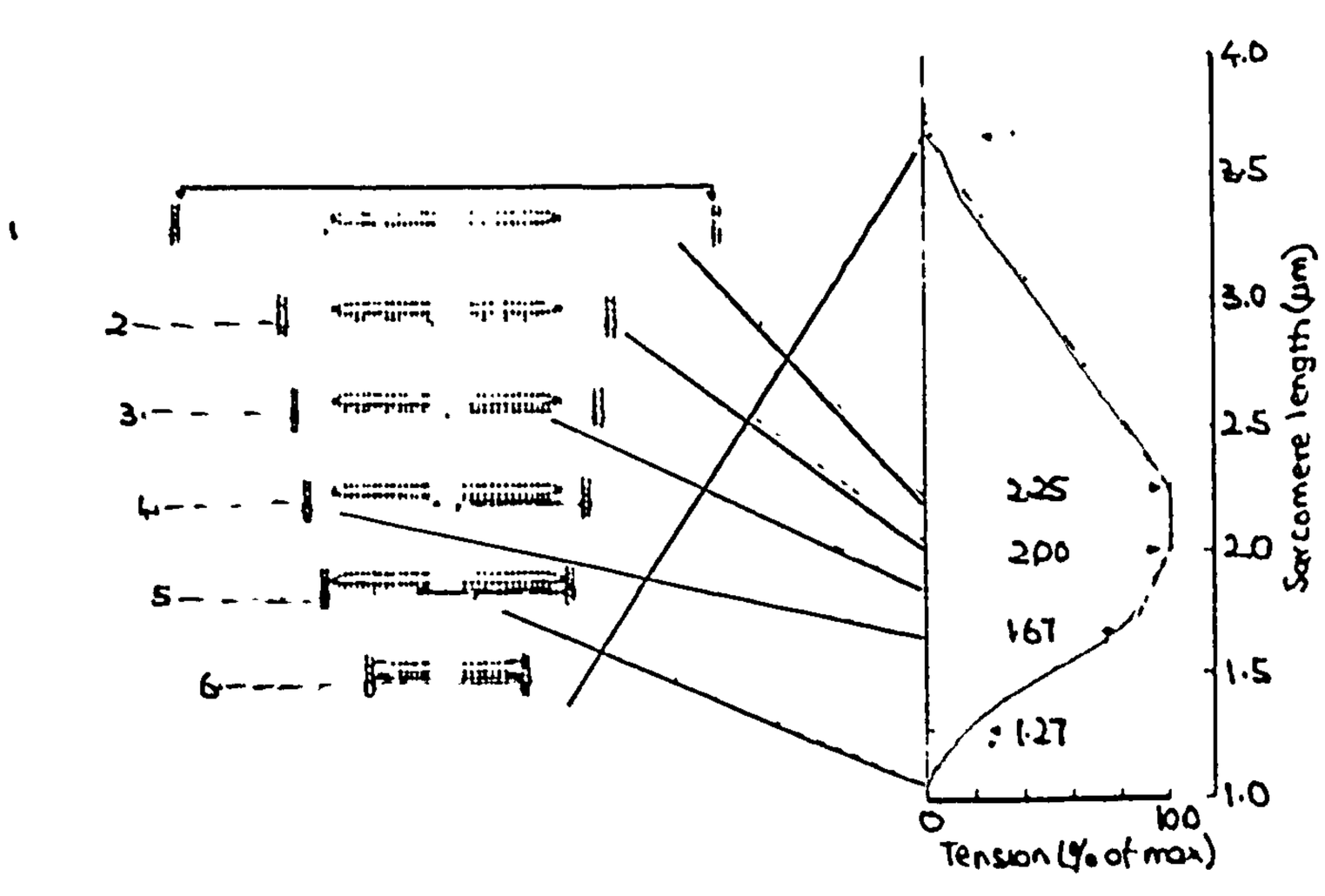


Fig. 5.1 Force during isometric contraction: study of Gordon, Huxley and Julian (1966). Muscle contraction is controlled using a twin beam oscilloscope and a servo mechanism. The lower diagram shows the tension developed with different degrees of overlap of the actin filaments over the central myosin rod. The arrows in 1 indicate the two Z lines. 2 - 6 show the different tensions developed at various overlap lengths. (from McComas, 1977)

5.2.2 Inaccuracies in the measurement of muscle contraction

Isometric study of the contraction of a whole muscle has inherent inaccuracies because the ends of a muscle fibre will tend to contract at the expense of the middle region and also because some of the force of contraction is absorbed in elastic fibres within the muscle tendon complex. Sica and McComas (1971) have studied the isometric twitches in the extensor hallucis brevis muscle of subjects of varying ages. Their results show a large dependence of the degree of tension developed on the age of the subject and the degree of stretch applied to the fibres before stimulation. The concept of elasticity in a muscle is very important in influencing the form of the muscle response developed during single twitch stimulation. McComas points out that if it were not for the series elastic component the tension developed during a single isometric twitch would rise very rapidly to the maximum value commensurate with the permitted degree of overlap between the actin and myosin filaments. Instead, much of the mechanical energy produced by the sliding action of the filaments is used in stretching the elastic component within the muscle and tendon rather than developing tension at the attachments of the tendon.

Replacing the single twitch by a repeated or tetanic stimulation gives sufficient time for the elastic component in the muscle to be fully stretched and for the full tension developed by sliding filaments to be recorded. McComas points out that the ratio of tension developed between a single twitch and a tetanic stimulus is 0.2 in cats but only 0.1 in humans. The existence of the elasticity component, which is thought to reside in the myosin cross bridges, has considerable consquences for the comparison of mechanical with electrical activity of the muscle.

5.3 Evoked muscle responses

Muscle responses to stimuli applied to the controlling nerve can be measured more accurately than voluntary activation. Practical analysis of the response can be made on (1) single twitches, at a frequency of 0.1 - 0.2 Hz; (2) tetanic stimuli at various frequencies; (3) post tetanic single twitches; (4) repeated stimuli given at a frequency of 2 Hz over two seconds. These stimuli are termed the Train of Four (TOF, Ali et al, 1971). Single twitches are of little value in assessing muscle block, because partial blockade of the post junctional receptors is only manifest as fade when the muscle itself is stressed by repeated stimuli. In some muscles it is possible to have a 100% twitch compared with the control when three quarters of the receptors are blocked (Paton and Waud, 1967). For this reason some form of assessment of muscle action based upon repeated stimuli is mandatory.

5.3.1 The TOF test

Single stimuli of muscle whose end plates are partially blocked with a non - depolarizing blocker may show no diminution in muscle response compared with the control. The degree of paralysis is only revealed by the stress of a repeated stimulus. The question of which challenge is the most appropriate therefore arises. Since most human muscles are activated at between 15 and 50 Hz many workers felt that only some form of tetanic challenge would be acceptable (Epstein and Epstein, 1973). However since assessment of muscle activity is often required on subjects who are awake, the considerable discomfort produced by such stimuli is usually unacceptable. The TOF test was devised as a compromise between the

single twitch and tetanic challenge. It is readily acceptable to subjects who are awake and has been extensively studied to correlate the fade produced with muscular blockade and clinical parameters mentioned above (Viby - Mogensen, 1982). Study of trains of stimuli greater than four has shown that further fade after the fourth response is minimal and so the four stimuli can be regarded as an optimum challenge (Lee, 1975). The test can be used clinically without calculating the fade ratio (defined as the height of the fourth compared with the first response). Lee (1975) showed that by simply counting the number of responses visible as muscle twitches a good estimate could be made of muscle blockade (see fig 5.2). If all four responses are clearly seen the block is less than 75%. When the fourth response disappears the block is 75%. The disappearance of the third and second twitches corresponds to 80% and 90 - 95% block respectively. This simple test, which requires only a muscle stimulator is of value in the operating theate. Where muscle response can be measured, with the TOF test no control value is required. Ali et al, (1971) showed that during recovery from paralysis induced during general anaesthesia there was a straight line response between fade and the first response of the four (see fig. 5.3). Further, the TOF ratio was found to be in direct proportion to the ratio of the first response to a measured control. The implication of this observation was that a set degree of TOF fade indicated a certain level of muscle blockade. At a ratio of 0.7 the first response was 100% of the control value and there was good correlation with the clinical signs of recovery from relaxation, such as head raising and inspiratory effort. Critics of TOF have pointed out that despite its convenience as a test, the information gained may be of doubtful relevance to a physiologically activated muscle. During a tetanus with fade, peak tetanic tension is

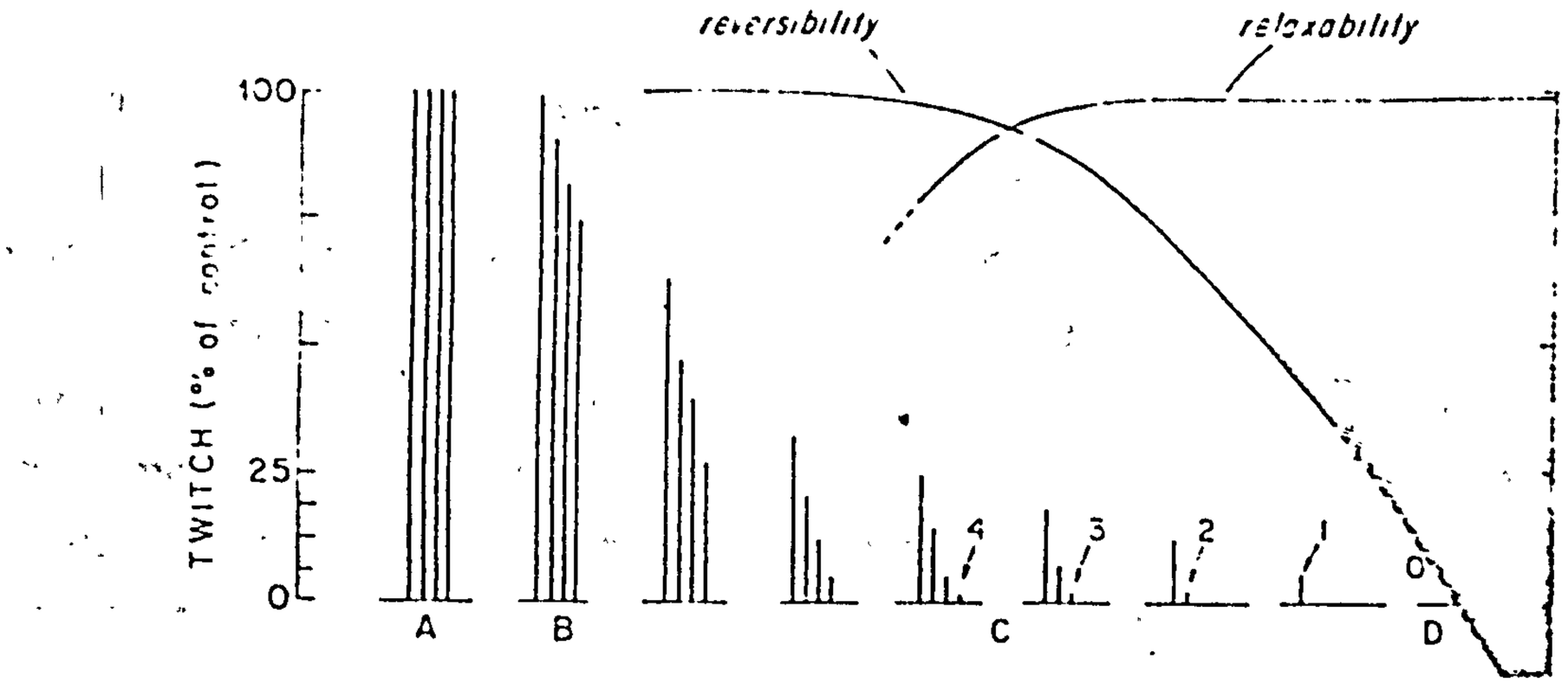


FIG. 4. "Train-of-four count", relaxability and reversibility of curariform block. A, B, C and D correspond to the equivalent points labelled with the same letters in figure 3. After the train-of-four ratio becomes zero, C, disappearance of the 4th twitch is followed by disappearance of the 3rd, the 2nd and finally the 1st twitch, as neuromuscular block (measured as diminution of the first twitch) progresses from 75% block to total block. Regarding relaxability, an 80% block should provide satisfactory abdominal relaxation, provided adequate anaesthesia and a clear airway are maintained (Katz, 1967a, 1971). Regarding reversibility, the difficulty to reverse the block will increase rapidly, as shown by the rapidly declining reversibility curve, if the block is more profound than 80% depression of the twitch, as can be seen from figure 5, it may become very difficult (Baraka, 1967), beyond point D To assess both profound block and reversibility, in the "grey zone" between C to D, use the "train-of-four count", 4, 3, 2, 1 and 0 as shown.

Fig. 5.2 The train of four count
(from Lee and Katz, 1980)

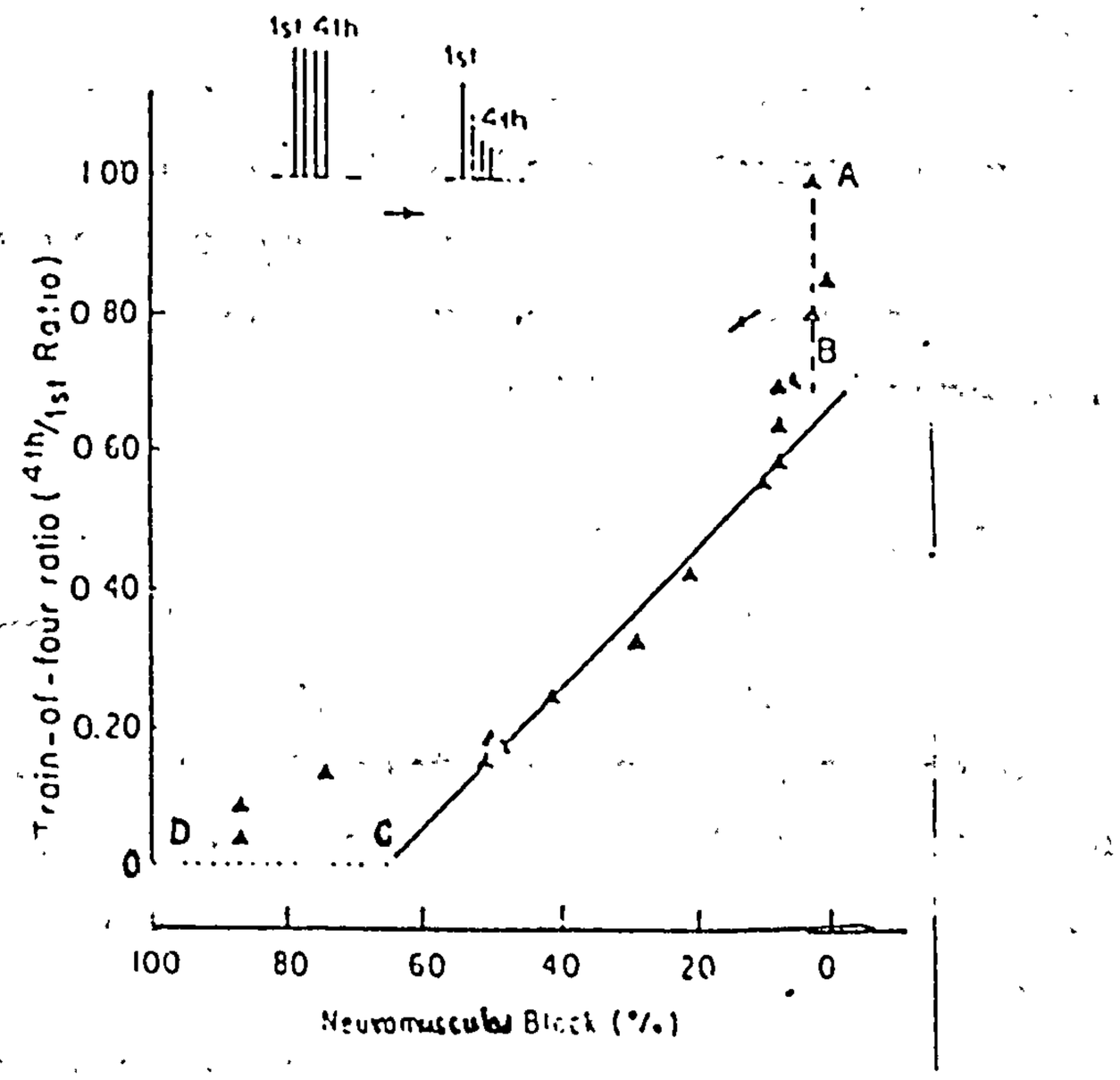


Fig. 5.3 Relationship between train of four ratio and muscle relaxation
(from Lee and Katz, 1980)

developed at a different time from minimal muscle response. TOF gives no indication about the the degree of force produced by a contracting muscle. Despite this, the nature of the test makes it particularly suitable for studies on volunteers who are undergoing trials with muscle relaxants and it has found wide application in monitoring of patients who are recovering from balanced general anaesthesia.

5.4 The isolated forearm technique

Most studies of the action of muscle relaxants have been carried out on subjects who are deliberately paralysed as part of a balanced anaesthetic technique. This means that much information has to be gained from patients who may be receiving quite different combinations of drugs and vapours apart from the muscle relaxant. Vapours such as halothane interfere with muscle studies since they alter the contractile process within the muscle fibre independently from events at the neuromuscular junction. Thus Katz (1973) commented for a need for standardization in the way muscle studies were done if valid comparisons were to be made.

The isolated forearm technique (Feldman and Tyrell, 1970) was devised to overcome the need for whole body paralysis and the necessary positive pressure ventilation by testing relaxant drugs in a tourniquet - isolated forearm. Small quantities of relaxants can be injected intravenously, diluted in normal saline. Most of the relaxant is fixed to the receptor sites within three minutes, at which time the cuff can be released. Feldman and Tyrell reported only occasional diplopia from unfixed relaxant which was introduced into the blood stream after release of the tourniquet. The effects of the relaxant can be followed by stimulating the ulnar nerve and recording the response as a thumb twitch produced by adductor pollicis. The

twitch is detected by a strain gauge. It was noted in section 5.2 that recording conditions must be strictly isometric, and this produces one of the main difficulties of the technique. Modern twitch analysers have features in their construction to ensure that this requirement is met (section 7.10.3). The isolated forearm technique has also been used in experiments where the muscle response is detected electrically as a compound electromyogram (Brown et al, 1975). This arrangement has the advantage that isometric conditions are not necessary for recording.

5.5 Comparison of mechanical and electromyographic measurement of fade

EMG has been used for some years as an alternative to twitch measurement or mechanomyogram (MMG, Crul, 1983) in assessing the degree of activity in a partially paralysed muscle (for reviews see Epstein and Epstein, 1975 and Calvey, 1984). The relative simplicity of recording the EMG signal, the accessibility for study of almost any muscle that could be stimulated and the lack of need for an isometric recording system seemed to offer considerable advantages over the conventional mechanical recording systems. EMG is finding increased use, particularly in the field of anaesthesia.

5.5.1 Measurement of muscle response from the compound EMG

The EMG arises as a resultant of many single fibre muscle action potentials. It can be recorded either from a concentric needle electrode in the muscle or from conducting pad electrodes placed over the covering skin. EMG is usually recorded from an

arrangement where one electrode is placed over the belly of the contracting muscle and the other over the relatively electrically silent area of the tendon. Several different techniques have been used to assess the compound EMG. From direct measurement either the single upward going phase of the wave or the double phase, as indicated in figure 5.5 may be measured. In addition, the wave may be integrated. Nightingale et al (1966) reported that the amplitude of the upward going phase of the EMG was closely correlated to the area under that part of the waveform and so could be taken as an estimate of the total electrical activity. Pugh et al (1984) measured a compound EMG in anaesthetised subjects and found good correlation between peak to peak (positive to negative) height and the area under the positive and negative phases.

5.5.2 Direct comparison of EMG with MMG

The clinical responses to the train of four test discussed in section 5.3.1 were derived from mechanical assessment of muscle fade. Studies comparing the EMG with the MMG have been carried out by several groups of workers with variable results. A major problem has been to compare results gained under different conditions of anaesthesia and test stimulations. De Jong and Freund (1967) recorded simultaneous EMG and MMG in adductor pollicis following a 5 second stimulation at 40 Hz in patients anaesthetized with nitrous oxide and halothane. Comparing tetanic muscle tension with single peak EMG height after paralysis with suxamethonium or decamethonium, they found that fatigue of muscle tension and fade of EMG amplitude changed proportionally with increasing doses of relaxant. The authors concluded that under the conditions of their study EMG and MMG could be used interchangeably. They also claimed a close association between EMG and MMG

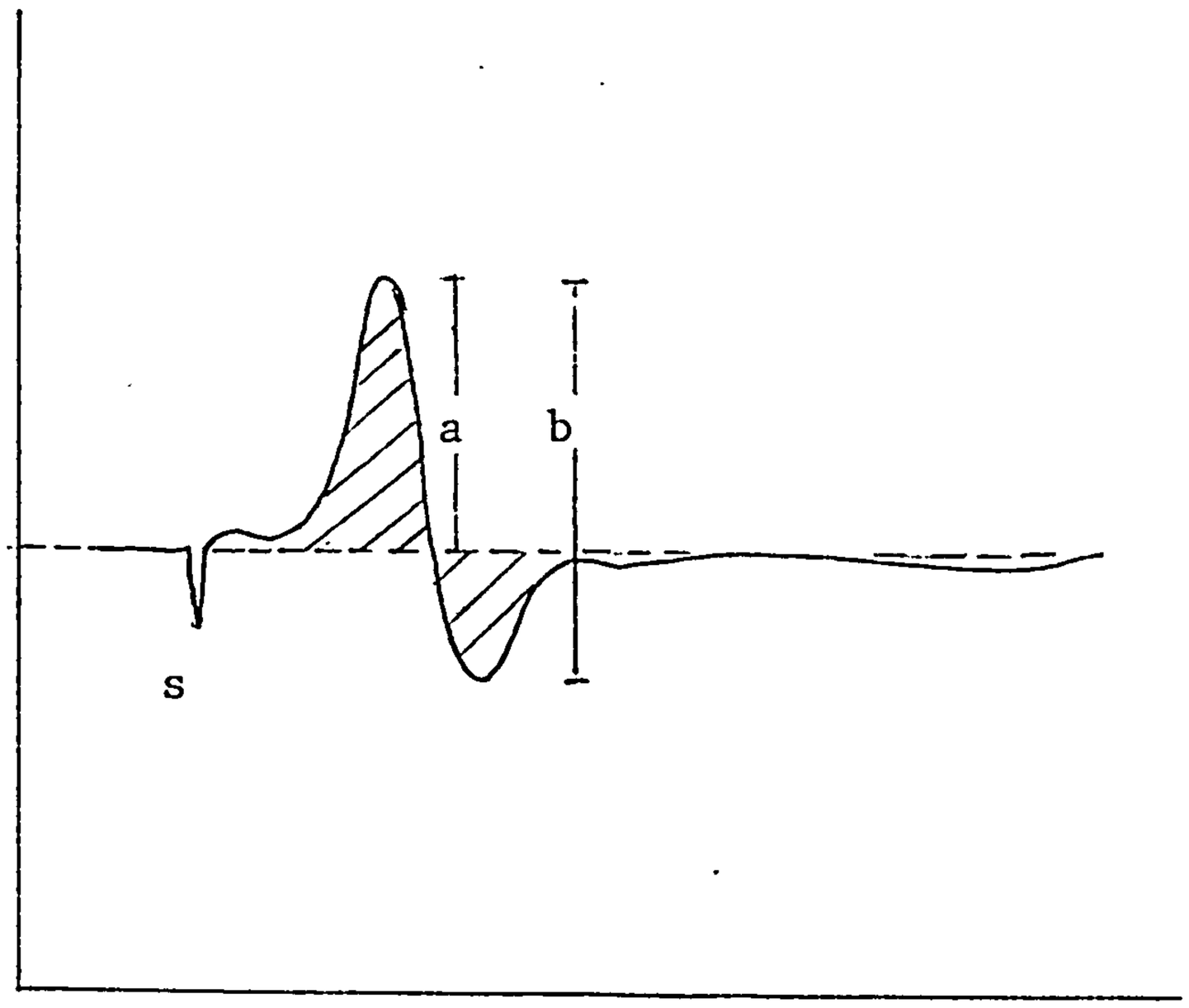


Fig. 5.4 Quantitative evaluation of the evoked electromyogram. Single peak height has been shown to be correlated to the area under the upward - going section of the wave. Peak to peak height has also been shown to be correlated to the areas under both parts of the wave.

a = single peak height

b = peak to peak height

s = stimulus artefact

in the unanaesthetized subject but gave no data. In his EMG and MMG study of adductor pollicis and abductor digiti minimi paralysed with suxamethonium, Katz (1973) found conflicting results for 0.1 and 50 Hz stimulations. The mechanical response was usually less sensitive to suxamethonium than was the thenar or hypothenar EMG. However, when the muscles were paraylsed with dTC the MMG was usually more sensitive than the EMG recorded from either muscle. From these findings Katz proposed that, wherever possible both the mechanical and electrical response to nerve stimulation should be studied due to the lack of comparability between the two systems in this study. Epstein and Epstein (1973) compared MMG and EMG recordings from adductor pollicis stimulated at 0.1 and 30 Hz for 5 seconds in patients anaesthetised with nitrous oxide and halothane. They noted a wide variation in the development of twitch tension and maximal depression of the EMG single peak in the eight subjects studied. Although mean MMG tension and EMG single peak height corresponded roughly to each other, the MMG amplitude depression was always significantly greater than the EMG depression. Following tetanic stimuli, EMG was always found to exceed MMG fade. In their discussion of these findings, the authors considered some of the problems of comparison between the EMG and MMG. An isometric system and correct positioning of the transducer at right angles to the direction of action of the muscles are important considerations. Disparity between EMG and MMG may exist because of the time scale of events involved. The generation of the muscle action potential is usually complete within 10 - 15 msec but the full contraction of the muscle fibre may take several times as long. Crul et al (1983) have published a comparison of integrated EMG with MMG in the adductor pollicis which shows good correlation for first response and TOF fade. No details of the numbers included in the study or the anaesthetic procedure

used are included in this paper. The authors claim that their results correlate agree with those of Epstein and Epstein (1973). However the latter state that 'while dTC depresses both mechanical and electrical responses we cannot confirm the claim that the two methods are equal. Although in our subjects there was a roughly linear relation between depression of the normalised mechanical twitch and the EMG, depression of the two to identical degrees was not seen.'

5.6 Conclusions

The response of a partially paralysed muscle can be assessed either mechanically or electrically. These measurements can be made during general anaesthesia or in the isolated forearm of conscious volunteers. The relationship between MMG and EMG has not been found to be entirely interchangeable and there are indications that the fade response, as measured by the TOF test may be quite different in each case.

Chapter 6: Single Fibre Electromyography

6.1 Introduction

In chapter 5 the evoked EMG was described as a means of measuring muscle activity. This chapter considers the measurement of muscle action potentials (MAP) from single muscle fibres in man and reviews the findings of the technique in several clinical conditions. The subject was reviewed extensively in the monograph by Stalberg and Trontelj (1979).

6.2 Conventional electromyography

Clinical EMG is recorded by pad (section 5.5.1) or needle electrodes. The concentric needle electrode, shown in figure 6.1 has a recording surface of about 150 x 580u and records an EMG arising from many individual muscle fibres. Human muscle fibres have a diameter ranging from 25 - 100u and the conventional electrode records from fibres both close and distant from the recording surface. In theory, the contribution to the EMG of an action potential from an individual muscle fibre decreases exponentially with its distance from the recording electrode. In practice however there are many distortions and the apparent amplitude of the closest fibre potentials is always lower that expected due to shunting of the isopotential lines within the tissue

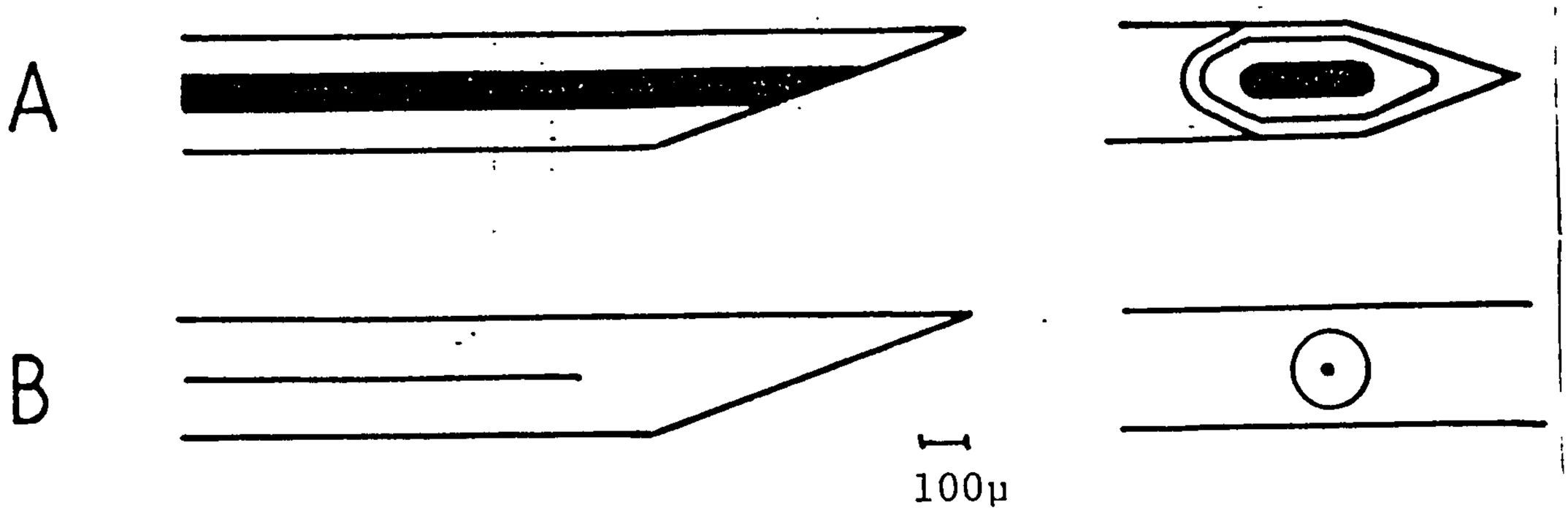
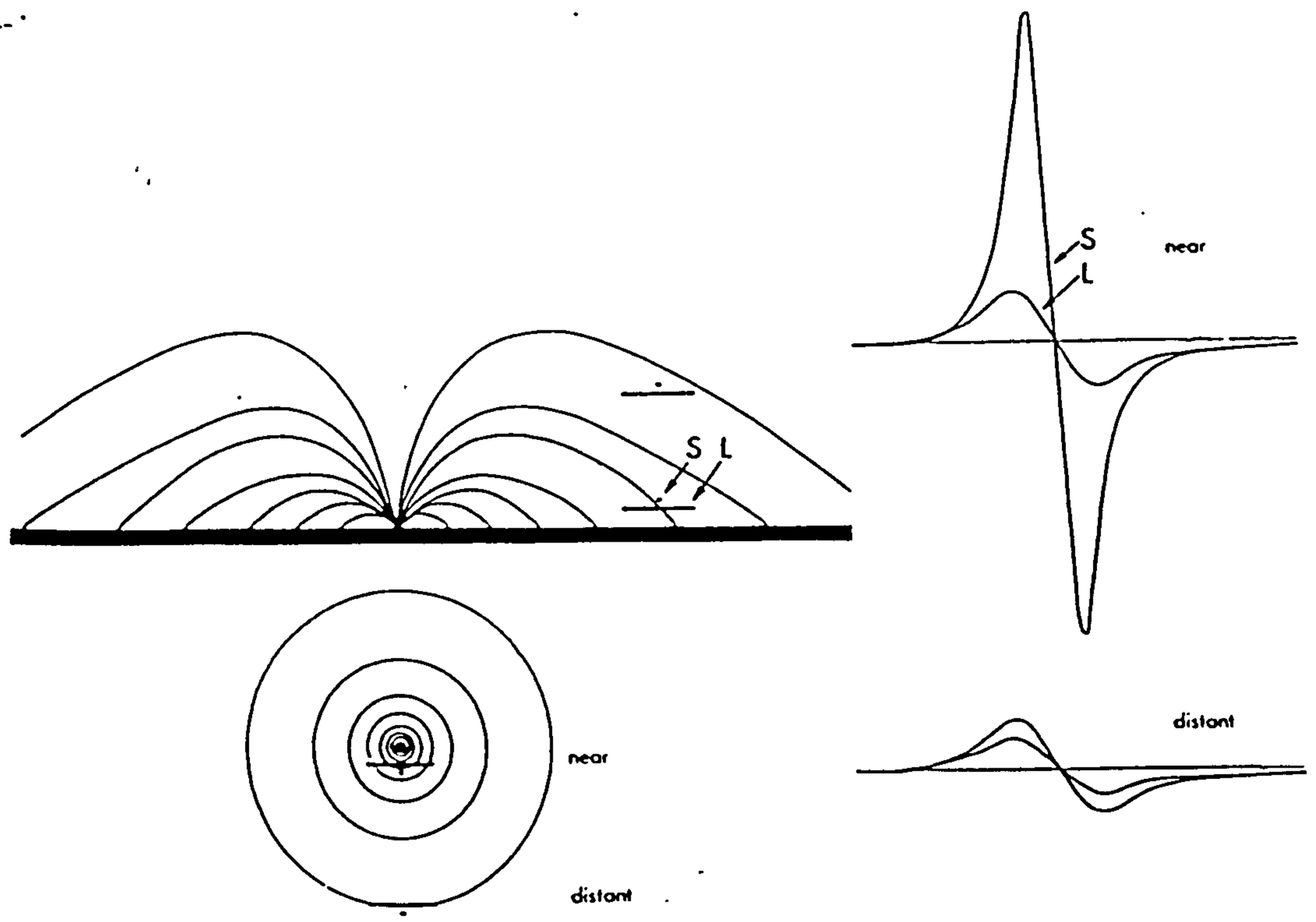


Fig. 6.1 (a) conventional and (b) single fibre electromyography electrodes



The electrical field around a muscle fibre recorded with a small (S) and a large (L) electrode surface. The large electrode shunts the isopotential lines at short fibre-electrode distances, but less so at longer distances. Thus at a short distance, the large electrode records lower amplitude of the action potential than the small electrode.

Fig. 6.2 Shunting of isopotential lines (from Stalberg and Trontelj, 1979)

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6.3 The development of the SFEMG electrode

6.3.1 Increasing the electrode resolution

The theoretical considerations behind the development of am electrode which could record individual muscle fibre action potentials have been reviewed by Stalberg and Trontelj (1979). Fig 6.2 shows the isopotential lines around a muscle fibre recorded with small and large electrode surfaces. It is apparent that the larger electrode shunts the isopotential lines at short fibre - electrode distances but less so at longer distances.

6.3.2 Design of the SFEMG electrode

From the preceeding considerations it is seen that recording with a smaller diameter electrode should increase the observed amplitude of the single fibre signal. The electrode, developed by Eckstedt (1964) is shown in figure 6.1. The recording surface has been reduced to 25u diameter, which is smaller than the mean muscle fibre diameter. The construction of a conventional EMG needle electrode is shown in the same illustration for comparison. One obvious difference is the the electrode, although built into the same size hypodermic needle casing as the conventional electrode (No. 23 gauge) records through a side port in the needle shaft rather than on the bevel. This is to reduce distortion of the muscle fibre during recording of the MAP. The signal is recorded with the steel casing of the needle shaft acting as a reference electrode.

During early studies of SFEMG, electrodes were constructed with up to fourteen individual SFEMG

electrodes within one needle casing. This arrangement was used to study the propagation velocity of the MAP along an individual fibre since it was thought that velocity propagation changes might be characteristic for certain types of myopathy (Stalberg, 1966). In fact this was not the case, but as is detailed below, during these studies important time - related changes were noted between MAP originating from the same motor unit which were shown to be related to transmission changes taking place at the neuromuscular junction.

6.4 Characteristics of the SFEMG signal

The preceding section showed that MAP could be recorded by a reduction in the size of the recording electrode to reduce shunting of the isopotential lines. In addition, selectivity of the electrode can be improved by careful setting of the filters in the amplifier (section 6.4). Although it is impossible for an electrode not to record more distant signals than those immediately of interest in the most proximal motor unit, in practice the distant signals consist only of low frequency components. This is because tissue acts as a high frequency or low pass filter. By setting the high pass filter of the recording amplifier to 500 Hz most of the unwanted distant signals can be removed without detriment to the SFEMG signal of interest (fig 6.3).
The typical SFEMG waveform is shown in fig 6.4.
Signals detected and recorded, as described below, are only acceptable if they conform to the criteria laid down by Eckstedt (1964). These may be summarized as follows;

(1) The characteristic SFEMG signal is a biphasic spike with a positive - negative going fast deflection having a rise time of 75 - 100 usec and a total duration of about one millisecond;

(2) The shape should be constant at consecutive

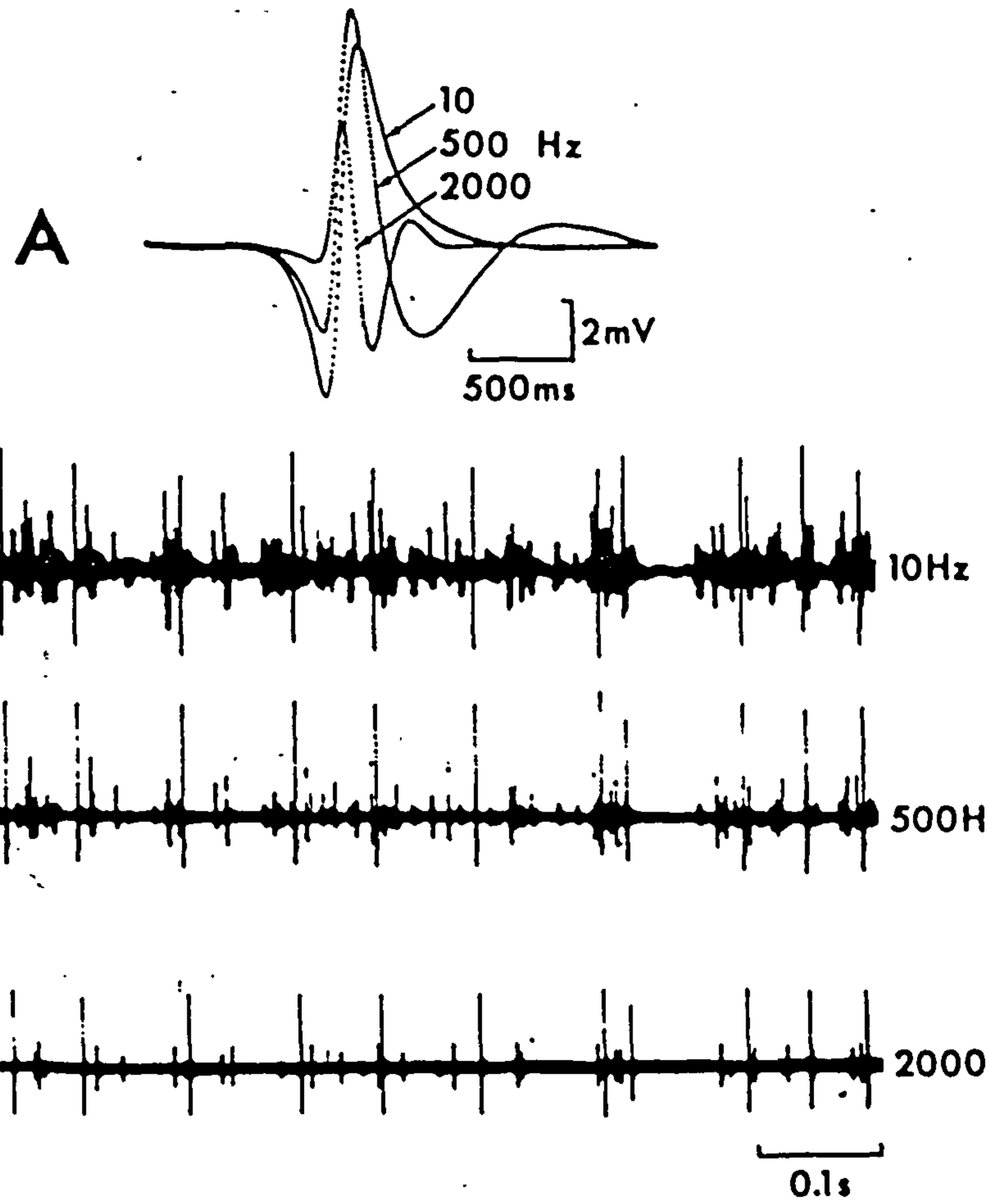

Fig. 6.3 Amplifier filtration in SFEMG
(from Stalberg and Trontelj, 1979)

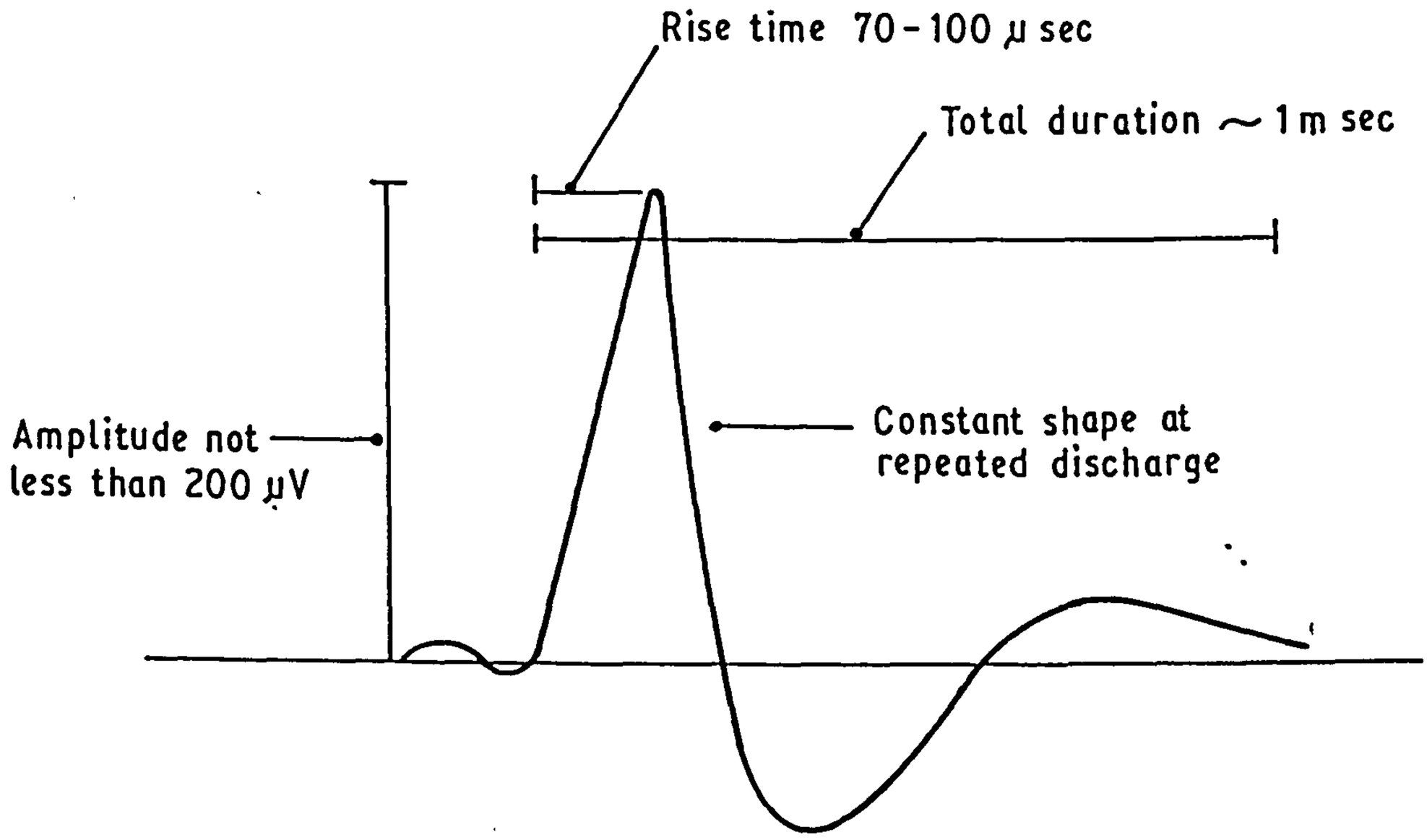

Fig. 6.4 Characteristics of the
single fibre electromyogram

discharges when the recording system has a resolution of 5 - 10 usec;
(3) The recorded amplitude of the signal should not be less than 200 uvolt.

The amplitude of the SFEMG signal is positively correlated with the diameter of the muscle fibre and is dependent on the distance of the recording electrode from the fibre. At the optimum recording position the recorded amplitude is usually between 1 and 7 mV but occasionally signals in excess of 15 mV are recorded. The spectral energy of the wave is concentrated between 100Hz and 10 kHz with a peak value at 1.61 $\pm$ 0.30 kHz and -3 dB points at 0.91 $\pm$ 0.19 kHz and 2.41 $\pm$ 0.53 kHz. The power spectrum of the wave changes with variation in distance of the the recording electrode from the point of origin.

6.5 The practical recording of SFEMG

6.5.1 Technical considerations

SFEMG can conveniently be performed on many muscle sites in man. Each muscle gives rise to signals with slightly different characteristics (Stalberg, Eckstedt and Broman, 1971). Extensor digitorum communis in the forearm has been studied extensively. The muscle can be activated easily by the subject through simple extension of the middle finger with the forearm outstretched. In this way it is possible to activate the muscle at a steady rate of between 15 and 20 Hz. A scheme of the essential equipment required for SFEMG recording is shown in figure 6.5. The signal from the electrode is first amplified using an amplifier of high input resistance and low input capacitance to avoid distortion of the signal. This is necessary because the electrode

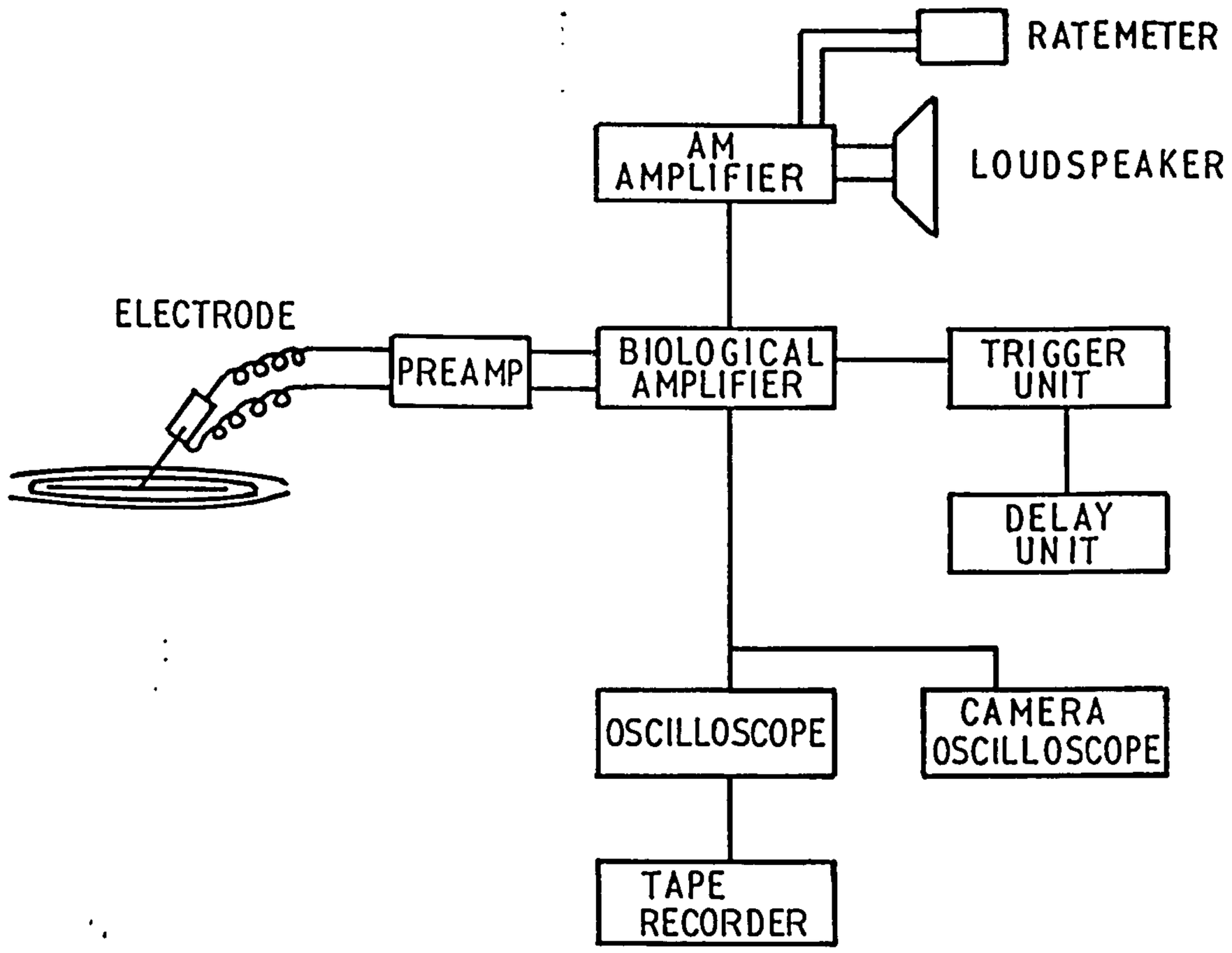

Fig. 6.5 Scheme for recording SFEMG. The AM amplifier and loudspeaker provide a characteristic audio signal which aids detection of the fibre pair (see text)

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itself, having a small recording surface has a high impedence. In practice, the signal is first led to a preamplifier and then to a main amplifier which has the filters set to between 500Hz and 16 kHz. In section 6.4, the high pass setting was emphasized for its importance in eliminating signals from distant fibres. The amplifiers used must be of high quality and extremely stable. Other units of the SFEMG recording system which are of importance, before the output stage from the main amplifier, are the rate meter and audio output from a loudspeaker. These help the subject to maintain a steady innervation during recording. The correct activation rate for successful SFEMG recording should be between 15 and 20 Hz. The loudspeaker is useful in monitoring this rate as the the operator as makes small manipulations of the recording electrode to find suitable SFEMG recordings. As will be shown later, most information during voluntary activation of muscle can be obtained by SFEMG recording of a pair of MAP with a common terminal innervation (the fibre pair). This signal has a very characteristic hollow sound when presented at audio frequencies and is easily detected often before appearing on the oscilloscope screen. In most SFEMG systems the output from the amplifier is recorded on reel to reel magnetic tape for off - line data processing.

During recording, the SFEMG signals are displayed on the main oscilloscope at a gain of about 1 mV/div and a time base of 0.1 mS/div, each division being 1.5 cm. Before recording SFEMG, a number of electronic refinements are necessary to a basic oscilloscope in order to display the features of the fibre pair.

6.5.2 Triggering

To examine any repeating waveform on an oscilloscope some form of triggering device must be

used. This means that before the waveform is traced by the beam dot across the screen in the x direction the trace must be started by the wave reaching a certain height in the y direction. With the selection of an appropriate x axis speed or time base, potential changes in the y direction can be plotted in a waveform which appears stationary on the screen. To analyse SFEMG two or more waveforms from the same motor unit, and therefore having a common terminal innervation, are examined. Each of these waveforms moves in time slightly with respect to the other. To examine this change on the oscilloscope it is necessary to trigger the sweep from one waveform. When this is achieved, the second and sometimes the third and fourth SFEMG waveforms appear in an approximately steady x relationship to the first, indicating that they are firing with a fixed time relationship with it and are therefore part of the same motor unit. In this situation all time variability between the component SFEMG waveforms of a fibre pair is displayed as variability of the second while the first is held stable. It is the analysis of this variability that is the basis of SFEMG analysis. For successful SFEMG recording the triggering should be as stable as possible. This means that the sweep must be triggered by successive discharges of the triggering waveform with great accuracy. This can be achieved by having the trigger level set by high quality electronics and by recording a stable waveform with a fixed upward - going sweep. The prerequisites for acceptable SFEMG stated in section 6.4 included the stipulation that the fast upward - going part of the waveform must have a rise time of between 75 and 100 usec. In practice, the triggering level is set at about one third of the way up this fast ascent.

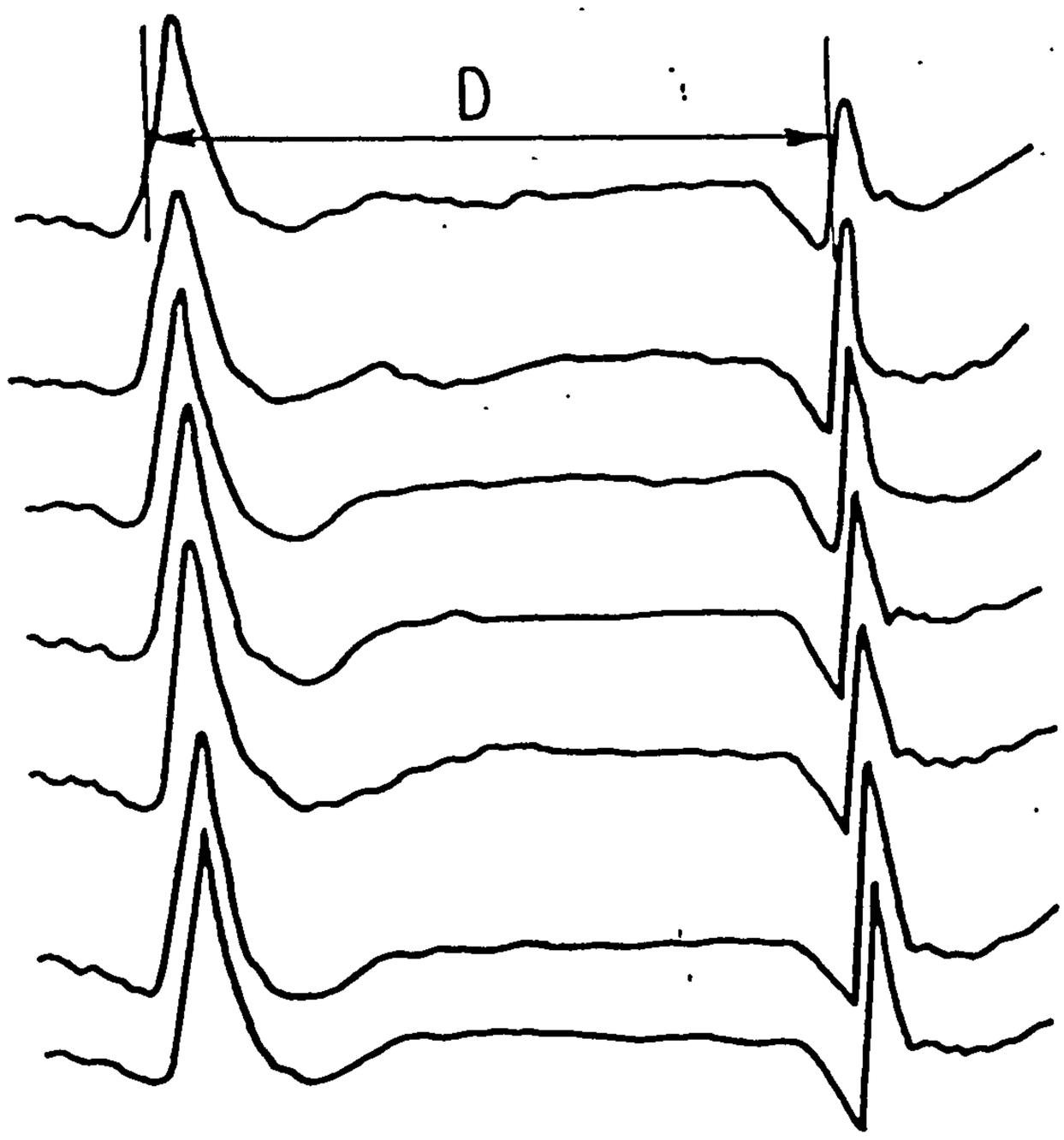
6.5.3 Delay Unit

Another important electronic refinement for successful SFEMG recording is the delay unit. This is a digital storage device which delays the display of the first waveform of a fibre pair and allows accurate inspection of the first part of the triggering waveform, a part which ordinarily would not be visible in a conventional oscilloscope display. The delay unit therefore has the effect of shifting the SFEMG trace across the screen to the right.

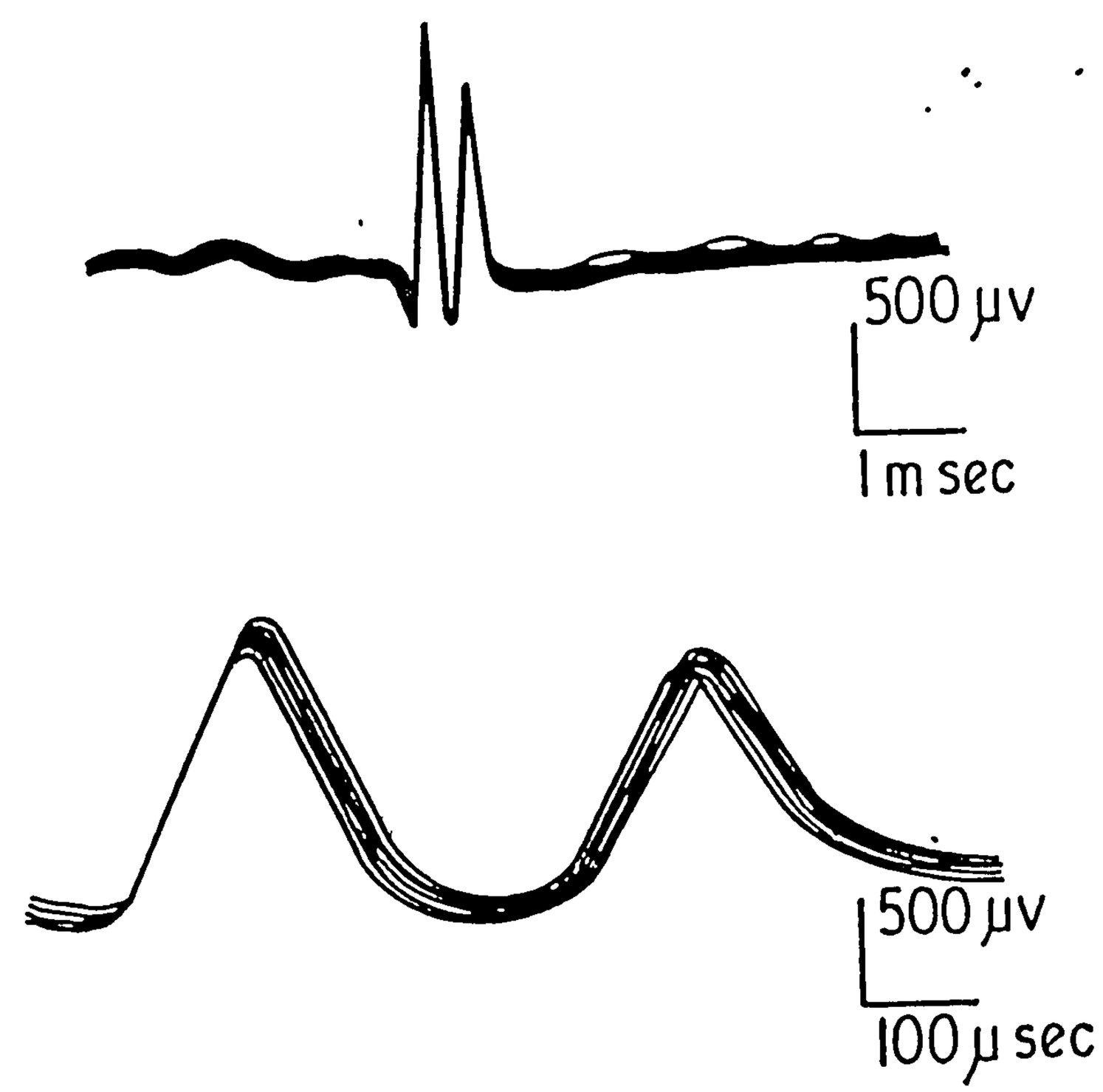
6.6 SFEMG parameters

6.6.1 Introduction

Figure 6.6 shows a recording of SFEMG from successive nerve discharges at a rate of 15/sec. The first action potential is fixed in time on the x axis because the trace is being triggered from it. However, the second action potential is seen to be moving backwards and forwards along the x axis with respect to the first. This effect is better seen in the superimposition of the traces shown in the same illustration. This x axis variation in the position of the second wave is termed 'jitter'. Jitter is a measure of the variability of the interpotential distance between the two SFEMG waveforms during successive discharges. The analysis of this variability has been considered in several ways (Eckstedt, Nilsson and Stalberg, 1974).



(a) Typical SFEMG signal from EDC displayed sequentially. The firing rate is 15 / sec.



(b) upper trace: ten superimposed sweeps
lower trace: the time base has been expanded ten times to show the jitter of the second action potential.

Fig. 6.6 The jitter phenomenon in SFEMG

6.6.2 The mean consecutive difference of jitter

Figure 6.6 shows that the distance between successive waveforms with a constant shape at a fixed point on the upsweep is called the interpotential interval (D). The distance is usually measured electronically from the trigger point on the first SFEMG waveform, parallel to the x axis to the intercept of the rising phase of the second wave . Originally, it was thought that the distribution of variability of the interpotential intervals followed a normal distribution and that jitter could be expressed by calculating the standard deviation of the mean interpotential interval (MIPI). However, Eckstedt et al (1974) showed that this approach was likely to lead to inaccuracy as there are sometimes slow trends in the interpotential intervals which are thought to be due to different degrees of slowing of the propagation velocity during continuous activity, or to minor changes in action potential shape resulting from slight displacement of the recording electrode. These authors felt that a better way to compute jitter was to use mean consecutive difference (MCD) which is given by the expression:

$$MCD = \frac{|D1 - D2| + |D2 - D3| + .. |Dn-1 - Dn|}{n - 1}$$

where D is the interpotential interval and the figure subscipts refer to successive discharges during voluntary activation. If D is expressed in time units the MCD is derived in microseconds. The expression is particularly suitable for analysis by microcomputer. Eckstedt and his co - workers therefore examined the relationships between jitter calculated as MCD and other expressions. They recommended that MCD should be calculated on not less than 40 consecutive SFEMG discharges, but in practice a good estimate of jitter

can be gained by measuring the time variation of five or ten superimposed consecutive discharges (see fig 6.7). The variations so demonstrated on the x axis are known as the R5 and R10 distances. Eckstedt at al showed that MCD = 0.49 x R5 = 0.37 x R10. In addition they have shown that the standard deviation (SD) of the the interpotential intervals is related to MCD by the expression MCD = 1.13 x SD. The same authors reported a correlation coefficient of better than 0.95 for manual against digital calculations of MCD.

6.6.3 Blocking

When consecutive discharges of a fibre pair are displayed on the recording oscilloscope the second MAP is occasionally absent. This phenomenon, illustrated in figure 6.8 is known as blocking. It is a feature of SFEMG in muscle that is affected pathologically (section 6.10) and is diagnostically very valuable. Blocking may be regarded as the microneurophysiological correlate of muscle weakness. This means that if an increasing proportion of muscle fibes is recorded as blocking by SFEMG reduced activity in the whole muscle will be detected in the height of the MMG and compound EMG. Blocking of a single MAP is thought to arise through a failure of the EPP to reach threshold in the end plate and is thus directly related to both pre and post junctional factors (section 6.8.2). Stalberg and Thiele (1975) have described a different form of blocking involving two or more MAP of a polyphasic recording where the block is thought to take place at the junction of a terminal nerve twig.

6.7 Errors and artefacts in SFEMG

SFEMG is a technique that records delicate

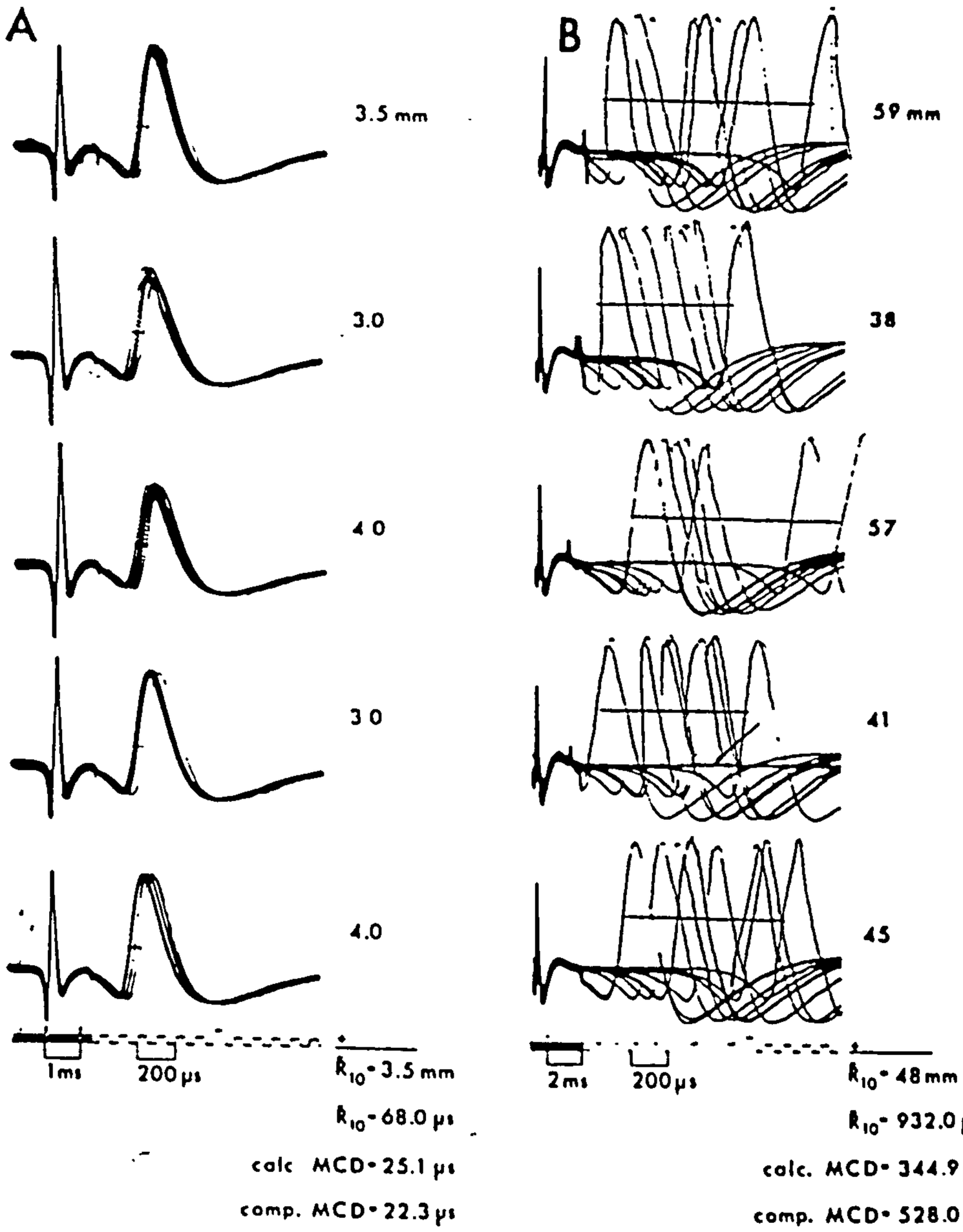


Fig. 6.7 Manual calculation of jitter in (A) a normal and (B) abnormal fibre pair. Five groups of 10 superimpositions are measured. The mean value of R10 is then calculated and multiplied by 0.37 to give an approximate value of MCD. (from Stalberg and Trontelj, 1979)

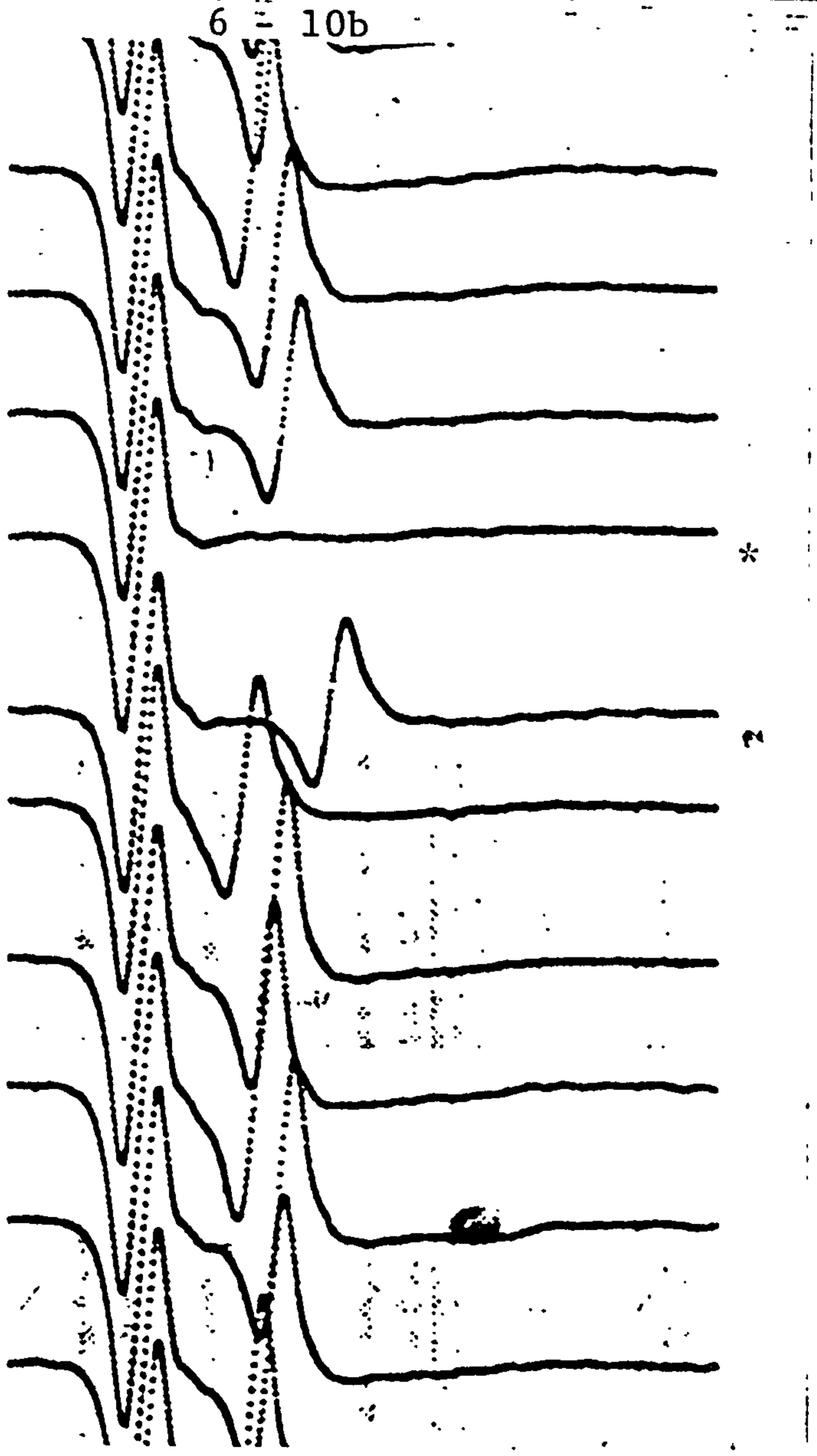


Fig. 6.8 Blocking in SFEMG. The second waveform is absent at *. Firing rate 15/sec. (Taken from an original recording.)

Fig. 6.9 SFEMG amplitude distortion due to changing the electrode position. (from Stalberg and Trontelj, 1979)

electrical signals with an operator controlled electrode. Because of this direct contact several artefacts can arise which have been reviewed by Stalberg and Trontelj (1979). Small movements of the electrode, which are essential in tuning the muscle fibre pair to provide the SFEMG signal, may also distort it during the resording period. The distortion is detected as a variation in the amplitude of the signal as shown in figure 6.9. Small variations of this nature may not be important but large changes can reduce the amplitude below the triggering threshold (section 6.5.2) and give rise to loss of both waveforms of the fibre pair or an apparent blocking (pseudoblocking) in the second. This should not be confused with the true neuromuscular block described in the previous section. Recently Davis and his co - workers have produced an improved computer - controlled triggering device which can follow movement related changes in amplitude and adjust the triggering point accordingly (Davis et al, 1983). The rules for the acceptance of SFEMG signals were stated in section 6.3.3. Occasionally signals such as that shown in figure 6.10 are seen. These are thought to originate from fibres damaged by previous insertion of an electrode. Careful SFEMG technique displaces the fibres without damage if the point of the needle containing the electrode is kept sharp. The point should therefore be checked carefully before each use to avoid muscle fibre damage.

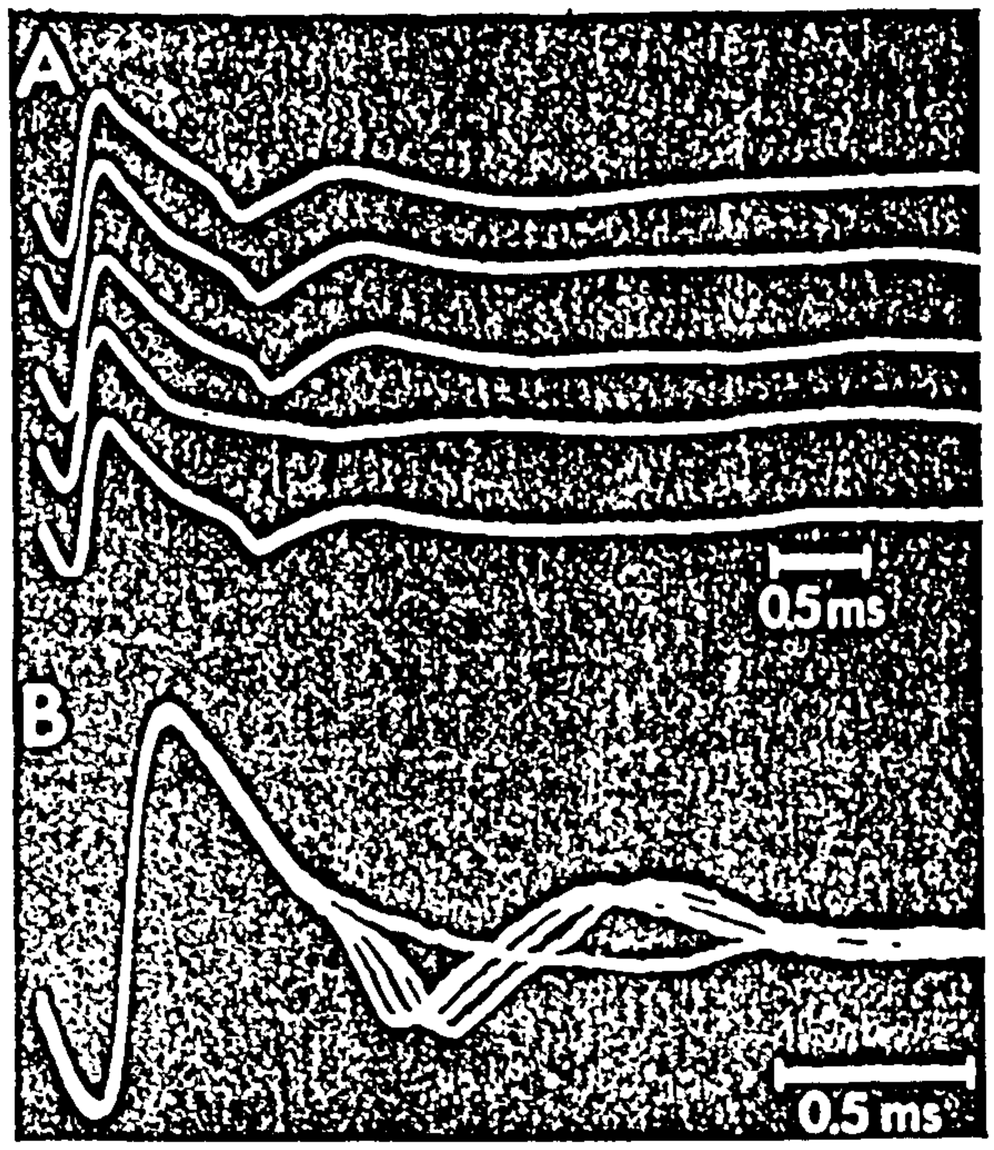


Fig. 6.10 SFEMG signals from damaged muscle fibres (from Stalberg and Trontelj, 1979)

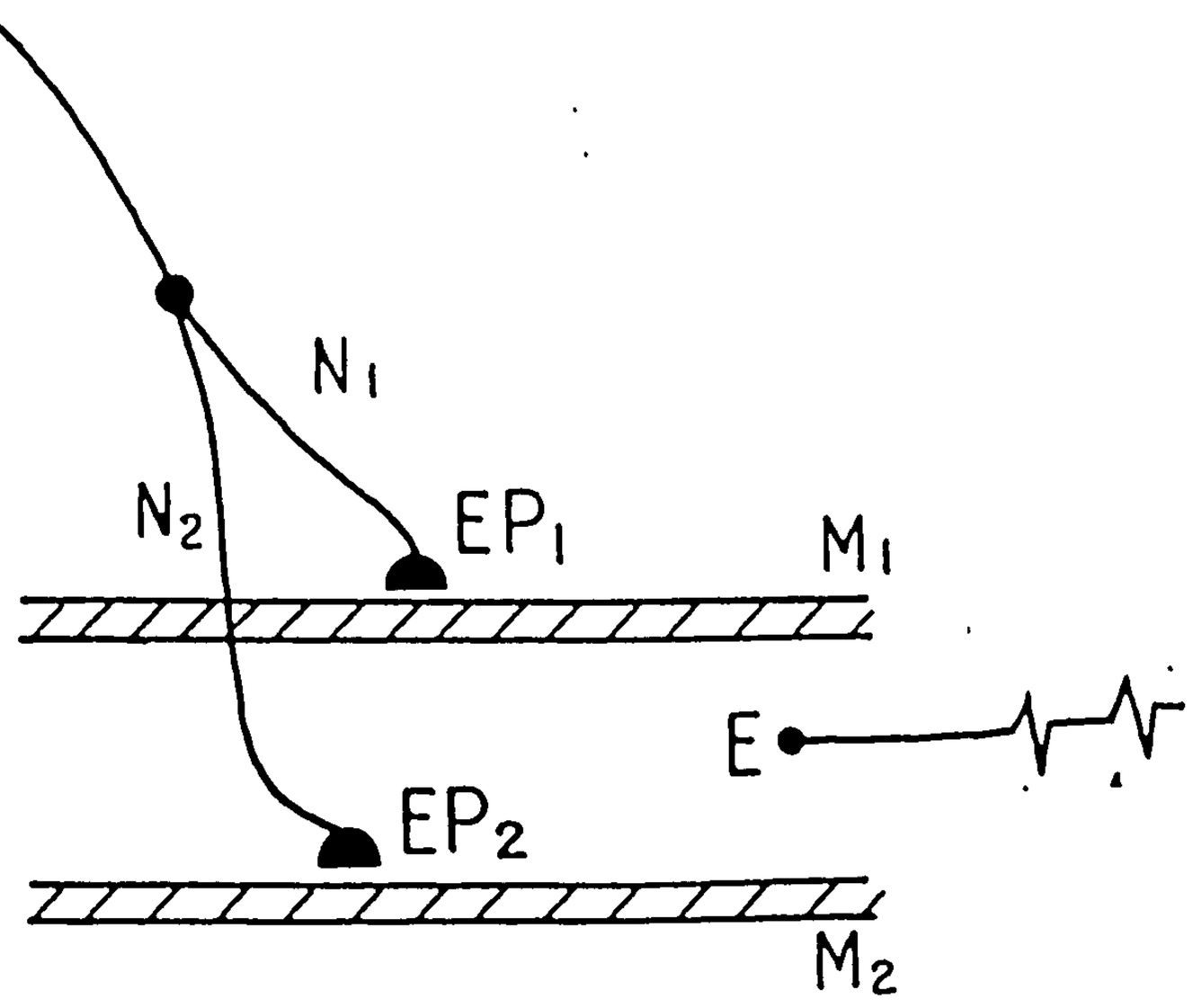


Fig. 6.11 Origins of the SFEMG signal. The activating nerve impulse is conducted down the terminal nerve fibres (N1 and N2) to two end plates (EP1 and EP2)in the same motor unit. The SFEMG electrode (E) records single muscle action potentials from the two fibres (see text)

6.8 The origins of the jitter phenomenon

6.8.1 Theoretical considerations

Figure 6.11 shows schematically the origins of an SFEMG fibre pair. The activating nerve impulse divides at point P and passes to the two controlling endplates EP1 and EP2. The propagated MAP from these junctions spread along the muscle fibres and are recorded at point E. The relative lengths of the terminal nerve fibres and the distances travelled by the MAP along the fibres before being recorded determine the MIPI. From this diagram it may be seen that variability in the time of recording the two action potentials could theoretically arise (1) in the non-myelinated nerve terminal fibres (2) in the endplates and (3) in the propagation velocity of the MAP along fibres A and B. The problem of the relative contributions of these three possibilities has been considered by Stalberg (Stalberg and Trontelj, 1979). It has been shown by microneurography that the variations occuring in the propagation of impulses down the terminal nerve fibres is very small and are not sufficient to account for observed jitter changes. Considering the propagation velocity of the MAP along the muscle fibres it is possible that variations here may account for jitter change. It is known from studies with multiple SFEMG electrodes (Stalberg, 1966) that the propagation velocity of an MAP along a muscle fibre changes in proportion to the preceeding interdischarge interval (the time between successive nerve impulses activating the fibre). For a short preceeding interval the propagation velocity is higher than for a long one. Difference in conduction time along the terminal nerve twigs gives rise to the observed MIPI. In practice, when the conduction time along the two muscle fibres from the end plates to the recording site is roughly the same the MIPI is small.

In most recordings this is actually the case with MIPI usually < 1 mS. Since both fibres considered belong to the same motor unit and are therefore both affected by the same interdischarge interval, the changes in the two conduction times will largely cancel each other out, if the propagation velocity changes are the same in both fibres. Hypothetical calculations made by Stalberg have shown that variability from this source adds less than 10 usec. to observed jitter. However, it should be noted that if the MIPI is long (>3mS) and is due to widely seperated motor end plates or a large difference in muscle fibre conduction velocities the conduction time changes along the muscle fibres will not cancel.

6.8.2 The end plate and jitter

In recordings with steady neuromuscular activation the contributions of variability in the terminal nerve branches and MAP propagation velocity are small. The main source of jitter is regarded as originating in the neuromuscular junction itself. The statistical nature of neuromuscular transmission with ACh being released as quanta was established by Fatt and Katz in 1951 using intracellular recording of EPP and MEPP (section 3.2.2). Elmqvist et al (1964) used intracellular recording of a human intercostal nerve - muscle preparation from both normal sources and patients having myasthenia gravis. Figure 6.12 shows compound EPP and MAP from their studies. The observation that there was wide variation in EPP rise time in myasthenia gravis was followed later (Stalberg et al, 1974) by the detection of large SFEMG jitter values in this condition. Stalberg and his co - workers therefore proposed that the jitter phenomenon is directly related to the variability of EPP rise time to the threshold of generation of MAP. The hypothesis that EPP variations caused jitter appeared

to be substantiated by observations of jitter abnormality in many pathological conditions which are known to affect the end plate, and by the effects of drugs which interfere with neuromuscular transmission. Lundh et al (1977) have provided the most recent contribution to this discussion in their study of jitter changes in experimentally induced botulism.

6.8.3 Normal jitter

In any SFEMG screening of a muscle between 10 and 20 fibre pairs are usually examined using about four or five penetrations of the skin. On average, a random insertion of an electrode produces a fibre pair in about 25% of recordings (Stalberg and Thiele, 1975). Thus fibre pairs must most often be detected by small tuning movements of the electrode as described above. Triplet waveforms are obtained from random insertion in far fewer insertions, usually about three percent.

Normal jitter has been studied in several human muscles under voluntary activation and the results of studies by Stalberg, Eckstedt and Broman (1971) are reproduced in table 6.1. The standard way of analysing jitter profiles from any muscle has been to take a mean value from an unmodified histogram and apply an upper limit of normality at three standard deviations above the mean jitter value. Stalberg and his co - workers concluded from a large series of recordings that in normal subjects one in twenty fibre pairs could be expected to have a jitter value greater than this range. In the case of extensor digitorum communis, a muscle widely studied in man the upper limit of jitter MCD normality is taken by most workers as being 55 usec. In practical clinical terms, two out of twenty fibre pairs is regarded as being borderline abnormal and any greater incidence as being of pathological significance. Stalberg has emphasized

Muscles	Number of potential pairs	MCD — pooled data mean, SD	SD of MCD values from individual subjects mean, SD	Upper normal limit close to mean + 3SD
frontalis (range of means for individual subjects)	258	20.4, 8.8 (15.7 – 29.2)	6.2, 2.3 (5.5 – 8.7)	45
biceps	125	15.6, 5.9		35
EDC (range of means for individual subjects)	759	24.6, 10.6 (16.5 – 32.0)	8.3, 3.2 (2.3 – 12.4)	55
rectus femoris	73	31.0, 12.6		(65)* 60
tibialis anterior	153	32.1, 15.0		(75)* 60
EDB	29	85.3, 68.6		none

Table 6.1 Values of normal SFEMG jitter in several human muscles (from Stalberg, Eckstedt and Broman, 1971)

Muscles	AGES			
	10–25 years m SD n	26–50 years m SD n	51–75 years m SD n	above 75 years m SD n
Frontalis	1.61 0.21 (11)	1.72 0.21 (15)		
Deltoid	1.36 0.16 (20)	1.40 0.11 (10)		
Biceps	1.25 0.09 (20)	1.33 0.07 (17)		
Extensor digitorum communis	1.47 0.16 (61)	1.49 0.16 (98)	1.57 0.17 (59)	2.13 0.41 (21)
1st dorsal interosseus	1.33 0.13 (14)	1.45 0.12 (6)		
Rectus femoris	1.43 0.18 (11)	1.57 0.23 (14)		
Tibialis anterior	1.57 0.22 (18)	1.56 0.22 (21)	1.77 0.12 (4)	3.8 (1)
Extensor digitorum brevis	2.07 0.42 (16)	2.62 0.30 (11)		

Table 6.2 Values of normal SFEMG fibre density and the variation with age (from Stalberg and Thiele, 1975)

that any SFEMG screen containing less than 20 pairs cannot be regarded as being sufficient for clinical comment. Mean jitter has also been suggested as being a good indicator of normality (Sanders et al, 1979, Cruz - Martinez et al, 1982) and in the case of EDC values of 34 - 40 usec. have been adopted as norms. It should be noted that there are two problems with this method of assessing normality of jitter. The first is that it takes no account of skew which occurs in the distribution. Comparison of means therefore using parametric statistical methods may therefore produce misleading results, particularly when assessing the action of a particular agent on jitter profile. Secondly, no account is taken of the finite probability of finding more than 1/20 high jitter readings in a normal subject when a small number of recordings has been made. These problems are considered further in chapter 8 and appendix 1.

6.8.4 Physiological variations in normal jitter

Variations in the spread of jitter values are known to exist both between different muscles within an individual and between the same muscle in different individuals. Moreover, it has been shown that normal and abnormal jitter values may be recorded within the same motor unit. The influence of propagation velocity of jitter was discussed above. Because of variations related to this parameter large values of MIPI tend to be associated with larger values of jitter. Below MIPI values of 4 mS there is no correlation however.
Some of the physiological factors which have been shown to affect jitter are:-

(1) site of recording; SFEMG is most often recorded in the main body of a muscle near the end plate zone. Recording near the tendon insertion has

been shown to produce a higher incidence of abnormal jitter and is therefore avoided during clinical screening;

(2) activation rate; normally, the rate of firing of the nerve controlling the muscle being examined under voluntary activation is kept at about 15/sec using the audible loudspeaker or a rate meter. If it is much lower than this frequency there may be marked changes in recorded jitter (Davis et al 1985);

(3) temperature; decrease in the intramuscular temperature below 35 deg. C increases jitter by 1 - 3 usec/deg C;

(4) age; Stalberg has shown that there are no age dependent changes below the age of 70 years when the fibre density (section 6.9.1) of the muscle is normal. The highest normal jitter values are found in tibialis anterior after the age of 50 and are associated with an increased fibre density. This would indicate that there are probable associated neurological changes (Stalberg et al, 1971);

(5) duration of recording; the duration of any one SFEMG recording has been shown by Stalberg to have less effect than might be expected on the jitter value. However, other workers (Swash, 1985 personal communication) have shown that there are changes in jitter after recording any one pair for several minutes. To eliminate this possible artefact jitter should be calculated from the early part of a recording under voluntary activation as soon as stable conditions are achieved;

(6) ischaemia; Dahlbeck and his associates (1970) showed that jitter was very sensitive to ischaemic conditions in the forearm. The mechanism of this finding must be closely linked with the

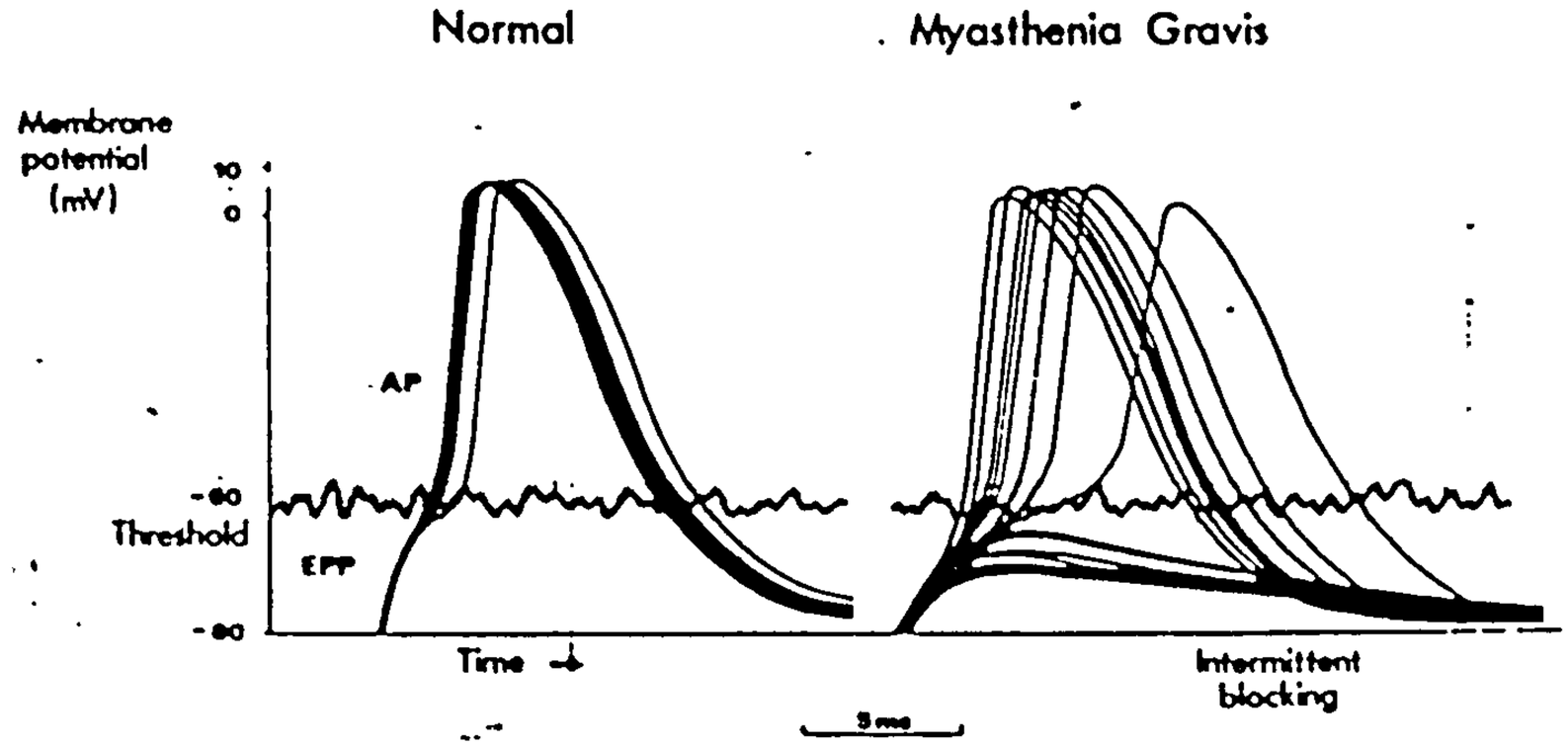


Fig. 6.12 EPP rise time as a cause of jitter in normal and myasthenic muscle (data redrawn by Stalberg and Trontelj, 1979 after Elmqvist et al, 1964)

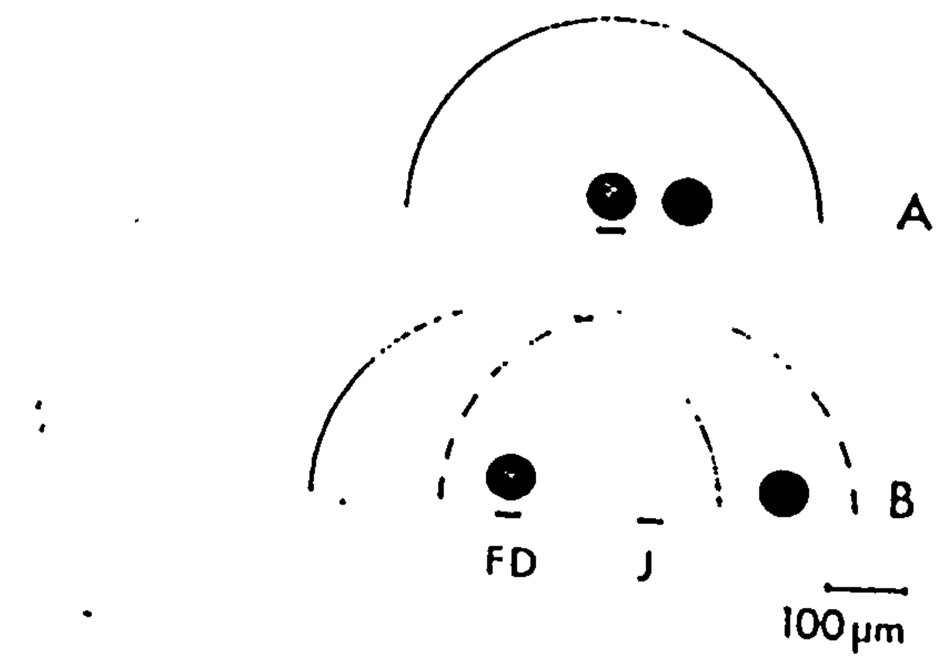


Fig. 6.13 Fibre density measurement. The electrode must be as close as possible to one active fibre indicated in A and as FD position in B. In B only one fibre is recorded (from Stalberg and Thiele, 1975)

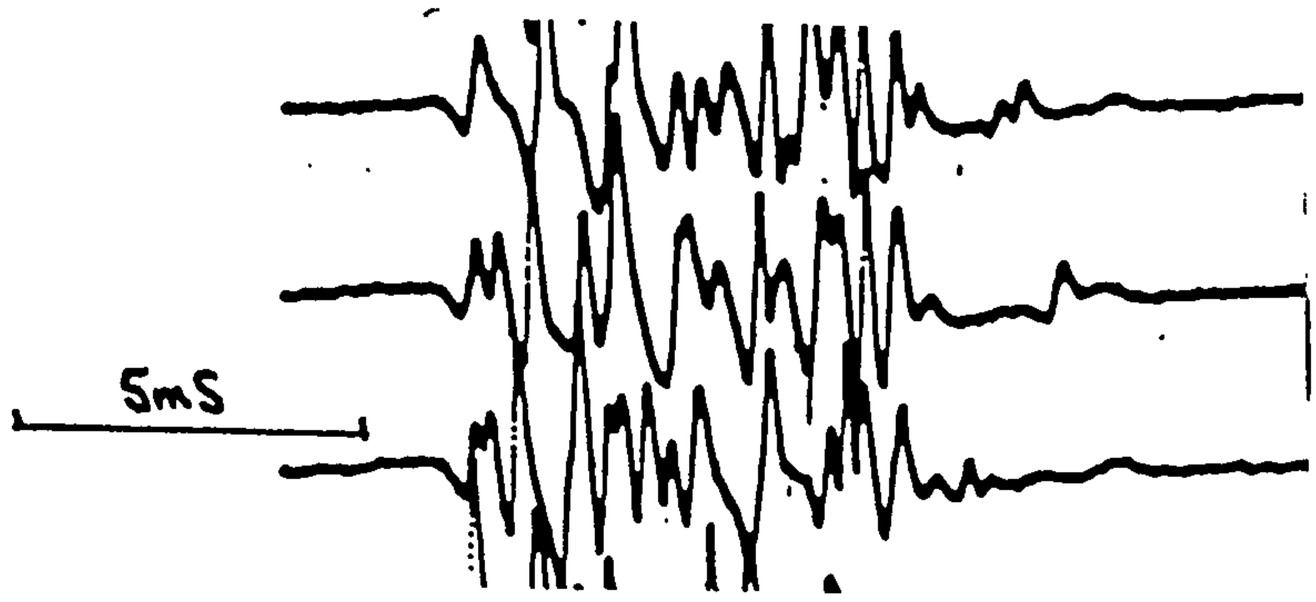


Fig. 6.14 Polyphasic SFEMG (from Stalberg and Trontelj,1979)

biochemical processes causing jitter and may be as much a function of electrolyte abnormalities (particularly calcium) in tourniquet - induced hypoxia as a directly oxygen - related process. All SFEMG recordings however should be made at as near normal physiological conditions as possible before any pathological significance can be ascribed to them.

6.9 SFEMG and muscle morphology

The review of SFEMG so far has concentrated on the ability of the technique to detect time variability between two muscle action potentials from the same motor unit which can provide valuable information about the end plates. In this section another use for SFEMG in determining muscle fibre density is described, together with some recent developments in the classification of fibre size and type.

6.9.1 Fibre density

The SFEMG electrode, because of its small recording area, has a limited range of detection. Stalberg (Stalberg and Trontelj, 1979) showed that the volume of resolution could be regarded as a sphere of about 300u diameter centered at the electrode. In effect this means that, using maximum amplifier sensitivity, all SFEMG signals detected which conformed to the rules given in section 6.5.1 can be regarded as coming from fibres within a single motor unit passing through this sphere (see fig. 6.13). Histological studies have shown that the fibres of individual motor units interdigitate and are not confined to a specific area of the muscle (section

6.9.4). There is therefore a good chance of picking up two fibres from the same motor unit following a random SFEMG electrode insertion. It follows that a count of the number of linked SFEMG signals from a random insertion can provide a measure of the fibre density in the muscle. This use of SFEMG has been reviewed by Stalberg and Thiele (1975).
To estimate fibre density, the sensitivity of the SFEMG amplifier is first set to the maximum possible without noise being too troublesome, which is 200 uV/division. The electrode is then inserted at random into the muscle and the signal triggered on the largest amplitude MAP recorded. A count is made of all time related peaks having an amplitude of at least 200 uV. To repeat the determination the electrode is either moved a new area of muscle, triggering from a new high amplitude signal, or reinserted. The mean of twenty or so such measurements gives a figure which is characteristic of the fibre density within the muscle. Values for normal muscles quoted by Stalberg and Thiele are given in table 6.2. In pathological conditions of the anterior horn cell or in reinnervation the fibre density values are increased. This means in practice that more polyphasic SFEMG signals are recorded following random insertion. An example is shown in figure 6.14. The determination of fibre density expands the diagnostic yield from the SFEMG technique.

6.9.2 Fibre type

Knowledge about the histological and biochemical characteristic of skeletal muscle has increased greatly since the introduction of the clinical muscle biopsy as a diagnostic technique. The subject has recently been reviewed by Swash and Schwartz (1984). All vertebrates have muscle fibres which are differentiated into red and white types,

corresponding to slow and fast contracting fibres. Red muscles are slow contracting and specially developed for postural activity. They contain few mitochondria but many lipid droplets and glycogen granules. Red fibres react strongly with oxidative enzymes such as succinic dehydrogenase and are designated type 1 fibres. White fibres contract faster and are found in muscles which are used for short bursts of intense activity. Unlike red fibres, they fatigue rapidly. These fibres are designated type 2 and are characterised by their reaction with non - oxidative enzymes such as myophosphorylase. In any human muscle, red and white fibres are mixed in a random pattern called the mosaic. The mosaic varies according to the work usually performed by the muscle. Diaphragm, for example, contains a preponderance of red fibres whereas soleus contains both red and white. Swash and Schwartz (1984) have summarised the properties of muscle fibre types according to standard histological methods. Type 2 fibres are subclassified into 2a, which are fast twitch, fatigue resistant, and 2b, which are fast twitch, rapidly fatiguing.

6.9.3 Fibre size

Brooke and Engel (1969) have demonstrated variation in mean muscle fibre size (usually taken as the lesser transverse diameter) dependent on age and sex. At the age of one year mean muscle fibre diameter is 16u and at age 40, 60u. The mean diameter increases by 2u for each year to age 5 years and then by 3u/year to age 9 years. Adult values are reached between the age of 12 and 15 years. In adult men most muscle fibres are within the range 40 - 80 u and in women 30 - 70u. Although in childhood type 1 and type 2 fibres are similar in size, in adulthood there are considerable differences. Polger et al (1973) have studied autopsy subjects and shown that for adult

males type 2 fibres were larger than type 1 in 90% of the muscles studied. This situation is the reverse of that obtaining in women where type 1 fibres are usually larger than type 2. This study showed that both fibre types were equally variable in size with moderately large ranges of diameter values in individual subjects. In some muscles, the fibres were larger in the deep than in the superficial zones.

6.9.4 The motor unit

The concept of a group of muscle fibres, termed the motor unit, which are controlled by a common branching motor nerve is central to the SFEMG technique. The number of fibres within a motor unit varies greatly. Thus extraocular muscles have a motor unit number of 9 whereas gastrocnemius may have as many as 1900 (Feinstein et al, 1955). Estimates have been made of the total number of motor units in the thenar muscle of about 250 - 340 units (McComas, 1977; Brown, 1972). All muscle fibres within a motor unit have the same histological and mechanical characteristics. In other words they are of the same fibre type. However, the type depends on the innervation and so a slow muscle aquires the characteristics of a fast muscle if the nerve to a fast muscle is transplanted into it. This point was demonstrated in the classic experiments of Buller, Eccles and Eccles (1960). It was originally assumed that a muscle fibre innervated by its original neurone would retain its characteristics if re - innervated artifically with a type 2 neurone. Buller et al showed that this assumption was incorrect. They studied one fast muscle, flexor digitorum longus (FDL) and one slow muscle (soleus) in the cat. The nerves to these muscles were divided and the central stump of one was sutured to the distal portion of the other. After regeneration the FDL became controlled by the

orignal soleus neurone, and soleus by the neurone originally controlling FDL. When twitch characteristics of the muscles were re - examined it was found that soleus had become fast and FDL slow. The experiments therefore indicated that the speed of contraction of any muscle fibre was determined by the neuronal control.

The final point of importance concerning the motor unit is the spatial distribution of the fibres. Motor unit fibres are not grouped close together in muscle, but are spread widely across the cross section of the muscle. Throughout this area there is considerable interdigitation with fibres of other motor units. The scattered anatomical arrangement of motor unit fibres has been demonstrated electrophysiologically in man (Stalberg and Eckstedt, 1973). Kugelberg et al (1970) have demonstrated the same finding histologically in animals.

6.9.5 The threshold of firing of motor units

Stalberg and Trontelj (1979) have reviewed the differences in threshold of firing of motor units. Thus high and low threshold units have been shown to have differences in histological (Wormolts and Engel, 1973) and mechanical characteristics (Edstrom and Kugelberg, 1968, Milner - Brown et al, 1973 and Burke et al, 1973). Milner - Brown et al also found correlation between threshold of activation and the size of the motor unit in man. In SFEMG the minimal voluntary activation used for recording recruits the low threshold postural fibres. These belong histologically to the type 1 classification.

6.9.6 Myopathic effects on fibre size and type preponderance

A characteristic of myopathic disorders is that many fibres which are larger or smaller than the normal range occur in a muscle biopsy. Histograms of fibre diameters in such biopsies demonstrate a wide range of diameters. The increasing variability in fibre size is thought to be due to increased load placed upon surviving healthy muscle fibres in which loss of functioning fibres has occurred (Swash and Schwartz, 1977) and to atrophy, which results from incomplete regeneration, necrosis or fibre splitting. In some myopathies (particuarly of a metabolic origin) selective type 2 atrophy may occur, but it is not a specific feature since it may occur in disuse atrophy, stroke and Parkinson's disease.

Type 1 fibre preponderance is not, in itself, regarded as being a specific abnormality, but a predominance (>55%) is in general associated with myopthies, particularly Duchenne dystrophy. By contrast, fibre type 2 predominance is often found in neurogenic disorders.

If fibre type predominance is found in a muscle such as vastus lateralis or biceps brachii, which normally contains equal numbers of type 1 , type 2a, and type 2b fibres, it assumes particular significance and a neurogenic disorder should be suspected.

6.10 SFEMG in diseased muscle

So far, discussion of SFEMG jitter has been confined to findings in normal muscle. We now consider changes which are found in pathologically affected muscle. The hypothesis that jitter at the skeletal neuromuscular junction is related to the rise time of the end plate potential (section 6.2.2) has been examined on a computer model by Stalberg (Stalberg, Schiller and Schwartz, 1975) to examine the effects of other factors at the SKNMJ. This showed that jitter can arise theoretically from variations in the threshold potential at the post junctional membrane (although there appears to be no published confirmation of this suggestion) and from modification of the release of ACh from the nerve terminal leading to changes in the quantal components of the end plate potentials. In section 6.11 SFEMG measurements in two conditions where ACh release from the nerve terminal is affected will be reviewed. Section 6.12 then considers SFEMG in conditions where there are changes in the post junctional membrane.

6.11 SFEMG and disease of the nerve terminal

Chapter 3 considered the mechanism of ACh release from the nerve terminal. Several stages in the release process may be affected by pathological conditions and drugs. SFEMG measurements have been made in two conditions, the Eaton Lambert syndrome and botulism, which are known to affect the nerve terminal.

6.11.1 SFEMG in the Eaton Lambert syndrome

This condition, also known as the myasthenic

syndrome, is a rare disease manifested as muscle weakness which was first described by Eaton and Lambert (1957). The main clinical symptoms are weakness and easy fatiguability of the proximal limb muscles, particularly in the leg. Unlike myasthenia gravis, involvement of the ocular and bulbar muscles is uncommon. Tendon reflexes are weak or absent and autonomic involvement is possible (Eaton and Lambert, 1957; Elmqvist and Lambert, 1968; Henriksson et al, 1977). The basic lesion in the disease has been shown to be release of a decreased number of ACh quanta from the nerve terminal (Elmqvist and Lambert, 1968). In a study of a biopsied intercostal nerve - muscle preparation, these authors showed that the end plates had normal miniature end plate potentials but reduced amplitude of end plate potential, some of which failed to reach the threshold potential (Elmqvist and Lambert, 1968; Lambert and Elmqvist, 1971). 70% of the cases studied have been shown to be associated with malignant neoplasms, especially small cell carcinoma of the bronchus. Recent work by Newsom Davis and his group indicates that the essential lesion in the nerve terminal may be an antibody to the Ca2+ ionophore (Lang et al, 1985).

The first SFEMG study of this condition was made by Schwartz and Stalberg (1975) in a case where no neoplasm could be demonstrated. Over a four month period 36 single fibre pairs were studied and all but one of these showed increased jitter. 29 pairs showed impulse blocking and this was always seen at an MCD value greater than 140 usec. There was considerable dependence of the jitter value on the frequency of activation of the muscle with all of the potential pairs showing improvement at higher (18 to 25 Hz) compared with lower (7 to 12 Hz) rates. At the higher rates impulse blocking was reduced. The authors commented that, compared with myasthenia gravis, there were very few clinical symptoms for the degree of SFEMG disturbance noticed. However, given the fact

that they demonstrated overall improvement in both jitter and blocking with increased innervation rate, it seems likely that patients suffering from the condition unconsciously put up their innervation rates to overcome the weakness. In myasthenia gravis this would be of no avail since the jitter and blocking worsen with increased activation (section 6.12.1.2). Henriksson et al (1977) studied two patients with myasthenic syndrome, again not associated with carcinoma. SFEMG in both cases showed increased jitter in the EDC associated with blocking. Increased frequency of innervation again showed improvement in both the jitter and blocking values. This study also examined the effects of guanidine on SFEMG. This drug has been used with some success in treating the myasthenic syndrome and acts by facilitating ACh release from the nerve terminal. In both cases a clear effect was demonstrated. In one, following withdrawal of the drug, there was increased blocking which was so severe that jitter values in many of the 50 potential pairs recorded could not be calculated. In the other case, guanidine caused a gradual reduction of the mean jitter value and the minimum innervation frequency necessary for a recording, during two months of administration. This study also demonstrated a beneficial effect of the short acting anticholinesterase edrophonium. This was thought to be a prejunctional effect which was not necessarily related to the anticholinesterase activity. A further interesting finding was that the affected fibres were largely type 2 (section 6.9.2) twitch, a finding which was suggested to be secondary to an impaired neuromuscular transmission in the type 2 motor units. Given however that SFEMG records only from postural (type 1 and intermediate) fibres the electrophysiological results indicate that these fibres too were affected, although not to the same morphological extent as the type 2 fibres. Henriksson et al suggest that there might be histochemical

adjustment of the type 2 fibres due to blocking of transmission leading to the production of type 1 fibres.

Cruz - Martinez et al (1982) studied a case of myasthenic syndrome which was associated with a clearly established carcinoma of the small cell type. SFEMG of the frontalis muscle showed increased jitter in all but two of 21 potentials analysed and 12 potentials were associated with significant degrees of blocking. All the blocking potentials had an MCD value greater than 130 usec. These authors too noticed that increased innervation rate was associated with a decrease in jitter and blocking. Guanidine appeared to reduce the overall mean jitter although the numbers of potentials compared was small and no allowance was made for a possible skew in the jitter distribution. All the papers mentioned confirm therefore that in the Eaton Lambert syndrome jitter and blocking are increased at low frequencies of activation and unlike these findings in myasthenia gravis are both reduced when the activation frequency is increased. The essential lesion of the syndrome at the nerve terminal causing reduced ACh release provides evidence that SFEMG is affected as much by events at the nerve terminal as by damage at the post junctional membrane with reduced receptor sites.

6.11.2 SFEMG in botulism

Poisoning with the toxin of Clostridium Botulinum leads to severe weakness and fatigability of muscle and symptoms of autonomic dysfunction. The essential lesion appears, like the Eaton Lambert syndrome, to be a defect in the release mechanism of ACh at the nerve terminal (Lambert and Elmqvist, 1971; Cull Candy et al, 1976). Two clinical studies of SFEMG in this condition have been published. Schiller and Stalberg (1978) studied two cases, one of which had

muscular weakness and the other none. In both cases SFEMG recordings were abnormal even after apparent total recovery. In the case with demonstrable weakness 15 of 19 pairs were abnormal two weeks after the intoxication and 12 of these had blocking. Blocking was seen at a threshold value of 65 usec. The authors noted that normal and abnormal jitter values could be obtained from different pairs in the same motor unit. The jitter was found to be dependent upon the discharge rate with the same inverse relationship applying as for jitter and blocking values in the Eaton Lambert syndrome. Further SFEMG studies showed a gradual reduction in the proportion of pairs with increased jitter and blocking leading to normality at four months after the intoxication. In the second case reported, the patient had no neuromuscular symptoms ten days after poisoning and decrement testing was normal. Decrement of the compound EMG is consequence of accumulative blocking at the single fibre level (section 6.6.3). In this case four out of twelve fibre pairs were abnormal. Further follow - up was not possible. The authors commented on the unusually low jitter value of 65 usec for the commencement of blocking and queried whether the explanation might be non - quantal release of ACh with a sudden decrease in the post junctional membrane potential. Such conditions have been shown to occur with intracellular recordings in the myasthenic syndrome (Tyler, 1963). Another clinical study of SFEMG in botulism was reported by Valli et al (1983). In EDC, SFEMG recordings made one week after the onset of muscular weakness showed that 11 out of 21 recorded pairs had abnormal jitter, with blockings present in four pairs. The blockings were present at jitter values greater than 100 usec. Two weeks later there was considerable improvement in the jitter profile with 4 out of 20 pairs having abnormal jitter. One pair is shown in the data as blocking at the low value of 65 usec. Lundh et al (1977) studied SFEMG jitter

and EPP in mild experimentally - induced botulinum poisoning in rats. They found that in partially paralysed muscle there was increased jitter and blocking. The blocking percentage decreased with increased activation frequency. EPP recorded using intracellular electrodes in muscle specimens in vitro were found to be reduced. This study confirmed that drugs which are known to increase ACh release reduced increased jitter.

The studies of SFEMG in botulism have confirmed the raised jitter and blocking associated with a lesion of the nerve terminal affecting ACh release. One important point to emerge from this work is the demonstration that blocking does not always occur at a fixed value of jitter and may be quite independent of it.

6.12 SFEMG and conditions affecting the post junctional membrane

6.12.1 SFEMG in myasthenia gravis

6.12.1.1 The disease process in myasthenia gravis

Myasthenia Gravis is a disease characterised by abnormal muscular fatigability (for a review of the clinical neurophysiology of this condition see McComas, 1977). It is a chronic condition starting in adolescence or early adulthood. For a long time weakness may be confined to, or be predominant in an isolated group of muscles. Later there may be permanent denervation and weakness of some muscles. Microscopic examination of the muscles involved in the disease shows simple atrophy and some

cellular exudate. There is infiltration of the muscle fibres by round cells called lymphorrages. The essential pathological lesion has been recognized as being of the SKNMJ, principally at the post junctional membrane, where there is simplification of the post junctional folds associated with widening of the synaptic cleft. The thymus gland is abnormal in about 75% of cases with myasthenia gravis and in 10% thymoma is present.

Myasthenia gravis is now regarded as having an immunological pathogenesis in which there is destruction of the postjunctional architecture and resultant loss of ACh receptor sites (Heilbron and Stalberg, 1978; Newsom Davis, 1983). The reduction in the number of ACh receptor sites is associated with the presence of a serum IgG antibody (the anti AChR antibody). The high correlation between the presence of these antibodies in myasthenia gravis together with the association with the HLAB8 gene suggest a genetically determined tendency for thymic cells which possess ACh receptors to produce antigens. At the post junctional site in the SKNMJ the number of AChR sites is characteristically reduced to about one third of normal. This gives rise to the principal pathophysiological abnormality which is a reduction in the amplitude of the EPP, some of which may fail to trigger MAP. The studies of Elmqvist et al (1964) which showed reduction in the quantal size of ACh release in an intercostal nerve - muscle preparation from patients with myasthenia gravis is not now accepted as being the whole explanation. The evidence for an immune basis for myasthenia gravis may be summarized as follows; (1) myasthenic immunoglobulin can transfer features of the disease when injected into experimental animals; (2) lowering serum antibody by plasma exchange causes a transient improvement in clinical symptoms and conversely a subsequent rise in the antibody titre is associated with clinical deterioration; (3) binding of antibody to AChR in

experimental animals is associated with an increased rate of internalization of AChR into the muscle cell and subsequent degradadation by lysozymal enzymes; (4) morphological studies show that IgG C3 and C9 bind to the postsynaptic membrane in a manner consistent with their participation in an autoimmune destructive process (5) myasthenia gravis has a high association with other autoimmune diseases such as systemic lupus erythematosus and rheumatoid arthritis.

6.12.1.2 SFEMG in myasthenia gravis

In myasthenia gravis, muscle weakness, unlike that of the Eaton Lambert syndrome, is not improved by increased effort of activation. The classical neurophysiological test was demonstration of decrement of muscle twitch or evoked compound electromyogram on repetitive stimulation. The microelectrophysiological correlate of this observation is failure in neuromuscular transmission defined as a failure in transmission at a proportion of motor end plates. Since SFEMG can detect both early and established failure at the neuromuscular junction as increased jitter and finally blocking, the technique has found considerable use in the study and early diagnosis of the disease. SFEMG has been studied in many muscle sites in myasthenia gravis by several groups of workers and there has been general agreement in the findings. Stalberg, Trontelj and Schwartz (1976) recorded SFEMG in 32 patients with clinically established myasthenia gravis. The series included 23 female and 9 male patients whose ages ranged between 12 and 75 years. The duration of symptoms ranged between six months and 23 years. 28 of the 32 patients were being treated with neostigmine. The SFEMG findings were that in any one muscle there could be (1) potential pairs with highly increased jitter (usually >100usec) and blocking; in

this situation the blocking was noted to increase with high jitter values (2) potential pairs with increased jitter but with no blocking; the jitter in this case was usually < 80 usec (3) normal fibre pairs. This series demonstrated clear improvement in the blocking percentage of fibre pairs after treatment with anticholinesterases. The reduction in the number of blocking pairs is presumably due to the anticholinesterase prolonging the action of ACh at the post junctional membrane causing a greater proportion of the EPP to reach the threshold value. It should be noted however that the short acting anticholinesterase edrophonium may cause improvement, no change or even deterioration in the SFEMG findings. This observation parallels the observed deterioration in overtreated myasthenics (section 2.4.1.1) and corresponds to the development of a partial depolarization block.
Stalberg et al also studied the relationship of blocking to innervation rate and established that jitter and blocking worsen with increased rate of innervation. They also found that patients in remission from the disease, although not showing any detectable muscle weakness still had an abnormal proportion of fibre pairs with abnormal jitter.
In a study of 168 single fibre pairs recorded from 127 patients with established myasthenia gravis Sanders et al (1979) found that 131 pairs showed increased jitter with blocking. The recordings were made in several muscles and the results emphasized the importance of examination of other muscles in cases where the diagnosis is in doubt. The authors question the assertion by Stalberg that if any muscle in the body is weak the EDC displays abnormal SFEMG. In 8 of 13 patients studied where the weakness was confined to the ocular muscles the EDC jitter was normal. Other muscles such as frontalis gave a more reliable indicator in this situation. These authors found that in 125 of 151 studies on the EDC of patients with myasthenia jitter was greater than two standard

deviations (>55usec) beyond the mean control value in 10% of fibre pairs. They added that the diagnostic reliability of the SFEMG is enhanced if the mean jitter of the tested muscle was also used as a criterion of normality. In the case of their normals the upper limit of mean normal jitter in EDC is taken as 34 usec.

6.12.2 The effect of non - depolarizing blockers on electromyographic jitter

Eckstedt and Stalberg (1969) applied SFEMG to a study of drugs and the motor end plate. Following the observation, reviewed in the previous section, that electromyographic jitter was increased in myasthenia gravis the authors investigated the effects of intravenous curare on jitter. They used two dose levels of the blocking drug, 15 and 30 ugm/Kg and recorded voluntarily induced SFEMG from five male subjects in biceps brachii and extensor digitorum communis. In all experiments they found that the jitter increased by about 5-15 usec after injection of curare at the lower dose. The peak value was obtained 3 - 5 minutes after the start of injection, following which the jitter declined slowly during the next fifteen minutes. No blocking was observed. At this dose level the subjects complained of disturbed eye coordination and heavy eyelids but there were no signs of muscular weakness in any other muscles. No measurements of overall tetanic fade were made in this study. The larger dose of curare was injected into subjects who had already received the low dose and usually before they had completely recovered. Again an increase in jitter was noted, in is case reaching a value of 40 - 80 usec with a peak effect 2 - 5 minutes after injection. Occasional blockings were noted but not until the jitter had exceeded 75 usec. At the higher dose diplopia and

ptosis were also noted but there was no general sign of muscle weakness. As before, no attempt was made to measure the gross paralytic effect of the curare. In the discussion of this study the authors used their observations of the effects of curare on jitter to support their hypothesis that the jitter phenomenon arises largely at the neuromuscular junction and not at the terminal nerve twigs or during propagation of the muscle action potential along the muscle fibre (section 6.8). The 1969 study showed only that an increase in jitter was associated with giving curare intravenously. Repeated doses seemed to have a greater effect than the first. However, since no measurement of the degree of paralysis caused by the curare was made using the conventional decrement method no comment could be made regarding the relationship of the jitter change to blocking and the occupancy of post junctional receptors by the drug. It should be noted that the doses of 15 and 30 ugm used, corresponding to 1 and 2 mg for a 70 Kg adult, are clinically very small. The standard first dose during a conventional general anaesthetic for this body weight is 30 mg. which achieves block in the majority of the muscle fibres in the body.

Stalberg, Schiller and Schwartz (1975) studied the relationship between curare and jitter in more detail. The doses used were 15 and 30 ugm/ Kgm. Decremental response of the surface EMG of extensor digitorum communis was measured in three out of a total of eleven subjects studied. At the lower dose, the initial jitter values ranged between 14 and 30 usec and these values increased by 2 - 7 usec at three minutes after injection. The jitters were normal after 10 minutes. The change in mean interpotential interval during the experiment was less than 100 usec. At the higher dose, the pretreatment jitter values ranged from 12 to 43 usec. The jitter increased maximally at three to five minutes after injection when the values ranged between 15 and 138 usec. The

greatest change was seen in potential pairs having the highest initial jitter. Blockings were observed when the value of the jitter exceeded 80 usec but the authors did not specify the degree. At neither dose level of curare could any decrement in the surface recording be observed with stimulation at 2 Hz. From this study the authors confirmed that there was a positive correlation between the change in jitter and the initial jitter value. In the discussion of the findings the authors state ' ....there are several hundred quanta of acetyl choline released with each nerve impulse, many times greater than that necessary to elicit a depolarization of the muscle fibre membrane. The safety factor expressed in this way is calculated to be four times in motor end plates in the rat diaphragm (Waser, 1967) and four to twelve times in the cat (Paton and Waud, 1967).' In stating the safety factor in terms of transmitter excess for a fixed receptor availability Stalberg, Schiller and Schwartz have in fact given a half view, based acetyl choline release only. This is suprising because in the same paper they go on to postulate that the observed effect of curare is related to reduction of avilable postjunctional receptors. In their paper Paton and Waud sum up their findings by stating that ' the margin of safety for neuromuscular transmission in the muscles studied is such that for single shocks every 10 seconds three quarters of the post - synaptic receptors must be occluded before transmission begins to fail at <u>some</u> junctions and that over nine tenths must be occluded before <u>all</u> junctions fail. On the other hand, this observation may indicate equally that only one quarter of the receptor pool is necessary for fully normal transmission at low rates of stimulation. Alternatively, the output of transmitter could be said to be four times greater than that necessary just to evoke a propagated response in every muscle fibre.' From this statement it emerges that safety factor overall can be viewed from the point of view of

transmitter release and post junctional receptor occupancy. Reduction of both will in theory lead to a change in the characteristics of the end plate potential and ultimately to the failure of that potential to reach the critical threshold value. When this occurs a blocking should be noticed if transmission is being recorded by SFEMG. In their further discussion of increased jitter associated with curare, Stalberg et al postulate that the obtained correlation 'may indicate that the principal factors determining the jitter value are correlated with safety factor. The motor end plates with high jitter probably have EPP with a slow rise time and a low amplitude.' They quote the work of Nastuk (1971) who found a fourfold variation in the rise time of the EPP in frog between different motor end plates. The main problem in this work is to decide whether the jitter effect with curare is caused by an effect at the nerve terminal, the post junctional membrane or both. Curare is known in small doses to affect the release of acetyl choline at the terminal (section 3.5.2), a point which was mentioned but not discussed further by Stalberg, Schiller and Schwartz (1975).

6.13 Conclusions

The clinical and experimental studies of SFEMG detailed in the previous sections have established the following points;

(1) SFEMG jitter can be increased by factors affecting both the nerve terminal and the post junctional receptor sites;

(2) both these sites can have an effect on the rise time of the end plate potential which is thought to be the main determinant of jitter;

(3) the blocking phenomenon bears a different relationship to jitter and activation frequency, depending on whether pre or post junctional pathology

is present;

(4) SFEMG is seen to be a sensitive indicator of disordered function at the neuromuscular junction.

The final conclusion indicates a possible role for SFEMG in experimental studies. Although strictly speaking a physiological tool the technique can provide information about pharmacological changes within the neuromuscular junction without invading the structure. It would therefore be possible to use it in human study of anticholinesterase related changes at the end plate. Chapter 7 describes the protocols for such a study, designed to examine the effects of GB after pyridostigmine pretreatment.

CHAPTER 7: Experimental Studies

7.1 Introduction

In chapter 2 the use of pyridostigmine was described as a prophylaxis against organophosphate poisoning. The use of the drug in this way has been the subject of studies at the Chemical Defence Establishment, Porton Down. Before pyridostigmine could be used in its prophylactic role current Service regulations require that it be granted a new product licence, even though it has been in clinical use for many years. The experimental studies reported in this thesis were part of this work.

The experiments were designed to answer the following questions:

(1) what is the effect of taking pyridostigmine according to the nerve agent pretreatment schedule (section 2.4.3) on the neuromuscular transmission of normal subjects as assessed by SFEMG?

(2) what is the effect on SFEMG of low doses of organophosphate in subjects taking pyridostigmine?

(3) what is the effect of pyridostigmine pretreament upon the action and monitoring of non - depolarizing muscle relaxants likely to be used in general anaesthesia in combat?

7.2 Pyridostigmine pretreatment and muscle function

The pyridostigmine pretreatment method, where an orally administered carbamate anticholinesterase can provide protection against otherwise lethal doses of nerve agents was described in section 2.4.3. The choice of pyridostigmine for study in man had many advantages. Considerable experience had been gained with the use of the drug over a long period in the treatment of myasthenia gravis (section 2.4.1). However, although the symptoms of autonomic disturbance and weakness produced by pyridostigmine overdoseage, both in patients with this condition and in misdiagnosed cases who also received treatment, were well - described the possible side effects of the relatively low dose pretreatment schedule in normal subjects were unknown.

The technique of single fibre electromyography was chosen as being a minimally invasive procedure which could be used to examine any subclinical neuromuscular disturbance in human volunteers. Previously published pharmacological studies using SFEMG (section 6.9.2) indicated that it was a neurophysiological technique which was very sensitive to drug action at the end plate. In addition, other pathological conditions, reviewed in chapter 6, which were known to affect the end plate caused marked changes in SFEMG, even in the subclinical state.

Evidence reviewed in chapter 4 from animal experiments indicates that, at certain dose levels, both carbamate and organophosphate anticholinesterases cause structural and electrophysiological changes at the neuromuscular junction. The protocol detailed in section 7.6 below therefore considered SFEMG changes in a double blind study of volunteers exposed to low levels of GB with pyridostigmine pretreatment. In addition, recordings were made after exposure to GB

alone at a higher dose level sufficient to cause 40% inhibition of red cell AChE.

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7.3 Pyridostigmine pretreatment and non - depolarizing relaxants

Non - depolarizing relaxants are an essential part of balanced anaesthesia using nitrous oxide, oxygen and an opiate analgesic with low concentrations of inhalational agents such as halothane or ethrane. Experience during the Falklands War (Bull et al, 1983) showed that this approach to general anaesthesia in the combat area was safe and fast, with a high volume of surgical casualties being treated in often primitive conditions where proper recovery facilities were unavailable. The success of such anaesthesia depends upon the extensive experience gained in more normal surroundings with the drugs used. In the case of those producing muscular relaxation, drugs such as alcuronium (Alloferin, Roche) have been used for many years and their action is therefore known with some degree of predictability. Anticholinesterases such as neostigmine are given at the end of operation to reverse the paralysis of non - depolarizing relaxants (section 2.4.2). However, the concept of inducing paralysis in a patient already taking a carbamate anticholinesterase for protection against possible nerve agent attack was a hitherto unknown anaesthetic problem. In a situation where the clinical characteristics of muscle relaxants must be as predictable as possible, the compromise in effectiveness which may be produced by pyridostigmine pretreatment clearly requires experimental study.
In clinical anaesthesia there is increasing need to monitor the action of non - depolarizing relaxants with a standard repetitive nerve stimulation technique (section 5.3). This is particularly so with a relaxant such as vecuronium (Norcuron, Organon) which

has a strictly limited duration of action and is best used with constant neuromuscular monitoring. Whether the fade relationships normally expected for any given degree of muscle relaxation could be expected to apply after pyridostigmine pretreatment was again not known. With these considerations in mind, a study of the fade characteristics of alcuronium with and without pyridostigmine pretreatment was made. Because of an unexpected finding in the relationship of fade and relaxation measured after onset of paralysis, this study was accompanied by another which examined the fade relationships of three different non - depolarizing relaxants.

7.4 Volunteer studies

The Chemical Defence Establishment at Porton Down has for many years conducted a human studies research programme using volunteers drawn from the three Services. All studies are carried out in accordance with the Declaration of Helsinki and the recommendations of the Royal College of Physicians (Royal College of Physicians, Nov 1984). All projects are scrutinised by an ethical committee within the establishment, and vetted finally by a medical committee drawn from senior civilian medical authorities. Volunteers are usually seconded to Porton over a two week period and are fed and accomodated within the Establishment. Medical administration and all screening tests are carried out by a special clinical research unit and an associated chemical pathology laboratory. All volunteers at Porton are fully briefed regarding the reasons for the studies in which they are about to take part and the exact nature of the experimental procedures to be performed. All have the right to withdraw from a study at any stage if they so wish without giving reasons and without any entry being made on the

service record. In such a case, a volunteer is replaced in a study and the incomplete data from the subject who has withdrawn set aside from the final statistical analysis. In the SFEMG (GB Ct5) studies one subject withdrew during the first (control) SFEMG session and was replaced. One other subject (036/83) was unable to attend for the final SFEMG session for domestic reasons and was again replaced in the double blind randomisation. In this case however the control SFEMG data were included in the analysis of pooled control data presented in section 8.2.1
During the studies all volunteers were housed in normal camp accomodation. Dietary and smoking restrictions were applied according to the experimental protocols detailed in sections 7.6 and 7.7.2.

7.5 Exposures to GB

Two of the experimental protocols described later relate to studies carried out on volunteers who were exposed to low doses of the organophosphate anticholinesterase sarin (GB, section 1.2). This agent can be absorbed via the respiratory, gastrointestinal or dermal routes. For experimental purposes administration is by inhalation. The dose given is calculated from a concentration - time product known as Ct. This gives a measure of the dose of agent inhaled by a subject over a set time while performing a standard task of marching in a circle at 96 paces per minute. Two levels of exposure were used:
(1) Ct5, where a concentration of 0.33 mg/cubic metre is inhaled for 15 minutes;
(2) Ct15, where a concentration of 0.5 mg/cubic metre is inhaled for 30 minutes. The Ct15 exposure is the highest permitted by the controlling ethical committees at Porton. All exposures were carried out

with direct medical supervision within the exposure chamber. Following exposure, all subjects were medically examined and blood taken for red cell AChE levels.

7.5.1 Side effects of GB exposure

At the Ct5 level some volunteers experienced a subjective sensation of tightening in the chest. This was more marked at the Ct15 level. No subject suffered any frank respiratory distress or withdrew from the study before or after exposure to GB. Volunteers exposed at the Ct15 level developed a marked miosis during exposure and complained of photophobia and dark sensitivity up to 48 hours afterwards. Accompanying the photophobia was an aching sensation behind the eye and marked conjunctival injection developed. Subjects were issued with dark glasses but none required specific analgesic medication.

7.6 Effects of pyridostigmine pretreatment and exposure to GB Ct5 on SFEMG in the extensor digitorum communis: Experimental method

Twenty four male volunteers, aged between 18 and 30 years, were studied. All were examined before entry into the trial and were found to be medically normal with no personal or family history of neuromuscular disorder. Full laboratory screening was carried out and each volunteer was confirmed as having a normal haematological profile as well as normal blood electrolytes, liver function tests (including ALAT, ASAT, bilirubin, alkaline phosphatase and gamma GT) and normal renal function. In addition blood sugar and muscle enzyme (serum creatine phosphokinase)

levels were confirmed to be within normal limits. Volunteers were excluded from the study if they regularly consumed more than four units of alcohol daily, each unit being equivalent to half a pint of beer. Smoking and alcohol were forbidden for twelve hours prior to and during the period of electromyography.

7.6.1 Experimental groups

Subjects were allocated to four groups and randomised between active and placebo oral pyridostigmine bromide 30mg (Roche) and GB exposure. This produced six subjects for each of the following groups:

Group	PYRIDOSTIGMINE	GB Ct 5
PP	placebo	placebo
AP	active	placebo
PA	placebo	active
AA	active	active

Each subject was studied over a 10 day experimental period according to the timetable given in table 7.1 . There were five separate sessions of SFEMG during this period to give recordings under the following conditions:

Session 1: control

Session 2: two hours after pyridostigmine 60 mg loading dose

Session 3: steady state (taking pyridostigmine 30mg eight hourly for a total of 15 doses) two hours after

Time	Procedure	Blood Analyses
Day 1	Medical screening	Screening Biochemistry and Haematology
Day 2	pm SFEMG Session 1 [Control]	Control AChE
Day 3	1400 SFEMG Session 2 [2 hours after pyridostigmine 60 mg]	AChE *
Day 5	0900 SFEMG Session 3 [steady state AChE inhibition 2 hours after 30 mg pyridostigmine]	AChE
Day 8	0800 Last dose of pyridostigmine 30 mg 1030 GB Ct 5 exposure # 1400 SFEMG Session 4 [3 hour post GB]	Pre GB blood AChE analysis
Day 11	1400 SFEMG Session 5 [3 days post GB]	AChE
Day 12	Discharge Medical examination	AChE

* Subjects continue taking pyridostigmine 30 mg at 0700, 1500 and 2300 h

\# Subjects exposed to a concentration of 0.33 mg/m³ GB for 15 minutes, marching at 96 paces per minute

Table 7.1 GB Ct5 / pyridostigmine study: experimental timetable

the morning dose
Session 4: three hours post GB exposure
Session 5: three days post GB exposure

7.6.2 Cholinesterase assays

Assays of red cell AChE levels were made at the start of any electromyographic session and the results corrected for individual PCV values. Results were expressed as red cell acetylcholinesterase corrected to a standard PCV of 0.45. AChE levels were analysed in the clinical biochemistry laboratory at CDE Porton Down by Mr. D. Parkes.

7.6.3 Practical SFEMG recording

Recordings were made of fibre pairs in extensor digitorum communis (EDC) in the right forearm. This is a convenient site which has been extensively studied by other workers (section 6.8.3). The muscle is easily activated and controlled voluntarily. The recordings were made with the subject lying comfortably on a bed with the right arm pronated and slightly flexed. The room temperature was kept at 23 deg C.
Electrodes (SF37, Medelec, England) were sterilized by soaking them in 2% glutaraldehyde for 12 hours to remove all possible spore and Australia antigen contamination. Following this, they were rinsed in sterile normal saline and cleaned electrolytically before each use by passing a current of 30uamp through the recording surface, rendered negative with respect to the steel case. The case also served as the reference electrode during recording. A Velcro earth band, soaked in normal saline, was placed around the wrist of the right arm during recording.
The segment of the muscle controlling the third finger

was identified using slight extensor movements. With the muscle totally relaxed a single fibre recording electrode was introduced through the skin and just into the muscle, about halfway along the length of the belly, near the motor point. Fibre pairs suitable for analysis were then identified by slight advancing and rotating movements of the electrode when the characteristic double waveform appeared on the oscilloscope screen of the recording myograph after triggering had been set on the first peak. Recordings were made with very slight extension of the third finger. Too much effort leads to recruitment of of other motor units and consequently a very noisy signal. For ideal SFEMG recording under voluntary innervation the discharge rate should be held between 15 and 20 Hz. Most subjects were able to do this by listening to the signal on the loudspeaker. In some cases a ratemeter with a visual display was used. The number of pairs recorded during each session varied. The detection of a voluntarily activated fibre pair is a delicate process and clear recordings depend on the degree of control of activation of EDC produced by the subject. During each session between 10 and 20 pairs were recorded during a one hour period. The subject was allowed to rest the muscle for a minute or so between insertions. This permitted better voluntary control during the extended recording period.

The Medelec MS6 electromyograph used in this study was specially designed for recording SFEMG and contained the features detailed in section 6.4 which are essential for successful SFEMG recording. Figure 7.1 shows the schematic diagram of a basic SFEMG recording arrangement. The signal from the electrode was passed through a preamplifier and then to an AA6M biostable amplifier with a preset bandpass of 0.5 - 16kHz. During detection of the signals the gain was usually set to 0.5 or 1 mV per screen division. The amplified signal was then passed internally to an SDS6 signal

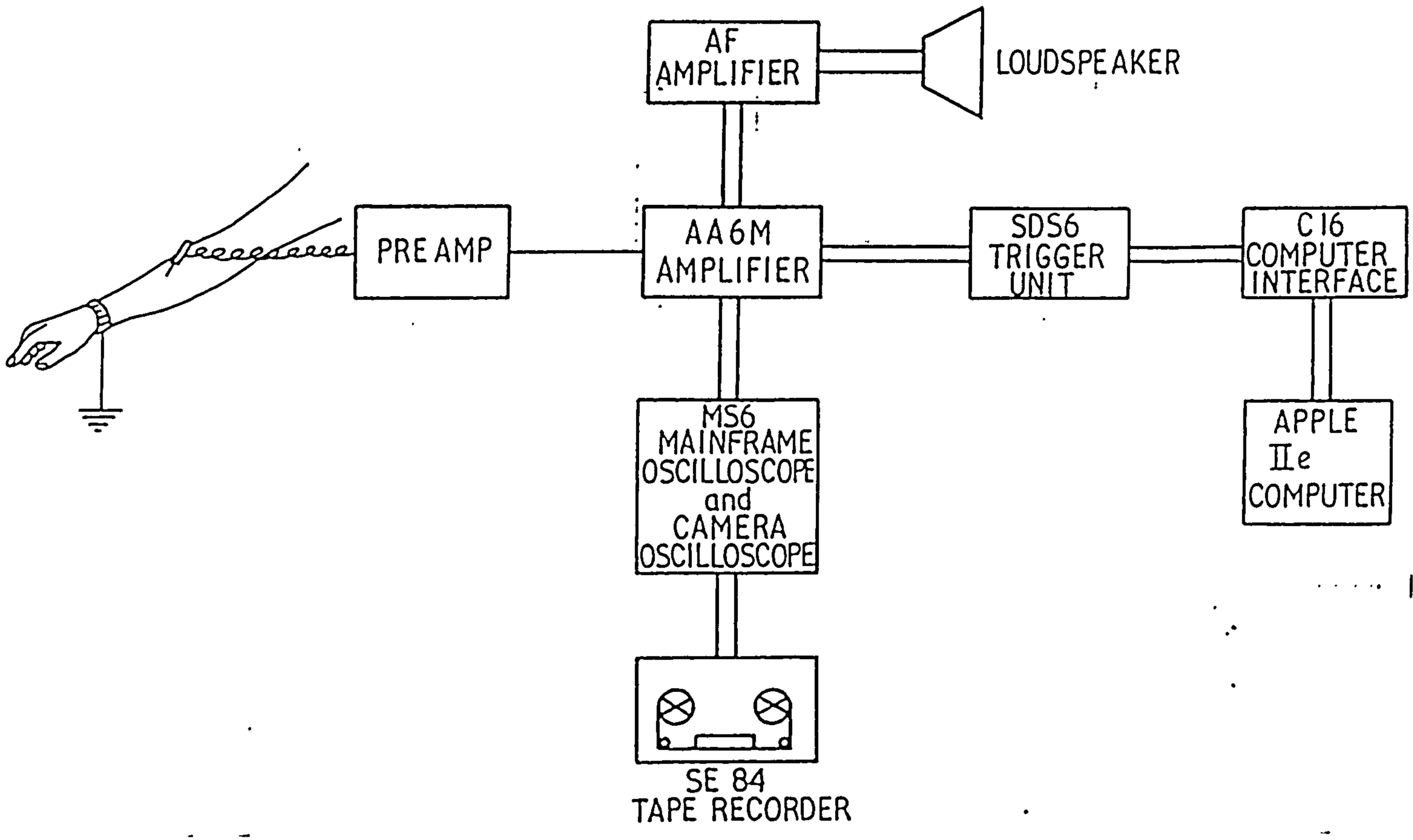


Fig. 7.1 Schematic diagram of SFEMG recording arrangements used during pyridostigmine, GB Ct5 and GB Ct15 studies

delay unit incorporating a trigger system which enabled the first of a pair of SFEMG signals to be held on the screen. Any other, time - locked waveform which then appeared on the trace must have originated from another muscle fibre with a common terminal innervation, in the same motor unit. In the SDS6 unit the trigger level could be set on either the upward or downward going phase of the first waveform. In these studies a trigger point about one third of the height of the upward going phase was used (figure 7.2). The SDS6 unit contains a delay line circuit which enabled the whole of the triggering waveform to be displayed on the screen to ensure that it fulfilled true SFEMG criteria (section 6.5.1). In addition the unit also has a 'window' feature which allows separation of a suitable fibre pair from superimposed but unrelated noise signals of larger ampiltude by triggering only on the smaller waveform.

In the MS6 system the processed signal was displayed on a main oscilloscope with a sweep velocity of 1 msec per division. A slave oscilloscope, contained in the system, enabled a photographic record of the trace to be preserved on UV light sensitive paper. Figure 7.3 shows a typical example of SFEMG signals from the study.

The time base is expanded ten times in this illustration to show more closely the time variation of the second peak. All signals detected during this study were recorded on an SE 84 FM reel to reel tape recorder (SE laboratories, England) at a speed of 15 inches per second for off-line processing.

7.6.4 Calculation of SFEMG parameters

Mean consecutive difference and the blocking proportion of recorded SFEMG jitters were calculated according to the methods given in section 6.5.2. In some cases MCD values were also calculated

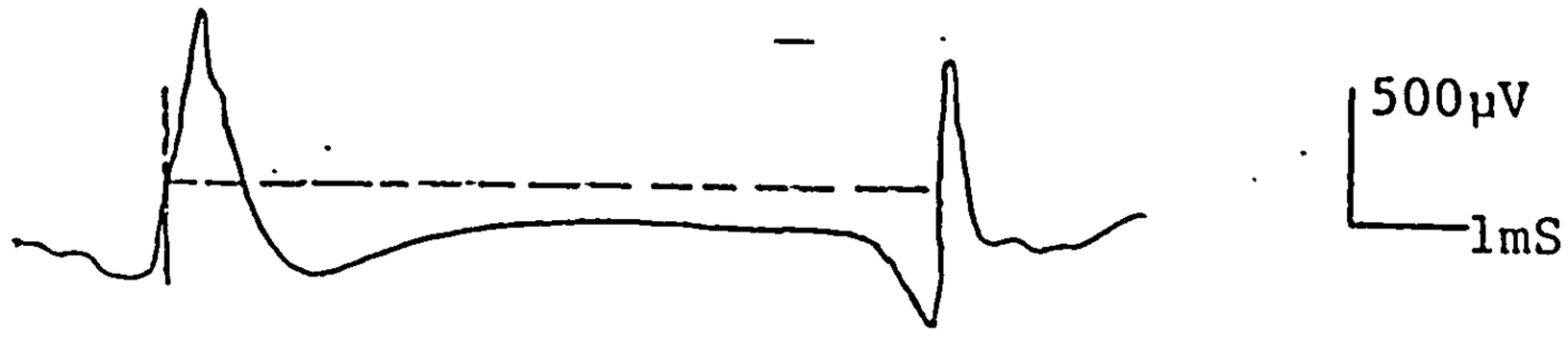


Fig. 7.2 SFEMG trigger point

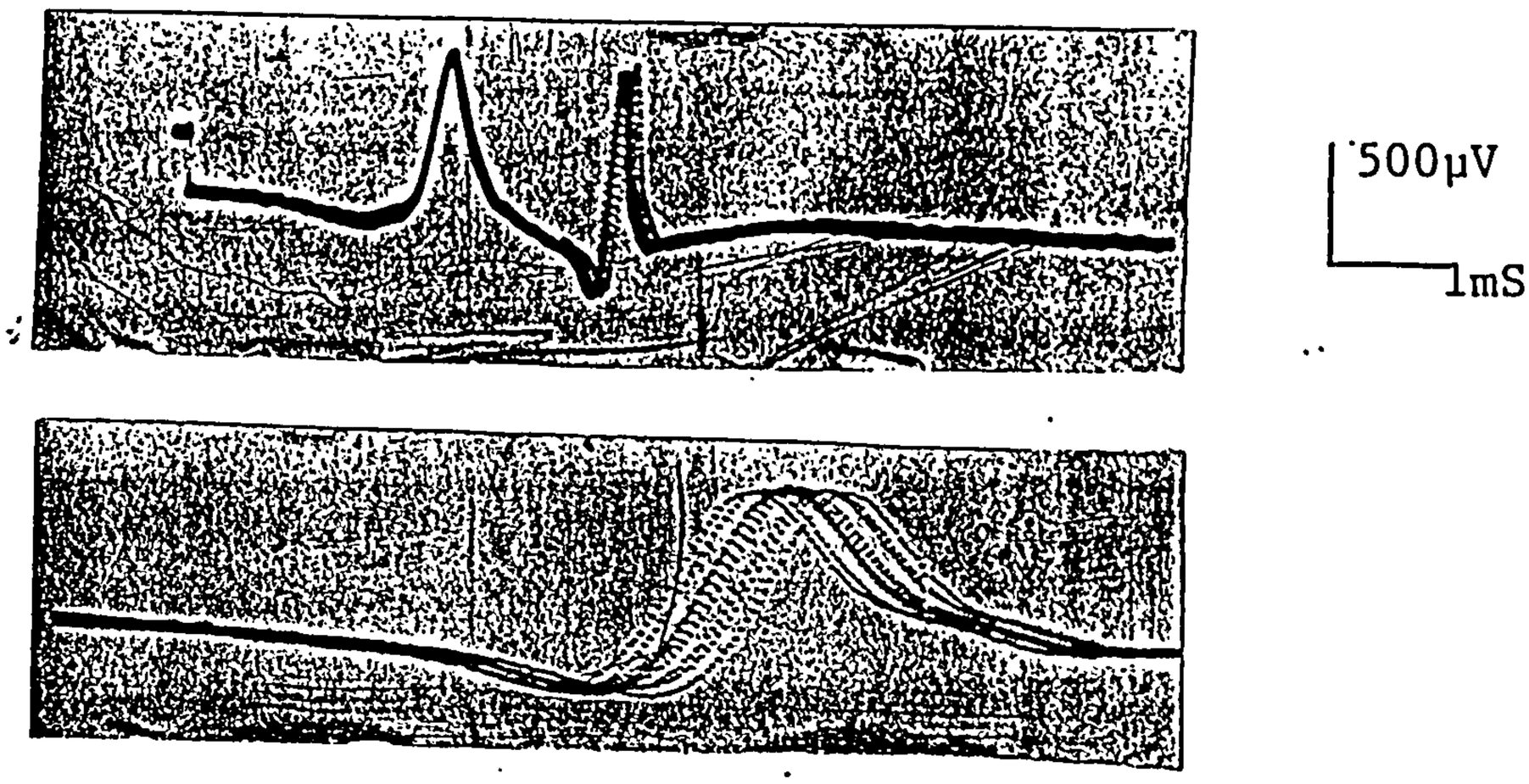


Fig. 7.3 Normal fibre pair with second potential expanded ten times showing jitter

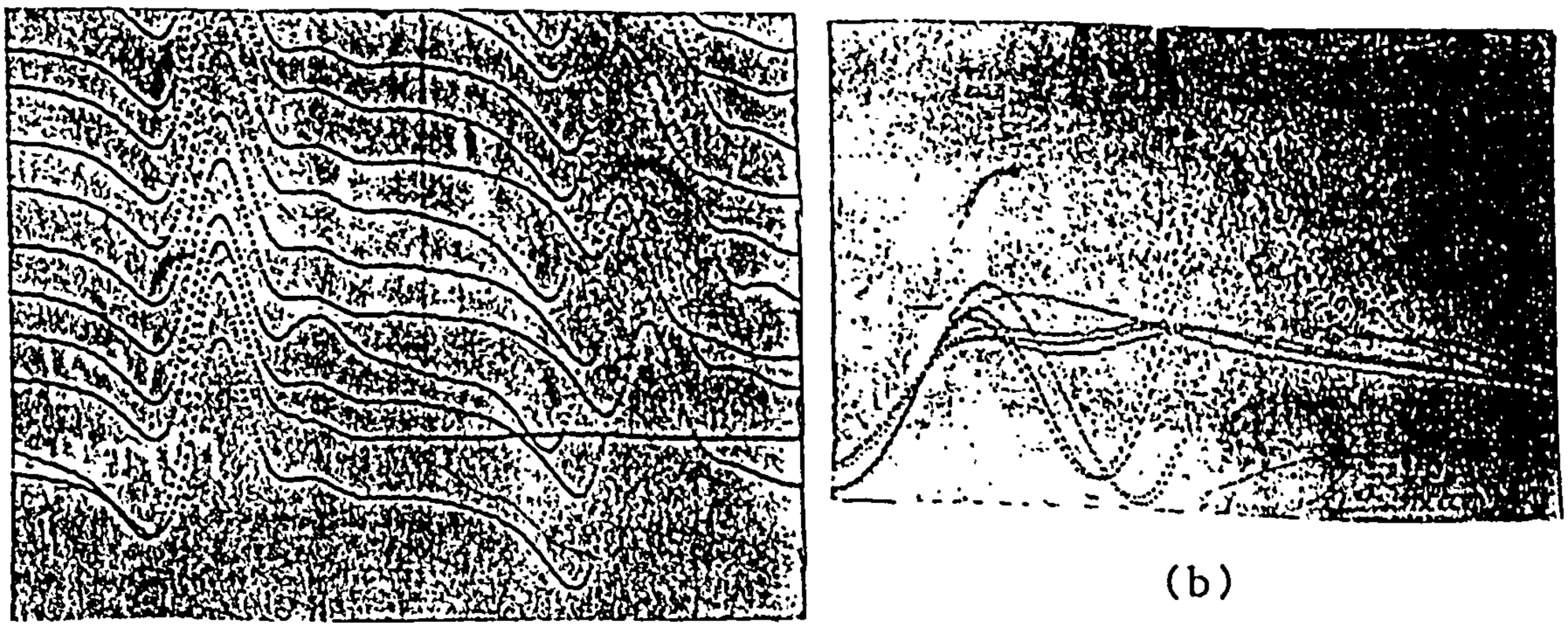
MCD = 29 µS; MIPI = 1.61 mS

from R10 sweeps (6.5.2) where the R10 distance bears a simple relationship to MCD. When blocking was observed the number of discharges with blocked second potentials was expressed as a percentage of the total discharges recorded. Care was taken to distinguish true from pseudo blocking (figure 7.4) caused by variability in the trigger due to amplitude variability in the first waveform.

7.6.5 Data processing

The results of this study were processed using a CI6 interface on the MS6 myograph. This unit linked the recording system to an Apple][e microcomputer which carried out the calculations of jitter using standard software (Medelec). In this system 40 consecutive discharges of a single fibre pair are stored and presented on a visual display unit. The desired reference for measurement of interpotential interval (IPI) can be set manually after inspection of the waveforms. MCD is then calculated automatically. Some data were processed using a DISA jittermeter (Dantec Electronics, Bristol), an alternative hard - wired SFEMG processing system. Both systems gave good agreement with each other in a cross calibration (figure 7.5).

The Medelec CI6 /Apple system was also used to check the inherent jitter of the tape recorder caused by tape speed variations. This was done by recording a triangular wave from a signal generator and calculating the MCD of the IPI measured from the beginning of the sweep. The tape jitter calculated in this way was found to be less than three usec. To maintain this, the recording and playback heads were cleaned carefully before each use.



(a) (b)

Fig. 7.4 True intermittent blocking of the second wave of a fibre pair (a) compared with pseudoblocking (b) caused by loss of triggering on the first waveform from movement of the electrode (taken from original recordings)

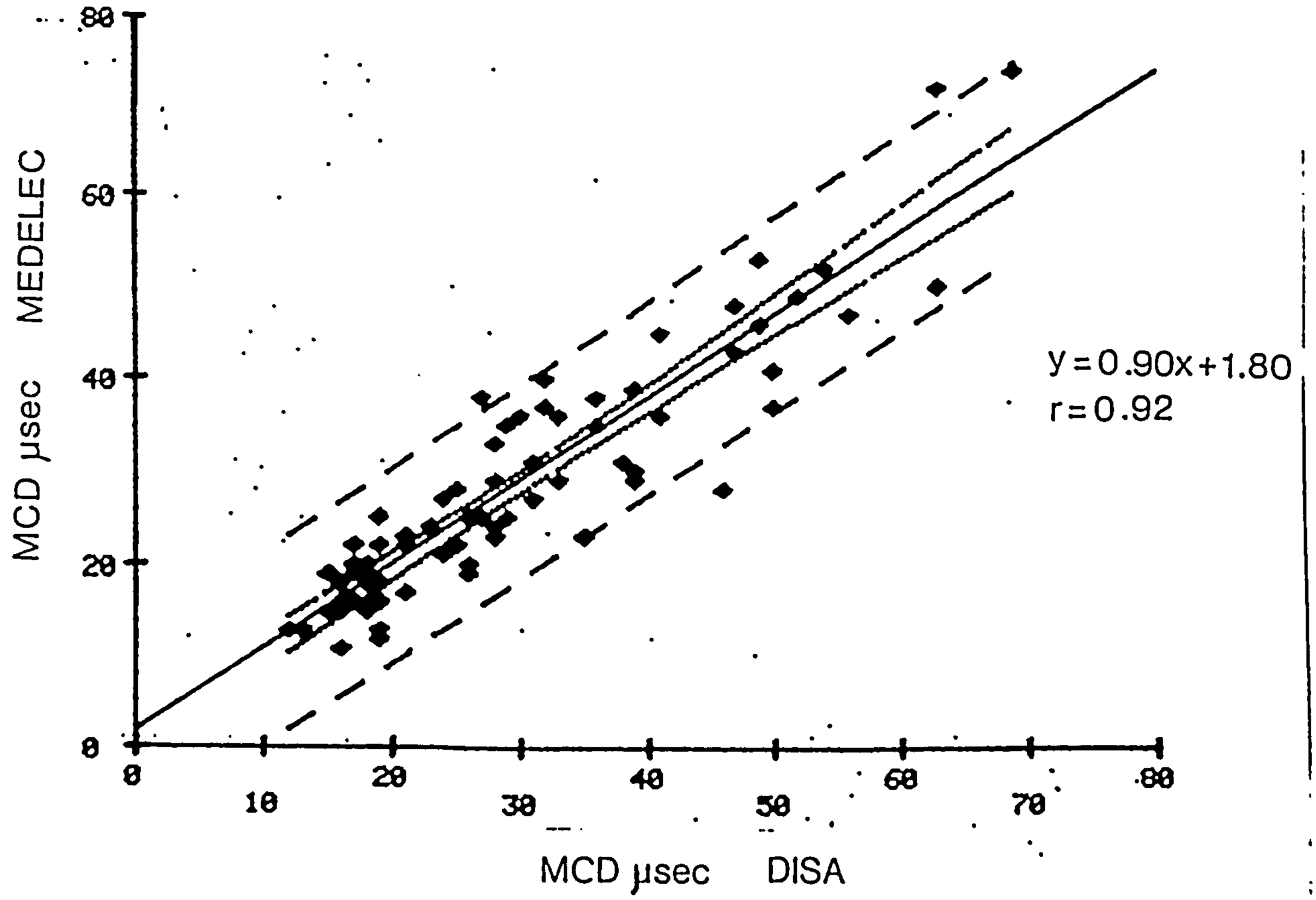


Fig. 7.5 MCD values of SFEMG jitter computed using two different system. There is good correlation between the DISA jittermeter which computes MCD from 100 fibre pair sweeps and the MEDELEC MS6/Apple 2e system which uses 40 sweeps.

7.7 Effects of GB Ct15 exposure on SFEMG in the extensor digitorum communis

The study of subjects exposed to GB Ct5 with pyridostigmine pretreatment was designed and conducted on a double blind basis. This was possible because GB at the Ct5 level causes only minimal miosis in the eye and minimal chest symptoms detectable by the volunteer. At the Ct15 level however, GB causes easily detectable myosis and conjunctival injection, rhinorrhoea and occasional tightness of the chest. In this situation the subjects during SFEMG recording are detectably different from those exposed to placebo agent, making a truly double blind study impossible. For this reason the design of the higher dose GB experiment could not incorporate a placebo exposure.

7.7.1 Experimental protocol

For the GB Ct15 study eight fit male volunteers aged between 18 and 30 years were used. The volunteers were again accepted and screened according to the process set out in section 7.6.1. The subjects used in the higher dose GB study also took part in an investigation into the effects of GB Ct15 on EEG and evoked potentials. SFEMG recordings were made at different times from EEG and there was no interference between the two experiments.

7.7.2 SFEMG recording

SFEMG was again recorded from the right extensor digitorum muscle under voluntary activation according to the method descibed in section 7.6.3. Each recording session lasted one hour during which time up to 20 fibre pairs were recorded. Four SFEMG

sessions were recorded in each subject under the following conditions:

Session 1: Control 1, day 2

Session 2: Control 2, day 4

Session 3: Three hours post GB Ct15 exposure, day 8

Session 4: Three days post GB Ct15 exposure, day 11

Session 5: Long term follow up to one year after exposure.

The timetable for the Ct 15 study is shown in table 7.2.
GB exposure at the Ct 15 level was conducted according to the protocol described in section 7.5.
No subjects experienced undue discomfort from the repeated recording sessions and there were no detectable clinical sequelae at the recording sites.
SFEMG was again processed through a Medelec MS6 electromygraph and recorded on magnetic tape for off - line processing to calculate MCD values of the fibre pairs using an Apple][e microcomputer.

7.8 Statistical analysis

Both SFEMG studies were analysed using the conventional method of determining the incidence of fibre pairs with high jitter and also using a new method of reciprocal transformation of jitter devised by Mr. N.C. Cross of the Department of Biostatistics at the Chemical Defence Establishment, Porton Down. Comparison of pyridostigmine and GB data was made using the conventional two tailed Student t test. Standard deviation was calculated using the formula

Study Day 1	Monday	am	Medical Examination ECG CXR Respiratory function PF, FEV, FVC, TLCO Laboratory Screening: blood + urine Blood cholinesterase (No 1) (2 samples) (Neurological examination (SFEMG
			Subjects who regularly drink more than 2 pints of beer or 4 shorts daily, or who would develop adverse symptoms on withdrawal of alcohol for 48 h will be excluded from the study.
Study Day 2	Tuesday	am	SFEMG(1): subjects 1 and 2
Study Day 3	Wednesday	12.00	Refrain from alcohol until 16.00 h Study Day 4
		22.00	Refrain from caffeinated beverages, smoking until 16.00 on Study Day 4
Study Day 4	Thursday	14.00	SFEMG(2): subject 1
		15.00	SFEMG(2):
Study Day 5	Friday		Weekend leave
Study Day 6	Saturday		
Study Day 7	Sunday	12.00	Refrain from alcohol until 16.00 h on Study Day 9 (Tuesday)
		22.00	Return to CDE
			Refrain from eating until 08.00 on Study Day 8
			Refrain from smoking and from caffeinated beverages until 16.00 h on Study Day 8
Study Day 8	Monday	08.00	Standardised breakfast
		09.00	Report to Clinical Research Unit Pre-chamber briefing
NB. No alcohol all day		10.15	Transport to exposure chamber
		10.30-11:00	Exposure to GB at level Ct 15 Subjects 1 and 2
		11.00	Blood cholinesterase (No 3)
		12.00	Blood cholinesterase (4)
		13.30	Neurological examination
		14.00	SFEMG(3): subject 1
		15.00	SFEMG(3): subject 2
		22.00	Refrain from smoking, alcohol and caffeinated beverages until 16.00 h Study Day 10
Study Day 9	Tuesday	08.00	Standard breakfast
		12.00	Blood cholinesterase (No 5) Subjects 1 and 2
Study Day 10	Wednesday	12.00	Blood cholinesterase (No 6) Subjects 1 and 2
		12.00	Refrain from alcohol until 16.00 h Study Day 11
		22.00	Refrain from smoking and caffeinated beverages until 16.00 h on Study Day 11
Study Day 11	Thursday	08.00	Standard breakfast
		12.00	Blood cholinesterase (No 7) Subject 1
		14.00	SFEMG(4): subject 1
		15.00	SFEMG(4): subject 2
Study Day 12	Friday	am	Laboratory-screening of blood and urine for post-study data
			Discharge medicals

Table 7.2 GB Ct15 study: experimnetal timetable. Subjects were studied in pairs over a two week period.

$$SD = \sqrt{\frac{\Sigma(x - \bar{x})^2}{n - 1}} \quad -$$

The standard statistical texts used in these studies were by Snedecore and Cochrane (1967) and Swinscow (1980).

7.9 SFEMG studies: technical comments

During these studies repeated SFEMG sessions were recorded in EDC in 33 volunteers. Although SFEMG recording in young volunteers has been recognised as being more difficult than in older subjects with pathological conditions, the studies showed that serial SFEMG is a feasible procedure in a pharmacological study. No volunteers complained of any sequelae except occasional aching in the forearm.

The two control sessions, introduced into the Ct15 study were to allow subjects to familiarize themselves with SFEMG without the pressure of trying to record an adequate number of control fibre pairs during the control session.

It was found that recording SFEMG in volunteers requires a good operator - subject relationship. Recording was easiest with the subject lying on a bed in quiet conditions. The presence of onlookers may be undesirable.

Special care was taken with regard to possible cross infection, particularly from Australia antigen during these studies. All subjects were screened for this agent and the glutaraldehyde sterilizing routine was used throughout, although long exposures to this substance caused a breakdown in the insulaton of the electrodes in some cases. Each electrode was therefore confirmed to have a resistance > 5 megohms after sterilization. There were no infective sequelae to SFEMG recording in these studies.

7.10 Pyridostigmine pretreatment and the action of non - depolarizing neuromuscular blockers.

The second group of studies on pyridostigmine pretreatment concerned the effect of the drug on the establishment and monitoring of muscle relaxation caused by drugs commonly used in general anaesthesia. The study was conducted using the isolated forearm technique (section 5.3.3) in non - anaesthetised volunteers and was in two parts. The first was to establish the pattern of train of four fade (section 5.3.1) against degree of paralysis for three commonly used muscle relaxants. The second part of the study considered the effect of pyridostigmine pretreatment on the established fade pattern during induction and recovery of paralysis. In both studies, muscle response was measured both electrically and mechanically and the results compared.

7.10.1 The isolated forearm technique: method

The isolated forearm technique used was based on that of Feldman and Tyrell (section 5.4). A commercially available frame (Radiometer, Copenhagen) was used to provide a rigid support for the stimulation and recording of the responses of adductor pollicis. The general experimental arrangement is shown in figure 7.6. Each subject was studied lying on a couch at an ambient room temperature of 22 deg C. A suitable vein on the dorsum of the left hand was cannulated using a no. 21 butterfly needle (Abbott). Patency was checked by injection of normal saline. Stimulating electrodes (no. 25 gauge) were inserted subdermally at the ulnar side of the wrist three centimetres proximal to the skin crease and halfway up the forearm (fig 7.7). The upper electrode was sited at least ten centimetres proximal to the lower one. A standard sphygmomanometer cuff was placed around the

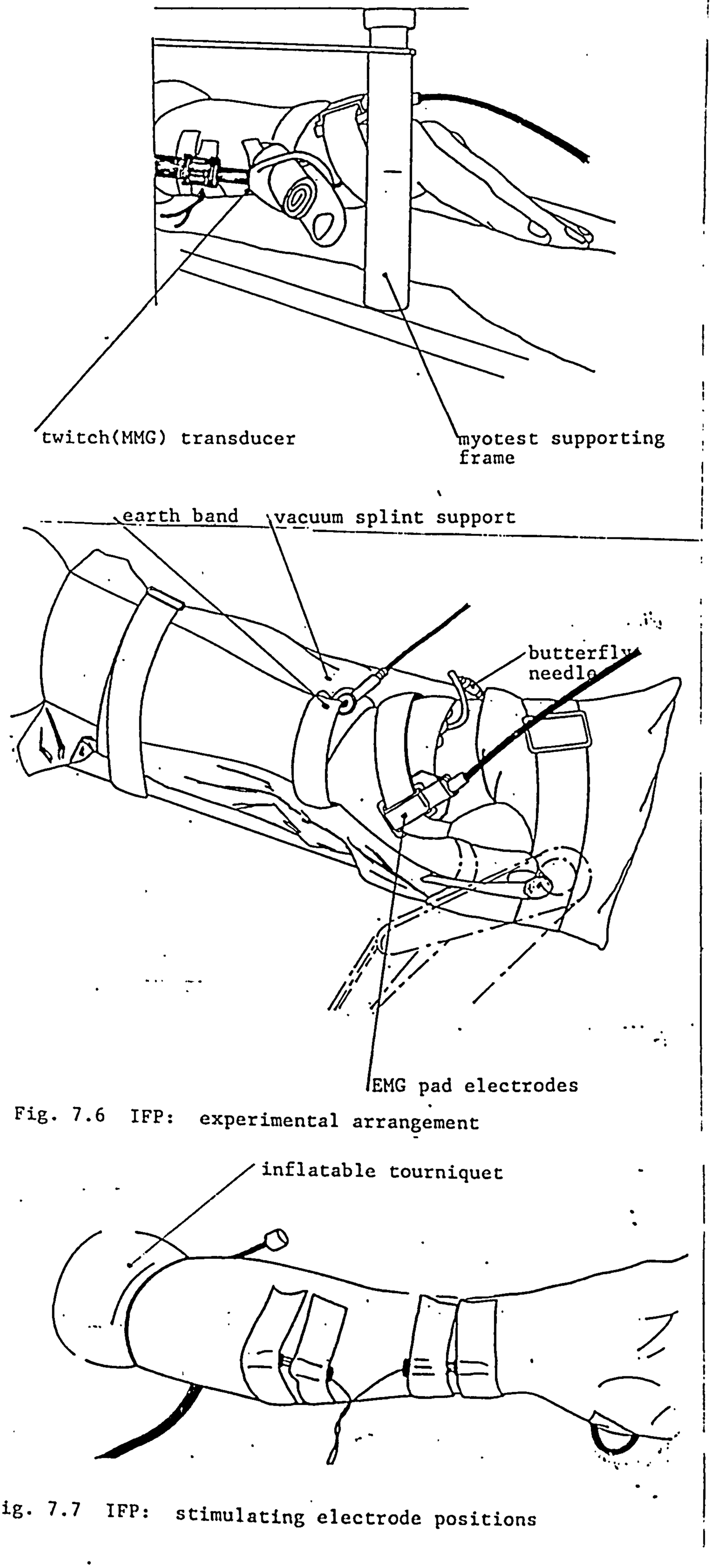


Fig. 7.6 IFP: experimental arrangement

Fig. 7.7 IFP: stimulating electrode positions

arm above the elbow. Felt pad EMG electrodes soaked in saline and covered with conducting gel mounted into a plastic holder (Medelec) were strapped over the dorsum of the hand laterally with the positive electrode lying over the adductor pollicis and the negative midway along the second phalanx. An earthing band was placed between the stimulating and recording electrodes. Trial stimuli of 0.2 msec. duration and up to 140 volts from the MS6 unit stimulator were then given and the electrode position adjusted to give the maximum single peak height in the evoked EMG as described below. After satisfactory signals had been obtained the arm was fixed, without moving the EMG electrodes into the arm frame assembly of the Myotest 2000 unit as shown in figure 7.6. The thumb was fixed securely into the ring of the strain transducer with gauze swabs to avoid slipping. The arm was fixed by evacuation of the surrounding air splint and velcro straps, and the transducer assembly adjusted for twitch recording as described in section 7.10.3.

7.10.2 EMG recording

EMG signals from the positive pad electrode may be regarded as being largely derived from the belly of adductor pollicis lying directly below (section 6.1). The signals were amplified and displayed on the main oscilloscope of a Medelec MS6 electromyograph system. Typical signals are shown in figure 7.8. Responses could be conveniently recorded with the time base set to 5 mS/division and the gain at between 5 and 10 mV/division. Amplification was through an AA6 biostable amplifier set to a bandwidth of 8Hz to 1.6kHz. Amplifier gain was set to present the signal so that the upward (negative) part of the trace covered two or three divisions of the screen and the gain was increased during the experiments as the signal amplitude fell to maintain this presentation.

Recording of the EMG was made using light sensitive paper adjacent to the recording oscilloscope of the MS6. During the test period before injection of relaxant signals were recorded first in a conventional single sweep mode and then in the continuous gated mode which presents the single peak height as a straight line which can conveniently be measured with a ruler (fig 7.9). In this study all trains of four were calculated from single peak heights measured from the origin to the maximum (upward going) negative peak height. At the end of each study further recordings of the EMG were made using a conventional XY plot to compare the final EMG shape with the initial one. Gross movement artefacts in the recording electrodes during an experiment would produce a distortion of waveform and consequent error in measuring the single peak height.

7.10.3 Muscle twitch recording

Thumb adduction twitches, or mechanomyograms (MMG) following stimulation were measured using the MYOTEST 2000 system, set up and operated by Dr G. Turner. This system, which was designed to monitor neuromuscular activity in the operating theatre and recovery room, presents signals from a strain gauge transducer as conventional twitches on a slow moving paper roll and also digitally using a liquid crystal display. Fade ratios from TOF stimuli at 2 Hz (section 5.3.1) are calculated by an embedded microcomputer and presented automatically (fig 7.10).

The arm was mounted in an evacuable splint so that the force of the thumb twitch was as nearly as possible at right angles to the pull direction of the transducer. The whole forearm was rigidly supported during stimulation. The direction of the isometric preload applied to the thumb is of great importance for .

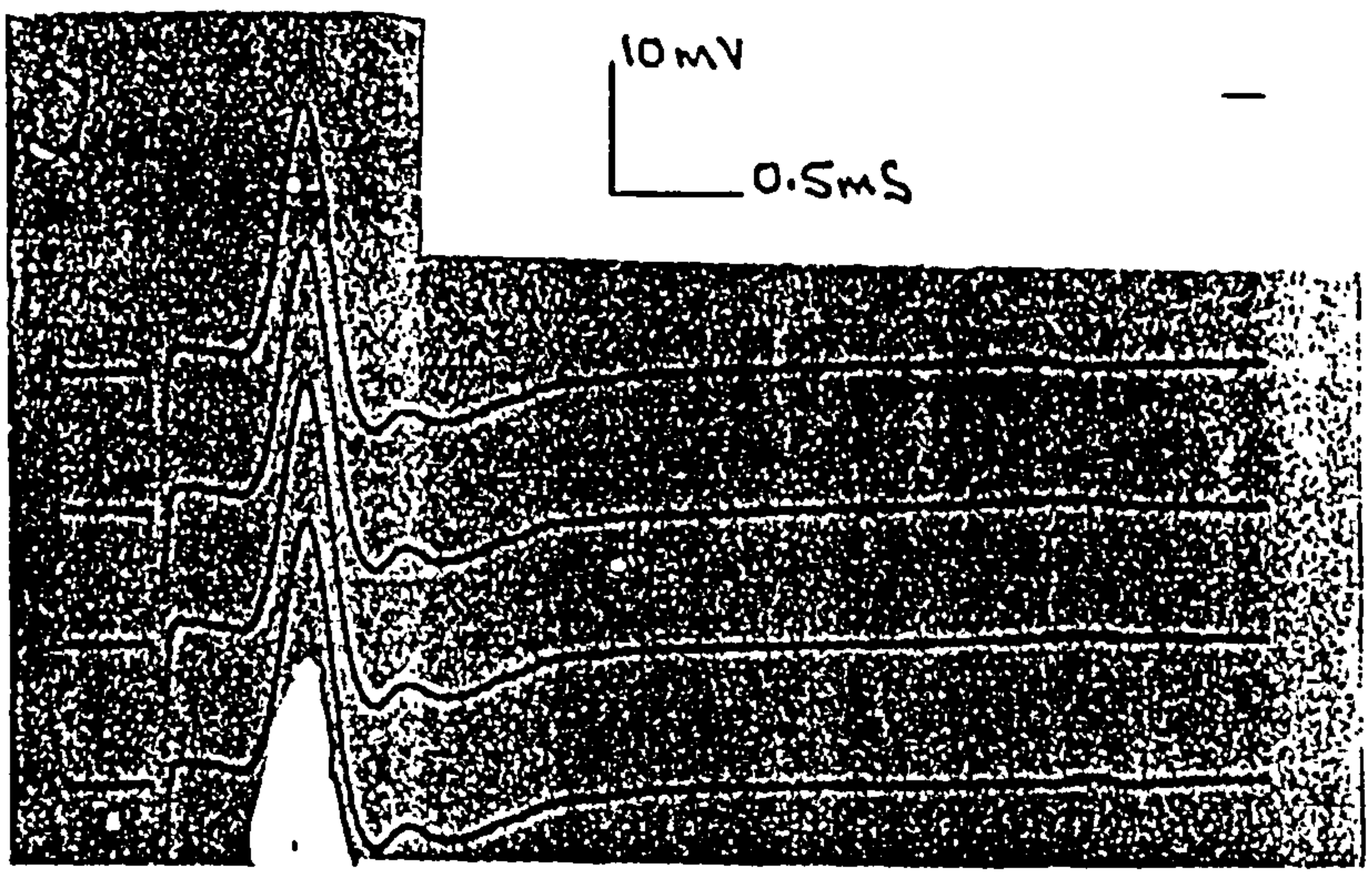


Fig. 7.8

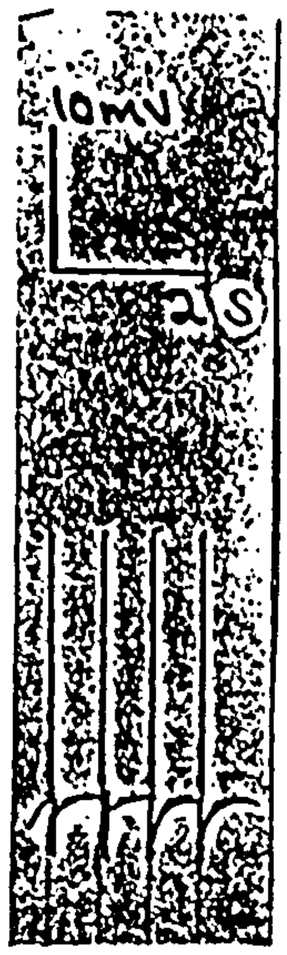


Fig. 7.9

Fig. 7.8 Evoked EMG recordings from adductor pollicis during a TOF at 2 Hz displayed sequentially.

Fig. 7.9 Signals from fig. 7.8 displayed using the continuous gated mode of the MS6 myograph. Single peak height can be conveniently measured. Note the absence of fade during this recording, which is of a control TOF.

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accurate recording of stimulated twitch tension. The preload was set in all experiments using the fine adjuster ring on the transducer, to 0.3 Kg. In the Myotest system this value can be read directly from the recording unit and was checked frequently while the experiment was in progress. After initial evacuation of the splint the preload was observed closely during the initial control period. Stability was usually gained after two or three minutes during which the subject became used to the nerve stimulation and associated twitch.

7.10.4 Nerve stimulation

TOF pulses at 2 Hz were generated using the Myotest stimulator which was inductively linked to the recording unit to trigger the automatic calculation of twitch height and fade ratio. The Myotest unit was linked to the MS6 electromyograph by taking the output of the Myotest stimulator and passing it via a Zenner diode to the stimulator unit of the MS6. In this way simultaneous triggering of both units was achieved. Signals were passed from the stimulator output of the MS6 to the subdermal ulnar nerve stimulating electrodes. Care was taken to ensure that the stimulating potential was supramaximal. The stimulating potential was gradually increased until the EMG and MMG responses were maximal and then increased by about one third of the maximal stimulation potential. Suitable values were found to be between 90 and 150 volts, and the pulse width 0.2 msec square wave duration.

7.10.5 TOF fade for three muscle relaxants

In this study eight volunteers were studied at the Chemical Defence Establishment Porton Down. All

subjects were screened and briefed according to the details described in section 7.4. No subject had any previous neurological history and no personal or family history of abnormal response to non - depolarizing relaxants. Each subject was studied at weekly intervals on a double blind basis using D-tubocurarine 5 mgm, alcuronium 1.5 mgm, and vecuronium 0.6 mgm diluted into 40 ml of normal saline. These doses were chosen to attempt to produce approximate equivalent relaxation in the isolated forearm and recovery within a maximum time of one hour. The weekly intervals were chosen to allow adequate washout of one relaxant before testing another. The experimental timetable is shown in table 7.3.

After setting up the isolated forearm and testing the reponse to pulses at 1 Hz, control values of TOF fade were measured for both EMG and MMG systems by delivering five trains of stimuli every minute, allowing ten seconds between the finish of one train and the start of the next. At time zero, the cuff was inflated to 250 mm Hg and the diluted relaxant injected via the butterfly needle. Each dose of relaxant was diluted into 40 ml of normal saline. Injection of diluted relaxant into the isolated forearm was complete by 40 seconds in all cases. The cuff was then left inflated for three minutes during which time TOF were measured at ten second intervals. This pattern of stimulation continued after the release of the cuff at three minutes up to ten minutes. After this time recordings were made at one minute intervals until return of the MMG and EMG to control values or to a maximum time of 50 minutes after the start of the experiment.

All subjects were observed closely for one hour following each experiment. The procedure was well tolerated in all cases and there were no clinical sequelae apart from occasional slight blurring of

1. Subject placed on standard examination couch.

2. Venous canulation of a small vein on the dorsum of the left hand using a no 21 gauge butterfly needle. Withdraw 0.5 ml blood.

3. No.25 gauge steel stimulating needles (sterilized by soaking for 12 hours in 2% glutaraldehyde solution) inserted subdermally over the ulnar nerve.

4. Surface EMG pad electrodes placed over Adductor Pollicis.

5. Test EMG recordings to stimuli of up to 130 volts and 0.2 msec duration.

6. Fix arm in the support frame of the Myotest 2000 twitch analyser and evacuate support splint.

7. Start stimulating with four impulses at 2 Hz every ten second and record control values of twitch and EMG train of four ratios.

8. At time zero inflate standard sphygmomanometer cuff around upper arm to at least 50 mm Hg above the systolic pressure.

9. Inject 1.5 mgm alcuronium diluted in 40 ml normal saline into the dorsal vein cannula.

10. Tourniquet released at three minutes.

11. Monitoring of twitch and train of four fade every ten seconds up to ten minutes after cuff inflation time and thereafter at minute intervals until recovery of control values.

12 Maximum total experimental time: one hour.

Each subject was studied at weekly intervals on a double blind basis using D-tubocurarine 5 mgm., alcuronium 1.5mgm, and vecuronium 0.6 mgm diluted into 40 ml of normal saline. These doses were chosen to provide an approximate equipotent relaxation in the isolated forearm and recovery within a maximum time of one hour.

Table 7.3 IFP study of three relaxants: experimental timetable

vision following release of the cuff and evidence of local histamine release in some experiments where dTC was used. There were no withdrawals from the study. Results were plotted as (1) MMG and EMG first response of the TOF against time from cuff inflation; (2) the MMG and EMG TOF fade against time from cuff inflation and (3) MMG and EMG first response against TOF fade. The results are presented in chapter 9.

7.10.6 Pyridostigmine effect on relaxant activity

The second study using the isolated forearm technique considered the effects of the pyridostigmine pretreatment regime. For this experiment the responses of adductor pollicis were studied in ten male volunteers aged between 18 and 40 years who were again screened and briefed according to the protocol set out in section 7.4. Time, and the availablity of volunteers did not permit study of the action of all three relaxants considered in the previous sections. Because of its established place in Service emergency anaesthesia alcuronium (Alloferin, Roche) was selected for further study.

The study was conducted on a double blind basis. Subjects were randomised to pyridostigmine or placebo pretreatment. Pyridostigmine was administered as an oral loading dose of 60 mg followed by 30 mg 8 hourly. Isolated forearm experiments identical to those described above were performed. MMG and EMG responses to 1.5 mg alcuronium were measured for control, post loading dose and steady state pyridostigmine sessions, according to the protocol given in table 7.4. Recovery of the partially paralysed forearm was followed up to 50 minutes after the inflation of the cuff. Results were presented for MMG and EMG recording as described in the previous section. There were no clinical sequelae from taking pyridostigmine before and isolated forearm procedure and no subjects

Study day 1	medical screening: ECG, CXR, blood and urine analysis (including plasma and RBC AChE control IFP (see table 7.3 for experimental details)
Study day 2	60 mg pyridostigmine bromide orally after standard breakfast IFP 2 2 hours after loading dose of pyridostigmine 0.5 ml blood for RBC AChE assay (and with each subsequent IFP) Subjects continue taking pyridostigmine 30 mg 8 hourly
Study day 3	pyridostigmine 8 hourly
Study day 4	final dose of pyridostigmine 30 mg two hours before IFP 3
Study day 5	discharge medical examinations repeat lab screening of blood and urine

Table 7.4 IFP study of the effect of pyridostigmine on alcuronium.
Each IFP session listed used 1.5 mg alcuronium diluted in 40 ml saline (see text)

withdrew from the experimental protocol.

7.11 Statistical methods

Data were analysed using standard correlation and regression equations given in the texts listed in section 7.8.

CHAPTER 8: SFEMG Studies: Results

8.1 GB Ct5 exposure and pyridostigmine pretreatment

Figure 8.1 shows MCD values of jitter for all fibre pairs recorded during this study. The numerical values of MCD are tabulated in appendix 2. In figure 8.1 data are presented for each of the five SFEMG sessions recorded from the 24 subjects studied, together with one subject who had to leave the study for domestic reasons before the final session could be recorded. He was replaced without disclosure by the allocation controller but the SFEMG data recorded were used for the analysis of control jitter in this study. In fig 8.1 any fibre pair showing more than 10% blocking is represented as a solid disc. Red cell AChE activity is shown as Ba45, with the activity corrected to a PCV value of 0.45. Figure 8.2 shows typical recordings displayed superimposed to show jitter in the second waveform. Examples of normal and abnormal pairs are given.

8.1.1 Control jitter values in EDC

The distribution of MCD values of jitter from 421 fibre pairs recorded during all sessions from 25 subjects with unmodified AChE levels are shown in

Fig. 8.1 (shown on the following eight pages)

MCD values of SFEMG jitter for all fibre pairs recorded during the study of GB Ct5 exposure and pyridostigmine pretreatment. Data are shown for six subjects in each of four experimental groups. Each experimental session (see text) is shown as SFEMG 1 - 5. The dotted line is at the upper limit of normal jitter for EDC at 55μsec.

SO2 02 GB Ct 5/Pyridostigmine
SUBJECT : 044/83
GROUP : PP

SFEMG 1
SFEMG 2
SFEMG 3
SFEMG 4
SFEMG 5
0 50 100 150 200 250
Jitter (MCD µsec)

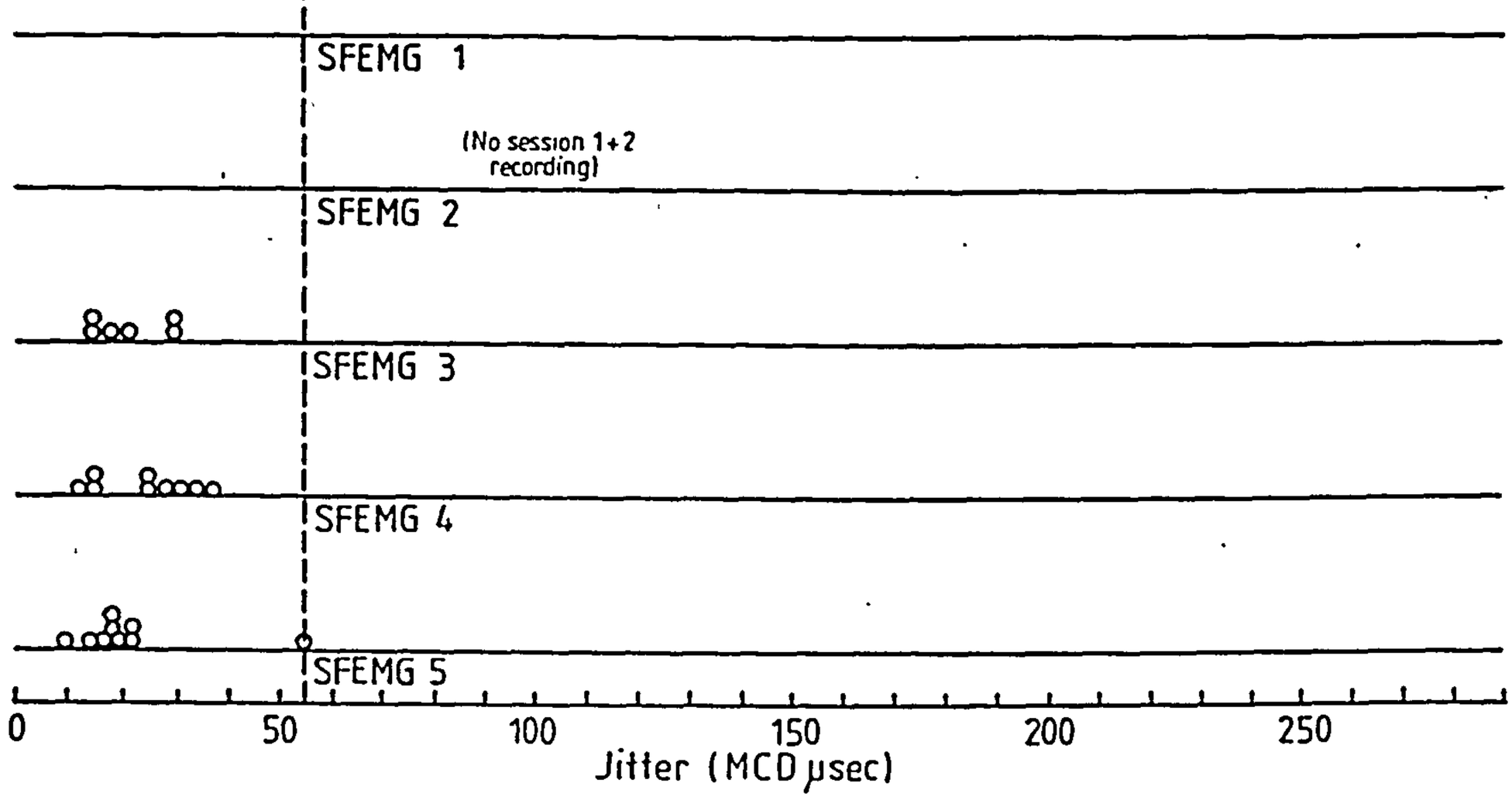
SO2 02 GB Ct 5/Pyridostigmine
SUBJECT : 046/83
GROUP : PP

SFEMG 1
SFEMG 2
SFEMG 3
SFEMG 4
SFEMG 5
0 50 100 150 200 250
Jitter (MCD µsec)

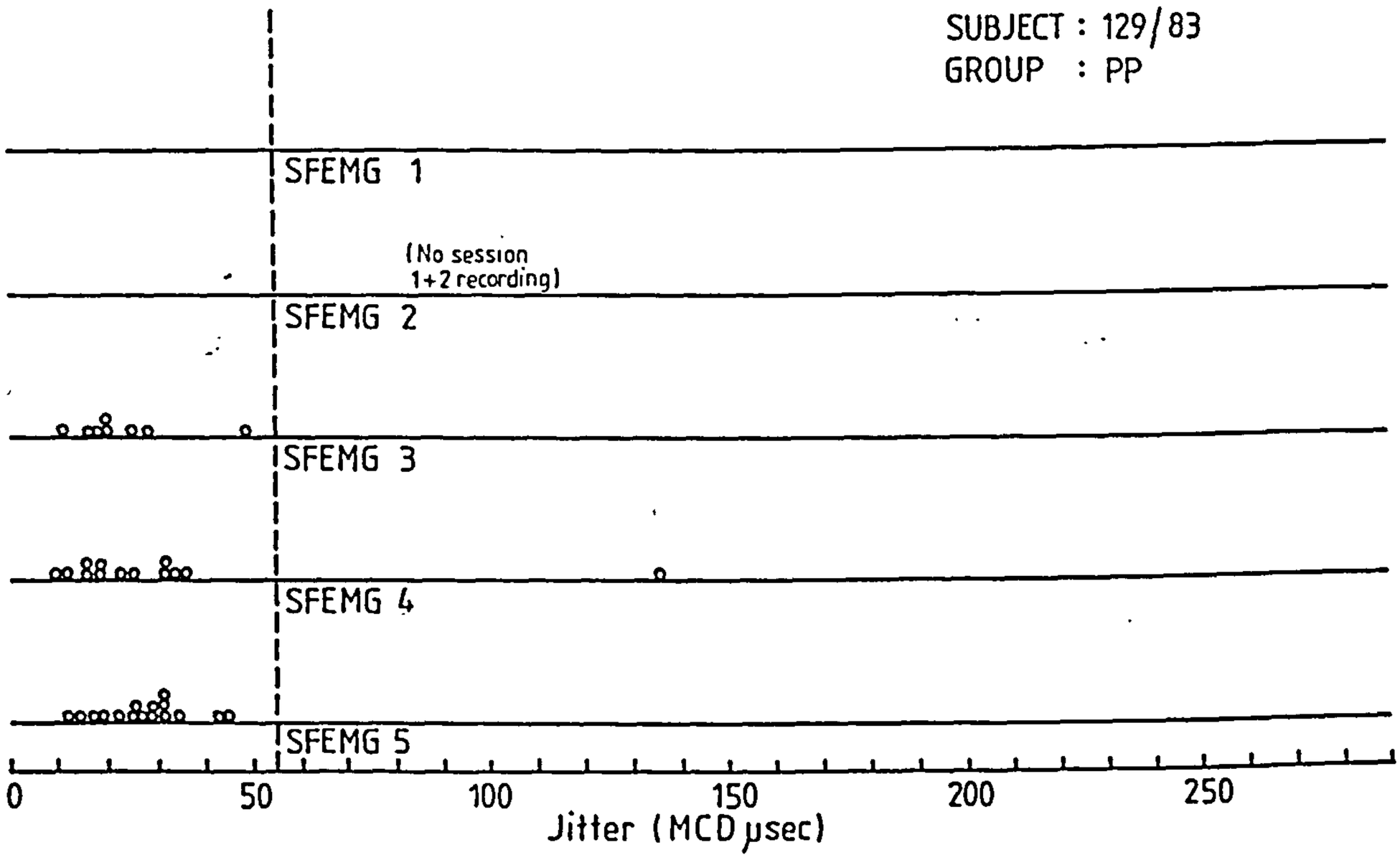
SO2 02 GB Ct 5/Pyridostigmine
SUBJECT : 141/83
GROUP : PP

SFEMG 1
SFEMG 2
SFEMG 3
SFEMG 4
SFEMG 5
0 50 100 150 200 250
Jitter (MCD µsec)

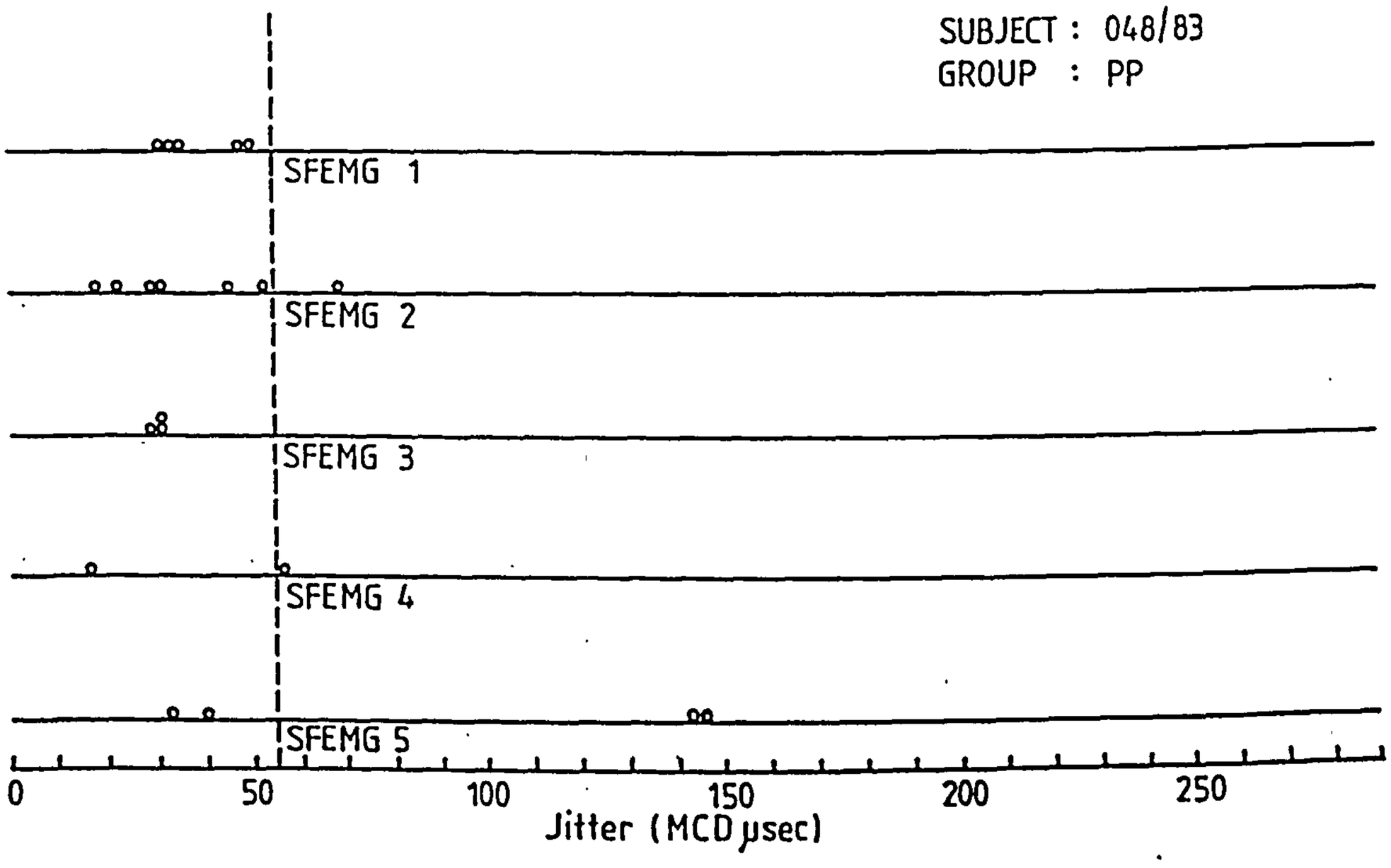
SO2 02 GB Ct 5/Pyridostigmine
SUBJECT : 130/83
GROUP : PP

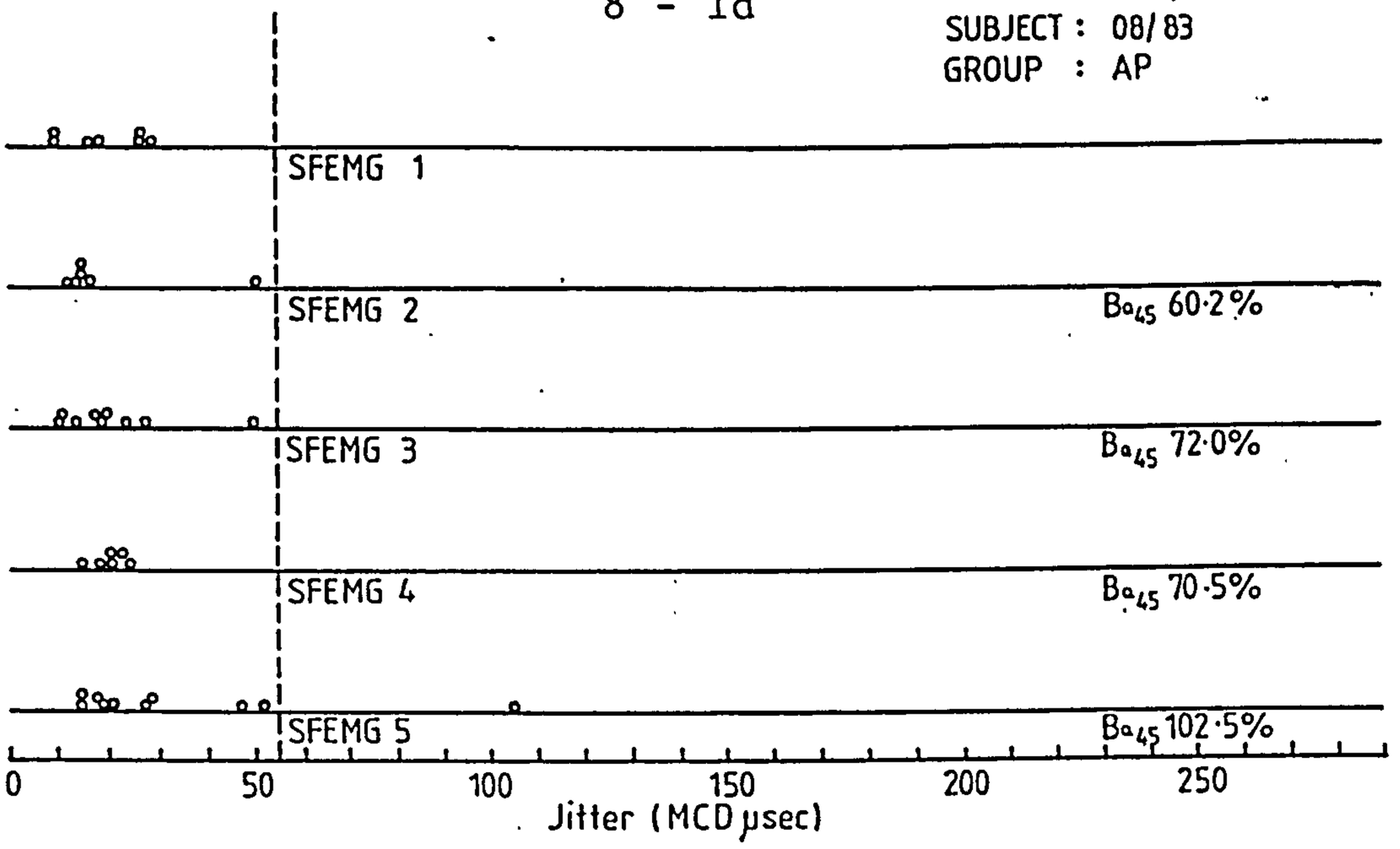


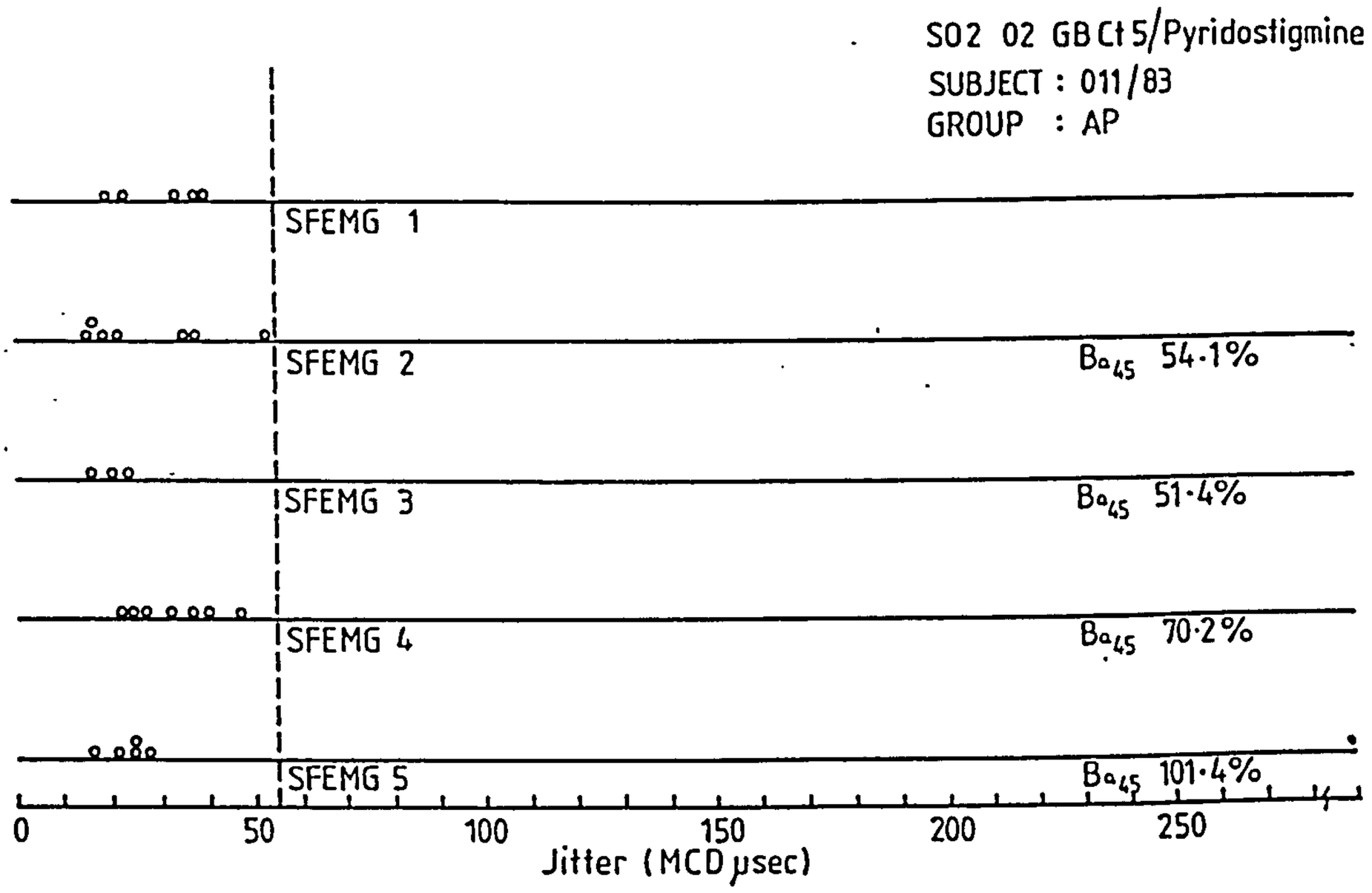
SO2 02 GB Ct 5/Pyridostigmine
SUBJECT : 129/83
GROUP : PP

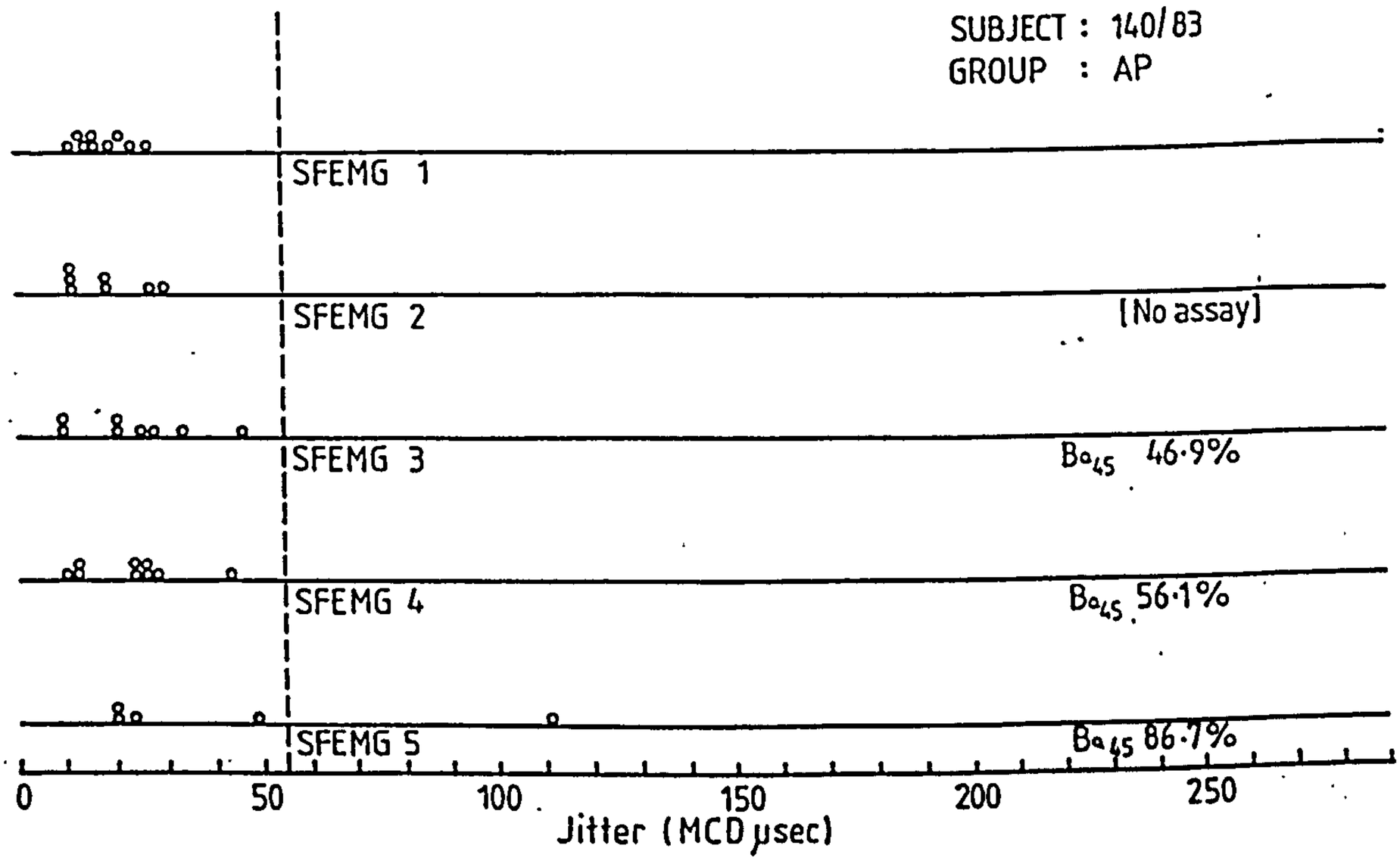


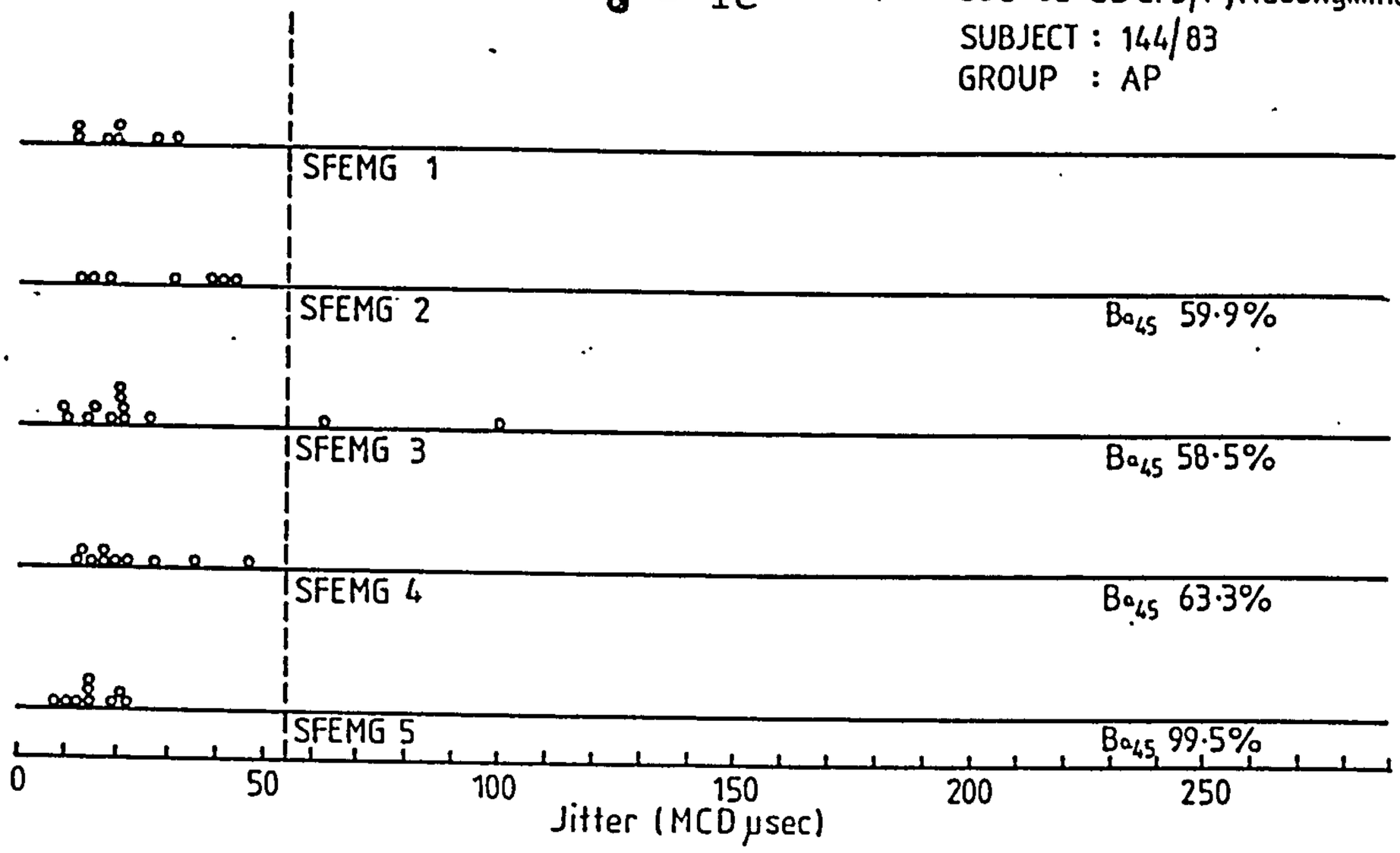
SO2 02 GB Ct 5/Pyridostigmine
SUBJECT : 048/83
GROUP : PP

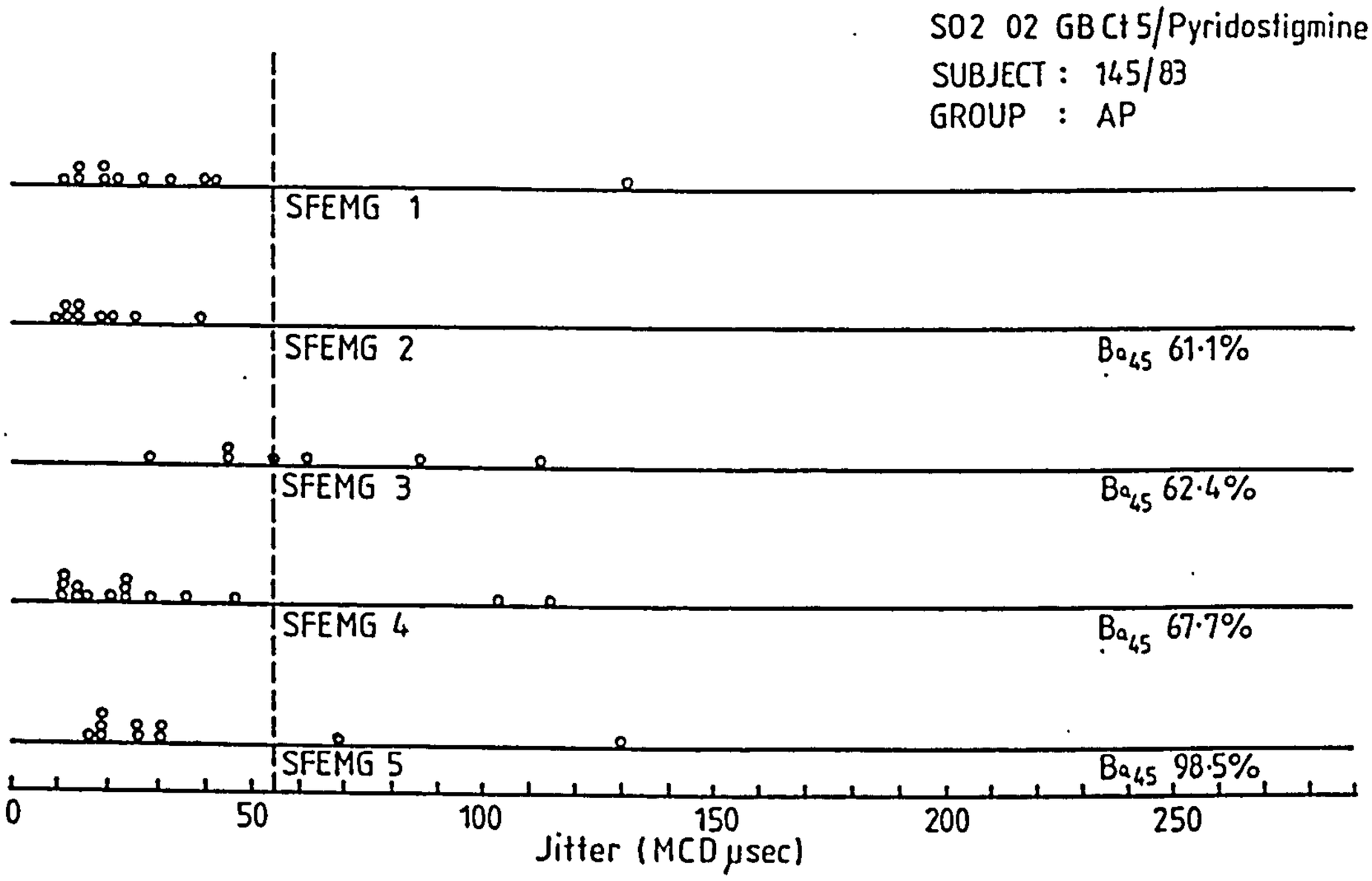


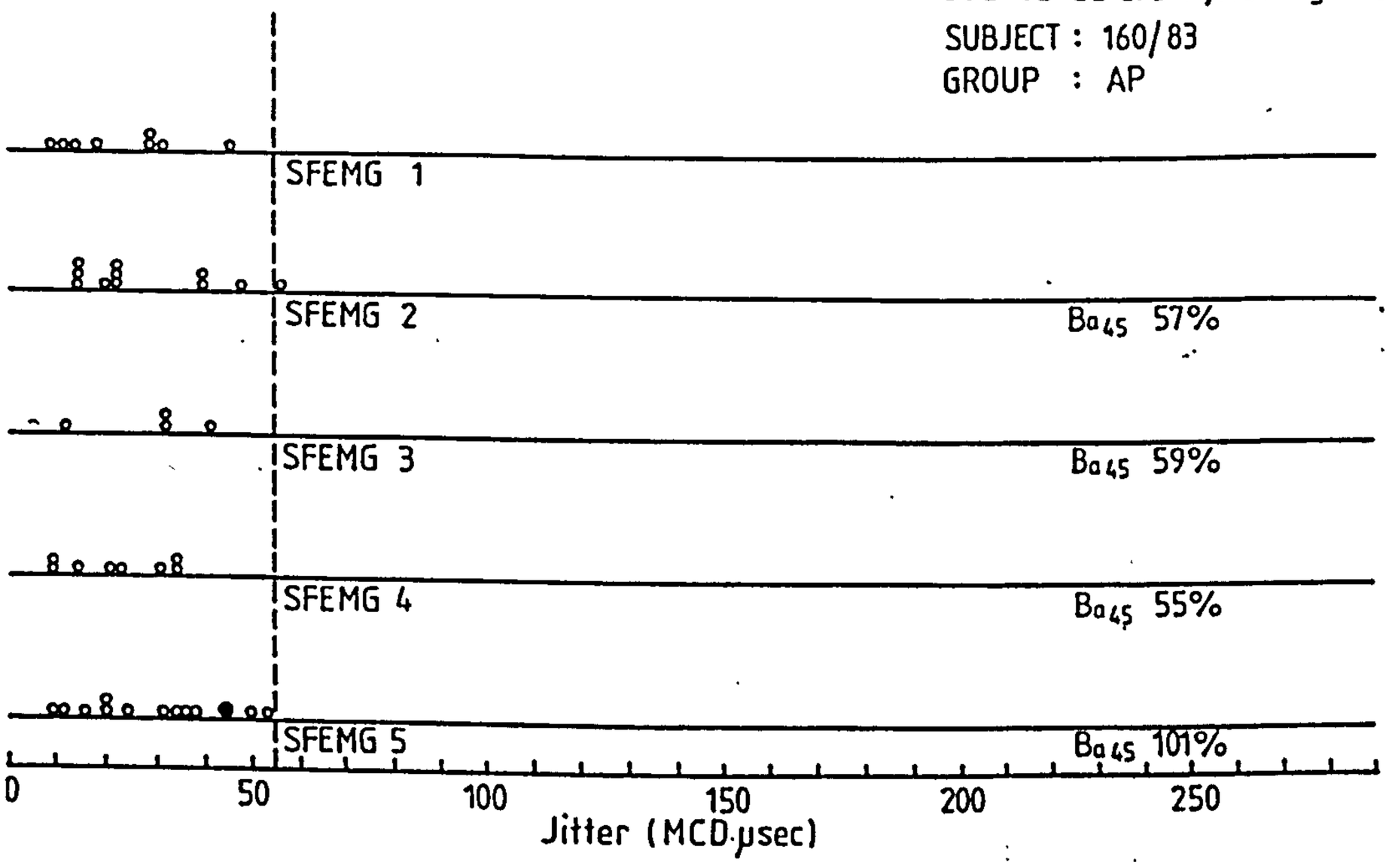

SO2 02 GB Ct 5/Pyridostigmine
SUBJECT : 08/83
GROUP : AP
SFEMG 1
SFEMG 2
Ba45 60·2%
SFEMG 3
Ba45 72·0%
SFEMG 4
Ba45 70·5%
SFEMG 5
Ba45 102·5%
0
50
100
150
200
250
Jitter (MCD µsec)


SO2 02 GB Ct 5/Pyridostigmine
SUBJECT : 011/83
GROUP : AP
SFEMG 1
SFEMG 2
Ba45 54·1%
SFEMG 3
Ba45 51·4%
SFEMG 4
Ba45 70·2%
SFEMG 5
Ba45 101·4%
0
50
100
150
200
250
Jitter (MCD µsec)


SO2 02 GB Ct 5/Pyridostigmine
SUBJECT : 140/83
GROUP : AP
SFEMG 1
SFEMG 2
[No assay]
SFEMG 3
Ba45 46·9%
SFEMG 4
Ba45 56·1%
SFEMG 5
Ba45 86·7%
0
50
100
150
200
250
Jitter (MCD µsec)


SO2 02 GB Ct 5/Pyridostigmine
SUBJECT : 144/83
GROUP : AP
SFEMG 1
SFEMG 2
Ba45 59·9%
SFEMG 3
Ba45 58·5%
SFEMG 4
Ba45 63·3%
SFEMG 5
Ba45 99·5%
0
50
100
150
200
250
Jitter (MCD µsec)


SO2 02 GB Ct 5/Pyridostigmine
SUBJECT : 145/83
GROUP : AP
SFEMG 1
SFEMG 2
Ba45 61·1%
SFEMG 3
Ba45 62·4%
SFEMG 4
Ba45 67·7%
SFEMG 5
Ba45 98·5%
0
50
100
150
200
250
Jitter (MCD µsec)


SO2 02 GB Ct 5 Pyridostigmine
SUBJECT : 160/83
GROUP : AP
SFEMG 1
SFEMG 2
Ba45 57%
SFEMG 3
Ba45 59%
SFEMG 4
Ba45 55%
SFEMG 5
Ba45 101%
0
50
100
150
200
250
Jitter (MCD µsec)

SO2 02 GB Ct 5/Pyridostigmine
SUBJECT : 041/83
GROUP : PA

SFEMG 1
SFEMG 2
SFEMG 3
SFEMG 4 Ba_{45} 82·4%
SFEMG 5 Ba_{45} 87·6%
0 50 100 150 200 250
Jitter (MCD µsec)

SO2 02 GB Ct 5/Pyridostigmin
SUBJECT : 096/83
GROUP : PA

SFEMG 1
SFEMG 2
SFEMG 3
SFEMG 4 Ba_{45} 79·5%
SFEMG 5 Ba_{45} 84%
0 50 100 150 200 250
Jitter (MCD µsec)

SO2 02 GB Ct 5/Pyridostigmine
SUBJECT : 042/83
GROUP : PA

SFEMG 1
SFEMG 2
SFEMG 3
SFEMG 4 Ba_{45} 87%
SFEMG 5 Ba_{45} 89%
0 50 100 150 200 250
Jitter (MCD µsec)

SO2 02 GB Ct5/Pyridostigmine
SUBJECT : 159/83
GROUP : PA

SFEMG 1
SFEMG 2
SFEMG 3
SFEMG 4 Ba_{45} 81 %
SFEMG 5 Ba_{45} 81%
SFEMG 6 (Extra recording session 8 days post GB) Ba_{45} 85%

50 100 150 200 250
Jitter (MCD µsec)

SO2 02 GB Ct 5/Pyridostigmine
SUBJECT : 097/83
GROUP : PA

SFEMG 1
SFEMG 2
SFEMG 3
SFEMG 4 Ba_{45} 87%
SFEMG 5 Ba_{45} 90%

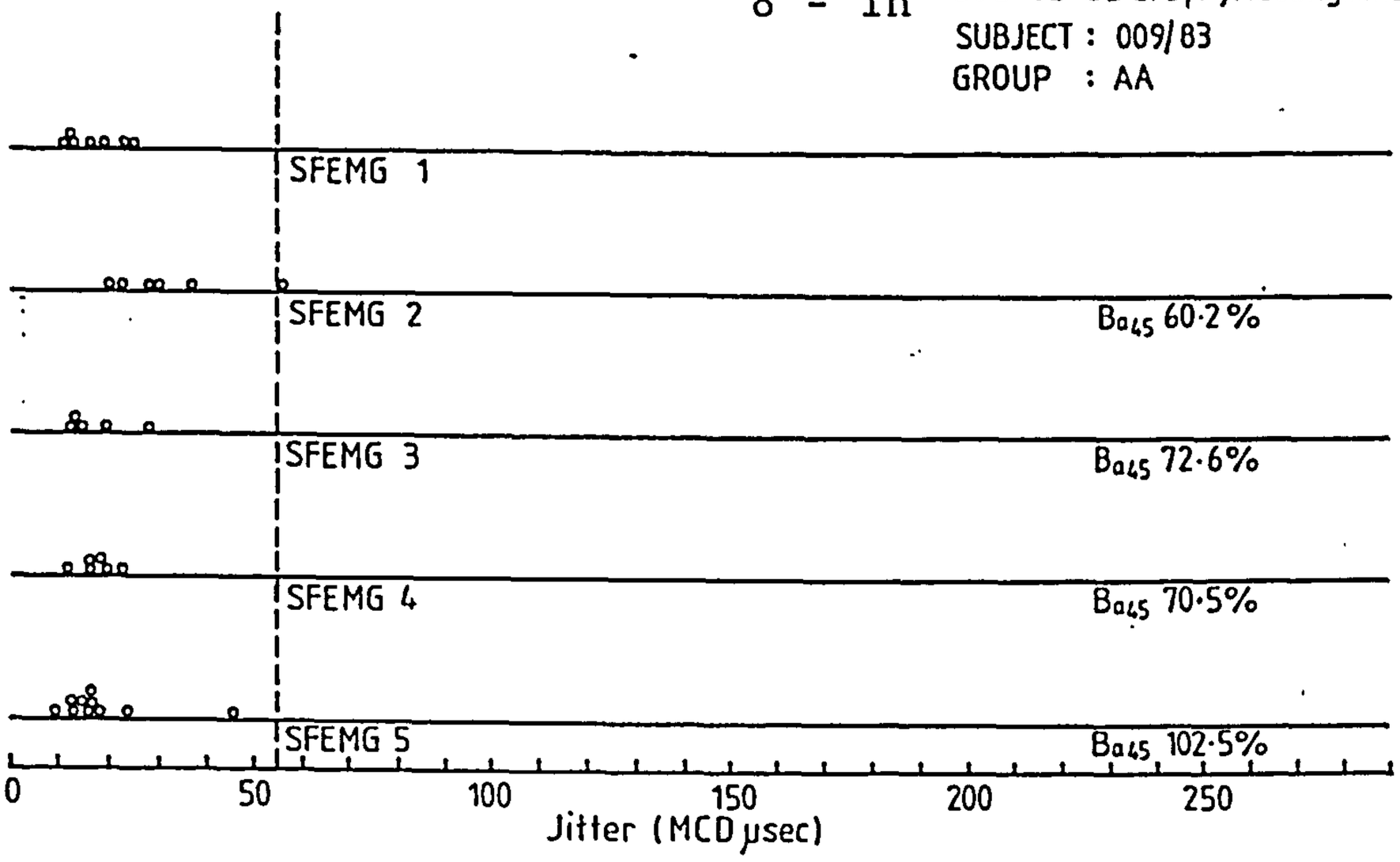
0 50 100 150 200 250
Jitter (MCD µsec)

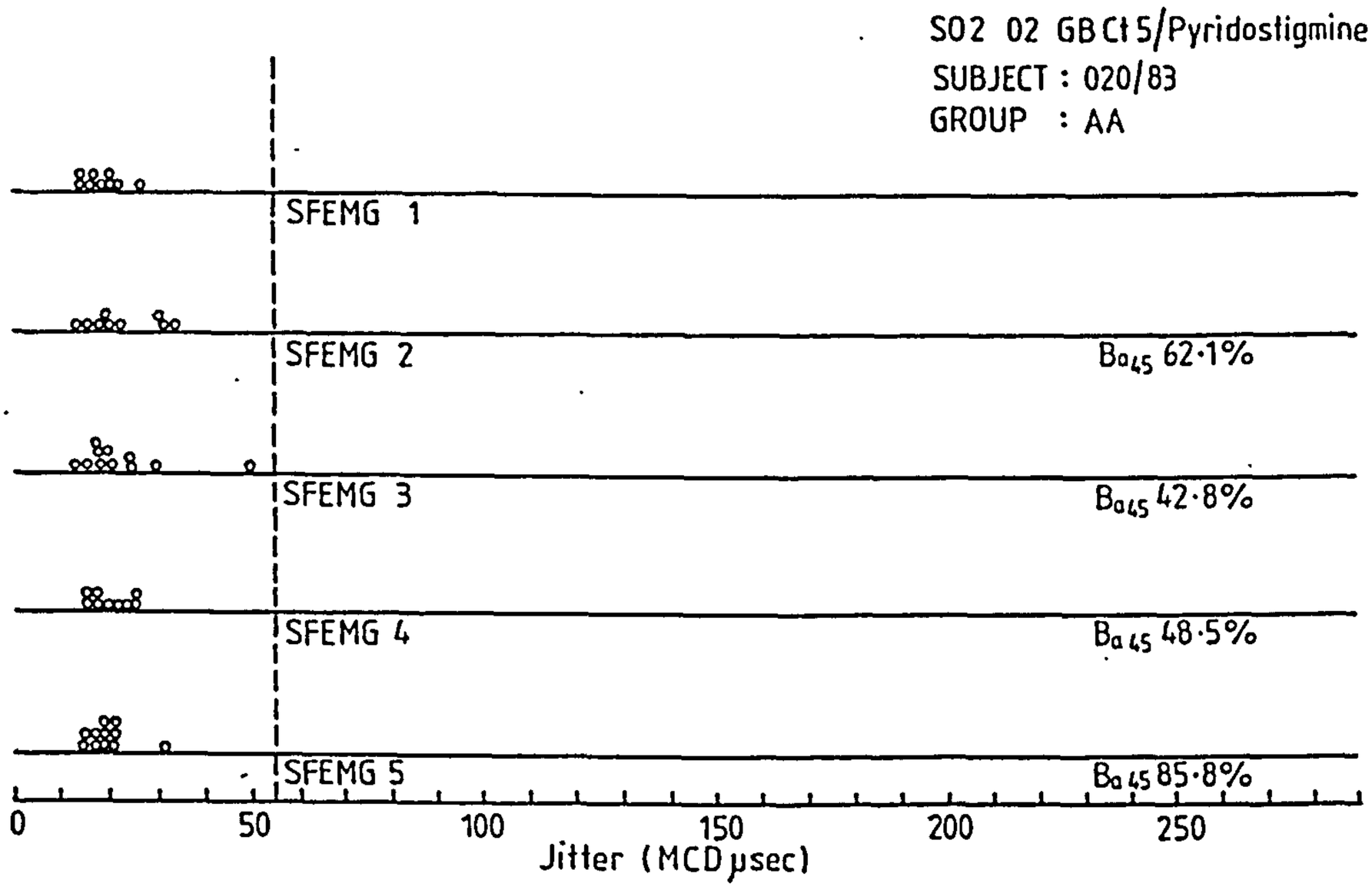
SO2 02 GB Ct 5/Pyridostigmine
SUBJECT : 172/83
GROUP : PA

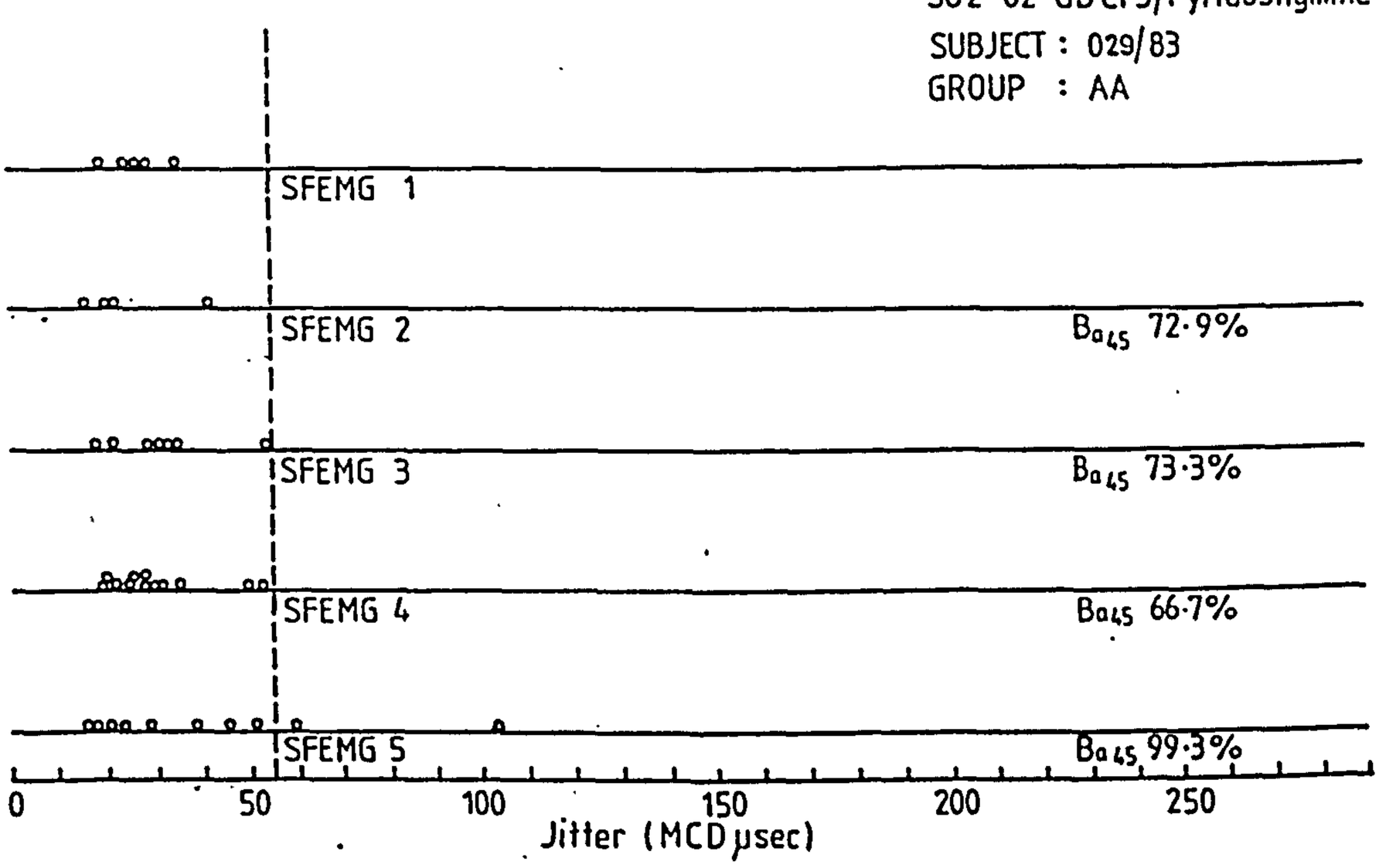
SFEMG 1
SFEMG 2
SFEMG 3
SFEMG 4 Ba_{45} 89%
SFEMG 5 Ba_{45} 89%

) 50 100 150 200 250
Jitter (MCD µsec)

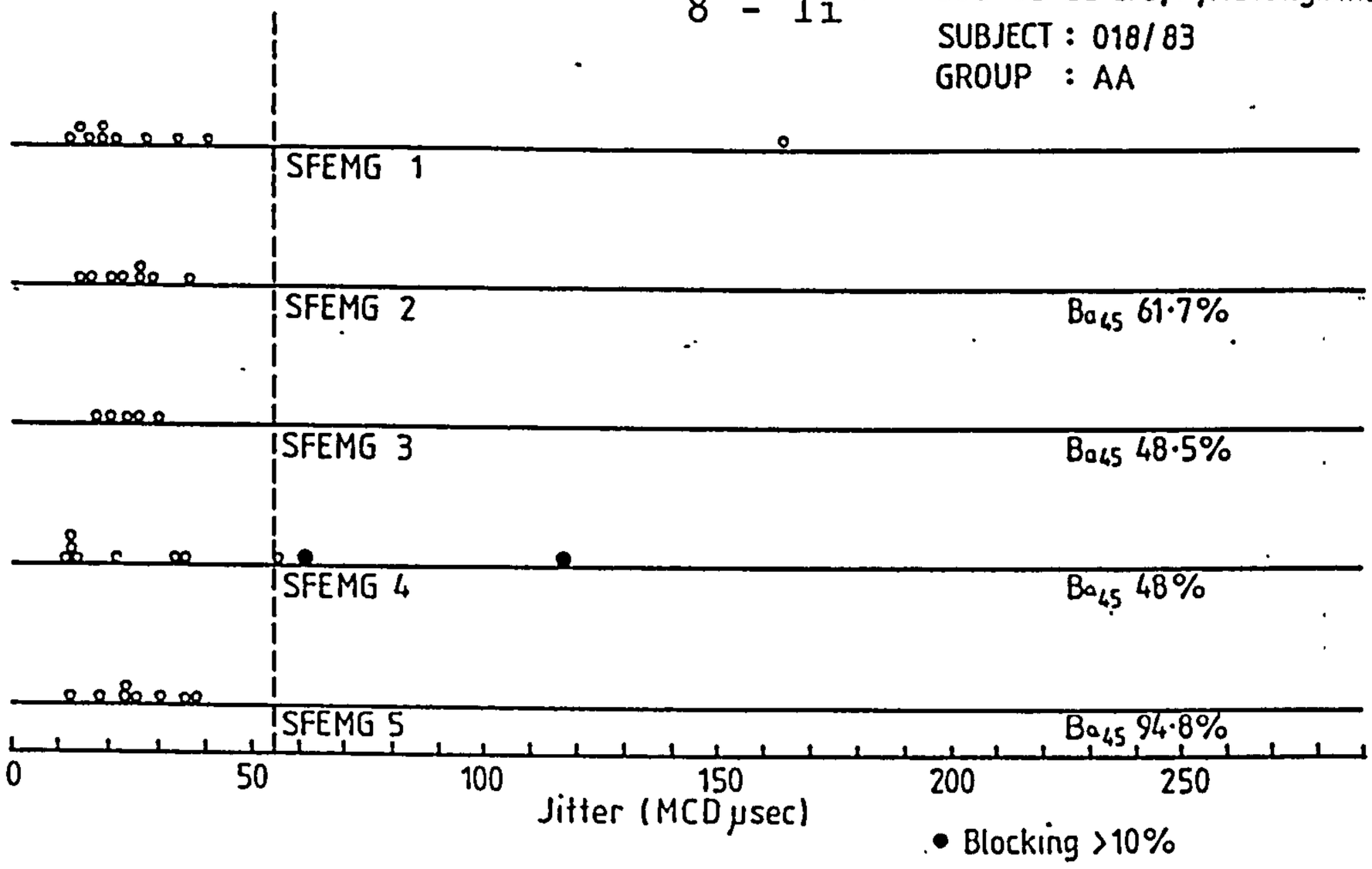
8 - 1h


SO2 02 GB Ct 5/Pyridostigmine
SUBJECT : 009/83
GROUP : AA
SFEMG 1
SFEMG 2
Ba45 60·2%
SFEMG 3
Ba45 72·6%
SFEMG 4
Ba45 70·5%
SFEMG 5
Ba45 102·5%
0
50
100
150
200
250
Jitter (MCD µsec)

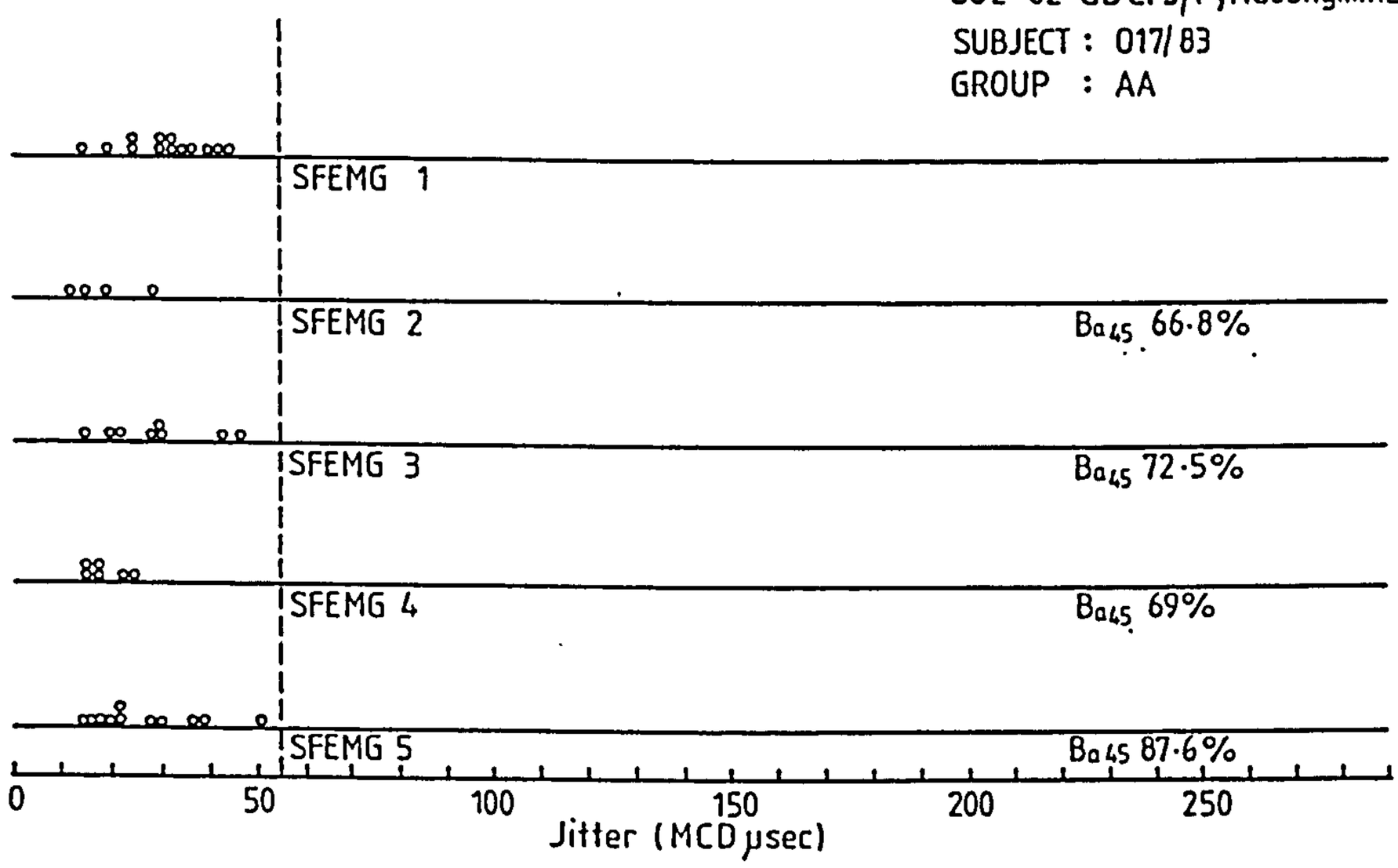

SO2 02 GB Ct 5/Pyridostigmine
SUBJECT : 020/83
GROUP : AA
SFEMG 1
SFEMG 2
Ba45 62·1%
SFEMG 3
Ba45 42·8%
SFEMG 4
Ba45 48·5%
SFEMG 5
Ba45 85·8%
0
50
100
150
200
250
Jitter (MCD µsec)


SO2 02 GB Ct 5/Pyridostigmine
SUBJECT : 029/83
GROUP : AA
SFEMG 1
SFEMG 2
Ba45 72·9%
SFEMG 3
Ba45 73·3%
SFEMG 4
Ba45 66·7%
SFEMG 5
Ba45 99·3%
0
50
100
150
200
250
Jitter (MCD µsec)

SO2 02 GB Ct 5/Pyridostigmine
SUBJECT : 018/83
GROUP : AA



SO2 02 GB Ct 5/Pyridostigmine
SUBJECT : 017/83
GROUP : AA



SO2 02 GB Ct 5/Pyridostigmine
SUBJECT : 012/83
GROUP : AA

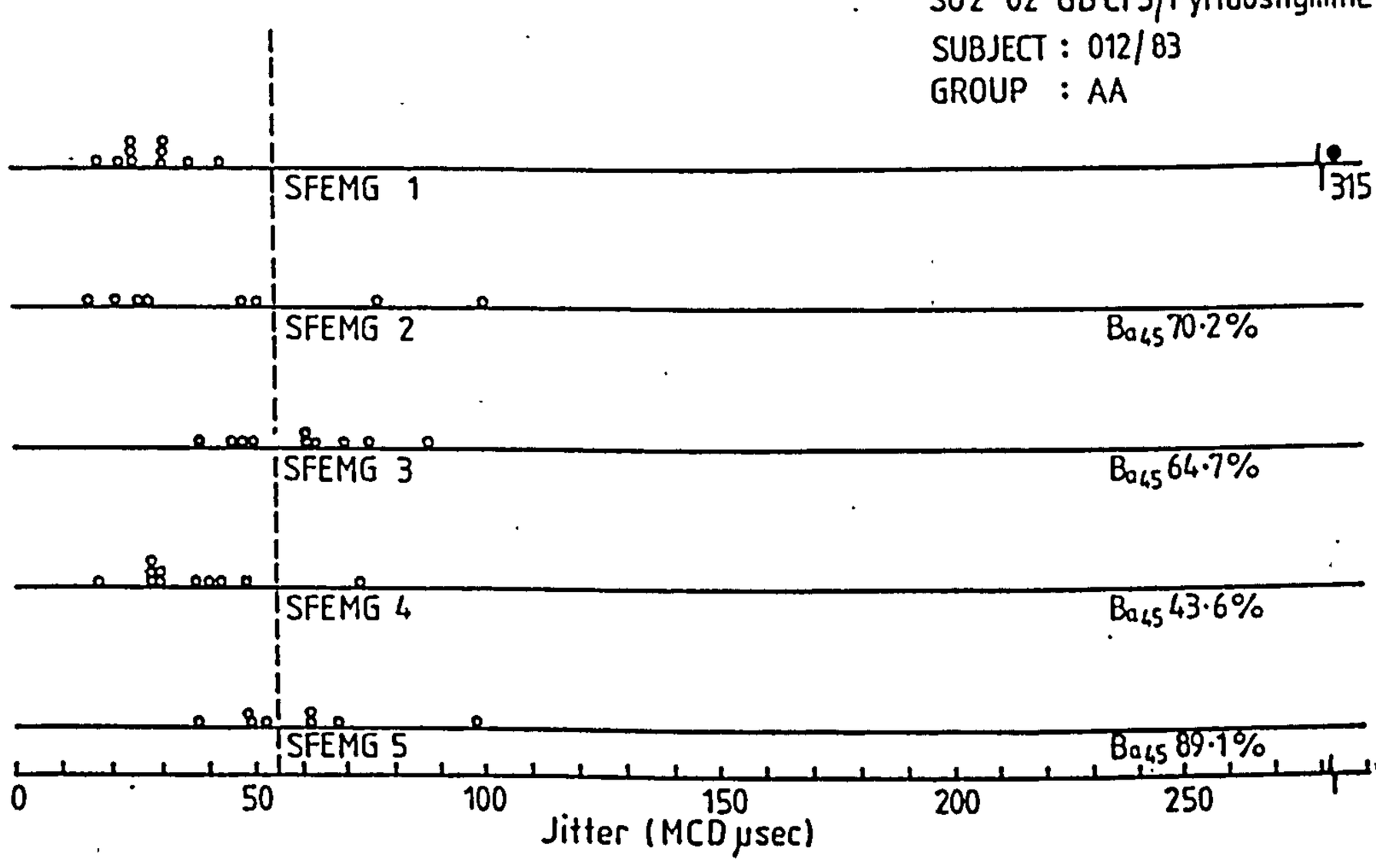


Fig. 8.2a Normal SFEMG fibre pair
MCD = 26 µsec

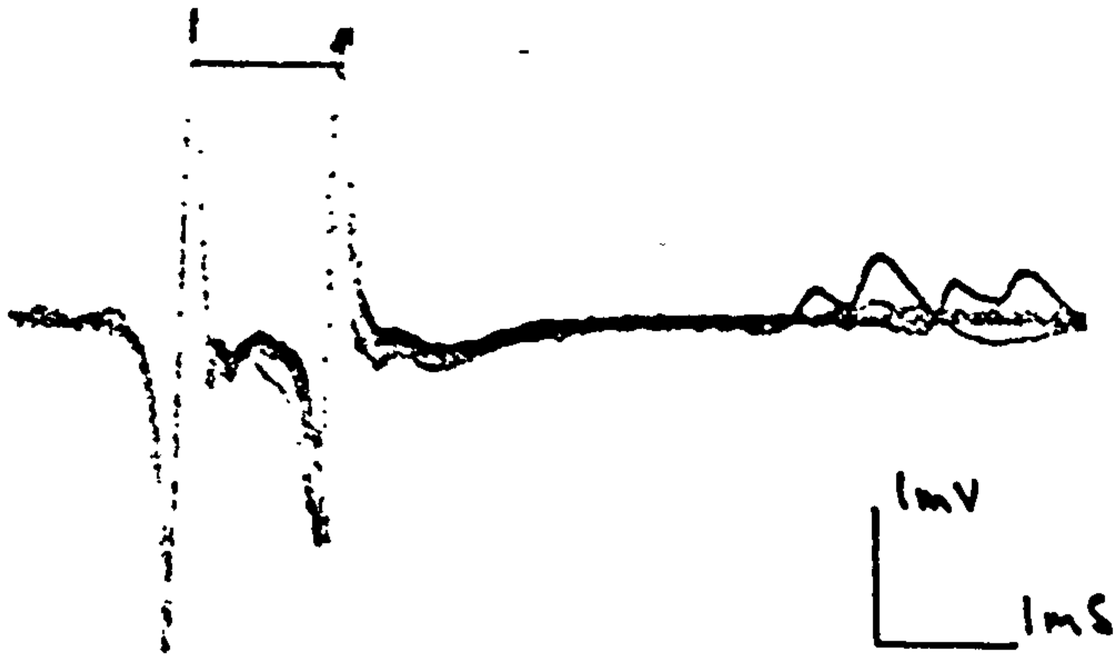


Fig. 8.2b Abnormal jitter. One blocking is seen in the ten superimposed sweeps shown at (a). Line (b) shows the R10 distance from which the MCD can be estimated at 200 µsec

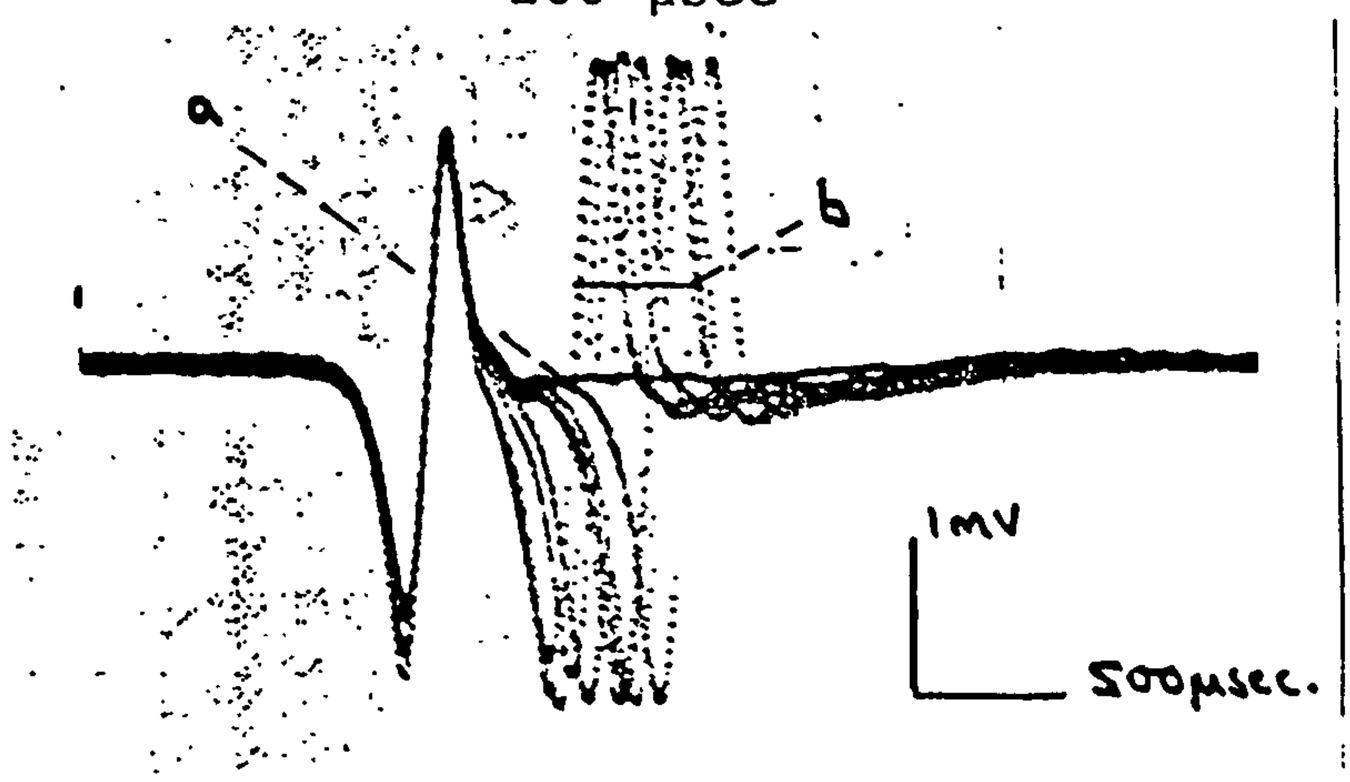


figure 8.3. The fibre pairs included in this figure are from group PP (sessions 1-5), group AP (session 1), group PA (sessions 1-3) and group AA (session 1). The overall mean MCD is 30.11 $\pm$ 29.97(SD) usec and the range of values 10 - 315 usec. Twenty fibre pairs (4.7%) have MCD values greater than 55 usec. Figure 8.4 shows figure 8.3 replotted with values greater than 55 usec truncated according to conventional practice (section 6.8.3). The mean MCD is now 25.37 $\pm$ 9.29 usec. Examination of the statistics of skewness, kurtosis and Kolmogorov D (see appendix 1) applied to figure 8.3 shows that the data do not follow a normal distribution. The truncated data in figure 8.4 have a closer fit to normal. Figure 8.5 shows data from figure 8.3 after application of the reciprocal transformation discussed in appendix 1. Reciprocal transformation has been adopted because, using this technique, it is possible to include all jitter values in an analysis of drug related effects without having to employ an arbitrary truncation of the distribution. Taking the reciprocal of MCD gives an expression which has the dimensions of 1/sec. In this study this quantity is called 'jitter frequency'. Appendix 1 shows that cumulative distribution plots of the control data transformed in this way indicate that the reciprocal transformation is a good fit to a theoretical normal distribution. The transformation enables comparison between study groups to be made using simple Student t testing. In the data shown in figure 8.5 the mean jitter frequency is 43.31 $\pm$ 17.88 kHz. For the analysis of the effects of pyridostigmine and GB Ct5 on jitter a paired analysis was used which used the control data from session 1. Figure 8.6 shows MCD values from 156 fibre pairs so recorded.

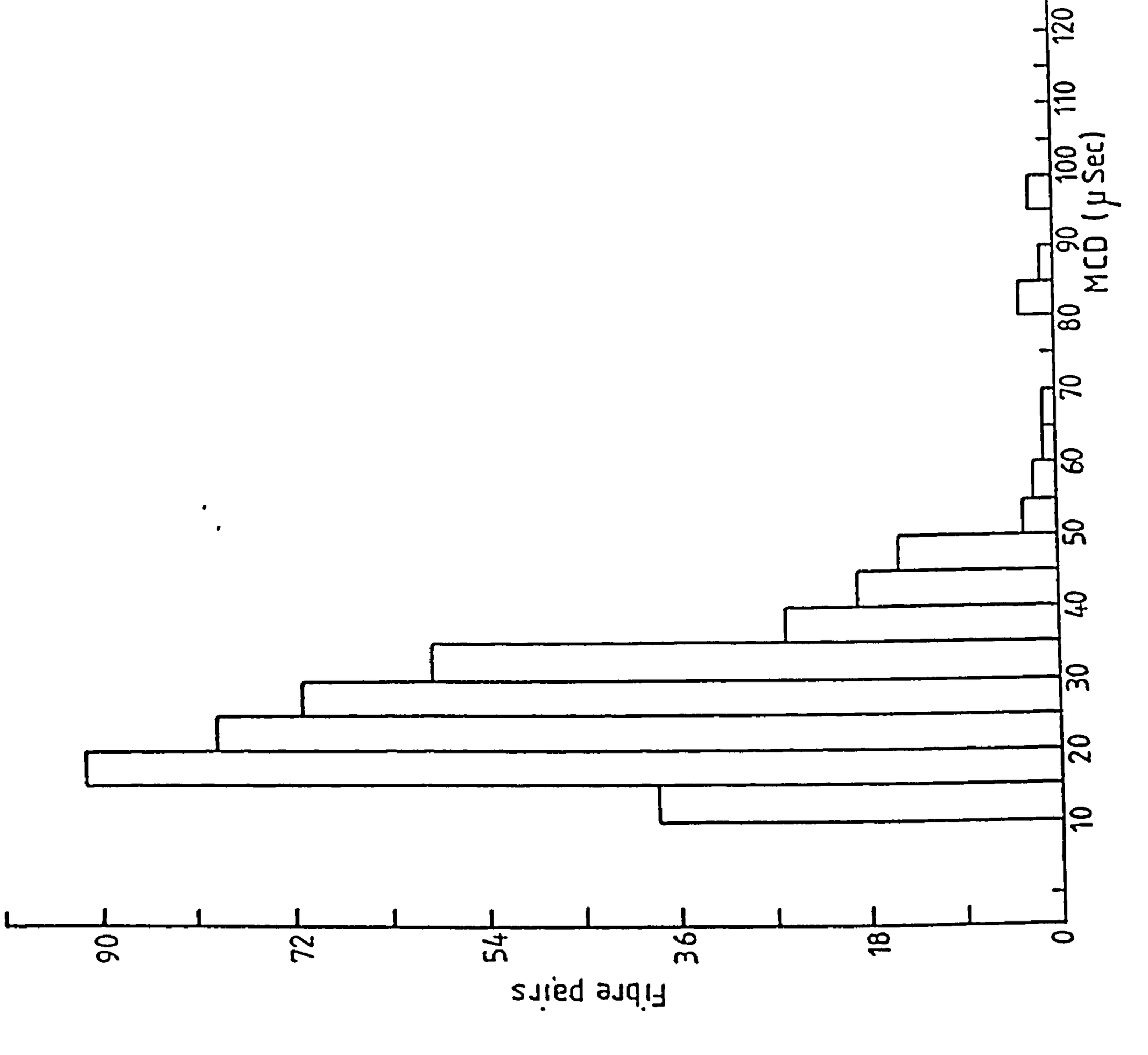


Fig. 8.3 GB Ct5/pyridostigmine study distribution of MCD values from 424 fibre pairs from subjects with unmodifed AChE (see text)

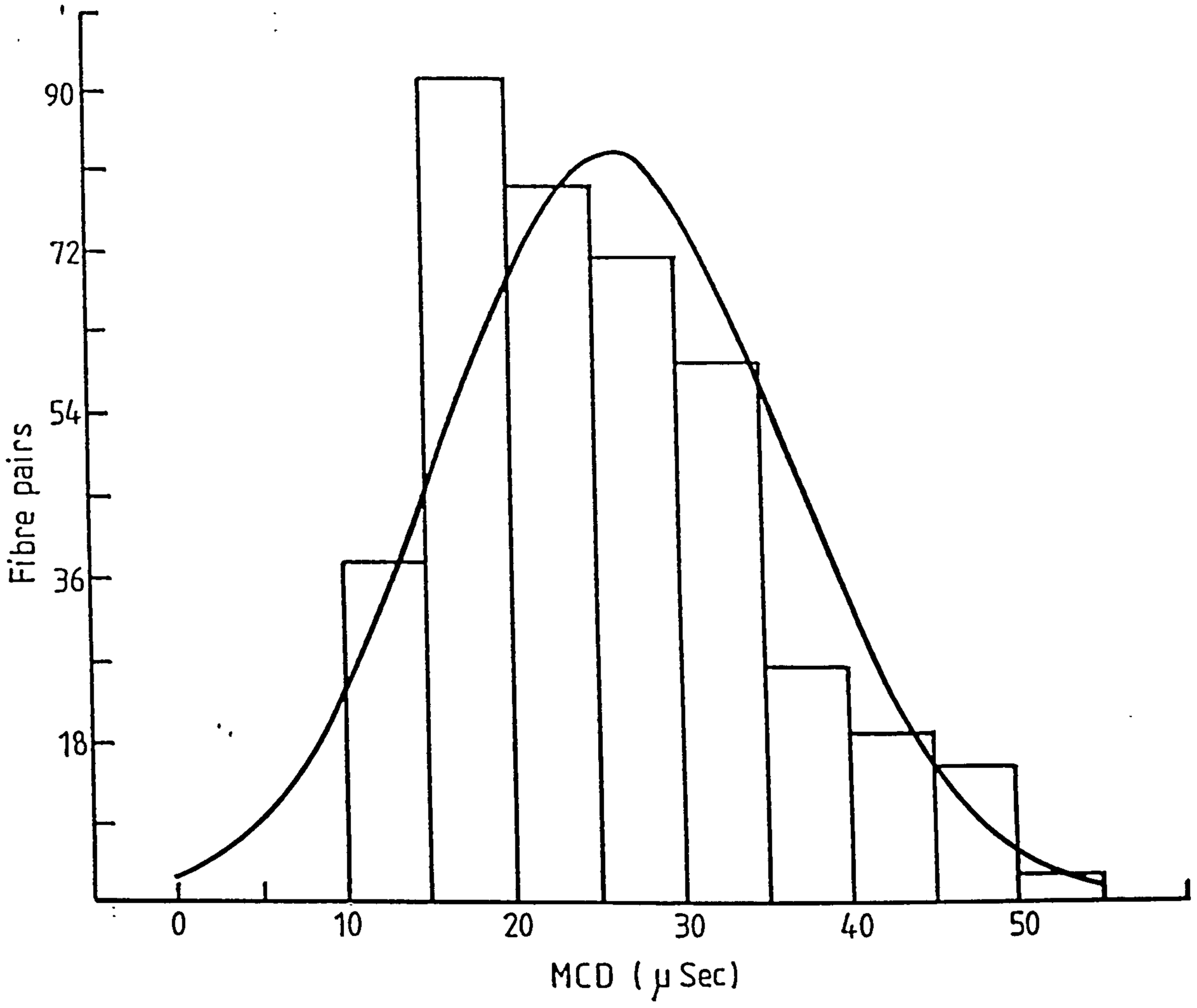


Fig. 8.4 MCD data from fig. 8.3 truncated at 55 μsec. The distribution is a closer fit to normal but remains skewed. Mean MCD = 25.37 ± 9.29 μsec.

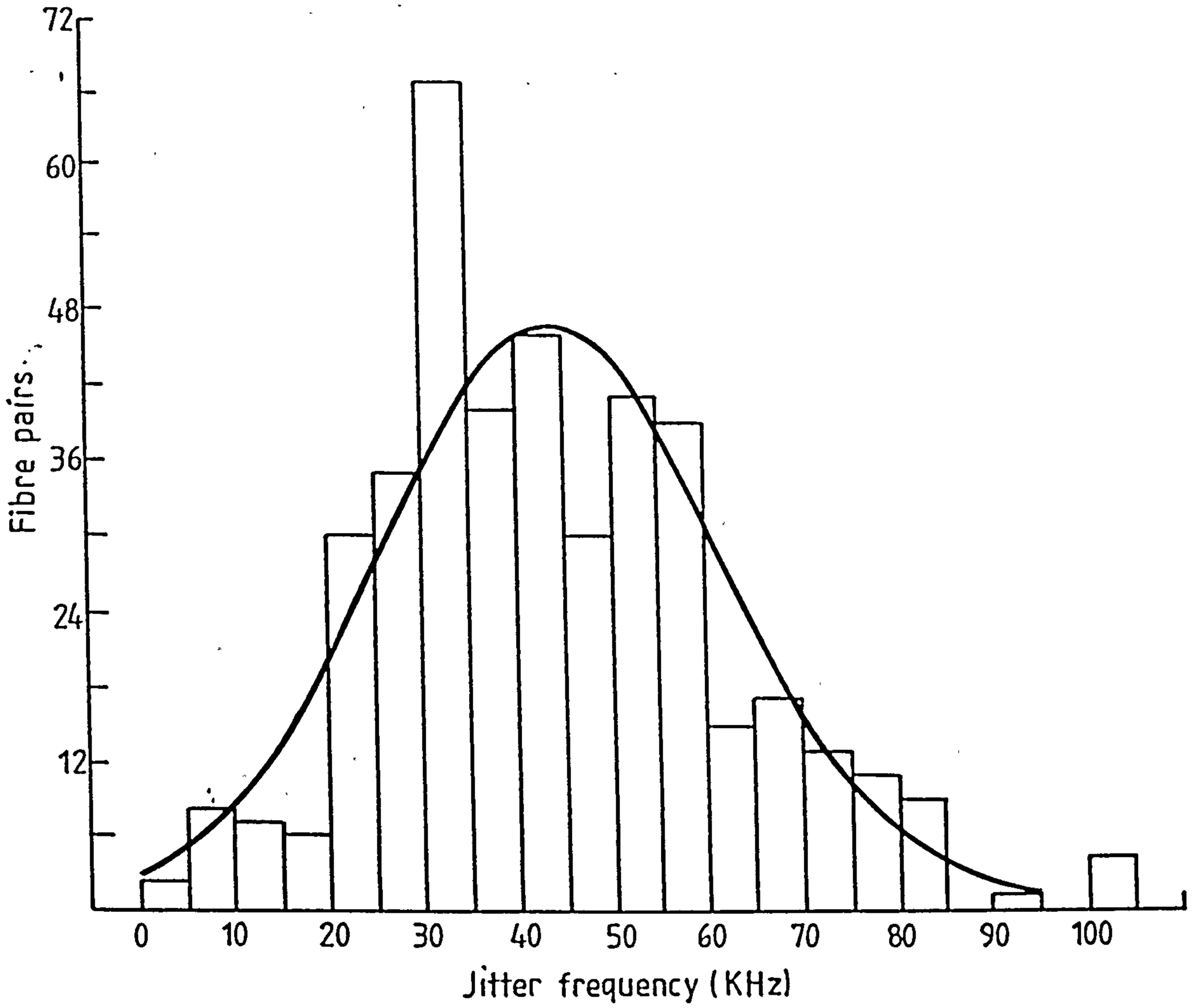


Fig. 8.5 Reciprocal transformation of MCD data shown in fig. 8.3. Mean jitter frequency = 43. 31 ± 17.88kHz The distribution of the data is closer to the true normal distribution shown superimposed (see text).

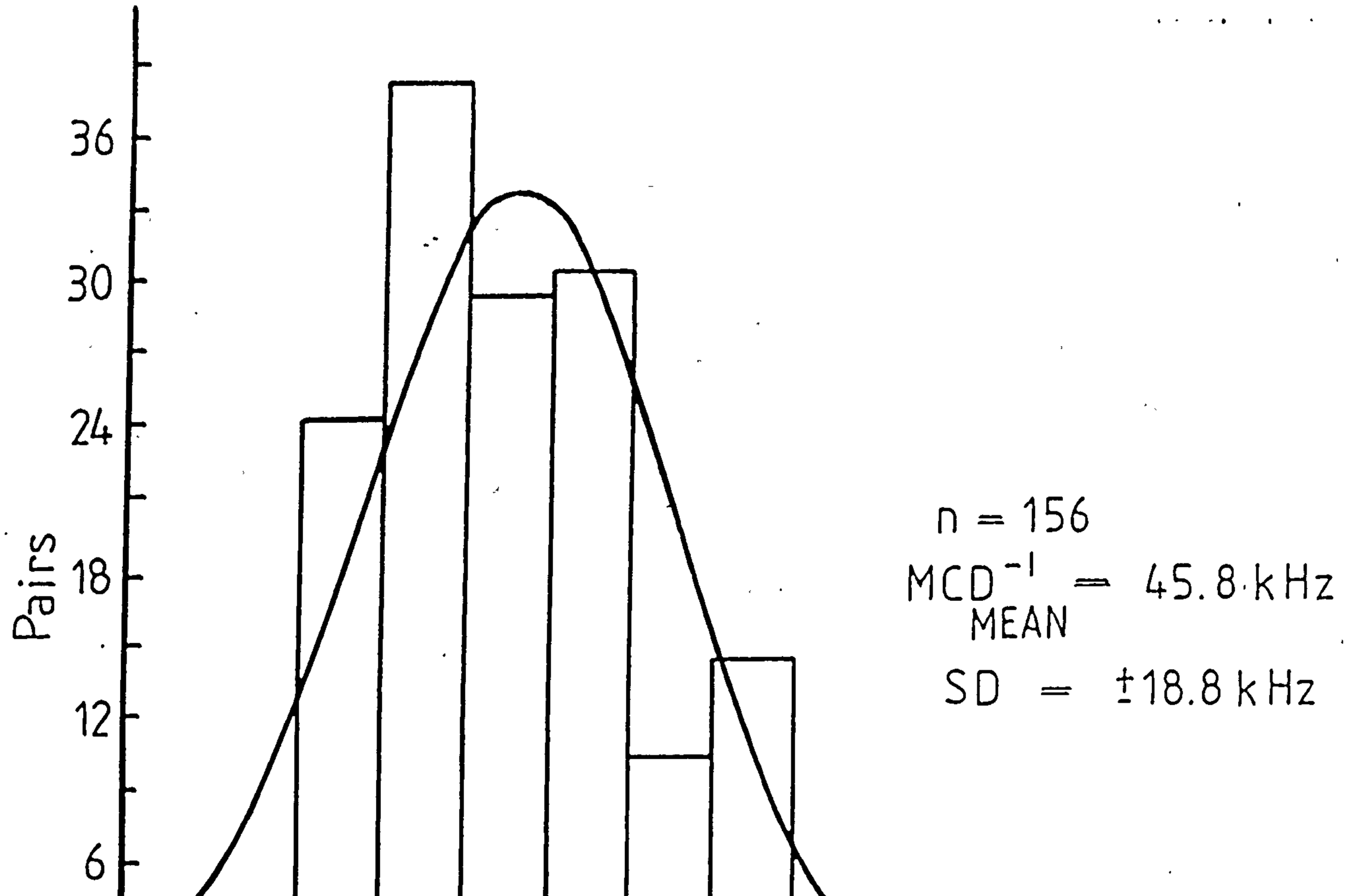


Fig.8.6 RECIPROCAL TRANSFORMATION OF CONTROL SESSION JITTERS FOR ALL SUBJECTS
THE TRUE NORMAL DISTRIBUTION IS SHOWN SUPERIMPOSED

8.1.2 Jitter values during inhibition of AChE by pyridostigmine

Table 8.1 shows the degree of AChE inhibition produced by pyridostigmine and GB Ct5 exposure in the subjects studied. Figure 8.7 shows the distribution of MCD for 215 fibre pairs measured in subjects taking pyridostigmine. The data were recorded from group AP (sessions 2, 3 and 4) and group AA (sessions 2 and 3). If the jitters are plotted as truncated distribution, as in figure 8.5, with values greater than 55 usec excluded, the mean jitter value is 24.7 $\pm$ 18.8 usec with 17/232 (7.3%) pairs having values greater than the cut - off value. Reciprocal transformation of the complete data, shown in figure 8.8 gives a mean jitter frequency of 45.8 $\pm$ 21.6 kHz which is equivalent to MCD = 21.8 usec within 95% confidence limits of 20.6 and 23.3 usec.

Two subjects taking pyridostigmine (145/84 and 012/84) developed an apparently abnormal proportion of high jitters. In both cases the mean jitter value and the high jitter count for their individual control determinations were within normal limits. The degree of AChE inhibition in these subjects did not differ significantly from the mean of all pyridostigmine treated subjects.

8.1.3 Jitter values after exposure to GB Ct5 with pyridostigmine pretreatment

Figure 8.9 shows the distribution of MCD values from unpretreated subjects (group PA) exposed to GB at the Ct5 level, together with their reciprocal transformation. The mean jitter frequency is 37.6 $\pm$ 18.5 kHz. Figure 8.10 shows the same plots for jitter

Sampling Time	Group		
	AP	PA	AA
2 hours after 60 mg pyridostigmine loading dose	58.4 ± 2.9 (5)	97 ± 3.1 (6)	66.9 ± 4.4 (6)
Pyridostigmine 30 mg 8 hourly for 48 hours	58.5 ± 8.9 (6)*	99.3 ± 2.9 (6)	62.7 ± 12.9 (6)*
3 hours after exposure to GB Ct 5	64.8 ± 8.3 (6)**	84.3 ± 3.9 (6)	60.2 ± 11.4 (6)** † p <0.001† ↓ 92.1 ± 7.5 (6)
72 hours after GB Ct 5 exposure	98.4 ± 5.9 (6)	86.9 ± 3.5 (6)	

† significance value between two results.

* samples taken 3 hours after 0700 h dose.

** sample taken 6.5 hours after last (0700 h) dose of pyridostigmine bromide.

Table 8.1 GB Ct5 / pyridostigmine study: degree of RBC AChE inhibition produced by pyridostigmine 30 mg t.d.s. and GB Ct5. AChE levels are seen to return to normal 72 hours after the final dose of pyridostigmine. The inhibition caused by GB Ct5 persists. The effect of pyridostigmine pretreatment is indicated by the significant recovery of the AChE level shown.
All values of AChE are corrected to a standard PCV of 0.45.

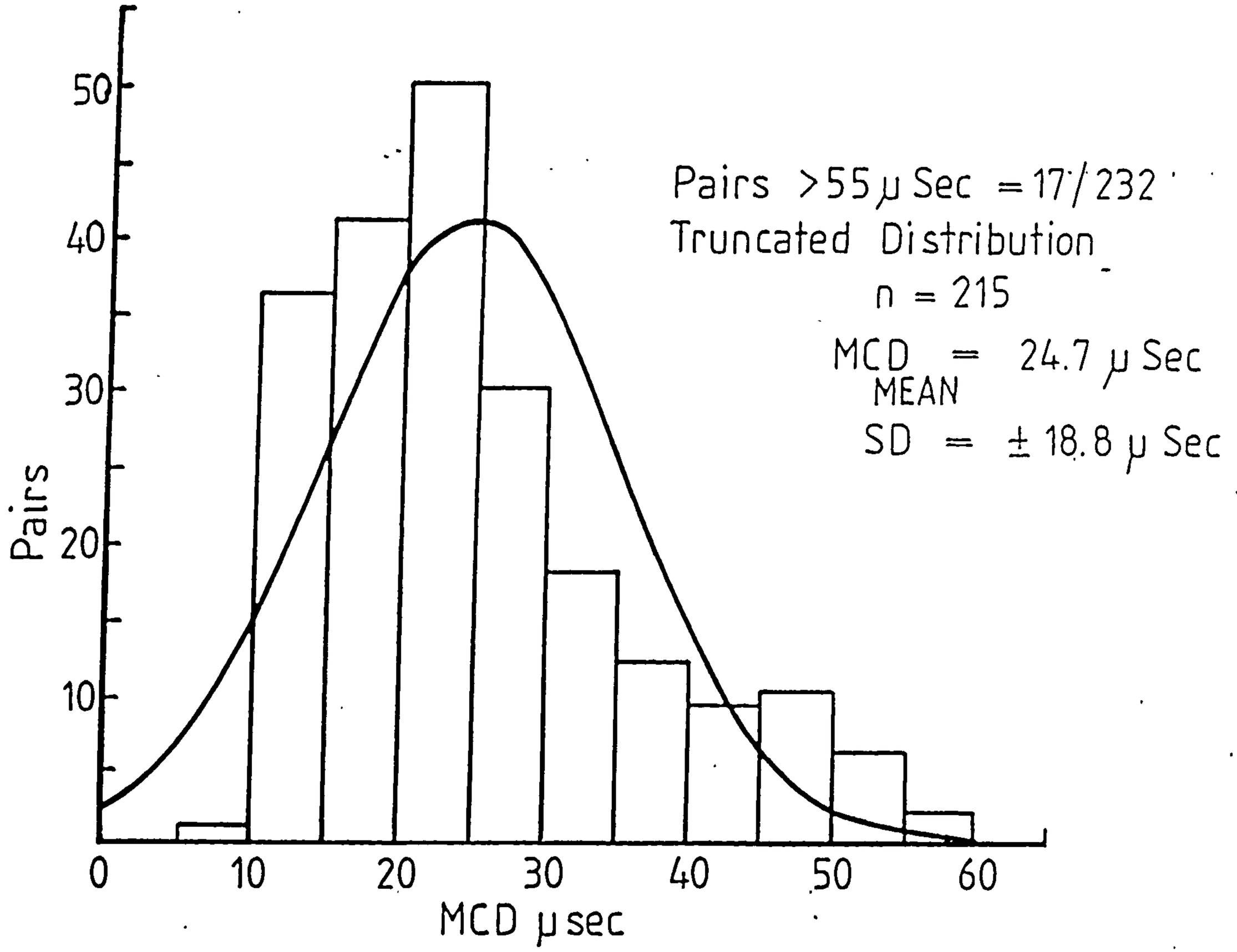


Fig. 8.7 JITTER DISTRIBUTION OF ALL PAIRS RECORDED WITH PYRIDOSTIGMINE MODIFIED AChE LEVELS. THE DISTRIBUTION HAS BEEN TRUNCATED (see text) A TRUE NORMAL DISTRIBUTION IS SHOWN SUPERIMPOSED

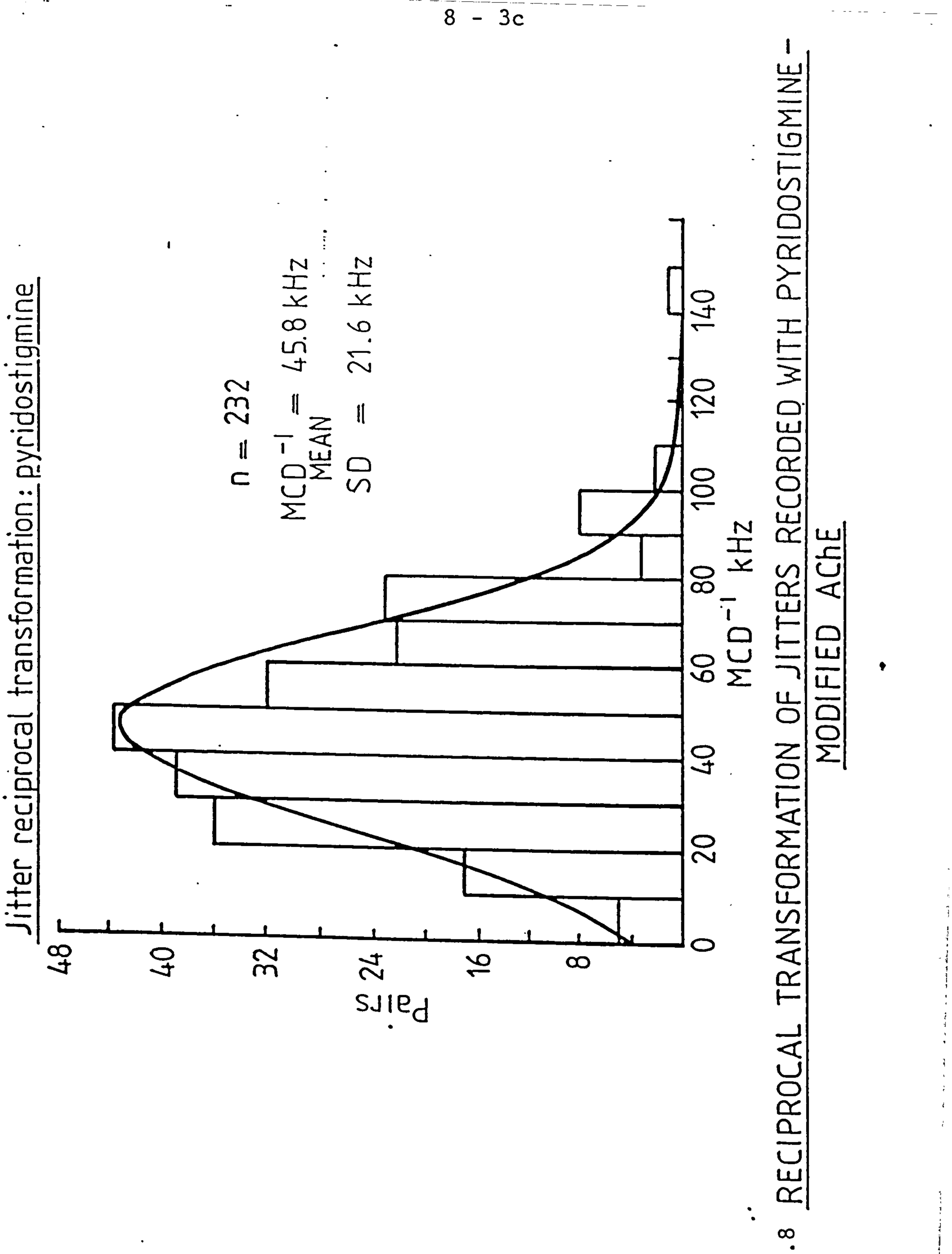

RECIPROCAL TRANSFORMATION OF JITTERS RECORDED WITH MODIFIED AChE - pyridostigmine
Jitter reciprocal transformation: pyridostigmine
n = 232
MCD⁻¹ MEAN = 45.8 kHz
SD = 21.6 kHz
Pairs
MCD⁻¹ kHz
0
20
40
60
80
100
120
140
8
16
24
32
40
48

Fig. 8.8

Jitter reciprocal transformation GB with no pretreatment

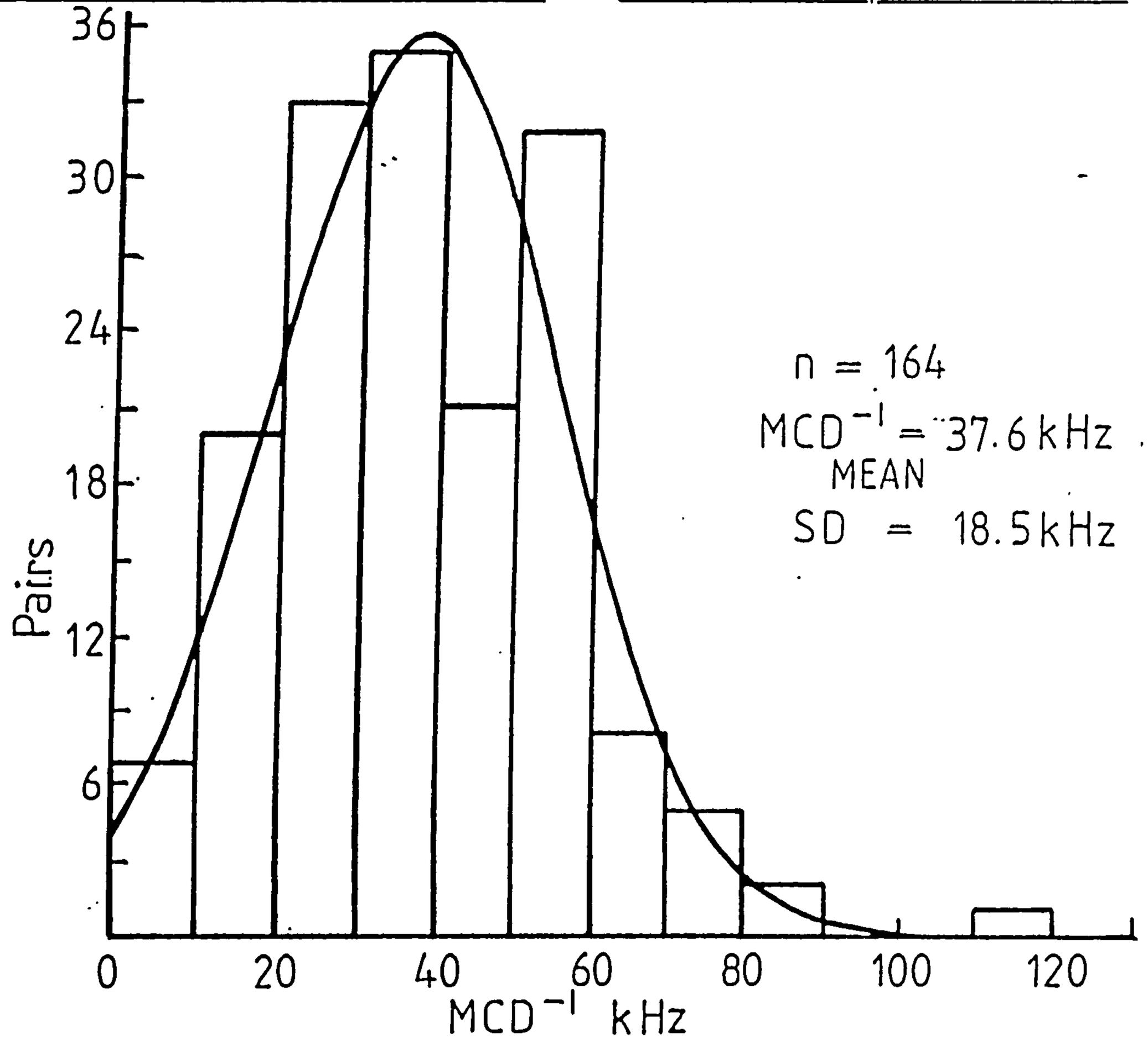


Fig. 8.9 RECIPROCAL TRANSFORMATION OF JITTERS FROM GB EXPOSED SUBJECTS WITHOUT PYRIDOSTIGMINE PRETREATMENT

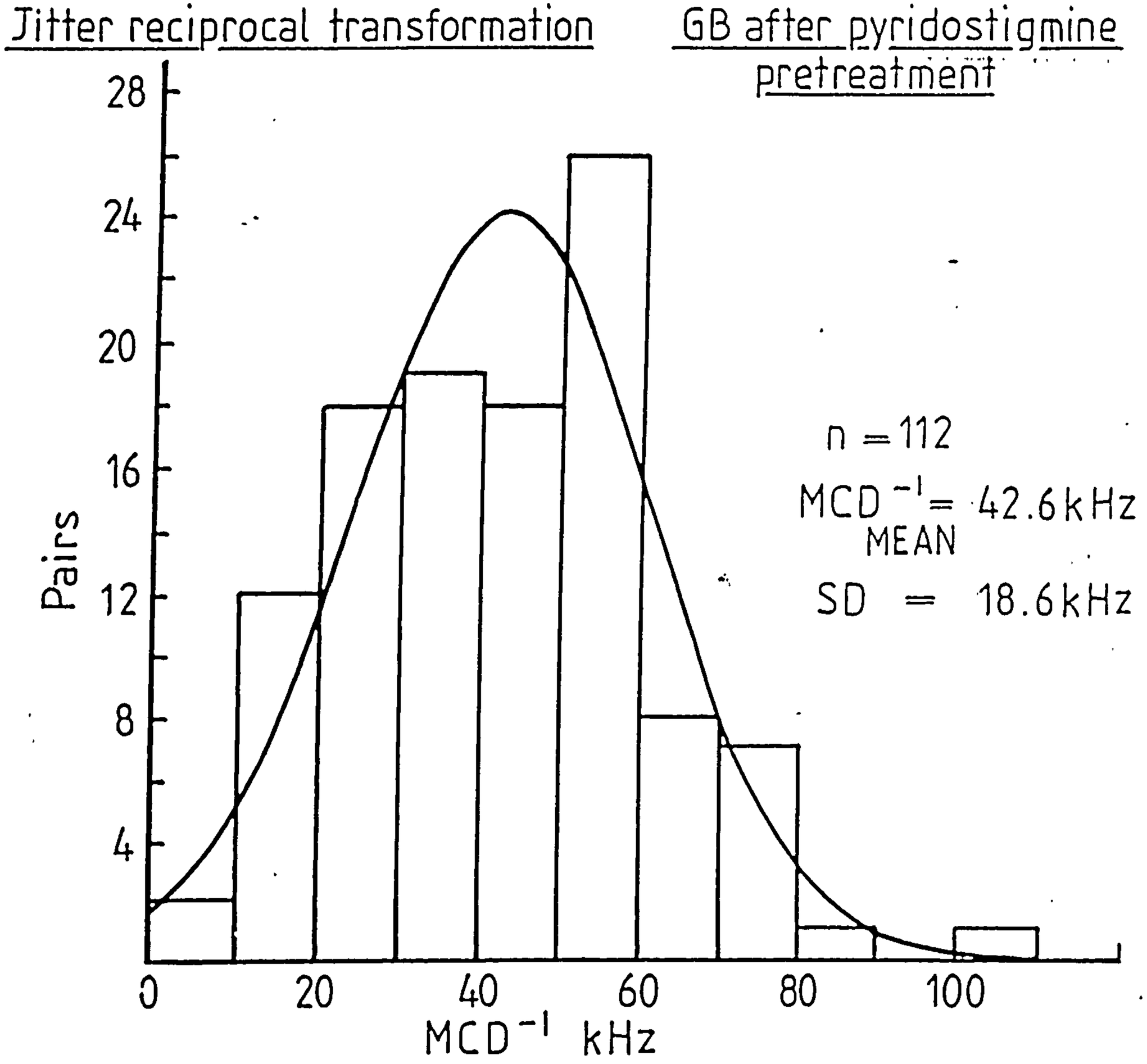


Fig. 8.10 RECIPROCAL TRANSFORMATION OF JITTERS FROM GB EXPOSED SUBJECTS WITH PYRIDOSTIGMINE PRETREATMENT

values from pairs recorded in subjects from group AA. The mean jitter frequency in this case is 42.6 $\pm$ 18.6 kHz.

8.2 Data analysis

8.2.1 Introduction

Analysis of the data from the pyridostigmine and GB Ct5 study proceeded using two methods (1) and examination of the incidence of fibre pairs having MCD > 55 usec following the conventional approach and (2) examination of shifts of mean jitter frequency after reciprocal transformation

8.2.2 The incidence of high values of jitter

Table 8.2 gives the incidence of recorded fibre pairs having MCD > 55 usec. To analyse the data, a series of chi - squared (or Fisher exact) tests were used to look for possible significant changes in the incidence of abnormal jitter values in different sub - groups. These are presented in table 8.3.

8.2.2.1 The integrity of controls

The consistency of the control data was confirmed by the non - significant (χ^2 = 1.99, p = 0.057) result obtained by comparing all the control data from the four study groups.

PP	10/162 (6.2%)
AP	1/47 (2.1%)

TABLE 8.2 THE INCIDENCE OF CLINICALLY ABNORMAL JITTER POOLED AND GROUPED FOR COMPARISONS

	Subjects	Session			
		1	2-3	4	5
PP	44/83 46/83 48/83 129/83 130/83 141/83	Control 10/162 (6.2%)			
AP	8/83 11/83 140/83 144/83 145/83 160/83	Control 1/47 (2.1%)	Pyridostigmine 8/147 (5.4%)		Post Pyrido 5/53 (9.4%)
PA	36/83* 41/83 42/83 96/83 97/83 159/83 172/83	Control 3/127 (2.4%) [6/164 (3.7%)]		GB-3 hr 8/69 (11.6%) [9/82 (11.0%)]*	GB-3 day 13/68 (19.1%)
AA	9/83 12/83 17/83 18/83 20/83 29/83	Control 2/51 (3.9%)	Pyridostigmine 9/85 (10.6%)	Pyrido+GB-3 hr 3/54 (5.6%)	Pyrido+GB-3 day 6/58 (10.3%)

* Subject 36/83 was not available for examination 3 days post GB.

PA	6/164 (3.7%)
AA	2/51 (3.9%)

8.2.2.2 The steady state effect of pyridostigmine on high jitter incidence

The effect of taking pyridostigmine 30 mg t.d.s. on the incidence of clinically high values of jitter was examined by considering data from groups AP and AA. Here the pooled session 1 incidence (3/98 or 3.1%) was compared with the pooled incidence from group AP, sessions 2-4 and group AA, sessions 2-3 (17/232 or 7.3%). It should be noted that although the high jitter incidence more than doubled after the administration of pyridostigmine the new incidence failed to achieve significance (χ^2 = 1.5, p = 0.22).

8.2.2.3 The effect of GB Ct5 three hours after exposure

Three hours after exposing pretreated subjects to GB Ct5 there was a marginally significant increase (χ^2 = 3.78, p = 0.052) in the incidence of high jitter values, shown by a comparison within group PA. Prior to exposure (sessions 1 - 3) the incidence was 6/164 (3.7%) but this increased to 9/82 (11%) three hours after exposure.

8.2.2.4 The effect of GB Ct5 three days after exposure

Further consideration of the data from group PA shows that the elevated incidence of abnormal jitter noted at three hours after exposure was increased three days later (13/68 or 19.1%). The

increased incidence after three days is not significantly different from the incidence after three hours (χ^2 = 0.97, p = 0.32). However, compared with the group control data the incidence of high jitters at three days after exposure showed a sustained, highly significant increase in abnormal jitter incidence (χ^2 = 14.2, p < 0.0001).

8.2.2.5 The effect of pyridostigmine pretreatment on high jitter incidence after GB Ct5 exposure

The effect of pyridostigmine pretreatment on the incidence of high jitters after GB Ct5 exposure was examined in group AA. Due to the small numbers of fibre pairs available for analysis in this group no significant differences could be demonstrated between the controls (2/51 or 3.9%), three hours (3/54 or 5.6%, p = 0.62) or three days after exposure to GB Ct5.

8.2.3 Reciprocal transformation analysis of pyridostigmine pretreatment and exposure to GB Ct5

The reciprocal transformation technique, described in appendix 1, is an effective way of examining the change of a whole jitter population by considering shifts in the mean jitter frequency. This technique was applied to the data using a paired analysis within groups to remove possible intergroup variations.
The groups were analysed as follows:

Group AP : Control -- pyridostigmine pretreatment -- placebo GB

Group AA : Control -- pyridostigmine pretreatment -- active GB

Group PA : Control -- placebo pretreatment -- active GB

For each subject within any group the mean jitter frequency was determined from jitters measured during each stage of the experiment condition. These values are listed in table 8.3. A balance in the number of fibre pairs was difficult to guarantee in this study because because of the difficulty of recording SFEMG in normal subjects. As many fibre pairs as possible were measured during each recording session of approximately one hour. Table 8.3 gives the average number of measurements per subject per group for each session. Table 8.4 shows individual mean jitter frequency changes associated with pyridostigmine pretreatment and GB Ct5 exposure.

8.2.3.1 Analysis of table 8.4

(1) Matching of experimental groups under control conditions

Group mean jitter frequencies under initial control conditions are:

Group	PP	AP	PA*	AA
Mean	40.15	51.50	45.27	45.59
SD	13.20	8.40	5.23	10.41

TABLE 8.3 INDIVIDUAL AND GROUP JITTER FREQUENCIES (kHz)

Time Lapse (Days)		2			2			3			3			5			
Session		1			2			3			4			5			6	
Grp	Subj No	n	f	sd	n	f	sd	n	f	sd	n	f	sd	n	f	sd	n	f	sd
PP	44	5	39.3	9.7	6	38.1	5.7	8	38.0	12.9	2	51.2	10.9	10	39.9	13.8			
	46	1	22.0	-	3	32.9	6.9	4	37.3	29.1	4	40.4	12.8	6	40.7	18.2			
	48	5	26.6	5.6	7	31.8	14.7	3	32.6	0.6	2	38.2	29.2	4	17.3	12.2			
	129	8	49.3	18.4	13	48.1	24.9	16	41.0	15.6									
	130	6	50.5	15.1	9	46.0	18.8	9	54.6	21.4									
	141	2	53.2	33.6	9	34.1	19.6	5	38.3	26.7	7	46.5	19.4	8	43.8	19.6			
	Grp	6	40.15	13.2	6	38.5	7.0	6	40.3	7.5	4	44.1	5.9	4	35.4	12.2			
AP	8	7	59.3	29.0	6	56.9	18.8	9	52.8	20.2	6	47.5	8.1	10	42.8	22.2			
	11	5	36.6	11.7	7	42.0	17.2	3	49.4	8.7	7	36.5	10.9	5	42.3	7.9			
	140	9	55.6	13.7	7	57.7	19.2	8	50.0	26.5	9	48.2	21.2	5	33.0	17.3			
	144	7	55.6	20.5	7	42.1	21.0	12	51.6	26.2	10	49.2	18.9	9	64.6	18.3			
	160	8	55.1	29.8	11	41.0	17.0	5	61.0	50.4	8	52.4	26.2	13	43.3	27.3	16	48.4	17.5
	145	11	46.7	25.3	9	63.2	23.4	7	19.2	8.4	16	54.0	28.0	10	37.7	17.2			
	Grp	6	51.5	8.4	6	40.4	9.9	6	47.3	14.4	6	48.0	6.2	6	44.0	10.9			
PA	41	3	49.0	19.5	8	45.1	16.5	6	54.3	11.7	6	41.0	17.4	7	42.6	9.5			
	42	6	45.8	12.4	7	40.7	16.1	12	39.8	15.5	9	36.7	18.7	8	34.2	11.9			
	96	4	29.8	18.6	13	44.7	33.5	7	42.2	20.9	15	34.5	16.3	8	27.9	13.9			
	97	3	41.2	14.0	5	31.2	8.3	3	49.7	6.0	6	45.2	15.8	16	25.2	9.6			
	159	9	42.7	12.7	11	50.4	13.9	10	60.8	31.3	17	42.0	13.1	17	39.3	29.4	14	47.1	16.8
	172	6	51.7	21.9	8	56.2	15.5	6	37.1	23.3	16	33.0	21.6	12	46.5	17.7			
	*36	14	41.7	11.1	14	38.0	16.9	9	29.8	10.5	13	36.1	18.9						
	Grp	7	43.1	7.1	7	43.8	8.2	7	44.8	10.7	7	38.4	4.4	6	36.0	8.4			
AA	9	7	60.3	13.1	6	34.0	10.8	5	59.9	17.4	6	57.8	13.8	10	60.6	20.7			
	12	11	33.6	13.4	8	31.4	18.4	10	17.5	4.3	11	30.8	11.0	8	17.7	4.7			
	17	10	38.2	14.2	4	58.0	17.9	8	38.8	15.1	6	56.1	10.0	11	41.8	15.1			
	18	9	46.4	23.6	8	44.4	13.1	5	43.8	8.9	10	44.1	26.6	8	42.3	16.3			
	20	9	55.2	10.7	9	47.1	14.3	11	48.6	14.4	9	49.5	10.1	11	53.3	10.2			
	29	5	39.8	8.4	4	46.0	16.2	7	35.0	12.2	12	35.5	9.8	10	33.5	16.3			
	Grp	6	45.6	10.4	6	43.5	9.7	6	40.6	14.2	6	45.6	10.9	6	41.5	15.1			

n = number of fibre pairs recorded in listed during designated recording session

f = mean jitter frequency for the session

GROUP	Subject Number	Condition				Control -Pyrido	Control -Gb(3hr)	Control -GB(3 day)	Pyrido -GB(3hr)	Pyrido -GB(3 day)	GB (3 hr) -Gb(3 day)
		Control	Pyrido	GB							
				3 hr	3 day						
PA	Av no of estimates	21		12	11						
	041	49.03		41.03	46.62		8.00	2.41			-1.59
	042	41.45		36.66	34.22		4.79	7.23			2.44
	096	41.49		34.53	27.90		6.96	13.59			6.63
	097	36.99		45.19	25.20		-6.20	13.79			19.99
	159	51.55		42.01	39.28		9.54	12.27			2.73
	172	49.13		32.97	46.46		16.16	2.67			-13.49
	Group mean	45.27		38.73	35.95		6.54	9.33			2.79
	SD	5.23		4.75	8.36		7.34	4.56			10.91
	P						0.08	0.004			0.56
AA	Av no of estimates	9	14	9	10						
	009	60.30	45.75	57.85	60.63	14.55	2.45	-0.33	-12.10	-14.86	-2.78
	012	33.57	23.69	30.84	17.70	9.88	2.73	15.87	-7.15	5.99	13.14
	017	38.19	45.20	56.11	41.82	-7.01	-17.92	-3.63	-10.19	3.38	14.29
	018	46.42	44.15	44.12	42.30	2.27	2.30	4.32	0.03	1.85	1.82
	020	55.19	47.90	49.45	53.33	7.29	5.74	1.86	-1.55	-5.43	-3.88
	029	39.84	38.98	35.50	33.52	0.86	4.34	6.32	3.48	5.46	1.98
	Group mean	45.59		45.65	41.55		-0.06	4.07	-4.70	-0.61	4.10
	SD	10.41		10.93	15.08		8.85	6.75	6.30	8.11	7.83
	P						0.99	0.20	0.13	0.86	0.26
				Post Pyrido			Control-3hr post pyrido	Control-3 day post pyrido	Pyrido-3hr post pyrido	Pyrido-3 day post pyrido	Post pyrido 3hr-3 day
				3 hr	3 day						
AA	Av no of estimates	7	25	9	9						
	008	59.35	52.47	47.51	42.82	6.88	11.84	16.53	4.96	9.65	4.69
	011	36.64	41.02	36.48	42.33	-4.38	0.16	-5.69	4.54	-1.31	-5.85
	140	55.63	51.58	48.20	33.00	4.05	7.43	22.63	3.38	18.58	15.2
	144	55.59	48.36	49.20	64.58	7.23	6.39	-8.99	-0.84	-16.22	-15.38
	145	46.65	48.99	54.02	37.74	-2.34	-7.37	8.91	-5.03	11.25	16.28
	160	55.12	48.93	52.36	43.39	6.19	2.76	11.73	-3.43	5.54	-8.97
	Group mean	51.50	46.56	47.96	43.98	2.90	3.54	7.52	0.60	4.56	3.99
	SD	8.4	4.04	6.16	10.85	5.04	6.68	12.46	4.30	12.12	12.44
	P					0.22	0.25	0.20	0.78	0.40	0.47
	Pooled mean	47.47	44.75			3.79					
	SD	8.36	7.73			6.21					
	P					0.06					

Table 8.4 GB Ct5 / pyridostigmine study: paired analysis of change in jitter frequency associated with GB exposure and pyridostigmine pretreatment. Group mean values are shown with standard deviations (SD) and the degree of significance(P) of the difference from the appropriate control.

* excluding subject 036/83

Consideration of these values shows that the most significant difference in group control mean jitter existed between groups AP and PA for which the t test was 1.54, p = 0.015. Otherwise the groups may be regarded as being well balanced.

8.2.4 Effect of pyridostigmine pretreatment on mean jitter frequency

An estimate of any change in jitter associated with taking pyridostigmine is assessed by considering changes in jitter frequency within individual experimental subjects. Two groups, AA and AP provide this data. The mean change in jitter frequency was found to be a reduction of 3.79 kHz (SD = 6.21, t = 2.114, p = 0.058). Therefore a small, marginally significant, reduction in individual mean jitter frequency (corresponding to an increase in mean jitter of 1.7 usec, with 95% confidence limits - 0.01 to + 3.91) is associated with taking pyridostigmine 30 mg t.d.s.

No significant changes from controls were observed three days after stopping pyridostigmine.

8.2.5 The effect of GB Ct5 on mean jitter frequency

The changes in jitter frequency of subjects exposed to GB Ct5 without pyridostigmine pretreatment may be determined from a consideration of group PA

(1) three hours after exposure where the mean

individual change in jitter frequency was found to be a reduction of 6.54 kHz (SD = 7.34, t = 2.18, p = 0.08. Although not highly significant this shift corresponds to an increase in derived mean MCD of 3.7 usec (95% confidence limits: 0.6 - 10.1 usec);

(2) three days after exposure where the mean individual change in jitter frequency was a reduction of 9.33 kHz (SD = 4.6, t = 5.01, p = 0.004). This highly significant change corresponds to an increase in derived mean MCD of 5.73 usec (95% confidence limits: 2.46 - 10.0 usec)
Direct comparison of the three hour and three day data did not show any statistically significant change, possibly because two subjects (041 and 172) showed some signs of recovery.

8.2.6 The effect of GB Ct5 exposure following pyridostigmine pretreatment on mean jitter frequency

Changes in mean jitter frequency following GB Ct5 exposure in pyridostigmine pretreated subjects were examined in group AA. No significant changes were observed.

8.2.7 Conclusions

The findings from the analysis of the GB Ct5 exposure with pyridostigmine pretreatment study were as follows:

1. The study groups were largely well balanced, both with respect to the incidence of high jitter as well as mean jitter frequency

2. Pyridostigmine produces a marginally

significant ($p = 0.058$) reduction in the mean jitter frequency but is not associated with any significant overall increase in the incidence of high jitter. No significant changes remain three days after discontinuing the drug

3. GB Ct5 exposure is associated with an increasingly significant reduction in mean jitter frequency. Three hours after exposure the reduction in jitter frequency is barely perceptible ($p = 0.08$) and the incidence of high jitters is marginally significant ($p = 0.052$). Three days after exposure the reduction in jitter frequency became highly significant ($p = 0.004$) as did the increase in high jitter incidence ($p < 0.0001$)

4. No changes of any significance were observed following GB Ct5 exposure after pyridostigmine pretreatment, either in jitter frequency or high jitter incidence.

These findings are summarized in the diagrams shown in figures 8.11 and 8.12.

8.3 High values of jitter and blocking

Table 8.5 shows the percentage of discharges which blocked during recording of fibre pairs with MCD values > 55 usec. Only in the unpretreated GB exposed group (group PA) was any significant blocking seen. In this group the correlation coefficient between jitter MCD and blocking percentage was < 0.6.

8.4 Effects of exposure to GB Ct15

BLOCKING IN FIBRE PAIRS WITH HIGH JITTER

Group	No of pairs > 55 µsec	% of pairs with blocking fraction > 0.1
Control	4/156	0
Pyridostigmine	17/232	0
Pyridostigmine/GB	0/112	0
GB alone	22/137	29

where control = all groups, session 1

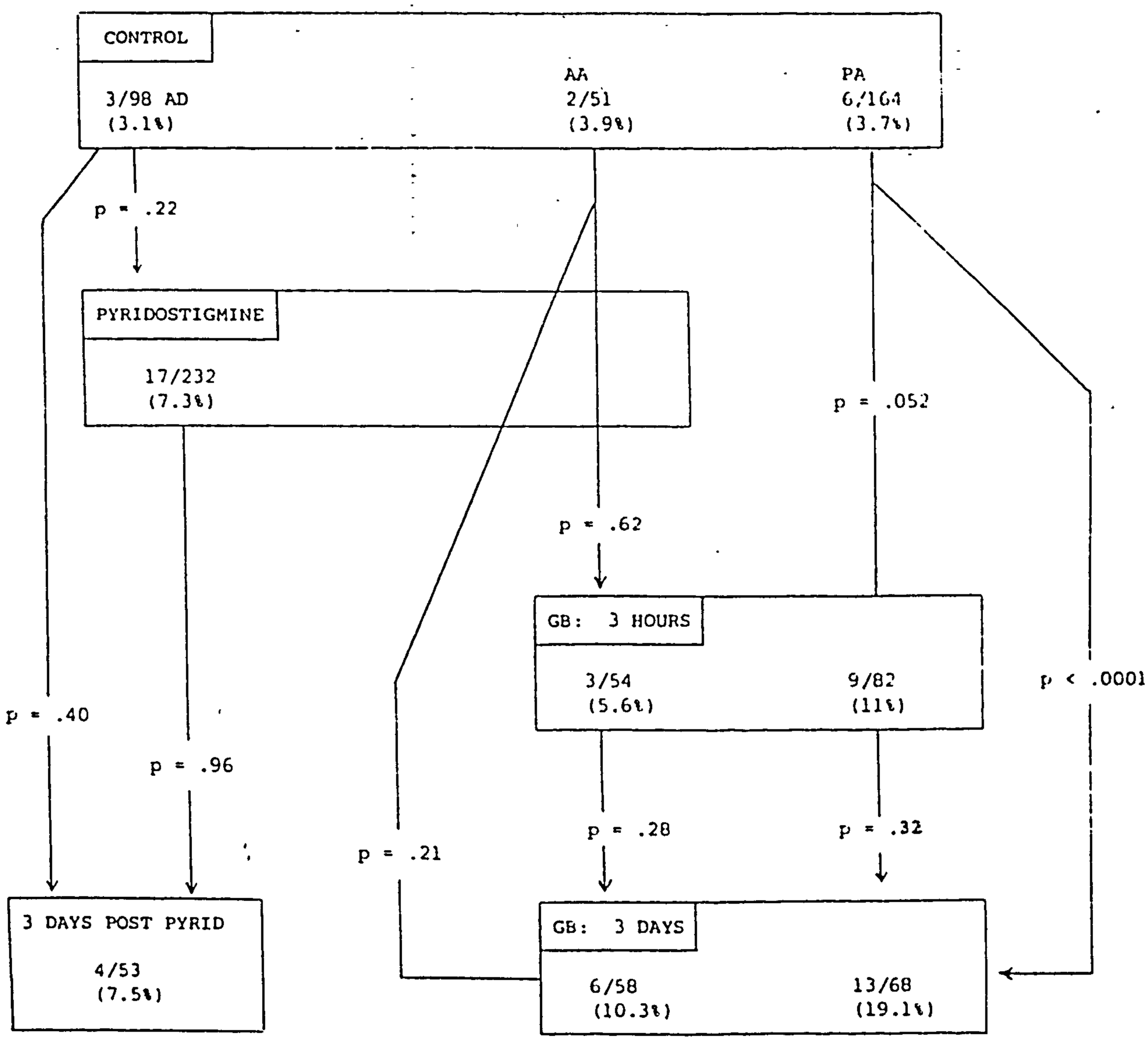
pyridostigmine = group AP, sessions 2, 3 and 4, and group AA, sessions 2 and 3

pyridostigmine/GB = group AA, sessions 4 and 5

GB = group PA, sessions 4 and 5

Table 8.5 GB Ct5/pyridostigmine study
SFEMG blocking in fibre pairs with high MCD values.

NERVE AGENT PRETREATMENT AND ABNORMAL JITTER INCIDENCE - OVERALL ANALYSIS



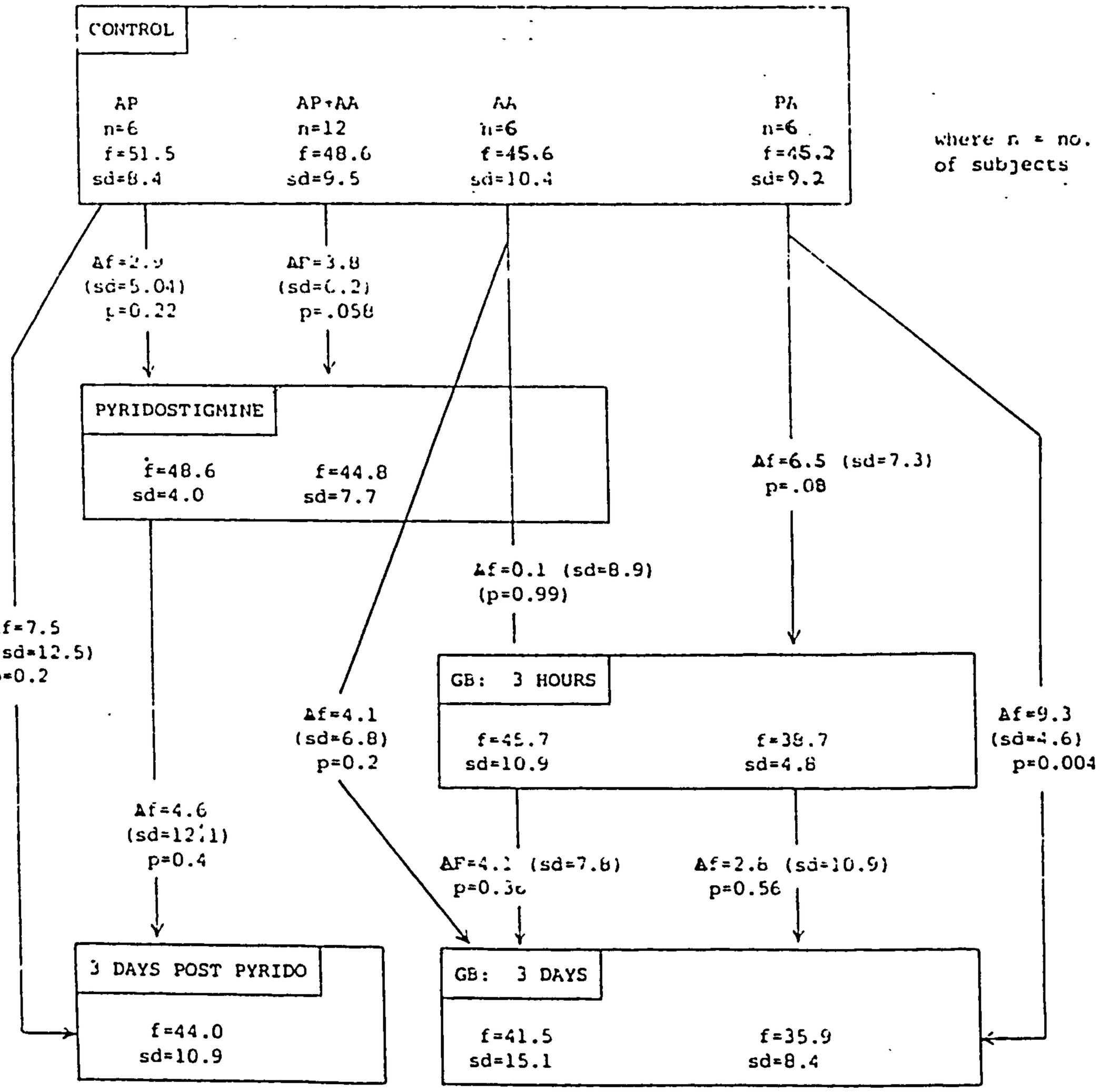
1. Treating subjects with pyridostigmine produces an insignificant increase ($p = .22$) in abnormal jitter incidence with no latent effects at 3 days after termination of treatment.

2. Subjects pretreated with pyridostigmine and then exposed to GB (Ct 5) show no significant increase in abnormal jitter incidence at 3 hours ($p = .62$) or 3 days ($p = .21$) after exposure.

Subjects exposed to GB without pretreatment experienced a marginally significant ($p = .052$) increase in abnormal jitter incidence by 3 hours which progressed to a highly significant level ($p < .0001$) by 3 days.

Fig. 8.11 GB Ct5 / pyridostigmine study: summary of changes in incidence of abnormal MCD values (see text)

NERVE AGENT PRETREATMENT AND JITTER FREQUENCY - OVERALL ANALYSIS



where n = no. of subjects

×10

Treating subjects with pyridostigmine produces a marginally significant (p = 0.058) reduction in jitter frequency but no significant (p = .48) changes persist to 3 days.

Subjects pretreated with pyridostigmine and then exposed to GB (Ct 5) show no significant reduction in jitter frequency at 3 hours (p = .99) or 3 days (p = .2).

Subjects exposed to GB without pretreatment experienced an insignificant reduction in jitter frequency at 3 hours (p = .08) which progressed to a highly significant level (p = .004) by 3 days.

Fig. 8.12 GB Ct5 / pyridostigmine study: summary of changes in jitter frequency (see text)

8.4.1 Jitter profiles

SFEMG measurements for all fibre pairs recorded from 8 subjects exposed to GB Ct15 are shown in figure 8.13. Data are displayed as MCD values and blocking, if greater than 10% is indicated by a solid symbol. The normal clinical upper limit of jitter is indicated by a dotted line.

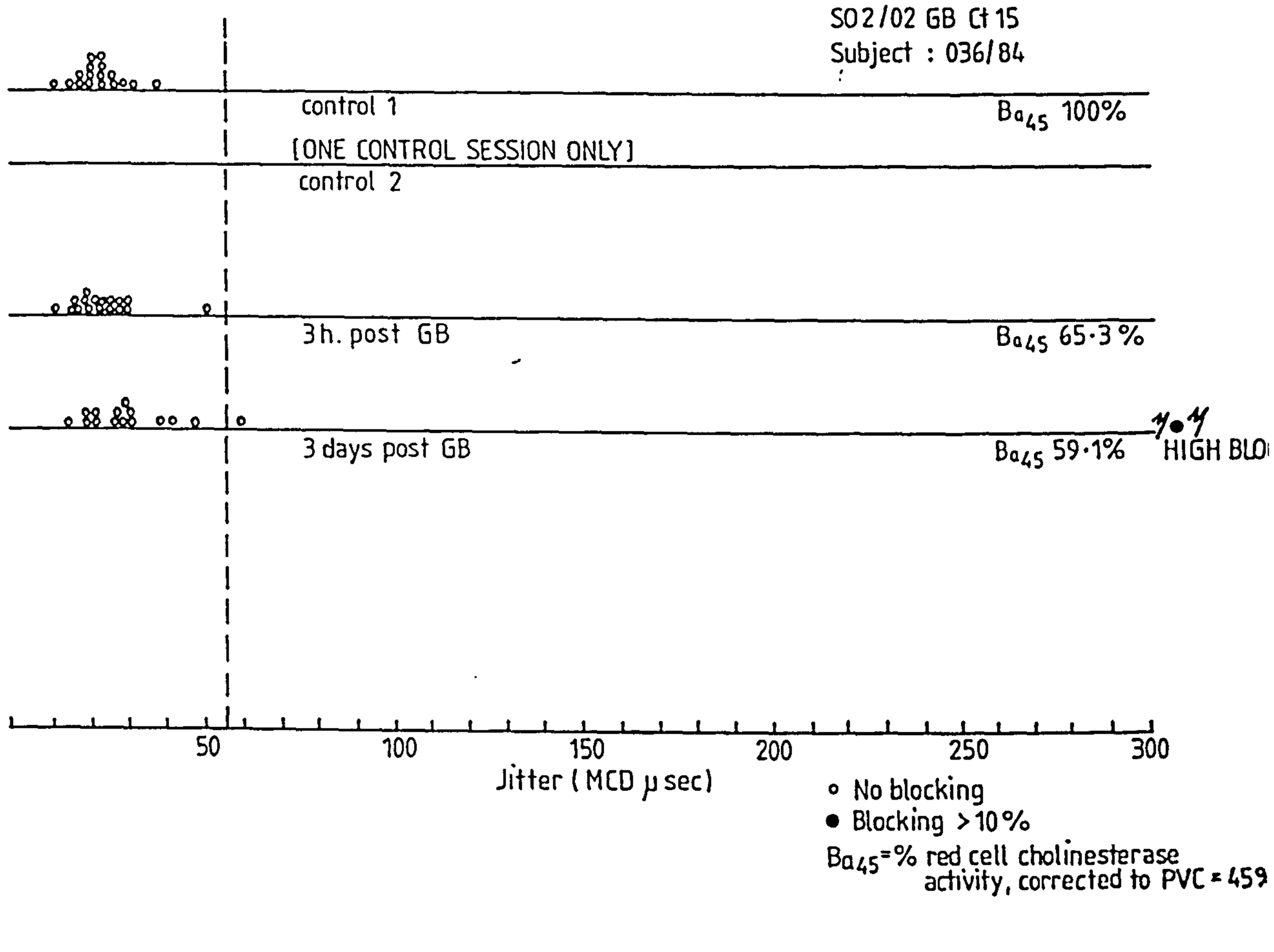
8.4.2 Controls: data and analysis

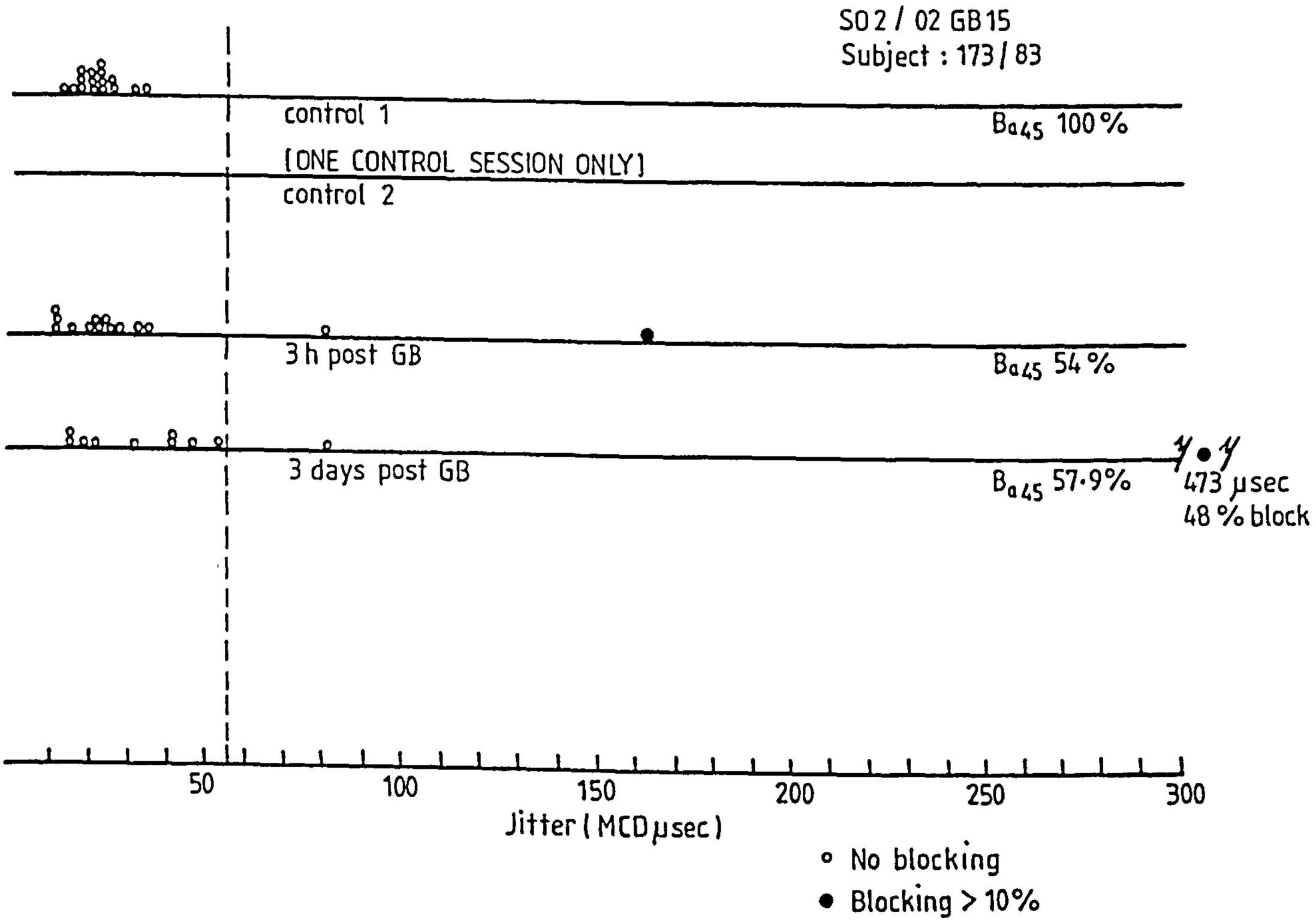
Table 8.6 shows mean jitter frequency values for the two control recording sessions in five of the eight subjects studied, and for the single control session from the remaining three. In the Ct15 study two control sessions were recorded in most cases. The aim of the first recording session was to familiarize subjects with the technique and so the number of fibre pairs recorded is less than that of the second. In three subjects it was not possible, because of availability, to record session 1 and in these cases all control data were recorded during a single session. It is possible from a consideration of the subjects where two control sessions were recorded to assess any statistical difference between the two. Since none of the p values attains significance there is no reason why the two control sessions where recorded should not be combined. Table 8.7 therefore lists control values of mean jitter frequency for each of the subjects after combination of sessions 1 and 2. The table the overall mean jitter frequency of control SFEMG recordings in the eight subjects was 44.8 $\pm$ 8.1 kHz. This is equivalent to a derived mean MCD value of 22.3 usec within 95% confidence limits of 19.4 and 26.3 usec.

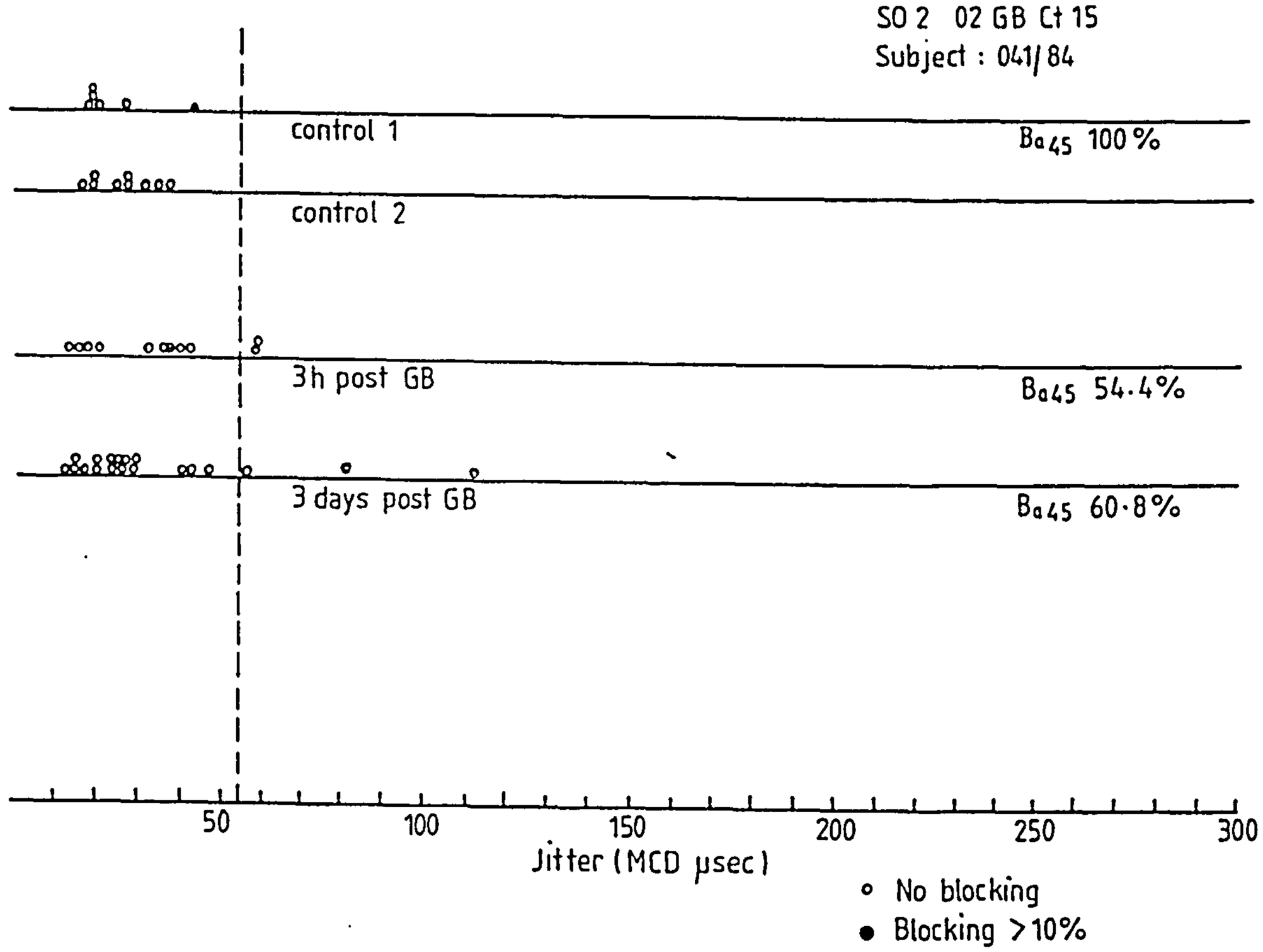
The low mean value for jitter frequency in subject 063/84 is a result of having 5/14 MCD values in the

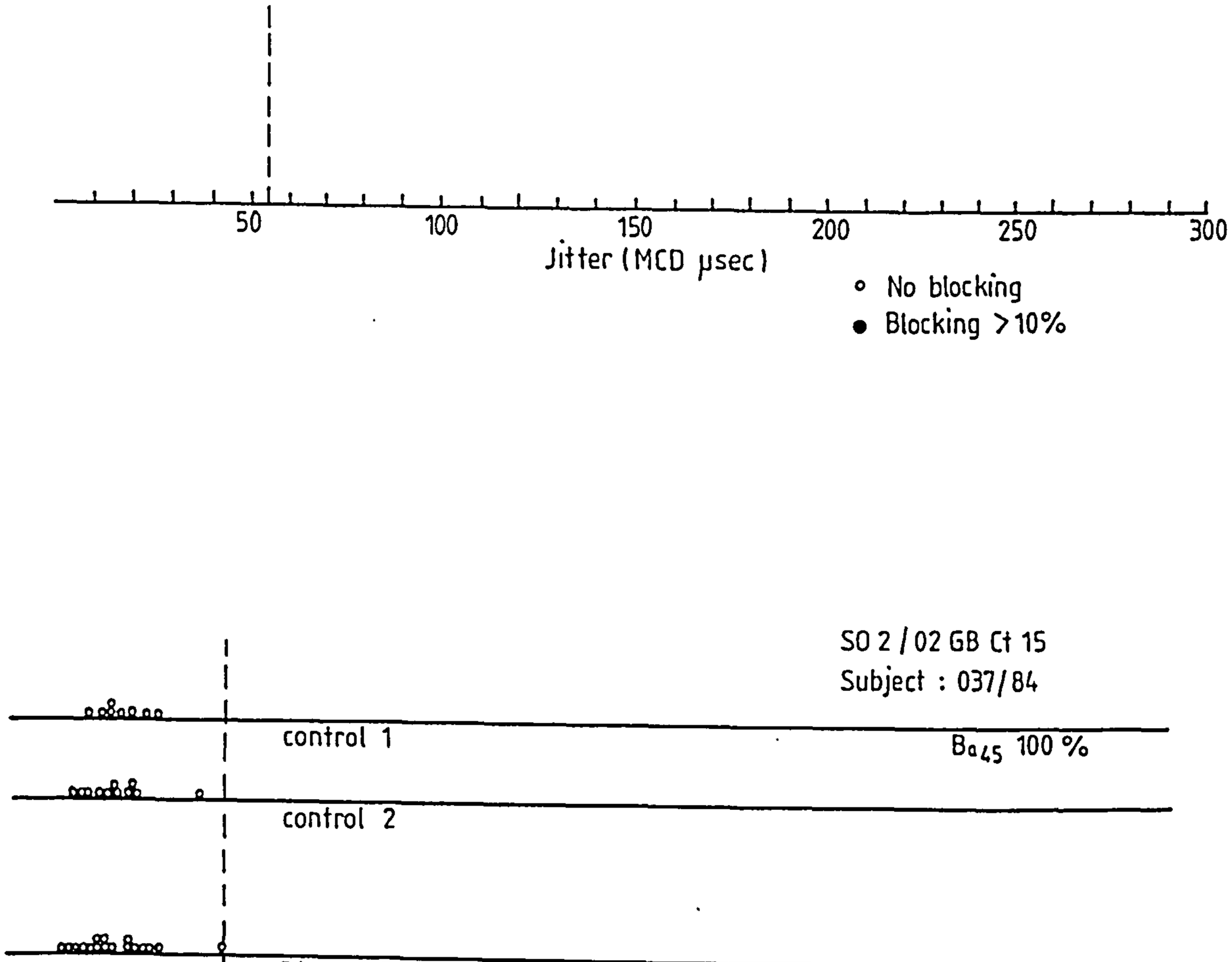
Fig. 8.13 (shown on the following four pages)

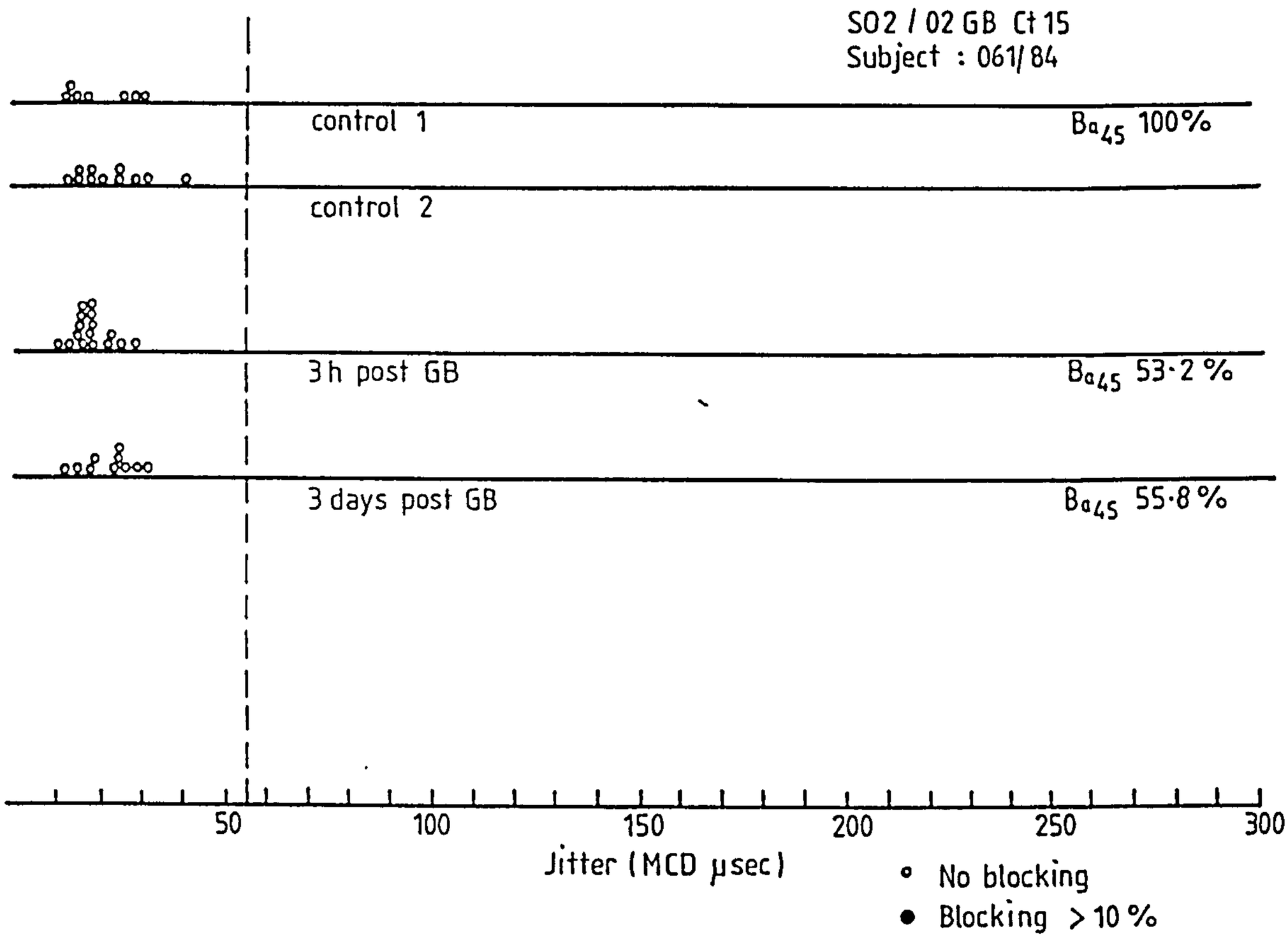
MCD values recorded in EDC from eight subjects exposed to GB Ct15. The dotted line is at the upper limit of normal jitter for EDC at 55 μsec. Blocking of the second waveform in more than 10% of discharges is shown as a solid circle. Red cell AChE activity is shown corrected to a standard PCV of 0.45.

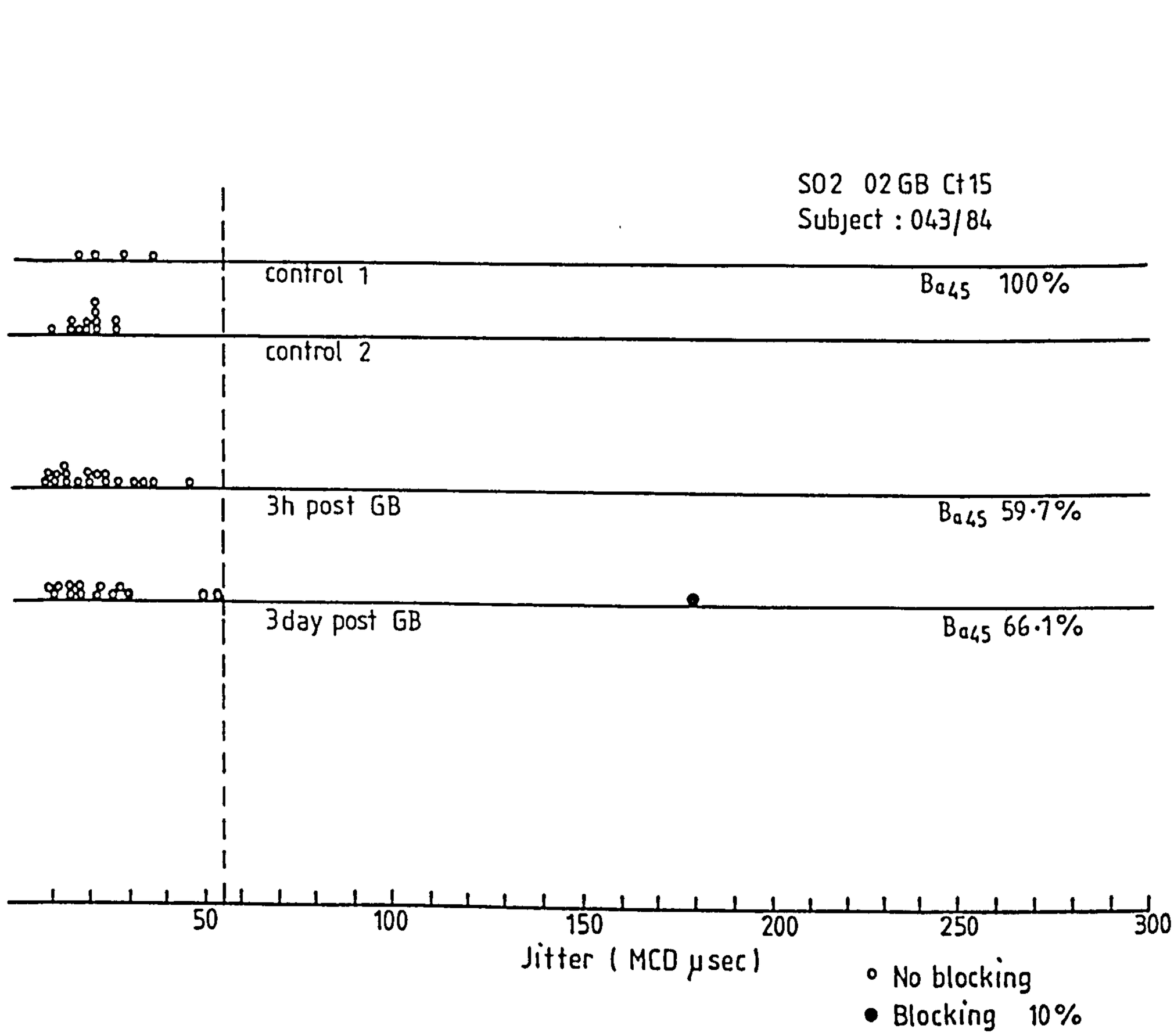

SO2/02 GB Ct 15
Subject : 036/84
control 1
Ba45 100%
[ONE CONTROL SESSION ONLY]
control 2
3h. post GB
Ba45 65·3 %
3 days post GB
Ba45 59·1%
HIGH BLO
50
100
150
200
250
300
Jitter (MCD μ sec)
No blocking
Blocking >10%
Ba45=% red cell cholinesterase activity, corrected to PVC = 459

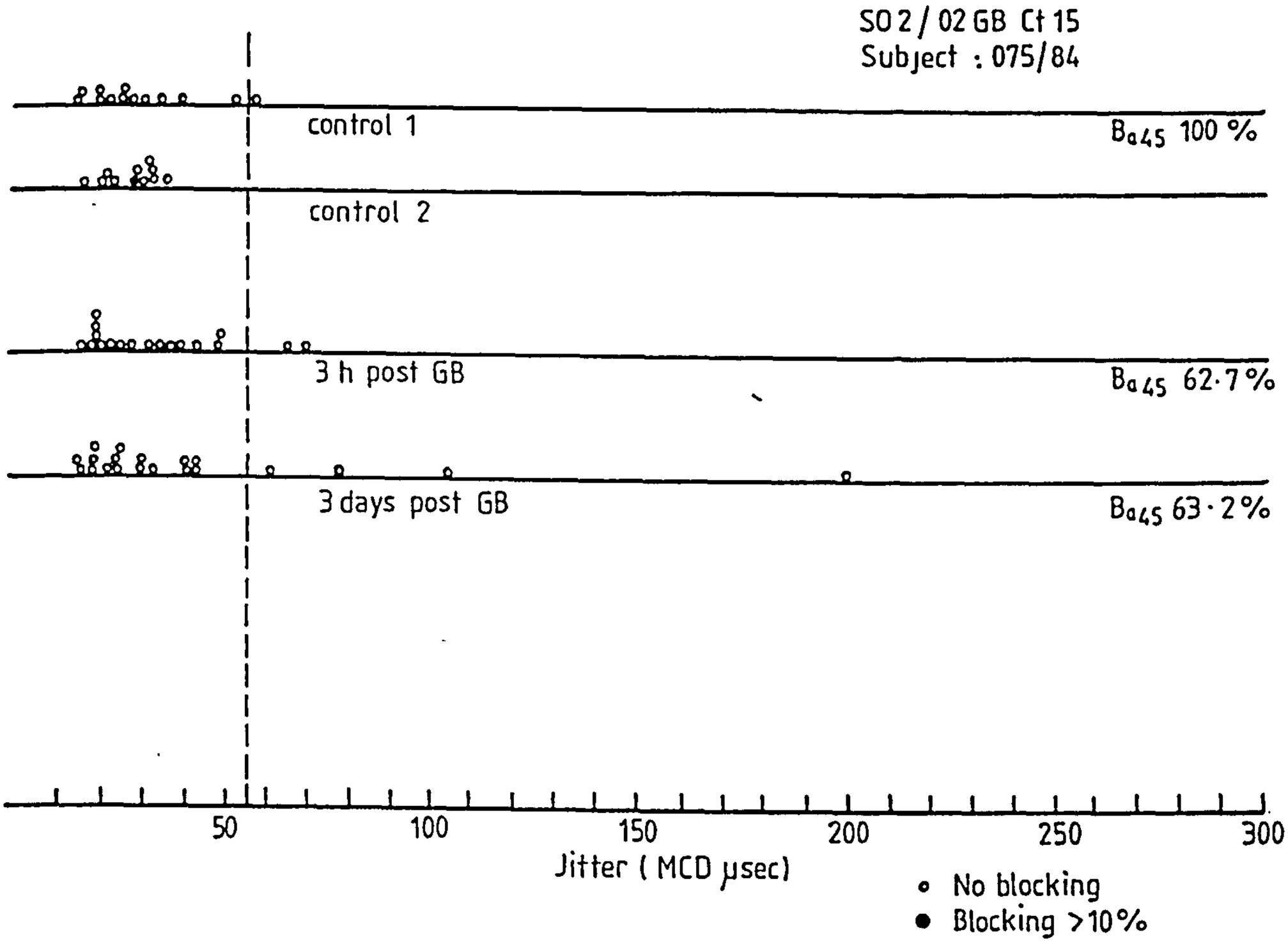

SO2 / 02 GB15
Subject : 173 / 83
control 1
Ba45 100 %
[ONE CONTROL SESSION ONLY]
control 2
3 h post GB
3 days post GB
Ba45 57·9%
473 μsec
48 % block
50
100
150
200
250
300
Jitter (MCD μsec)
No blocking
Blocking > 10%


SO 2 02 GB Ct 15
Subject : 041/84
control 1
Ba45 100 %
control 2
3h post GB
Ba45 54.4%
3 days post GB
Ba45 60·8%
50
100
150
200
250
300
Jitter (MCD µsec)
No blocking
Blocking >10%


SO 2 / 02 GB Ct 15
Subject : 037/84
control 1
Ba45 100 %
control 2
3h post GB
Ba45 51·4 %
3 days post GB
Ba45 59·9%
50
100
150
200
250
300
Jitter (MCD µsec)
No blocking
Blocking > 10%


SO2 / 02 GB Ct 15
Subject : 061/84
control 1
B_{a45} 100%
control 2
3h post GB
B_{a45} 53·2 %
3 days post GB
B_{a45} 55·8 %
50
100
150
200
250
300
Jitter (MCD µsec)
No blocking
Blocking > 10 %


SO2 02 GB Ct15
Subject : 043/84
control 1
B_{a45} 100%
control 2
3h post GB
B_{a45} 59·7%
3day post GB
B_{a45} 66·1%
50
100
150
200
250
300
Jitter (MCD µsec)
No blocking
Blocking 10%


SO2 / 02 GB Ct 15
Subject : 075/84
control 1
Ba45 100 %
control 2
3 h post GB
Ba45 62·7 %
3 days post GB
Ba45 63·2 %
50
100
150
200
250
300
Jitter (MCD µsec)
No blocking
Blocking >10%

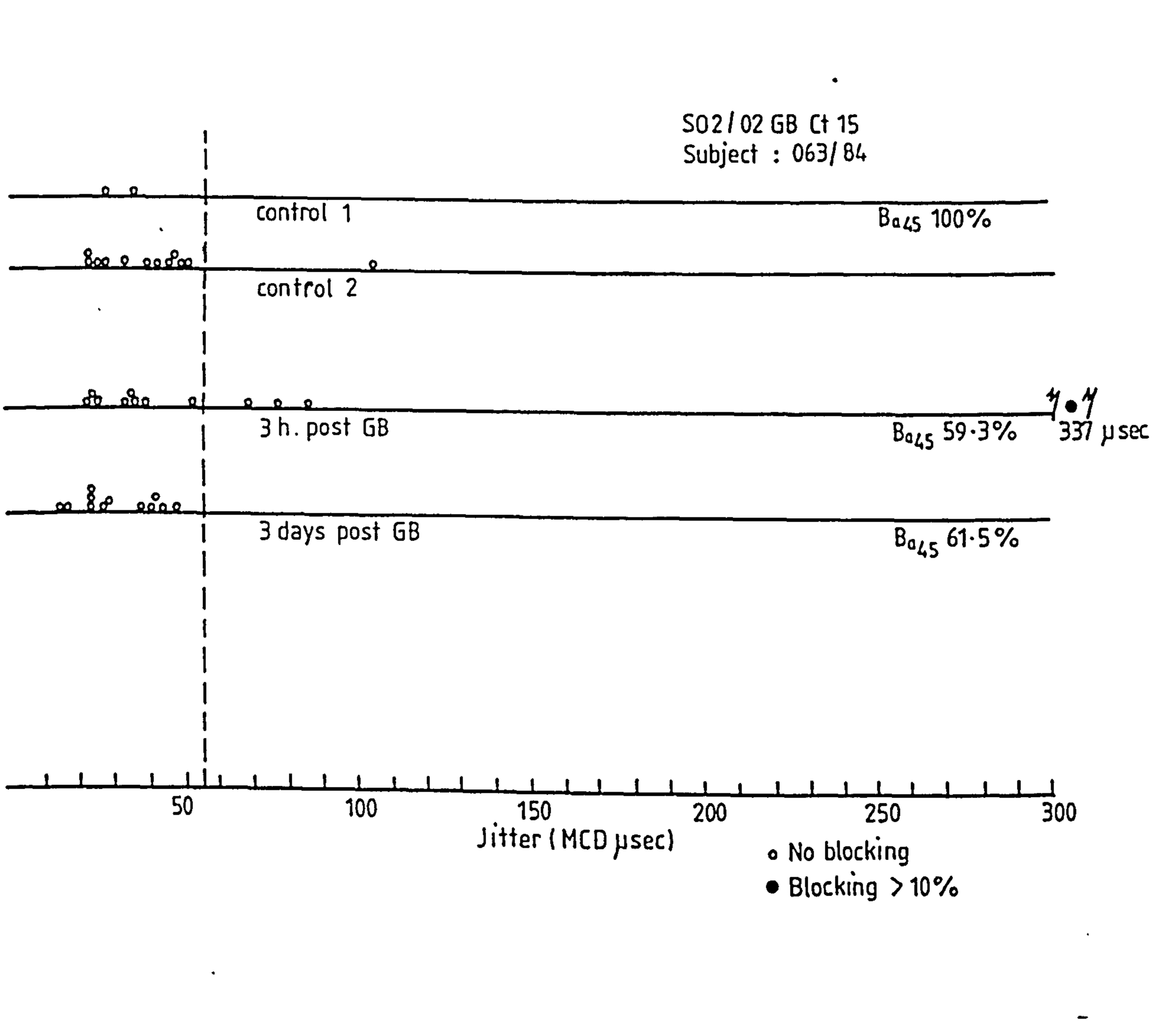

SO2 / 02 GB Ct 15
Subject : 063/ 84
control 1
Ba45 100%
control 2
3 h. post GB
Ba45 59·3%
337 µsec
3 days post GB
Ba45 61·5%
50
100
150
200
250
300
Jitter (MCD µsec)
No blocking
Blocking > 10%

Table 8.6 GB Ctl5 study: mean jitter frequencies after reciprocal transformation for control recording sessions in each subject

Subject	Session 1	Session 2	F	t
173/83		49.6 ± 11.4(17)		
036/84		50.3 ± 18.7(18)		
037/84	38.2 ± 7.9(8)	40.8 ± 13.1(12)	2.75 (p=0.11)	0.5 (p=0.63)
041/84	47.9 ± 14.3(6)	42.3 ± 13.7(9)	1.09 (p=0.43)	0.76 (p=0.46)
043/84	42.4 ± 13.8(4)	54.7 ± 17.1(12)	1.54 (p=0.46)	1.31 (p=0.21)
061/84	59.3 ± 22.6(7)	50.0 ± 16.4(11)	1.90 (p=0.19)	1.02 (p=0.32)
063/84		29.5 ± 11.0(14)		
075/84	40.7 ± 16.5(13)	38.9 ± 10.3(11)	2.57 (p=0.08)	0.31 (p=0.76)

Data are listed as mean jitter frequency in kHz ± standard deviation (number of fibre pairs recorded). F is the variance ratio which compares the dispersion of the sample values.

t = Student's "t" test

p = probability of obtaining the result by chance alone.

Table 8.7 GB Ctl5 study: mean jitter frequencies for combined control sessions in each subject. The equivalent MCD of the mean control jitter frequency is shown in μsec together with the values of 95% confidence.

Subject	Mean Control Jitter Frequency (kHz)	Derived Mean MCD (μsec)	95% Conf.
173/83	49.6 ± 11.4(17)	20.2	18.0 28.8
036/84	50.3 ± 18.7(18)	19.9	17.0 24.8
037/84	39.8 ± 11.1(20)	25.1	22.2 28.9
041/84	44.5 ± 13.7(15)	22.4	19.2 27.1
043/84	51.6 ± 16.9(16)	19.4	16.5 23.5
061/84	53.4 ± 19.0(18)	18.7	15.9 22.7
063/84	29.5 ± 11.0(14)	33.9	27.9 43.2
075/84	39.9 ± 13.7(24)	25.1	21.9 29.3
Mean 26.3	44.8 ± 8.1	22.3	19.4
Mean (exc 063/84)	47.01 ± 5.6	21.3	19.1 23.9

where 95% conf. = 95% confidence limits on the derived mean MCD in μsec.

original recordings which were greater than 45 usec. The individual recorded jitter values for this subject were as follows:

35 27 27 48 53 47 42 39 22 23 104 32 45 46

It is seen that only one jitter value is greater than the usually accepted upper level of clinical abnormality of 55 usec in EDC. Excluding this the mean value of jitter using Stalberg's assessment is 37.4 $\pm$ 10.4 usec. Following the usually accepted clinical criteria subject 063/84 cannot be regarded as being within normal control limits. In addition, the mean value is outside that established for controls during the pyridostigmine/GB Ct5 study. For this reason subject 063/84 was excluded from the analysis given below of the effect of GB Ct15 on normal jitter. The results for this subject are presented separately. Excluding the control values of subject 063/84 from the overall mean control jitter frequency gives a mean value of 47.0 $\pm$ 5.6 kHz. This is equivalent to a derived mean MCD value of 21.3 usec within 95% confidence limits of 19.1 and 23.9 usec.

8.4.3 Exposure to GB Ct15: analysis

Table 8.8 shows mean jitter frequencies for all subjects, excluding 063/84 derived from MCD values recorded during each session of the study. Changes in mean jitter frequency between (1) control and three hours after GB exposure, (2) three hours after exposure and three days after exposure and (3) control and three days after exposure are shown in the right hand column. Group mean values for each of the jitter frequency changes are then given, together with simple t test and the corresponding probability values. Jitter frequency changes for subject 063/84 are shown

Table 8.8 GB Ctl5 study: jitter frequency changes for each subject following GB exposure. Values in the first three columns are of mean jitter frequency in kHz. Subject 063/84 is treated separately due to abnormal control jitter values (see text)

Subject	Control	GB+3h	GB+3d	Jitter Frequency Shift		
				C/GB3h	GB3h/GB3d	C/GB3d
173/83	49.6	46.3	33.4	3.3	12.9	16.2
036/84	50.3	49.9	38.7	0.4	11.2	11.6
037/84	39.8	41.9	30.7	-2.1	11.2	9.1
041/84	44.5	37.5	39.1	7.0	-1.6	5.4
043/84	51.6	57.3	50.2	-5.7	7.1	1.4
061/84	53.4	57.7	48.2	-4.3	9.5	5.2
075/84	39.9	36.1	34.5	3.8	1.6	5.4
			Group mean	0.34	7.41	7.76
			sd	4.64	5.45	4.93
			t	0.20	3.60	4.16
			p	0.85	0.01	0.006
063/84	29.4	25.5	38.0	3.9	-12.5	5.4

separately. Table 8.9 shows the incidence of fibre pairs with clinically abnormal jitter following GB Ct15 exposure. Table 8.10 shows AChE levels following GB Ct15 exposure.

The figures presented in table 8.8 show that exposure to GB Ct15 is associated with a reduction in mean jitter frequency at three hours post exposure of 0.34 $\pm$ 4.64 kHz ($p = 0.85$) which increases to 7.76 $\pm$ 4.93 kHz at three days post exposure. These changes are reflected in the increased incidence of high MCD. values in five subjects three days after exposure. Table 8.10 shows that GB Ct15 exposure produces about 40% inhibition of red cell AChE which is persistent three days post exposure.

8.4.4 Blockings after GB Ct15 exposure

Table 8.11 shows the fraction of the recorded fibre pair waveforms used to compute jitter where the second wave of the pair failed to appear. The blocking precentage is listed for sessions recorded three hours and three days after exposure to GB Ct15. A blank entry indicates that no fibre pairs showing high jitter were recorded during that particular session. Figure 8.14 shows a plot of the blocking percentage against jitter from the data for both sessions presented in table 8.11. The correlation coefficient for this plot is $r = 0.83$ ($p < 0.001$). However this correlation, quoted in isolation is misleading because of the cluster of data points with MCD < 100 usec. From an inspection of figure 8:14 it appears that the increase in blocking with MCD takes place after a threshold value of about 100 usec . The correlation coefficient in this case is reduced with $r = 0.695$ and is significant at the $p < 0.05$ level.

Table 8.9 GB Ctl5 study: incidence of clinically abnormal jitter during recording sessions for each subject before and after GB exposure. HB indicates a fibre pair with a high degree of blocking. Figures in brackets are the numbers of recorded fibre pairs included in the mean.

Subject Number	Control MCD Mean ± SD µsec	Proportion of fibre pairs with MCD >55µsec		
		Session 1 & 2	Session 3	Session 4
173/83	21.2 ± 5.2 (17)	0/17	2/15	2/12
036/84	21.9 ± 6.6 (18)	0/18	0/18	1/15
037/84	26.8 ± 7.6 (20)	0/20	1/17	5/17 +3xHB
041/84	24.8 ± 8.5 (15)	0/15	2/11	3/20
043/84	21.1 ± 6.3 (16)	0/16	1/18	3/15
061/84	21.1 ± 8.0 (18)	0/18	0/16	0/10
063/84	35.2 ± 10.5 (13)	1/14	4/12	0/12
075/84	26.8 ± 10.6 (23)	1/24	2/16	4/20

Subject	AChE level: % of individual control	
	Session 3 (3 h post GB Ct 15)	Session 4 (3 d post GB Ct 15)
173/83	54.0	57.9
036/84	65.3	59.1
037/84	51.4	59.9
041/84	54.4	60.8
043/84	59.7	66.1
061/84	53.2	55.8
063/84	59.3	61.5
075/84	62.7	63.2
Mean	57.5 ± 5.0(8)	60.5 ± 3.2(8)

Table 8.10 GB Ct15 study: degree of AChE inhibition produced by GB exposure at three hours and three days after exposure. The persistence of the inhibition is apparent.

Values are RBC AChE levels corrected to a PCV of 0.45.

Table 8.11 GB Ctl5 study: degree of SFEMG blocking in fibre pairs with high MCD values. B indicates a fibre pair with a high degree of blocking where the MCD value cannot be calculated with accuracy.

Subject	Session 3	% Blocking	Session 4	% Blocking
	MCD µsec		MCD µsec	
173/83	80	0	54	0
	163	14	81	0
			473	48
036/83	50	8		
	59	5		
	B	B		
037/84	55	3	B	B
			B	B
			B	B
			183	40
			58	0
			145	10
041/84	59	0	112	3
042/84			54	0
			178	35
			50	0
061/84			61	0
			54	0
063/84	76	3		
	68	3		
	52	3		
	337	25		
075/84	65	0	200	3
	59	0	104	0
	78	0	61	0

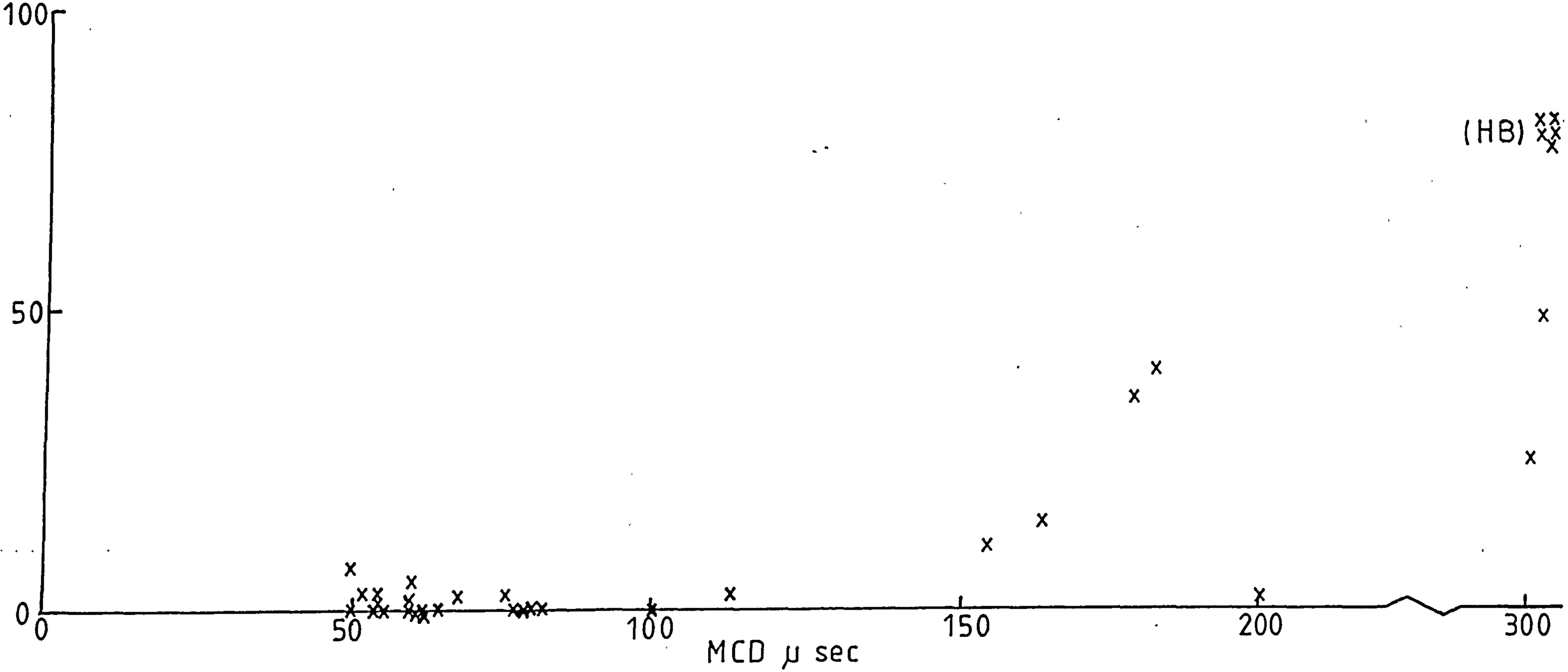


Fig. 8.14. GB Ct'15 study : high jitter values and degree of blocking (HB = high degree of blocking where MCD values cannot be accurately calculated)

8.4.5 GB Ct15 study: summary of findings

The summary of findings from the analysis of jitter after GB Ct15 exposure were as follows:

1. Changes in mean jitter seen following GB Ct5 exposure were repeated at a higher dose of GB but without a proportional dose - related effect

2. The significant change in mean jitter frequency seen at three hours after exposure to GB Ct5 was not seen at the higher dose; it may be noted however that four of the subjects exposed to GB Ct15 had an apparently abnormal incidence of clinically abnormal(> 55 usec) MCD values at this time

3. In five subjects the SFEMG changes at three days after exposure were also seen as an apparently abnormal incidence of clinically abnormal (> 55 usec) MCD values

4. High MCD values of jitter were associated with increased blocking at a threshold value of about 100 usec.

CHAPTER 9: Isolated forearm studies: results and analysis

9.1 Fade during onset and recovery of paralysis induced by three non - depolarizing relaxants

9.1.1 Presentation of data

The results from this study produced a large amount of data for MMG and EMG changes associated with the three relaxants tested in the isolated forearm. The data are therefore presented graphically to show the following relationships:

(1) MMG and EMG first response with respect to time from inflation of the tourniquet;

(2) MMG and EMG train of four responses with respect to time from inflation of the tourniquet;

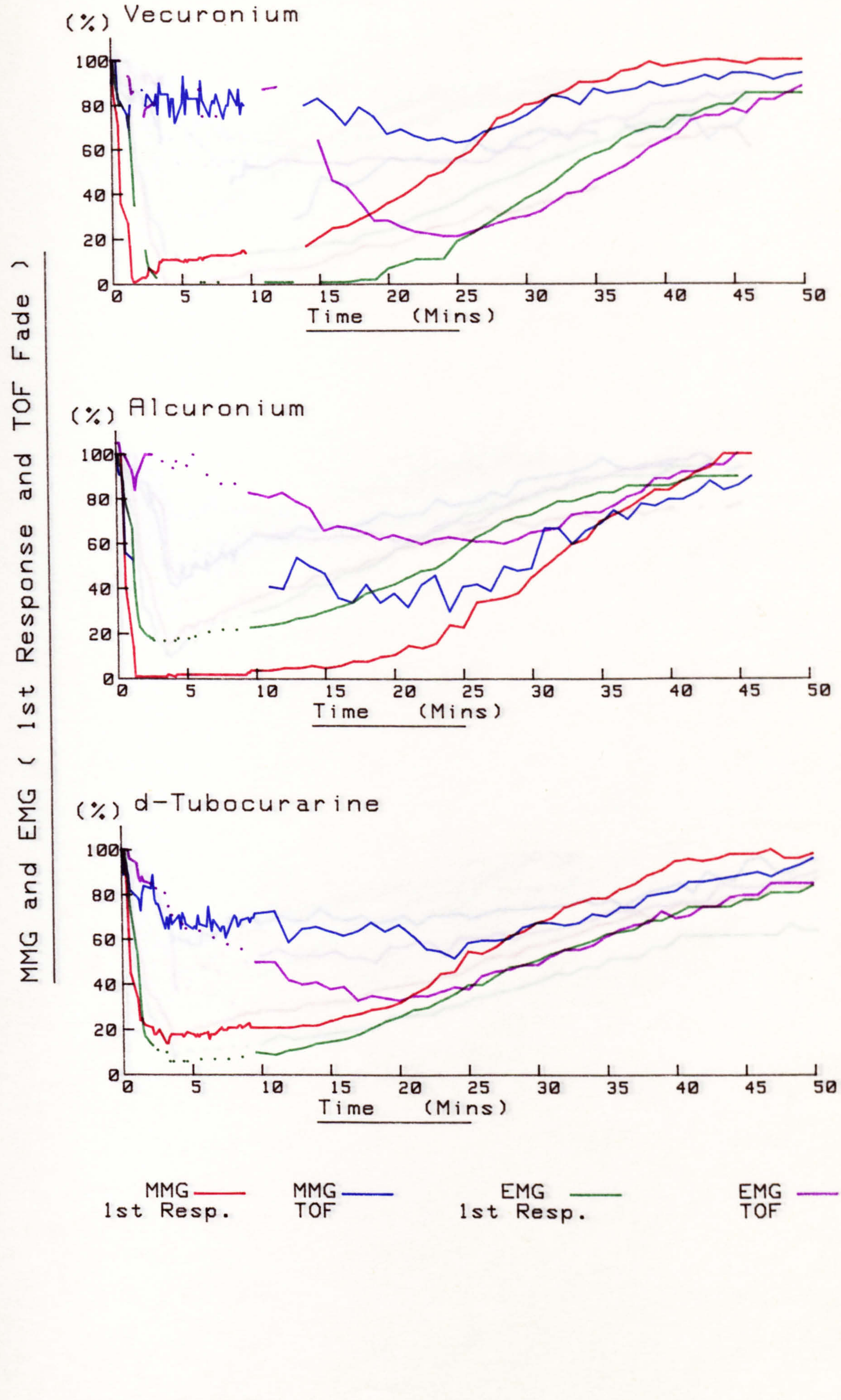
(3) MMG and EMG first response with respect to train of four fade

9.1.2 The relationships between MMG, EMG and time

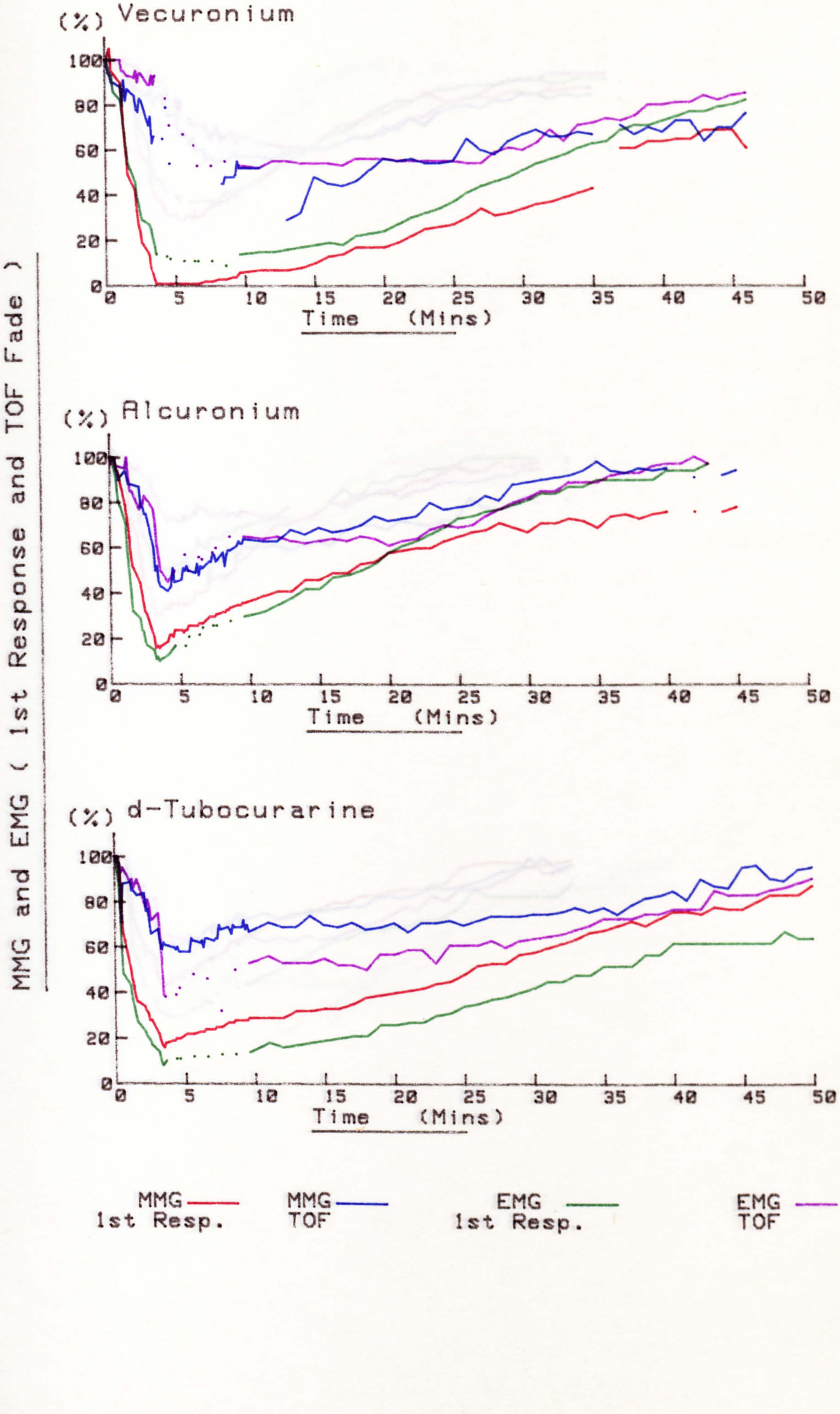
Figure 9.1 shows plots of MMG and EMG data against time from all six subjects used in the study. The first response of the TOF and the TOF fade ratio are plotted between the same axes as a percentage of

Fig. 9.1 Isolated forearm study: Data plots (on the following six pages) from six subjects treated with vecuronium alcuronium and d - tubocurarine. The onset and recovery of muscle relaxation (indicated by the degree of first response of the TOF) and fade during TOF for MMG and EMG recording are shown as colour - coded lines(see text).

Protocol S03/1A. Subject No 3 / 84



Protocol S03/1A. Subject No 17 / 84



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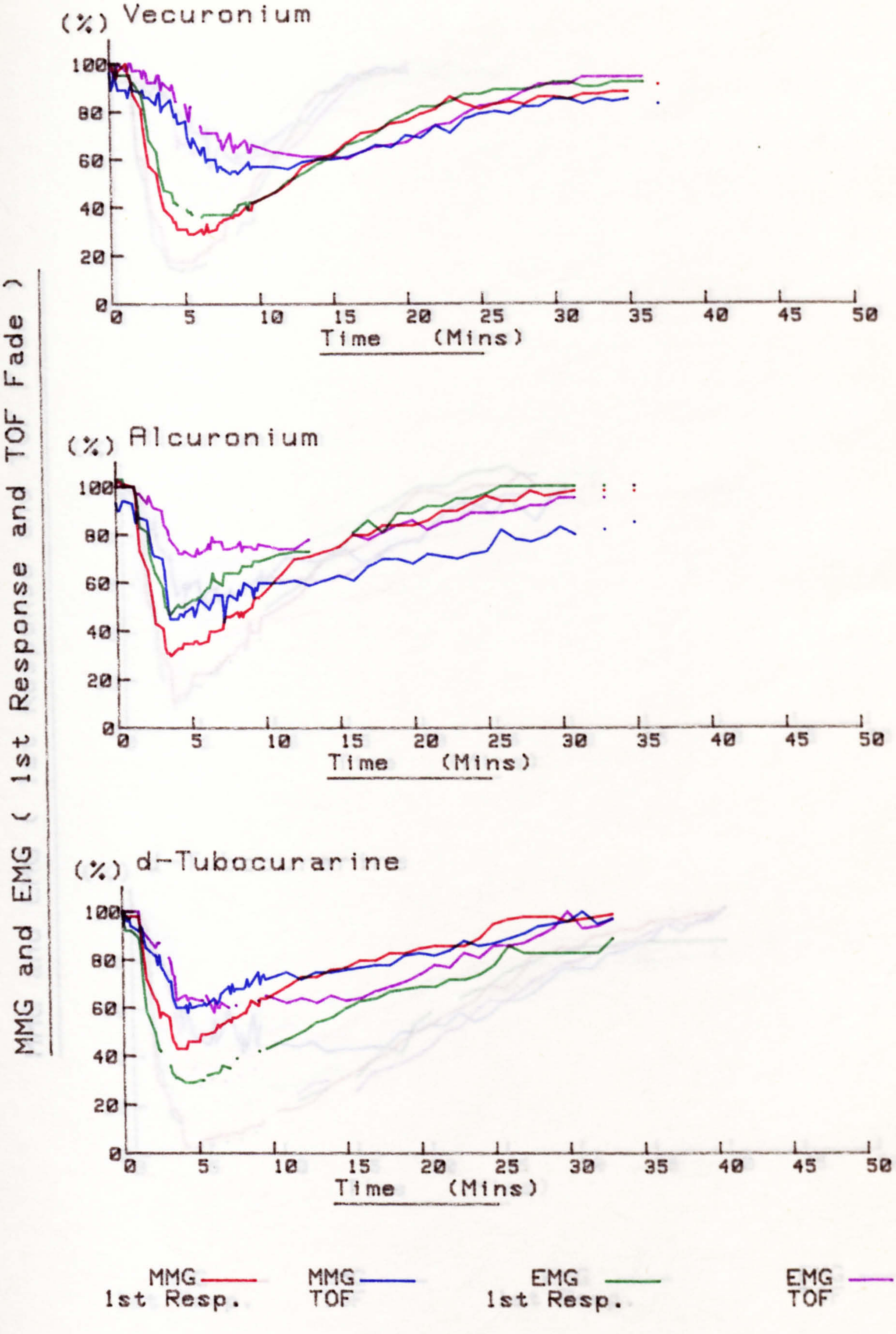

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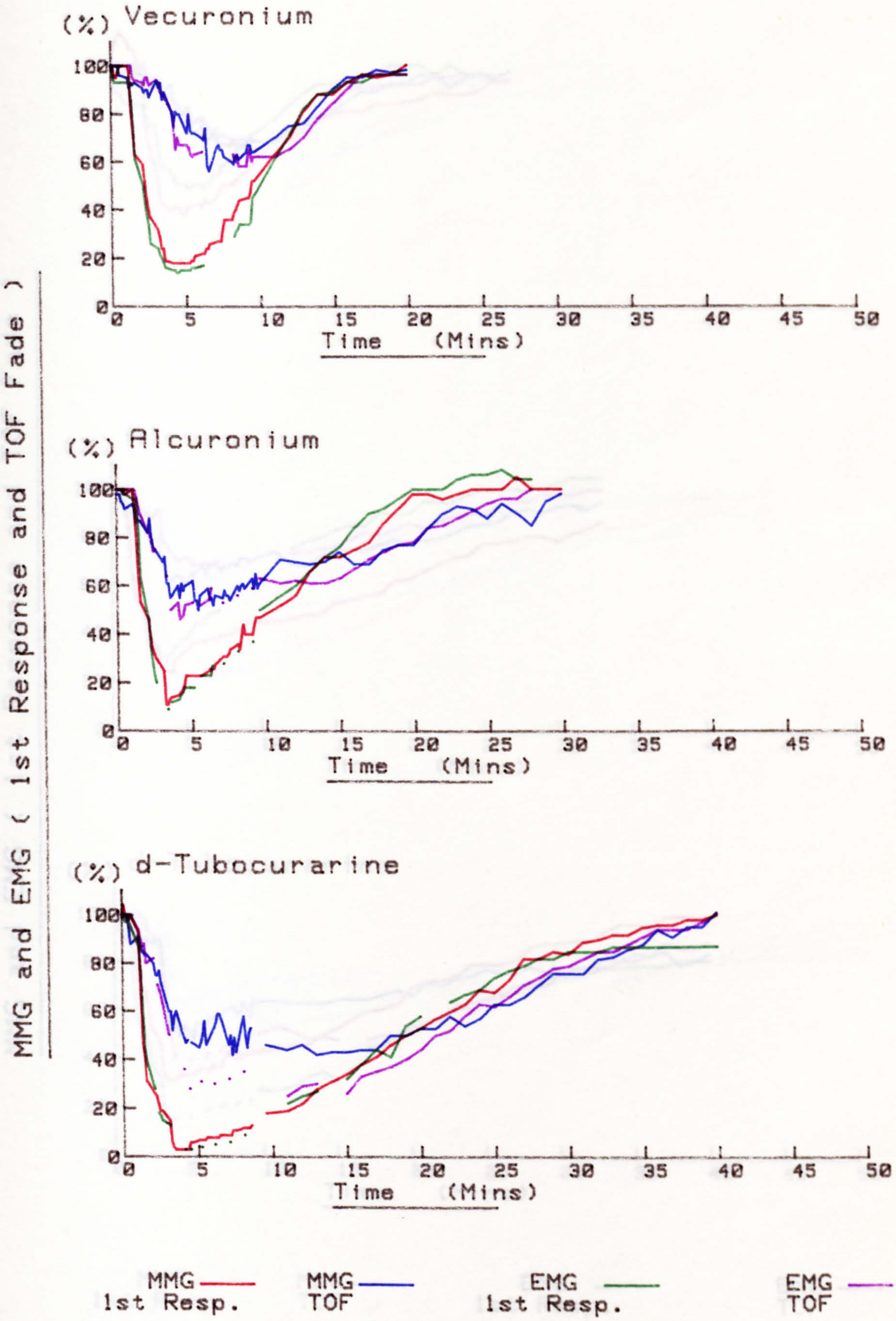
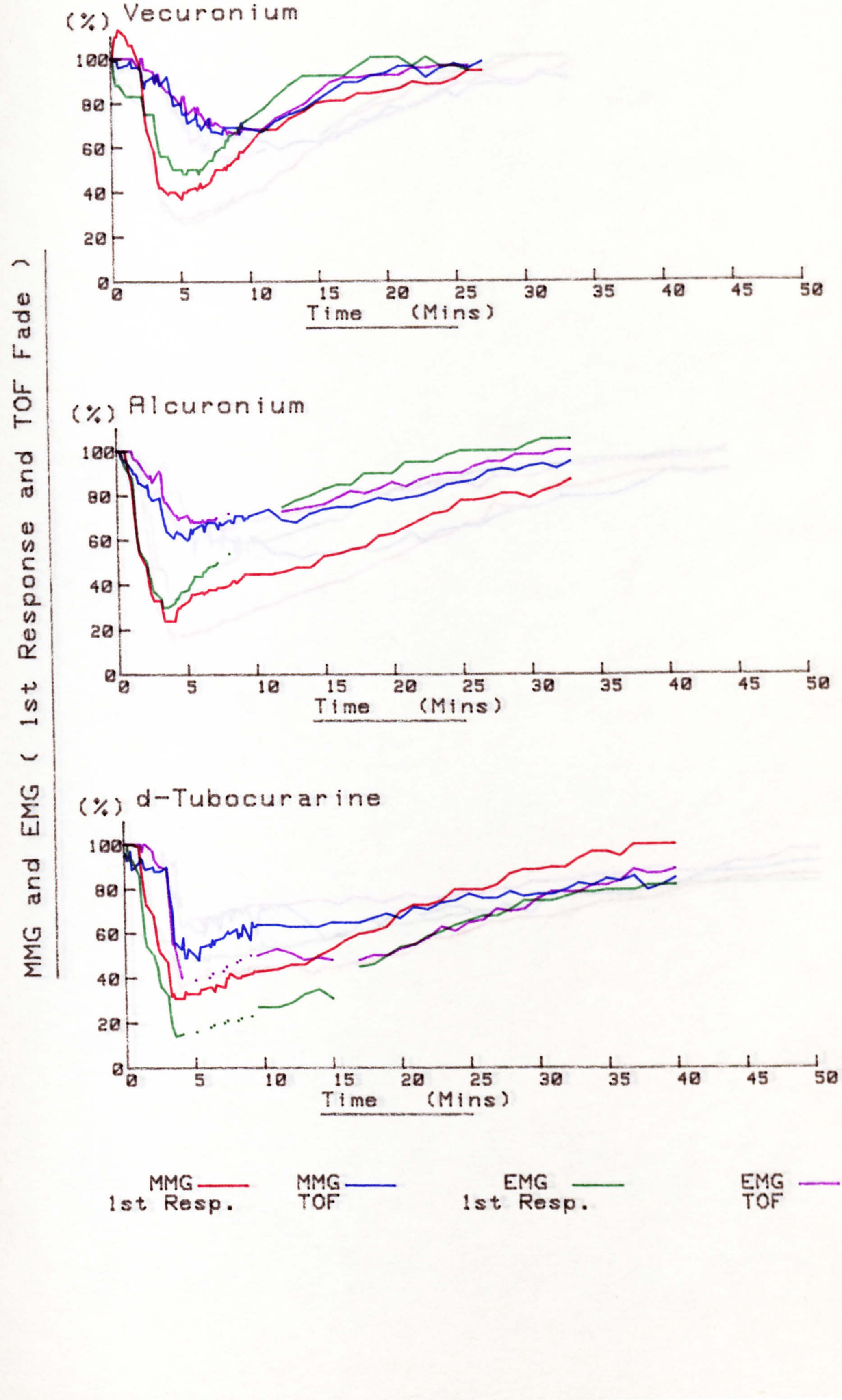


Protocol S03/1A. Subject No 56 / 84



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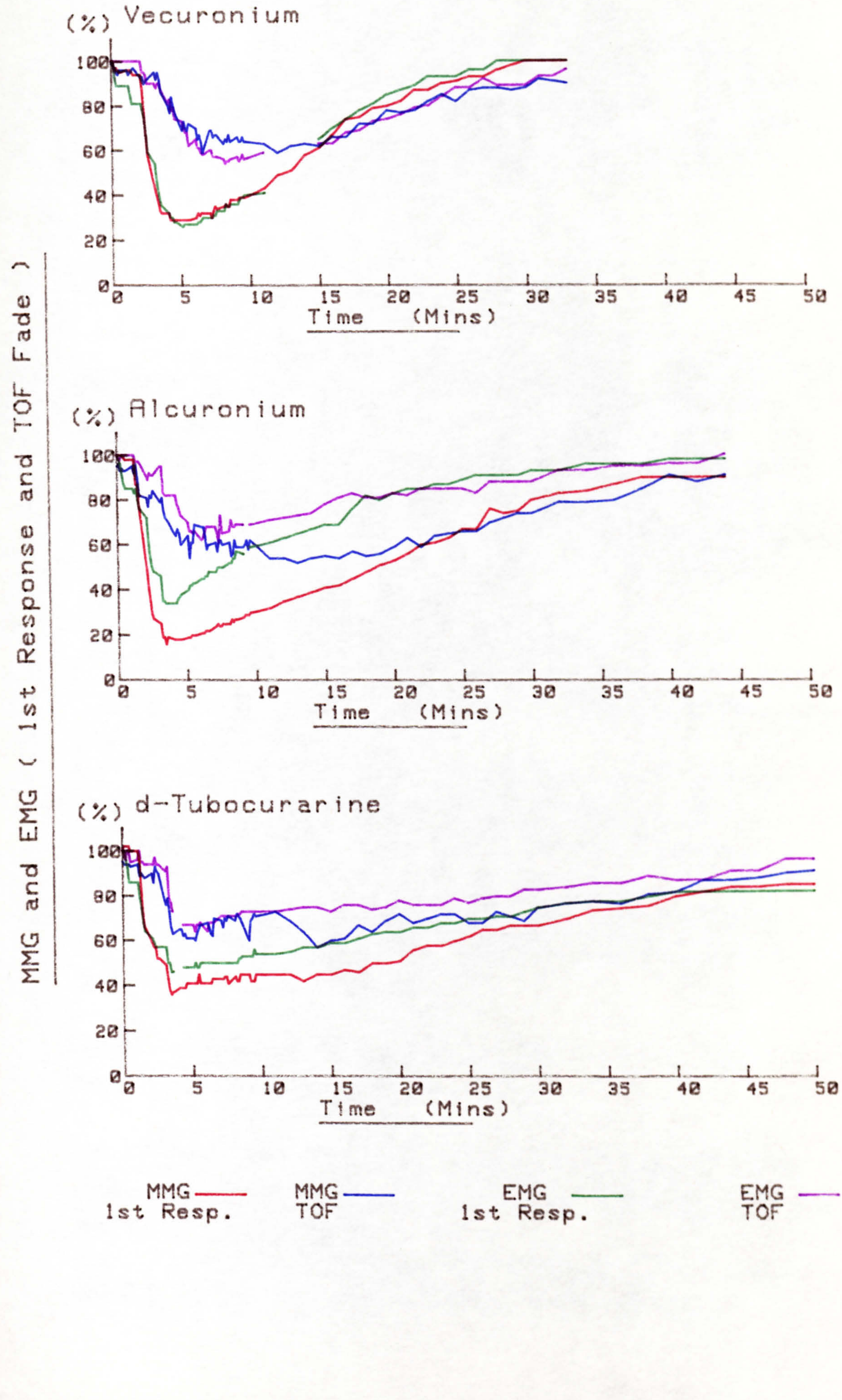

the control values in each case. Figure 9.2 shows a typical MMG recording from one experiment showing development of paralysis and fade after injection of the muscle relaxant at time zero. Figure 9.3 shows a typical compound EMG recording from the MS6 myograph with the measured single peak height used in this study.

9.1.3 Analysis of muscle relaxation

Examination of figure 9.1 shows that there was considerable individual variation in the responses to the three relaxants of the six subjects studied. To analyse these responses and to compare MMG with EMG the following features of the curves were considered:

(1) peak paralysis, which is defined as the lowest recorded percentage first response of MMG and EMG compared with their control values;

(2) onset, defined as the length of time in minutes from the injection of the relaxant to a value of MMG or EMG first response not greater than 5% above the minimum observed

(3) duration, defined as the period in minutes during which the MMG or EMG first response was consistently below a value 5% above the observed minimum;

(4) recovery, defined as the period in minutes taken to recover between 25% and 75% of the control MMG or EMG first response.

These parameters are listed in table 9.1 for each of the six subjects examined.

To analyse the first response against time variation the four parameters listed above were subjected to the following procedures:

(1) correlation, where the data were examined to establish how well MMG and EMG first responses

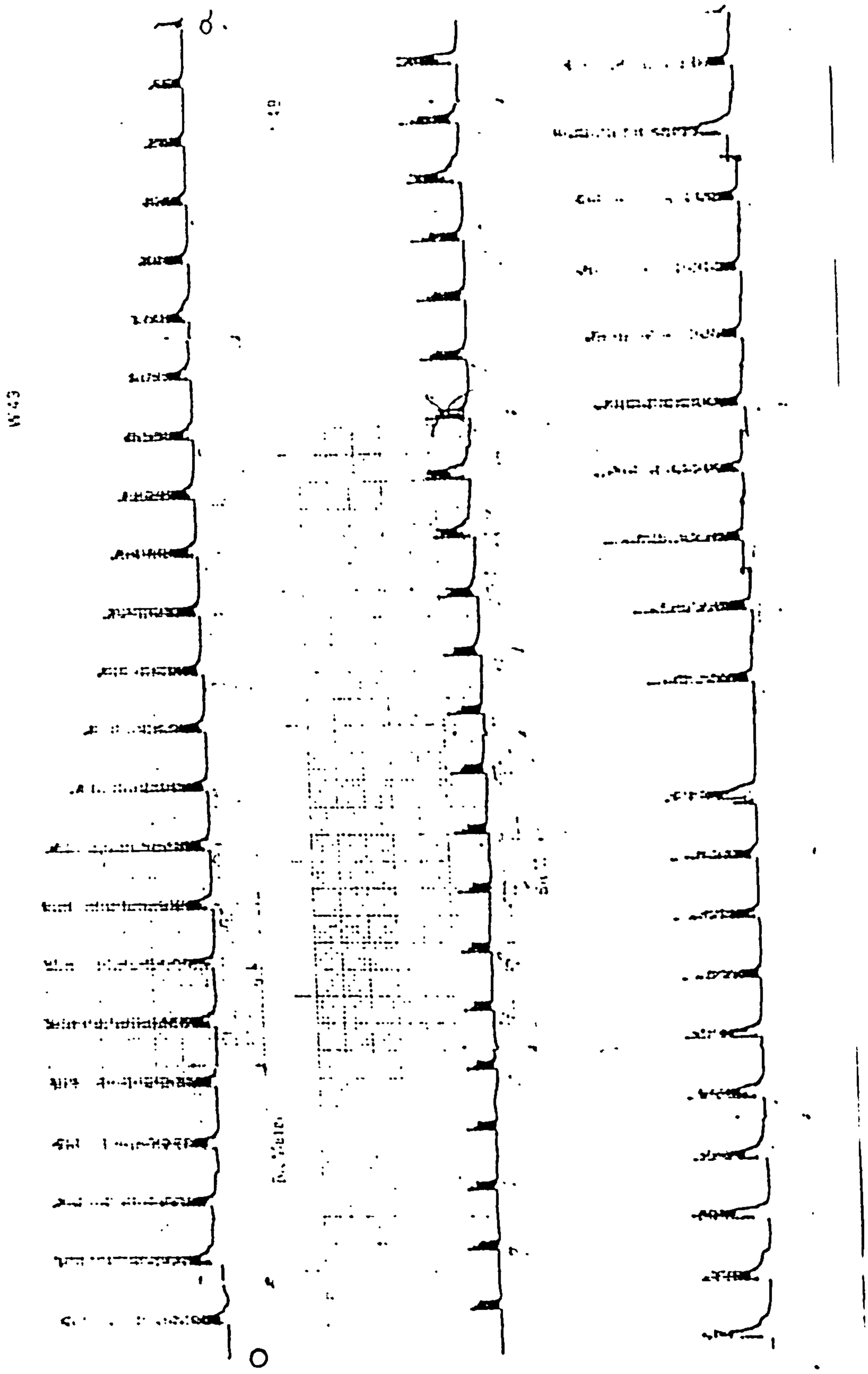

Fig. 9.2 Isolated forearm study: typical MMG recording from one experiment showing the development of relaxation and TOF fade from time 0. TOF fade is seen to be more marked during the recovery phase after point a (see text)

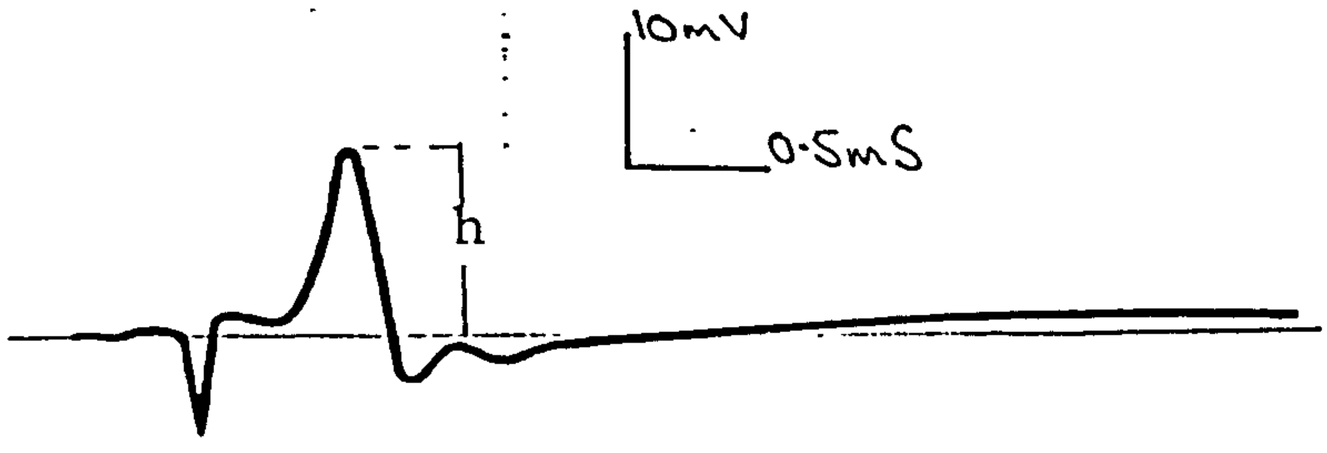

Fig. 9.3 Isolated forearm study: evoked EMG recording from adductor pollicis showing the single peak measurement used in this study. The stimulus artefact is visible at t.

PARAMETER (See text for definitions)	Drug	M.M.G Subject 03/84	17/84	47/84	48/84	56/84	60/84	E.M.G. Subject 03/84	17/84	47/84	48/84	56/84	60/84
Peak Paralysis (%)	ALC	1	16	30	11	31	29	17	10	47	9	14	26
	TBC	14	16	43	3	37	18	6	8	29	3	48	34
	VEC	1	1	29	18	24	39	1	9	37	14	30	46
Induction (mins)	ALC	1.2	3.2	3.4	3.2	3.6	3.8	2.0	3.2	3.4	3.5	3.6	4.4
	TBC	2.4	3.2	3.6	3.2	3.8	3.4	2.8	3.2	3.4	3.5	4.5	3.4
	VEC	1.6	3.6	4.4	4.0	3.4	3.4	3.0	4.0	4.4	3.8	3.2	3.4
Duration (mins)	ALC	8.6	1.6	2.6	1.2	3.2	4.2	7.8	1.4	1.8	1.0	3.6	3.4
	TBC	4.8	2.0	1.8	3.2	3.2	3.6	9.2	6.2	3.4	4.6	2.4	1.6
	VEC	1.8	6.4	3.4	2.4	1.4	4.2	17.0	6.0	5.6	2.8	1.6	5.2
Recovery (mins)	ALC	12	16*	9	5	13.5	9.5	11.5*	17	12	5	12	7
	TBC	13.5	24*	12	13.5	6*	14*	16.5*	25*	12	9.5*	4	13
	VEC	12.5	18*	8.5*	5	16.5*	16*	11*	25*	8.5*	3	10.5	17*
Full recovery	ALC	34	>40	25	20	33	22	>35	38	21	23	>33	20
	TBC	40	>45	28	34	>20	>36	>38	>51	>26	32	16	35
	VEC	36	>36	> 29	16	>36	>43	>30	>40	>26	10	27	>42

Table 9.1 Isolated forearm study: parameters of relaxation/time data shown in fig.9.1 for alcuronium(ALC) d-tubocurarine(TBC) and vecuronium(VEC). > = experiments terminated before full recovery
* = recovery to less than 95% of initial value

correlated with each other

(2) regression; if the correlation between MMG and EMG first response was significant, regression analysis was performed to see whether there was a 1:1 relationship between the two methods of measurement.

9.1.4 Correlation between MMG and EMG first response

Table 9.2 lists the correlation coefficients for the peak effect, onset, duration and recovery variables defined above. The final column gives the correlation coefficients averaged from those calculated for all three relaxants in each individual. The table shows good correlation between MMG and EMG first responses during the induction and recovery periods.

9.1.5 Regression analysis

Table 9.3 examines further the closeness of a linear relationship between MMG and EMG measurements established by the correlation coefficients given in table 9.2 to see how closely the relationship approached 1:1. Regressions were performed for MMG(x) on EMG(y) and MMG(y) on EMG(x) for the first responses of each train of four. Tests of significance were applied to two null hypotheses;

(1) $H : Slope = 0$

All twelve regressions for slope were significant on this hypothesis confirming that a significant linear relationship exists between MMG and EMG first response assessments of induction and recovery.

(2) $H : Slope = 1$

	ALCURONIUM	d-TUBOCURARINE	VECURONIUM	Mean of all three drugs
Peak Paralysis	0.51	0.77	0.96 **	0.98 ***
Induction	0.94 **	0.81 *	0.82 *	0.99 ***
Duration	0.98 ***	0.38	-0.12	0.85 *
Recovery	0.89 *	0.95 **	0.84 *	0.95 **

Table 9.2 Correlation between MMG and EMG assessments of parameters listed in table 9.1.

* = significant at p = 0.05, ** = significant at p = 0.01 and *** = significant at p = 0.001

		EMG(y) on MMG(x)			MMG (y) on EMG(x)		
		ALC	TBC	VEC	ALC	TBC	VEC
Induction	Slope (m)	0.77	0.95	0.45	1.13	0.70	1.49
	s.e.	0.15	0.34	0.16	0.21	0.25	0.52
	Intercept (c)	0.98	0.37	2.11	-0.73	0.84	-2.02
	s.e.	0.46	1.12	0.55	0.73	0.87	1.92
Recovery	Slope (m)	0.98	1.15	1.26	0.81	0.78	0.57
	s.e.	0.25	0.20	0.40	0.21	0.13	0.18
	Intercept (c)	1.47	-2.64	-3.51	2.13	3.46	5.67
	s.e.	2.86	2.88	5.43	2.37	1.94	2.58

Table 9.3 Regression analysis of the relationship between MMG and EMG assessment of the induction and recovery phases of fig. 9.1

Table 9.3 shows that the slopes calculated by the method of least squares for induction and recovery regressions range between 0.45 and 1.49. The only slope to differ significantly from unity was for the induction period when EMG was regressed on MMG for vecuronium. This finding is weighted by the dominance of an extreme result obtained in one subject (03/84). With vecuronium as with the other two drugs he was the quickest to respond to injection of relaxant and peak paralysis estimated by MMG was achieved in half the time indicated
by EMG. For the remainder of the slopes therefore the absence of a demonstrable difference of the regression slopes from unity indicates that the 1:1 hypothesis is valid and that EMG and MMG first response measurements are related in this way.
The slopes given in table 9.3 may also be examined to see whether there is a difference in the relationship between MMG and EMG first responses depending on induction or recovery of paralysis. This is done by comparing regression lines in pairs corresponding to the onset and recovery stages. The result is shown graphically in figure 9.4. Analysis of the pairs of regression slopes using a simple t test shows that no significant difference could be demonstrated.
This result seems suprising in the case of vecuronium where the induction slope appears very different from the recovery. To explain why no statistically significant difference can be demonstrated between the induction and recovery relationships for this drug, the data are replotted in figure 9.5 on the same axes. This approach reveals two problems;
(1) the scatter (or variance) of the recovery data is very much greater (by a factor of 30) than that of the induction
(2) induction of paralysis was complete over a much shorter time period of five minutes compared with the

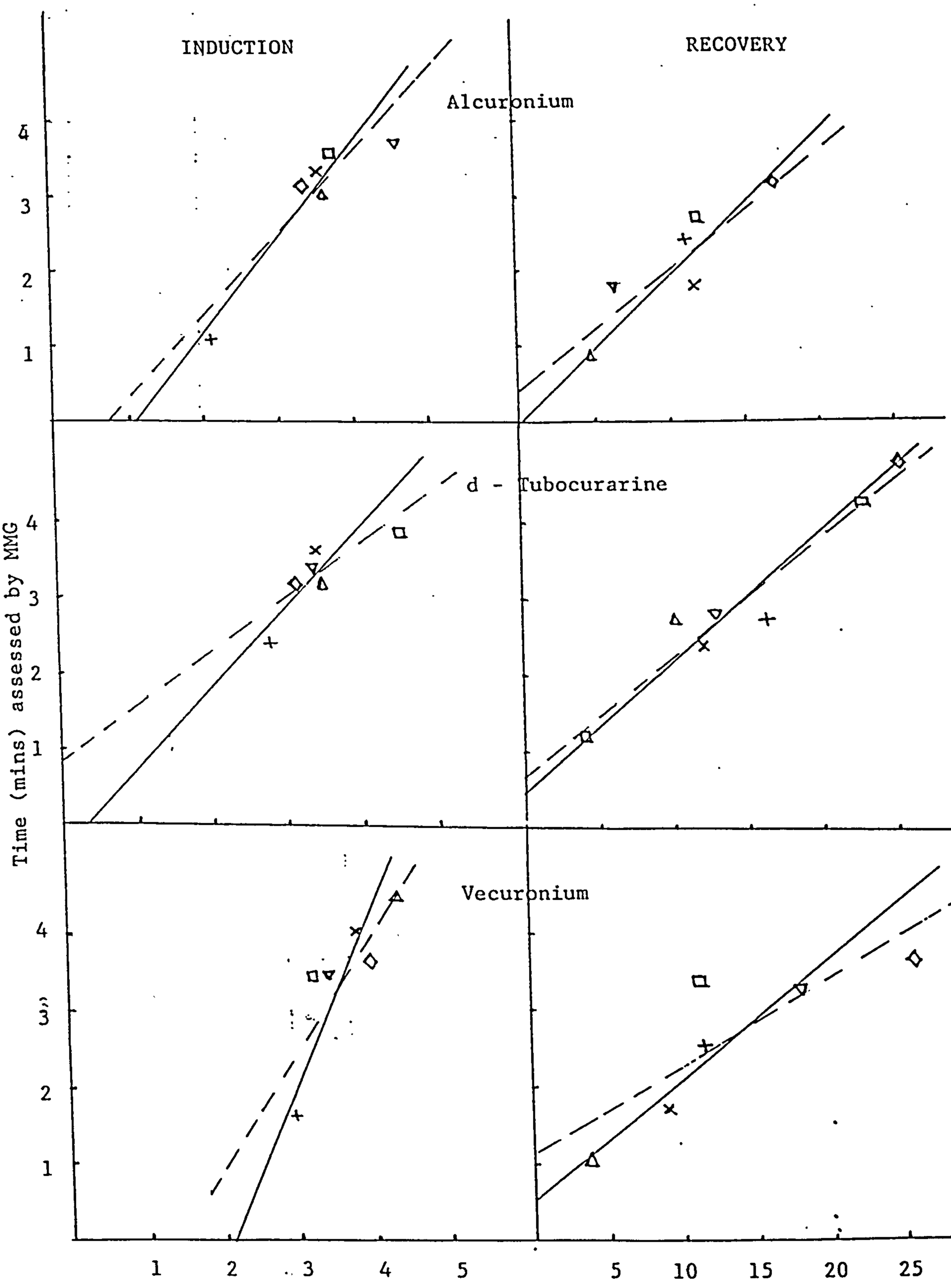

Fig. 9.4 Linear regression lines for induction and recovery times of relaxation during IFP

Key: + 031/84; ◇ 17/84; × 47/84; △ 48/84; □ 56/84; ▽ 60/84;

– · – – – MMG on EMG; ———— EMG on MMG

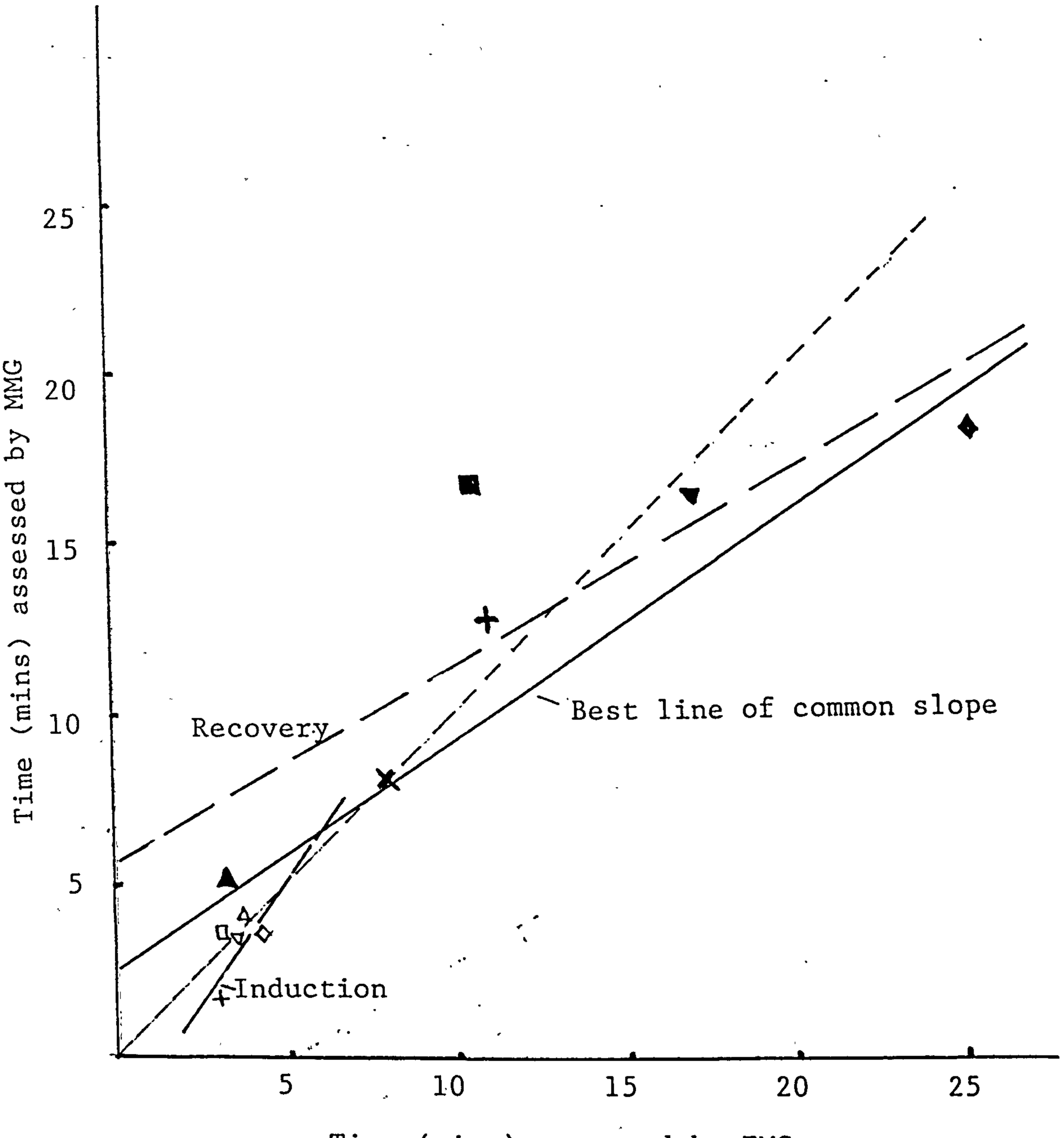

Fig. 9.5 Linear regression lines for induction and recovery of paralysis in the IFP produced by vecuronium. Bold symbols refer to recovery and faint to induction. The dotted line is that of the theoretical 1:1 relationship between MMG and EMG assessed times.

Subject symbols are those detailed in fig.9.4

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25 minutes of recovery. These two factors indicate that the smaller variability in the induction data could easily be engulfed by that of the recovery data without appreciably altering the slope.
Therefore in the case of vecuronium it is not possible to demonstrate a difference between the induction and recovery MMG/EMG relationship at the level of sensitivity of this experiment.

9.1.6 Comparison of MMG and EMG assessment of TOF fade against time

In figure 9.6 the method of calculating the TOF ratio for MMG and EMG is shown. EMG TOF values are calculated after transposition to a single peak height using the repeater oscilloscope. In figure 9.1 the TOF responses for MMG and EMG were plotted against time from injection of the relaxant for each of the six subjects studied. Examination of these curves shows the following immediately identifiable features:
(1) there is wide individual variation between responses for both drugs and subjects;
(2) in some cases using MMG, the first response of the train of four was low and the fourth response was either absent or indeterminate. This feature was never seen with the EMG recordings where the train of four count was always four.
Table 9.4 shows the minimum values of fade recorded in each subject for each relaxant. There is no correlation between MMG and EMG estimates of fade at this point. The scatter diagram in figure 9.7 shows equal numbers of points on either side of the theoretical 1:1 relationship with no dominance of one system over the other;
(3) there was a good correlation ($r = 0.86$) between the time to minimum value of train of four assessed by MMG or EMG;
(4) there was a wide spread in the individual time

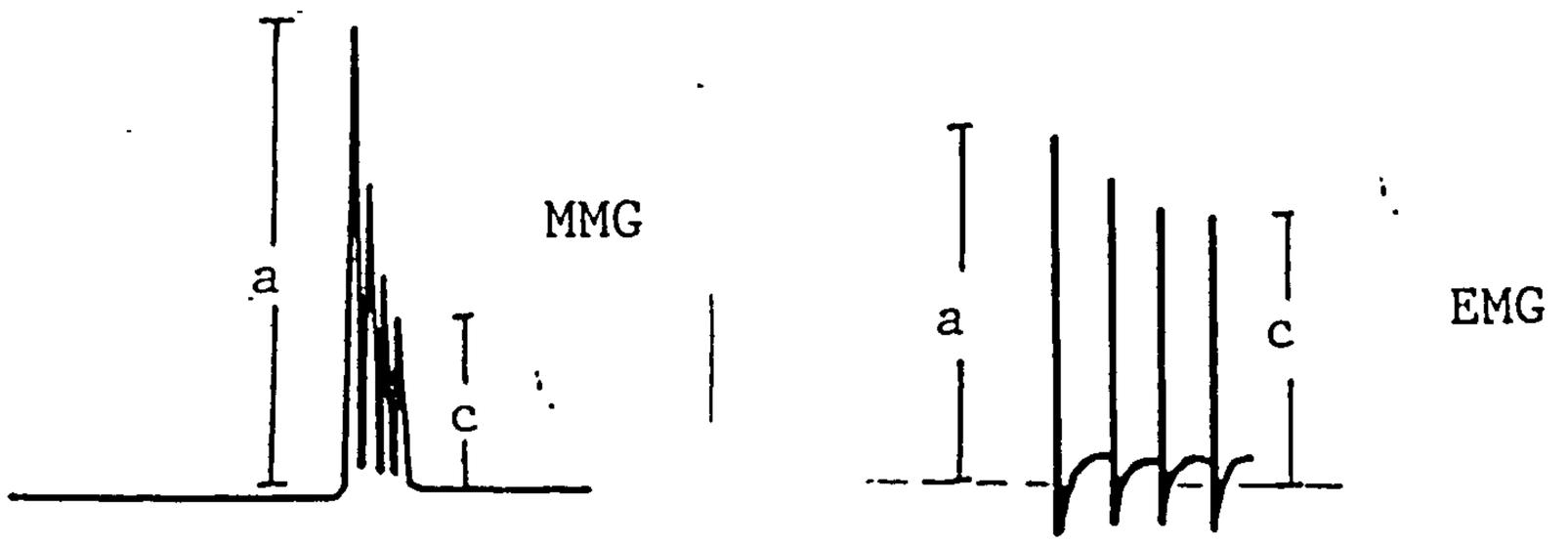


Fig. 9.6 Isolated forearm study: method of calculating TOF ratio for MMG and EMG. TOF is defined as c/a .

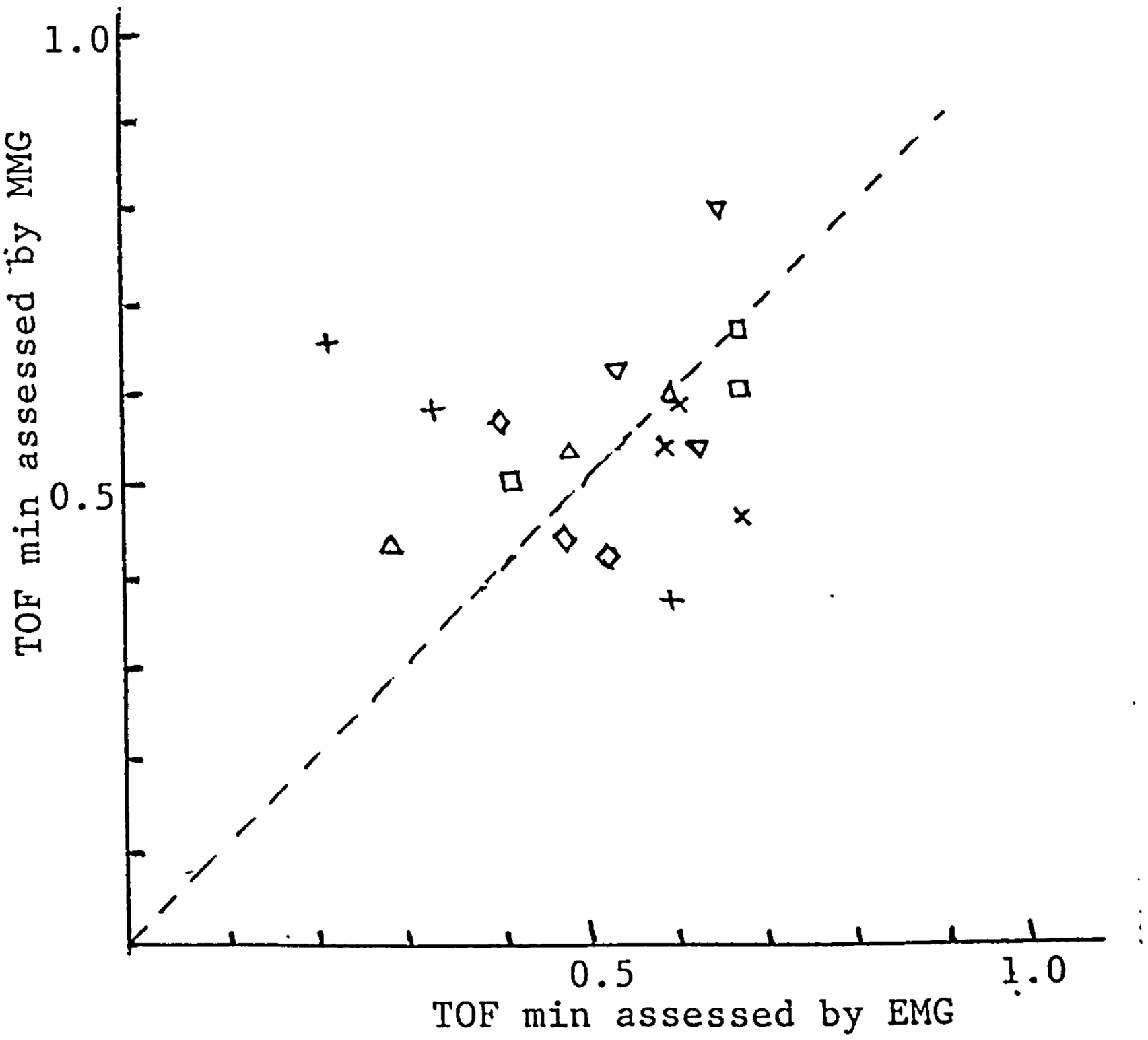


Fig. 9.7 Isolated forearm study: minimum values of TOF for the three relaxants in six subjects assessed by MMG and EMG. The dotted line shows a theoretical 1:1 relationship. There is no dominance of one method over the other.

Key: + 03/84; ◇ 17/84; × 47/84; △ 48/84; □ 56/84; ▽ 60/84.

Subject	Alcuronium		Vecuronium		dTC	
	MMG	EMG	MMG	EMG	MMG	EMG
03/84	0.38 20	0.61 25	0.66 24	0.23 24	0.59 24	0.35 20
17/84	0.45 4	0.49 5	0.43 15	0.54 10	0.59 5	0.42 6
47/84	0.55 8	0.62 12.5	0.47 4	0.72 5	0.60 5	0.62 6.5
48/84	0.55 6.5	0.50 4.5	0.62 8	0.61 9	0.44 13	0.30 9
56/84	0.62 4.5	0.69 6	0.68 8	0.67 9	0.51 5	0.43 4
60/84	0.55 15	0.65 6	0.63 11	0.56 8	0.81 5	0.67 5

Table 9.4 Correlation between MMG and EMG assessments of minimum TOF for three relaxants in the IFP.

For each subject/drug combination two values are shown. The upper is the lowest TOF value recorded and the lower the time taken to reach this value.

For all TOF values EMG/MMG r = 0.04 (no correlation

For all times to minimum TOF values

r = 0.86

variation of development and recovery of fade. One subject (03/84) showed a marked difference in the time taken to develop maximum paralysis and consequently longer recovery than the others. There is no obvious technical explanation for this discrepancy which was seen in all three drugs tested.

9.1.7 The relationship between MMG and EMG first response and fade

Figure 9.8 shows the TOF data from figure 9.1 replotted against first response. This presentation is seen to result in an hysteresis loop in every case. The direction of the curves, following the progression through onset and recovery of paralysis is indicated by the crosses at the end of the broken line. The data have been smoothed by taking running mean of eight data points. It was noted in section 9.1.5 that different subjects have widely differing responses to the relaxants used. In order to compare the first response/TOF fade loops normalization of the responses was necessary. This was provided by the following relationship:

$$f_{norm} = \frac{f - f_{min}}{1 - f_{min}}$$

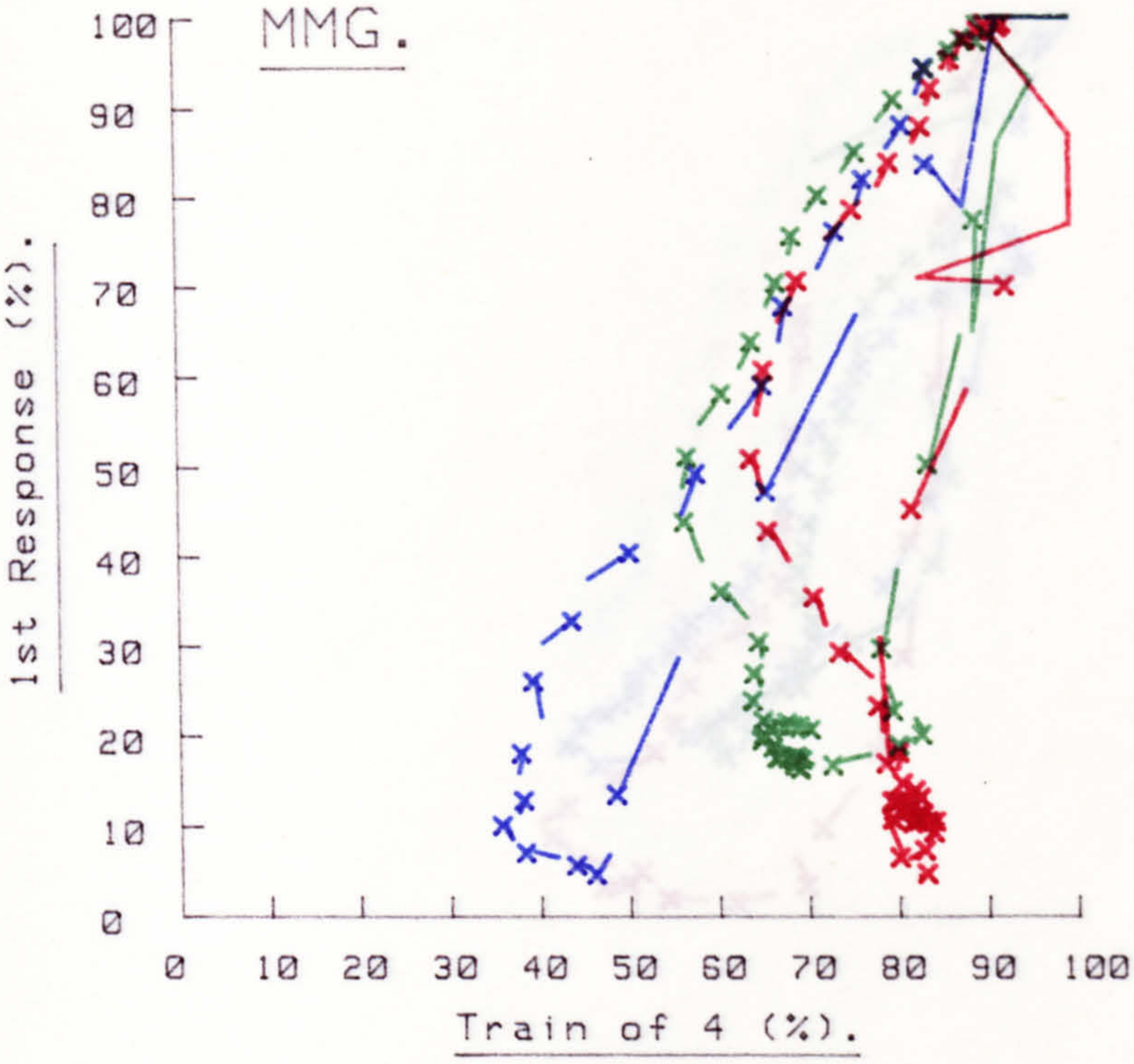
where f = TOF fade , or level of first response.

Figure 9.9 shows normalized hysteresis loops for a typical subject. The loops show that even after normalization there appears to be considerable individual variation in response. An attempt was made to establish possible characteristic loop patterns for the three relaxants before breaking the drug

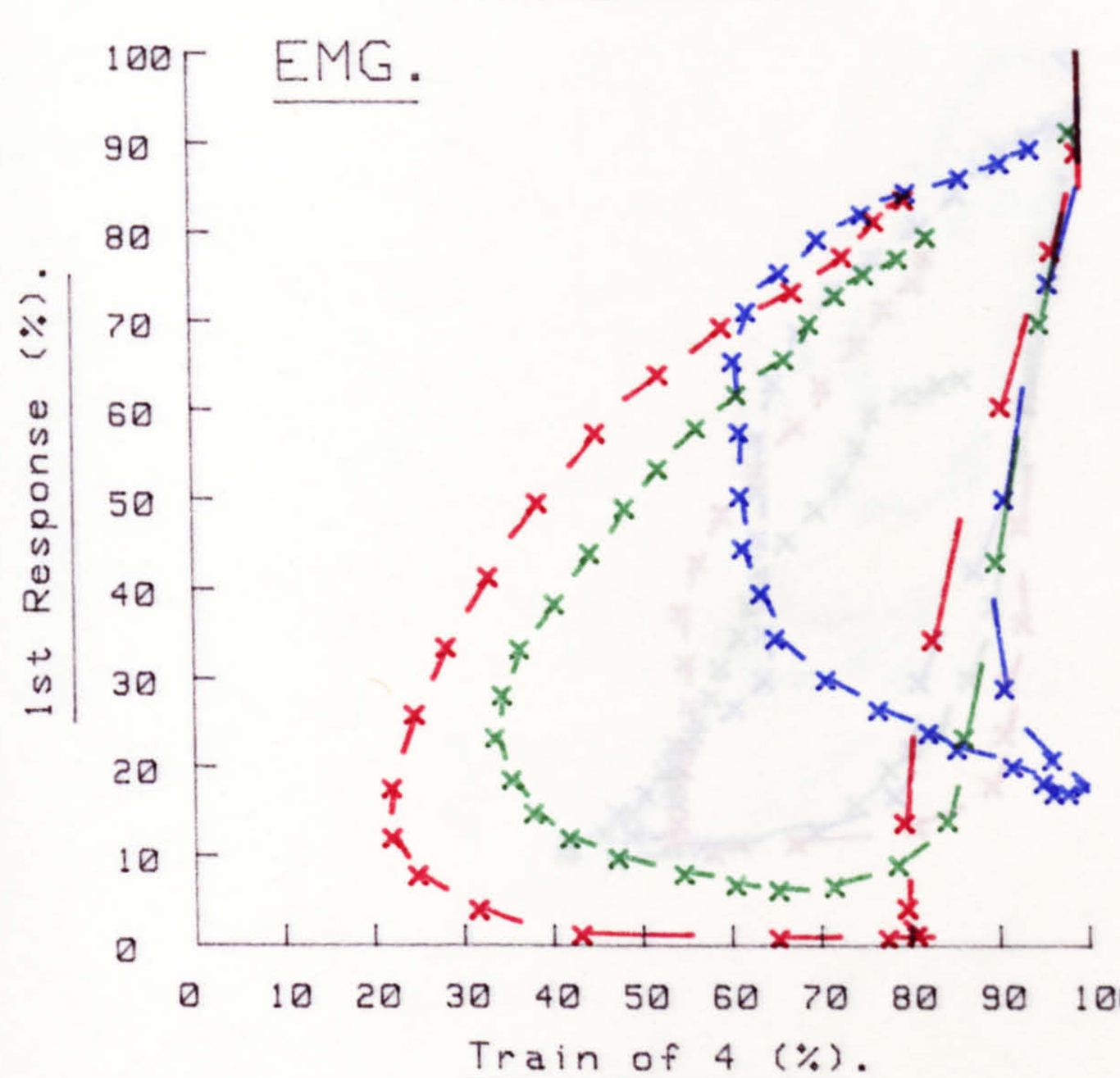
Fig. 9.8 (shown on the following six pages)
Isolated forearm study: data from fig. 9.1 have been replotted to show the relationship of degree of muscle relaxation (indicated by the first response of the TOF) against TOF fade. Data for three relaxants are shown by the appropriate colour code as in fig. 9.1. The position of the cross at the end of the line indicates the direction of the experiment from induction to recovery. Data points are running means of eight recorded experimental points.
In each case the relationship of relaxation to fade during induction is different from that during recovery, giving rise to a loop - shaped curve (see text).

Protocol S03/1A. Subject No 3 / 84

MMG.

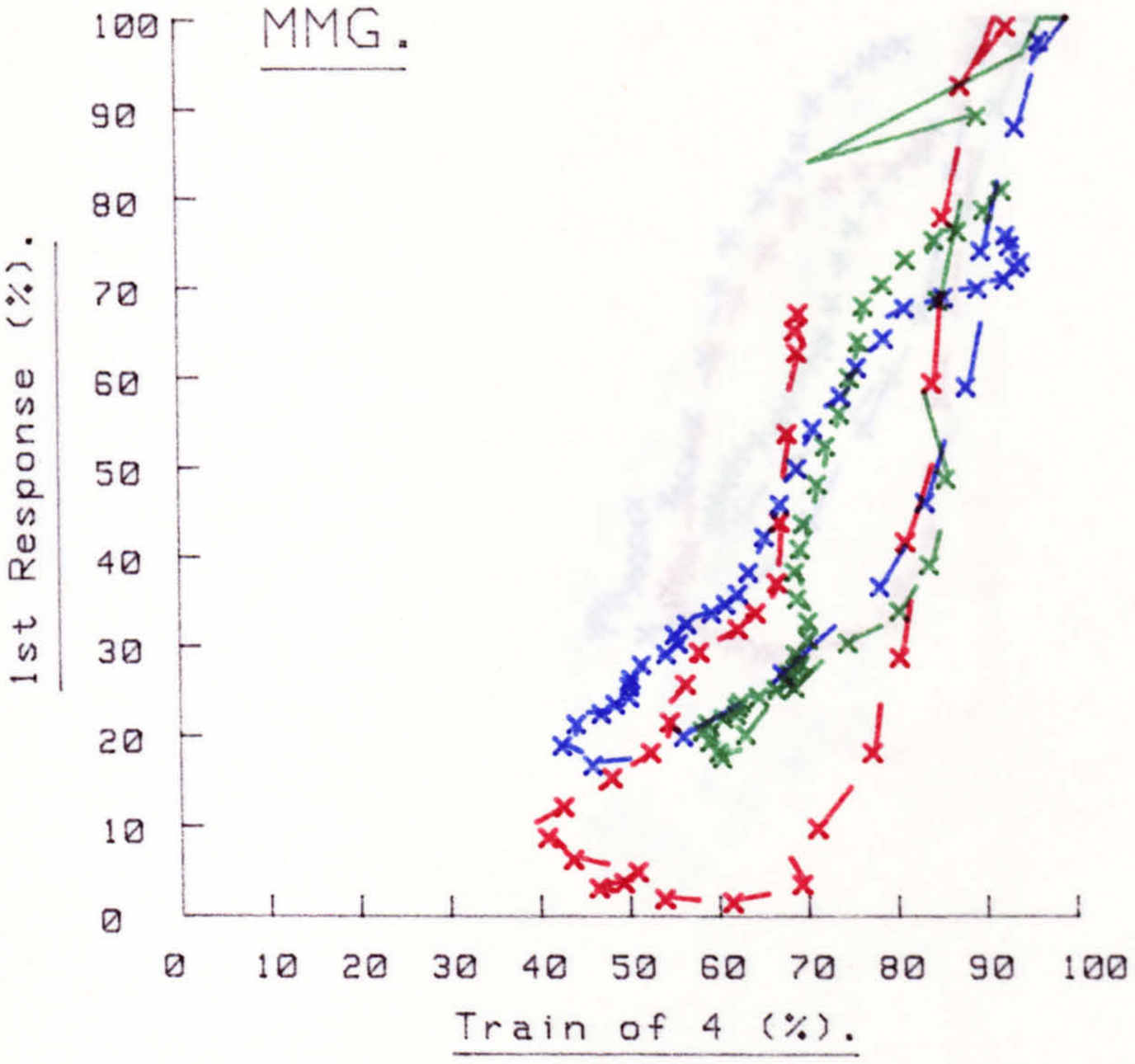


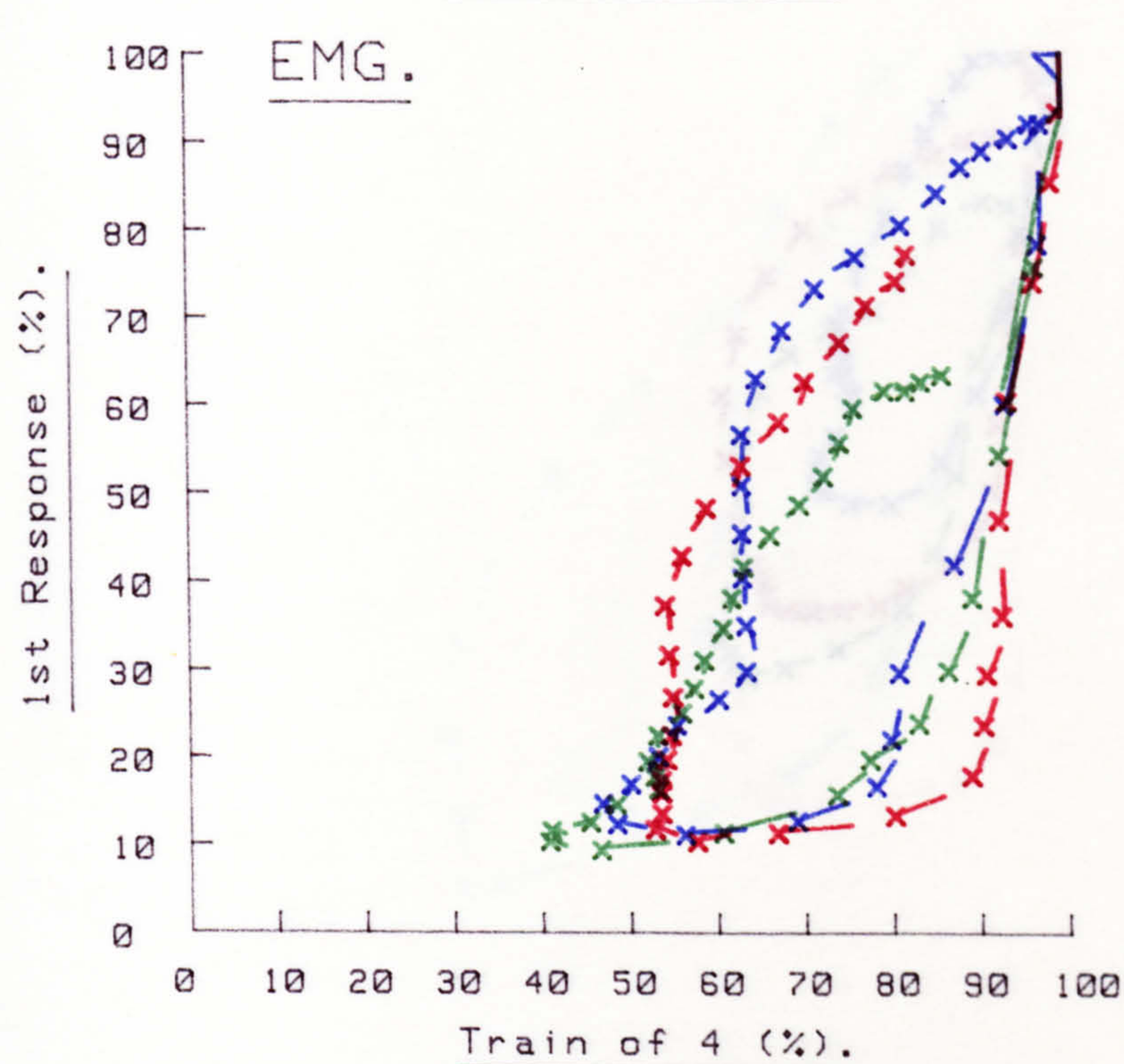
EMG.



— Vecuronium. — Alcuronium. — d-Tubocurarine.

Protocol S03/1A. Subject No 17 / 84





— Vecuronium. — Alcuronium. — d-Tubocurarine.

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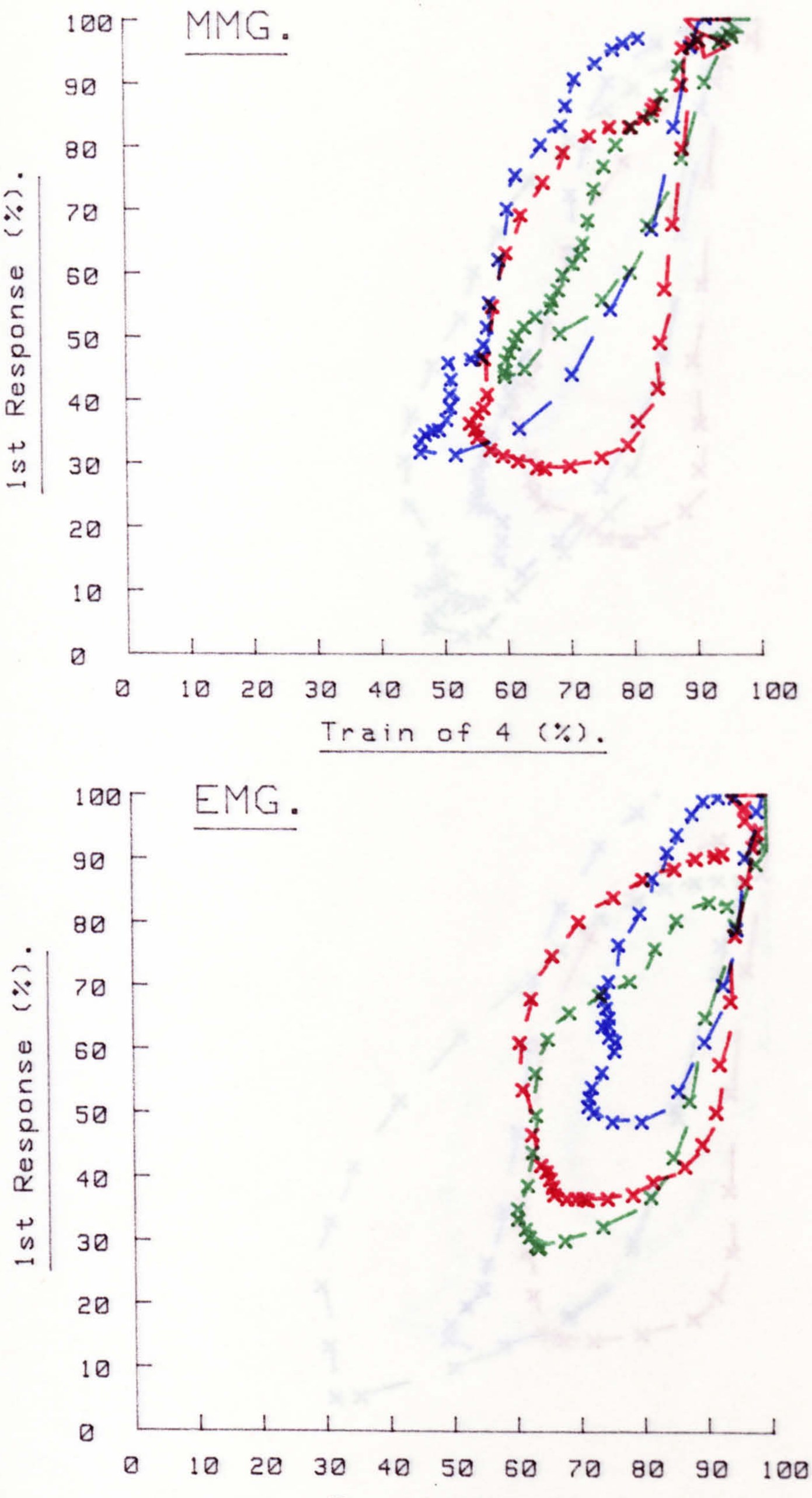

— Vecuronium. — Alcuronium. — d-Tubocurarine.

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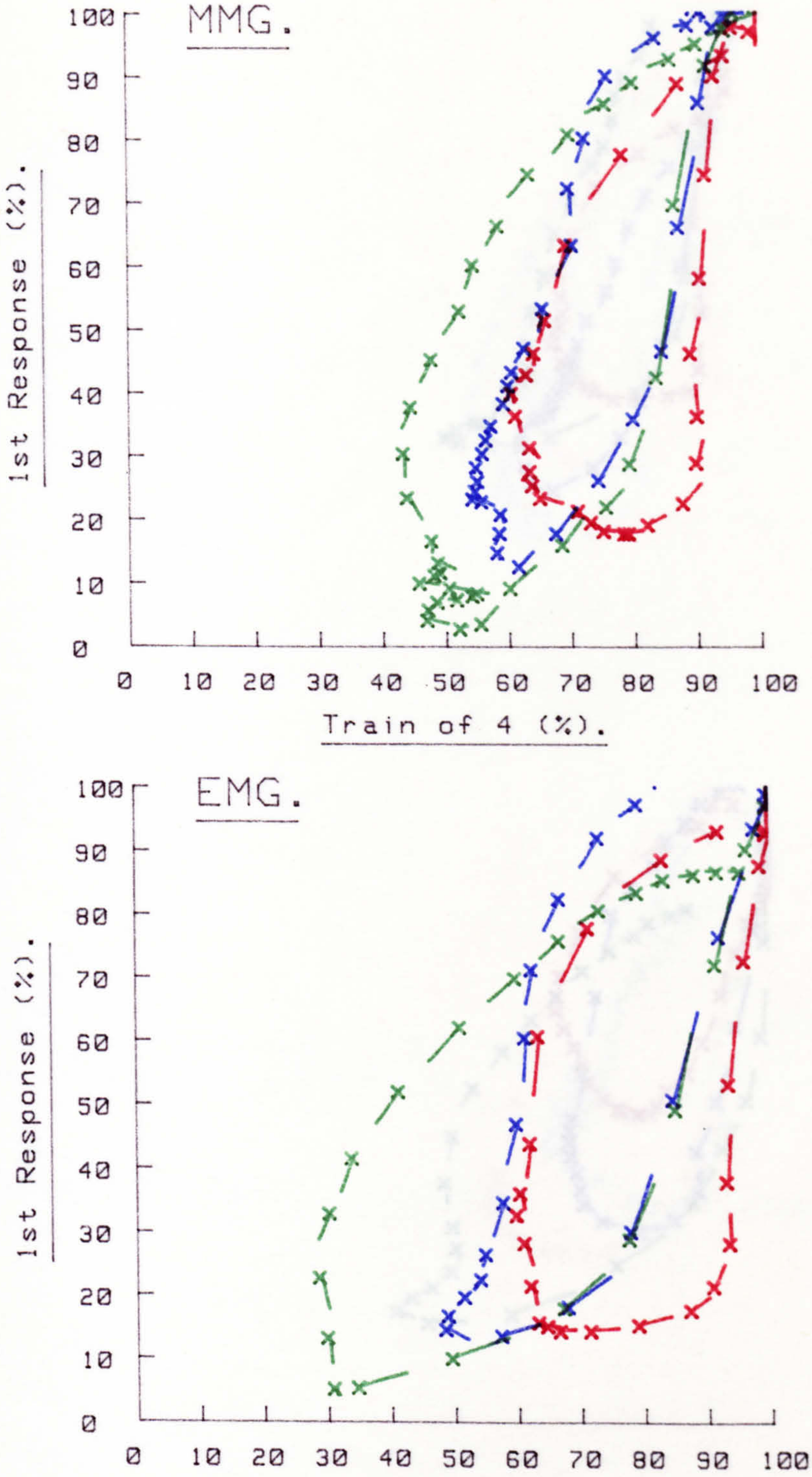
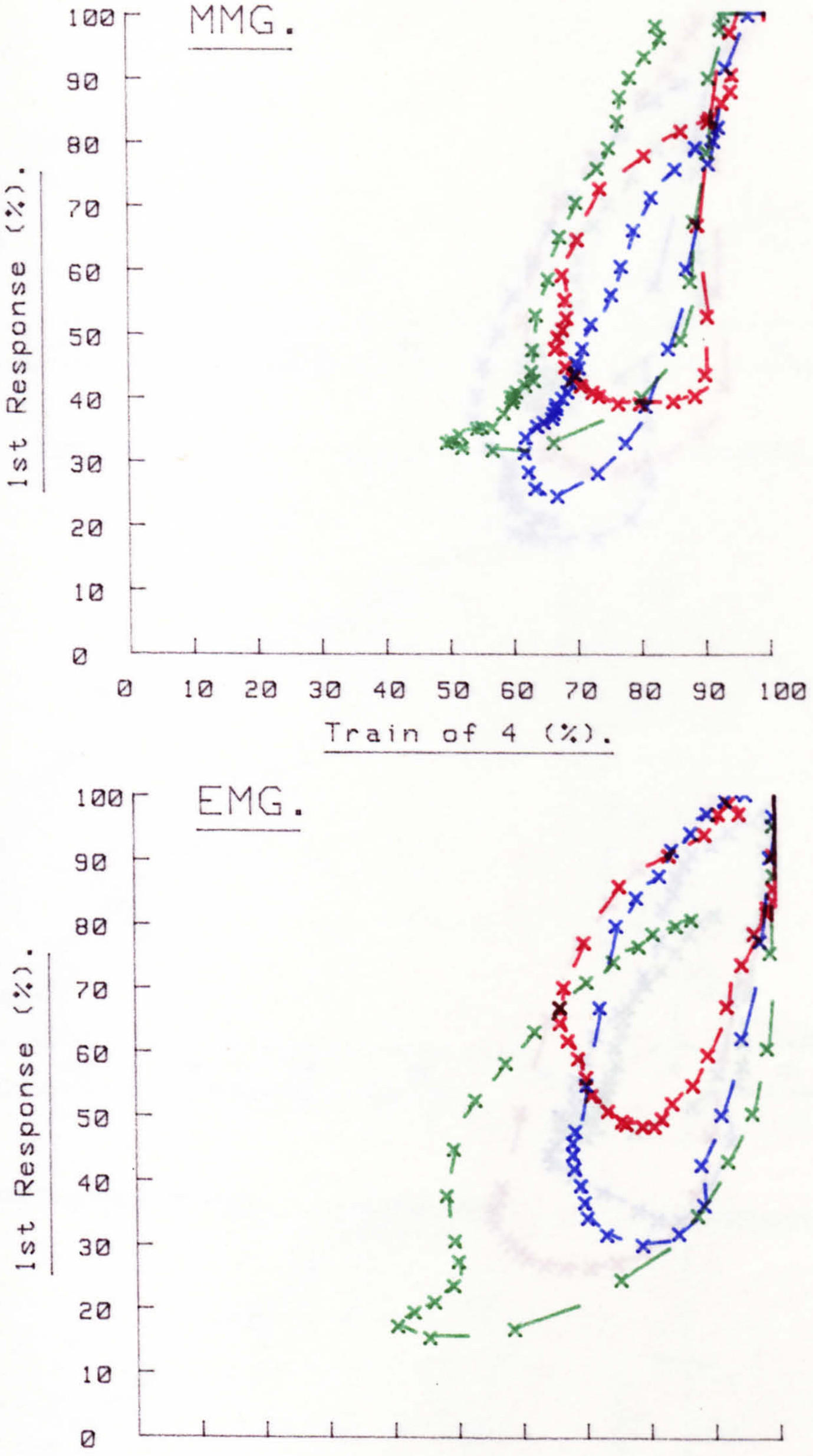


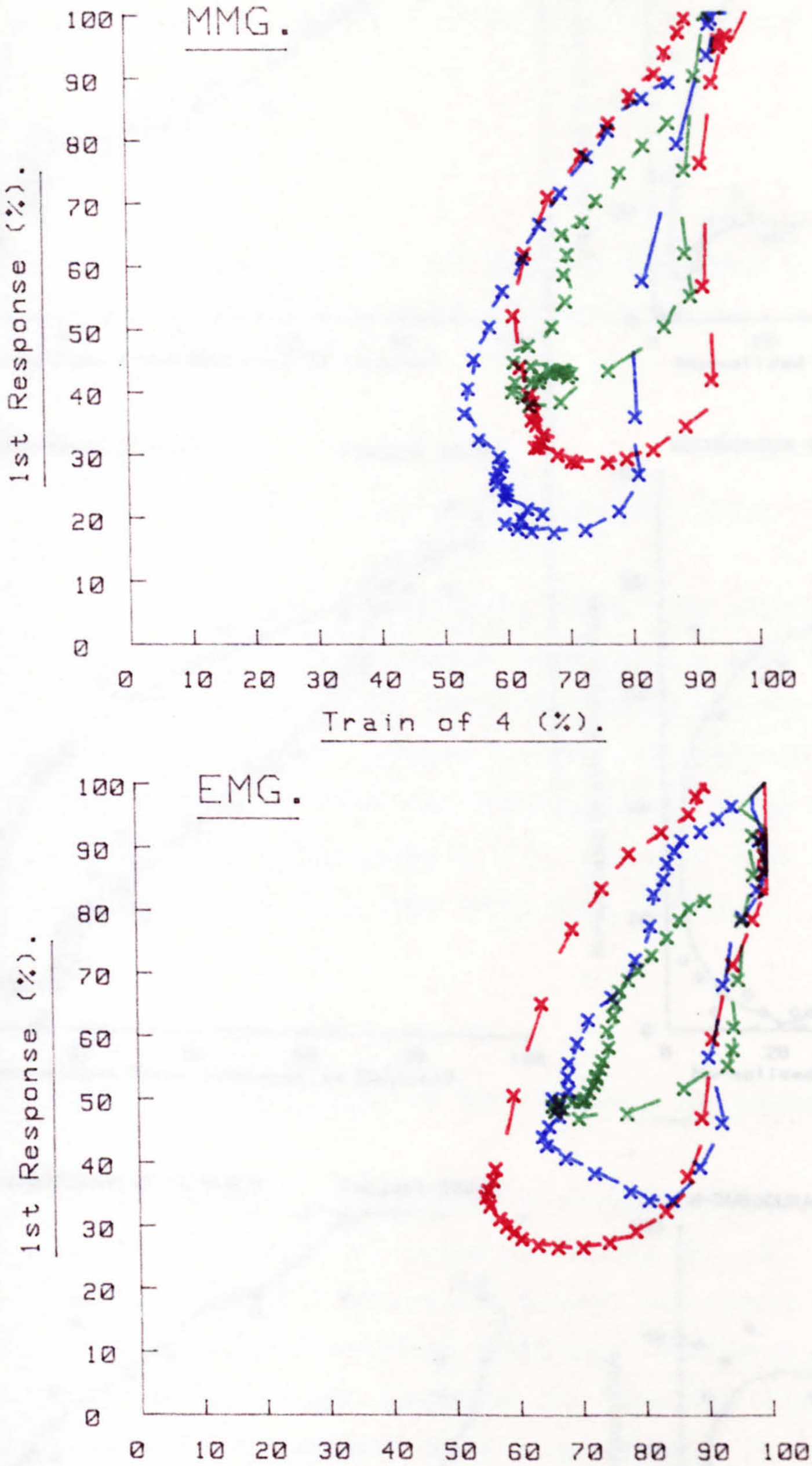
— Vecuronium.— Alcuronium.— d-Tubocurarine.

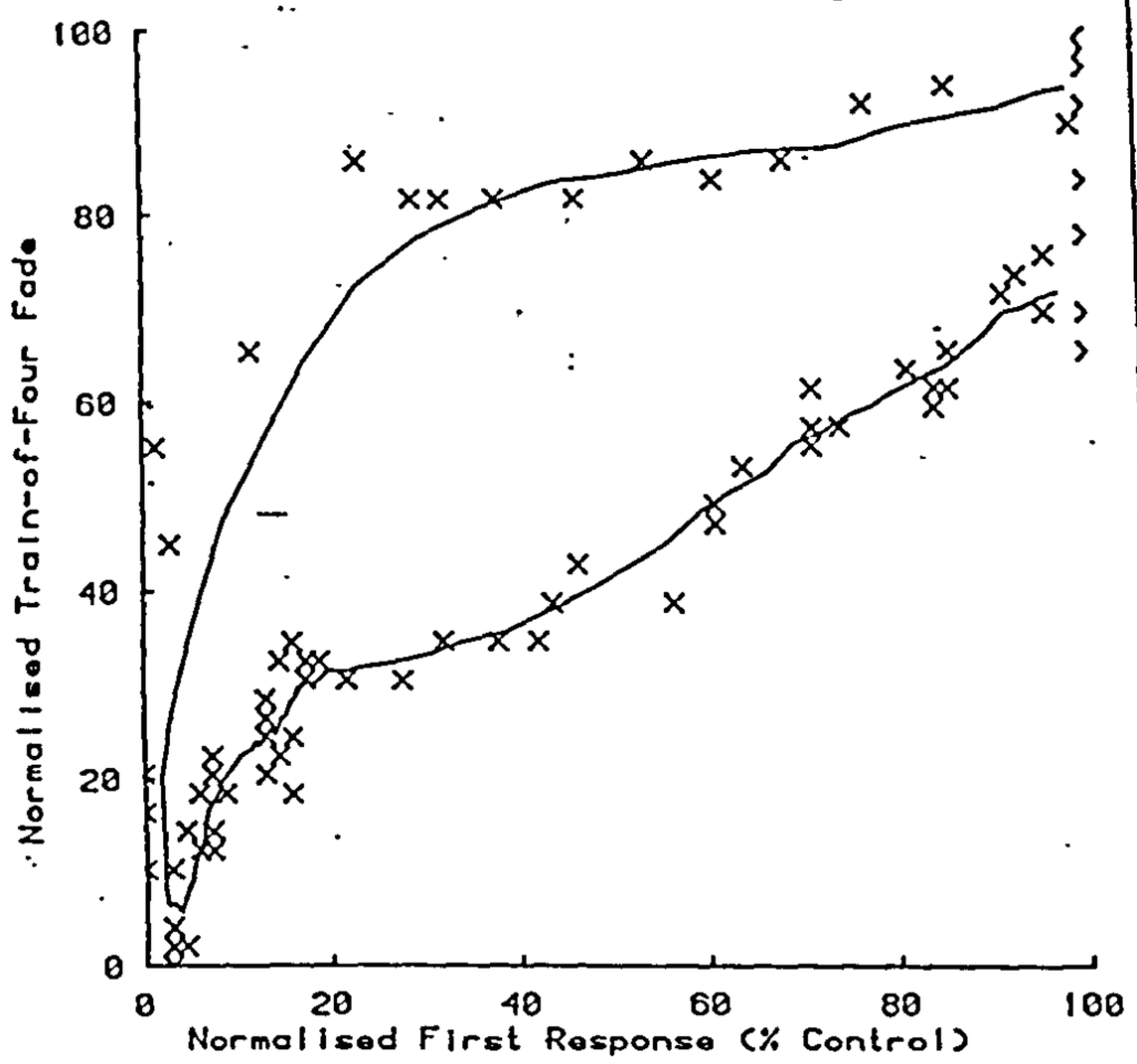
Protocol S03/1A. Subject No 56 / 84

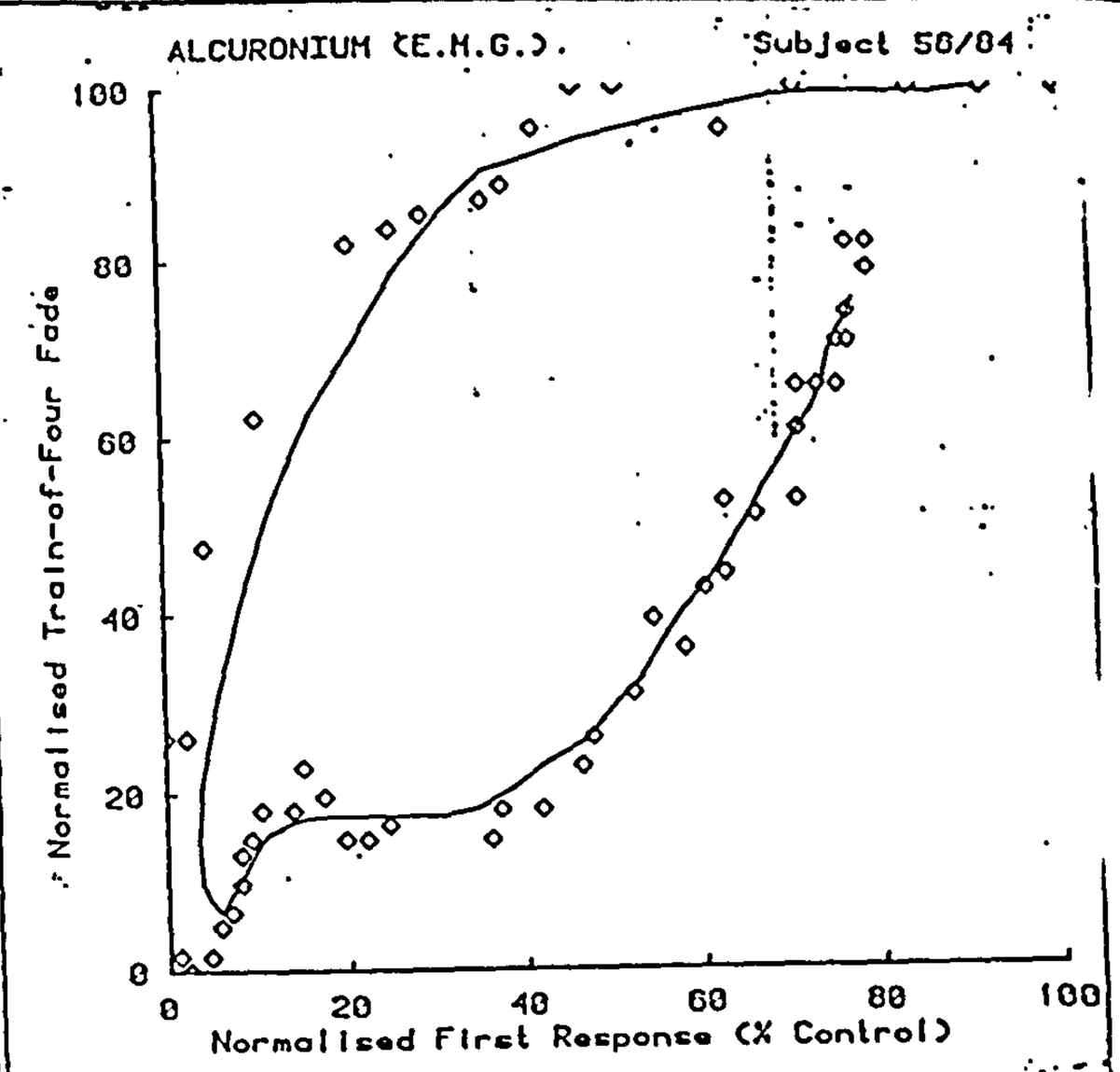


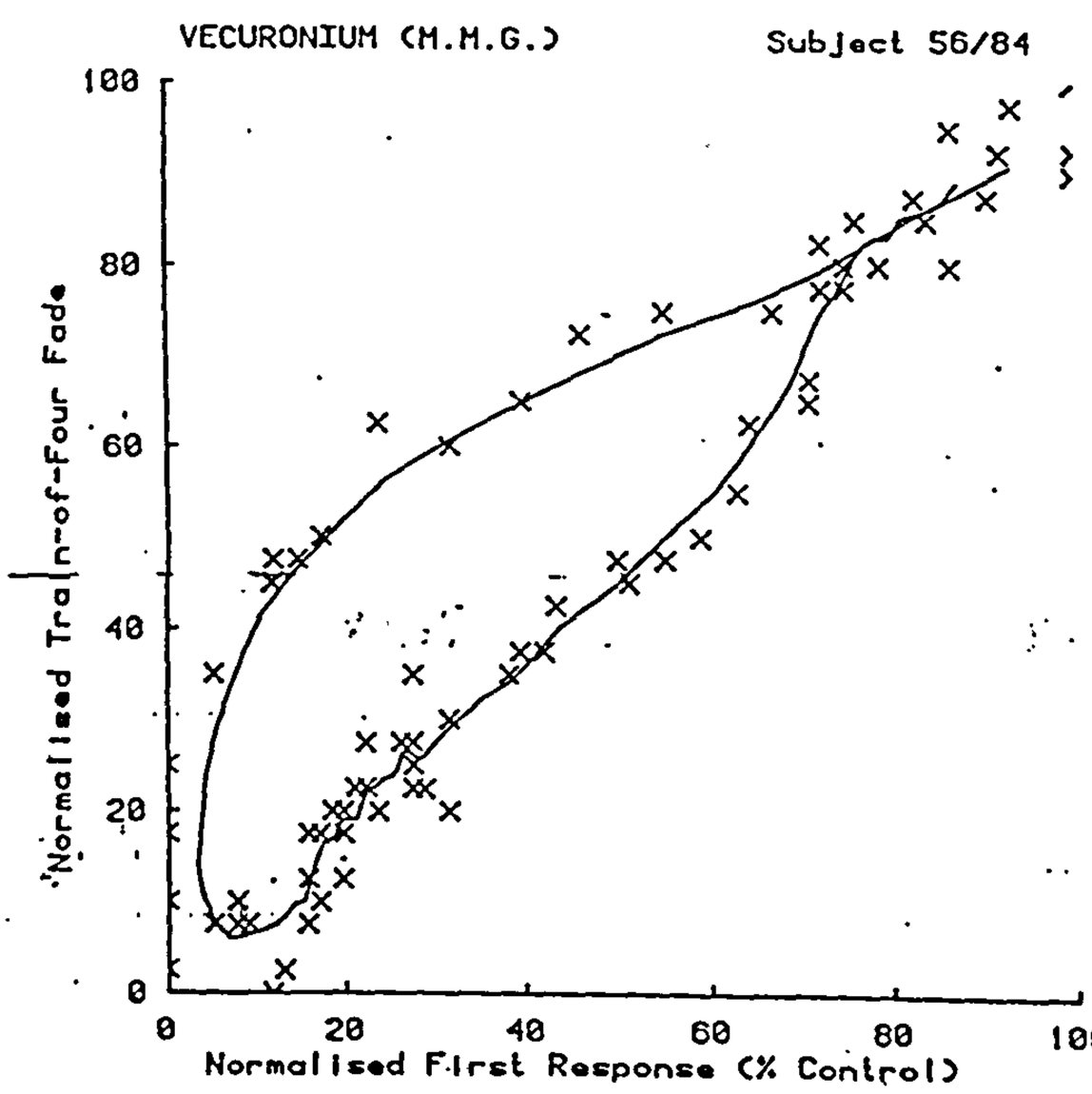
— Vecuronium. — Alcuronium. — d-Tubocurarine.

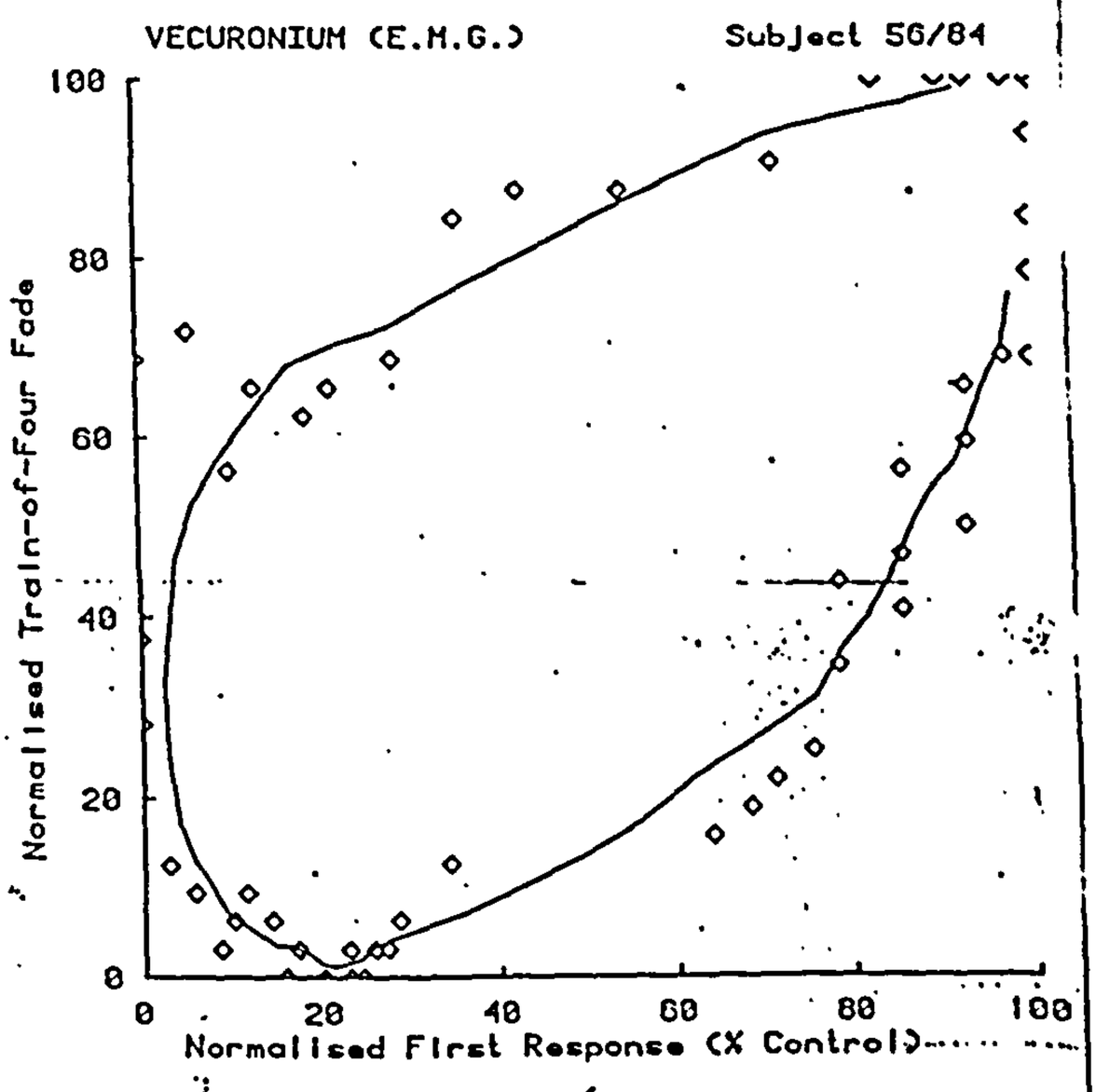
Protocol S03/1A. Subject No 60 / 84

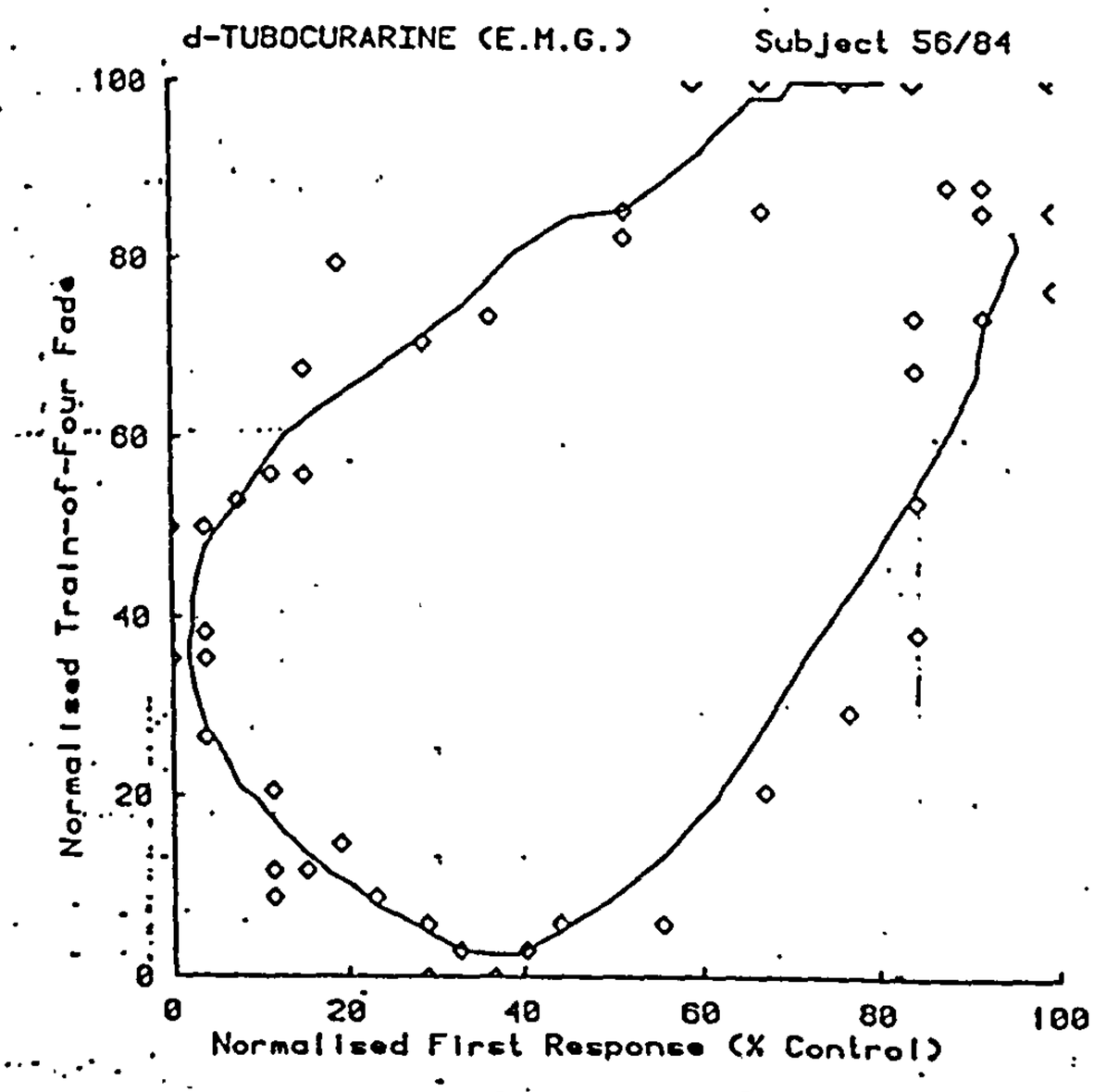












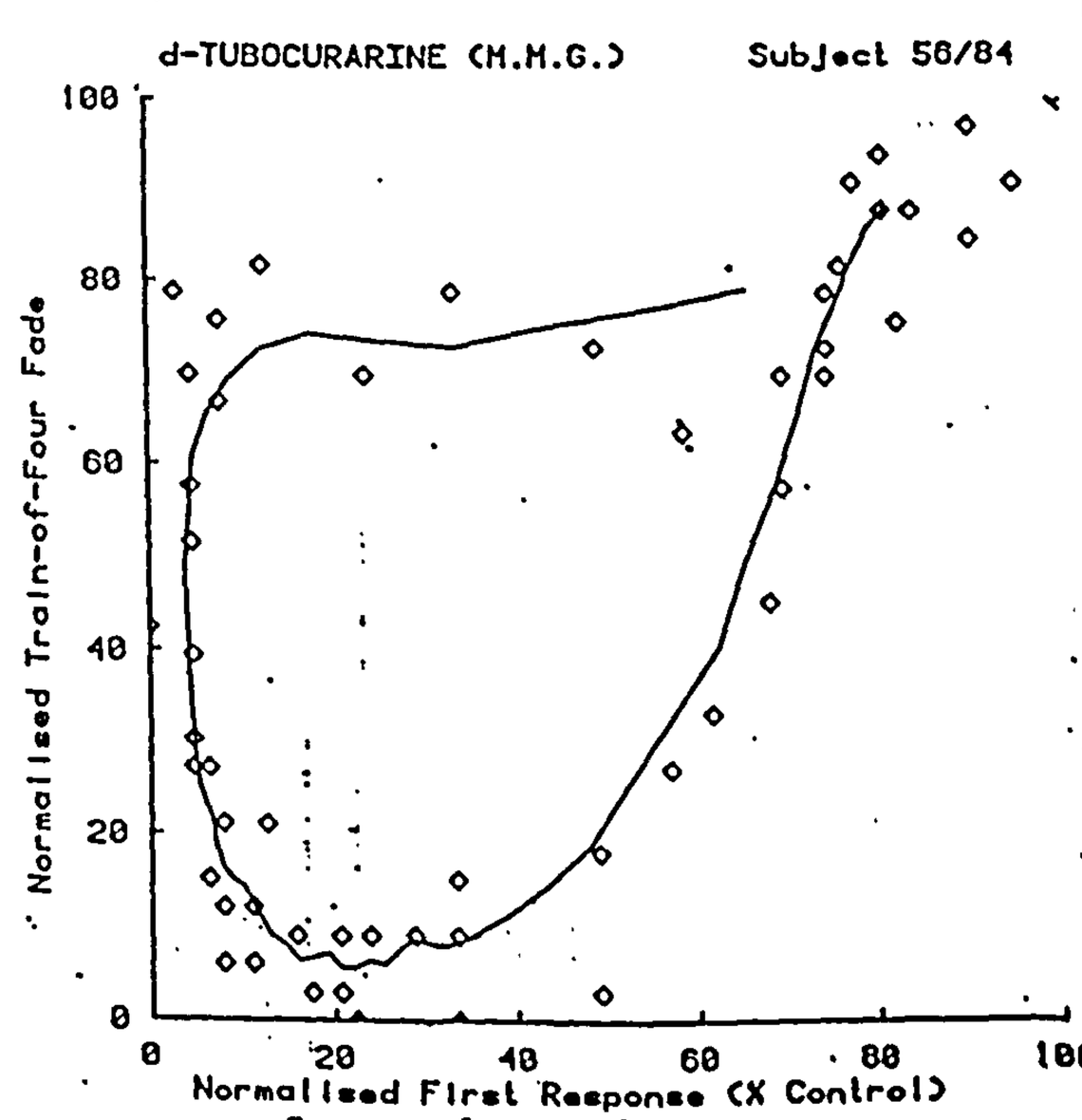


Fig. 9.9 Normalized first response plotted against TOF fade for one subject from fig. 9.1

allocation code. On a simple scoring basis however, estimating loop area, two observers were unable to identify any drug - related loop shape.

9.2 The effect of pyridostigmine pretreatment on the action of alcuronium

9.2.1 Presentation of data

The data from the study of the effect of pyridostigmine pretreatment on the action of alcuronium in the isolated forearm are presented graphically in figure 9.10. As before, first response and TOF data are plotted against time between the same axes. Data are shown for each of the three sessions recorded in the ten subjects studied.

9.2.2 Analysis of the effect on paralysis

The effects of pyridostigmine pretreatment were examined by consideration of (a) the peak paralysis (b) the time taken to reach peak paralysis and (c) the recovery period. Table 9.5 shows these values for each session in the ten experimental subjects together with the change in the parameter between sessions 1 and 2 and 1 and 3 on a paired basis with individual control values. In this analysis recovery was measured from the minimum recorded value of the first response to 90% of control. These results are shown diagrammatically in figure 9.11. Inspection of the values shows the following points:

Fig. 9.10 Isolated forearm study: effect of pyridostigmine. Data plots (shown on the following ten pages) from alcuronium IFP in ten subjects treated with pyridostigmine. Subject 84/84 had a further control experiment (84-91/84) before the steady state recording. The onset and recovery of muscle relaxation (indicated by the degree of first response of the TOF) and fade during TOF for MMG and EMG recording are shown as colour - coded lines (see text).

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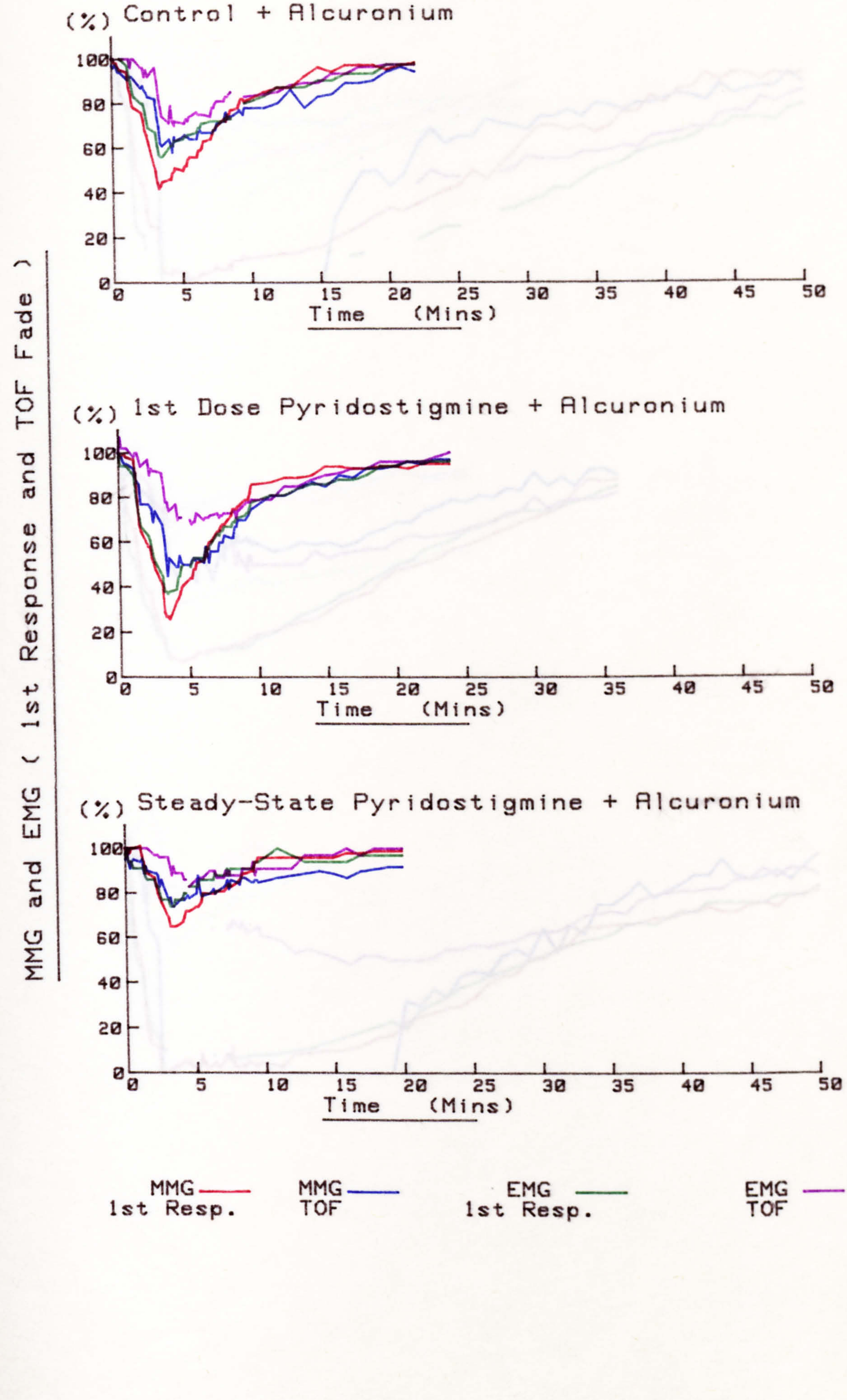

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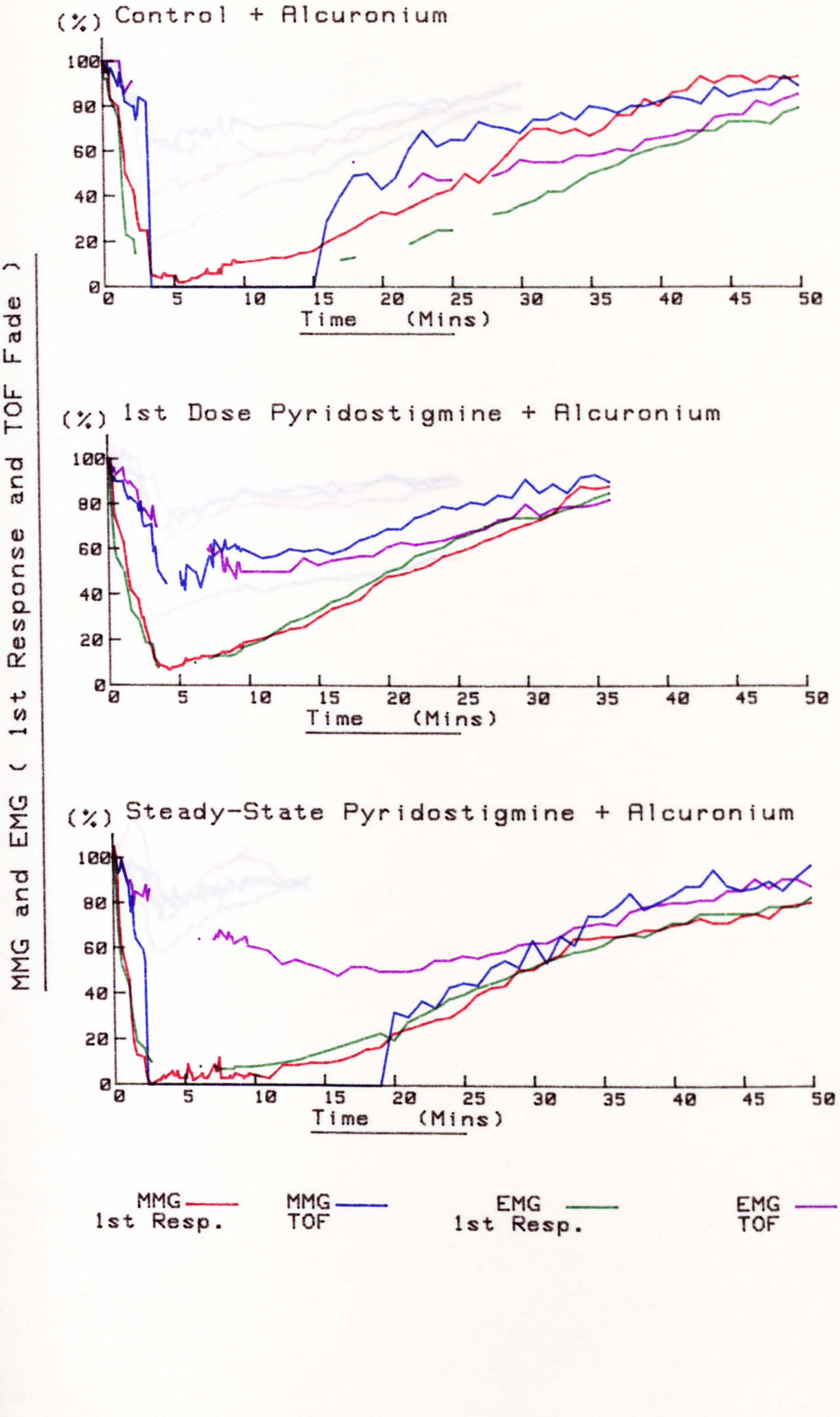

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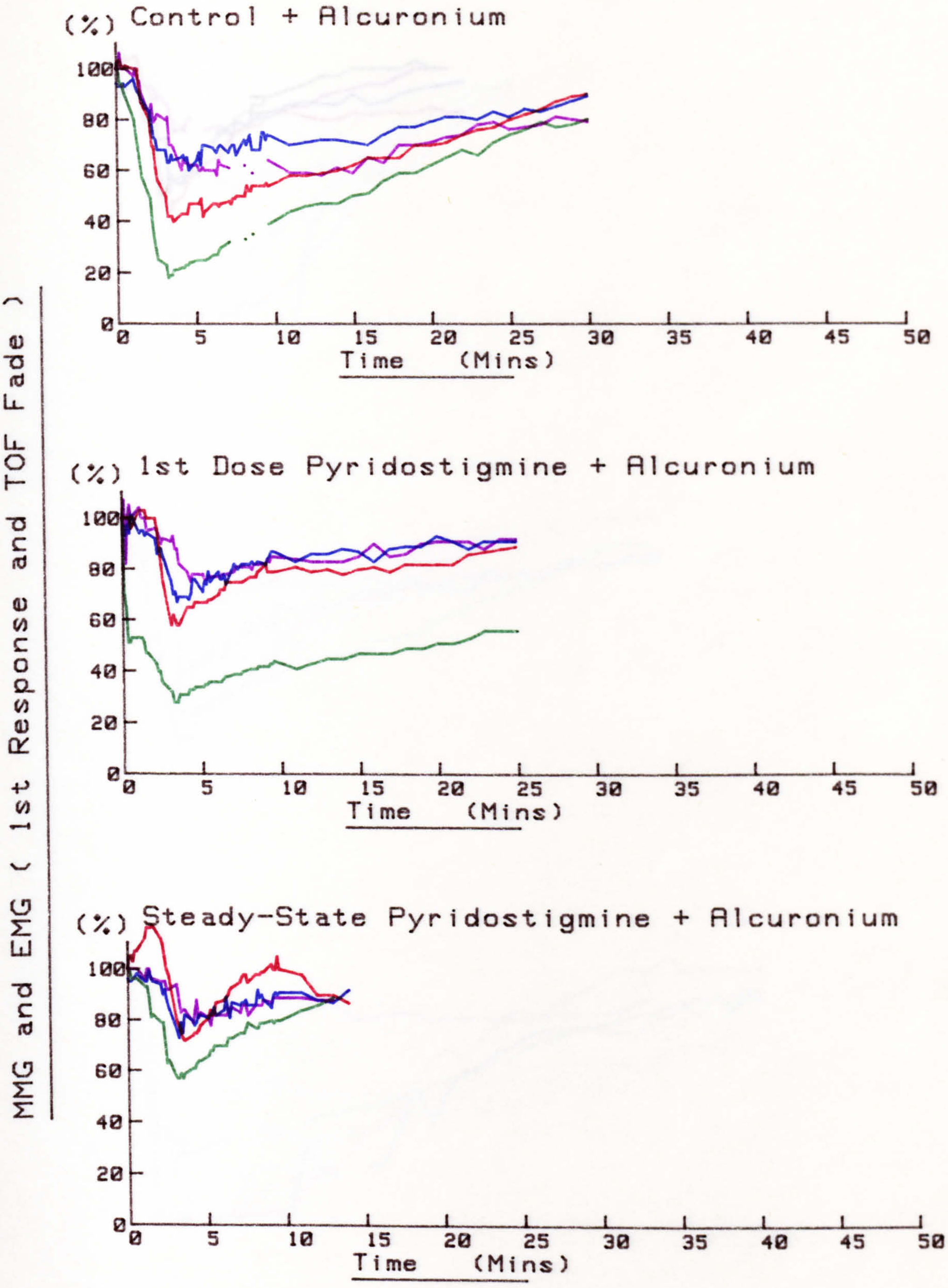

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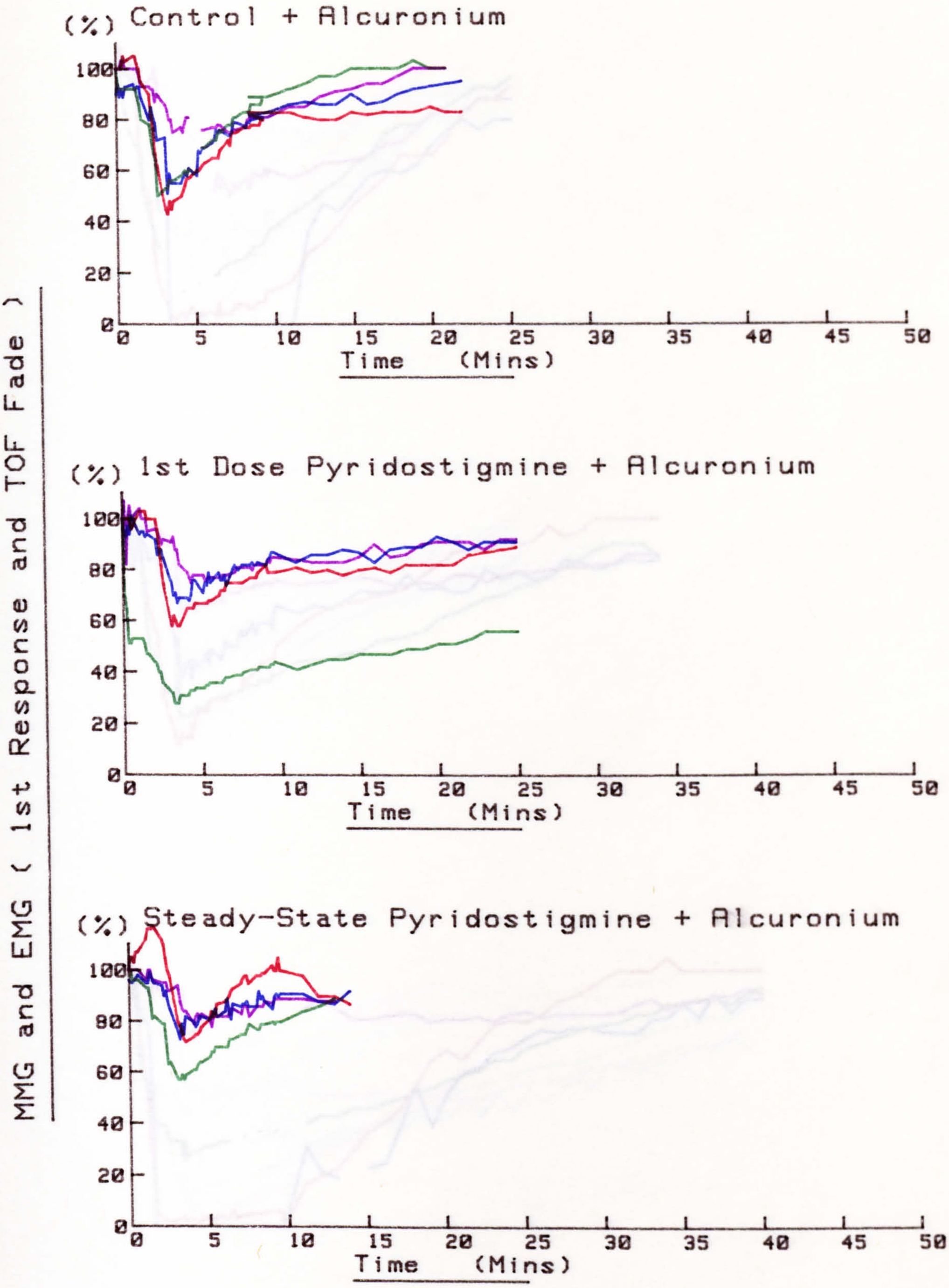

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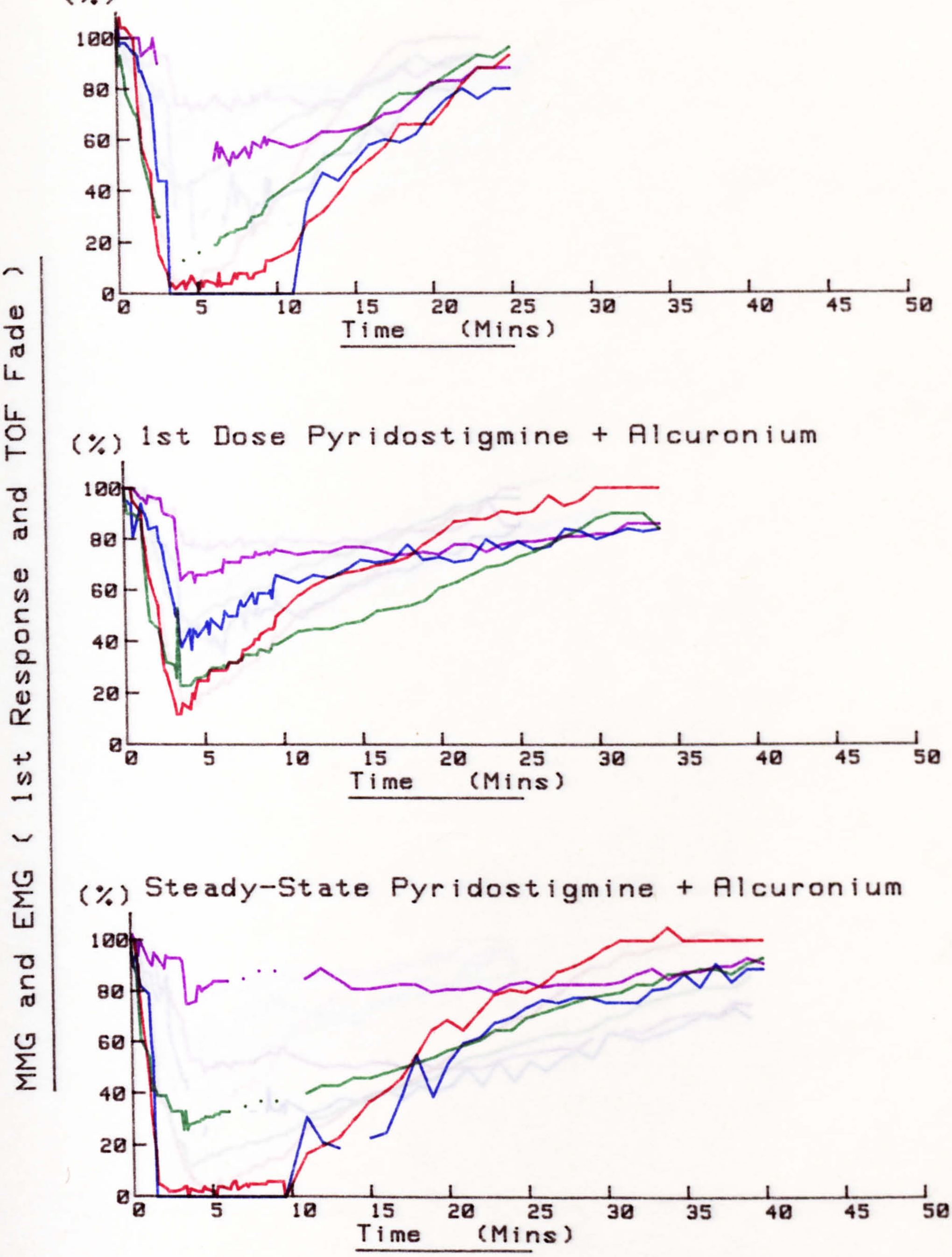

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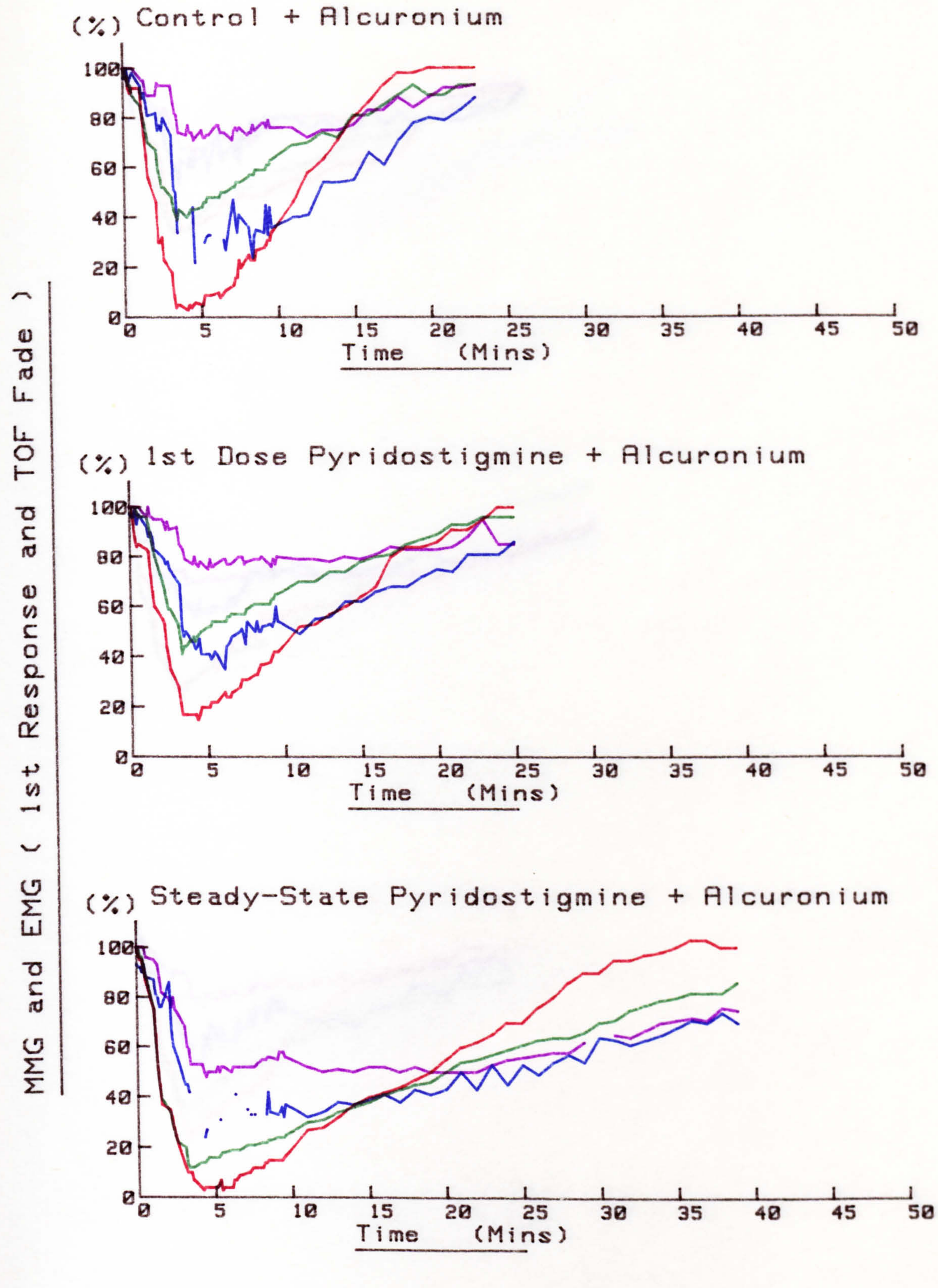

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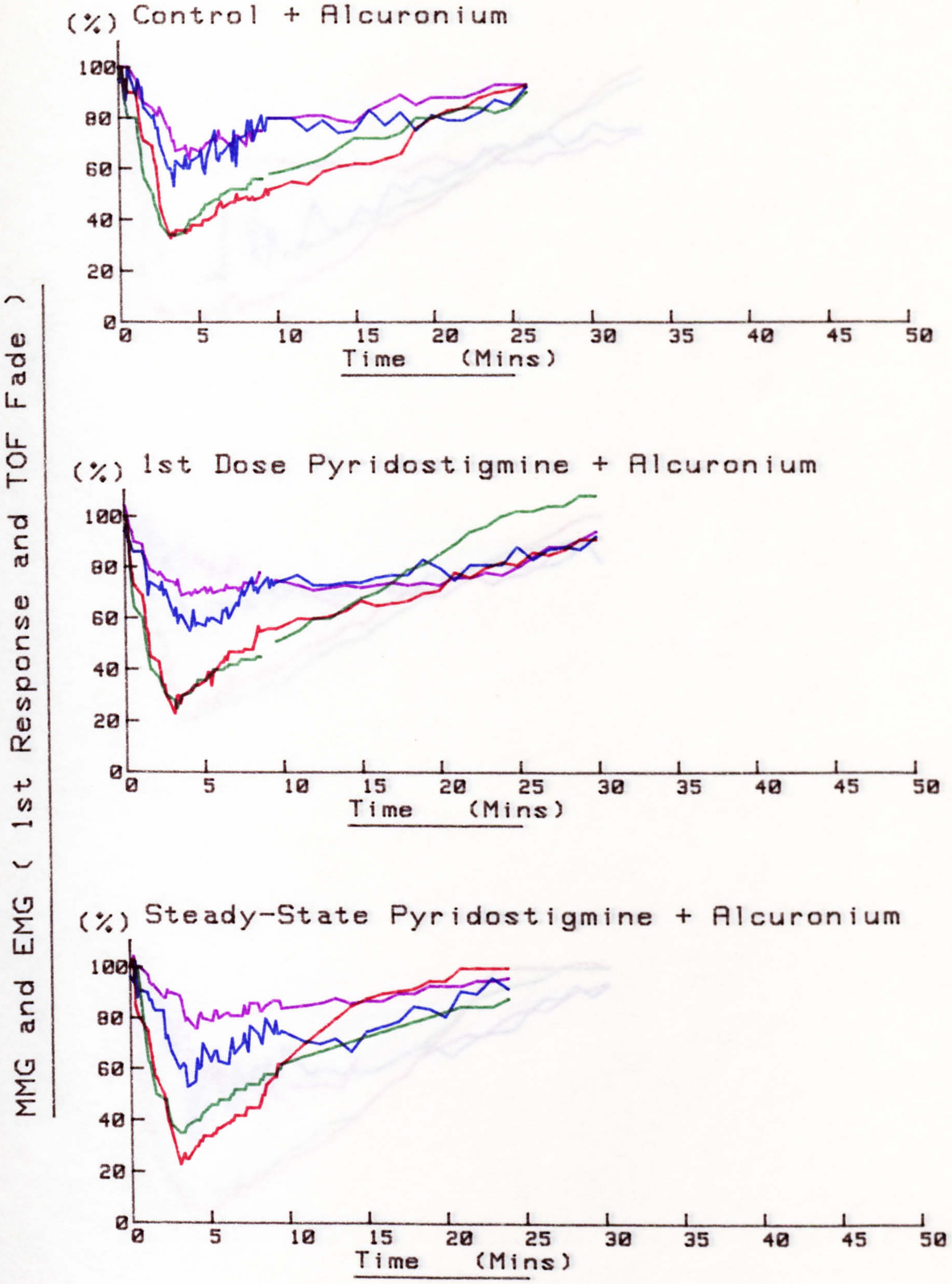

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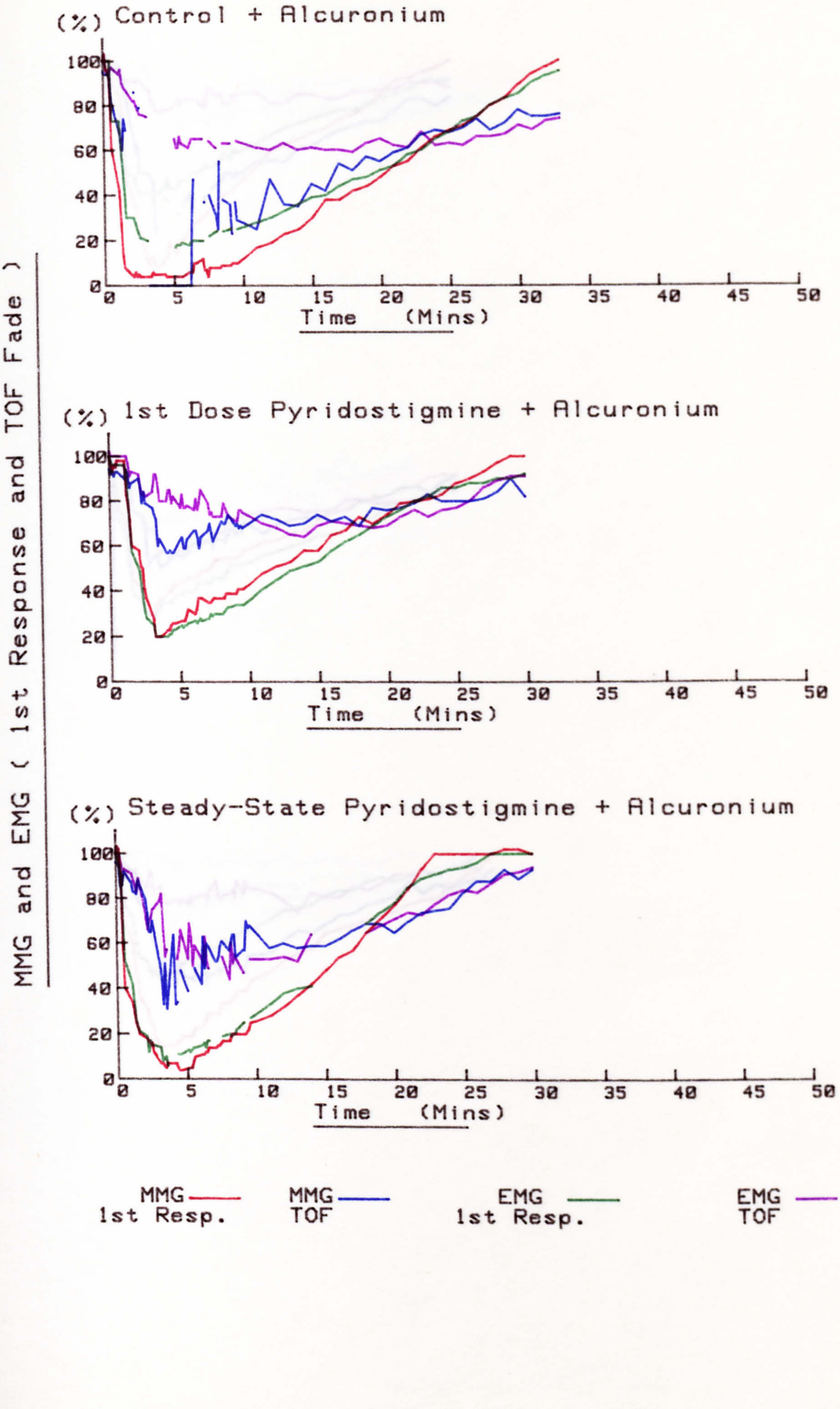

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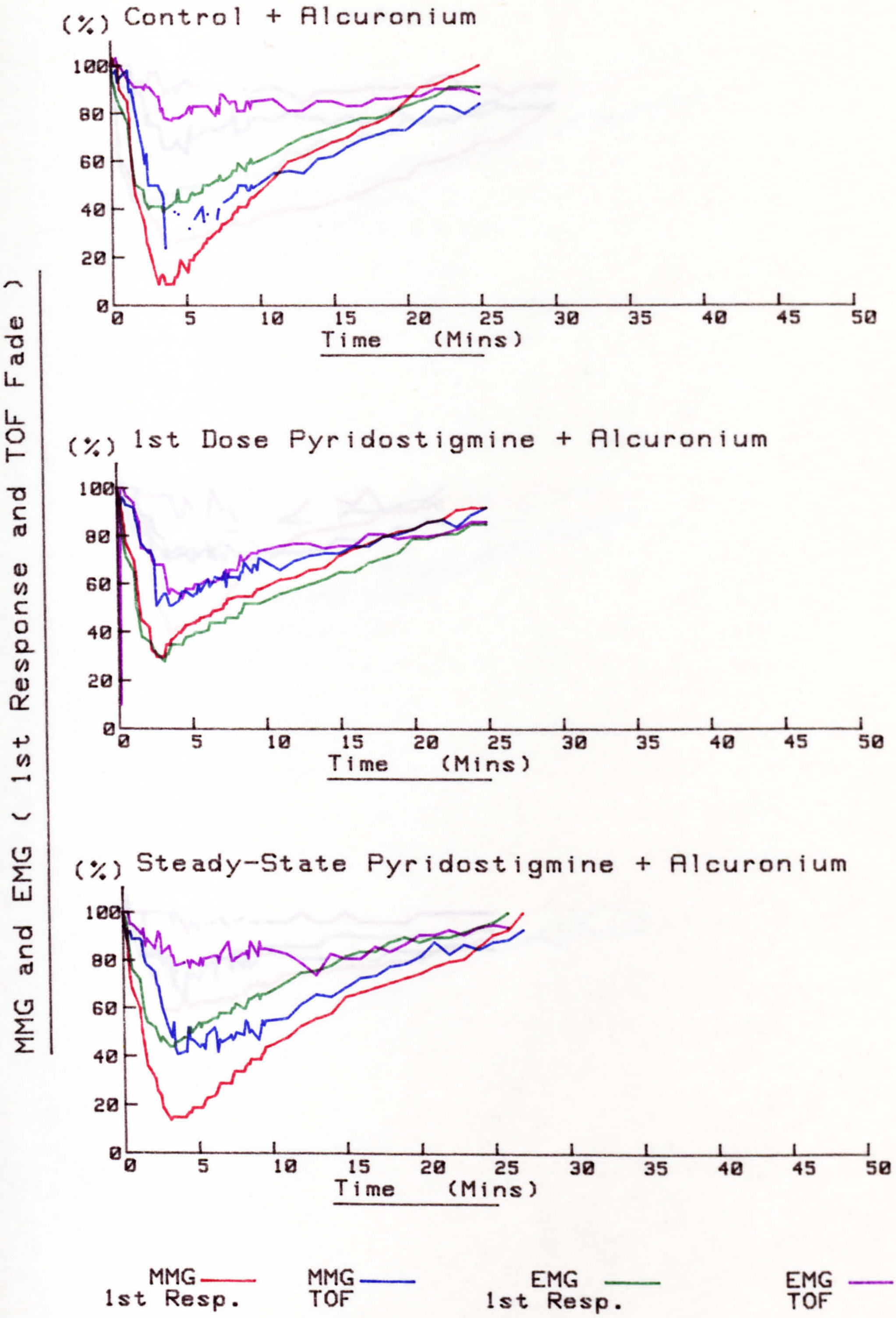

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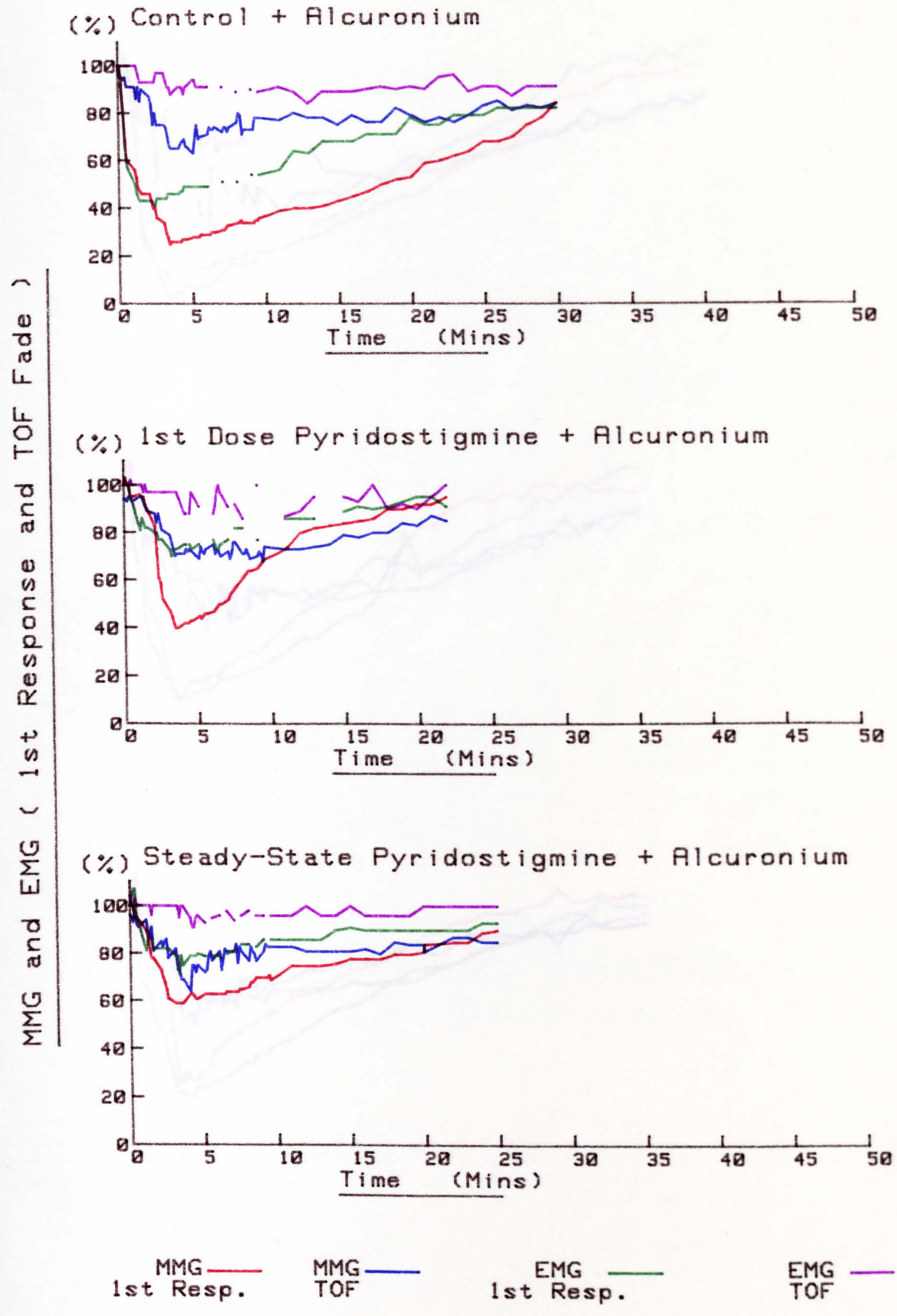

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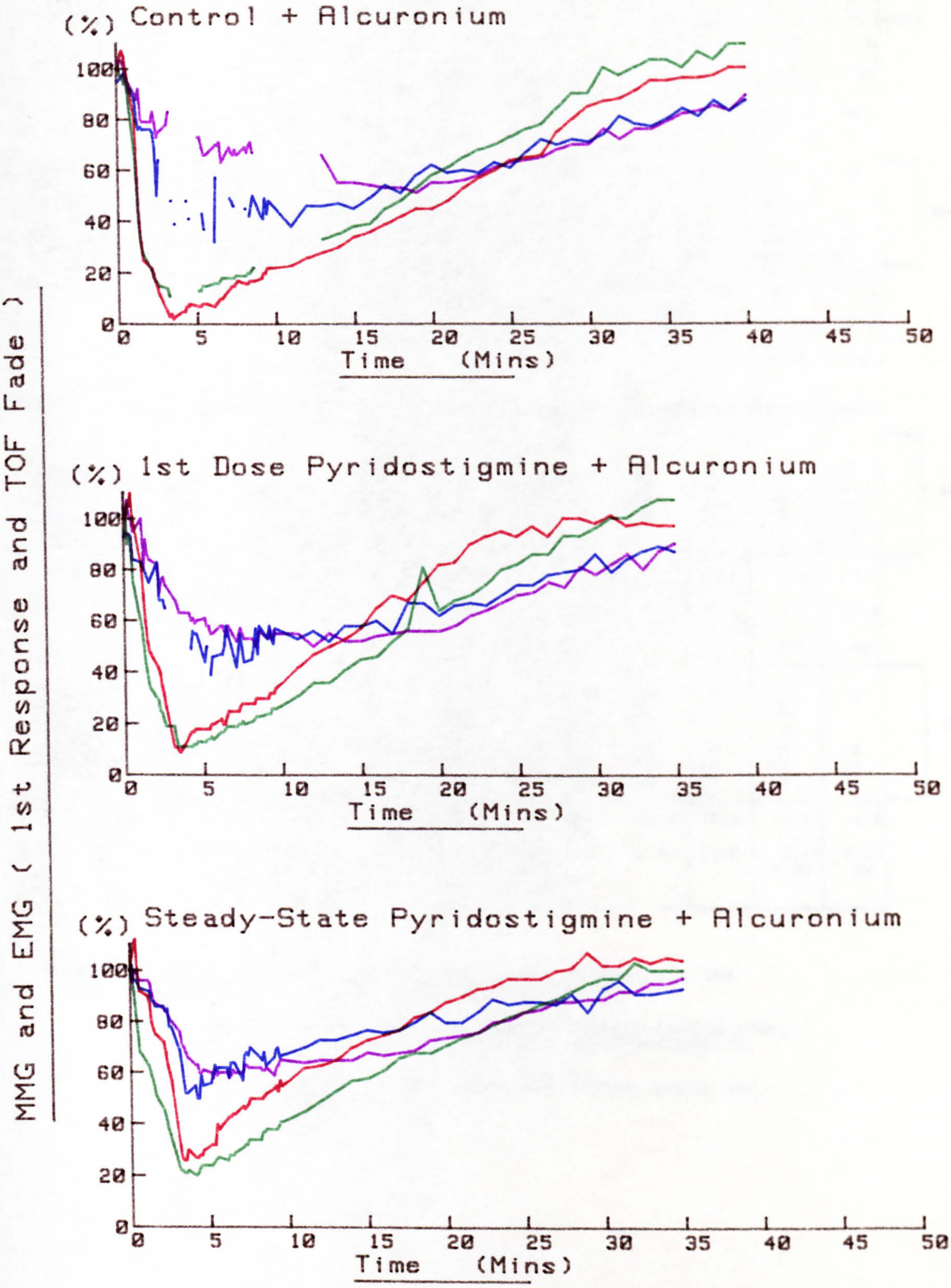


	Time(min)to max paralysis					Minimum first response (%)					Time(min) to 90% recovery					
Subject	C	I1	I2	1-C	2-C	C	I1	I2	1-C	2-C	C	I1	I2	1-C	2-C	
069/84	3.57	3.97	3.37	-0.4	-0.2	42	26	65	-16	23	14	14	9	0	-5	MMG active
085/84	3.97	3.37	2.37	-0.6	-1.4	2	12	2	10	0	24	25	28	1	4	
086/84	4.17	3.57	4.37	-0.6	0.2	3	17	3	14	0	17	21	29	4	12	
087/84	3.37	3.17	3.17	-0.2	-0.2	33	23	23	-10	-10	24	29	16	5	-8	
092/84	3.17	2.97	3.17	-0.2	0	9	30	14	21	5	21	23	25	2	4	
Mean	3.65	3.41	3.29	-0.24	-0.32	17.8	17.8	21.4	3.8	3.6	20	22.4	21.4	2.4	1.4	
SD	0.41	0.38	0.72	0.41	0.63	18.5	5.1	29.9	16	12.1	4.42	5.56	8.62	2.07	7.99	
P				ns	ns				ns	ns				<.1	ns	
083/84	5.57	4.32	2.97	-1.2	-2.6	2	7	0	-5	2	44	36	50	-8	6	MMG placebo
084/84	3.97	3.77		-0.2		40	58		18		30	25		-5		
091/84	3.37		3.97		-3.6	43		72		29	22	6.34		-18.5		
088/84	2.17	3.37	4.77	-1.2	-2.6	4	20	4	16	0	30	26	22	-4	-8	
093/84	3.77	3.77	3.17	0	-0.6	25	40	59	15	34	30	18	25	-12	-5	
113/84	3.77	3.77	3.57	0	-0.2	2	9	26	7	24	33	22	21	-11	-12	
Mean	3.77	3.77	3.69	-0.5	-1.9	19.3	26.8	32.2	10.2	17.8	31.5	25.4	24.9	-8	-7.5	
SD	1.10	0.37	0.72	0.62	1.45	19.3	21.8	32.3	9.5	15.6	7.15	6.7	15.8	3.53	9.1	
P				ns	<.05				<.1	<.1				<.01	ns	
069/84	3.77	3.77	3.37	0	-0.4	56	37	74	-19	18	15	18	7.5	3	-7.5	EMG active
085/84	4.17	3.36	3.37	-0.8	-0.8	13	26	26	13	13	22	31	39	9	17	
086/84	3.77	3.57	3.34	-0.2	-0.2	39	41	12	2	-27	18	20	39	2	21	
087/84	3.17	3.37	3.17	-0.2	0	34	25	35	-9	1	26	21	24	-5	-2	
092/84	3.77	3.17	3.17	-0.6	-0.6	39	28	44	-10	5	23	25	21	2	-2	
Mean	3.73	3.45	3.28	-0.36	-0.4	36.2	31.4	38.2	-4.6	2	20.8	23	26.1	2.2	5.3	
SD	0.36	0.23	0.10	0.33	0.32	15.4	7.2	23.2	12.3	17.5	4.32	5.17	13.3	5	12.8	
P				<.1	0.05				ns	ns				ns	ns	
083/84	2.34	3.77	2.97	1.4	0.6	15	8	10	-7	-5	50	36	50	-14	0	EMG placebo
084/84	3.34	3.37		0		18	28		10		30	25		-5		
091/84	2.97		3.17		0.2	50		57		7	10	13		3		
088/84	4.17	3.37	3.97	-0.8	-0.2	17	20	6	3	-11	31	28	23	-3	-8	
093/84	2.77	3.37	3.57	0.6	0.8	41	70	71	29	30	30	17	14	-13	-16	
113/84	3.37	3.57	4.17	0.2	0.8	11	11	20	0	9	29	28	27	-1	-2	
Mean	3.16	3.45	3.57	0.28	0.44	25.3	27.4	32.8	7.7	6	30	26.8	25.4	-7.2	-4.6	
SD	0.63	0.18	0.51	0.81	0.43	16.1	25.4	29.8	13.7	15.8	12.7	6.8	15.0	5.93	7.53	
P				ns	<.1				ns	ns				<.05	ns	

Table 9.5 Effect of pyridostigmine on paralysis parameters of the action of alcuronium in the isolated forearm.

C = control IFP; I1 = IFP after pyridostigmine loading dose; I2 = IFP during pyridostigmine 30 mg t.d.s. (see text for details).

P values relate to paired differences between control and I 1 and 2 recordings.

ns = $p > 0.1$

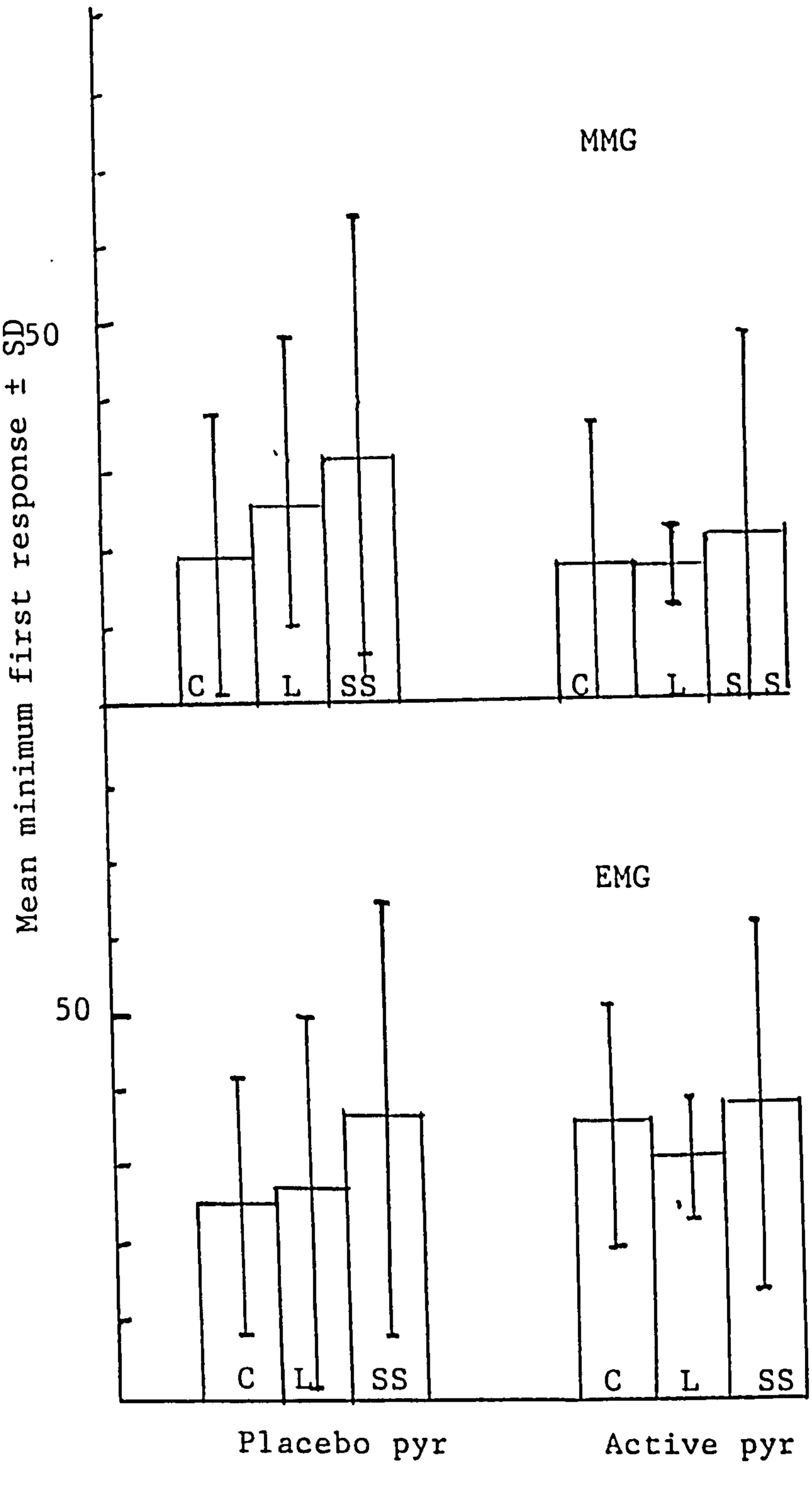


Fig. 9.11a Effect of pyridostigmine on degree of relaxation produced by alcuronium in the IFP. Data from table 9.5. Control(C), loading dose(L) and steady state(SS) data have been averaged (see text)

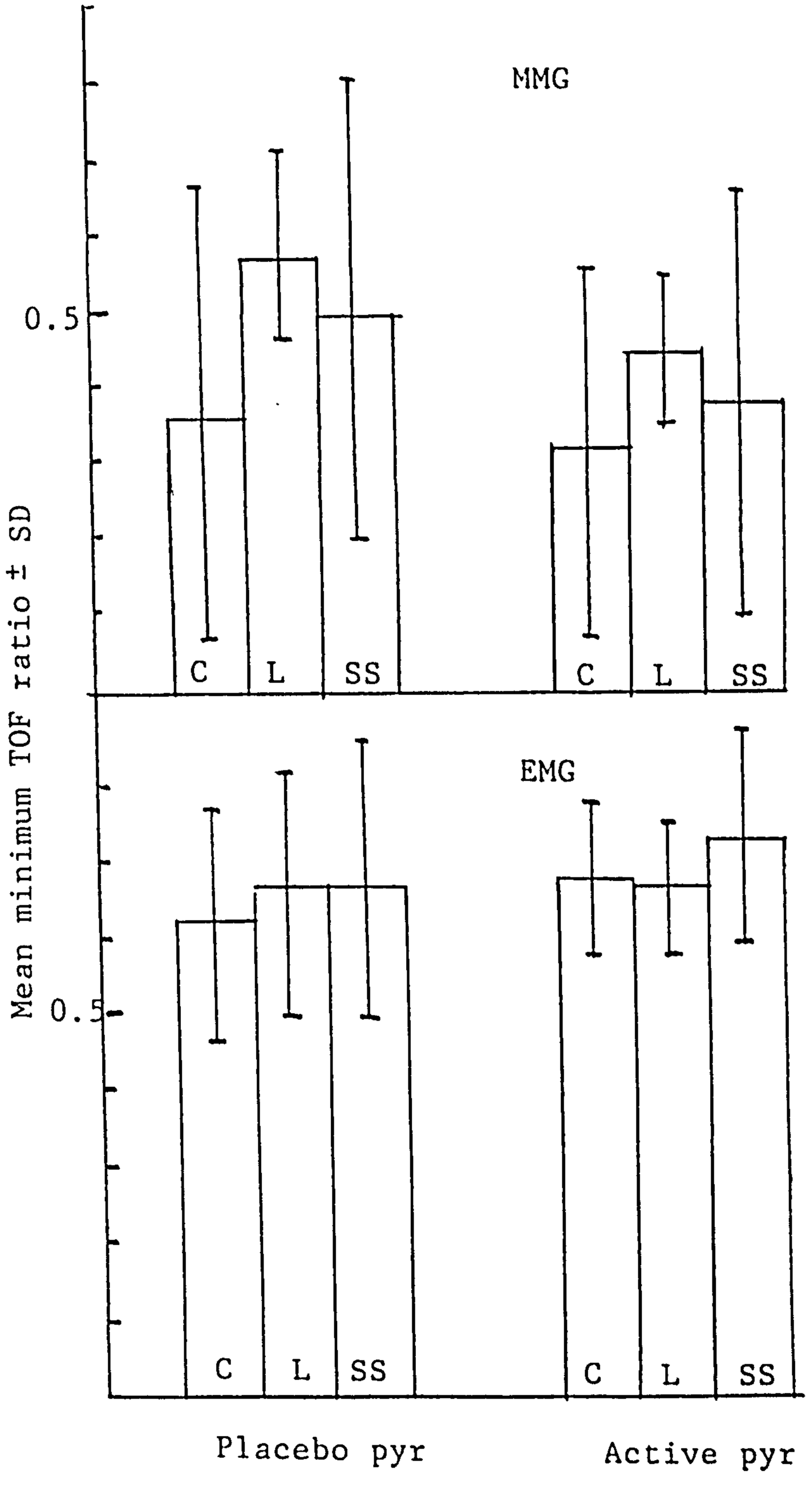


Fig. 9.11b Effect of pyridostigmine on degree of TOF fade produced by alcuronium in the IFP. Data from table 9.5. Control(C), loading dose(L) and steady state data have been averaged (see text).

(1) not all the subjects showed paralysis of the adductor pollicis brevis below 25% of control after 1.5 mg alcuronium. Of the control (IFP 1) data 6/10 subjects paralysed to below 25% as estimated by MMG and 4/10 as estimated by EMG. It should be noted that all subjects received alcuronium from the same production batch.

(2) there was wide variation in peak paralysis both in subjects receiving active and placebo pyridostigmine

Values of inhibition of AChE for subjects taking active pyridostigmine are shown in table 9.6. There is good agreement in the degree of inhibition produced in sessions 2 and 3.

(3) MMG showed a marginally significant reduction in the degree of paralysis achieved in IFP 2 and 3 in the placebo but not the active group;

(4) there was a marginally significant reduction in the time taken to reach peak paralysis in IFP 3, as assessed by both MMG and EMG for the placebo group and by EMG in the active group.

(5) the time taken to achieve 90% recovery was significantly reduced in IFP 2 for the placebo but not the active group

9.2.3 Effect of pyridostigmine on fade

Plots of TOF fade against first response are shown in figure 9.12. As before the data are shown as running means of 8 points with the cross on the broken line indicating the direction of onset and recovery of paralysis. Inspection of the curves shows that the loop shape detected during the previous experiment was present in data from all subjects.

Visual estimation of the loop area before breaking the allocation code gave correct assessment of a reduction in 4/5 subjects taking pyridostigmine as assessed by

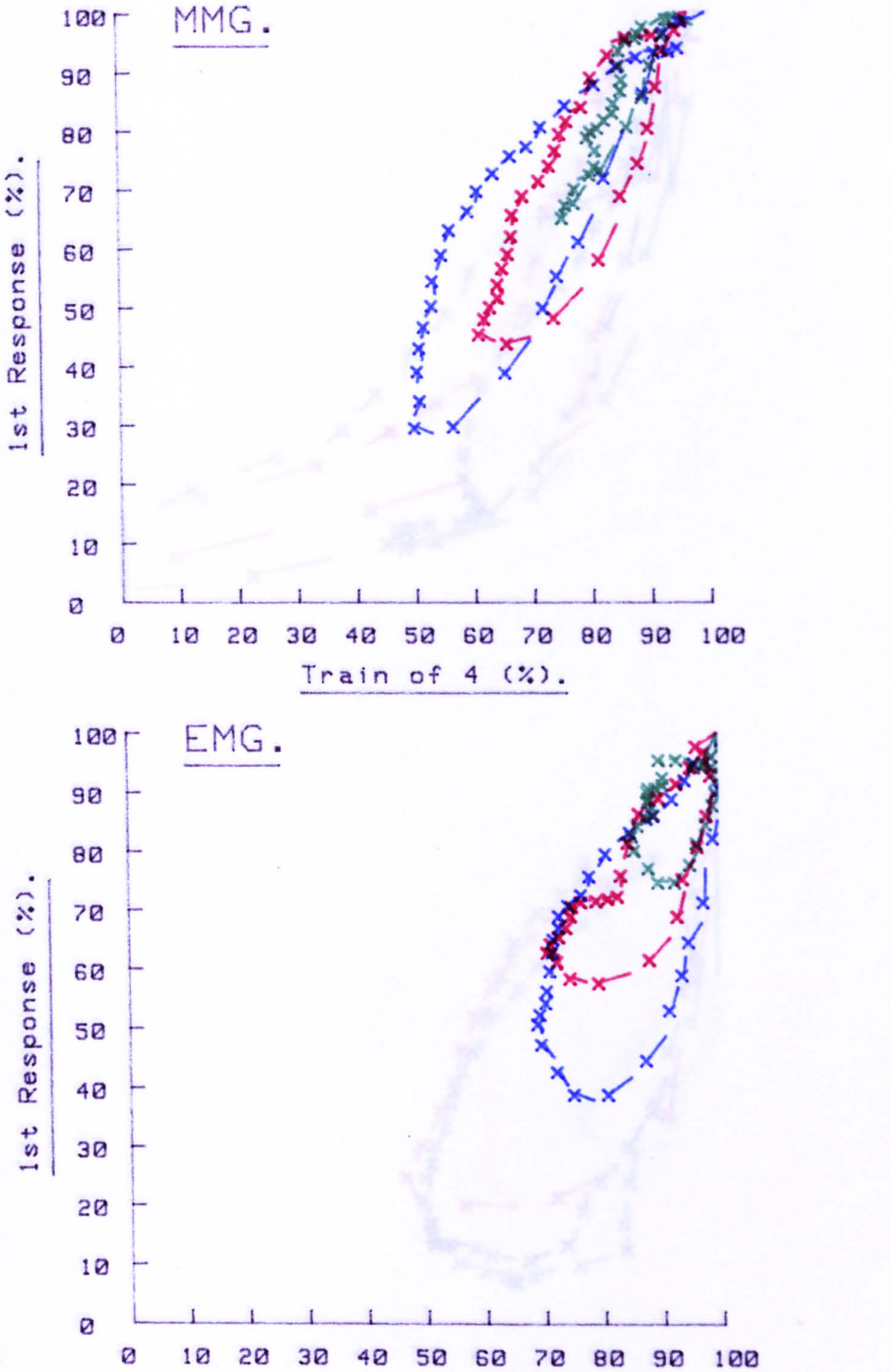
Subject	IFP 1	IFP 2	Allocation
069/84	59.0	56.4	A
083/84	104.6	99.6	P
084/91/84	105.7	102.5	P
085/84	60.0	55.6	A
086/84	57.4	54.0	A
087/84	58.5	59.9	A
088/84	103.3	100.7	P
092/84	65.1	69.2	A
093/84	103.5	*	P
Mean active pyr AChE activity	60.0 ± 3	59.0 ± 6.1	

Table 9.6 AChE inhibition by pyridostigmine in the alcuronium IFP study. All figures are enzyme activity as a percentage of control. IFP 1 and 2 refer to recordings made after the loading dose of pyridostigmine 60 mg and the steady state of 30 mg t.d.s respectively.
A = active drug
B = placebo drug

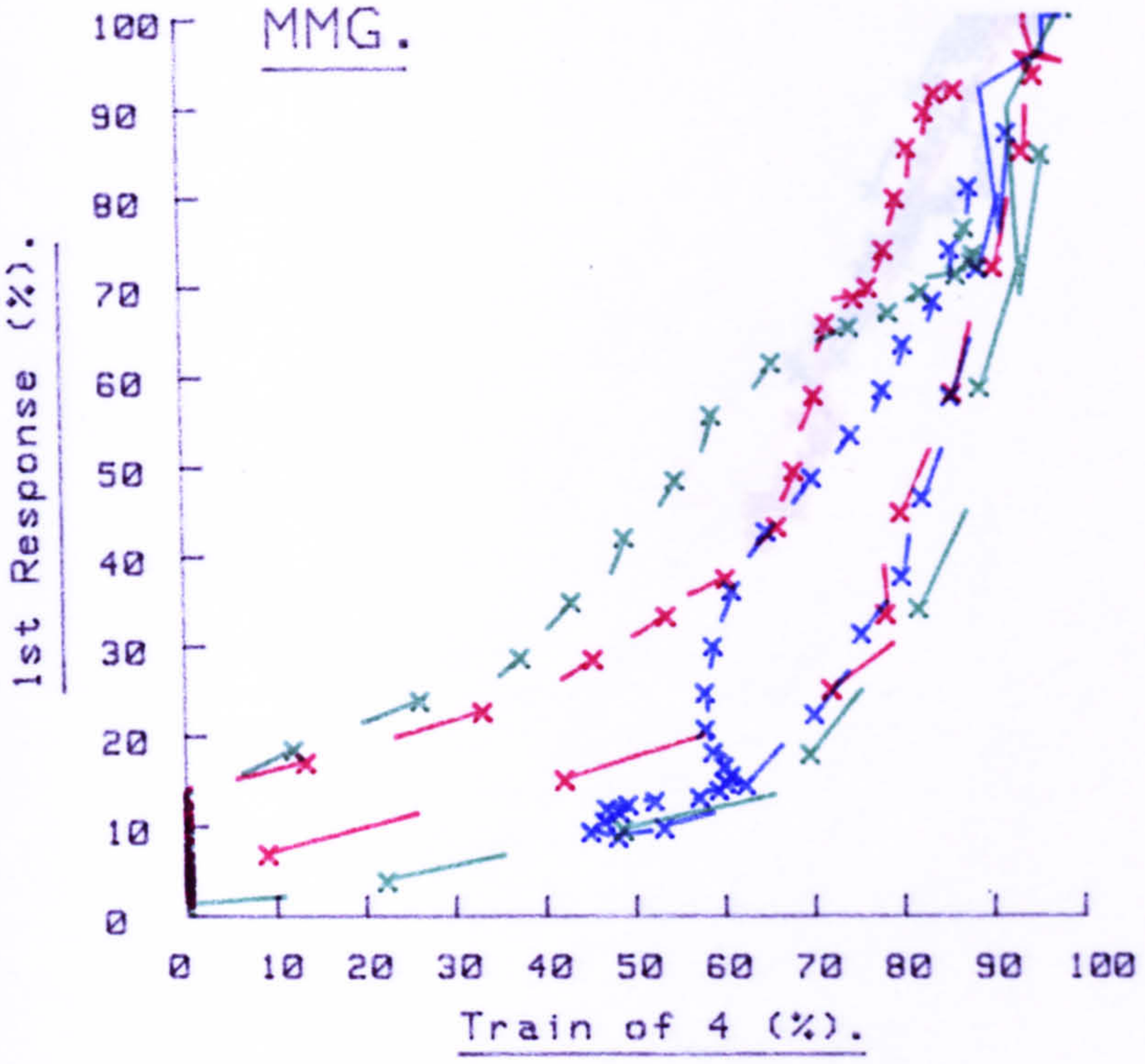
The sample at * could not be analysed.

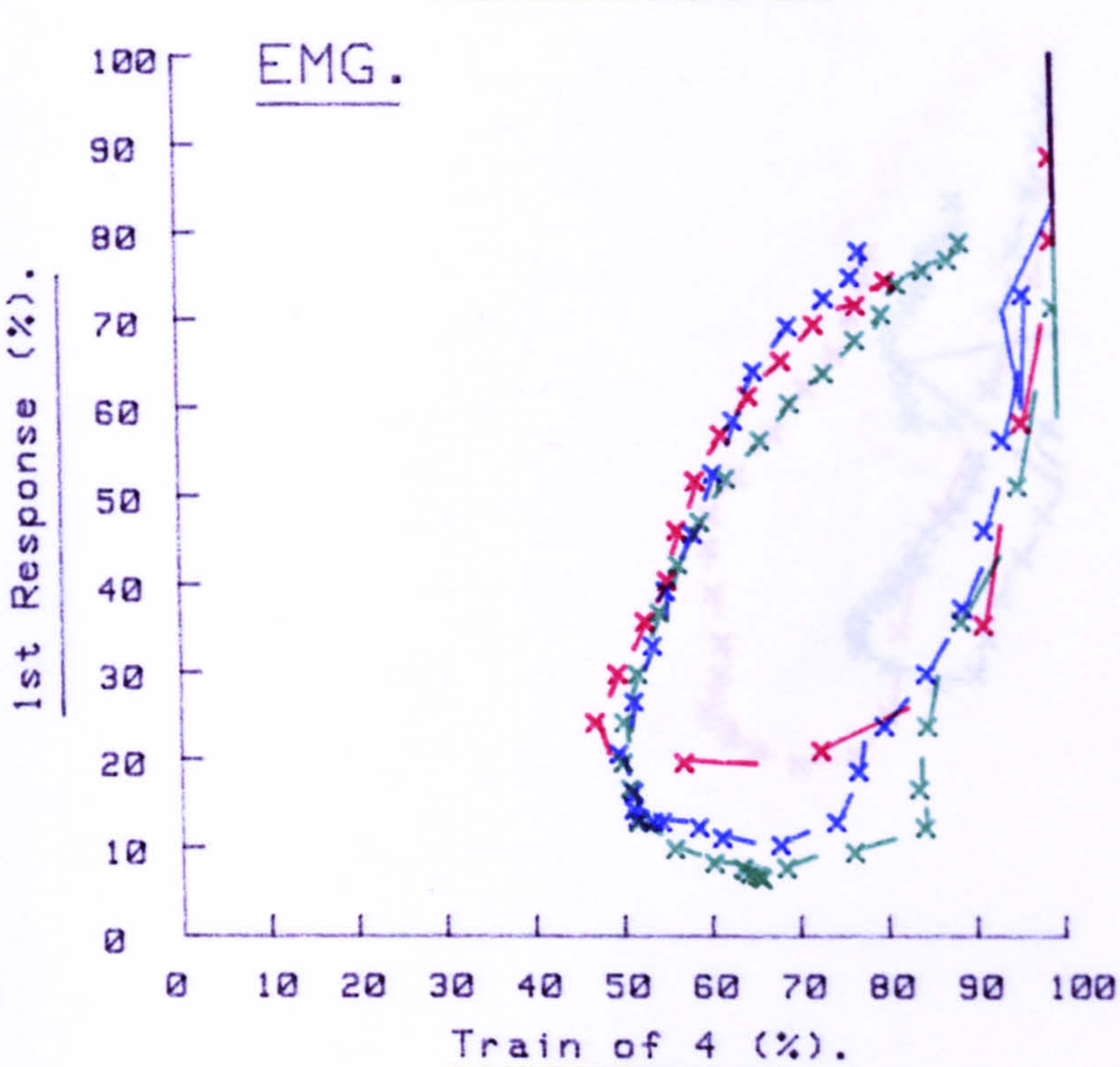
Fig. 9.12 (shown on the following ten pages) IFP and pyridostigmine study: data from fig. 9.10 shown plotted after the method of fig. 9.8 (see text)

Protocol S03/1B. Subject No 69 / 84



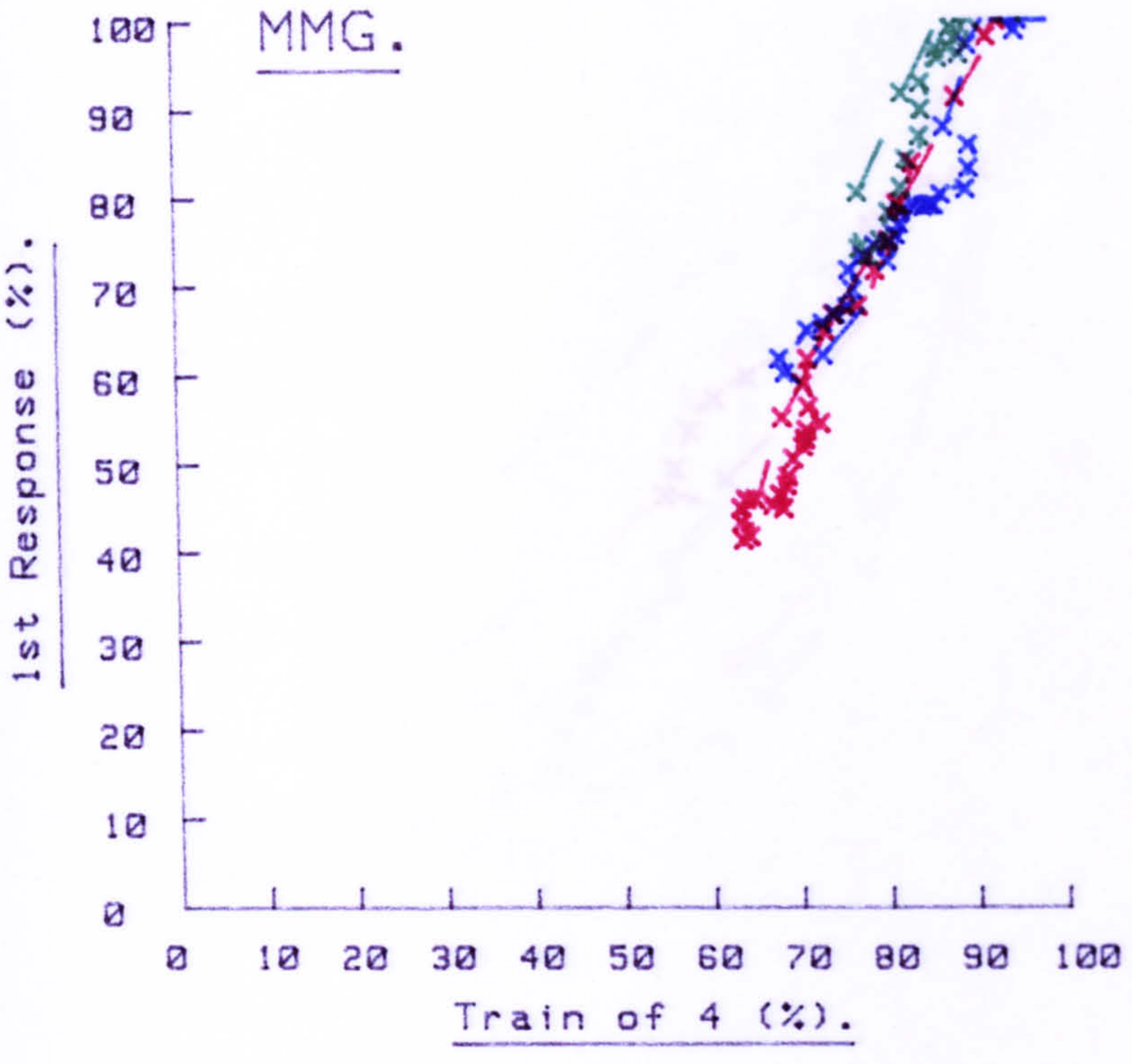
Protocol S03/1B. Subject No 83 / 84

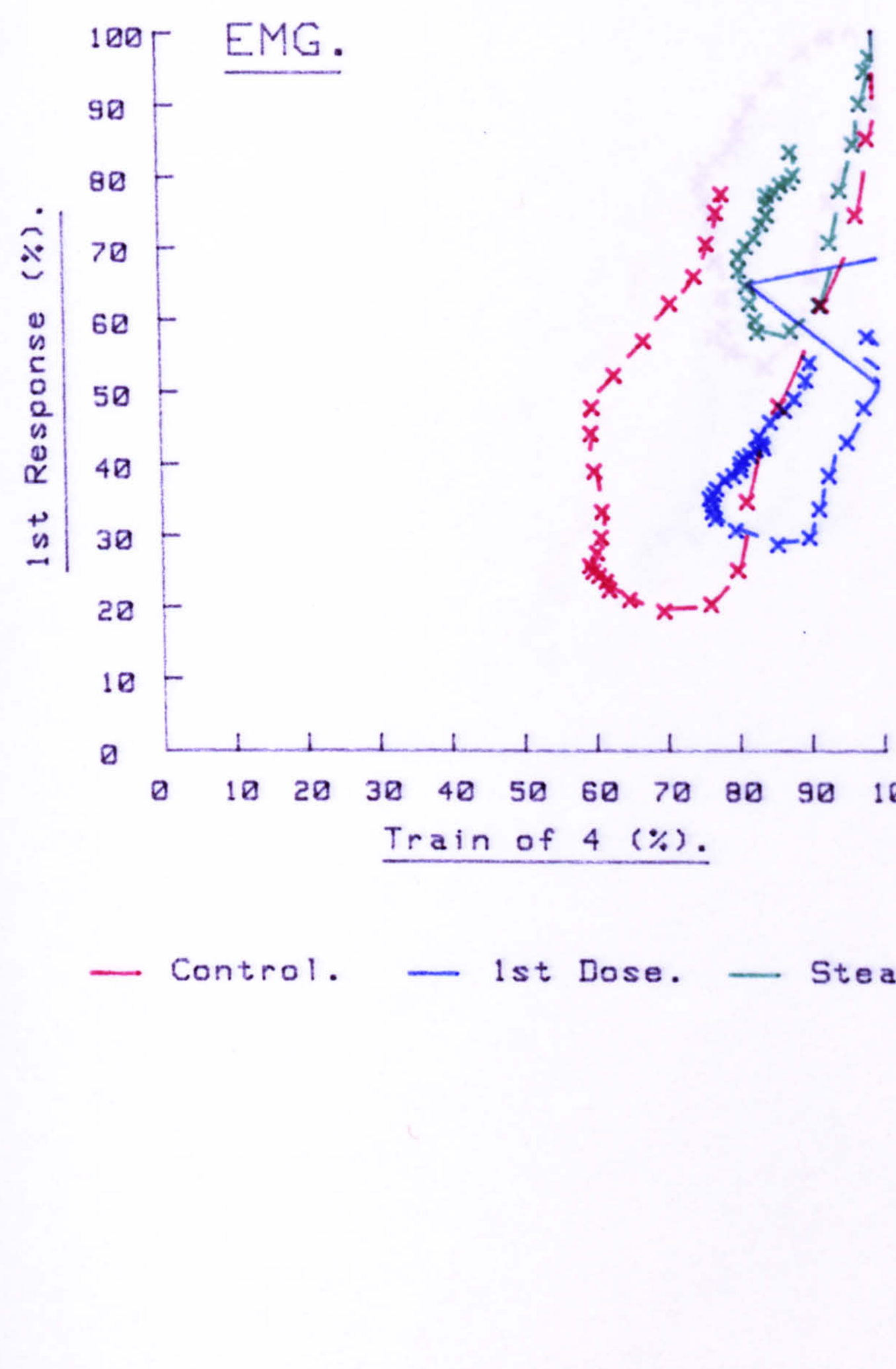




— Control. — 1st Dose. — Steady-State.

Protocol S03/1B. Subject No 84 / 84





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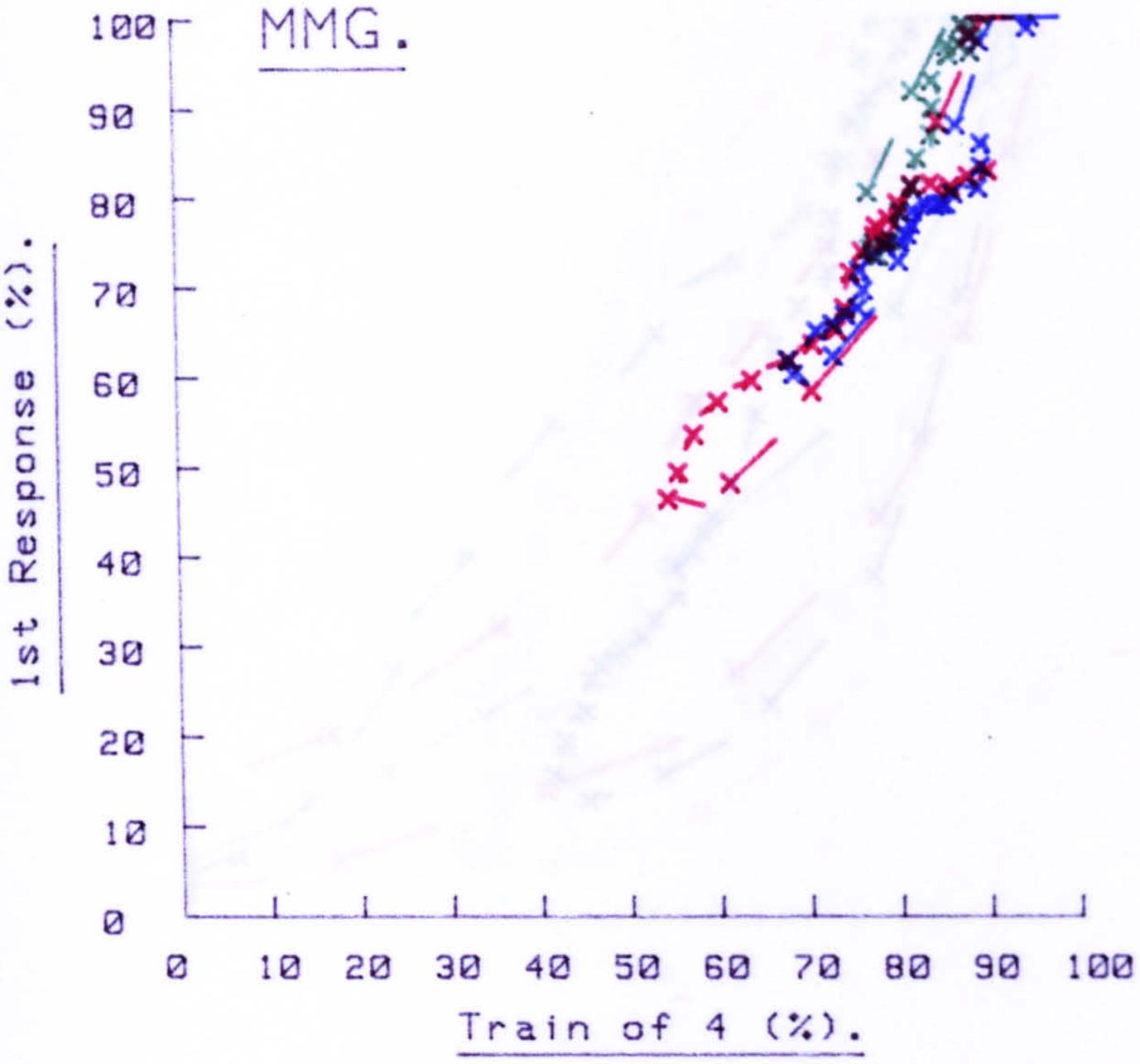
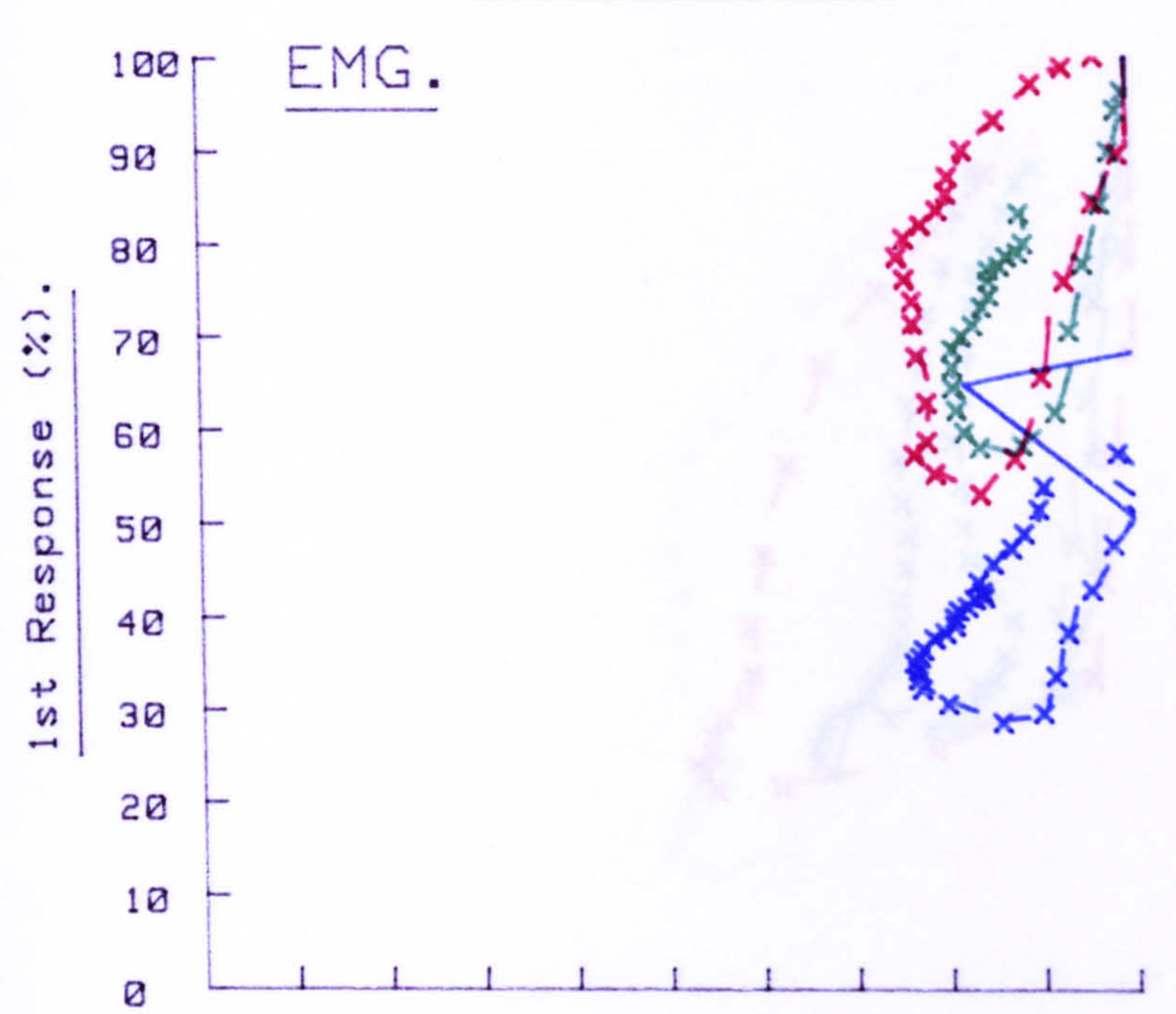
Protocol S03/1B. Subject No 8491 / 84





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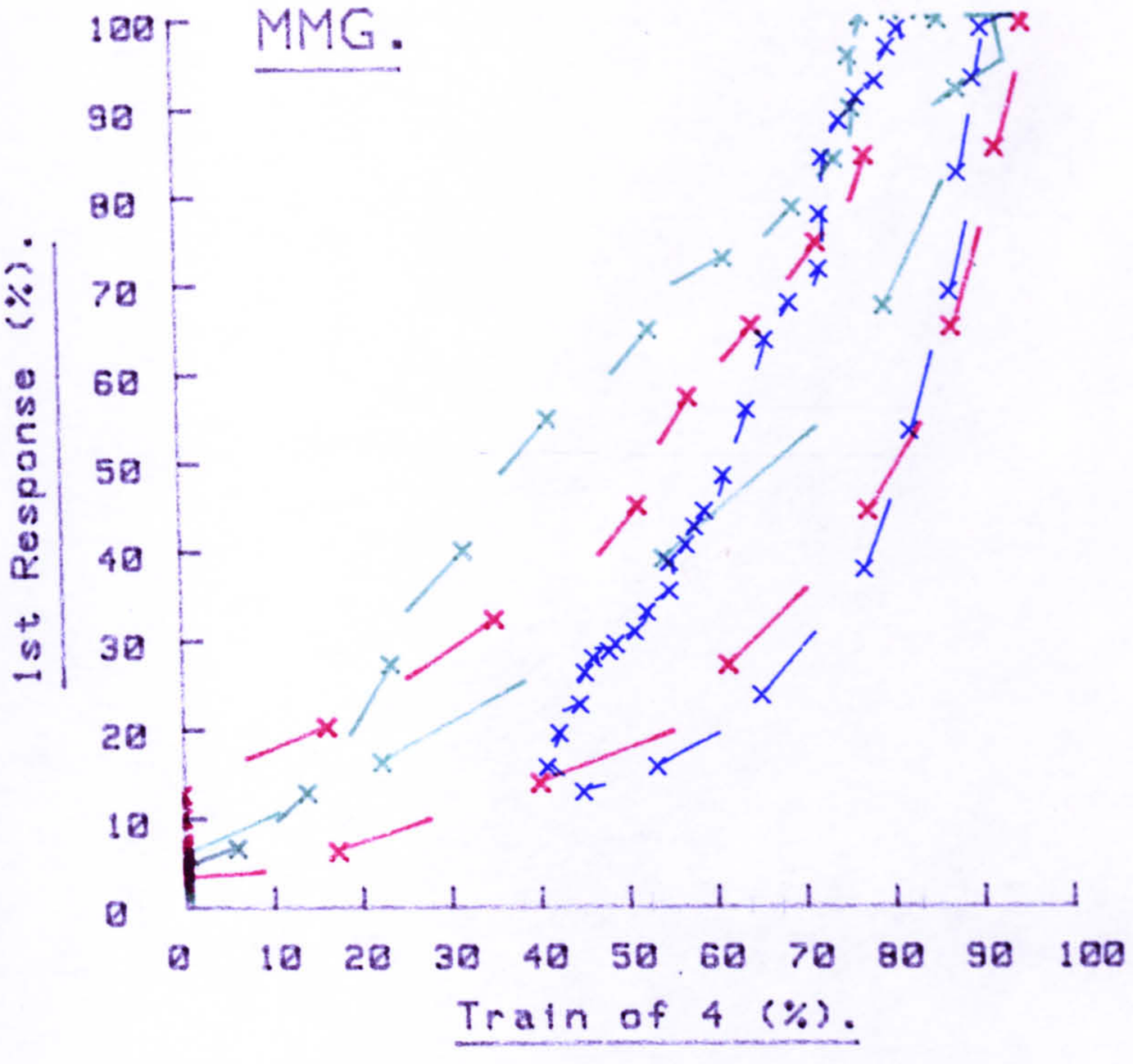
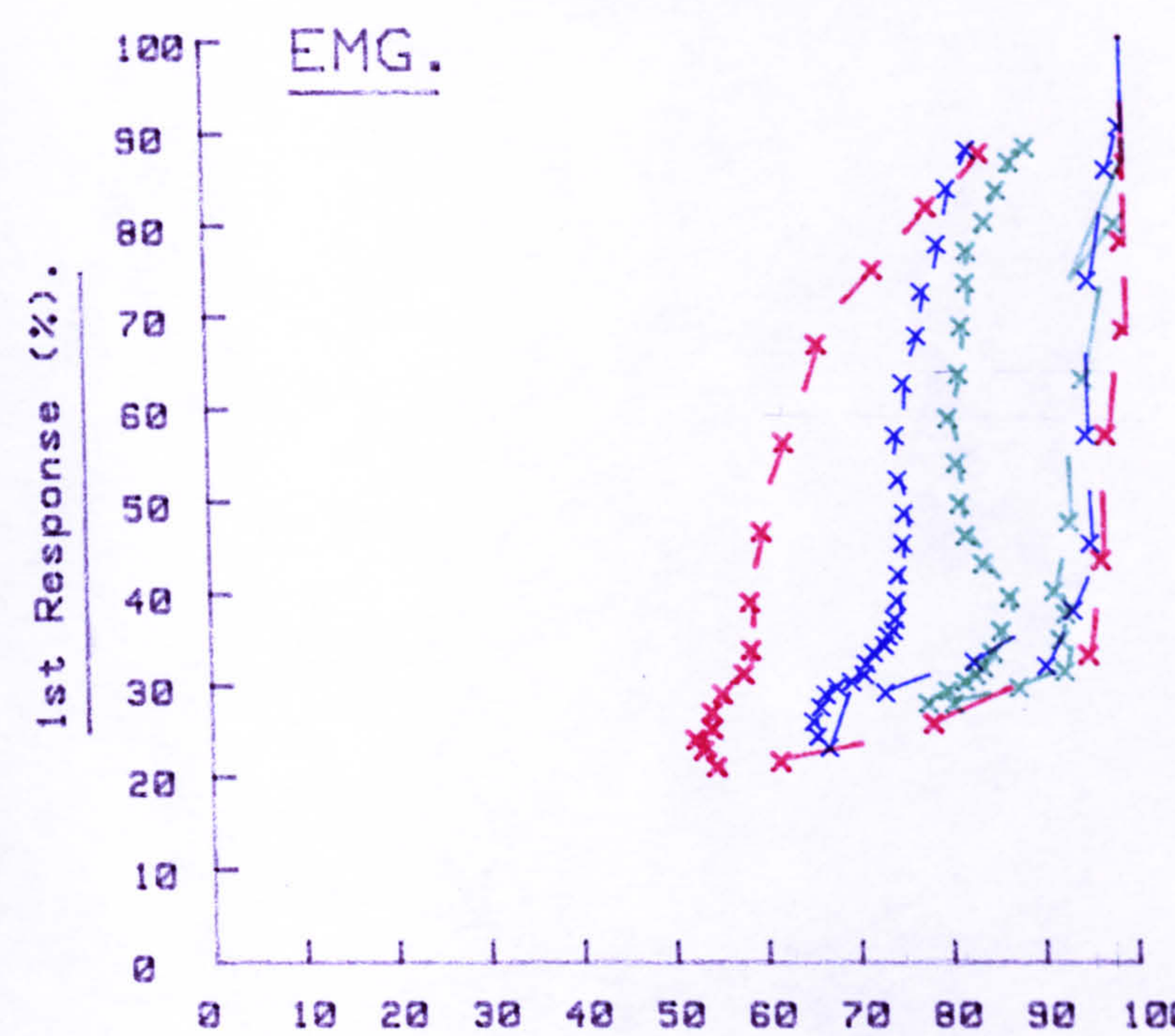
Protocol S03/1B. Subject No 85 / 84





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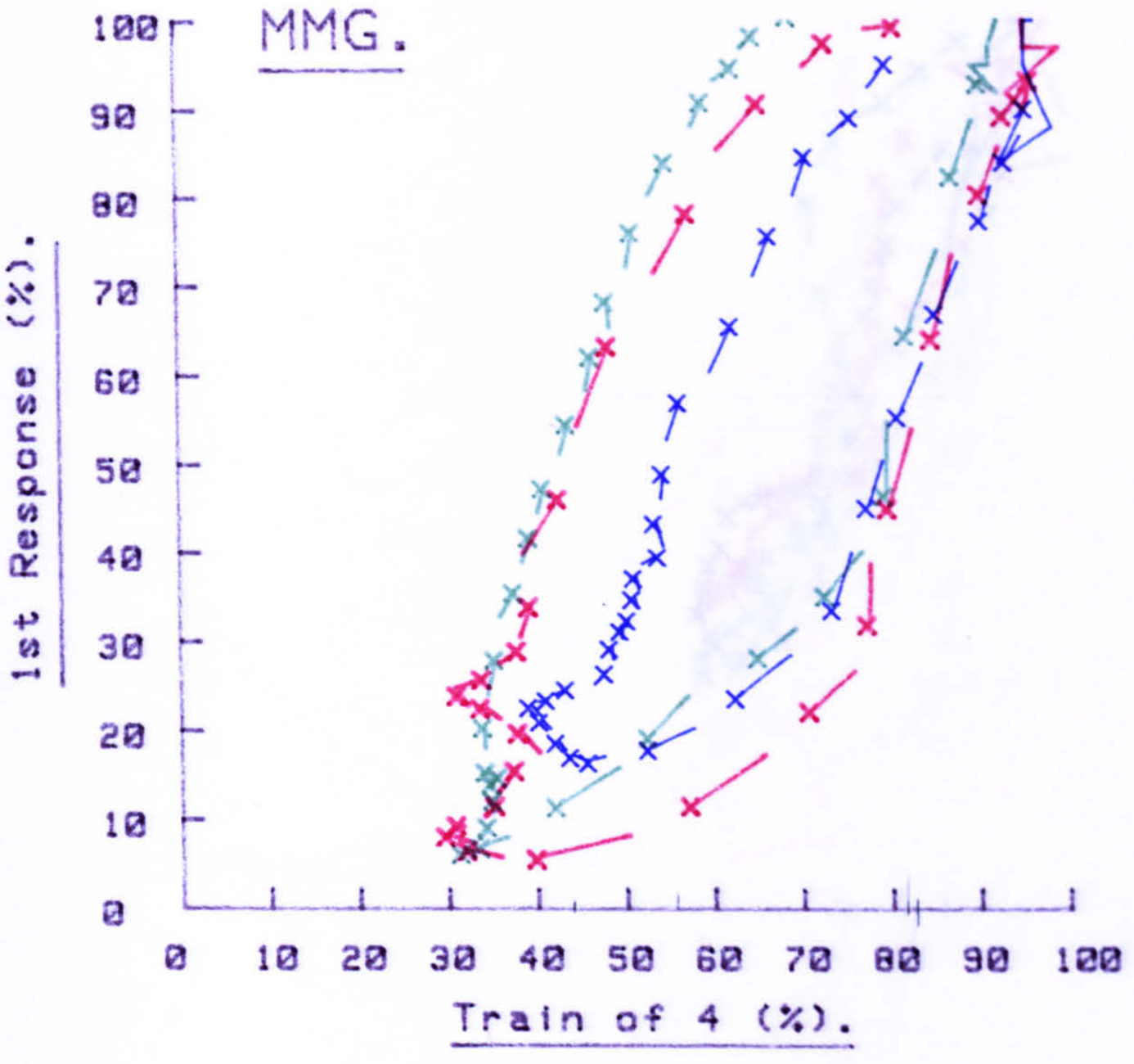

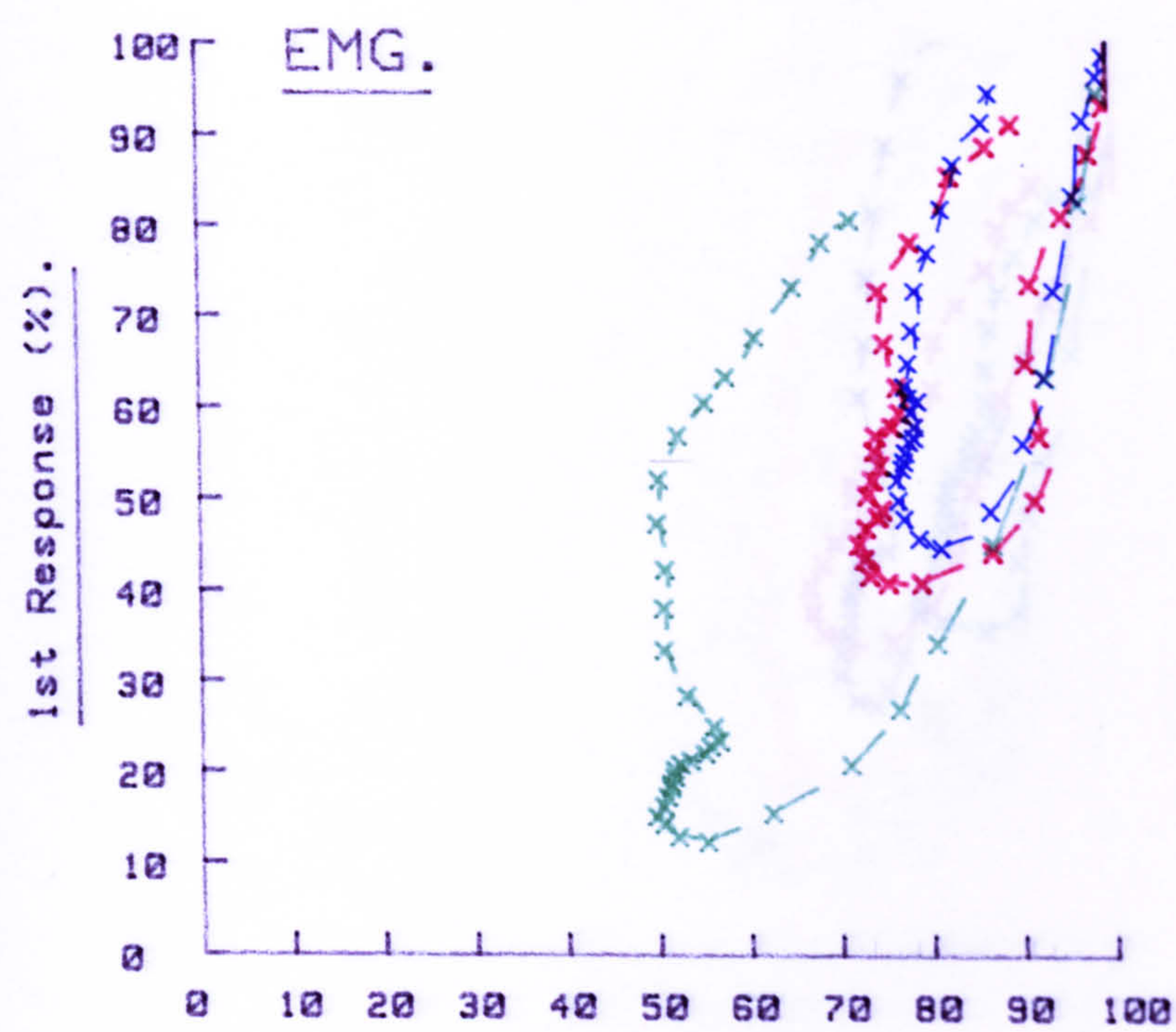

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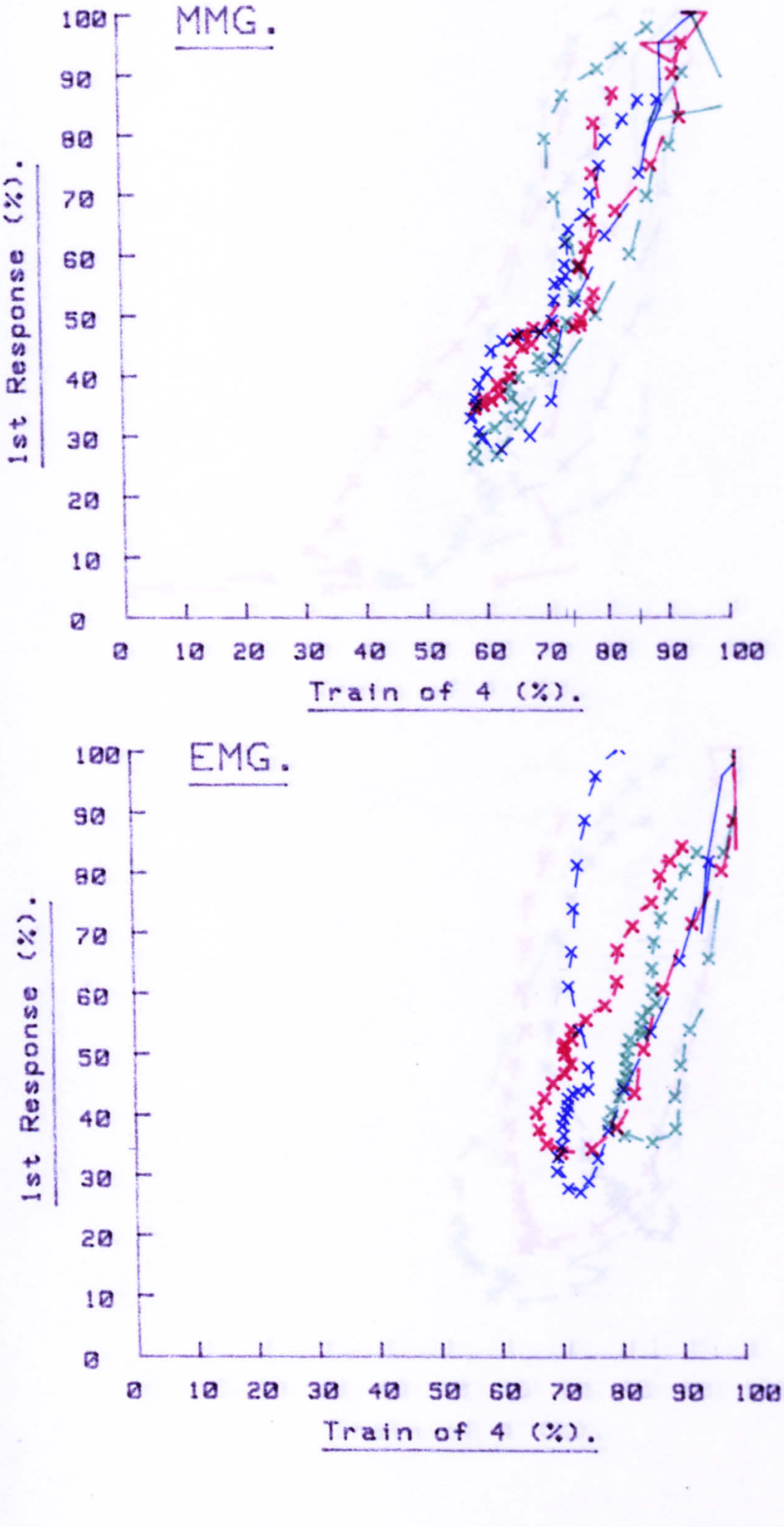

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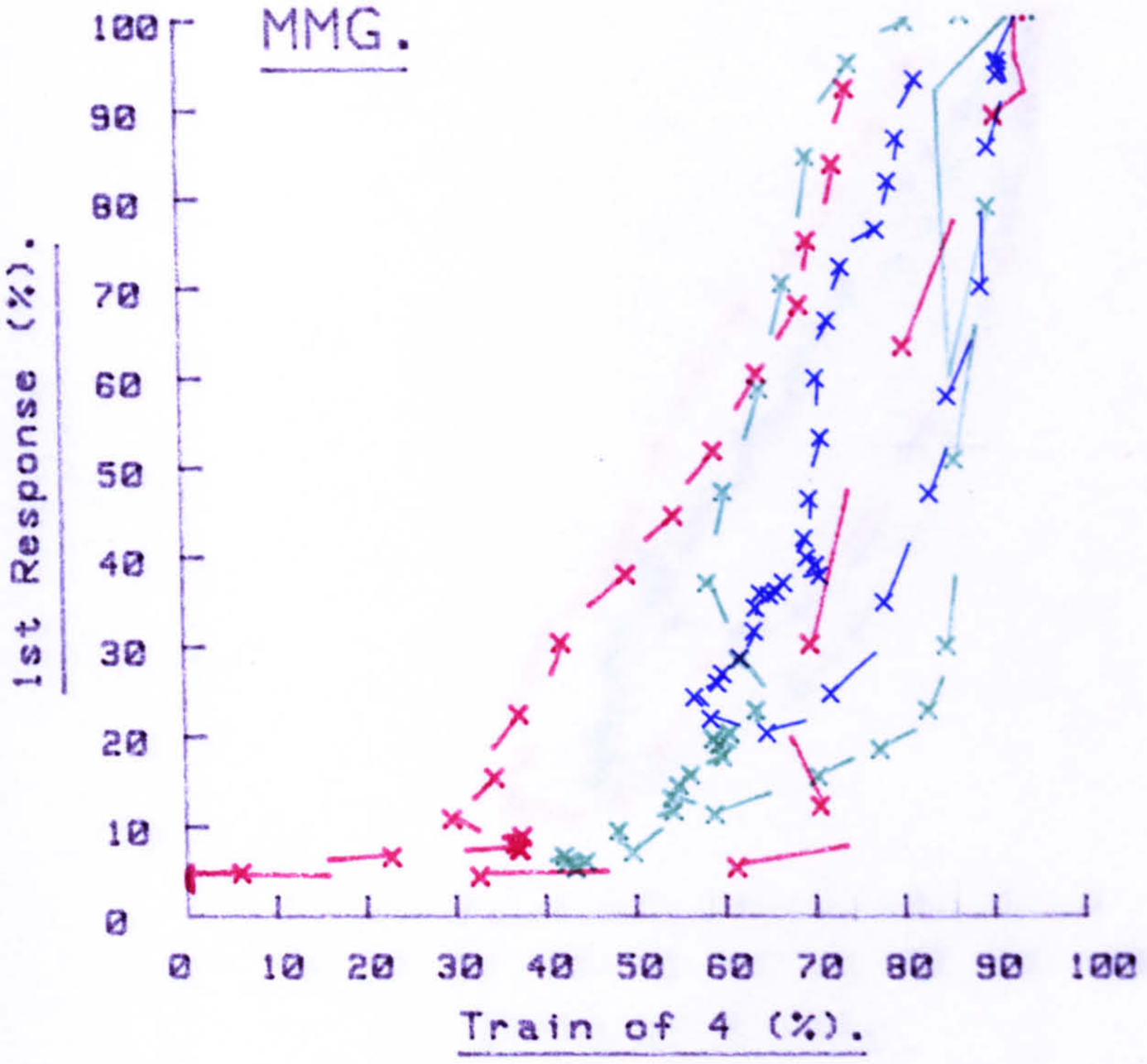

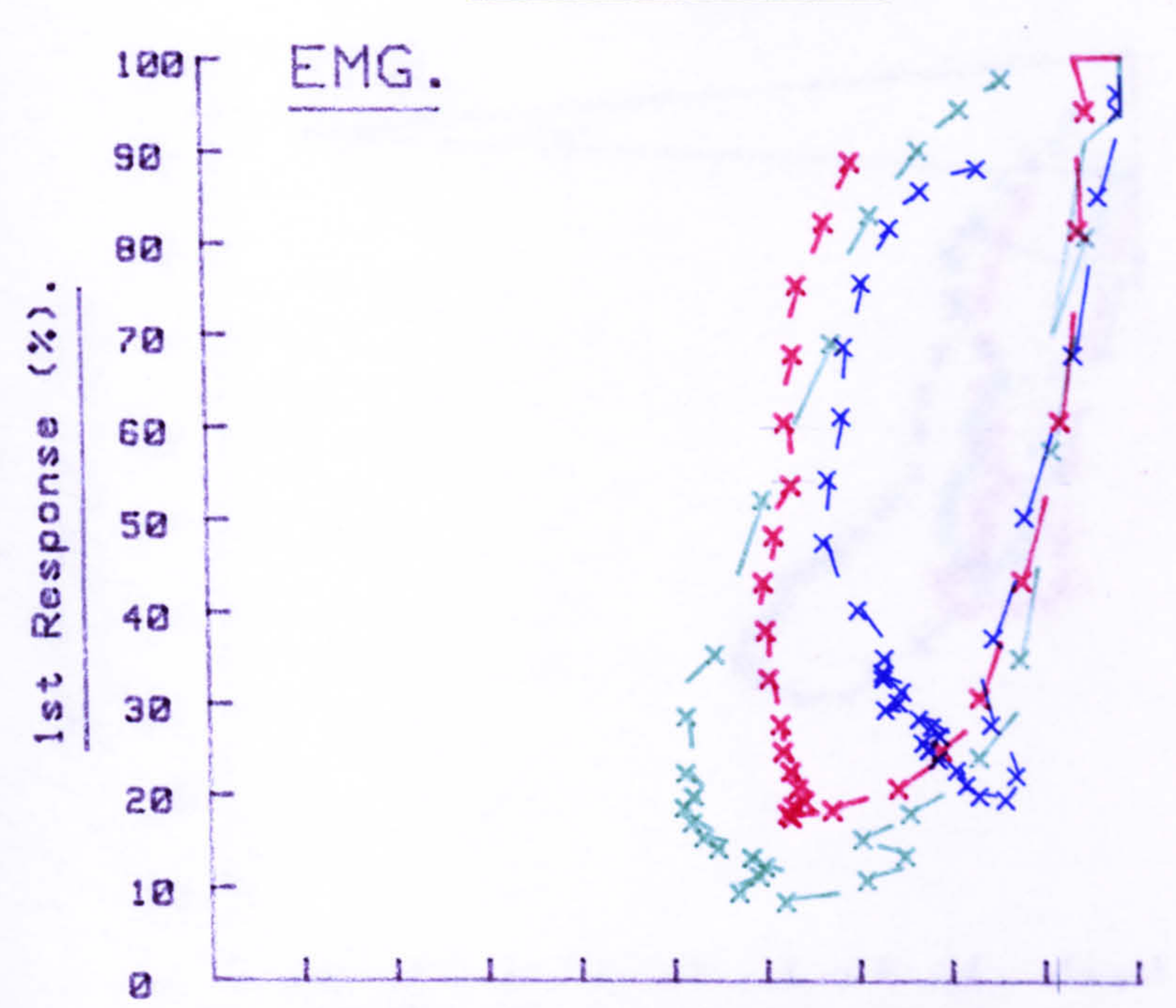

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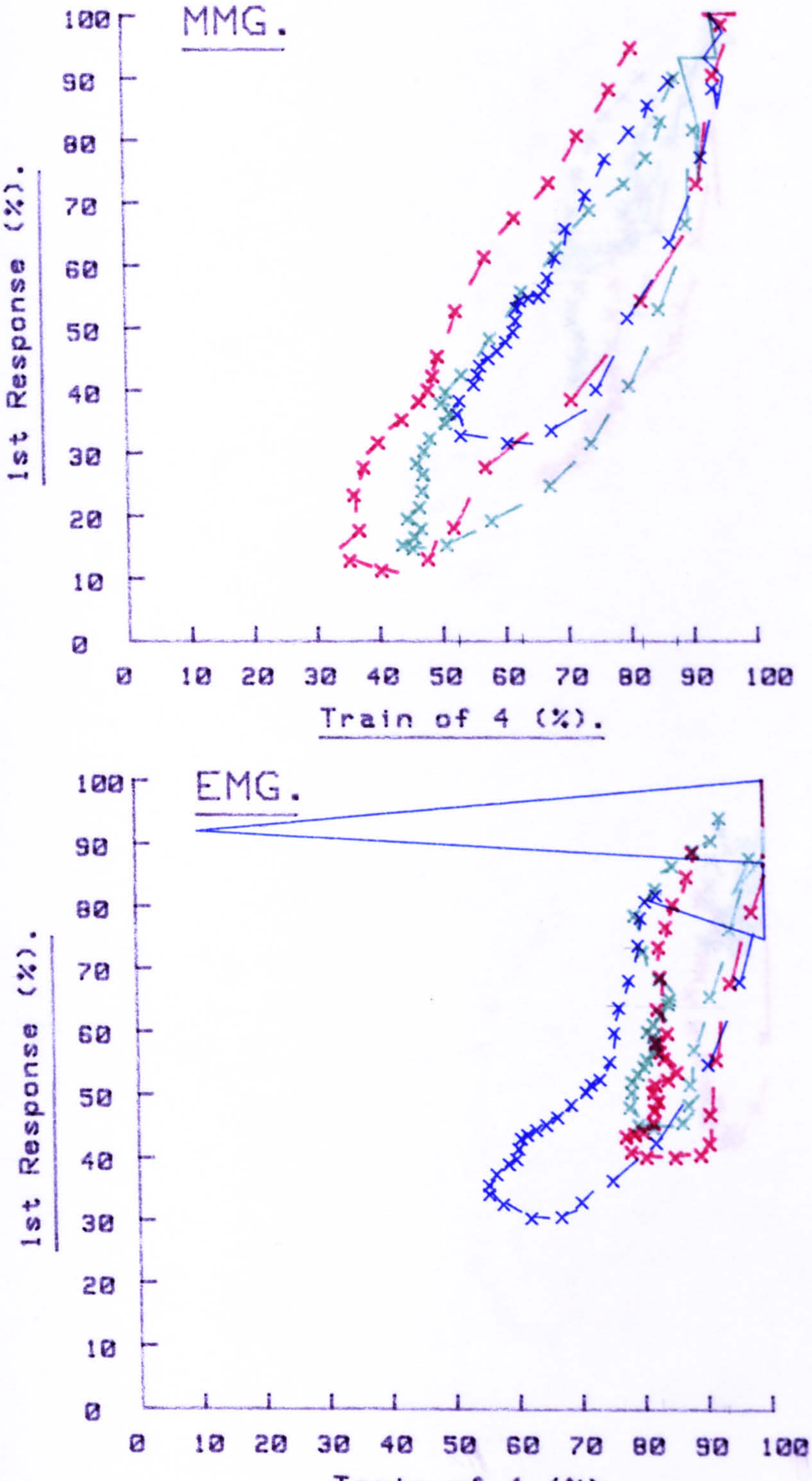

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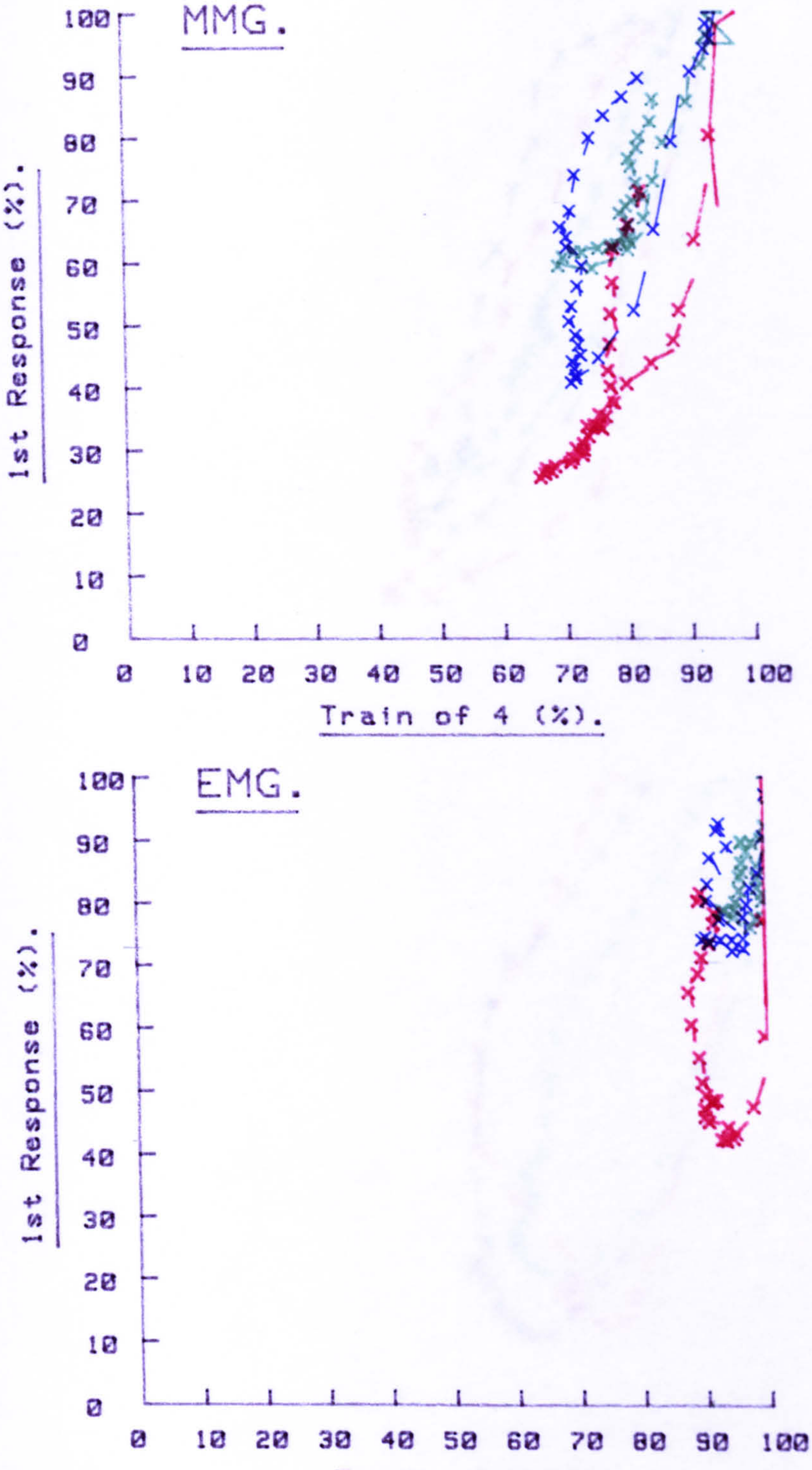

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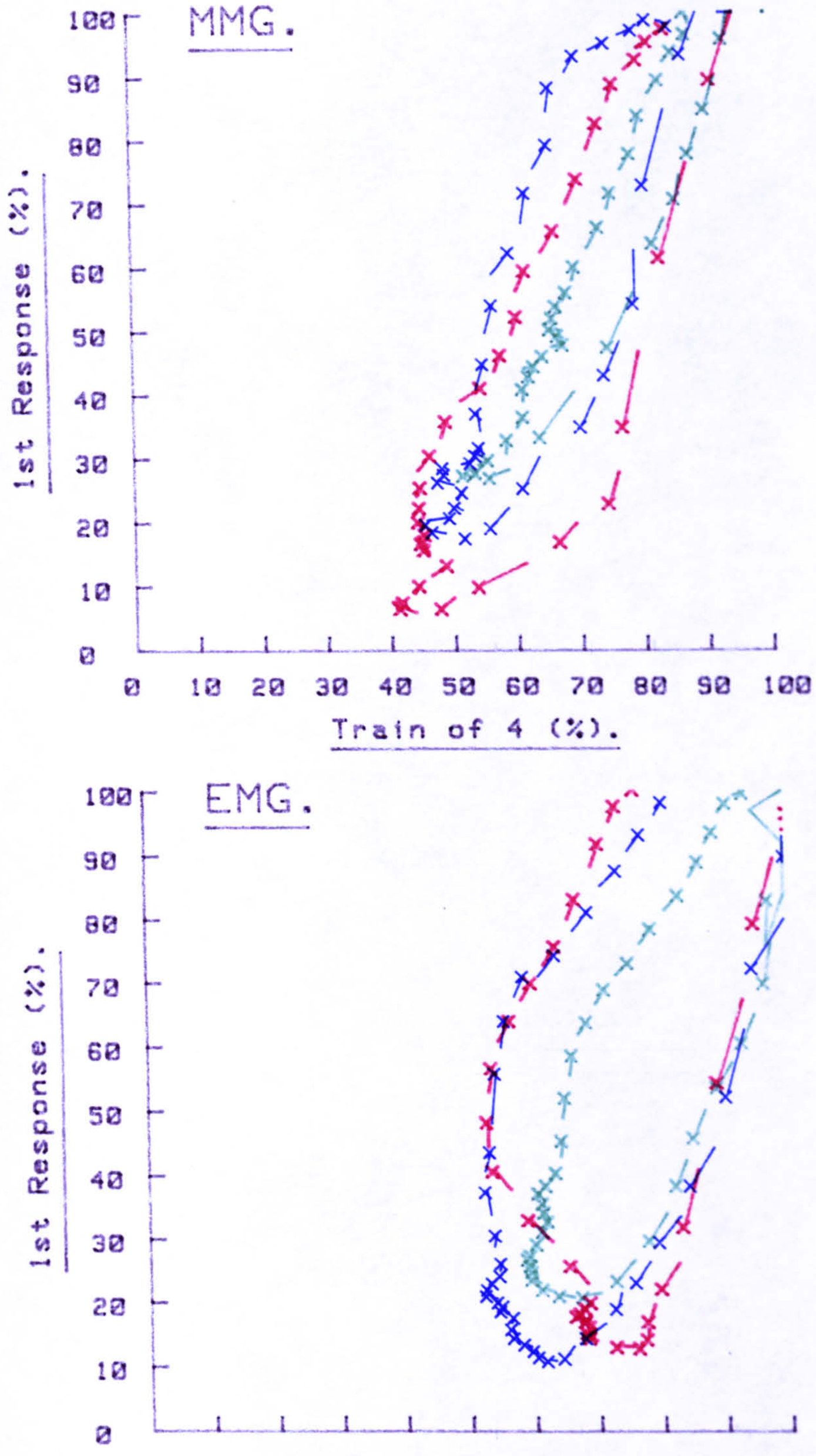


EMG and 2/5 subjects as assessed by MMG. In addition 3/5 subjects taking the placebo were assessed as having no change in loop area from the EMG data and 3/5 from the MMG data. The data appear therefore to indicate a reduction in the area of the TOF/first response loops associated with pyridostigmine. Values of maximum TOF fade and the time taken to reach this point are presented in table 9.7. The data are again analysed on a paired basis. The analysis of changes in fade alone detected no significant pyridostigmine - related effects.

Subject	C	I1	I2	1-C	2-C	C	I1	I2	1-C	2-C
	Time (mins) to min TOF					Min TOF (% of control)				
MMG Active										
069/84	4.6	3.8	3.6	1.2	1	58	45	74	13	-16
085/84	3.4	4.0	1.8	-0.6	1.6	0	38	0	-38	0
086/84	5	6.2	4.6	-1.2	0.4	22	35	24	-13	-2
087/84	3.8	4.2	4.0	-0.4	-0.2	53	55	53	-2	0
092/84	4.0	2.3	4.0	1	0	24	51	41	-27	-17
Mean	4.1	4.2	3.6	0.0	0.6	31.4	44.8	38.4	-13.8	-7
SD	0.7	1.2	1.1	1.0	0.7	24	8.4	28.1	20.1	8.7
P				ns	ns				ns	ns
MMG Placebo										
083/84	3.6	6.0	2.6	2.6	-1	0	42	0	42	0
084/84	7.2	3.8		-4.6		67	67		0	
091/84	3.4		3.4		0	51		73		22
088/84	3.4	4.2	3.6	0.8	0.2	0	57	33	57	33
093/84	5.2	5.2	4.2	0	-1	63	69	64	6	1
113/84	6.2	5.8	4.4	1.6	-2.2	32	39	50	7	18
Mean	4.8	5.0	3.6	0.1	-0.8	36.5	54.8	44	21.8	14.8
SD	1.6	1	0.7	2.8	1	30.1	13.9	28.9	25.9	14.2
P				ns	ns				ns	ns
EMG Placebo										
083/84	22	8.4	19	13.6	-3	44	50	50	6	6
084/84	6.6	4.6		-2		58	75		17	
091/84	4		4.2		0.2	75		79		4
088/84	18	14	8.2	-4	-9.8	59	64	44	5	-15
093/84	13	4.2	5.2	-8.8	-7.8	84	88	93	4	9
113/84	19	8.6	5.4	-10.4	-13.6	51	50	58	-1	7
Mean	13.8	8.0	8.4	-2.3	-6.8	61.8	65.4	64.8	6.2	2.2
SD	7.2	4.0	6.1	9.5	5.5	15	16.4	20.6	6.6	9.8
P				ns	0.05				ns	ns
EMG Active										
069/84	4.8	5.4	5	0.6	0.2	70	68	83	-2	13
085784	6.2	4	3.8	-1.8	-2.4	52	64	75	12	23
086784	5	5.2	4.8	0.2	-0.2	71	75	48	4	-23
087784	4.8	4	4.2	-0.8	-0.6	64	69	7.6	5	12
092/84	4.2	4.6	4.8	0.4	0.6	77	54	77	-23	0
Mean	5	4.6	4.5	-0.3	-0.5	66.8	66	71.8	-0.8	5
SD	0.8	0.7	0.5	1.0	1.2	9.5	7.8	13.7	13.4	17.6
P				ns	ns				ns	ns

Table 9.7 Effect of pyridostigmine on TOF Fade parameters in the alcuronium IFP.

C = control IFP; I1 = IFP after pyridostigmine loading dose; I2 = IFP during pyridostigmine 30 mg t.d.s. (see text for details)
Subject 084/091 could not complete the protocol during during one week and recorded a second control session with a second experimental number before recording I2 (see text).

P values relate to paired differences between control and I1 and 2 recordings.
ns = $p > 0.1$

CHAPTER 10: Discussion

10.1 The experimental use of SFEMG

In the studies reported in this thesis, SFEMG measurements have been made in the EDC muscle of 24 male volunteers exposed to GB Ct5 with pyridostigmine pretreatment. A further eight volunteers have been studied before and after exposure to GB Ct15. All subjects received at least four, one hour sessions of SFEMG in the same muscle over a ten day period. The investigation was well tolerated in all cases. Almost all subjects learned very quickly to relax the forearm and produce a steady activation of EDC by extension of the middle finger enabling clear fibre pair recordings to be obtained. There were no clinical sequelae from any of the recording sessions. The sterilization procedure of soaking the electrodes for 12 hours in 2% glutaraldehyde appeared effective, although electrode insulation failure did occur in some instances because of it. These electrodes were not used for SFEMG recording. Stalberg has commented on the difficulty of recording SFEMG from young, fit subjects and we have found that a good operator - subject relationship is essential for successful recording. Experience gained recording SFEMG in neurophysiological clinic with patients has confirmed that SFEMG is more easily recorded in pathologically affected muscles.
The EDC muscle was chosen for use in this study because of its ease of access and activation, bearing

in mind that the studies were being carried out in volunteers. A further important advantage of choosing EDC was that it has been extensively studied by other workers and a large amount of control data therefore exists (section 6.8.3). The question of fibre type mosaic of EDC was considered in section 6.7.2. Since no control or serial muscle biopsies were possible in these studies we were not able to confirm that the mosaic was identical in all cases. It has been shown that mosaic can change with training (Jansson and Kaiser, 1977, Saltin et al, 1976). It should be noted however even in the highly athletic volunteers studied EDC was not notably hypertrophied, as standard forearm training regimes tend to develop the flexors rather than the extensors. Further, there appeared to be no clinically obvious differences in SFEMG recorded from these subjects, apart from a difficulty in some cases in producing the minimal activation of EDC required for recording.

SFEMG has had a steady but limited application over the past decade in clinical diagnosis. Its use in clinical neurophysiology clinics has been limited by the time taken for making recordings, given that between 10 and 20 fibre pairs have conventionally been required for analysis. Considerable experience is necessary before successful SFEMG recordings can be made. A good liason is required between subject and operator, so critical is the recording upon small electrode movements which can radically alter the amplitude of the signal. This artefact in turn leads to a failure of the triggering. Recent work (Davis et al, 1983) has produced improved recording of SFEMG by application of computer techniques to the triggering. A variety of different triggering options are now available and the amplitude artefact can be minimised using a potential following circuit which reduces the triggering level in proportion with the amplitude reduction. However, even without sophisticated electronics good SFEMG recordings can be made

providing quiet relaxed conditions are observed at all times.

All data from the studies reported were recorded at 15 inches/sec onto a reel to reel FM tape recorder for off - line processing. This method of storage has proved satisfactory over a long period and the jitter error introduced by tape flutter was minimal. It is important however to keep all tape heads clean if this condition is to be maintained. Stalberg recommended the use of a commercial AM tape recorder which covers the frequency bandwidth of the SFEMG signal well. Two automatic computerised data processing systems have been used to calculate jitter. These were (1) the Medelec MS6 / Apple 2e system and (2) the DISA jittermeter which is a hard - wired microcomputer. In a comparative study where the tapes were processed through both systems, a good correlation was found (figure 7.5). Results have also been checked using R10 variation measured on printed traces from the repeater oscilloscope. This technique was discussed by Eckstedt et al (section 6.6.2) and found to be well correlated to MCD measured by sequential measurements of interpotential distance at low MCD values. At higher values the correlation was poorer. The R10 method has been found to be useful for a rapid estimate of jitter and is an acceptable method of processing if computerised facilities are not available.

10.2 Control SFEMG data in EDC

10.2.1 The reciprocal transformation

A large number of control SFEMG recordings were made in the studies presented. The jitter values obtained showed a marked skewness of their distribution (section 8.1.1 and appendix 1).

This finding confirmed the results of previous workers who have all assessed normality of SFEMG jitter by truncating the upper tail of the distribution and calculating the incidence of jitter values greater than three standard deviation above the mean of the remaining truncated distribution. Values of greater than 2/20 pairs with MCD values greater than 55 usec have been regarded as abnormal. This approach has been acceptable in clinical practice where jitter changes are often marked and there is co-lateral clinical evidence of disease. Examination of the tables of exact confidence however shows that to be 95% certain that the observed incidence of 1/20 represents the true incidence in a population of recorded fibre pairs at least 72 pairs must be examined. In practical terms this is impossible during any single recording session. It was evident therefore that a different approach to jitter change was required in a study designed to look for small pharmacological effects on neuromuscular function.
The reciprocal transformation technique, described in appendix 1, removes the skew from the distribution of MCD values and allows comparison of overall population shifts to be made using simple parametric statistics such as the t test. All SFEMG data presented here have been analysed in this way. In addition, further analysis based on determination of high jitter incidence was employed.
The reciprocal transformation was adopted on the assumption that any drug effect sought would apply to all MCD values within a population. What the transformation cannot do is to say whether all values are equally affected. It may not be the case that all fibre pairs are equally affected by organophosphate. As an example of possible variability at end plates Stalberg et al (1975) showed that pairs with initially high MCD values were more affected by small doses of non - depolarizing blockers. This point is considered further in section 10.2.3.

10.2.2 Control jitter values in EDC

The analysis of 421 control fibre pairs recorded during the pyridostigmine - GB Ct5 study showed a skewed distribution with 20 pairs (4.3%) having MCD values greater than 55 usec. This incidence is in good agreement with the results of several other groups (section 6.8.3) as is the uncorrected mean of the distribution truncated at 55 usec. The mean value obtained was 25.37 $\pm$ 9.29 usec. After reciprocal transformation the mean value of all the control data from this study, except for three fibre pairs with values less than 10 usec which may arise from a split fibre artefact, was 43.3 $\pm$ 17.9 kHz. The control values obtained from the higher exposure GB Ct15 study were in good agreement with the GB Ct5 data and also showed that there was no significant difference in data recorded during control sessions separated by two days. The question of whether repeated SFEMG recording can alter the characteristics of the muscle is important. Stalberg has described the artefact SFEMG recording from a damaged fibre (Stalberg and Trontelj, 1979) and such recordings cannot be included in the final analysis. They were seen only rarely during these studies.

10.2.3 The homogeneity of control data

Of the 32 subjects studied in the two SFEMG studies only one (063) had control values which were not within normality as assessed by reciprocal transformation and untransformed mean MCD values from other workers' studies. The proportion of MCD values > 55 usec was normal. Although many minor pathological conditions such as viral myositis (Stalberg and Trontelj, 1979) can give rise to jitter abnormalities

no such explanation could be discovered in the case of subject 063/84. Because of the abnormality in control values subject 063 was not included in the analysis of the effects of GB Ct15 on jitter given in section 8.4.2.

The question of variability of fibre type mosaic has been mentioned previously. SFEMG recorded under voluntary activation derives largely from type 1 fibres. Duchen and Gale (1985) have shown that the neuromuscular junctions may be differentiated according to fibre type morphologically. The possibility exists that the junctions may also be differentiated functionally. Safety margin, as defined by Paton and Waud, may be fibre dependent and not uniform through all human populations. Little is known of the actual values of safety margin in human muscles and the subject requires further study.

The reciprocal transformation technique may be more sensitive in detecting drug related changes occurring in a recorded population of end plates because it can detect changes in the complete distribution. In a sampling technique such as the SFEMG method described, changes occuring in an individual pair of end plates which may have particular characteristics, such as safety factor, that are affected by a pharmacological agent cannot be determined. Attempts to follow the fate of individual fibre pairs after drug treatment (eg Schwartz and Stalberg, 1975) considered only short term changes and then with difficulty. Section 6.8.3 reviewed possible explanations for the existence of a normal range of jitter. The sampling SFEMG technique cannot determine which of this range is more susceptible although further mathematical examination of the distribution may reveal changes in shape which reveal differential effects on sub populations.

10.3 The effects of pyridostigmine on SFEMG

10.3.1 Clinical screening of the pretreatment regime

In the double blind study reported in section 7.6, pyridostigmine bromide 30 mg t.d.s. was taken before exposure to GB Ct5 by 24 fit male volunteers who had SFEMG recordings in the EDC. The drug regime is that proposed for pretreatment protection of Service personnel who may be at risk of exposure to nerve agents (section 2.4.3). No side effects of pyridostigmine were reported by the subjects studied. This finding is in agreement with the results of the product licence study carried out at CDE Porton Down which reported only mild gastrointestinal upset from the drug when used in this dose. Compared with the doses which are used in the treatment of myasthenia gravis (section 2.4.1) the pretreatment schedule doseage is modest. In occasional misdiagnosed cases, where non - myasthenic patients have received large doses of pyridostigmine, marked weakness and fasciculation has been observed (section 2.4.1). This 'cholinergic crisis' corresponds to the beginning of a chronic depolarization block due to the presence of excess ACh. Whether histological and functional damage is caused to the end plates in humans, similar to that reported in animal studies (section 4.2) is not known. From the outset of this study there was no clinical indication that pyridostigmine, in the pretreatment doseage, caused any neurologically detectable damage to normal end plates. The SFEMG measurements were made to look for evidence of possible subclinical lesions, using a minimally invasive technique. Although <u>in vitro</u> studies of ultrastructure and electrophysiology comparable to those described in chapter 4 would be revealing they are not easy to set up with volunteers. In view of the changes

demonstrated by Dettbarn and his co - workers, although at higher levels of AChE inhibition than in the studies reported, an ultrastructural examination of human end plates after anticholinesterases seems an important future study.

10.3.2 Degrees of AChE inhibition produced by pyridostigmine

The Ellman method is regarded as being the most accurate available for the analysis of AChE (Ellman et al, 1961) and has been adopted as the standard technique at Porton Down. The method can estimate red cell AChE and may be corrected for the individual subject variation in PCV. The resulting corrected red cell AChE levels have been used as a marker of enzyme inhibition in these studies. At a steady state while taking pyridostigmine 30 mg eight hourly, the mean level of corrected red cell AChE two hours after a single dose is 58.5% of normal. GB Ct5, by comparison, produces a level of 84% of normal. Two points concerning AChE estimations in these experiments require comment. Firstly, the reversible complex formed by pyridostigmine, and with all carbamates, breaks down at a significant rate compared with the time taken to produce an analysis of AChE. Thus Dettbarn and his co - workers (section 4.2) felt unable to give an accurate AChE level in blood during carbamate inhibition. The method used in these studies was however regarded as being fast enough to enable data about AChE inhibition by pyridostigmine to be provided at various points in the protocol. With GB inhibition of AChE the problem does not arise since the rate of reversibility of the inhibition is very slow.

The second point about measurement of the AChE levels in these experiments is that there is no clear relationship between the corrected red cell values

obtained and the true value of the end plate AChE .The latter can only be estimated using a tissue homogenised in vitro . Although, ideally, the end plate AChE should have been measured in our experiments it was feasible only to measure red cell values. Thus any interpretation of the data in cholinergic terms should bear in mind possible discrepancies between the two sites of location of AChE. The resolution of the effects on the recently recognised 4S, 10S and 16S forms of AChE in red cells is unknown (section 4.1.3.4). It is apparent that the measurement of AChE in red cells can only be regarded as a crude indicator of values within the SKNMJ. It must also be remembered that anticholinesterases have other actions apart from their effects on the cholinergic system and observed physiological and pharmacological actions may not always be explicable in relation to AChE inhibition (section 1.4.2.2).

10.3.3 Pyridostigmine and SFEMG jitter

The results and analysis presented in section 8.2 show that pyridostigmine produces a minimal change in the jitter of the fibre pairs recorded. On a paired analysis the change in corrected mean MCD produced was less than 2 µsec. The change was not present three days after stopping pyridostigmine. Although this result was obtained from analysis of twelve subjects taking pyridostigmine two subjects had a significant increase in the proportion of fibre pairs with high jitter. In one of these there was also significant pyridostigmine - associated change in the mean jitter frequency. The high jitter pairs did not demonstrate any appreciable degree of blocking. The two subjects concerned were homogeneous in every respect with other volunteers and there appears to be no immediate explanation for the apparently anomalous findings. Muscle biopsy studies of their EDC mosaic

were not possible, but such a study might reveal an abnormality. Stalberg has shown that relatives of patients suffering from myasthenia gravis have subclinical jitter changes (Stalberg et al, 1976). Recently a congenital condition has been described where the AChR channel opening time is prolonged (slow channel myopathy, Engel, 1985). It may be possible that subclinial variants of this condition exist where a drug such as pyridostigmine might have a significant channel blocking effect, leading to increased jitter. Further SFEMG measurements on larger numbers of volunteers taking pyridostigmine may resolve the problem. Such a study is underway at the time of writing.

From the point of view of the classical postjunctional action of pyridostigmine the slight increase in jitter caused by the drug does not fit completely with the known observation that small doses of non - depolarizing relaxants also cause increase jitter (section 6.12.2). This finding was explained by Schwartz and Stalberg (1975) as being related to occupancy of postjunctional receptors. Since carbamate anticholinesterases, in low dose, tend to stabilize and prolong the EPP (section 3.4.2) and are so used to reverse the blocking effects of non depolarizing relaxants, it might be thought that, on a purely postjunctional analysis, pyridostigmine should stabilise any end plates with reduced safety factor. It seems likely that whatever mechanisms are offered in the future for the effects of pyridostigmine on neuromuscular jitter they will have to encompass the possible prejunctional electrophysiological changes mentioned in section 4.2.2 and ion channel blocking. Both these effects could lead to changes in the rise time of the EPP. This point is considered further below.

10.4 The effects of experimental GB exposure on SFEMG

10.4.1 Clinical sequelae of GB exposure

In chapter 1 the clinical effects of exposure to high doses of organophosphates were described. Most of the evidence presented comes from clinical studies of self - poisoning and is associated with degrees of inhibition of AChE of 70% or more. Experimental studies in man have only been conducted to a maximal degree of ACh inhibition of about 40% produced by GB Ct15. In the studies reported here, twelve volunteers were exposed to GB at the Ct5 level, six of them after pyridostigmine pretreatment, and a further eight were exposed to GB at the Ct15 level. At both dose levels subjects reported a subjective sensation of tightening in the chest during exposure, and at the higher dose level a significant miosis developed over the ensuing twenty four hour period. Subjects were both photophobic and dark sensitive. The ocular symptoms and signs resolved after 48 hours and all subjects were clinically normal on discharge from the study. There were no detectable long term sequelae in neurological examination of subjects up to one year after exposure. The clinical effects noted here are compatible with descriptions of the earliest stages of organophosphate poisoning described in section 1.5.

10.4.2 The effects of GB exposure upon EDC jitter

In the studies reported at both the Ct5 and Ct15 levels, GB exposure has been shown to have a

small but statistically significant effect on SFEMG jitter in EDC. GB Ct5, which produces 15% AChE inhibition, caused a reduction in mean jitter frequency of 6.5 ± 7.3 kHz (p = 0.08) at three hours post exposure, increasing to 9.3 ± 4.6 kHz (p = 0.004) three days after exposure. GB Ct15, which produces 40% AChE inhibition, caused a reduction in mean jitter frequency of 0.34 ± 4.64 kHz (p = 0.85) at three hours post exposure, and a reduction of 7.76 ± 4.93 (p = 0.006) at three days post exposure. These figures indicate that GB exposure caused increased jitter in the recorded fibre pair populations from EDC. In terms of AChE inhibition there was no obvious dose response relationship. At both exposure levels the mean jitter frequency changes, although small, reached a high degree of significance three days after exposure. The GB effects were revealed using both the conventional method of determining the incidence of high jitter and changes in the whole fibre pair population after reciprocal transformation.

10.4.3 Jitter changes in relation to the degree of AChE inhibition

The results discussed in the previous section may be compared with those for AChE inhibition caused by pyridostigmine discussed in section 10.3.4. At a comparable degree of AChE inhibition to that caused by GB Ct15 exposure, pyridostigmine pretreatment caused only a minimal increase in jitter. Pretreatment of subjects with pyridostigmine who were then subsequently exposed to GB Ct5 protected against the jitter changes seen in unpretreated subjects. Thus it appears that two anticholinesterases of differing types have different effects on the neuromuscular junction which are not directly related to their ability to inhibit AChE. This point is discussed further below.

10.4.4 Time scale of jitter changes induced by GB

We have seen that the jitter changes induced by GB at the Ct15 exposure level were not apparently time related. The shift in mean jitter frequency at three days post exposure was greater than that calculated from measurements made three hours post exposure and showed considerable ($p = 0,006$) statistical significance. Analysis of the GB Ct5 exposure however indicated that that a significant mean jitter frequency shift occured at three hours after exposure. Subject 063/84, who had an initially low control jitter frequency was atypical in that the mean jitter frequency remained low at three hours post GB Ct15 exposure but was normal at three days post exposure.

10.4.5 The possible effect of GB at the neuromuscular junction

In seeking an explanation for the action of GB on SKNMJ jitter not only the observed jitter change must be considered but also the increased blocking percentage. The results in section 8.2.4 and 8.3.4 have shown that blocking at raised jitter levels is seen with GB but not at comparable levels of raised jitter caused by pyridostigmine. This interesting finding may provide a link with changes seen in conventional EMG in workers chronically exposed to organophosphates. Roberts (1976) found that the mean height of compound EMG was reduced in this situation. Burgess (1985, personal communication) has produced evidence which confirms these findings. Reduced mean compound EMG height was shown to recover over a period of six months. Both these studies were retrospective and uncontrolled.

Since compound EMG is composed of a summation of

individual single fibre action potentials, a reduction in height, or evidence of fade in evoked compound EMG indicates increased blocking of members of individual motor units. The greater sensitivity of SFEMG might be expected to demonstrate this point in the industrial studies. In the experimental studies reported here blocking has been seen after both GB Ct5 and Ct15 exposures.

The nature of the GB - related lesion which produces jitter changes is a matter for speculation. Chapter 4 reviewed the electrophysiological and ultrastructural changes caused by organophosphate exposure in the rat. Preusser, (1967*), Fischer (1968 and 70*) and Ariens et al (1969) showed that rats injected with near lethal doses of DFP paraoxon or soman developed necrotic lesions in the end plate region which could be prevented by treatment with dTC. The work was extended by Dettbarn and his colleagues (section 4.1) by performing detailed electrophysiological and structural studies on end plates treated with paraoxon. Hobbiger (1976) commented that it seemed important to establish whether these necrotic lesions are produced by all anticholinesterases, how and where they are repaired and whether they might lead to some impairment of muscle function which would escape notice in acute experiments solely linked to testing effects on classical parameters of neuromuscular transmission. It is possible that SFEMG studies reported here may provide a subclinical indication of that muscle impairment. Few other SFEMG studies have been published for comparison. Stalberg (Stalberg et al, 1978) made a non - controlled retrospective study of workers involved with spraying organophosphate insecticides. No abnormalities were found but the nature of the study makes direct comparison with the present study impossible.

A detailed study of juvenile myopia in Japan (Saku disease, section 1.5.7) has been shown to be associated with chronic organophosphate exposure. A

*(Germ., quoted in Laskowski and Dettbarn, 1977)

parallel study using beagles has confirmed the development, at an equivalent dose of organophosphate, myopathic lesions in the ciliary muscle. The indications are therefore that organophosphate exposure, both chronic and acute may be causing hitherto unrecognised subclinical neuromuscular damage. The problem is to relate clinical observations with the experimental ultrastructural and neurophysiological findings from animal studies, and to extensive current knowledge about ACh release and combination with the AChR. SFEMG provides a useful and minimally invasive electrophysiological link for this task.

10.5 A model for possible anticholinesterase effects on SFEMG jitter

10.5.1 Introduction

To explain the observed effects of anticholinesterases on SFEMG it is necessary to examine the possible sites of action of the agents in relation to models of the genesis of the SFEMG parameters. Any hypothesis about the technique has to explain both the jitter and blocking phenomena. It must also take account of the possible contributions of factors outside the neuromuscular junction.
Considering the origins of jitter, Stalberg (section 6.6) concluded that the proportion of jitter originating from the terminal axons and from the variability of propagation of MAP along the muscle fibres was small in comparison with jitter originating from the neuromuscular junction. This only held however if the interpotential interval was small.
More recently, Davis et al (1985) have demonstrated the importance of variability of interdischarge interval causing variations in propagation velocity in fibre pairs with a large interpotential interval.
They have produced a computerised correction factor for jitter in these situations.
Under the conditions of the experiments reported here the steady activation frequency and small interpotential intervals recorded permit discussion of the jitter and blocking phenomena in terms of events occuring at the neuromuscular junction. For the following discussion this site is regarded as being the primary source of these SFEMG parameters.

10.5.2 The existing model for the origins of jitter

The statistical nature of the origins of the

EPP at the postjunctional membrane has been clearly demonstrated by thirty years of work using intracellular electrodes. Almost all such recordings have been made in vitro using animal muscle. However Elmquist et al (section 3.4.2.4) did record combined EPP and MAP from human intercostal muscle. These recordings showed that there was a variability in the rise time of the EPP to reach the threshold of firing of the muscle fibre. The variability was markedly increased in muscle from patients with myasthenia gravis. Stalberg concluded that this rise time variability could explain the jitter phenomenon (Stalberg, Trontelj and Schwartz, 1976). Blocking was viewed as being the failure of generation of a muscle action potential by an EPP which failed to reach the threshold value.

In computer simulation studies (Schwartz and Stalberg, 1975) it was shown that, theoretically, the rise time variability could come from both pre and postjunctional origins. Although the rate of generation of the EPP is a function of the number of receptor sites which are available to contribute to the depolarization, it could also be a function of the rate of release of the ACh from the nerve terminal. Stalberg did not discuss this important possibility further but Lundh et al, comparing jitter and EPP from muscle taken from rats exposed to botulinum toxin showed that increased jitter was associated with reduced amplitude of the EPP. In this condition (section 6.9.2) jitter and blocking were shown to be reduced by increased nerve stimulation and by drugs which increased the rate of ACh release from the nerve terminal. From their measurements Lundh et al (1977) reasoned that a reduced mean height of EPP would be associated with a wider range of its rate of rise. These authors therefore concluded that this factor was the explanation for the cause of jitter.

One further aspect of the conventional model of jitter may be mentioned. The existence of normal jitter was

explained by Stalberg in relation to the rising EPP reaching a threshold value that was subject itself to normal variation (figure 6.13). The muscle fibre action potentials are generated with a different latency. However the postulated variation in threshold value appears to receive no support in the literature and has not been discussed further by other workers in SFEMG.

10.5.3 Tests of the established jitter model

Since generation of the EPP is a function of the available ACh receptor sites at the postjunctional membrane and the release of ACh from the nerve terminal, any factor affecting these should in turn affect jitter. Such modifications can be provided by pathological processes and drugs. The current views concerning the origins of neuromuscular jitter may therefore be tested in the light of knowledge about such modifying factors.

10.5.4 Pathological modifications of jitter

Sections 6.8 - 6.10.1 reviewed the known pathological conditions which give rise to jitter and blocking. Conditions such as botulism and the Eaton Lambert syndrome which affect ACh release fit well with the concept of jitter being due to rise time variability and blocking as the result of an EPP of insufficient height. Thus increased nerve activation frequency and drugs which can increase ACh release produce an improvement in both jitter and blocking. In myasthenia gravis however the position is not so clear. This condition was originally thought to have a prejunctional lesion but considerable evidence shows that there is destruction of postjunctional receptors by an immune process (section 6.10.1.1). In contrast

to prejunctional lesions, increased muscle activation frequency in myasthenia gravis produces a worsening of the jitter and increased blocking (section 6.12.1.2). Although increased ACh mobilisation may occur in these circumstances it does not produce improvement in the jitter. This observation warrants further consideration. In myasthenia the reduced number of receptors will quickly become bound to ACh and will remain in that condition for the refractory period of the channel. Thus blocking may be expected to start at a lower level of jitter than in the prejunctional conditions and to bear a fixed relation to the jitter. In prejunctional conditions however, since the postjunctional receptors are normal the rising EPP although slow, will continue to rise to threshold value over a longer period but will have greater chance of reaching the critical value than in myasthenia gravis. Blocking in this situation will be a consequence of reduced ACh release and ACh removal by AChE. A possible conclusion from this argument is that blocking is an inevitable event in postjunctional pathology but is not necessarily so in prejunctional pathology.

The phenomenon of ACh release has recently been considered in an extensive review by Dunant (1985) which underlines the extreme complexity of the process and challenges some of the classical quantal ideas. From the viewpoint of SFEMG detailed information relating the generation of the EPP to ACh release is required and would form the basis of a useful further study.

10.5.5 Pharmacological modifications of jitter

The effects of the non - depolarizing blocking drugs such as curare on jitter and blocking were reviewed in section 6.10.2. Stalberg and his co - workers concluded that the safety margin at the

postjunctional site was being reduced by the drugs and the jitter increases seen were thus a consequence of reduced receptor availability. From the argument presented in the previous section blocking should therefore have been a relatively early feature accompanying the increased jitter. However, the evidence for the prejunctional actions of small doses of curare - like drugs (section 3.5.2) indicates that there may be activation frequency dependent reductions in ACh release. It may be therefore possible the the jitter changes seen were of prejunctional origin. In this case blocking would be expected to occur with higher jitter values and to be reduced by increased nerve activation. Thus the experiments with non - depolarizing drugs cannot in themselves confirm that jitter is a measure of safety factor in the postjunctional sense of the term as stated by Paton and Waud. However safety factor can also be viewed as an excess of ACh for a given number of receptors. Thus 'safety factor' should be specified as either pre or postjunctional in discussing processes which give rise to jitter alterations.

10.5.6 Further consideration of the jitter hypothesis

The SFEMG results presented in chapter 8 indicate that there is a small but significant effect of GB on jitter. An examination of the known characteristics of jitter and blocking in pathological conditions where the pathological process is established as being either pre or postjunctional may provide important clues about the site of action of organophosphate and carbamate cholinesterases at the neuromuscular junction.

10.5.7 Jitter and blocking in postjunctional pathology

Myasthenia gravis was originally thought to be caused by a defect in the release of ACh but is now regarded as being a consequence of reduction of receptor sites at the postjunctional membrane (section 6.12.1.1). Stalberg, Trontelj and Schwartz (1976) showed that above 75 usec blocking became significant in four untreated myasthenics. Twenty out of 27 recorded pairs showed blocking at a standard activation frequency of 15 Hz. Blocking was seen to increase significantly with increased activation frequency. The importance of blocking in SFEMG recorded in myasthenia gravis was recognised by Sanders et al (1979) who used blocking percentage of discharges recorded as part of their diagnostic index for the disease. In some cases of myasthenia the patient is unable to provide sufficient voluntary activation to produce a successful SFEMG recording. Schwartz and Stalberg (1975) established the association of blocking with myasthenia gravis using nerve stimulation to produce SFEMG rather than voluntary activation. All these studies have demonstrated the presence of SFEMG blocking in an essentially post - junctional condition and the worsening of blocking in recordings where where voluntary activation frequency (f act) could be increased.

10.5.8 Jitter and blocking in prejunctional pathology

In their study of two cases of human botulism Schiller and Stalberg (1978) commented on the high blocking percentage of discharges at low values of jitter with an activation frequency of 15 Hz. The blocking threshold was found to be as low as 65 usec

and the blocking percentage in one case was 60%.
Valli et al (1983) however found that, of 11 abnormal jitters recorded in 21 fibre pairs, five exhibited blocking with a threshold of 110 usec.
Henriksson et al (1978) who studied SFEMG in the Eaton Lambert syndrome again showed significant blocking at low jitter with a threshold value of < 55 sec. In the same disease however Schwartz and Stalberg (1975) showed that 21/35 pairs blocked in a three month study of one patient with a threshold of 140 usec. Cruz - Martinez et al (1982) did not indicate the blocking threshold in their results from one patient with Eaton Lambert syndrome but stated that 'jitter and blocking were worse after rest and improved with increasing activation frequency.' This statement emphasises the finding from all SFEMG studies in the Eaton Lambert syndrome, namely that increased f act produces decreased jitter and blocking. This is the reverse of the situation found in myasthenia gravis.

10.5.9 Blocking as a function of pathological process

The previous sections show that (1) prejunctional pathology is associated with significant blocking at low jitter in 2/4 studies; blocking and jitter are both improved by increased f act;
(2) in postjunctional pathology one study has shown a threshold value for blocking of 75 usec but all studies agree that jitter and blocking increase with increased f act.
From these findings it seems likely that (1) jitter threshold for blocking cannot itself be regarded as pathognomonic (2) blocking tends to occur at lower jitter values in prejunctional pathology and (3) a specified activation frequency is critical in all diagnostic evaluation.

10.5.10 EPP rise time and jitter:
modifications of the hypothesis

Given the differences between the effects of increased f act on jitter and blocking in pre and postjunctional conditions we may consider the jitter hypothesis further. A possible extension of the idea founding jitter on the variability of EPP rise time is shown in figure 10.1. Normal neuromuscular transmission involves ACh combining with the AChR postjunctionally. The safety margin is 4 or 5 to 1 and a depolarization of about 15 mV is required to reach the firing threshold. The distribution of EPP amplitude for repeated firings has been shown by Boyd and Martin (1956) to be distributed in a skewed way with peaks corresponding to between one and four times the value of the MEPP. It may be possible that the rates of rise of the EPP may too be regarded as following a similar distribution. In this view there is no need to postulate variability of the threshold level as being the source of normal jitter, as Stalberg has done (section 6.8.2).

Figure 10.2 shows the possible effect on EPP rise time when prejunctional pathology is present. Although the number of postjunctional receptors is normal, release of ACh at low f act is impaired. This gives rise to a widening of the distribution of the rising EPP as shown and possibly also an early incidence of blocking. Both effects are removed by increased release of ACh with increased f act and are therefore use - dependent.

In the case of reduction of receptors at the postjunctional site, shown in 10.3, the safety margin may be severely reduced. In this situation only a few receptors will be available for combination with the transmitter. In any one neuromuscular junction the capacity of the synapse to deal with repeated

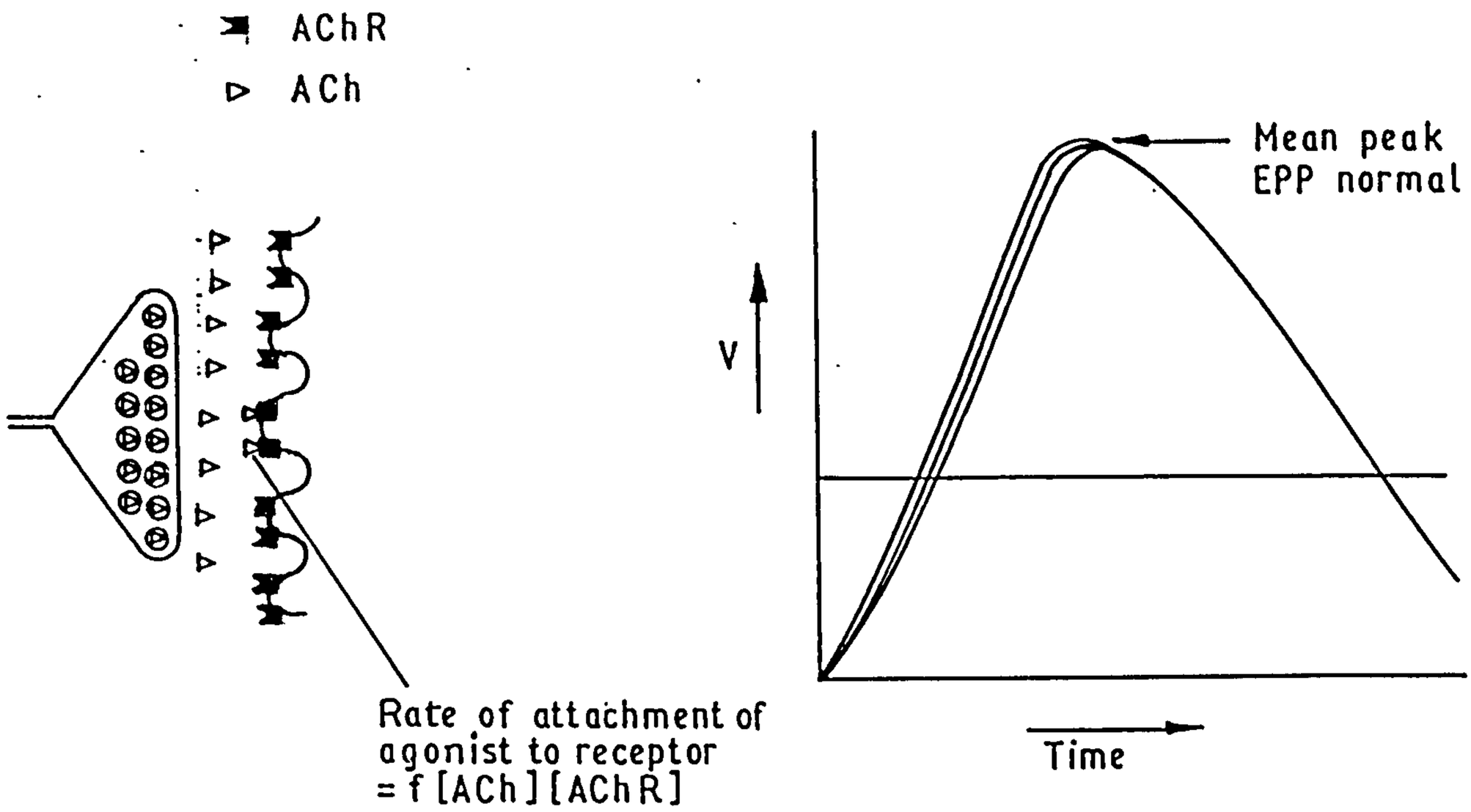


Fig.10.1 EPP rise time and MAP during normal neuromuscular transmission The generation of the EPP is a function of ion channel opening caused by combination of ACh released from the nerve terminal with the AChR at the post-junctional membrane. Since ACh release is subject to statistical variation with time it follows that the rate of EPP rise will be subject to a similar variation, causing a latency in the generation of the MAP. This is detected as SFEMG jitter.

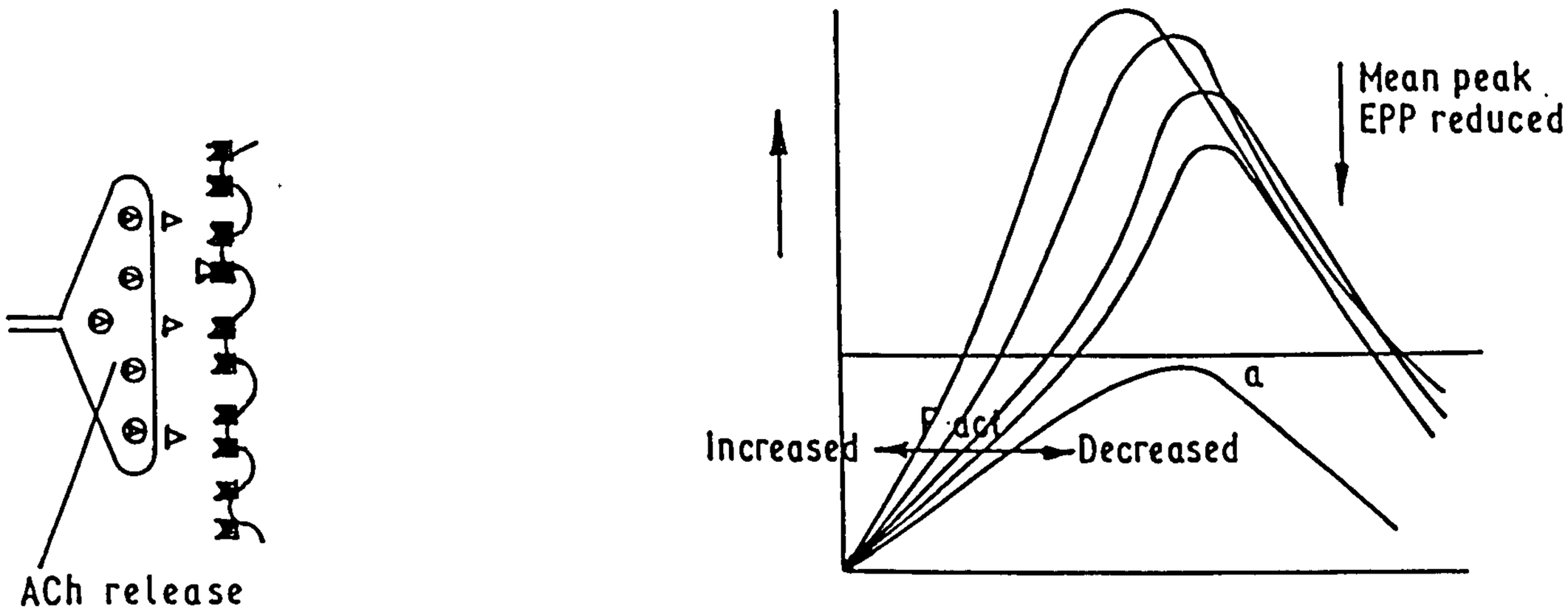
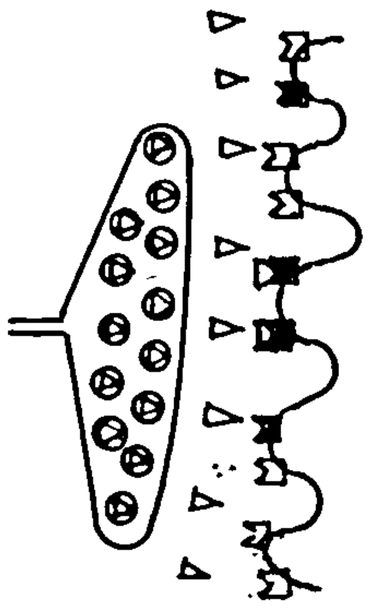


Fig. 10.2 EPP rise time and MAP during defective ACh release. ACh reaching the AChR opens the ion channels normally but local [ACh] is reduced. The distribution of the rate of EPP is widened and is a function of the rate of activation of the nerve terminal (Fact). The greater variability is latency is reflected as increased jitter. At (a) the EPP has failed to reach the firing threshold. Increasing Fact causes greater release of ACh and a consequent shift of the EPP rise times and hence jitter towards normal.



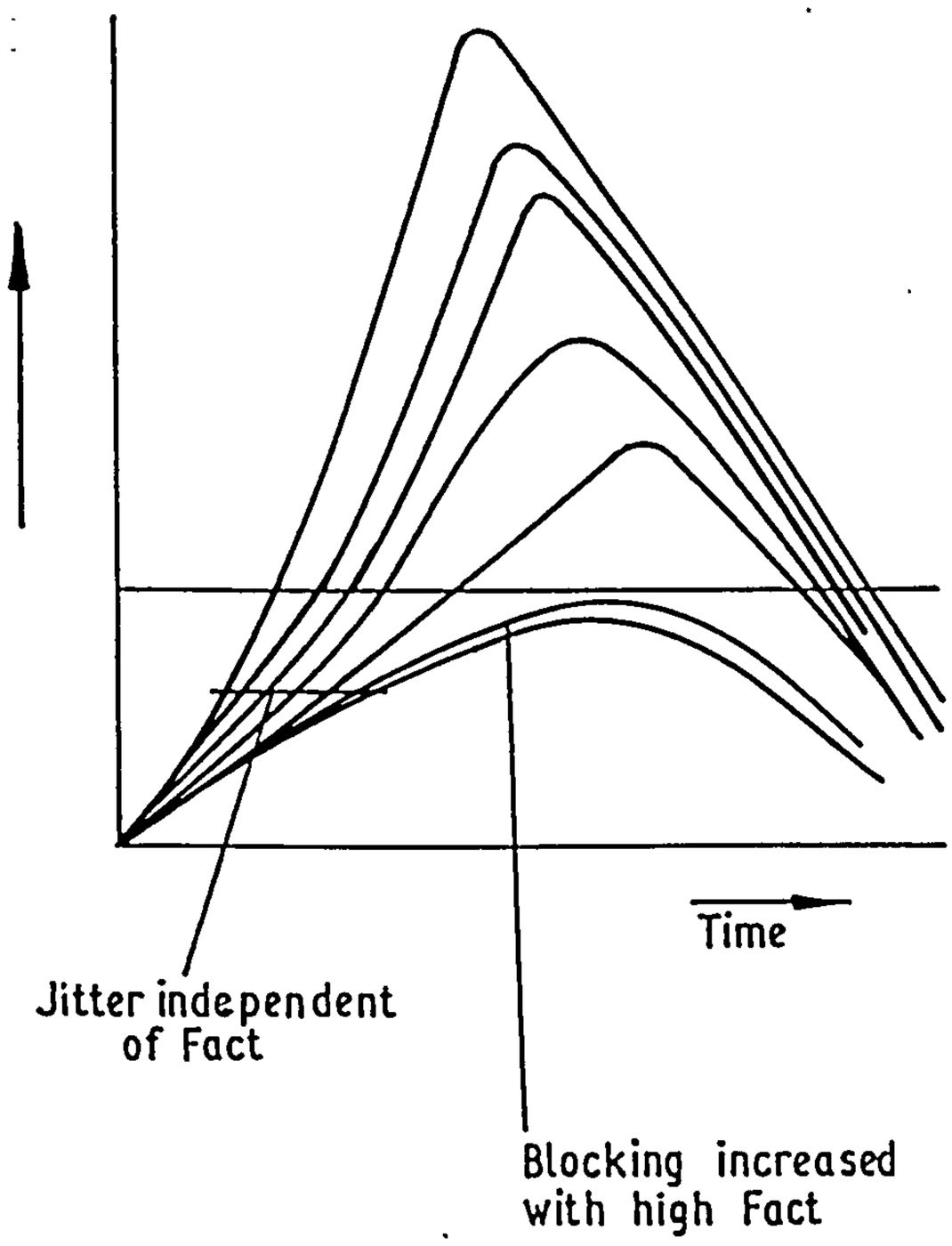


Fig. 10.3 EPP rise time and MAP with reduced numbers of normal ACh R. The normal safety margin of neuromuscular transmission is reduced. With fewer ACh R available to combine with ACh the distribution of the rate of EPP rise time is widened, leading to increased jitter and blocking. Increasing F act to produce a greater [ACh] does not correct the SFEMG. abnomalities and blocking is increased at high activation rates (see text)

stimulation will depend upon remaining available AChR if the occupation time of the receptors by ACh becomes significantly long with respect to the time between stimulations. At a critical point, determined by both receptor availability and firing rate, the junction must block. Up to this point the EPP height may vary according to a skewed normal distribution leading to increased jitter. This situation therefore leads to increased jitter and block with increased stimulation. The jitter modification due to loss of AChR may also be viewed in terms of the classical and prejunctional positive feedback hypotheses of ACh release. On the classical model, with repeated stimulation ACh release is decreased and with a reduced safety margin generation of EPP becomes more difficult. On the positive feedback model where ACh release is maintained, the refractory time of the ACh - AChR complex may be important. With fewer AChR available EPP rise time variability will increase with increasing f act. As the system approaches saturation there will be increased jitter and blocking.

10.6 The effects of anticholinesterases on the relationship between jitter and blocking

Having considered the origins of jitter and blocking in terms of both pre and postjunctional factors we may now look for possible explanations of the observed effects of pyridostigmine and GB presented earlier. It is known that anticholinesterase drugs such as pyridostigmine and neostigmine have the ability to improve muscle power in myasthenia gravis (section 2.4.1). To understand why a drug which prolongs the concentration of ACh at the postjunctional membrane should be able to do this, particularly in view of the receptor time factor suggested as being important in the previous section

it is necessary to look at the nature of the end plates on any myasthenic muscle. If all end plates had the same reduction of safety factor, any increase in activation should, from the argument of section 10.5.4.4 produce block in all of them. Moreover, since the receptors might become saturated and refractory with increased f act any increase in the concentration of ACh at the postjunctional site should have no effect. However, the evidence is that in any myasthenic muscle there is a spectrum of safety factors because each end plate appears to be affected differently by the disease (section 6.12.1.1). It is possible for some end plates not to be affected at all and for normal and compromised end plates to co - exist within the same motor unit. In terms of muscle fibres firing to a repeated stimulus therefore, the situation may be represented by figure 10.4.a. With the presence of anticholinesterase at any set f act the population of fibres induced to fire may be shifted to the right. Hence in figure 10.4b there will be less fade at the end of the train , and this will be represented by increased muscle power. This overall situation must be distinguished from SFEMG measurements in muscle fibre pairs where anticholinesterase will only improve jitter and blocking with increased f act if there are sufficient receptors available for combination with the increased ACh concentration in the time available. With very poor safety factors the improvement could therefore be minimal. The SFEMG appraisal of the situation after giving anticholinesterase will depend upon sampling of several fibre pairs. For a large number the jitter profile should shift to the left, as shown in figure 10.5.

10.6.1 The effects of pyridostigmine 30 mgm tds and GB on jitter and blocking

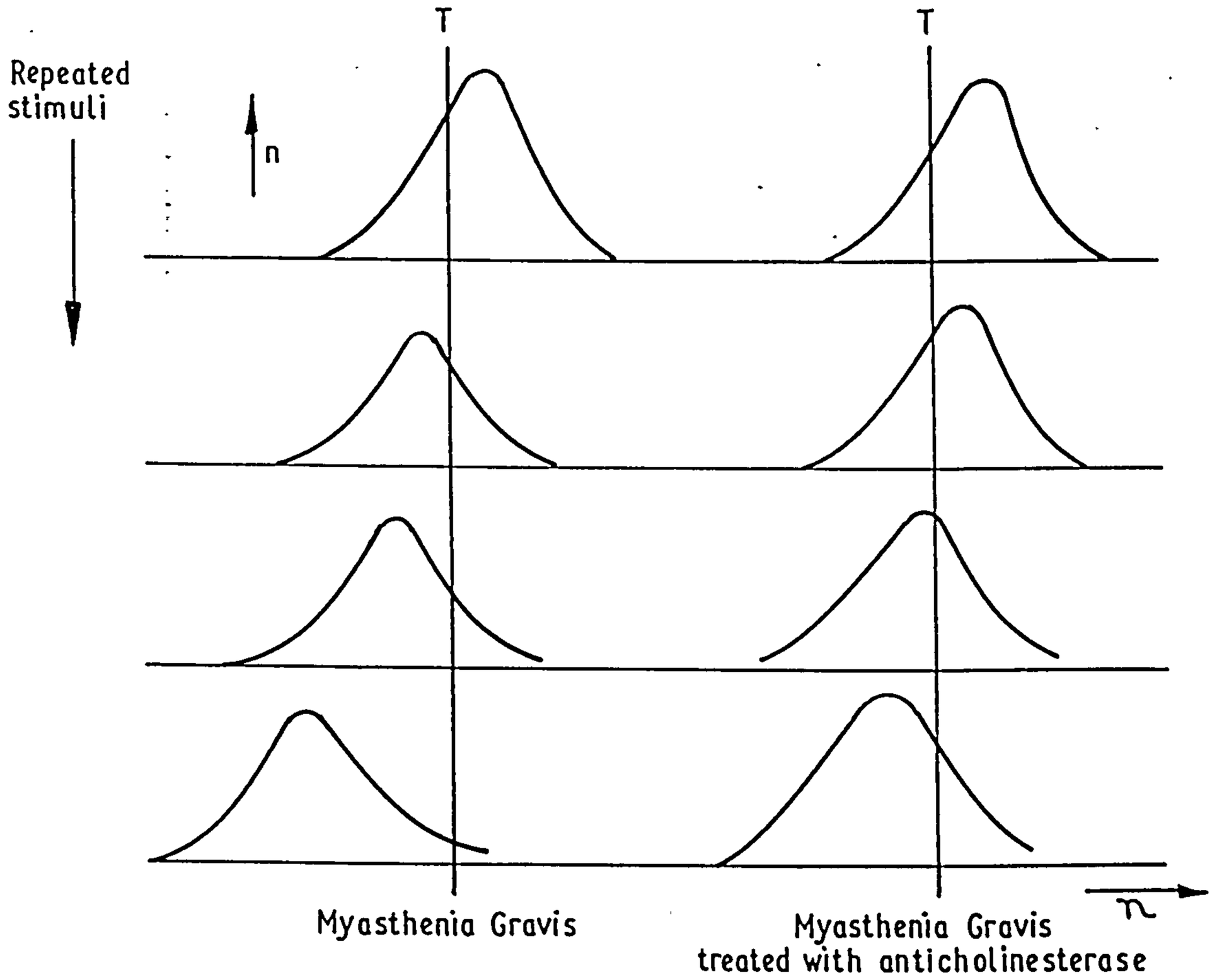


Fig. 10.4 Diagrammatic representation of the number of end plates (n) in a myasthenic muscle reaching the firing threshold during repeated stimuli. In (a) an increasing number fail giving rise to progressive weakness. In normal muscle the safety margin is such that all median points are above threshold. In (b) the increased lifespan of ACh in the presence of AChE causes an increased chance of any one end plate reaching threshold despite a reduced safety margin. The distributions on repeated stimulation are consequently shifted to the right.

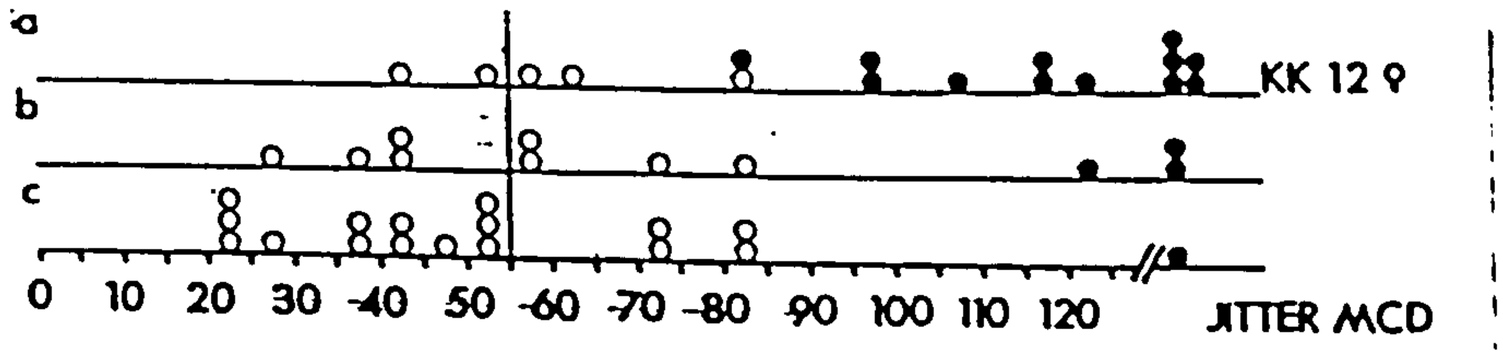


Fig. 10.5 Jitter profiles from muscle affected by Myasthenia gravis (a) before and (b) after anticholinesterase treatment. (Taken from Stalberg and Trontelj, 1979).

The results presented in chapter 8, analysed have shown that pyridostigmine causes only a very slight change in the jitter frequency of all volunteers who received it. In two subjects, where high jitter MCD values were recorded following pyridostigmine, blocking was absent in every case. GB Ct15 exposure however, which produced an equal degree of AChE inhibition was associated with the development of a significant increase in jitter after three days, accompanied by blocking. These effects were also seen after exposure to GB Ct 5 which produced only 15% AChE inhibition. The following conclusions emerge from an analysis of blocking after GB exposure:

(1) it was insignificant below 100 usec (section 8.2.4)

(2) above 100 usec there was a positive correlation with increased jitter (r = 0.69)

(3) the percentage of fibres showing a blocking fraction > 0.1 was 29% for GB Ct5 and 26.3% for GB Ct15, three days after exposure.

(4) the blockings were all seen at a fixed f act of 15 - 20 Hz. In isolated cases however where f act was varied deliberately jitter was shown to worsen with increased activation. We were not able to conduct a study of jitter and blocking variation with f act. This would form the basis of a useful future project.

10.7 Possible explanations for the anticholinesterase associated SFEMG findings

10.7.1 Relation to ultrastuctural changes

The results of the study indicate that there is a time - dependent change in the jitter

profile of EDC in volunteers exposed to low concentrations of GB. There was however no detectable dose response relationship. The failure to demonstrate such a relationship may reflect the small number of volunteers who were exposed to GB Ct15. One subject (063/84) who was not included in the analysis had changes in jitter in the opposite direction from the others. He was excluded because of his abnormal control values. However, the possibility remains that apparently homogeneous subjects may not be so in terms of fibre type and their SFEMG responses to anticholinesterases. More subjects are required to clarify this point.

A small overall jitter change was detected following treatment with pyridostigmine although two subjects had a more detectable response to this drug. Pyridostigmine itself protects against the jitter change associated with GB exposure at the Ct5 level. These findings are seen against the background of a large body of evidence indicating that anticholinesterases can cause structural changes at the neuromuscular junction (sections 4.1 and 4.2). The changes in animal models have been linked to possible postjunctional accumulations of ions following the persistent opening of ion channels by abnormally high concentrations of ACh (Dettbarn, 1984). While this may be one explanation it should not be forgotten that organophosphates have biochemical actions other than that on AChE. Notable amongst these is the known attack on serine linkages (Marquis, 1985). The action of GB at the end plate receptor site may therefore be a direct one which alters the ability of the receptor proteins to produce a open channel configuration (section 3.3). This may render the receptor partially or totally inactive. The GB Ct5 study showed more significant jitter changes following GB alone, which caused only 15% inhibition of RBC ACh, than following pyridostigmine, which caused 40% inhibition. The jitter change was

sustained when GB Ct15 caused 40% inhibition, indicating that the jitter change was agent, rather than AChE specific. This view must be qualified by the knowledge that the relationship between RBC and endplate AChE is not known. It is possible that chemically different anticholinesterases inhibit AChE to a variable degree at different sites. The importance anticholinesterase action on different types of AChE (section 4.1.3.4) must also be considered. In relation to the animal work described in chapter 4 it must also be remembered that the degrees of ACh inhibition were about twice those induced in the experiments reported here and often sustained by repeated doses of organophosphate.

10.7.2 Relation to electrophysiological changes

Knowledge about the relationship of the observed jitter change after pyridostigmine and GB to electrophysiological changes at the SKNMJ is limited by the lack of experimental data on EPP rise times. Linking the rate of rise to pre and postjunctional factors is difficult. Stalberg and his colleagues produced a hypothetical computer simulation but no algorithm appears to exist which relates rise time to factors such as ACh release and postjunctional AChR availability. The evidence reviewed in chapter 4 shows that rats given carbamates exhibit (1) MEPP frequency unchanged or reduced initially (2) quantal content reduced or unchanged and (3) reduced MEPP size, with one study showing reduced EPP height. Taken with the observation of necrosis and reduced numbers of AChR the results indicate the possibility of long term jitter change but of little short - term disturbance. Paraoxon on the other hand produced short - term pre and postjunctional changes of increased MEPP frequency and raised EPP height indicating increased ACh release. Longer term actions

were predominantly those of postjunctional change associated with fibre type - related myopathic change. The organophosphate - related electrophysiological changes taken with the structural changes indicate a progressive electrophysiological modification which may be compatible with the jitter changes seen afer single exposure to GB seen on this study. Section 4.1.3.3 showed that there was evidence of a difference between the recovery of pre and postjunctional lesions associated with organophosphate exposure. It is possible that both factors may act antagonistically in the early period after exposure with the return to normality in one revealing the lesion in the other as increased jitter. An electrophysiological study of the pre and postjunctional factors concerned with the rise time of the EPP is required to resolve this problem. At present, it appears that there are connections of anticholinesterases with electrophysiological changes that may be detected as SFEMG abnormalities, but further animal work, at lower levels of AChE inhibition is required to elucidate the nature of the links.

10.8 What is the clinical significance of the anticholinesterase related jitter changes?

The studies with GB and pyridostigmine have shown small but statistically significant changes in SFEMG. There have been no neurological or other clinical sequelae from either of the two anticholinesterases. The clinical significance of the results would appear to be slight but may be the first indications of changes that would be clearer at higher dose levels. The clinical significance of the jitter changes associated with GB exposure must be viewed in the context of jitter changes in established clinical conditions (section 6.11 and 6.12). SFEMG is not a diagnostically specific technique and all jitter

changes must be considered in the context of the overall clinical presentation. Myasthenia gravis in remission, for example, will still demonstrate a change in jitter profile even though clinical signs may be absent. SFEMG has been suggested as a way of detecting potential relapse and possible pre or subclinical myasthenia (Stalberg et al, 1976).

Pharmacologically - related jitter changes are usually temporary and totally reversible. From Dettbarn's studies (section 4.1) it is possible that even the low GB Ct15 dose may be causing end plate damage. To detect this a study of AChR antibodies might be feasible in human volunteers but a more invasive muscle biopsy and electronmicroscopic study might be harder to justify to an ethical committee.

Further studies are taking place using a rat model and evoked SFEMG in extensor digitorum longus. This study can be conducted to high degrees of AChE inhibition and will be accompanied by electronmicroscopic studies of the endplate. The advantage of this study is that, by using evoked muscle stimulation, the temporal variability of the MAP can be studied at any defined section of a train of stimuli and just at random, as in the clinical SFEMG recording. The aims of the animal studies are to examine the dose respone relationship of the GB effects on SFEMG and to attempt to correlate these with ultrastructural changes.

10.9 Conclusions

In summary, the SFEMG studies reported have shown jitter changes in EDC in man following small doses of GB. The doses were not sufficient to inhibit AChE to an extent which would produce any neuromuscular signs or symptoms. The jitter changes were not observed when AChE was inhibited to the same degree by pyridostigmine. Treatment with pyridostigmine 30 mg t.d.s prevented the jitter change

associated with exposure to GB Ct5. The clinical significance of the results is uncertain but should be viewed against the considerable background evidence of the actions of anticholinesterases on the ultrastructure and electrophysiology of the neuromuscular junction.

It is concluded that pyridostigmine pretreatment causes no significant subclinical neuromuscular sequelae.

10.10 The effects of pyridostigmine pretreatment on the degree and assessment of induced muscle relaxation

10.10.1 Practical considerations in comparing EMG and MMG

Chapter 5 reviewed the methods currently in use for assessing muscle response to single and repeated stimuli. In practice, the information about the the responsiveness of a muscle is governed by (1) the detection method used (2) the type of nerve stimulus presented to it. The study reported in chapter 9 has used both isometric mechanical and electrical methods of measuring muscle activity. Previous studies have shown that only in certain circumstances are the EMG and MMG related to each other (section 5.5). For a proportional relationship to apply, measurements must be made under isometric conditions (section 5.2) and under conditions where the muscle contractile mechanism can be expected to function properly. It is known that certain drugs and anaesthetic agents can interrupt the actin - myosin system, causing a progressive failure of contraction, although the individual MAP are conducted normally via an intact neuromuscular transmission mechanism.

Isometric conditions are not necessary for the measurement of muscle activity electrically. In practice, electromyography can be used to measure muscle response in situations where direct twitch measurement would be impossible. Section 5.5.1 showed that EMG recording of almost any muscle can be achieved by placing a recording electrode in proximity to the contracting belly of the muscle and recording with respect to a distant point, usually over the muscle tendon. Certain factors can however influence the EMG assessment of muscle activity. The first is the shape of the compound waveform which is modified

by the bandpass of the filter in the recording amplifier. The settings must therefore be carefully specified. The second point of error is in the way the height of the waveform is measured. Section 5.5.1 pointed out that both single and double peak heights have been used, together with integration of the wave envelope. The success of the integrative approach relies heavily on the waveform not changing shape as the amplitude decreases. In the present study this was not always found to be the case and so single peak height was adopted for EMG measurement, being a convenient measurement which could be translated easily into a single line in the MS6 myograph using the continuous mode recording in the repeater oscilloscope.

The isolated forearm method has been used in the past to assess muscle relaxation either by mechanical or electrical means. In the experiments reported in this study both methods have been used simultaneously, in non - anaesthetised subjects, enabling comparison of the recorded responses. The method of mechanical recording enabled frequent checking of the preload to be made to ensure that strict isometric conditions were maintained. The experiments have shown that the method can successfully be used in human volunteers receiving supramaximal stimuli to the ulnar nerve at 2 Hz over 2 seconds for periods up to an hour.

10.10.2 Frequency of stimulation

Section 5.3 discussed the essential importance of the frequency of the challenge stimuli and the preconditioning effect of the time interval between the repeated trains of stimuli.

Physiologically, all muscles are controlled by a tetanic stimulation with more motor units being recruited as the frequency of stimulation increases. Average stimulation frequencies in man vary according

to the muscle considered. EDC, under the minimal effort required to record SFEMG, is activated at 15 - 20 Hz while diaphragm is activated with rhythmic bursts of activity at 50 Hz. In assessing muscular activity in the passive situation, for example during general anaesthesia, external stimuli must be used. The question then arises about the pattern of applied stimuli used which most closely mimics normal physiological activity. Suggestions have been made to use tetanic stimuli at between 30 and 100 Hz but the stimulus pattern which has been most widely adopted is the train of four stimuli at 2Hz (section 5.3.1). This test has been studied extensively and is particularly suitable for studies on conscious subjects, since the degree of discomfort produced is minimal. All tests of fade in clinical neurophysiology are subject to preconditioning by a previous impulse. The phenomenon of post - tetanic facilitation of twitch in a normal muscle and post tetanic decurarisation in a partially paralysed muscle are examples of artefact in measuring true neuromuscular transmission. These are avoided by using the train of four test. An interval of ten seconds between stimuli has been found to be sufficient to allow for the ACh mobilization in the nerve terminal to return to normal and therefore standardize the postjunctional response.

Fade of response following a train of four stimulus is not seen electromyographically in normal muscle (Lee, 1975). This finding has been confirmed by the results of this study. The apparent mechanical fade of normal muscle when assessed by the MMG technique has also been confirmed. There is therefore a disparity between fade measured electrically and mechanically. The reason for mechanical fade during control conditions lie in the partial failure of the muscle

contraction mechanism itself and not in the neuromuscular transmission, which is guarded by the safety factor of the excess of postjunctional receptors.

10.10.3 Relationship of MMG and EMG

From the subjects studied in the two studies reported here it has been possible to compare MMG and EMG assessment of the activity of adductor pollicis using (1) the degree of relaxation of the muscle by comparison of the first response of each TOF with the height of the appropriate control response and (2) the degree of fade at a set time after the injection of a non - depolarizing relaxant. The results have shown that, while there is good agreement between the two techniques in estimating the first response, the relationship between the two methods in estimating fade is poor (section 9.1.6). Several previous groups of workers have examined the relationship between MMG and EMG in this context and have arrived at variable conclusions (section 5.5). The present results show that measurements of muscle fade measured mechanically or electrically cannot be regarded as being equivalent, nor can they be used interchangeably. The relationship between TOF during recovery from anaesthesia and clinical signs such as head raising and tidal volume has been discussed by Viby Mogensen (1982). His findings, based on mechanical TOF measurements are widely used in assessment of residual paralysis post - operatively. However, caution is required in applying TOF measurements from EMG in the same situation.
Lee (1975) has linked the disappearance of the third and fourth responses of the TOF with fixed degrees of paralysis induced by relaxant drugs. The number of impulses appearing is called the TOF count. This finding is widely used as a method of estimating

paralysis clinically without actually measuring the numerical TOF ratio. In the measurements reported here the third and fourth EMG responses of the TOF were not observed to disappear at any stage when the first was still detectable, an observation noted by other workers. Thus the application of the TOF count method to EMG data may lead to confusion in assessng the degree of clinical paralysis.

10.10.4 Relationship between TOF fade and first response

There have been indications in the literature that the fade/first response relationship established for the recovery phase of a muscle treated with non - depolarizing blocking drugs may not hold for the onset of paralysis (section 3.5.3). The results reported here (sections 9.1.2 and 9.2.3) have established for three such drugs that the degree of recorded fade is indeed dependent on whether recording is taking place during onset or recovery. The phenomenon, which gives rise to an hysteresis loop relating the parameters concerned, might be termed 'differential fade.' The finding is irrespective of whether the muscle reponse is recorded using MMG or EMG. In the first experiment, where the loop relationship was established for alcuronium, vecuronium and dTC attempts to fit a specific loop shape to the drug used failed on an assessment test made by simple visual scoring before the experimental allocation code was broken. It was concluded therefore that if such a relationship exists, the experimental design and technique was too insensitive to detect it. The placebo data from the second study showed that the pattern of paralysis in the isolated forearm can not always be repeated (section 10.10.1) which would tend to confirm this point.

Previous studies (Williams et al, 1980 and Pearce et

al, 1985) have detected differences in the rate of development of fade in patients paralysed during general anaesthesia. The experimental studies on non - anaesthetised subjects reported here have removed the possibility that the anaesthetic agents may, in some way, have been responsible for differential fade. Most of the work on clinical assessment of muscle fade has, of necessity, been conducted during general anaesthesia. In such cases a variety of techniques have been used which were dictated by operative considerations. This makes comparison of results between different groups very difficult. However, the straight line relationship established by Ali and his co - workers (Ali et al, 1971) has been widely adopted in clinical practice as a basis for monitoring neuromuscular recovery. As mentioned previously, uncritical translation of this finding into monitoring by other means may introduce significant error. Lee et al (1975) for example, showed that first response plotted against fade during recovery monitored by electromyography produced a curvilinear response in some cases. The present studies have shown that an approximately linear response exists during the recovery phase for the relaxants tested but that the slope is very dependent on whether MMG or EMG was used for the recording.

10.11 The effects of pyridostigmine pretreatment on differential fade

10.11.1 The repeatability of the IFP

The design of the study of the effects of the pyridostigmine pretreatment regime on the action of alcuronium involved the use of placebo pyridostigmine. This allowed comparison of loops from

five subjects which were essentially three repeated control sessions to see whether the IFP method is repeatable. Early studies of the technique did not clarify this point (Feldman and Tyrell, 1970). For a comparative study of the action of relaxant drugs the repeatability of the technique is important and is the main determinant of its sensitivity. Many factors determine the spread of the relaxant in the isolated forearm. Relative masses of fat and muscle will affect the pharmacokinetics of the distribution. The temperature of the limb is important, as is the degree of exsanguination before injection of the drug. Before starting IFP studies the spread of the relaxant, diluted into 40 ml of normal saline was observed in one case using isotopic labelling and gamma camera tracking. Use of the dorsal veins of the hand as an entry route was shown to give even distribution of the fixed drug throughout the occluded forearm. Injection higher up however, does not give such as satisfactory result (Brown and Charlton, 1975). Due to constraints of the experimental protocol we were not able to examine the repeatability of the distribution of labelled relaxant in the same subject.

The repeatability of the technique may be assessed from the first response against time plots of those subjects receiving placebo pyridostigmine before alcuronium 1.5 mg. For each subject three plots are available. The analysis of the data in section 9.2.2 shows that both in terms of peak paralysis and recovery IFP are not easily repeatable. Not all the subjects in the second study actually paralysed below 25% of the control with 1.5 mg alcuronium although the previous study had indicated that this would be a suitable dose. Since the second study was conducted on a double blind basis there was no mechanism for modifying the dose once the study had begun and insufficient volunteers to provide allocated replacements if sufficient paralysis did not occur.

The poor repeatability of IFP could not be attributed to experimental conditions or technique, both of which were standardised.
The possibility that alcuronium has a cumulative agonist action at the AChR may be considered. If such an atypical action were to have a significantly longer half life than the conventional antagonist action, the time period allowed in the protocol for washout of the drug may not have been adequate. Such a possibility can only be speculative in terms of the experiments reported here.
If the results are significant, explanation in terms of conventional neuromuscular theory is difficult.

10.11.2 The effect of pyridostigmine pretreatment on the degree of muscle relaxation produced by alcuronium.

The curves of first response against time shown in figure 9.10 and the peak paralysis values shown in table 9.5 show no significant effect of pyridostigmine pretreatment on paralysis caused by alcuronium in the isolated forearm although there is a small reduction in the time taken to reach maximum fade. In terms of the classical action of the anticholinesterase at the postjunctional ACh receptor the degree of resistance anticipated would depend on the degree of AChE inhibition. The receptor occupation by non - depolarizing muscle relaxants is competitive, so the local increase in concentration of ACh caused by the carbamate tends to overcome occupation of the alpha protein moeties by alcuronium. In terms of simple mass action this means that at any moment a receptor is more likely to be occupied by ACh, which puts the receptor into an open state (section 3.3) than by alcuronium. There is therefore a greater chance of the end plate potential reaching

the threshold of depolarization and for the muscle fibre which it controls to fire. In practical terms, this physiological event is expressed as a reduction in the degree of paralysis produced overall by the muscle relaxant. In normal clinical practice, where carbamate anticholinesterases are used to reverse neuromuscular paralysis this effect is observed as a matter of course. The results given here seem to indicate that there is no difference in the effect of pyridostigmine on the first response if the anticholinesterase is given in the pretratment doseage before the relaxant. This observation however requires qualification because of the unexpected placebo pyridostigmine findings. Since pyridostigmine, by its action on AChE, presumably causes an increase in ACh concentration at the postjunctional membrane there is at least a theoretical possibility that the drug may cause weakness through a prolonged depolarization block. Repeated doses of neostigmine have been noted to do this and Payne et al (1980) have warned against excessive use of the drug during reversal of non - depolarizing blockade. These authors did not publish the degree of AChE inhibition produced by neostigmine so direct comparison with the pyridostigmine data is not possible. However, since SFEMG measurements in volunteers treated with pyridostigmine 30 mg 8 hourly have shown only minimal changes there is no evidence that the pretreatment regime causes any depolarizing block or muscle weakness. SFEMG will detect these as increased jitter and blocking long before any frank clinical expression.

10.11.3 The effects of pyridostigmine pretreatment on differential fade

The data presented in section 9.2.3 show that, for subjects 069, 085, 086 and 092 who received

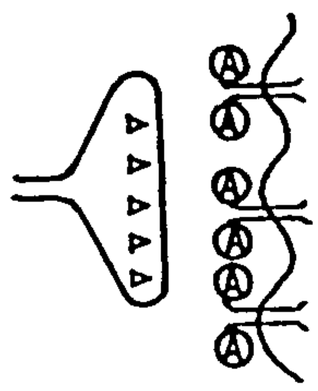
active pyridostigmine the differential fade pattern was not abolished by reduction of AChE. To what extent it may have been modified is difficult to determine owing to the limited sensitivity of the IFP. 4/5 subjects shown a reduction in loop size assessed by EMG and 2/5 asessed by MMG. Section 10.9.2 showed the poor relationship between EMG and MMG which may explain this discrepancy. The overall appearance is of a reduction in loop area. The rates of onset of fade shown in figure 9.13 show that in some subjects taking pyridostigmine the onset of fade during the IFP is delayed with respect to the control

10.12 Explanations for the differential fade phenomenon.

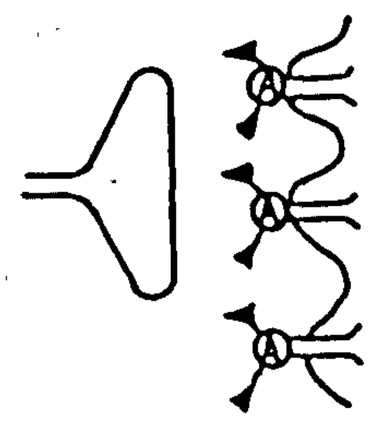
In the review of neuromuscular transmission given in chapter 3 the classical explanation of fade was linked to a gradual reduction in the amount of mobilized and released ACh during a train of repeated stimuli. With the excess number of receptor sites at the postjunctional membrane demonstrated by Paton and Waud (1967) the reduction in ACh release would not normally be noticeable in terms of numbers of EPP failing to reach the threshold level for firing of the MAP until about three quarters of the receptors were blocked by a non - depolarizing relaxant. Then, a proportion of the third or fourth stimuli may be expected not to reach threshold and the fibres would not fire. Scaled up into whole muscle terms, this fibre failure would be exhibited as fade. Section 3.5 summarized the alternative hypotheses of fade caused by non - depolarizing relaxants as (1) the blockage of the prejunctional cholinergic positive feedback receptor leading to a failure to sustain ACh release and (2) the blockage of ion channels by the physical presence of the molecules of relaxant drug after the

channels are activated by ACh (ion channel blockade). Any hypothesis of the differential fade phenomenon must explain why exposure of the neuromuscular junction to relaxants for a period of time allows observed fade to develop when initially it was absent. Bowman has suggested that the rates of attachment of the relaxant to the pre- and postjunctional cholinergic receptors are different, with the prejunctional attachment being slower. He sees ACh output as being maintained during a train of stimuli by positive feedback ensuring that roughly the same proportion of muscle fibres being stimulated will fire with each stimulus. If the postjunctional safety factor is removed however the sum total first response will be smaller than the control. In other words a fixed proportion of the muscle fibres will fail to fire with each stimulus and the muscle will appear partially paralysed but no fade will appear until the relaxant binds to the prejunctional receptor. The ion channel blocking view of these events is that as the attachment of the non - depolarizing drugs to the alpha receptor sites becomes less secure due to pharmacokinetic reasons the channels will come more under the influence of ACh which, acting competitively will tend to open the ion channels at the first of the train of stimuli. However, because the relaxant is still present in the vicinity of the ion channel the molecules will still be able to block the activated channel even though they are not present in sufficient concentration to combine with the receptors.

The effect of pyridostigmine pretreatment may be considered in terms of the hypotheses of fade presented. Figure 10.6 summarizes three situation in terms of the proposed mechanisms. Passive run down of ACh release cannot alone explain differential fade without the implication of another factor such as ion channel blockade. The effects of pyridostigmine on ion channel blockade may therefore be considered first. Pyridostigmine, by its action on the AChE at



Stimulus 1

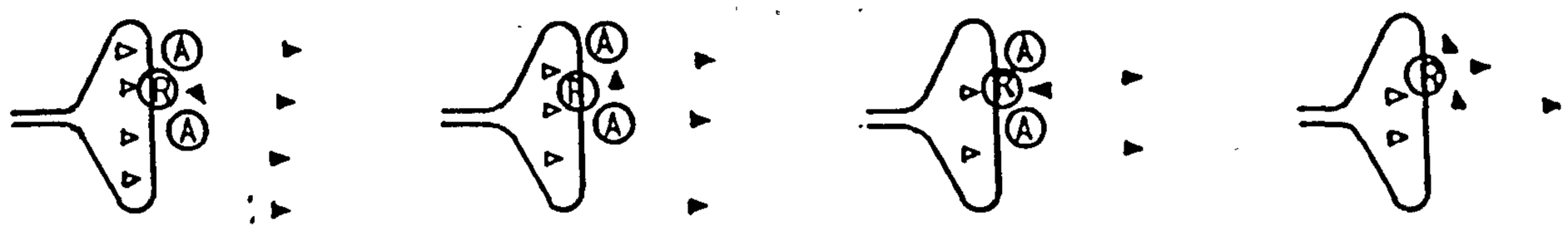


Stimulus 4

Ⓐ = Alcuronium

▲ = ACh

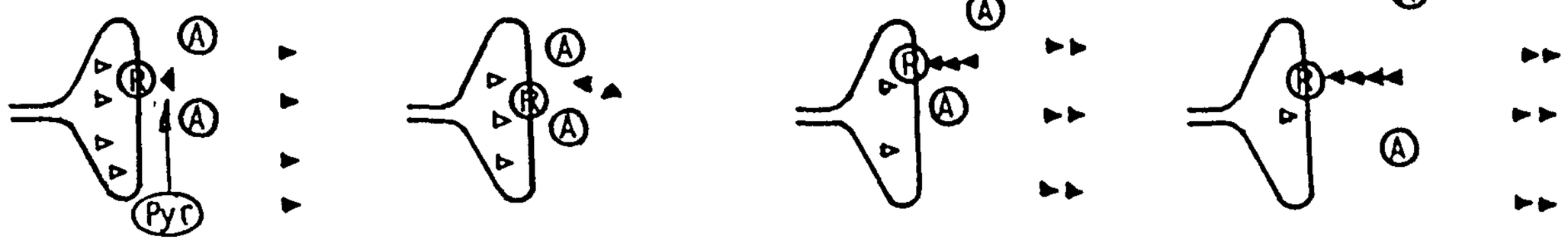
(a) Possible action of pyridostigmine on fade with ion channel blocking. Excess ACh at the AChR sites causes prolonged opening of the channels antagonising the blocking action of alcuronium (A). However, the relaxant molecules are present at the funnel-shaped mouth of the receptor and enter while the channel is in the open state. They cause interference with the ion flow and block in a use-dependent manner.



Stimulus 1 2 3

Ⓡ = Prejunctional positive feedback receptor

(b) Possible prejunctional action of alcuronium on ACh release in the absence of pyridostigmine. With repeated stimulation the ACh controlled positive feedback mechanism is blocked and ACh release progressively reduced in a use-dependent manner.



Stimulus 1 2 3 4

Ⓟyr = Pyrodistigmine

(c) Modification of the blocked positive feedback mechanism by pyridostigmine. [ACh] is increased in the prejunctional area immediately after release overcoming competitively the attachment of the relaxant mechanism to the prejunctional receptor. The positive feedback mechanism is thus restored.

Fig.10.6 POSSIBLE ION CHANNEL BLOCKING AND PREJUNCTIONAL RECEPTOR CONTRIBUTIONS TO FADE DURING TOF STIMULATION

the postjunctional membrane may be expected to cause an increase in the lifespan of ACh when first released and therefore antagonise the postjunctional actions of the non - depolarizing relaxant on a competitive basis. It would therefore be expected that for any given dose of relaxant after pretreatment the first response would be less. In other words, there would be a resistance to paralysis. However because the channels are more likely to be open in the presence of the excess ACh produced by pretreatment, according to the ion channel theory it would be expected that more channel blocking and hence fade would be seen during an IFP procedure. In other words, the differential fade phenomenon would tend to disappear. However, the results of these studies show that the differential fade effect is still present after pyridostigmine pretreatment.

We may now consider the effects of pyridostigmine on the prejunctional receptor theory. Pyridostigmine may be assumed to have an action at both the pre and postjunctional sites causing local increase in the life of released ACh. Thus, apart from the classic postjunctional activity there would tend to be a persistance of the positive feedback effect at the prejunctional site. Bowman's explanation of differential fade hinged upon the different rates of attachment of the relaxant molecules to these receptors (section 3.5.4). With repeated stimulation the normal positive feedback mechanism is blocked and ACh release is reduced in a use - dependent manner. In the face of increased prejunctional ACh concentrations therefore, it might be expected that the rate of attachment of relaxant at this site would be slowed. This might then be expressed as a delay in the onset of fade. From this hypothesis a shift in the differential fade curve would be expected but the effect itself would ultimately be seen.

The essential difference between the prejunctional receptor and ion channel hypotheses may be that, after

pyridostigmine pretreatment, on the ion channel explanation more fade should be seen for a given first response earlier in the IFP procedure whereas on the prejunctional receptor explanation less fade should be evident. The fade results presented here would seen therefore to support the prejunctional receptor theory. However, given the complex nature of neuromuscular transmission the true explanation of the differential fade phenomenon is likely to involve facets of all the hypotheses put forward so far. Further experiments in more sensitive animal and <u>in vitro</u> systems will be needed before further discussion is possible. Endplate current studies to trace the changes in activity after pyridostigmine pretreatment would be particularly valuable. At present the differential fade phenomenon must be noted as a clinical reality and care taken in the interpretation of TOF data during the early stages of partial paralysis induced by non - depolarizing relaxants. In general anaesthesia pyridostigmine pretreatment may be expected to have a minimal effect on degree of paralysis produced by subsequent administration of non - depolarizing relaxant but the interpretation of TOF measurements after pyridostigmine requires especial caution.

10.13 Conclusions

The study of the effect of pyridostigmine pretreatment on the action of non - depolarizing relaxants has produced the following conclusions:

(1) there is reasonable agreement between MMG and EMG in measuring the degree of muscle relaxation but not the TOF fade

(2) in all IFP recordings there was a difference in the degree of fade recorded during onset compared with recovery of paralysis; the resulting hysteresis loop has been termed differential fade

(3) pyridostigmine pretreatment at 30 mg t.d.s. produces little effect on the degree of paralysis produced by alcuronium but the fade characteristics may be modified.

Pyridostigmine pretreatment would therefore seem to present no contraindication to the conventional use of alcuronium in general anaesthesia. The studies show however that the established fade responses with respect to degree of paralysis need to be applied with caution, particularly if electromyography is used to measure muscle action.

Final Summary and Suggestions for Further Study

Experiments are reported which investigate the effect of taking oral pyridostigmine 30 mg t.d.s. on the single fibre electromyographic (SFEMG) jitter of extensor digitorum communis (EDC) in man and the effects on the pattern of subsequent muscle relaxation by a non - depolarizing relaxant in the isolated forearm. In addition, SFEMG studies are reported in man after exposure to GB Ct5 with pyridostigmine pretreatment and GB Ct15. Analysis of SFEMG changes was made (a) conventionally, by determining the incidence of clinically abnormal jitter values (> 55 usec) and (b) by using a new technique of reciprocal transformation which allows the change in jitter of a whole population of recorded fibre pairs to be assessed by parametric statistical methods. The transformation enables detection of small overall changes in population jitter which may not be revealed by conventional analysis.

SFEMG findings

(1) Control jitter values from 25 normal subjects in one study and a further eight subjects in the other were in good agreement and showed a skewed distribution of jitter values. The incidence of mean consecutive difference (MCD) values > 55 usec was 5%. If the values above 55 usec were truncated the distribution approximated more closely to normal with

a mean jitter value of 25.4 $\pm$ 9.3 usec. These findings are in accordance with those published for EDC by other workers. There was no significant difference between mean jitter values in EDC from two groups of measurements made in the muscle at an interval of two days.

(2) Pyridostigmine 30 mg t.d.s. produced only a minimal change in jitter, equivalent to a change in mean MCD of 1.7 usec, in 12 subjects. There were high jitter pairs seen in two subjects from this group but all changes returned to normal three days after stopping the drug, when acetyl cholinesterase (AChE) levels had returned to normal.

(3) Exposure to GB Ct5 produced a marginally significant increase in the incidence of clinically abnormal fibre pairs in unpretreated subjects three hours after exposure which became more significant after three days. These changes were also seen after reciprocal transformation of the jitter data. The change at three days, equivalent to a shift in mean jitter of 6 usec is small in comparison with SFEMG changes seen in pathological conditions such as myasthenia gravis which affect the neuromuscular junction. Pyridostigmine pretreatment protected against the changes in jitter produced by GB Ct5.

(4) Exposure to GB Ct15 produced significant changes in the proportion of recorded abnormal jitter values and in the mean shift of the jitter population but not in proportion to the increased degree of AChE inhibition caused by the exposure. In this study only the changes seen at three days after exposure were significant.

(5) Little SFEMG blocking was seen with high MCD values after treatment with pyridostigmine but more blocking was associated with GB exposure. Below 100 usec the incidence was not significant but above this value there was a positive correlation with increased jitter. Increased activation rate appeared to increase jitter and blocking after GB exposure.

Further SFEMG studies

The following studies are suggested to clarify the SFEMG findings of this study:

(1) A study of the distribution of control jitter values in relation to fibre type mosaic in a muscle. The possibility of end plates with different safety margins associated with fibre type sub - classes may explain the skew seen in populations of jitter. Such a study may reveal apparently normal subjects as having sub - clinical neuromuscular conditions.

(2) A study of repeated SFEMG recordings on the distribution of jitter in a muscle. Although there is some evidence of increased incidence of typically 'damaged' fibres in a repeatedly - recorded muscle, the stability of control distributions requires establishing for the future use of SFEMG as a pharmacological tool.

(3) A further study of larger numbers of patients taking pyridostigmine would be useful. If heterogenicity exists in muscle sensitivity the group of 12 subjects studied may have been too small to show up sub - group susceptibilities.

(4) Animal SFEMG studies are required to show dose

- time effects for a wider range of GB exposure than is possible in man. Studies could be performed in the isolated rat hemidiaphragm preparation which has been used by other workers for electrophysiological study after organophosphate exposure. This study should be accompanied by ultrastructural electron microscopic examination to look for possible pre and postjunctional changes at the GB doses used in the reported human investigations.

(5) An algorithm is required to relate rise time of the end plate potential with pre and postjunctional factors. The study of variability of rise time has not received as much experimental attention as the decay.

(6) Clinical SFEMG studies are required in patients exposed to organophosphate, both acutely and chronically. Such data may come from a country such as India where there is a high incidence of accidental exposure.

Isolated forearm study findings

Isolated forearm procedure (IFP) studies in 16 subjects have investigated the pattern of muscle relaxation produced by alcuronium, dTC and vecuronium and the effect of pyridostigmine pretreatment on alcuronium. All studies were performed on normal awake subjects without the complication of interference from anaesthetic agents. The findings were:

(1) Repeated IFP studies can be made in awake human subjects receiving train of four (TOF) stimuli every minute for up to one hour without undue

discomfort. TOF stimuli may be delivered every ten seconds for three minutes at the start of the experiment.

(2) Repeated IFP studies with alcuronium over a one week period have shown that there is considerable variation in response which limits the sensitivity of the technique as a pharmacological tool to examine the effects of drugs modifying the action of non - depolarizing blocking agents. The results may indicate a possible anomalous cumulative agonist action of alcuronium at the postjunctional membrane.

(3) Fade during TOF stimulation before the injection of relaxant is seen in mechanical (MMG) but not electrical (EMG) recordings. This reflects the artefact of progressive failure of the contractile mechanism with repeated stimulation.

(4) There was good agreement between MMG and EMG recordings in assessing degree of muscle relaxation from the first response of each TOF but poor agreement in assessing fade. This emphasises the caution which should be applied in the translation of mechanically - derived TOF relationships for predicting clinical relaxation to electrical measurements.

(5) The relationship between first response and TOF fade was different between onset and recovery of paralysis. Ths effect has been termed 'differential fade' and was seen for all three relaxants studied. No specific loop shape could be accorded to a relaxant by simple inspection after normalization of responses.

(6) Pretreatment with pyridostigmine 30 mg t.d.s. produced little effect on the degree and pattern of relaxation in the IFP. Use of non - depolarizing relaxants in subjects pretreated with pyridostigmine

who may subsequently require a general anaesthetic is therefore possible without loss of potency.

(7) Pyridostigmine pretreatment does not abolish differential fade produced by alcuronium but may modify it.

Further IFP and related studies

The following further studies are suggested:

(1) Study of the onset of TOF fade with respect to relaxation during general anaesthesia. Although the experiment would be complicated by possible interference from anaesthetic agents there would be no need for the IFP procedure since general paralysis supported by intermittent positive pressure ventilation is produced. Careful studies may reveal a characteristic shape of differential fade loop for a specific relaxant.

(2) Facilities and time available did not permit a computerized analysis of changes in the differential fade loops during the studies reported. An integrative analysis may reveal differences which were not apparent on simple inspection.

(3) The use of an animal model may allow examination of the differential fade phenomenon for a wide range of relaxants known to have differing actions at pre and and postjunctional sites. This may clarify the possible contributions of prejunctional cholinergic receptors and ion channel blockade in producing the effect.

APPENDIX 1: Reciprocal transformation of SFEMG jitter

A.1.1 Deviation of data from a normal distribution

During the study reported in section 8.1.1 421 technically acceptable fibre pairs were recorded during control SFEMG recording sessions in 25 subjects. These data were pooled to give the distribution of MCD values shown in figure 8.3. Figure 8.4 shows the data from figure 8.3 replotted with values of MCD > 55 μsec truncated. To examine the closeness of these distributions to the normal, data from figures 8.3 and 8.4 are plotted as conventional cumulative frequency plots in figure A.1a and A1b. In this method a normal distribution appears as a straight line. The marked deviation from linearity in both non - truncated and truncated data indicates a departure from the normal distribution in both. This departure is confirmed by examination of the skewness, kurtosis and Kolmogorov D statistics shown in table A.1.1.

A.1.2 Reciprocal transformation

The standard practice in dealing with data which do not fit a normal distribution is to subject them to a mathematical transformation and then examine the normality of the distribution of the modified

values. The most usual transformation with biological data is logarithmic. This approach was tried but produced no improvement in the data presented here. Reciprocal transformation however was shown to produce considerable improvement. In this process MCD values were transformed according to the relationship:

$$MCD^{-1} = 1000 / MCD$$

The reciprocal transform for each jitter value has the dimension of 1/T and may conveniently be expressed in kHz when the MCD is in μsec. The reciprocal MCD parameter has been termed 'jitter frequency.' Figure A.1b shows the distribution of data from figure 8.3 after reciprocal transformation. The mean value of jitter frequency is 43.3 $\pm$ 17.9 kHz (421) and the range of values is 3.2 - 100 kHz. The cumulative frequency plot of the transformed data, shown in figure A.1c, now displays little departure from linearity. Inspection of the statistics for the transformation shows that transformation produces marked reduction in the degree of skewness displayed by the original data and the values of kurtosis and Kolmogorov D are reduced to insignificance. In comparison with the statistics for the truncated distribution, reciprocal transformation is seen to produce a closer fit to a normal distribution while including all the technically acceptable data.

Statistic	Untransformed data		Reciprocal transformation All data
	All data	Truncated data	
n	421	401	421
	MCD (μ sec)		Jitter frequency (kHz)
Mean	30.11	25.37	43.31
SD	29.97	9.29	17.88
Range	10–315	10–54	3.2–100
Skewness	5.78, $p < .001$	0.69, $p < .001$	0.50, $p < .001$
Kurtosis	47.48, $p < .001$	2.91, $p = .36$	3.23, $p = .17$
Kolmogorov D	0.228, $p < .01$	0.096, $p < .01$	0.08, $0.01 < p < .05$

Table App.1 Statistics for control jitter data from fig. 8.3 after truncation and reciprocal transformation. The significantly reduced values of skewness and Komogorov D statistic after reciprocal transformation of all data points confirm the close approximation of the transformaed data to a normal distribution seen in fig. App.1(c).

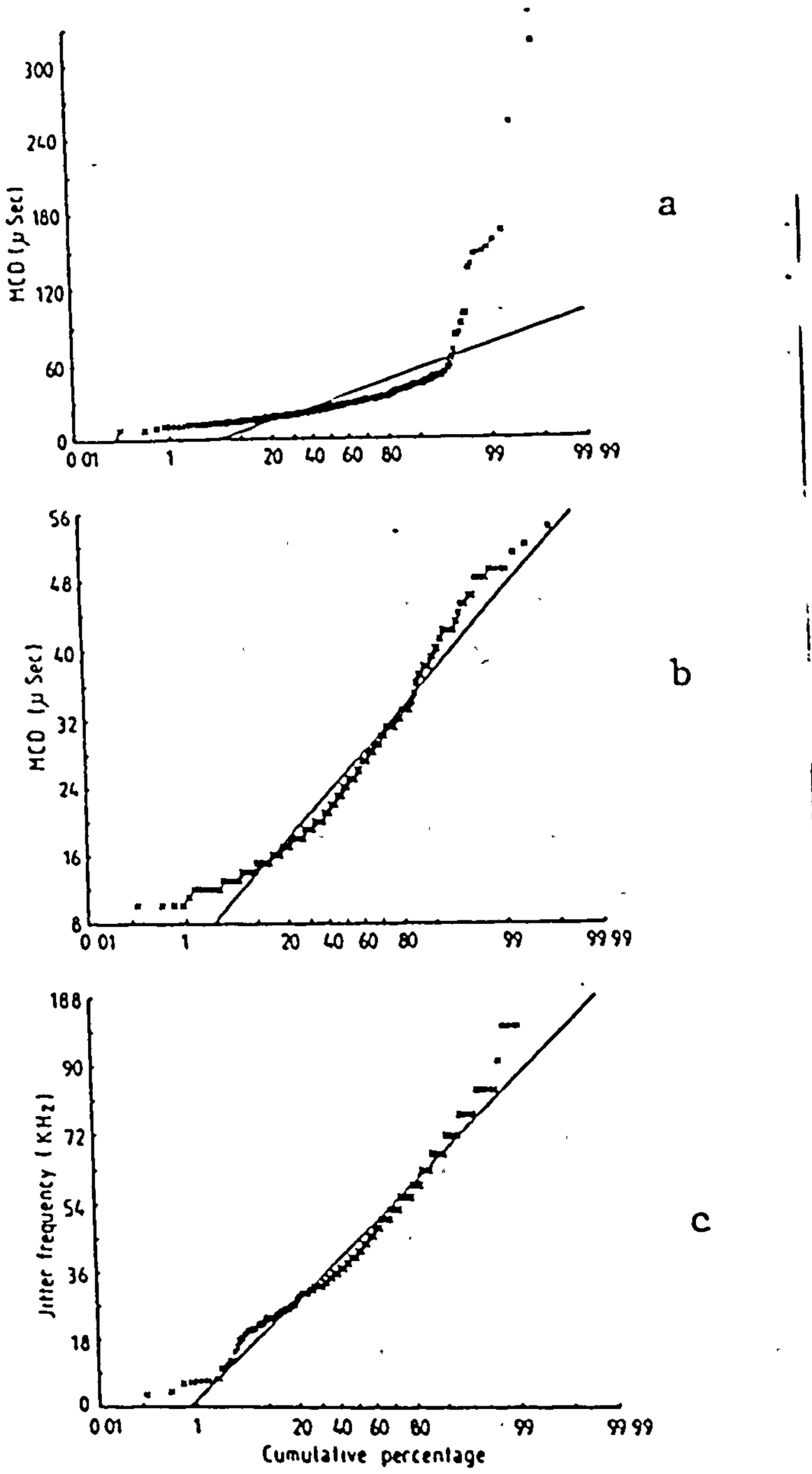


Fig. App.1 Effect of truncation and reciprocal transformation of the normality of the distribution of a jitter population. Control data from fig. 8.3 are plotted (a) unchanged (b) truncated at 55 μsec and (c) after reciprocal transformation on a cumulative frequency plot. The superimposed straight line is of a normal distribution. The close approximation of the reciprocal distribution, which includes all data points, to normal is seen in (c).

Illustrations

Figure A.1a Cumulative frequency plot of the distribution of MCD values presented in figure 8.3. The data do not follow a normal distribution. The non linearity of the data is evident in comparison with the superimposed straight line of a normal distribution.

Figure A.1b Cumulative frequency plot of truncated data in figure 8.4 Although high jitter values have been eliminated there is still a departure from a normal distribution.

Figure A.1c Cumulative frequency plot of reciprocally transformed data from figure 8.3 There is closer approximation to a normal distribution than in figure 8.4 although all technically acceptable data points have now been included. Comparison with figure A.1a shows the closeness to normal distribution produced by reciprocal transformation of the complete data set.

Figure A.1d Reciprocal transformation of data from figure 8.3. Transformed MCD values are shown as f(kHz) corresponding to 1000/usec.

Appendix 2: GB Ct5/pyridostigmine study;
MCD values for group PP

Treatment	Session / Subject	1	2	3	4	5
N11	44/83	19 23 27 27 38 22 31 33 34 48 49 12 17 18 20 20 25 28 48 15 15 18 21 30 30 13 34	Placebo 21 24 23 29 30 25 18 22 38 31 45 (68) 10 12 14 16 19 23 32 33 (136) 13 15 22 25 25 38 32 34 14 20 20 32 32 38 46 (80 82)	Pyrido 16 23 23 26 27 30 40 49 13 25 46 (96) 30 31 31 13 15 17 18 23 25 26 27 29 30 31 31 31 35 43 44 10 16 17 18 18 20 22 22 (55) 12 27 33 35 (80)	Placebo 17 23 17 18 24 25 41 17 (57) 14 14 22 22 23 38 45	19 24 42 28 38 18 23 42 26 31 40 (145 20 25 (155) 17 23 29 48 13 29 33 146) 13 20 27 (1
GB	46/83					
GB	48/83					
Pyrido	129/83					
Pyrido	130/83					
Placebo	141/83					

Values quoted are MCD in µsec
Abnormal jitters, MCD > 55 µsec, are in parentheses
BL = Blocking pair

Appendix 2: GB Ct5/pyridostigmine study
MCD values for group PA

Treatment	Nil			Placebo			Pyrido			GB+3 hr			GB+3 day		
Session / Subject	1			2			3			4			5		
41/83	14	26	27	15	15	18	15	16	17	14	21	26	19	19	20
				21	28	29	18	21	29	28	30	51	23	28	29
				35	39								34		
42/83	15	20	22	17	17	19	15	17	18	15	18	20	20	21	26
	22	28	32	25	39	41	20	26	27	22	32	35	26	29	39
				41			28	28	32	42	54	(99)	43	(63)	
							33	38	(89)						
96/83	20	26	42	7	16	17	12	21	22	17	18	20	20	28	29
	(144)			19	24	25	23	33	42	22	23	24	30	31	(73
				28	28	31	46			24	25	28	83	86)	
				33	42	49				40	41	44			
				(250)						46	(159	182)			
97/83	19	22	39	24	30	31	18	20	23	17	18	19	25	26	27
				33	54					19	31	52	28	33	34
													36	41	43
													43	49	50
													(58	66	71
													148)		
159/83	16	18	21	12	18	18	7	14	16	16	16	16	9	12	15
	21	24	24	18	19	20	16	19	19	20	20	20	16	18	22
	28	32	49	20	21	26	20	21	29	20	24	24	22	24	30
				29	32		32			26	27	33	38	48	48
										33	34	36	52	(70	75
										39	41		256	256)	
172/83	14	14	15	14	15	15	12	26	36	14	14	18	12	17	18
	20	37	42	15	18	18	37	42	45	20	20	27	18	19	21
				25	37					30	30	35	23	24	25
										39	49	59)	26	33	(99)
										90	100	131			
										196)					
36/83*	16	19	19	15	16	18	23	24	29	15	16	17			
	21	21	22	22	22	23	31	31	33	19	21	32			
	22	24	26	25	28	31	42	51	(97)	34	37	46			
	29	29	33	33	38	39				49	49	52			
	34	48		(63	149)					(85)					

* Not available for final recording session (see text)

Appendix 2: GB Ct5/pyridostigmine study
MCD values for group AP

Treatment	Nil			Active Pyrido						Placebo			GB		
Session / Subject	1			2			3			4			5		
8/83	10	10	18	14	15	16	12	13	15	16	20	22	14	14	16
	19	27	28	16	17	51	19	20	20	22	24	25	19	20	28
	29						25	28	50				29	47	52
													(105)		
11/83	19	22	33	16	17	19	17	21	24	20	21	23	18	23	26
	36	37		22	35	37				28	33	37	26	28	BL
				53						47					
140/83	13	14	16	13	13	13	11	11	21	12	14	14	21	21	25
	17	17	20	19	20	27	22	26	28	25	26	28	48	(112)	
	23	24	27	30			34	36		28	29	43			
144/83	12	12	18	14	15	19	10	11	15	13	14	16	10	12	14
	20	21	27	32	39	42	16	20	21	19	19	21	16	16	16
	31			43			21	22	22	23	28	36	20	22	23
							27	(63	>100)	47					
145/83	11	13	14	10	12	13	29	45	45	11	11	11	17	19	19
	19	20	21	14	14	19	55	(60	85	13	13	14	19	26	27
	27	33	40	21	25	39	113)			14	20	22	31	32	(69
	42	(132)								22	22	27	140)		
										34	43	(101			
										112)					
160/83	9	13	14	16	16	16	7	13	33	11	11	16	9	12	17
	18	28	28	21	23	24	33	41		23	24	31	21	21	25
	31	45		24	40	40				35	35		32	35	36
				48	(56)								38	44	50
													53		

Appendix 2: GB Ct5/pyridostigmine study
MCD values for group AA

Treatment	Nil			Active Pyrido						GB+3 hr			GB+3 day		
Session / Subject	1			2			3			4			5		
09/83	13	13	16	21	23	29	13	14	15	12	17	17	10	13	14
	17	19	19	30	37	(56)	20	29		19	20	23	16	17	17
	24												17	18	24
													46		
12/83	18	23	25	16	21	25	39	47	48	18	28	28	39	49	50
	25	25	31	27	47	50	52	(59	60	28	29	30	52	(63	63
	31	31	37	(78	102)		61	69	78	38	40	43	69	99)	
	43	(315)					90)			49	(73)				
17/83	14	19	24	13	15	19	15	20	21	15	15	17	15	17	18
	27	29	30	28			29	30	30	18	22	23	20	22	23
	31	36	38				43	46					29	30	37
	40												39	51	
18/83	12	15	16	15	17	21	18	20	24	13	14	14	13	19	24
	18	21	25	22	27	27	26	30		14	22	33	24	26	31
	33	39	(163)	29	36					34	51	(61	36	37	
										117)					
20/83	14	15	16	14	16	19	14	16	18	16	16	18	15	15	17
	17	19	20	20	20	23	18	18	19	18	20	23	17	18	18
	20	21	27	31	32	33	21	25	25	25	26	27	19	21	21
							30	50					22	32	
29/83	19	24	26	16	20	21	18	22	29	20	21	22	17	19	21
	27	34		42			31	33	35	25	26	28	24	29	36
							55			28	30	31	38	51	(60
										35	50	53	104)		

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